APPLICATION NOTE

Bruker Proteomic Product Suite for NK Cells

Bruker's functional proteomics reveals unique secretomic signatures and insights into NK Cells

In this Application Note we outline:

- Overcoming challenges in NK Cell research
- Comparing subtle gene edits in NK Cell therapies
- Immunotherapy-induced NK Cell and T Cell activation provides early translational insights
- Off-the shelf CAR-NK manufacturing optimization
- Revealing mechanisms of immune evasion in solid tumors through assessing NK Cell potency
- Uncovering transcriptional regulators of NK Cell cytotoxic potency

BRUKER

Prep, Run, Analyze

High Level Challenges and Applications

Application 1: Comparing Subtle Gene Edits in NK Cell Therapies

Application 2: Immunotherapy-induced NK Cell and T Cell Activation Provides Early Translational Insights

Application 3: Off-the Shelf CAR-NK Manufacturing Optimization

Application 4: Revealing Mechanisms of Immune Evasion in Solid Tumors Through Assessing NK Cell Potency

Application 5: Uncovering Transcriptional Regulators of NK Cell Cytotoxic Potency

Bruker Product Types that Address These Challenges:

Single-Cell Secretome (Human)

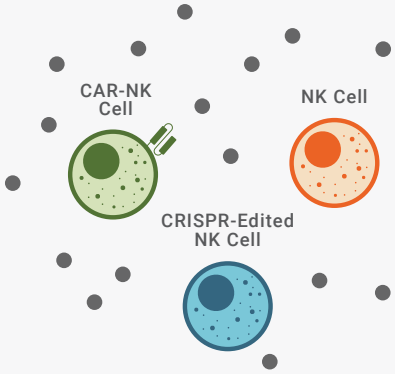
Single-Cell Secretome (Mouse)

Overcoming Challenges in NK Cell Research

One of the newer therapies gaining attention, natural killer (NK) cell therapy, has the potential to lower the occurrence of immune-related adverse effects. As a part of the innate immune system, NK cells are essential to immune responses as one of the first lines of defense against foreign invaders. However, challenges such as limited persistence and tumor evasion test the durability and efficacy of these novel therapies*. Understanding the functional mechanisms of NK cell potency and persistence is critical to overcoming this hurdle.

Immune cells with high polyfunctionality are considered to be the cells with "superpowers," which can be beneficial or detrimental in terms of their function in different disease indications. The presence of "superhero" cells have correlated to cell therapy persistence and potency in various studies while "supervillain" cells have been shown to drive inflammation and toxicity. Identifying these cellular subsets is critical for understanding the biomarkers of immune response and disease progression.

Cell Types and Cytokines Implicated

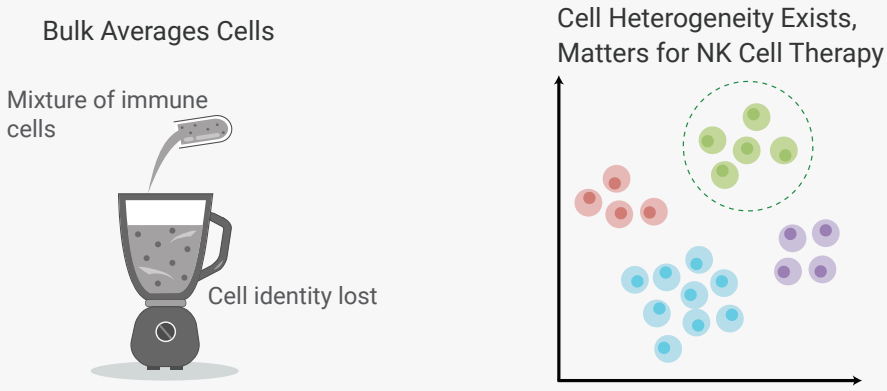


Functionally defining NK cells

- **Challenges 1: Require Mouse Single-Cell Secretome Solution**
- **Challenge 2: Require Human and Mouse Single-Cell Secretome Solution**
- **Challenges 3-5: Require Human Single-Cell Secretome Solution**

*Liu, S., Galat, V., Galat4, Y. et al. NK cell-based cancer immunotherapy: from basic biology to clinical development. JJournal of Hematology & Oncology 14, 7 (2021). https://doi. org/10.1186/s13045-020-01014-w

Why Cell Subsets for Multiplexing Cytokines Matter for NK Cell Therapy



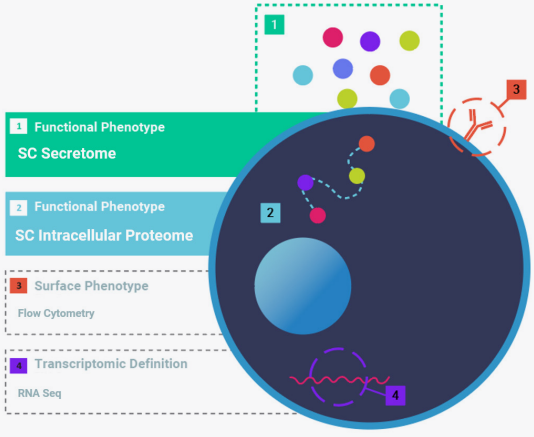
Traditional technologies average serum protein information from all cells. Bruker's cellullar functional phenotyping uncovers cellular differences to identify functional mechanisms in NK Cell therapies.

Understanding Cellular Immune Function is Critical for Understanding Response in NK Cell Therapy

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, essential functional extracellular phenotypic differences that reveal the biological drivers of patient response may be missed. 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 phenotypic differences that reveal the biological drivers of patient response may be missed.

Prep, Run, Analyze

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.

Application 1 – Comparing Subtle Gene Edits in NK Cell Therapies

Single-Cell Functional Secretome Reveals Differences in CISH-Deleted (CISH-/-) iPSC-NK Cells for NK Cell Therapy Development to Enhance Decision Making

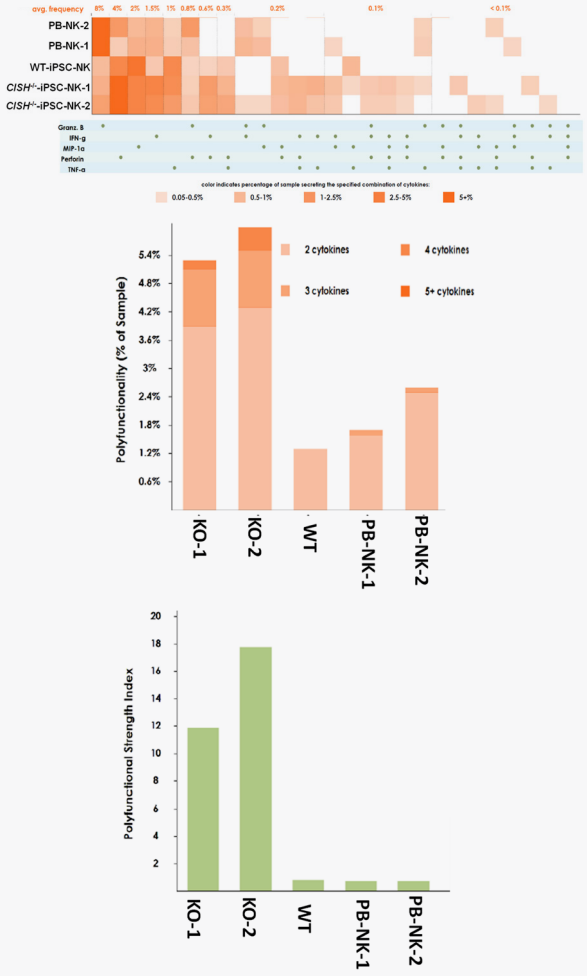
Products Used

Single-Cell Secretome (Human)

Highlights of Insights into CISH-/- iPSC-NK cells for NK Cell Therapy Development:

- Single-cell phenotyping metrics underscore significant differences between CISH-/- iPSC-NK cells and wild type cells.
- CISH-/- cells showed enrichment of polyfunctional cell subsets compared to the wild type samples.
- Single cell metrics of the CISH-/- cells were substantially higher than those of the wild type cells, indicating that CIS plays a key role in regulating NK cell activation-induced exhaustion and that Notch activation prevents this exhaustion and enables production of functionally hyperactive NK cells.
- At day 35, CISH-/- iPSC-NK demonstrate better anti-tumor activity *in vivo*; results are consistent with 10x higher *in vitro* Polyfunctional Strength Index (PSI) of CISH-/- iPSC-NK cells relative to wild type cells.

CISH-/- iPSC-NK Cell Product Analyses: *in vitro* Correlates to Mouse Model



Critical potency differences between CISH-/- and wildtype cells correlated with *in vivo* mouse response to therapy

Zhu et al., 2020, Cell Stem Cell 27, 224–237 August 6, 2020

Application 2 – Immunotherapy-induced NK Cell and T Cell Activation Provides Early Translational Insights

Bruker's Single-Cell Proteomics Demonstrates NK Cell and T Cell Persistence in Response to a Novel PEG-IL2 Agonist in Solid Tumor

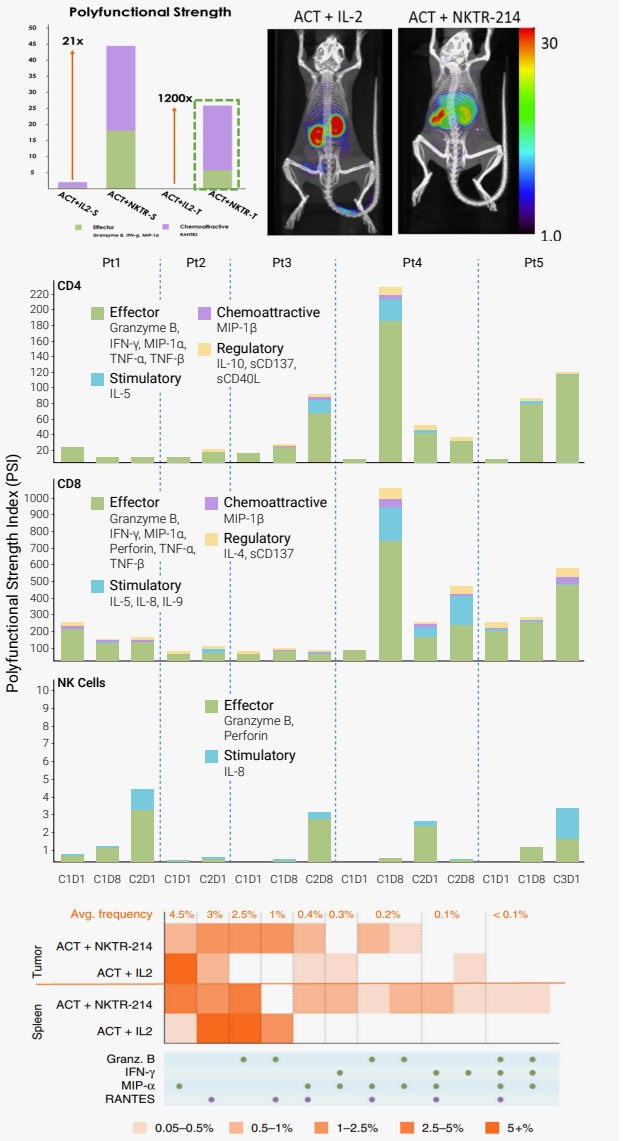
Products Used

Single-Cell Secretome (Mouse and Human)

Highlights of Applying Single-Cell Functional Proteomics to Predict Anti-Tumor Response

- The Bruker platform showed mechanistic upregulation of patients' NK cells after a novel PEG-IL2 agonist therapy, NKTR-214, corresponding with persistence in solid tumors.
- Single-Cell Secretome technology was used to profile translational insights between early-stage development of combination NKTR-214 with adoptive cell transfer therapy. In response to NKTR-214, researchers saw upregulated polyfunctionality in mouse CD8+ T cells after adoptive transfer, which correlated with increases of proliferation, homing, and anti-tumor T cell persistence *in vivo*.
- In patients with melanoma receiving NKTR-214, researchers observed superior anti-tumor activity as well as an increase in polyfunctional T and NK cells in peripheral blood.

Predicting the Anti-Tumor Response of a Novel PEG-IL2 Agonist



In response to PEG-IL2, researchers saw upregulated polyfunctionality in mouse CD8+ T cells after adoptive transfer, which correlated with increases of proliferation, homing, and anti-tumor T cell persistence *in vivo*. The enhanced PSI was also observed in blood T cells and NK cells from melanoma patients who received PEG-IL2 therapy.

Parisi G, et al. Persistence of adoptively transferred T cells with a kinetically engineered IL-2 receptor agonist. Nature Communications 11: 660, 2020

Application 3 – Off-the Shelf CAR-NK Manufacturing Optimization

Bruker's Technology Provides a Product Quality Metric for Optimizing the Manufacturing Method of an Off-the Shelf CAR-NK Cell Therapy

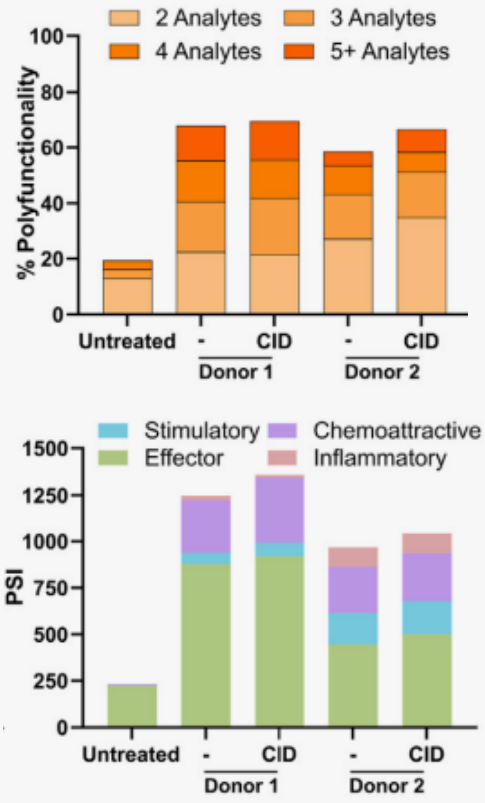
Products Used

Single-Cell Secretome (Human)

Highlights of Applying Single Cell Functional Proteomics to CAR-NK Manufacturing

- Bruker's Single-Cell Secretome Solution was used to assess cytokine secretion profiles of HER2-CAR-NK cells, in comparison to unmodified NK cells. CAR-NK cells secreted a range of effector cytokines including: granzyme B, perforin, TNF-α, MIP1-β, MCP-1, RANTES, and IL-8. In contrast, less than 13% of unmodified cells secreted granzyme B and less than 3.5% secreted the other cytokines. Additionally, unmodified NK cells secreted 2–3 effector molecules on average, while CAR-NK cells were capable of producing up to 10 effector molecules.
- Data from this study suggests that inhibition of TBK1 and IKKε significantly improves the transduction of NK cells and results in a fully functional cell product with a superior cytotoxic capacity.
- Utilizing data uncovered with Bruker' single cell proteomics platform, the authors were able to characterize reliable generation of genetically modified NK cells using VSV-G LVs. The study's NK cell transduction method could potentially be adapted to clinical production of allogeneic CAR-NK cell therapies, which has large implications in the field of off-the shelf cell therapy research.

Cytokine Secretion Profiles of HER2-CAR-NK Cells vs. Unmodified NK Cells



Single-Cell Functional Proteomics reveals inhibition of TBK1 and IKKε improves potency and cytotoxic capacity of CAR-NK cells.

Chockley P, et al. Transient blockade of TBK1/IKKε allows efficient transduction of primary human natural killer cells with vesicular stomatitis virus G-pseudotyped lentiviral vectors. *Cytotherapy* 2021; 23(9).

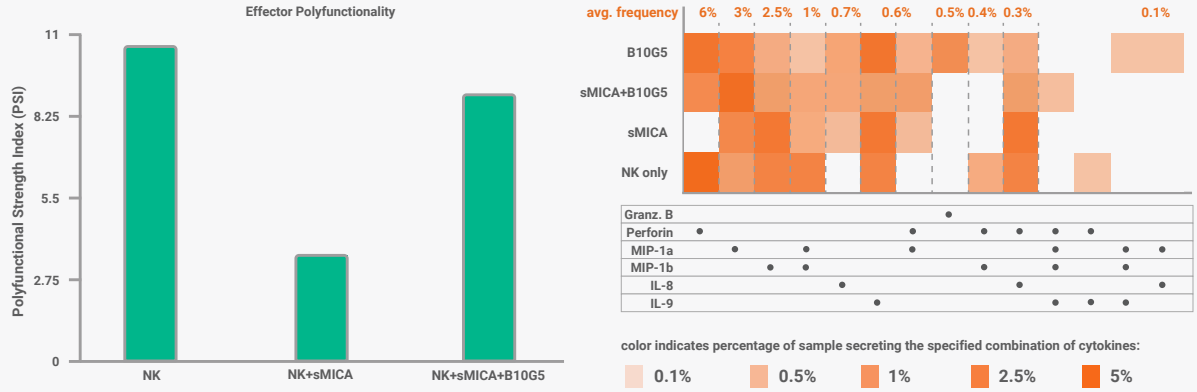
Application 4 – Revealing Mechanisms of Immune Evasion in Solid Tumors Through Assessing NK Cell Potency

Single-Cell Proteomics Reveals a Molecular Mechanism that Mediates Immune Suppression Demonstrated by Loss of NK Cell Polyfunctionality

Products Used

Single-Cell Secretome (Human)

Single-Cell Functional Proteomics Discovers a Potential Target for Enhancing NK Cell-Based Cancer Immunotherapy



Single-cell polyfunctional data revealed the role of soluble MIC (sMIC) on NK cell polyfunctionality and the ability to restore polyfunctionality through the use of an sMIC-clearing antibody (B10G5).

Highlights of Single-Cell Secretome Data as a Pre-Clinical Choice Metric

- Single-cell functional proteomics data revealed that NK cells treated with soluble MIC (sMIC), a molecule often found in the tumor microenvironment, showed a reduction in their polyfunctional strength compared to untreated NK cells, as well as a depletion in the secretion of cytotoxic effector cytokines (functional heatmap).
- The depletion in polyfunctional strength index (PSI) and effector cytokines by sMIC interaction could be recovered through the addition of the antibody B10G5 that specifically clears sMIC.
- This study demonstrated that sMIC regulates NK cell cytotoxicity through the CBM signalosome pathway and that interaction with sMIC reduces NK cell polyfunctionality, providing insight into how the anti-cancer function of NK cells is mediated by inhibitory and activating signals, and how the inhibitory signals which enable cancer progression can be counteracted.

Dhar P, et al. Tumor-derived NKG2D ligand sMIC reprograms NK cells to an inflammatory phenotype through CBM signalosome activation. *Communications Biology* 2021; 4(905).

Application 5 – Uncovering Transcriptional Regulators of NK Cell Potency

Single-Cell Functional Proteomics Reveals the Functional Impact of Transcription Factor on NK Cell Polyfunctionality

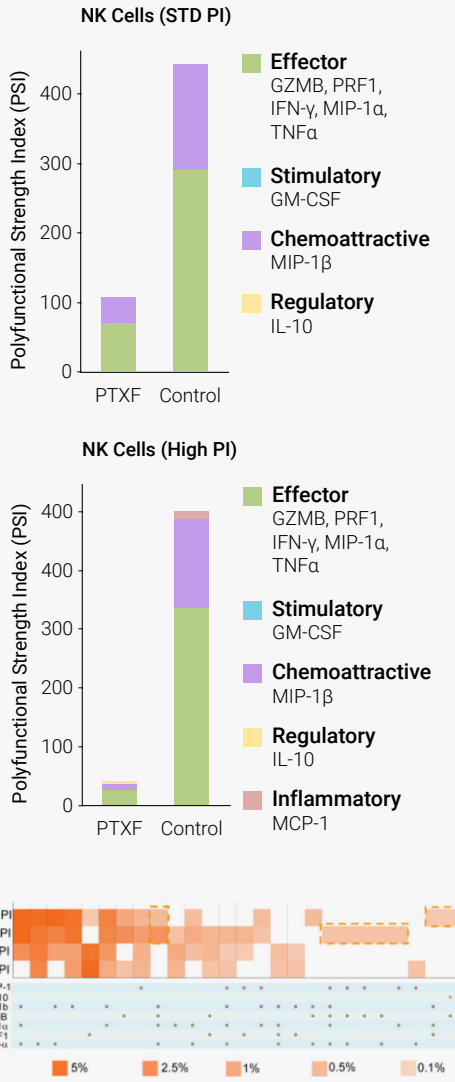
Products Used

Single-Cell Secretome (Human)

Highlights of Single-Cell Functional Proteomic Data on UncoveringRole of Transcription Factors on Cell Function

- Although it is known that the transcription factor c-Rel plays a role in T and B cell activation, c-Rel's role in NK cells was unknown.
- Single-cell functional proteomics revealed that when c-Rel was inhibited, NK cells had reduced polyfunctionality and a less heterogenous cytokine secretion profile, including a reduction in granzyme B secretion (functional heatmap).
- The reduction in polyfunctionality was also correlated with a reduced cytotoxic capacity in c-Rel deficient NK cells.
- These results suggest that c-Rel regulates the anti-tumor functionality of NK cells and that therapeutically targeting c-Rel could potentially restore the cytotoxic capacity of dysfunctional NK cells.

Bruker' Unique PSI is Critical for Understanding Functional Impacts of Transcriptomic Level Changes on NK Cell Cytotoxicity



c-Rel inhibition yields reduced polyfunctionality and less heterogenous secretion profiles in NK cells

Vicioso et al. NF-κB c-Rel Is Dispensable for the Development but Is Required for the Cytotoxic Function of NK Cells. Frontiers in Immunology. 2021.

Prep, Run, Analyze

Challenges & Applications

Application 1: Comparing Subtle Gene Edits in NK Cell Therapies

Application 2: Immunotherapy-induced NK Cell and T Cell Activation Provides Early Translational Insights

Application 3: Off-the Shelf CAR-NK Manufacturing Optimization

Application 4: Revealing Mechanisms of Immune Evasion in Solid Tumors Through Assessing NK Cell Potency

Application 5: Uncovering Transcriptional Regulators of NK Cell Potency

Solutions

- Single-Cell Functional Secretome Reveals Differences in CISH-Deleted (CISH-/-) iPSC-NK Cells for NK Cell Therapy Development to Enhance Decision Making
- Bruker's Single-Cell Proteomics Demonstrates NK Cell and T Cell Persistence in Response to a Novel PEG-IL2 Agonist in Solid Tumor
- Bruker's Technology Provides a Product Quality Metric for Optimizing the Manufacturing Method of an Off-the Shelf CAR-NK Cell Therapy
- Single-Cell Proteomics Reveals a Molecular Mechanism that Mediates Immune Suppression Demonstrated by Loss of NK Cell Polyfunctionality
- Single-Cell Proteomics Reveals the Functional Impact of Transcription Factor on NK Cell Polyfunctionality