APPLICATION NOTE

Bruker Proteomic Product Suite for Oncology & Tumor Functional Phenotyping

Bruker's functional proteomics reveals unique multi-omic signatures and insights into oncology

In this Application Note we outline:

- Overcoming challenges in oncology
- Resolving tumor heterogeneity to reveal independent trajectories of drug tolerance
- Informing targeted combination therapy to overcome resistance with single-cell intracellular proteomics
- Understanding cancer cell communication to infer a strategy to inhibit tumor cell metastasis
- Revealing aberrant cytokine signatures of progenitor myeloid cells in disease progression in mice

BRUKER

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High Level Challenges and Applications

Application 1: Resolving Tumor Heterogeneity to Reveal Independent Trajectories of Drug Tolerance

Application 2: Informing Targeted Combination Therapy to Overcome Resistance with Single-Cell Intracellular Proteomics

Application 3: Understanding Cancer Cell Communication to Infer a Strategy to Inhibit Tumor Cell Metastasis

Application 4: Revealing Aberrant Cytokine Signatures of Progenitor Myeloid Cells in Disease Progression in Mice

Bruker Product Types that Address These Challenges:

- Single-Cell Intracellular Proteome
- Single-Cell Metabolome
- CodePlex Secretome
- Single-Cell Secretome

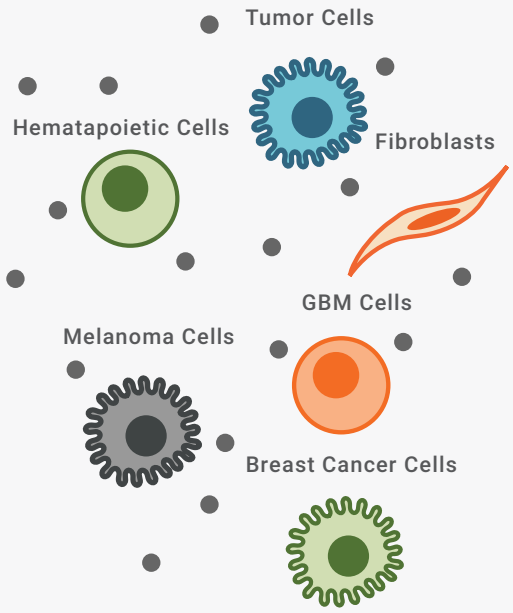
Overcoming Challenges in Oncology

Cancer cells in the tumor microenvironment secrete cytokines that interact with other cells, such as immune cells, in a 3D extracellular matrix, which facilitates intracellular communications and jointly moderates pathophysiological processes, including cancer-induced angiogenesis and metastasis. Secretomic profiles of cancer cells advance as they multiply and become more invasive, which suggests that secreted factors may be associated with proliferation and migration.

Bruker detects the key intracellular pathways in tumor cells that can uncover critical and unseen targets in oncology, helping you understand earlier in development the impacts of your therapies and the mechanisms behind patient resistance in oncology research.

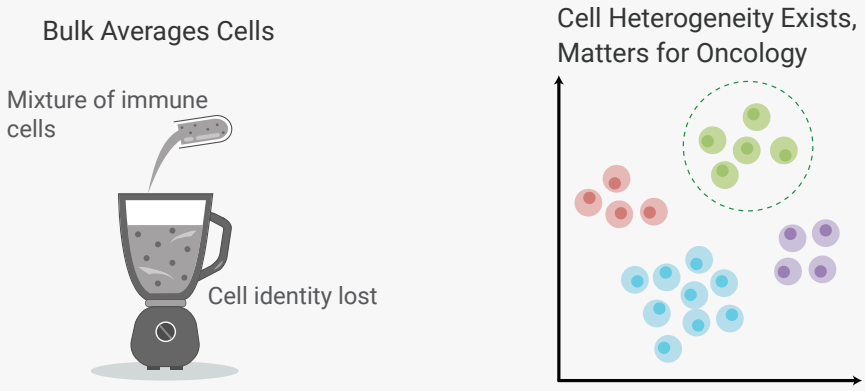
- **Challenge 1: Requires Single-Cell Intracellular Proteome & Metabolome Solutions**
- **Challenge 2: Requires Single-Cell Functional Proteomics and CodePlex Secretome Solution**
- **Challenge 3 & 4: Require Single-Cell Functional Proteomics Solution**

Cell Types and Cytokines Implicated



Functionally defining each cell type involved in the immune response in oncology

Why Cell Subsets for Multiplexing Cytokines Matter in Oncology



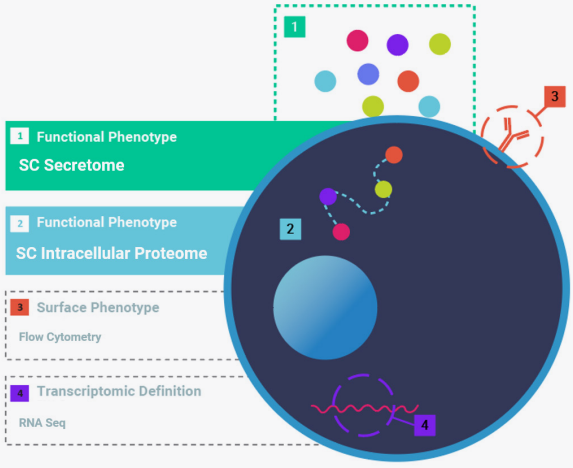
Traditional technologies average serum protein information from all cells. Bruker's cellullar functional phenotyping uncovers cellular differences to identify functional mechanisms in oncology.

Understanding Cellular Signaling Pathways is Critical for Understanding and Overcoming Resistance and Disease Progression in Oncology

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 and Overcoming Resistance in Oncology

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 oncology.

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

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

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, 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)

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

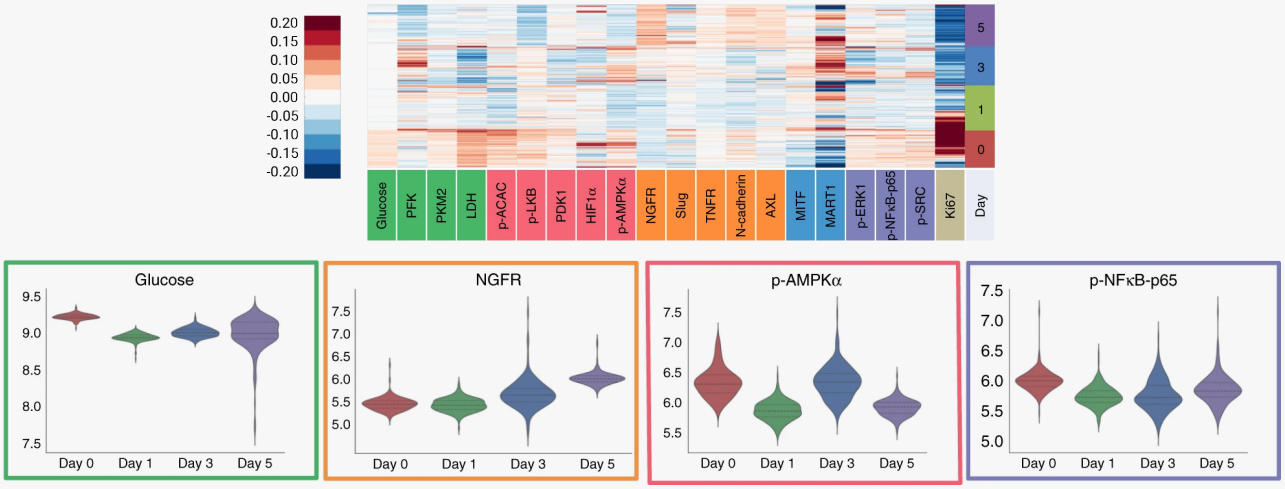
Application 1 – Resolving Tumor Heterogeneity to Reveal Independent Trajectories of Drug Tolerance

Multi-Omic Analysis of Signaling, Phenotypic, and Metabolic Regulators to Overcome Drug Resistance in Solid Tumor

Products Used

- Single-Cell Intracellular Proteome
- Single-Cell Metabolome

Capturing Metabolites, Cytoplasmic Proteins, and Phosphoproteins from Single Cells with Bruker's Proteomic Barcoding Technology



Single-Cell Intracellular Proteome and Single-Cell Metabolome were used to analyze mutant melanoma cancer cells (BRAFV600E M397) to gain further information about the transition from drug responsive to drug tolerance [1].

Highlights of Multi-Omics Analysis for Drug Resistance in Solid Tumor

- Drug resistance is a major problem in cancer treatment. Although many gene expression studies have tried to elucidate the mechanism of drug resistance in solid tumors, no previous studies have truly mapped the proteomic path(s) to resistance at the single-cell level.
- Using a well-characterized melanoma cell line that is known to rapidly develop drug resistance, multiple impactful analyses revealed changes in functional phenotype over time in individual cells.
- Bruker's Single-Cell Intracellular Proteome/Metabolome technology revealed multiple independent paths that tumor cells take in the development of drug resistance.

Su Y, et al. Multi-omic single-cell snapshots reveal multiple independent trajectories to drug tolerance in a melanoma cell line. Nature Communications, 2020.

Application 2 – 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

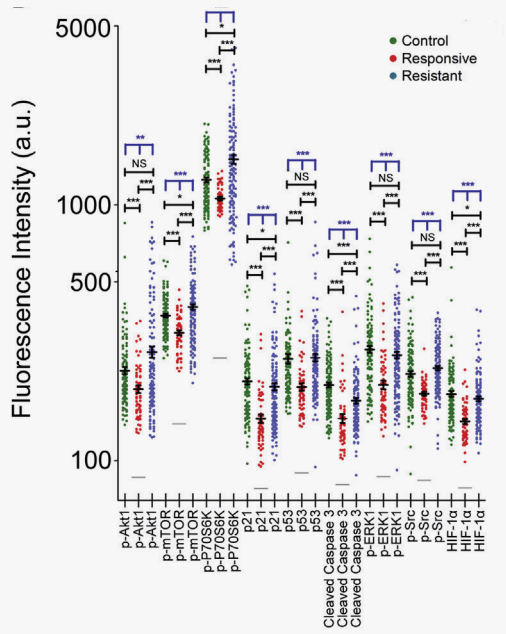
Single-Cell Intracellullar Proteome

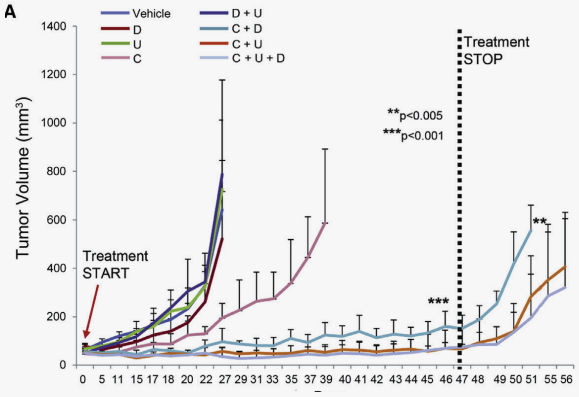
Highlights of Informing Targeted Combination Therapies with Single-Cell Intracellular Proteomics

- 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 [2].

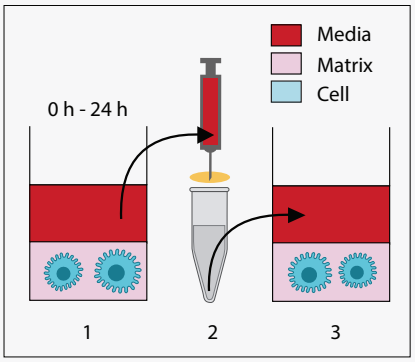
Application 3 – Understanding Cancer Cell Communication to Infer a Strategy to Inhibit Tumor Cell Metastasis

Bruker's Functional Proteomics Reveals Unique Synergistic Paracrine Signaling Pathway Promoting Cell Migration

Products Used

CodePlex Secretome

Secreted Cytokines Promote Tumor Metastasis



	HT1080WT						
	10	20	50	100	120	150	R^2
EGF							0.07
FGF							0.01
HGF							0.01
VEGF							0.40
PDGF							0.02
MIF							0.82
MCP1							0.09
RANTES							0.57
MIP1a							0.01
IFNg							0.17
TNFa							0.22
TNFb							0.68
GMCSF							0.01
IL1a							0.17
IL1b							0.10
IL2							0.09
IL4							0.14
IL5							0.34
IL6							0.85
IL8							0.83
IL10							0.00
IL12							0.03
IL13							0.25
FITC							0.41
0							57.7AU

	MDA-MB-231				
	10	20	50	100	R^2
EGF					0.91
FGF					0.86
HGF					0.03
VEGF					0.53
PDGF					0.06
MIF					0.27
MCP1					0.11
RANTES					0.00
MIP1a					0.00
IFNg					0.00
TNFa					0.38
TNFb					0.93
GMCSF					0.60
IL1a					0.11
IL1b					0.02
IL2					0.39
IL4					0.21
IL5					0.61
IL6					0.99
IL8					0.82
IL10					0.00
IL12					0.30
IL13					0.40
FITC					0.24
0					8.9AU

The secretome mediated pathway of cell migration is an adaptive process dictated by cell signaling. Understanding this mechanism provides a potential strategy towards decreasing the metastatic capacity of cancer cells [3].

Highlights of Bruker's Functional Proteomics and Secretomic Signatures in Tumor Cell Migration

- Functional phenotyping revealed IL-6 and IL-8 were secreted at high concentrations in a specific ratio and density-dependent manner.
- Proteins typically associated with promoting tumor metastasis and progression were not elevated, suggesting that IL-6 and IL-8 are responsible for driving the density-dependent cell migration within 3D matrices.
- The data reveals a possible mechanism for the promotion of tumor cell migration while inferring an approach to reduce metastatic capability of tumor cells.

Jayatilaka H, et al. Synergistic IL-6 and IL-8 Paracrine Signalling Pathway Infers a Strategy to Inhibit Tumour Cell Migration. Nature Communications, 8, 15584, 2017

Application 4 – Revealing Aberrant Cytokine Signatures of Progenitor Myeloid Cells in Disease Progression in Mice

Bruker Single-Cell Secretome Technology Reveals Distinct Cytokine Profiles Associated with Pathogenesis

Products Used

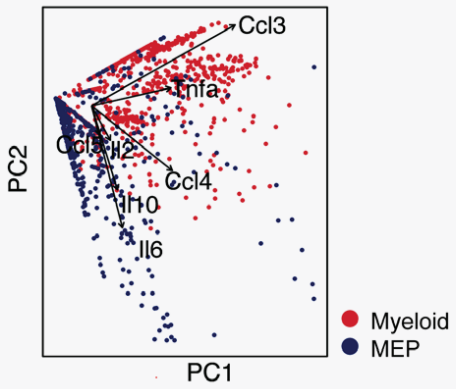
Single-Cell Secretome

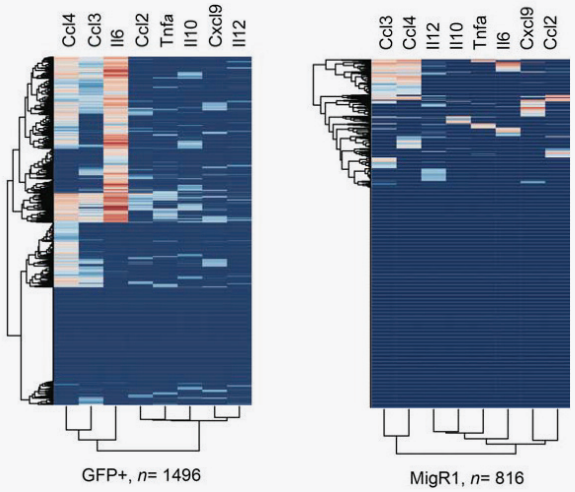
Highlights of Identifying Aberrant Cytokine Signatures in Mature and Progenitor Myeloid Cells

- Data generated with the single-cell secretome solution demonstrated that JAK1/2 inhibition leads to a rapid, potent reduction in serum cytokine levels, consistent with the rapid clinical benefits seen with JAK inhibitor therapy and demonstrating this is a direct effect of JAK kinase inhibition on cytokine production.
- Hematopoietic cells in Myelofibrosis (MF) show significant up-regulation of a spectrum of pro-inflammatory cytokines, elevation of cellular heterogeneity in cytokine secretion, and increased multifunctional cytokine production, which are not observed in normal hematopoietic cells.
- Both mature and progenitor myeloid cells contribute to increased cytokine production, and more interestingly they show distinct cytokine profiles suggesting their different roles in MF pathogenesis.

Kleppe M, et al. JAK-STAT Pathway Activation in Malignant and Non-Malignant Cells Contributes to MPN Pathogenesis and Therapeutic Response. Cancer Discovery 5: 316-331, 2015.

Pathogenic Secretion of Multiple Cytokines by MF Cells





Top: PCA analysis of single cell cytokine secretion data from MEP and myeloid cells identified two principal components, largely defined by production of Il6 and Il10 (PC1, MEP) Ccl3, and Tnfa (PC2, myeloid). Bottom: Single-cell secretomic analysis of Bone Marrow (BM) cells revealed a striking increase in the cytokine production levels and heterogeneity [4.]

Prep, Run, Analyze

Challenges & Applications

Application 1: Resolving Tumor Heterogeneity to Reveal Independent Trajectories of Drug Tolerance

Application 2: Informing Targeted Combination Therapy to Overcome Resistance with Single-Cell Intracellular Proteomics

Application 3: Understanding Cancer Cell Communication to Infer a Strategy to Inhibit Tumor Cell Metastasis

Application 4: Revealing Aberrant Cytokine Signatures of Progenitor Myeloid Cells in Disease Progression in Mice

Solutions

- Single Cell intracellular proteome and metabolome reveals multiple independent pathways to drug tolerance in solid tumor
- Single-Cell Phosphoproteomics identifies adaptive mechanism of resistance
- CodePlex Secretome reveals unique synergistic paracrine signaling pathway promoting cell migration
- Single-Cell Secretome technology reveals distinct cytokine profiles associated with pathogenesis

References

1. Su Y, et al. Multi-omic single-cell snapshots reveal multiple independent trajectories to drug tolerance in a melanoma cell line. Nature Communications, 2020.
2. Wei W, et al. Single-Cell Phosphoproteomics Resolves Adaptive Signaling Dynamics and Informs Targeted Combination Therapy in Glioblastoma. Cancer Cell, 2016
3. Jayatilaka H, et al. Synergistic IL-6 and IL-8 Paracrine Signalling Pathway Infers a Strategy to Inhibit Tumour Cell Migration. Nature Communications, 8, 15584, 2017
4. Kleppe M, et al. JAK-STAT Pathway Activation in Malignant and Non-Malignant Cells Contributes to MPN Pathogenesis and Therapeutic Response. Cancer Discovery 5: 316-331, 2015.