APPLICATION NOTE

Bruker Proteomic Product Suite for CAR-T Therapy

Bruker's functional proteomics reveals unique secretomic signatures and insights into CAR-T therapy

In this Application Note we outline:

- Overcoming challenges in CAR-T therapy
- Overcoming antigen escape in Multiple Myeloma
- Single-cell proteomics in persistence and overcoming antigen escape
- Comparing subtle gene edits in NK cell therapies
- Precision analytics in automated CAR-T manufacturing with single-cell proteomics
- Preinfusion biomarkers for predicting clinical outcome in CAR-T cell treatment

BRUKER

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High Level Challenges and Applications

Application 1: Development of Next-Generation CAR-T Products for Solid Tumor

Application 2: Overcoming Antigen Escape in Multiple Myeloma

Application 3: Comparing Subtle Gene Edits in NK Cell Therapies

Application 4: Single-Cell Proteomics in Persistence and Overcoming Antigen Escape

Application 5: Precision Analytics in Automated CAR-T Manufacturing with Single Cell Proteomics

Application 6: Preinfusion Biomarkers for Predicting Clinical Outcome in CAR-T Cell Treatment

Bruker Product Types that Address These Challenges:

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

Overcoming Challenges in CAR-T Therapy

CAR-T therapies have quickly shifted the direction of treatments for aggressive diseases, such as blood cancers, where previous treatments were limited. Determining cellular fitness is a top challenge in moving the next generation of cell therapy treatments forward.

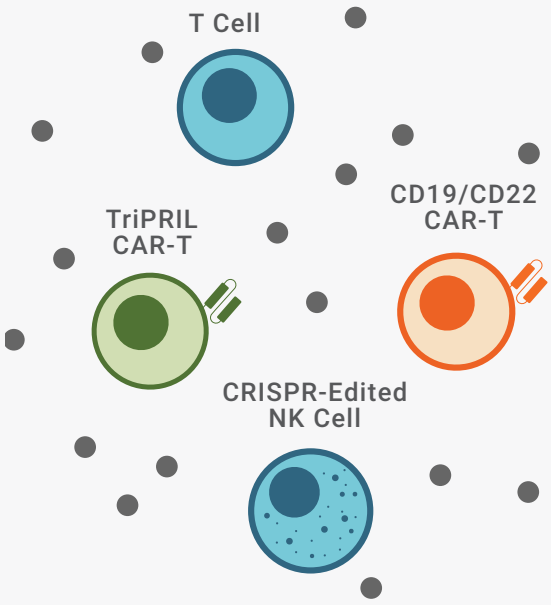
Because cell therapy relies on immune cells from patients or healthy donors in the case of allogeneic therapies, development and production is more complicated. To ensure that these cell products are potent and effective, knowing how to engineer potency and durability throughout the development and bioprocessing stages is crucial.

Challenges 1-3: Require Mouse Single-Cell Secretome Solution

Challenge 4: Requires Human and Mouse Single-Cell Secretome Solution

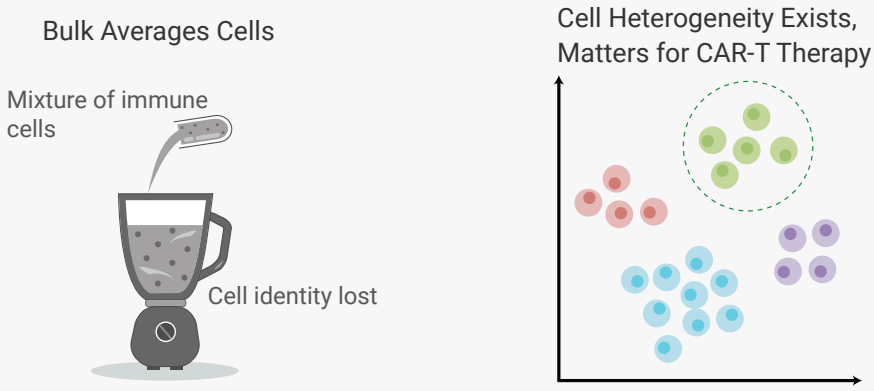
Challenges 5-6: Require Human Single-Cell Secretome Solution

Cell Types and Cytokines Implicated



Functionally defining each cell type involved in the immune response for CAR-T therapy

Why Cell Subsets for Multiplexing Cytokines Matter in CAR-T Therapy



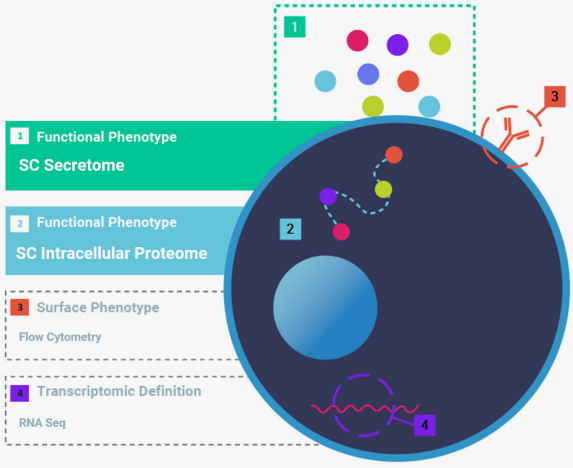
Traditional technologies average serum protein information from all cells. Bruker's cellullar functional phenotyping uncovers cellular differences to identify functional mechanisms in CAR-T therapies.

Understanding Cellular Immune Function is Critical for Understanding Response in CAR-T 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 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 CAR-T Therapy

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 constructs in CAR-T therapy.

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

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)

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

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).

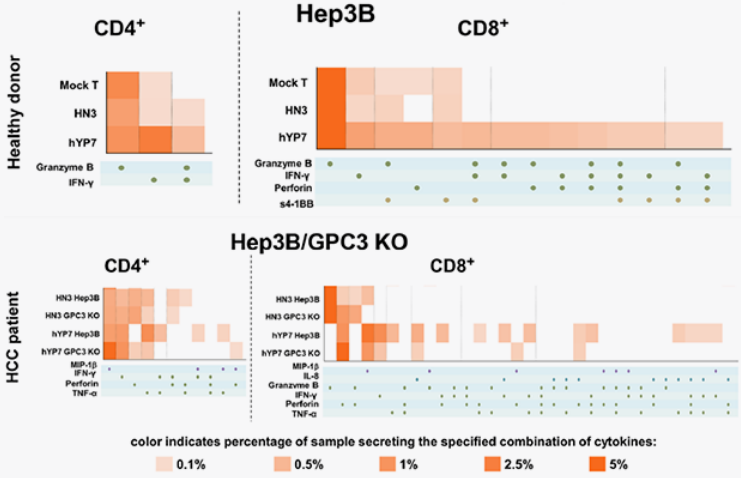
Application 1 – Development of Next-Generation CAR-T Products for Solid Tumor

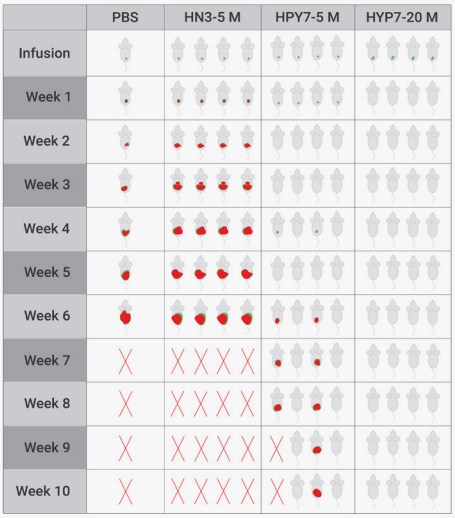
Single-Cell Functional Phenotyping Predicts Persistence in Cutting-Edge Cell Therapies for Difficult to Treat Liver Cancer

Products Used

Single-Cell Secretome (Mouse)

Functional Phenotyping Reveals Critical Functional Drivers That Correlated to CAR-T Cell Persistence in Solid Tumor





66% of mice treated with the hYP7 CAR-T cells had their tumors eliminated by week 3, while mice treated with the HN3 CAR-T cells did not have a reduction in tumor burden. The hYP7 CAR-T treated mice also continued to be free of tumors after additional Hep3B cells were introduced [1].

Highlights of Insights into Cutting-Edge CAR-T Therapy Development:

- Demonstration of a uniquely positive correlation of enhanced polyfunctionality by single-cell IsoCode proteomics with persistence of T cell response and *in vivo* killing capacity of GPC3-specific humanized YP7 (hYP7) CAR-T cell products to HCC.
- Single-cell functional phenotyping revealed the superior antitumor mechanisms of GPC3-targeted hYP7 CAR-T cell products in inhibiting Wnt signaling and promoting the perforin/granzyme-mediated apoptosis in tumor cells through enhanced polyfunctional CD8+ T cells subsets identified by single-cell functional proteomics.
- Single-cell proteomics demonstrates the antigen-driven persistence and expansion of polyfunctional hYP7 CAR-T cells in the tumor microenvironment which cause tumor regression in HCC mouse models, providing a novel therapeutic candidate of hYP7 CAR-T products for the treatment of patients with liver cancer.

Li D, et al. Persistent Polyfunctional Chimeric Antigen Receptor T Cells That Target Glypican 3 Eliminate Orthotopic Hepatocellular Carcinomas in Mice. Gastroenterology, 2020

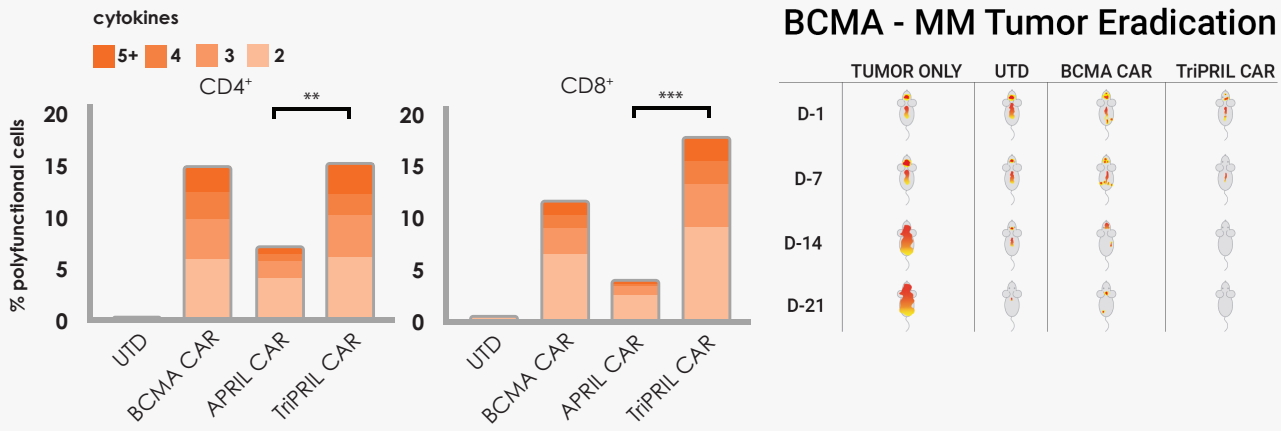
Application 2 – Overcoming Antigen Escape in Multiple Myeloma

Assessing CAR-T Cell Durability and Adaptability with Functional Single-Cell Proteomics

Products Used

Single-Cell Secretome (Mouse)

Polyfunctionality of Therapeutic Candidates for Multiple Myeloma



Single-cell polyfunctional data revealed the superiority of BCMA/TACI-specific polyfunctional response of TriPRIL CAR-T cells to the cell products with a monomeric, APRIL format [2].

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

- Single-Cell Secretome data demonstrated the positive association of single-cell functional phenotypic metrics to the functional enhancement of trimeric APRIL (TriPRIL) CAR-T cells. These metrics helped correlate uniquely with tumor killing activity against both human cells and mouse models *in vitro* and *in vivo* and show improvement difference of TriPRIL CAR-T cells versus April and conventional BCMA CAR-T cells.
- Functional phenotyping Identified highly upregulated polyfunctionality in TriPRIL CAR-T cell products that target both B-cell maturation antigen (BCMA) and Transmembrane activator and CAML interactor (TACI).
- Demonstrated the impact of polyfunctional metrics by single-cell IsoCode chip proteomics in precisely profiling dual-specific TriPRIL CAR-T cells for the prevention and treatment of patients with relapsed/refractory Multiple Myeloma.

Schmidts A, et al. Rational design of a trimeric APRIL-based CAR-binding domain enables efficient targeting of multiple myeloma. Blood Advances, 2019

Application 3 – Comparing Subtle Gene Edits in NK Cell Therapies

Single-Cell Functional Secretome Reveals Differences in Gene Edited NK Cell Therapies to Enhance Decision Making

Products Used

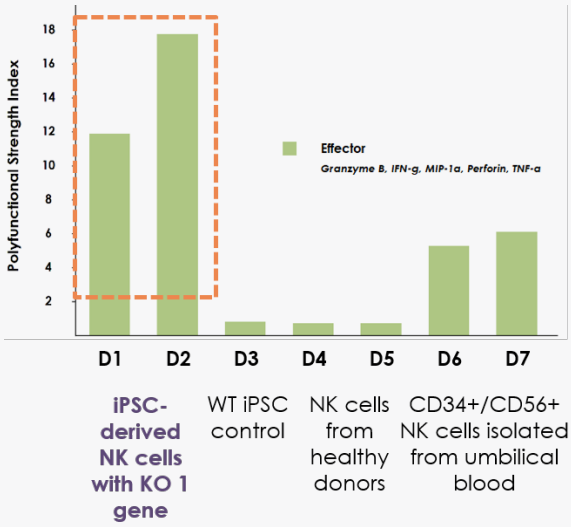
Single-Cell Secretome (Mouse)

Highlights of Insights into CRISPR-Edited NK Cell Therapy Development:

- Single-cell phenotyping metrics underscore significant differences between gene-edited KO-iPSC-NK cells and wild type cells.
- CISH-/- knock- out cells showed enrichment of polyfunctional cell subsets compared to the wild type samples.
- Single cell metrics of the knock-out cells were substantially higher than 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* PSI of CISH KO-iPSC-NK cells relative to wild type cells.

Zhu et al. Notch Activation Rescues Exhaustion in CISH-Deleted Human iPSC-Derived Natural Killer Cells to Promote In Vivo Persistence and Enhance Anti-Tumor Activity. ASH Annual Meeting 2018

CRISPR-Edited NK Cell Product Analyses: *in vitro* Correlates to Mouse Model



Critical potency differences between edited and wildtype cells correlated with *in vivo* mouse response to therapy [3].

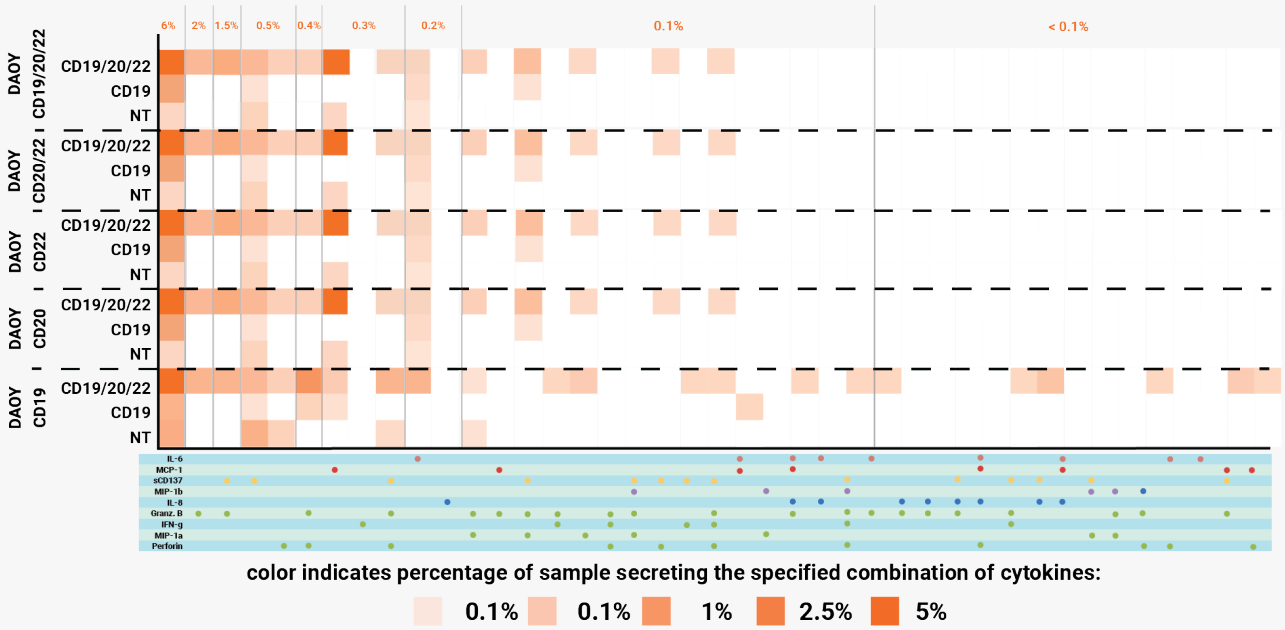
Application 4 – Single-Cell Proteomics in Persistence and Overcoming Antigen Escape

Single-Cell Secretome Reveals Biomarkers for Persistence in CAR-T Therapy

Products Used

Single-Cell Secretome (Mouse and Human)

CAR-T Cells That Target Acute B-Lineage Leukemia Irrespective of CD19 Expression



Single-Cell Secretome was used to characterize novel cell therapy products for effectiveness and potency [4].

Highlights of Applying Single-Cell Secretome to Persistence in CAR-T Therapy

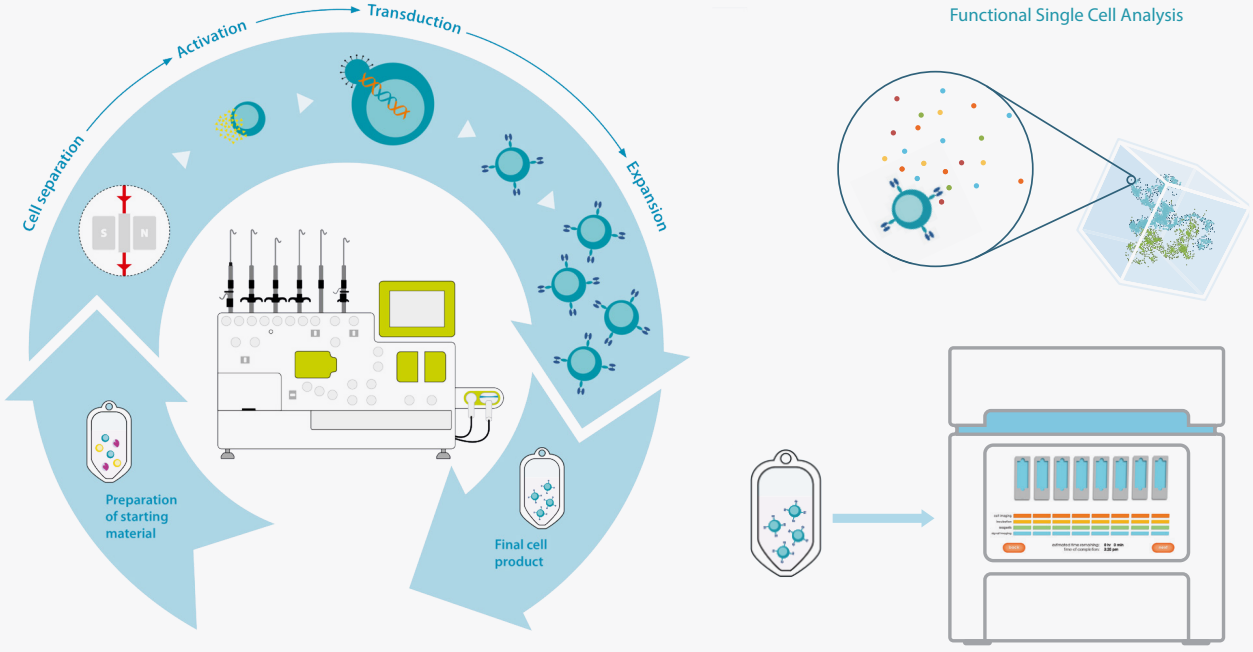
- Single-Cell Secretome reveals the enhanced polyfunctional profiles of CD19/20/22CAR-T cells with the robust anti-tumor activity in response to CD19+ and CD19- tumor cells, supporting a potential advantage of CD19/20/22CAR as salvage or front-line CAR therapy for patients with recalcitrant disease such as CD19-escape BL-ALL.
- Functional proteomics reveals insight into various challenges associated with delivering growth factors and cytokines for various therapeutic applications.
- Polyfunctional CD19/20/22CAR-T cells effectively target both CD19+ and CD19- B-lineage acute lymphoblastic leukemia (BL-ALL) and demonstrate superior anti-leukemic activity *in vivo* against CD19- and CD19+ BL-ALL.

Fousek, K et al. CAR T-cells that target acute B-lineage leukemia irrespective of CD19 expression. Leukemia, 2020.

Application 5 – Precision Analytics in Automated CAR-T Manufacturing with Single-Cell Proteomics

Optimizing CAR-T Bioprocessing Workflows with Miltenyi CliniMACS Prodigy and Bruker's IsoLight

Automated End-to-End CAR-T Cell Processing and Functional Analysis



Overview of the CliniMACS Prodigy® T Cell Transduction (TCT) Process workflow for CAR-T manufacturing (left, image credit: Miltenyi Biotec). Single cell cytokine profiling of functional quality and potency of the engineered cell product with the IsoLight System (right).

Focus on Quality with a Comprehensive End-to-End Solution from Miltenyi Biotec and Bruker:

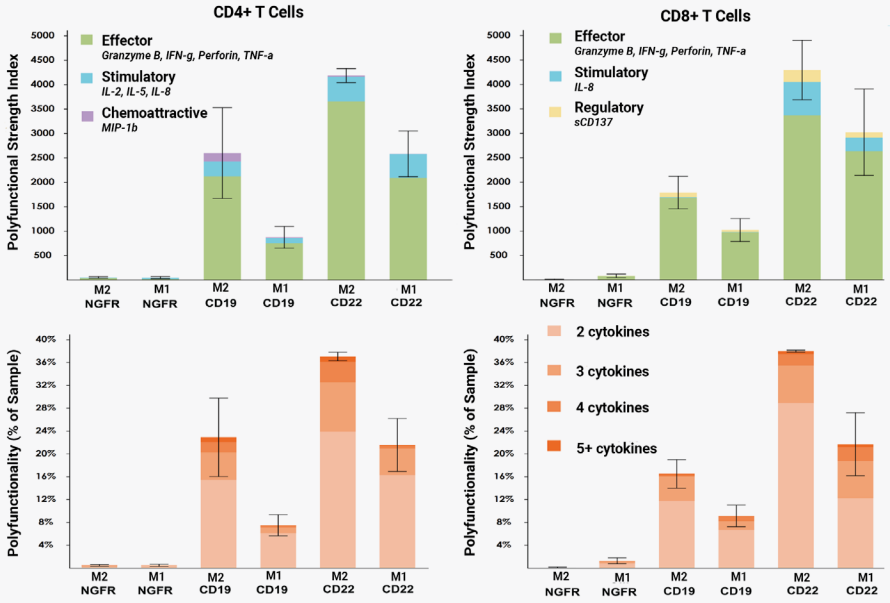
- The process of production of CAR-T products is complicated by the high requirements, numerous GMPs, instruments, and scientific personnel with significant and highly specific expertise. Therefore, there is an urgent need for utilizing an automated process which incorporates both cell product processing and functional evaluation.
- The CliniMACS Prodigy® represents the next generation in automated cell processing, offering advanced integrated solutions to streamline cell processing workflows – from cell separation through cell culture to formulation of the final product.
- In combination with Bruker's Single-Cell Secretome platform, it can minimize the need for human involvement in numerous steps, reducing human resource utilization and the risk of cell contamination, and eventually may be used to ensure successful translation from cell therapy development to clinical approved products.

Application 5 - Precision Analytics in Automated CAR-T Manufacturing with Single Cell Proteomics Cont.

Products Used

Single-Cell Secretome (Human)

Consistently More Robust Polyfunctional Response of CD4+ or CD8+ CAR-T Cell Products to CD19 or CD22 with Method 2 (M2) compared to Method 1 (M1)



Upper panel: Polyfunctional Strength Index of CD4+ (left) and CD8+ (right) CAR-T products. Lower panel: Polyfunctionality of CD4+ (left) and CD8+ (right) CAR-T products [5].

Highlights of Bioprocessing Optimization with Single-Cell Secretome:

- Comparing two CAR-T manufacturing methods, method 1 (M1) and method 1 (M2), single-cell polyfunctional data clearly demonstrates that M2 significantly improved the overall polyfunctional responses of bispecific CD19/CD22 CAR-T cell products compared to previously used M1.
- CAR-T cell products manufactured by M2 have much higher secretions in multiple secreted proteins; the enhanced polyfunctional metrics were mainly driven by effector and stimulatory cytokine secretions.
- Chemoattractive (in CD4+) and regulatory (in CD8+) cytokine secretions were also enhanced in M2 group, indicating that M2 improves the quality of CAR-T cell products in multiple dimensions and is superior in both quantities and qualities.

Srivastava SK, et al. Abbreviated T-cell activation on the automatic CliniMACS Prodigy device enhances bispecific CD19/22 Chimeric Antigen Receptor T-Cell viability and fold expansion, reducing total culture duration. ASH Annual Meeting, 2018

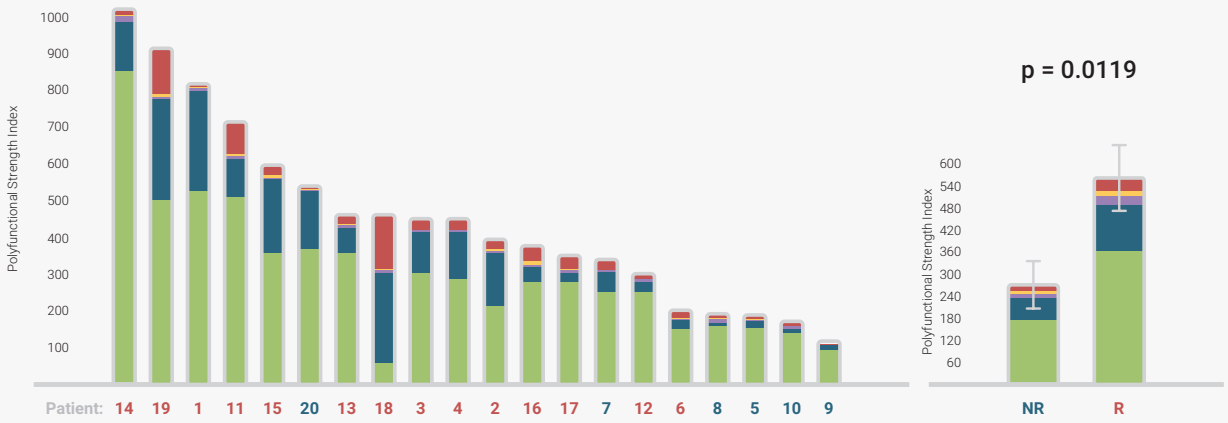
Application 6 – Preinfusion Biomarkers for Predicting Clinical Outcome in CAR-T Cell Treatment

Preinfusion CAR-T Cell Product Metrics Uniquely Correlate in vivo *Clinical Outcome in Non-Hodgkin Lymphoma Patients*

Products Used

Single-Cell Secretome (Human)

Single-Cell Secretomic Data Correlated with Outcomes in Non-Hodgkin Lymphoma



Preinfusion CAR T-cell product polyfunctional profiles are associated with clinical response [6].

Highlights of Bruker's Functional Proteomics and Predictive Biomarkers in CAR-T Therapy

- Single-cell functional phenotypic metrics outperformed other preinfusion metrics, including IFN-γ co-culture cytokine intensity, ratio of CD4+ to CD8+ T cells, and various T cell phenotype frequencies, and was the only metric that statistically differentiated responding from non-responding patients.
- Single-cell secretomic metrics in responding patients' CD4+ CAR-T samples were driven by multiple, non-redundant cytokines, including the effector/anti-tumor cytokines IFN-γ, and MIP-1, stimulatory cytokine IL-8, and inflammatory cytokine IL-17A.
- These results indicate single-cell secretomic data's potential as a biomarker for guiding personalized CAR-T cell treatments and potentially predicting therapeutic efficacy.

Rossi J, et al. Preinfusion Polyfunctional Anti-CD19 Chimeric Antigen Receptor T Cells Associate with Clinical Outcomes in NHL. Blood, 2018

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Challenges & Applications

Application 1: Development of Next-Generation CAR-T Products for Solid Tumor

Application 2: Overcoming Antigen Escape in Multiple Myeloma

Application 3: Comparing Subtle Gene Edits in NK Cell Therapies

Application 4: Single-Cell Proteomics in Persistence and Overcoming Antigen Escape

Application 5: Optimizing CAR-T Bioprocessing Workflows with Miltenyi CliniMACS Prodigy and Bruker's IsoLight

Application 6: Preinfusion Biomarkers for Predicting Clinical Outcome in CAR-T Cell Treatment

Solutions

- Single-Cell Secretome predicts persistence in cutting-edge cell therapies for difficult to treat liver cancer.
- Single-Cell Secretome data correlates uniquely with tumor killing activity against both human cells and mouse models *in vitro* and *in vivo*
- Single-Cell Secretome reveals differences in gene edited NK cell therapies to enhance decision making
- Single-Cell Secretome reveals biomarkers for persistence in CAR-T therapy
- Optimization of CAR-T bioprocessing workflows with Miltenyi CliniMACS Prodigy and Bruker's IsoLight
- Preinfusion CAR-T cell product metrics uniquely correlate *in vivo* clinical outcome in non-hodgkin lymphoma patients

References

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3. Zhu H, Blum R, Wu Z, Bahena A, Hoel HJ, Ask EH, Guan K-L, Malmberg K-J, and Kaufman DS., Notch Activation Rescues Exhaustion in CISH-Deleted Human iPSC-Derived Natural Killer Cells to Promote In Vivo Persistence and Enhance Anti-Tumor Activity. Presented at ASH, 2018
4. Fousek K, Watanabe J, Joseph SK, George A, An X, Byrd TT, Morris JS, Luong A, Martinez-Paniagua MA, Sanber K, Navai SA, Gad AZ, Salsman VS, Mathew PR, Kim HN, Wagner DL, Brunetti L, Jang A, Baker ML, Varadarajan N, Hegde M, Kim Y-M, Heisterkamp N, Abdel-Azim H, and Ahmed N. CAR T-cells that target acute B-lineage leukemia irrespective of CD19 expression. Leukemia, 2020.
5. Srivastava SK, Panch SR, Jin J, Shalabi H, Shah NN, Flynn B, Zhou J, Mackay S, Highfill SL, Stroncek DF. Abbreviated T-cell activation on the automatic CliniMACS Prodigy device enhances bispecific CD19/22 Chimeric Antigen Receptor T-Cell viability and fold expansion, reducing total culture duration. ASH Annual Meeting 2018
6. 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