APPLICATION NOTE

Bruker Proteomic Product Suite for Tumor Resistance

Bruker's Single-Cell Signaling Reveals Coordinated Signals from Tumor Cells Driving Resistance

In this Application Note we outline:

- Overcoming Challenges in Tumor Resistance
- Informing Targeted Combination Therapy to Overcome Resistance in Glioblastoma (GBM)
- Resolving Tumor Heterogeneity to Reveal Independent Trajectories of Drug Tolerance
- Early Signs Of Drug Resistance In Rare Melanoma Cells
- Elucidating Mechanisms of Tumorigenesis

BRUKER

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High Level Challenges and Applications

Application 1: Informing Targeted Combination Therapy to Overcome Resistance in Glioblastoma (GBM)

Application 2: Resolving Tumor Heterogeneity to Reveal Independent Trajectories of Drug Tolerance

Application 3: Early Signs of Drug Resistance in Rare Melanoma Cells

Application 4: Elucidating Mechanisms of Tumorigenesis

Bruker Product Types that Address These Challenges:

Single-Cell Signaling

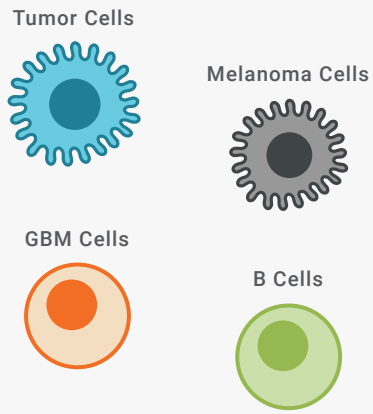
Overcoming Challenges of Tumor Resistance

A major challenge in the field of cancer immunology is that tumor cells can develop resistance to targeted therapies. Researchers are striving to identify pathways involved in cell state transition toward drug resistant phenotypes. Measuring and identifying early activation pathways is critically important when tackling drug resistance.

In the early stages of resistance, some cells go through adaptive rewiring. Bruker detects the key coordinated signals in tumor cells that can uncover critical and unseen targets in oncology, helping researchers understand the impacts of their therapies earlier in development, and the mechanisms behind therapy resistance in oncology research.

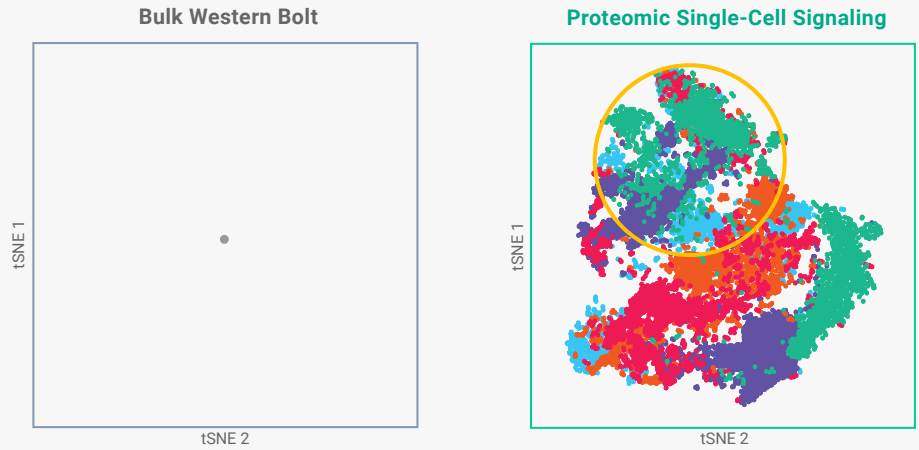
- **Challenges 1-4 Require Single-Cell Signaling Solution**

Cell Types Implicated



Functionally defining tumor cells.

Why Single-Cell Signaling Coordination Matters in Oncology



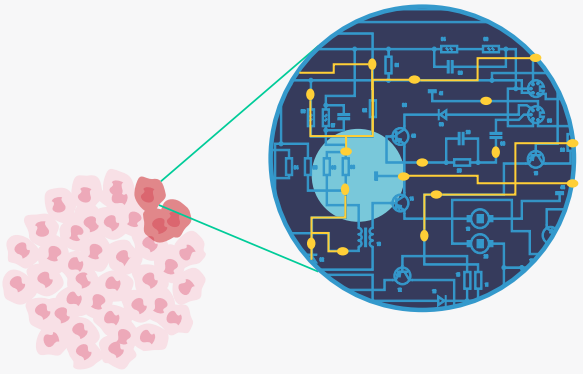
Traditional technologies, including western blot, average cell signals to compare broad trends. Bruker's Single-Cell Signaling identifies intracellular resistance pathways by coordinating phosphoproteomic signals from rare cells.

Understanding Single-Cell Signaling Coordination is Critical for Overcoming Resistance and Disease Progression In Oncology

Traditional technologies, including western blot, average protein information from all cells. Responder and non-responder are not related to ICP at this moment. Data shows that the specific proteins that are produced by each heterogenous cancer cell matter, and Bruker's Single-Cell Signaling uncovers these cellular differences.

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

Coordinated Signals from Rare Cells are Required to Identify Early Pathway Activation



Western blot misses coordinated signals from rare cells because all cells are averaged together. Bruker's Single-Cell Signaling reveals coordinated signals from rare tumor cells driving resistance.

Bruker's Proteomic Single-Cell Signaling Coordinates Phosphoproteomic Signals from Rare Cells

The award-winning Single-Cell Signaling Solution uniquely coordinates proteomic signals directly from each single cell, across thousands of single cells in parallel, for the first time. The leap over existing technologies like western blot, mass spectrometry, and flow cytometry, is the Single-Cell Signaling solution's ability to quantify highly multiplex 15+ phosphoproteins simultaneously from each cell. This enables the detection of critical activation pathways in rare cells and cell subsets, providing insights into rewiring the phosphoproteome to fight cancer.

Bruker's platform analyzes up to four Single-Cell Signaling chips simultaneously, connecting intracellular proteins back to each individual cell and unlocking the next level of resolution in cellular and immune research. The IsoLight and IsoSpark measure phosphoprotein intensity, thus enabling insights into intracellular pathways, disease progression, and mechanisms of therapy resistance.

Single-Cell Signaling Panels

Tumor Signaling Panel	Human Single Cell Signaling - Adaptive*	Human Single Cell Signaling - Myeloid*
P-Met	P-MEK1/2	P-MEK1/2
P-p44/42 MAPK	P-NF-kB P65	P-NF-kB P65
P-p90RSK	P-Stat3	P-Stat3
P-MEK1/2	P-Stat1	P-Stat1
P-NF-kB P65	P-Stat5	P-Stat5
P-Stat3	Granzyme B	GM-CSF
P-Stat1	IFN-y	IL-8
P-Stat5	Perforin	IL-10
P-S6 Ribosomal	TNF-a	TNF-a
P-eIF4E	GM-CSF	IL-1B
P-PRS40	IL-2	IL-6
Cleaved PARP†	IL-7	MIP-1a
P-IkBα	IL-8	MIP-1B
Alpha Tubulin	IL-10	MCP-1
	MIP-1a	
	MIP-1B	

* In Pipeline
† Inquire about availability

Kinases | Translation Factors | Cytokines | Apoptosis Marker
Transcription Factors | Signal Suppressor | Chemokines | Transcription Factor Inhibitor

Identify Adaptive Signaling Networks: Accelerate development of targeted therapies to overcome resistance & metastases

Highly Multiplexed per Cell: Targets 15+ intracellular proteins from each cell

Pathways Revealed: See multiple coordinated protein pathways engaged for first time

Fully Automated Proteomics Workflow

Published: In a variety of peer-reviewed journals & indication types

Our proprietary & patented "Proteomic Barcoded" IsoCode Chip uniquely captures single cells and detects the full range of phosphoproteins, enabling functional characterization of the signaling networks and resistance pathways of cancer cells.

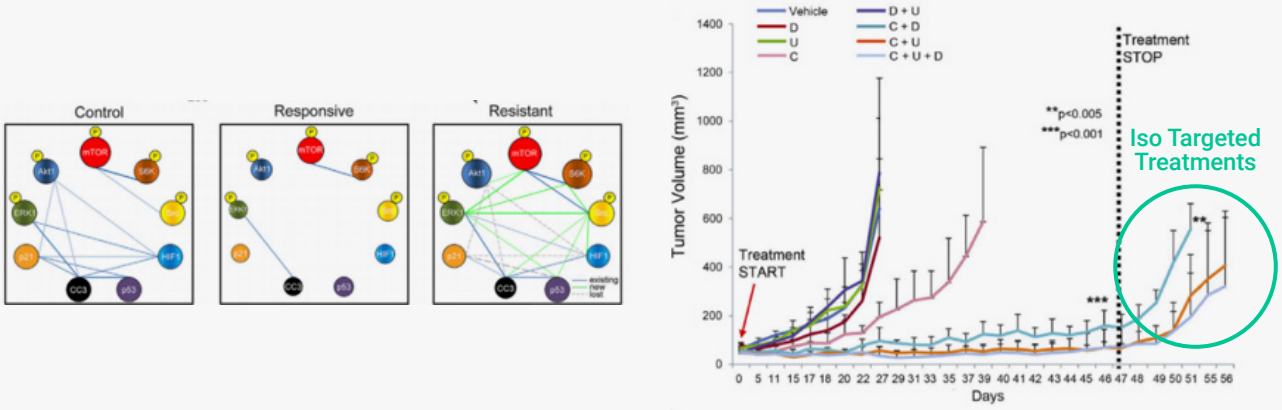
Application 1 – Informing Targeted Combination Therapy to Overcome Resistance in Glioblastoma (GBM)

Single-Cell Signaling Identifies Adaptive Mechanism of Resistance

Products Used

Single-Cell Signaling

Informing Better Combination Therapies to Overcome Resistance



Left: mTOR, ERK and SRC Pathway were detected by Bruker's system in rare single cells through Signal Coordination. Right: Results for the seven monotherapy or combination therapies based upon the predictions from the Single Cell Signaling. All seven predictions proved correct

Highlights of Informing Targeted Combination Therapies with Single-Cell Signaling

- Single-Cell Signaling uncovers rewiring of signaling pathways, revealing dominant mechanism of resistance.
- Single-Cell Signaling identifies changes in signaling nodes missed by genomic analysis and western blot.
- Targeting these signaling nodes before treatment blocks resistance, demonstrating the importance of signaling and network rewiring 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

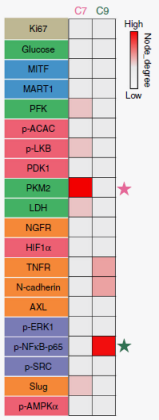
Application 2 – Resolving Tumor Heterogeneity to Reveal Independent Trajectories of Drug Tolerance

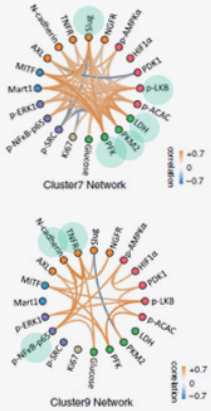
Bruker's Single-Cell Technology Reveals Early Coordinated Signals of Tumor Resistance in Melanoma

Products Used

Single-Cell Signaling

Capturing Phosphoproteins from Single Cells with Bruker's Proteomic Barcoding Technology





Single-Cell Signaling was used to analyze mutant melanoma cancer cells (BRAFV600E M397) to gain further information about the transition from drug responsive to drug tolerance.

Highlights of Identifying Adaptive Resistance Pathways in Melanoma Cell Line

- 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.
- Bruker technology identified separate significant pathways involved in the BRAFi-induced cell-state transitions to the drug-tolerant state and thus provided novel targets for drug treatment.
- Bruker revealed that these distinct targeted pathways involve different cell types, have different regulators, and are independently druggable to prevent long-term 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* 11: 2345, 2020.

Application 3 – Early Signs Of Drug Resistance In Rare Melanoma Cells

Single-Cell Signaling Reveals the Emergence of Drug-Activated Signaling at the Initiation of Adaptive Transition

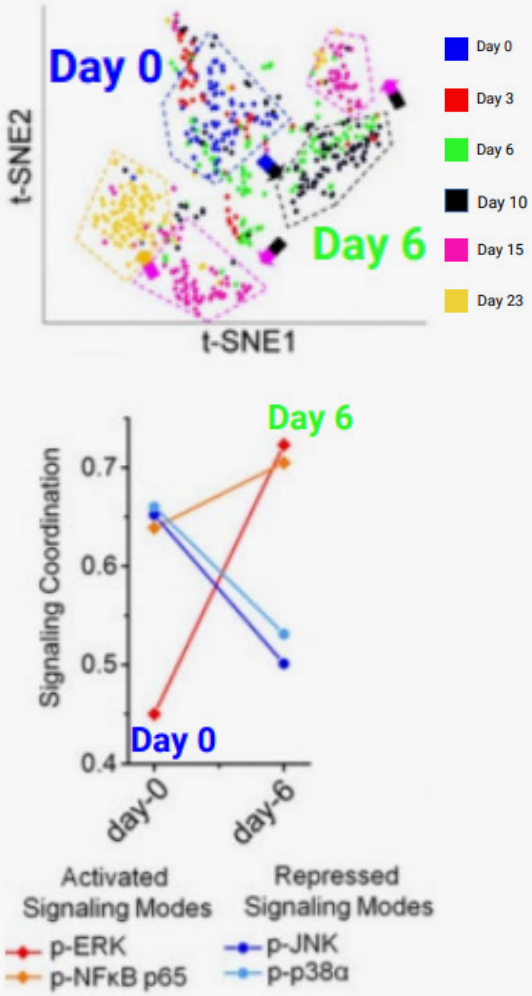
Products Used

Single-Cell Signaling

Highlights of Interrogating Signaling Pathways Directly Targeted by BRAFi

- Bruker's Single-Cell Signaling predicted early markers of resistance based on coordinated ERK and NFkB p65, JNK and p38 signal.
- Early markers of resistance were detected before transcriptomic changes & appearance of drug resistance.
- Bruker reveals that combining BRAFi with MEK and NFκB p65 inhibition might prevent the adaptive cell state transition toward drug-resistant phenotypes.

Single-Cell Proteomic Profiling of Melanoma Cell Line Provides Insight Toward Drug-Resistant Phenotypes



Single-Cell Signaling identifies proteins that participate most strongly in the signaling coordination at adaptive cell state transition

Su, Y. et al. Single-cell analysis resolves the cell state transition and signaling dynamics associated with melanoma drug-induced resistance. PNAS 114, 13679–13684 (2017)

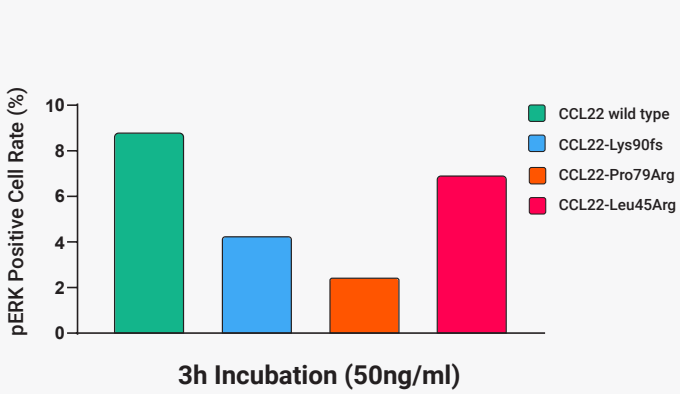
Application 4 – Elucidating Mechanisms of Tumorigenesis

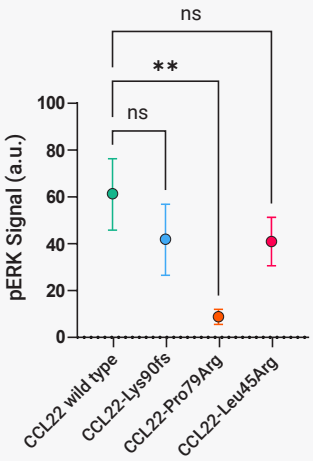
Bruker's Single-Cell Phosphoproteomic Technology Uncovers Pathway Activation in Cancer Cells

Products Used

Single-Cell Signaling

Single-Cell Signaling Shows Reduced Phosphorylation of ERK in Mutant CCL22





Ba/F3-CCR4 cells were stimulated with exogenous recombinant wild type or mutant CCL22. Single cell phosphoproteomic detection showed lower positive cell rate of phosphorylated ERK (left) and also decreased mean pERK signal intensity in mutant CCL22 treated cells (right).

Highlights of Using Single-Cell Signaling to Uncover Pathway Activation in Cancer Cells

- Somatic mutations in the chemokine gene CCL22 have been identified as the hallmark of a distinct subset of CLPD-NK and those mutations illustrate a unique mechanism of tumor formation in which gain-of-function chemokine mutations promote tumorigenesis by biased GPCR signaling and dysregulation of microenvironmental crosstalk.
- Ba/F3-CCR4 cells were stimulated with exogenous recombinant wild type or mutant CCL22. Single cell phosphoproteomic detection via IsoLight discovered lower positive cell rate of phosphorylated ERK and also decreased mean pERK signal intensity in mutant CCL22 treated cells.
- Bruker intracellular proteomic panel validated the reduction of ERK phosphorylation in mutant CCL22 treated cells. The result may explain attenuation of CCR4 internalization, which supports the notion of CCL22 mutation-specific, biased CCR4/GPCR signaling through impaired β-arrestin recruitment and signaling.

Baer C, et al. CCL22 mutations drive natural killer cell lymphoproliferative disease by deregulating microenvironmental crosstalk. Nature Genetics, 2022.

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Challenges & Applications

Application 1: Informing Targeted Combination Therapy to Overcome Resistance in Glioblastoma (GBM)

Application 2: Resolving Tumor Heterogeneity to Reveal Independent Trajectories of Drug Tolerance

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Application 4: Elucidating Mechanisms of Tumorigenesis

Solutions

- Single-Cell Signaling Identifies Adaptive Mechanisms of Resistance
- Bruker's Single-Cell Technology Reveals Early Coordinated Signals of Tumor Resistance in Melanoma
- Single-Cell Signaling Reveals the Emergence of Drug-Activated Signaling at the Initiation of Adaptive Transition
- Bruker's Single-Cell Phosphoproteomic Technology Uncovers Pathway Activation in Cancer Cells-