APPLICATION NOTE

Characterizing Adoptive Cell Therapies using Bruker's Proteomic Barcode Technology

Bruker's proteomic barcode technology measures single-cell cytokine secretions to provide unique functional insights into cancer immunotherapy.

In this Application Note we outline:

- The importance of polyfunctional cells in adoptive cell therapy
- The Bruker IsoCode® Single-Cell Secretome platform and Workflow
- Pre-clinical product characterization for early insights into efficacy and function
- Tracking cellular functionality to optimize manufacturing
- Discovering predictive clinical biomarkers that correlate with clinical outcomes

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Highly Potent Functional Cells in Adoptive Cell Therapy

Adoptive cell therapy (ACT) for cancer is a rapidly growing field that is providing new hope and improved outcomes for cancer patients. These therapies, such as CAR-T, CAR-NK, TCR-T, and TIL, involve harvesting immune cells, modifying, expanding, and/or activating them, and then infusing the cells into a patient. While thousands of ACT clinical trials have been performed or are currently ongoing, only a small handful have achieved FDA approval.* In part, this can be attributed to the fact that unlike traditional medicines and therapeutics, cell therapies are living products that are inherently difficult to characterize and predict with current technologies. Consequently, novel functional metrics are needed to better understand therapeutic behavior and improve ACT development and optimization.

Multiple studies in recent years have found that polyfunctionality, a single-cell characteristic describing the ability of a single immune cell to secrete multiple cytokines, is a critical metric that corresponds with cellular strength and functionality[1, 2]. Although polyfunctional cells are typically rare, they can exert significant physiological effects. In cancer immunotherapy, the multiple cytokines secreted by polyfunctional cells enact coordinated efforts to directly induce tumor death or modulate immune responses against cancer (Figure 1).

Identifying and characterizing these potent polyfunctional cells requires a technology that can measure highly multiplexed cytokines secreted from live single cells. Bruker IsoCode® Single-Cell Secretome platform utilizes microchambers and Bruker Proteomic Barcode Technology to detect 32 cytokines from individual single-cells in a fully automated fashion[2]. In this application note, we discuss how the IsoCode platform provides the necessary insight to make critical decisions at multiple stages in the ACT development process.

Bruker IsoCode® Single-Cell Secretome platform and Workflow

Bruker's IsoCode platform can be used to provide valuable functional insights at any stage of the ACT manufacturing process (Figure 2). Two different stimulation types can be used for activation depending on when cells are obtained for analysis. When analyzing starting materials (e.g. TIL) a general stimulation such as PMA/Ionomycin, anti CD3/CD28 for T cells, or R848 for NK cells can be used to gain an overall view of immune fitness (Figure 2; Workflow A). After genetic engineering and retargeting, co-culture stimulation with a relevant target cell expressing the target tumor antigen for CAR-T or target peptide pulsed cells for TCR-T can be used to mimic physiologically relevant scenarios (Figure 2; Workflow B). After stimulation, cells are then stained, loaded onto IsoCode® chips, and then inserted into the IsoLight® system or IsoSpark™ system.

Incubation, fluidics, and imaging are performed in a fully-automated and hands-off fashion within the instrument, producing high dimensional functional cytokine data from individual cells. All data is automatically imported into IsoSpeak® Data Analysis Software, which enables data interpretation and generation of publication ready visualizations. Polyfunctional strength index® (PSI®), Bruker's biomarker for immune cell potency , combines the percent polyfunctionality with cytokine signal strength to provide an overall indication of a sample's polyfunctional cytokine secretion potency. Other metrics and visualizations such as percent polyfunctionality, heatmaps, principal component analysis (PCA), and t-distributed stochastic neighbor embedding (t-SNE) graphs enable researchers to clearly interpret multidimensional single cell proteomic data[2].

By using IsoCode platform to simultaneously measure the functional secretion of 32+ cytokines from single-cells, researchers can obtain critical insights into biomarkers that are inaccessible using other proteomic platforms.

The sections below specifically highlight some of the many ways Bruker technology has been applied in the field of cell therapy.

Highly Potent Polyfunctional Cells

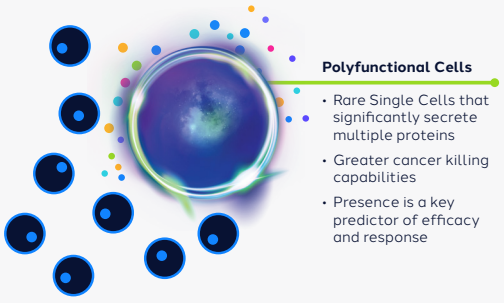

Figure 1: Highly potent polyfunctional cells that significantly secrete multiple cytokines are rare subsets of immune cells with greater cancer killing potential than other immune cells.

* Saez-Ibañez AR, Upadhaya S, Partridge T, Shah M, Correa D, Campbell J. Landscape of cancer cell therapies: trends and real-world data. Nat Rev Drug Discov. 2022 Sep;21(9):631-632. doi: 10.1038/d41573-022-00095-1. PMID: 35650421.

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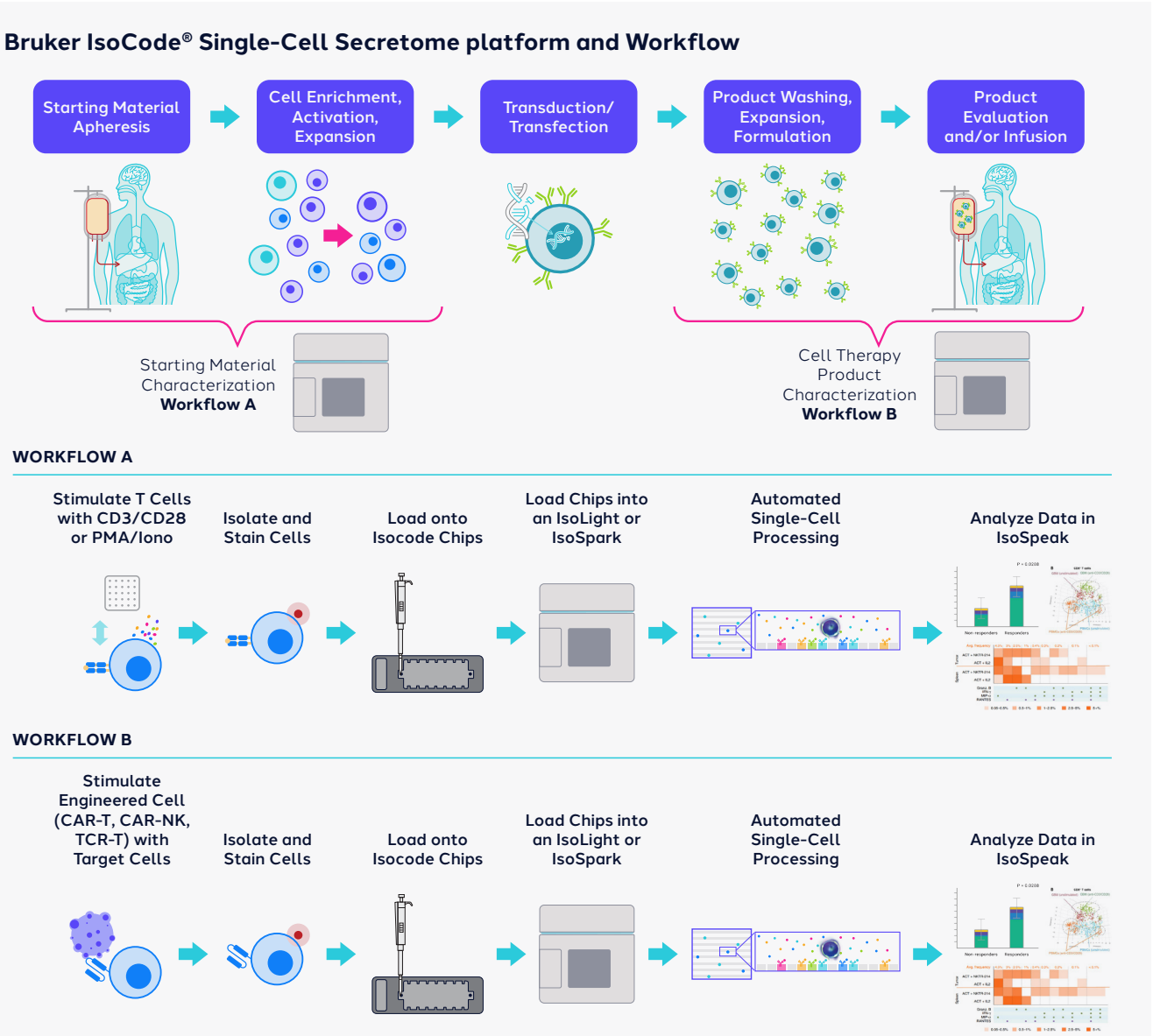

Figure 2: Bruker's proteomic barcoding platform and workflow are appliable at multiple stages in the ACT creation process.

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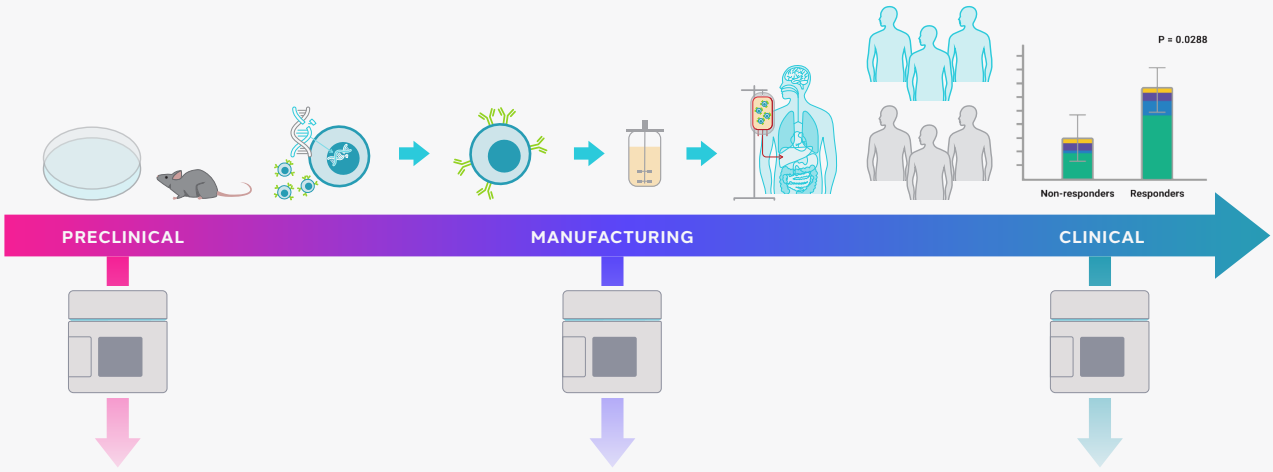
ACT Development Process Overview

Preclinical research, manufacturing optimization, and clinical evaluation are key stages involved in the development of all ACTs (Figure 3). At the preclinical stage, researchers typically explore and test different modifications, techniques, and designs using *in vitro* and ***in vivo*** animal models. It is critical to identify promising targets and treatment approaches and characterize their function during the therapeutic development process. Manufacturing optimizations are then needed to ensure consistency, scalability, and functionality. This stage commonly involves the exploration of new techniques and compounds which can affect functional phenotypes. Finally, clinical studies in patients are needed to evaluate performance in humans. At this stage, donor heterogeneity will result in product and response heterogeneity which necessitates a deeper understanding of ACT performance through clinical biomarkers. The sections below highlight a small handful of examples where Bruker technology has provided critical insights into cellular function across all stages of ACT development.

Development of ACT Therapies



- Select the best candidate
- Understand the drivers of efficacy

- Optimize manufacturing process
- Test new techniques and compounds for scale-up

- Identify biomarkers for clinical outcomes
- Characterize product and patient heterogeneity

Figure 3. Development of ACT therapies requires preclinical research, manufacturing optimization, and clinical evaluation. Bruker IsoCode® Single-Cell Secretome platform can provide valuable insights into different questions that arise during each of these development stages.

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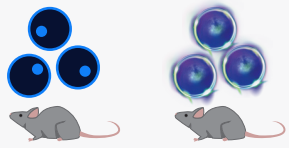
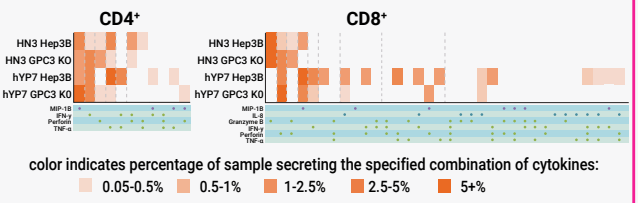
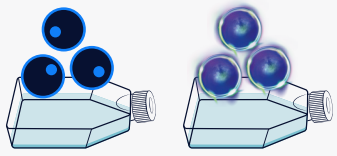
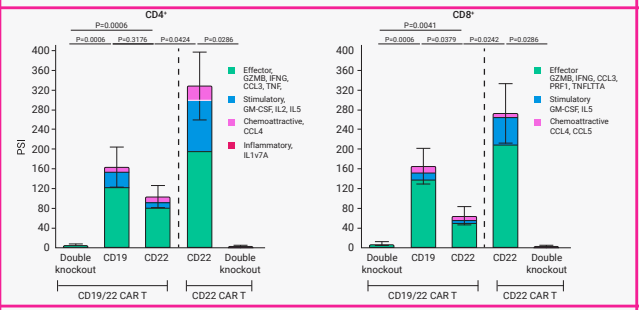
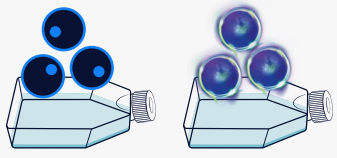
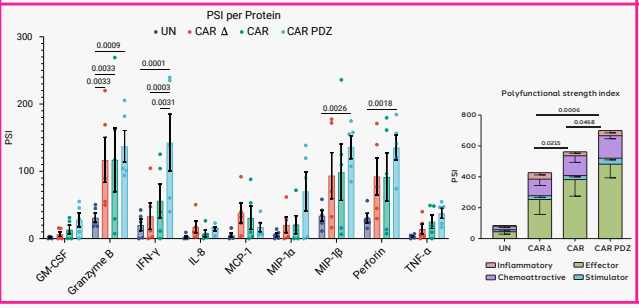
Pre-Clinical Characterization

Case studies using Bruker IsoCode® Single-Cell Secretome platform at the preclinical stage.

PRECLINICAL → MANUFACTURING → CLINICAL

SETUP	CASE STUDY DATA	IMPACT
 Product 1: HN3 GPC3 CAR-T Product 2: hYP7 GPC3 CAR-T		Bruker technology predicted the efficacy of 2 different GPC3 solid tumor CAR-T designs to identify the best candidate [3]
 Product 1: CD19/CD22 Bispecific CAR-T Product 2: CD22 Monospecific CAR-T		Comparing a CD19/CD22 bispecific CAR-T versus a CD22 monospecific revealed that the bispecific CAR-T had weaker PSI which necessitated improvements to mitigate the risk of relapse [4]
 Product 1: Control CAR-NK Product 2: PDZ Modified CAR-NK		Altering the PDZ binding motif to improve synapse formation successfully improved CAR-NK efficacy and function [5]

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Identify the Best Performing Candidate

Functional single-cell metrics are useful for comparing the efficacy of different CAR-T products. For instance, GPC3 is highly expressed in 70% of all hepatocellular carcinoma cases, making it an ideal CAR target. However, multiple binding sites exist on GPC3, which posits a question regarding which binding site to target for maximum CAR product effectiveness. In a study published in Gastroenterology, researchers at the NCI developed two CAR products targeting GPC3 –a HN3 CAR targeting the N-lobe of GPC3 and a hYP7 CAR targeting the C-lobe of GPC3[3]. The Bruker technology revealed that hYP7 CARs had significantly higher polyfunctional cytokine secretions and a higher frequency of polyfunctional profiles than HN3 CARs. This corroborated with *in vitro* and *in vivo* results demonstrating the increased cancer killing capabilities of hYP7 CARs. Together, the data collectively was used to greenlight the hYP7 CAR for clinical studies that are currently ongoing.

Obtain Early Insight into Downstream Clinical Results

Different targets of a multi-specific CAR-T may have varying levels of CAR-T engagement leading to altered efficacy dynamics. In a study published in Nature Medicine, researchers at Stanford used Bruker technology to compare a CD19/CD22 bispecific CAR-T versus a CD22 monospecific CAR-T[4]. Results revealed that the bispecific CAR-T had comparatively fewer polyfunctional cytokine secretions against CD22 – suggesting that the CD22 portion of the bispecific CAR exerted suboptimal immune pressure against cancer cells. These results directly corresponded with clinical studies showing CD22+ relapse in patients that received the bispecific treatment. Improvement of the CD22 epitope binding efficiency or transduction activation may provide mechanisms towards reducing relapse in the future.

Characterize the Effect of Product Modifications

CARs are constructed from multiple domains that can be modified to further improve efficacy. For example, changing antigen-binding, transmembrane, co-stimulatory, or intracellular domains can drastically alter a CAR's effectiveness. A specific study published in Nature Biotechnology from St. Jude's Research hospital investigated how improving the binding strength between a CAR and its antigen could be used to create a more effective therapy[5]. Researchers integrated a PDZ binding motif into their CAR construct to create CAR.PDZ-NK cells. They then used Bruker technology to test these modified cells and compare them against control CAR-NKs. Results showed that their PDZ modified CAR-NK cells had the highest percentage of polyfunctional cells and had a significant increase in Perforin, GranzymeB, MIP-1b, and IFN-g secretion. Applying Bruker technology allowed the researchers to easily assess the effect of their modification to and provided unique insights into cell function.

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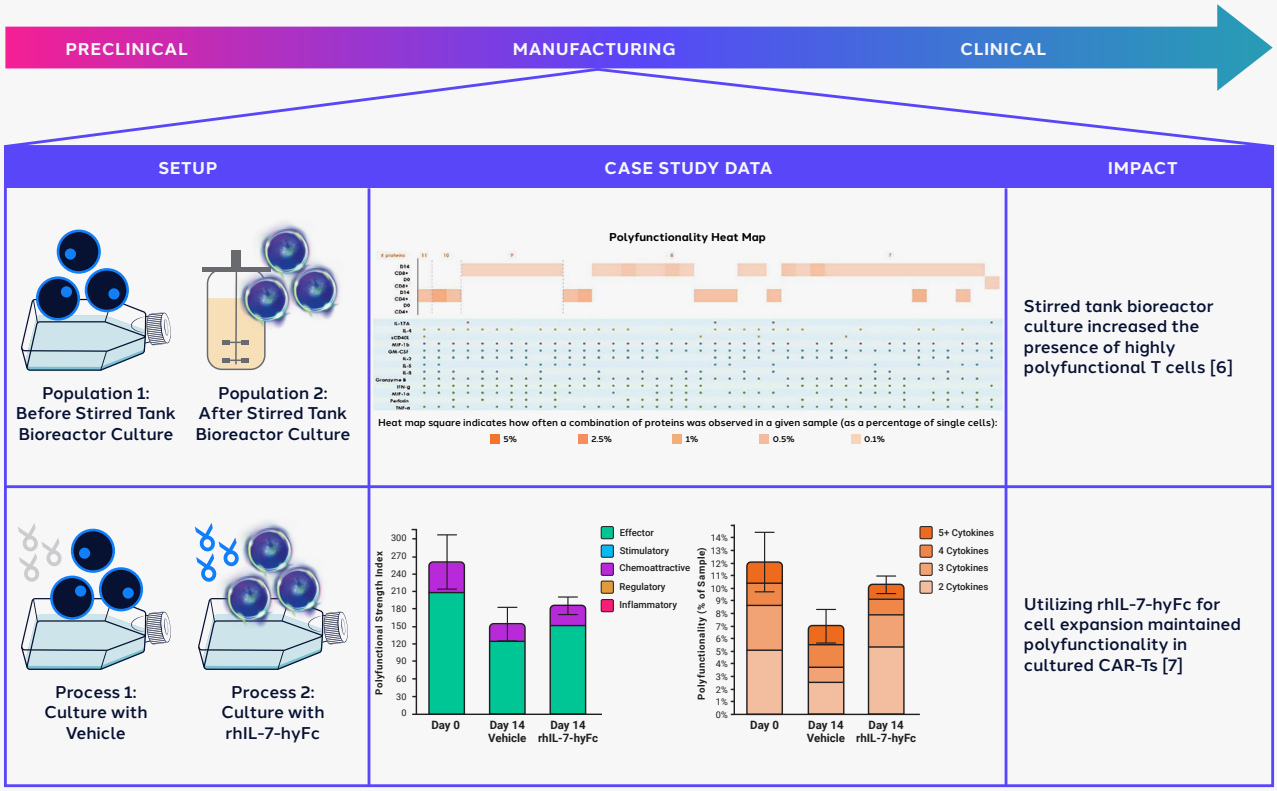
Manufacturing Optimization

Case studies using Bruker IsoCode® Single-Cell Secretome platform for manufacturing optimization.



Define the Effect of New Manufacturing and Bioprocessing Techniques

Clinical demand for cell therapies requires efficient and scalable techniques for T cell activation and expansion. However, integrating new techniques may have unforeseen effects on therapeutic performance. In a study published in Frontiers in Medical Technology, Lonza investigated the effect of stirred tank bioreactors on cellular polyfunctionality by comparing cells before and after stirred-tank culture[6]. Results showed a marked increase in CD4 and CD8 cells secreting 7 or more cytokines simultaneously after 14 days of stirred tank culture with many cells secreting combinations of effector, stimulatory, chemoattractive, regulatory, and inflammatory functions. This type of detailed functional information can validate cell functionality when deciding whether to adopt new manufacturing and bioprocessing technology.

Optimize Cell Expansion Strategies

Implementing various types of strategies to maintain or enhance product quality during manufacturing can generate products with more desirable performance. A specific case study published in Nature Communications from Washington University investigated the use of rhIL-7-hyFc, a modified IL-7 engineered with improved pharmacokinetics, to enhance universal CAR-T cell expansion and persistence[7]. Results showed that culture using rhIL-7-hyFc preserved polyfunctional cytokine secretions to a greater degree than culture with a negative control vehicle. Bruker technology can be used to validate any manufacturing strategies by directly assessing product performance from cell functionality data.

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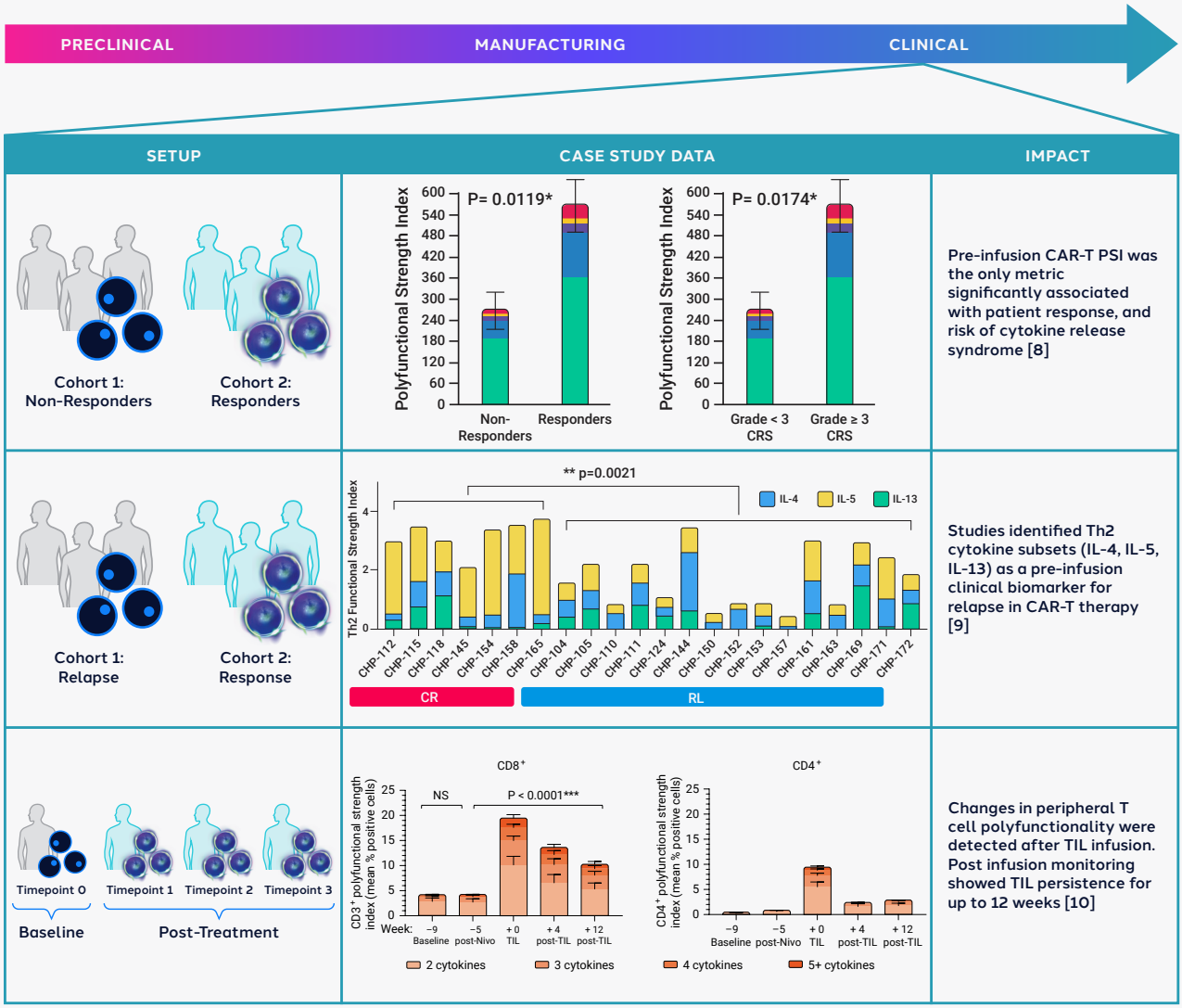
Clinical Biomarker Discovery and Immunomonitoring

Case studies using Bruker IsoCode® Single-Cell Secretome platform at the clinical stage.



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Identify Biomarkers that Correlate with Clinical Metrics

Autologous ACTs from different donors are highly heterogenous in clinical settings. Detailed single-cell functional analysis is required to fully characterize the complexities that underly clinical ACT studies. A study published in Blood from Kite Pharma directly investigated this by assessing pre-infusion CD19 CAR-T cells using Bruker technology[8]. Results showed that Bruker's PSI metric was the only metric that significantly correlated with patient response and risk of cytokine release syndrome. Other metrics generated from bulk cytokine analysis or flow cytometry failed to correspond with clinical outcomes. Only Bruker IsoCode® Single-Cell Secretome platform revealed a subset of polyfunctional cells capable of secreting multiple cytokines to generate a robust immune response that effectively eliminated cancer cells. This was the first study to associate a pre-infusion CAR-T measurement with clinical outcomes and established Bruker technology as a potent predictor of cancer immunotherapy strength.

Pinpoint the Cytokine Drivers of Clinical Response

Certain polyfunctional cells with specific secretion profiles may disproportionately contribute to clinical response. Bruker technology can be used to identify and characterize these profiles to provide a deeper understanding about ACT products. For example, a study published in Science Advances from University of Pennsylvania and Yale University used Bruker technology to assess pre-infusion CD19 products[9]. They found that patients who received CAR-Ts with functional cells secreting Th2 cytokines, namely IL-4, IL-5, and IL-13, had complete responses while patient receiving cells lacking these signatures experienced relapse. These results established that Th2 cell subsets were the primary drivers of robust clinical response. The multiplexed nature of Bruker technology allows researchers to identify specific cytokines from single-cells as key biomarkers for clinical outcomes.

Track Performance with Immunomonitoring

ACT products are living drugs with dynamic performance after infusion. Multiple aspects such as survival, proliferation, exhaustion, and persistence can be monitored to track post infusion therapy performance. A study published in Nature Medicine from Moffit Cancer Center used Bruker IsoCode® Single-Cell Secretome platform for immunomonitoring in patients before and after they received TIL therapy[10]. Results demonstrated a marked increase in PSI and polyfunctional cell levels after TIL infusion – showing that the treatments successfully increased the number of polyfunctional T cells circulating in the patients. Furthermore, tracking multiple timepoints after infusion allowed researchers to explore the temporal dynamics of TIL infusion. A tapered but persistent increase in polyfunctional cytokine secretions was detected for up to 12 weeks post TIL infusion.

Bruker as a Critical Guide Throughout the ACT Development Workflow

Successfully developing an ACT necessitates extensive knowledge of cellular behavior that can only be obtained from single-cell functional proteomics. IsoCode platform is a fully automated platform that makes single-cell functional proteomics more widely accessible than ever before. Using cutting edge technology, Bruker produces valuable metrics that have been shown to correlate with significant outcomes in pre-clinical, manufacturing, and clinical settings.

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References

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6. Gatla, H., et al., *Enabling Allogeneic T Cell-Based Therapies: Scalable Stirred-Tank Bioreactor Mediated Manufacturing.* Front Med Technol, 2022. **4**: p. 850565.
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