APPLICATION NOTE

# Integrated Biomarker Discovery and T Cell Analysis using the IsoSpark™ and Beacon® Platforms

Bruker's proteomic barcode and optofluidic technologies synergize to provide researchers with comprehensive understanding of T cell biology

**In this Application Note we outline:**

- Bruker's ecosystem for functional T cell profiling using the IsoSpark and Beacon
- The IsoSpark's ability to measure highly multiplexed cytokine secretions from single cells
- Workflows to identify correlative biomarkers on the IsoSpark for focused analysis on the Beacon
- How the Beacon uses optofluidic technology to evaluate cytokine secretion and cytotoxicity, with the ability to recover cells of interest
- Workflows to create therapies using the Beacon and analyzing potency of these novel therapeutics on the IsoSpark

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# Prep, Run, Analyze

## The Bruker Ecosystem for Functional T Cell Profiling

To harness the power of the immune system for therapeutic development, the ability to reliably profile individual T cell function is essential. Across fields such as cancer immunotherapy, vaccine development, infectious diseases, autoimmune disease and organ transplantation, new biomarkers to identify the most potent T cells are urgently needed. Many of today's solutions create a disconnected view of T cell biology, preventing researchers from leveraging the full potential of the phenome.

Bruker's full single-cell functional multiomic platform suite fills this gap by offering connected insights into the true in vivo biology of single T cells to improve the prevention and treatment of complex diseases and disorders.

In this application note, we'll outline how Bruker's suite of integrated tools, including the IsoSpark™ and Beacon® platforms, can enable you to unlock the functional drivers of T cell potency to gain a deeper understanding of T cell biology (Figure 1).

**The Bruker Ecosystem for Functional Cell Profiling**

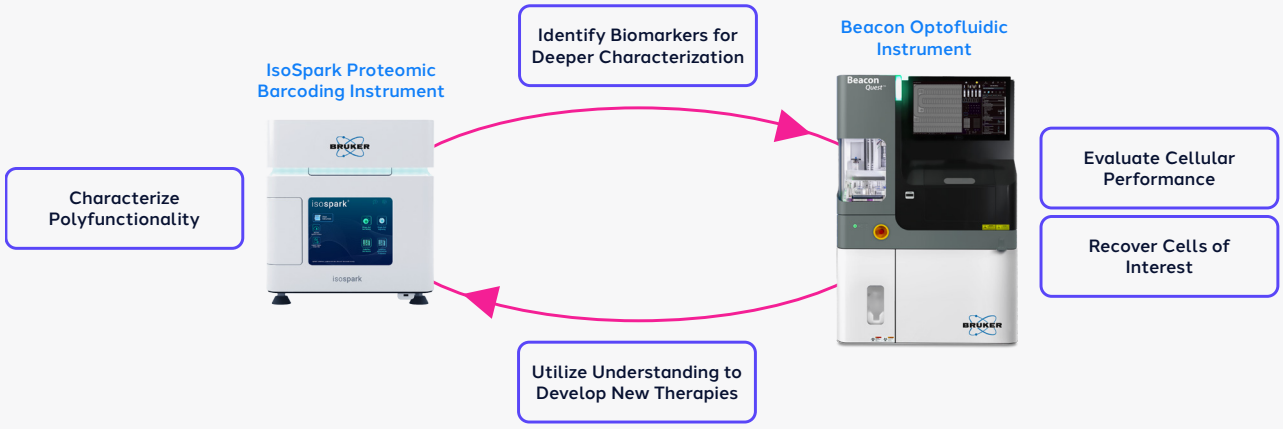



Figure 1 The Bruker Ecosystem for Functional Cell Profiling: PheonomeX's two analysis platforms each have strengths that complement each other. By utilizing both instruments, researchers gain the ability to characterize the phenome of cells with unprecedented depth to unveil impactful insights into cellular function.

## The IsoSpark Platform– Highly Multiplexed Single-Cell Proteomics for Biomarker Detection

The Bruker IsoCode® Adaptive Immune chip for the IsoSpark system enables highly multiplexed analysis of functionally secreted cytokines from single cells. Each IsoCode chip contains thousands of microchambers with capture antibodies spatially separated into a barcode pattern. Functionally secreted cytokines from single cells inside the microchambers are captured on these proteomic barcodes and the IsoSpark automatically processes chips to measure cytokine secretions (Figure 2).

The Adaptive Immune IsoCode chip is used with T cells and detects 32 cytokines spanning across effector, chemoattractive, inflammatory, stimulatory, and regulatory classifications. Simultaneously measuring the secretion of 32 cytokines from single cells allows researchers to detect polyfunctional cells – rare single-cells capable of significantly secreting multiple cytokines. These polyfunctional cells are highly impactful in multiple applications. Beneficial polyfunctional cells have been shown to drive higher potency in adoptive cell therapy products, greater patient response to checkpoint inhibitors, and better vaccine efficacy while dysfunctional polyfunctional cells are determinants of poor outcomes in autoimmune disease, infectious diseases, and transplant rejection[1-6].

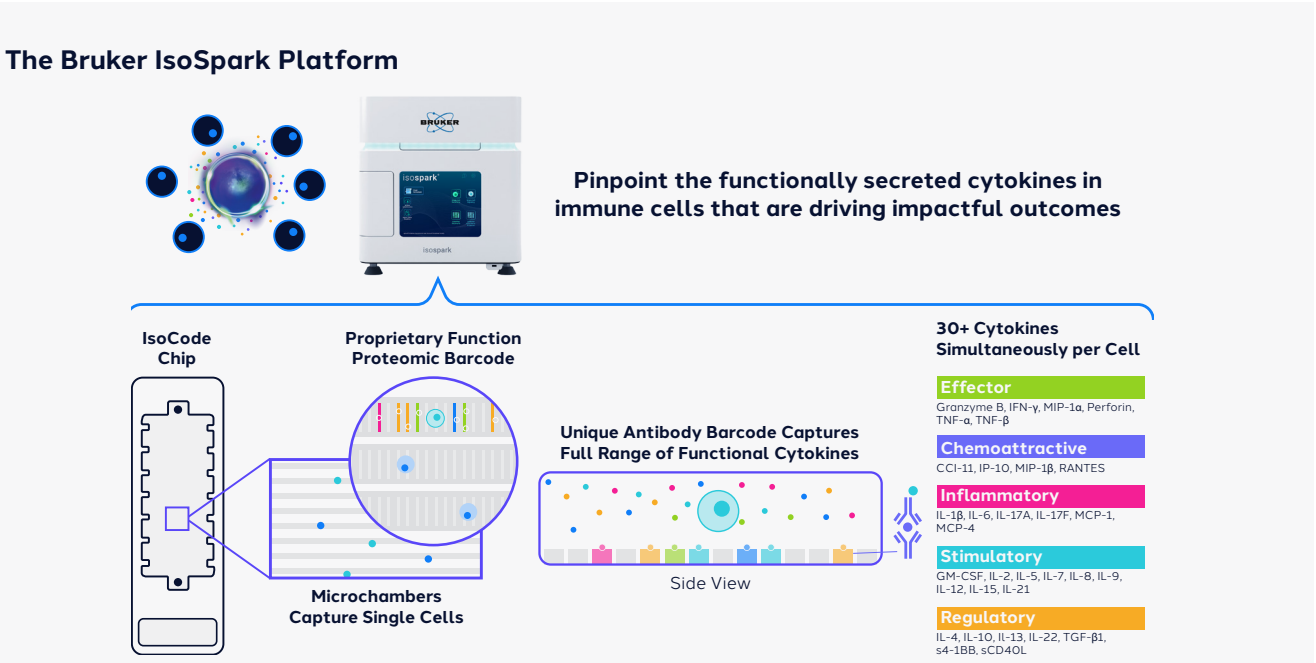



Figure 2 The Bruker IsoSpark Platform: Highly multiplexed analysis via proteomic barcoding technology determines cytokine drivers of outcomes such as potency and therapeutic response.

## The IsoSpark Platform Screens for Impactful Cytokine Subsets

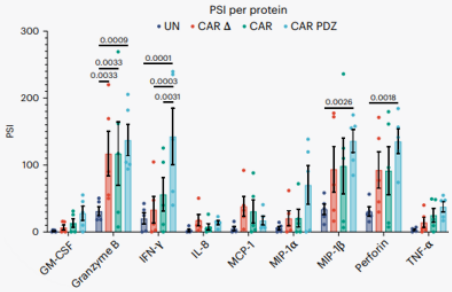
Characterizing polyfunctional cells on their own is a useful metric but not all subsets of polyfunctional cells are equally important. Certain polyfunctional cell profiles and specific single-cell cytokine secretions can be more correlative of various end results. Determining the importance of certain cytokine profiles requires highly multiplexed analysis to screen for impactful biomarkers. Multiple publications in high impact journals such as *Nature Biotechnology*, *Blood*, *Science Advances*, and *Cytotherapy* have used Bruker's IsoCode platform to discover cytokine biomarkers in preclinical and clinical settings (Figure 3).

Cytokine drivers in research projects can vary depending on the product, application, and setup. In a study investigating CAR constructs modified to improve synapse binding, modified CARs were found to enhance polyfunctional secretions of Granzyme B, IFN-g, and Perforin – cytokines directly involved in cytotoxicity (Figure 3a)[7]. In clinical settings, various biomarkers for different products have emerged to predict clinical response and side effects. A study in collaboration with Kite Pharma investigated the drivers of response in CD4 and CD8 CAR-T populations and found that responders had elevated levels of IL-8, IL-5, and IL-17A in CD4 CAR-Ts and IL-8 IFN-g, and MIP-1a in CD8 CAR-Ts (Figure 3b)[1]. Another example is a publication from Yale University and University of Pennsylvania investigating relapse biomarkers. Results showed that Th2 related functional cytokine secretions of IL-4, IL-5, and IL-13 were statistically significant predictors of relapse-free response (Figure 3c)[8]. Finally, a study from the Medical College of Wisconsin specifically analyzed CD4 CAR-Ts and found that TNF-b and IL-17A predicted response while increased levels of IL-8 and MCP-1 correlated with higher severity cytokine release syndrome (Figure 3d)[9].

Revealing polyfunctional cells and the cytokine secretions that correlate with biological outcomes is only the first step toward a more complete understanding of cellular phenotypes. After using the IsoSpark platform to characterize their cells, researchers may be interested in retrieving cells with specific cytokine profiles for further analysis. This is where the Bruker's Beacon platform, comes into play (Figure 4). The Beacon platorm uses cutting-edge optofluidic technology to detect select cytokine secretions from single cells to measure polyfunctionality, T cell killing of target cells to characterize cytotoxicity, and surface markers to identify cellular subsets. Cells with desirable traits can then be retrieved for downstream analysis.

## Case Studies for Determining Biomarkers using the IsoSpark™ Platform

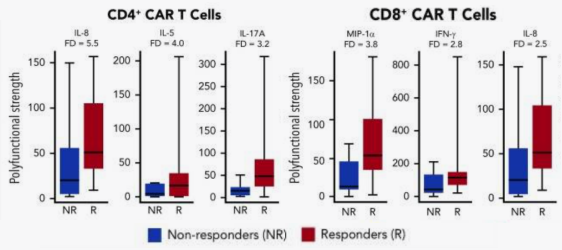
**A.** **Nature Biotechnology 2023**




Pre-clinical study investigating CAR binding modification revealed stronger levels of Granzyme B, IFN-g, and Perforin secretion in PDZ modified CARs

**Modified CAR Biomarkers**


**B.** **Blood 2018**

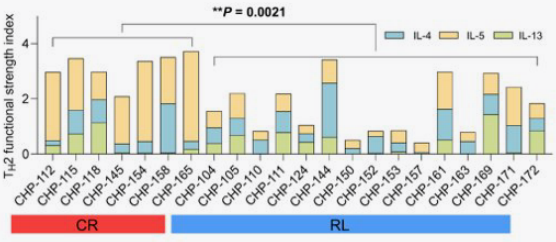



CAR-T Clinical Study found that responders had upregulated polyfunctionality of IL-8, IL-5, and IL-17A in CD4+ CAR-Ts and of MIP-1a, IFN-g, and IL-8 in CD8+ CAR-Ts

**CD4 CAR-T Clinical Response Biomarkers**

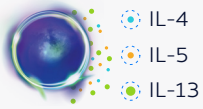

**CD8 CAR-T Clinical Response Biomarkers**


**C.** **Science Advances 2022**

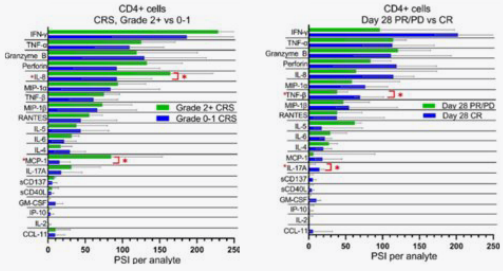



Clinical Study CAR-Ts showed that patients with CAR-Ts containing low levels of IL-4, IL-5 and IL-13 (Th2 associated cytokines) were more susceptible to relapse

**CAR-T Clinical Response Biomarkers**




**D.** **Cytotherapy 2022**




Clinical Study of CAR-Ts uncovered that CD4+ TNF-B and IL-17A were potent biomarkers for complete response while CD4 IL-8 and MCP-1 were indicators of CRS

**CD4 CAR-T Clinical Response Biomarkers**


**CD4 CAR-T Cytokine Release Syndrome Biomarkers**


Figure 3 Case Studies for Determining Biomarkers using the IsoSpark™ Platform: Measuring functional cytokine secretions from single-cells in a multiplexed manner reveals the specific profiles of cells that contribute to functional outcomes. In each case, specific cellular subsets secreting 2-3 cytokines were found to correlate with preclinical and clinical outcomes.

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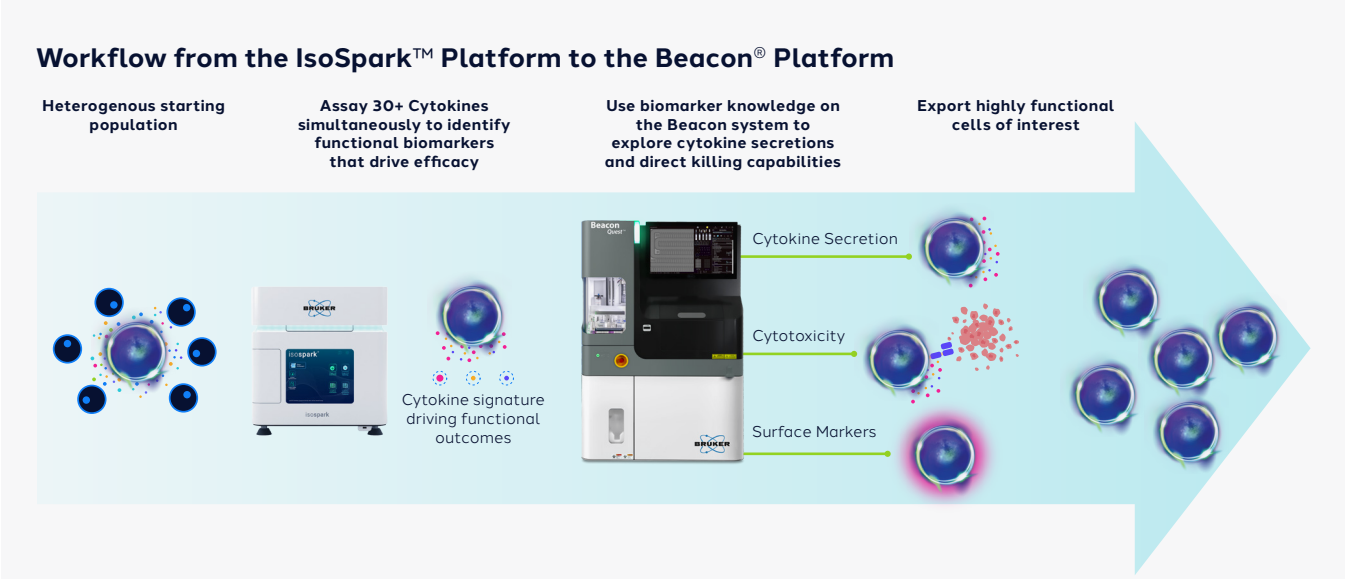


Figure 4 Workflow from the IsoSpark™ Platform to the Beacon® Platform: The IsoSpark platform specializes at identifying the specific cytokines that are mechanistically driving consequences. Information gained from the IsoSpark platform can be used to narrow down which cytokines to focus on when using the Beacon platform.

## The Beacon Platform– Optofluidics Enables Characterization of Single-Cell Phenotypes

The Bruker's Opto® T Cell Profiling workflow with the Beacon instrument enables comprehensive T cell characterization using optofluidic technology (Figure 5). T Cells are loaded into an OptoSelect® nanofluidic chip containing thousands of NanoPen® chambers. Optoelectropositioning (OEP®) is then used to move individual T cells into each NanoPen chamber. To assess polyfunctionality, up to 3 different capture beads designed to detect select cytokines of interest are loaded into each NanoPen chamber. As discussed in the preceding section, deciding which 3 functional cytokines to focus on can be determined by using the IsoCode platform on the IsoSpark instrument. Time lapse brightfield and fluorescence imaging on the Beacon are then used to assess whether individual cells secrete 0, 1, 2, or all 3 cytokines to characterize polyfunctionality[10]. Cells in NanoPen chambers can also be stained for surface markers to focus analysis on cells with activation markers or other surface identifiers. After profiling each individual cell, OEP can be used to export single cells out of the chip for downstream analysis such as RNAseq (RNA sequencing) and TCRseq (T cell receptor sequencing).

Furthermore, the Beacon platform also allows researchers to coculture single T cells with target cells, such as cancer cells or dendritic cells, to stimulate T cells and directly assess cytotoxicity using stains such as Caspase-3[11]. The assays for cytokine secretion, cytotoxicity, and surface staining can be combined in a single experiment to comprehensively interrogate the phenotype of single T cells. A multitude of other potential applications such as proliferation, serial killing, reporter cell assays, and ligand blocking are also possible using OptoSelect® chips for the Beacon system  but require optimization and validation.

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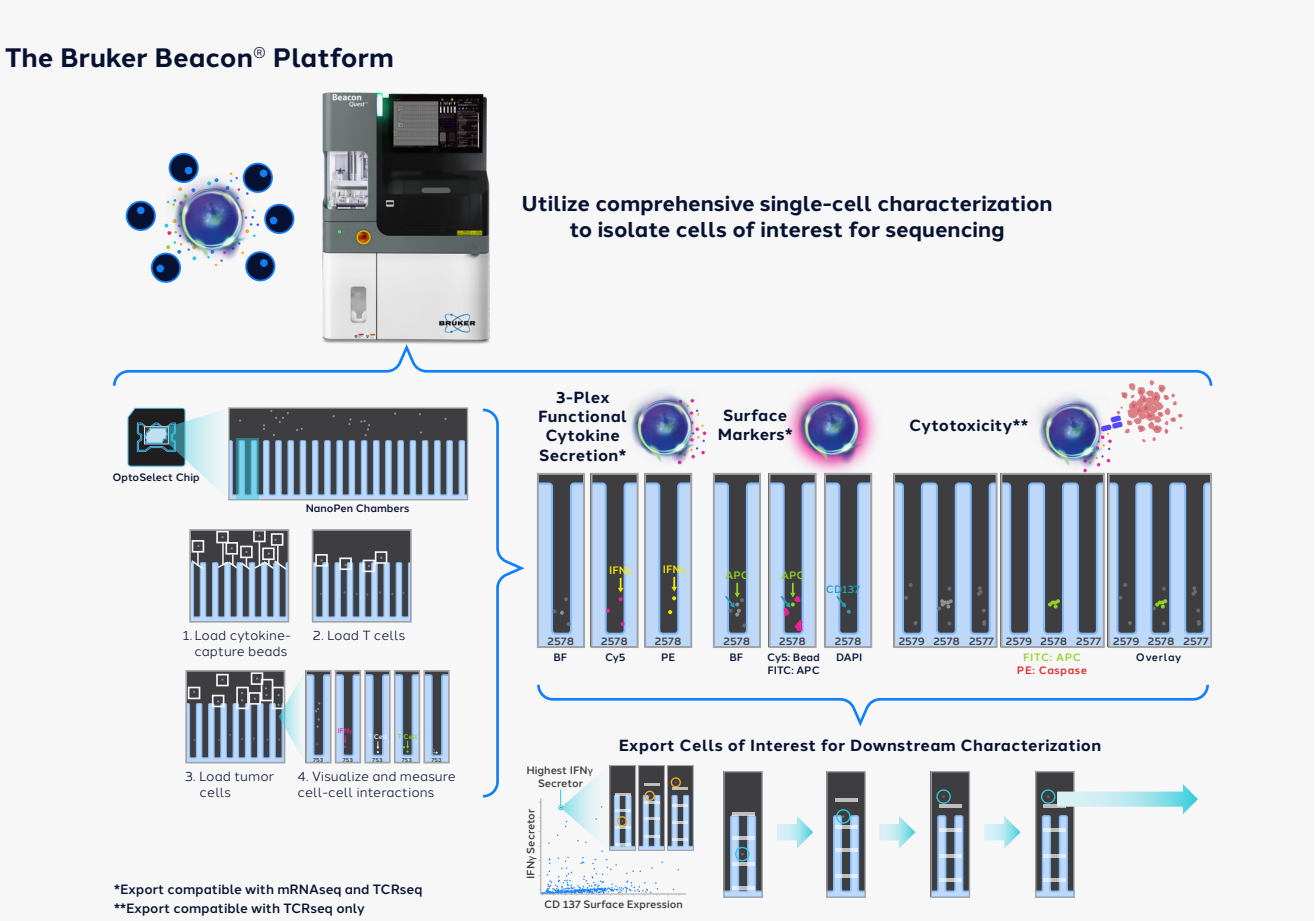


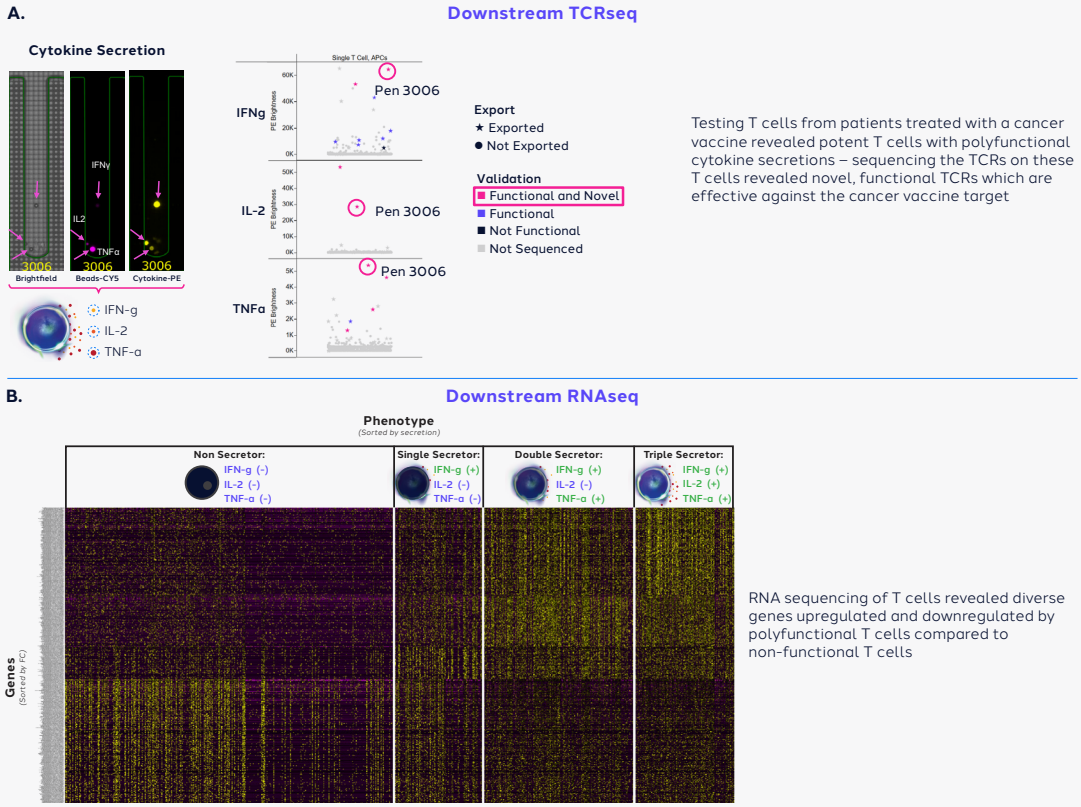

Figure 5 The Bruker Beacon® Platform: The Beacon platform uses optofluidics to manipulate cells and beads into NanoPen® chambers inside OptoSelect® chips. This can be used to set up a variety of assays to assess cytokine secretion, cytotoxicity against cancer cells, and surface marker staining to identify surface phenotypes. After assays are performed, optofluidics can also be used to extract cells of interest for downstream analysis.

## The Beacon Platform Connects Single-Cell Proteomic Polyfunctionality to Transcriptomics, Immunogenomics, and More

The cell export capabilities of the Beacon platform allow for various downstream analysis pathways such as RNAseq or TCRseq to provide further insights into underlying biological processes. Linking the phenotypes measured on the Beacon system with gene expression or immunogenomics can be used to reveal the underlying drivers of functional phenotypes.

In a Beacon T cell receptor study, T cells were obtained from a patient who was previously treated with a cancer vaccine. The Beacon system isolated individual T cells into NanoPen chambers and cocultured the T cells with peptide pulsed dendritic cells to identify functional T cells. After assaying thousands of individual T cells, cells with polyfunctional cytokine secretions of IFN-g, IL-2, and TNF-a using on a separate platform. These TCR sequences were then re-expressed and validated resulting in several novel functionally validated sequences (Figure 6a). By identifying specific TCR sequences associated with desirable phenotypes, researchers can leverage the TCR sequences into therapies. For example, identifying TCR sequences with high levels of polyfunctionality against cancer cells can be used to develop effective TCR-T therapies.

## Case Studies for Analysis of Cells on the Beacon® Platform with Downstream Analysis




| Upregulated Genes in Polyfunctional T Cells | | | | Downregulated Genes in Polyfunctional T Cells | | | |
|---|---|---|---|---|---|---|---|
| Gene | Pathway / Function | Gene | Pathway / Function | Gene | Pathway / Function | Gene | Pathway / Function |
| BOLA2B | Interleukin-12-mediated signaling pathway | MCM5 | DNA replication / cell cycle | AES | Innate immune system | LAPTM5 | Lysosome |
| CCL1 | Chemokine super family | MCM7 | DNA replication / cell cycle | AHNAK | Phospholipase-C pathway | LCK | Ret signaling |
| CCL13 | Chemokine super family | MIR4435-1HG | lncRNA gene regulation | CCSER2 | Microtuble binding | MALAT1 | Cell cycle regulation |
| DUSP4 | Ret Signaling / TLR4 signaling | NME1 | Metabolism / translation control | CD3D | T cell receptor signaling | PTPRC | T cell receptor signaling / innate immune system |
| DUT | Metabolism | PCNA | DNA repair | CD3G | T cell receptor signaling | PTPRCAP | TNFa signaling pathway |
| FABP5 | Innate immune system | PKM | Metabolism | CYTIP | Family of immune cell regulators | S100A4 | Ca, cAMP, and lipid signaling |
| H2AFZ | Epigeneic regulation | PPIA | Innate immune system | EMB | MAPK ERK pathway | S100A6 | Ca, cAMP, and lipid signaling |
| HSPD1 | Transcriptional regulation | RGS16 | GPCR signaling / MAPK ERK signaling | EVL | Cytoskeletal signaling | SATB1 | Chromatin regulation |
| IRF8 | IFNg signaling | RPS26 | rRNA processing | FTH1 | Innate immune system | STK17B | NF-kB signaling |
| JUNB | Cytokine signaling in immune system | SMS | Circadian gene expression | GNLY | Innate immune system / allograft rejection | TPT1 | Cytoskeletal signaling, Ca, cAMP, and lipid signaling |
| LINC00152 | Cell proliferation | TYMS | Metabolism | IKZF1 | Dendritic cell development / innate lymphoid differentiation | VIM | Cytokine signaling in immune system, ERK signaling |
| | | | | ITGA1 | Focal adhesion / ERK signaling | | |

Figure 6 Case Studies for Analysis of Cells on the Beacon® Platform with Downstream Analysis. Functional profiles of cells can be linked to transcriptomic, genomic, and immunogenomic data to reveal the drivers of polyfunctionality.

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RNAseq can also be used on specific cells to reveal key insights into functional phenotypes. After measuring IFN-g, TNF-a, and IL-2 cytokine secretions from T cells exposed to dendritic cell stimulation, the Bruker's OptoSeq® Single Cell 3' mRNA kit was used to profile gene expression of individual T cells using mRNA capture beads on chip. After cell lysis, mRNA capture, amplification, and sequencing, Bruker's PrimeSeq® software was used to associate T cells in specific NanoPen chambers to the sequencing data[12]. This allowed researchers to identify specific genes that were upregulated or downregulated by polyfunctional cells. Upregulated genes included genes involved in cytokine and chemokine signaling, metabolism, cell proliferation, and transcriptional regulation while downregulated genes included those involved in cAMP signaling and cytoskeletal signaling. Understanding the underlying mechanisms of T cell functionality empowers researchers to design strategies to control the polyfunctionality of T cells for various therapeutic applications.

The ability to link phenotypic data to gene expression and TCR sequences provides a way to reveal the key factors contributing to T cell functionality. This data can then be used to create new targets, therapies, and drugs that leverage the insights gained from the Beacon platform. Fully understanding the potency and mechanisms of these new therapies requires comprehensive analysis of functional cytokine secretions available by using the IsoSpark system (Figure 7). For example, the IsoSpark platform can validate the functionality of novel TCR-T cells created using TCR sequences identified on the Beacon platform. Together, the IsoSpark platform and the Beacon platform synergize to provide researchers with the ability to characterize and develop T cell therapeutics (Figure 1).

### Workflow from the Beacon® Platform to the IsoSpark™ Platform

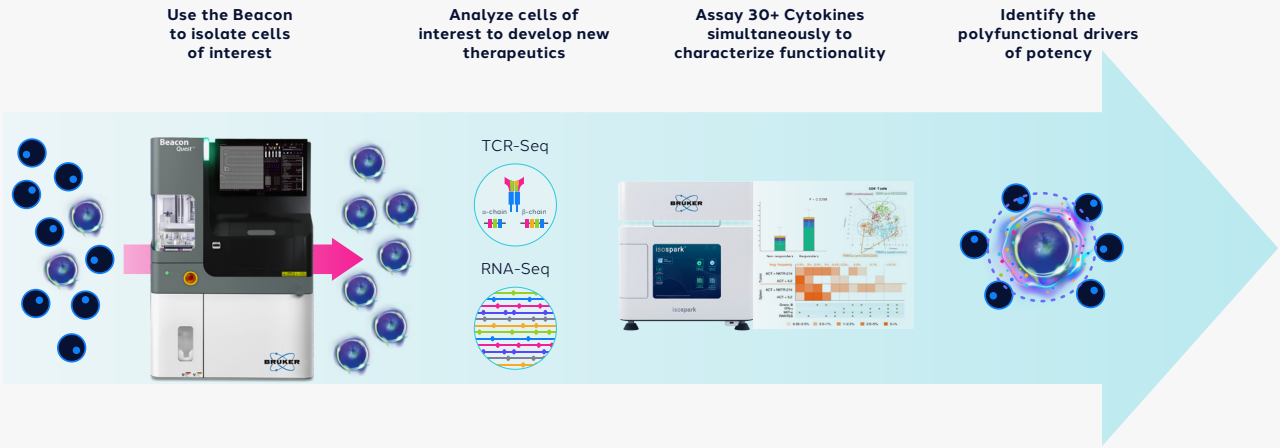


Figure 7 Workflow from the Beacon® Platform to the IsoSpark™ Platform: The Beacon platform specializes at directly characterizing multiple functional attributes of single cells including cytokine secretion, cytotoxicity, and surface marker expression. Cells of interest with specific profiles (such as cells that secrete cytokines and have a specific activation surface marker) can be recovered and analyzed using TCR-seq and RNA-seq to identify the root causes of their functional phenotype. This information can then be used to generate new therapeutics which can be tested for polyfunctional potency on the IsoSpark platform.

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## Summary

Highly multiplexed analysis on the IsoSpark platform reveals polyfunctional biomarkers and cytokine drivers of biological function while the Beacon platform delivers a multitude of ways to assess T cell functionality and analyze specific phenotypes of interest. Workflows combining the strengths of both instruments are uniquely positioned connect multiplexed discovery, functional analysis, and downstream retrieval of cells of interest. Bruker's instruments use patented proteomic barcode and optofluidic technology to unveil impactful insights inaccessible using other technologies. These unique capabilities provide researchers with a deeper understanding of T cell functionality to guide the analysis and development of therapeutics across a wide range of applications.


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