TECHNICAL NOTE

Employing Bruker's PSI™ to summarize complex single-cell cytokine data

PSI has correlated with objective response in a variety of cancer immunotherapies and is being used from discovery to development

Highlights

- Bruker's Polyfunctional Strength Index (PSI) is a powerful, novel metric for measuring the potency of a variety of immune cell types in cancer immunotherapy, based on the ability to uniquely isolate & detect potent functional cell subsets.
- PSI is defined as the percentage of polyfunctional cells per sample and the cytokine secretion intensities of each of those cells.
- Polyfunctionality alone is a meaningful indicator of potency in a variety of immunotherapy types, including CAR T therapy, where PSI has significantly outperformed other pre-infusion metrics.
- Many researchers are also using sub-components of the PSI to develop a sub-component polyfunctional strength cell subset driver, such as IL-17 or Stimulatory driven Polyfunctional Strength.
- The PSI Index reveals aggregate differences that guide your deep dive into polyfunctional cell subsets, via single-cell heat maps and cellular analyses like PAT PCA and t-SNE, that can reveal biomarkers and development insights.
- PSI is calculated using data created on Bruker systems.

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What is PSI?

PSI (Polyfunctional Strength Index), developed by Bruker, provides a unique measurement for enabling discovery, optimizing bioprocessing/manufacturing procedures and predicting clinical response. PSI is generated by identifying the polyfunctional single cells (those cells secreting two or more cytokines) and measuring the intensity of the cytokines they secrete (Figure 1, 3). The presence of polyfunctional cells is a strong indicator of a sample's overall functional potency and has been shown to help us understand the basic biology of immune system [1], discover the underlying mechanism for disease development [3] or vaccine efficacy [4], evaluate the quality of immunotherapeutic products [5] and predict clinical response [2] while significantly outperforming other metrics from traditional potency measurement technologies (Figure 7).

Phenotypically identical cells, though functionally highly heterogeneous

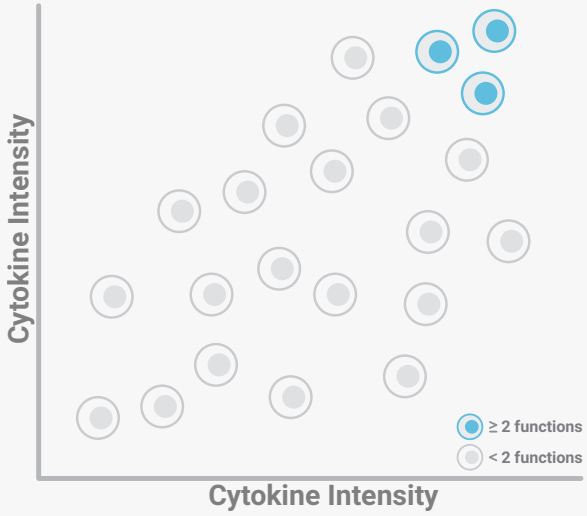


Figure 1 | Highly Polyfunctional cell subsets revealed by Bruker systems enable visualization of immune cells with a high degree of polyfunctional inflammation (cells that secrete two or more cytokines per cell, at a high intensity) that correlate to disease progression.

The polyfunctional strength index (PSI) overweights to uniquely identify highly potent, polyfunctional single T-cells

The four factors that PSI uses to uncover the correlative highly multiplexed subsets

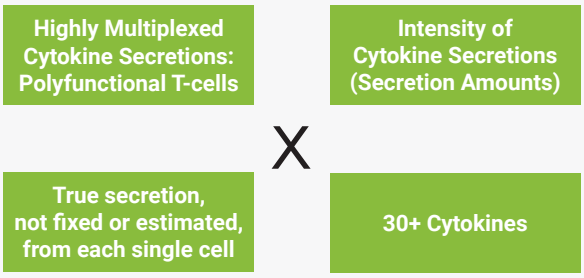


Figure 2 | PSI simultaneously encompasses (1) the breadth of cytokine secretions (polyfunctionality) and (2) the intensity of cytokine secretions (secretion amounts), which are (3) both found at a single-cell level across (4) 30+ cytokines

PSI is defined as the product of the polyfunctionality of the sample (the percentage of profiled single cells secreting two or more cytokines) and the intensity of the cytokines secreted by these polyfunctional single cells (Figure 3). The units of PSI are arbitrary, and more recently have been normalized to compare the highly multiplexed and intense cell subsets across runs, across time. *

The four factors that make PSI such an effective sample potency metric are that it simultaneously encompasses (1) the breadth of cytokine secretions (polyfunctionality) and (2) the intensity of cytokine secretions (secretion amounts), which are (3) both found at a single-cell level across (4) 30+ cytokines (Figure 2). Put together, the broad span and high resolution of these factors make for a powerful, all-encompassing measurement of sample potency that doesn't require a priori knowledge about the sample response.

 *PSI version 1.2, the current version of the PSI, includes normalization, which reduces the influence of outliers on the metric.

Bruker PSI™: polyfunctionality of a sample combined with the intensity of each cell's secreted cytokines

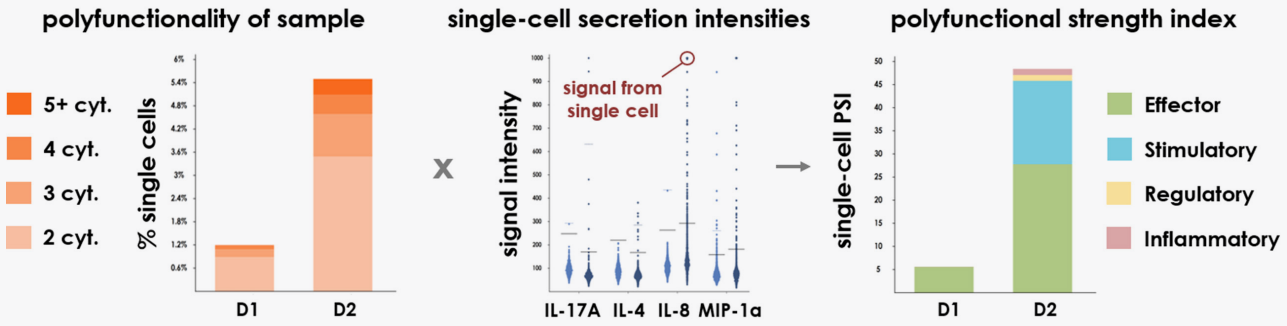


Figure 3 | PSI (Polyfunctional Strength Index)) is defined as the percentage of polyfunctional single-cells (secreting two or more proteins, i.e. left panel) in a sample, multiplied by the average & normalized signal intensity of the secreted proteins from individual functional groups (middle panel) from each cell. Each cell's strength, across 1000+ cells, is then aggregated and simplified into the readout at right. This PSI measurement provides a comprehensible visualization of the potent cell subsets, and the cytokine types driving these potent cell subsets.

In fact, the way in which these factors are combined is less important than the fact that they are used in conjunction with each other. For example, a change in potency (change in PSI) may be a more appropriate metric in some cases, such as measuring the increase in PSI over a baseline PSI measurement. Once a study is completed and a polyfunctionality index is used to assess the potency of a set of samples, along with potential associations to clinical outcome, the metric may then be further dissected into its contributors, to determine the drivers of this potency.

How is PSI generated?

PSI is a composite index generated from data obtained on Bruker's systems, which allow in-depth profiling of many functional secreted proteins that orchestrate the immune response, from greater than a thousand single cells in parallel (Figure 4).

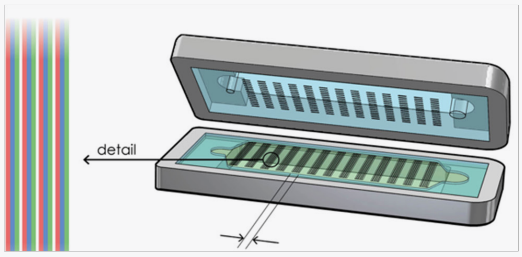
What is the logic behind PSI and why is it a uniquely valuable metric?

PSI consolidates high-dimensional, single-cell protein secretion data into a single metric that represents the overall activity of a sample. It captures two critically relevant factors uniquely:

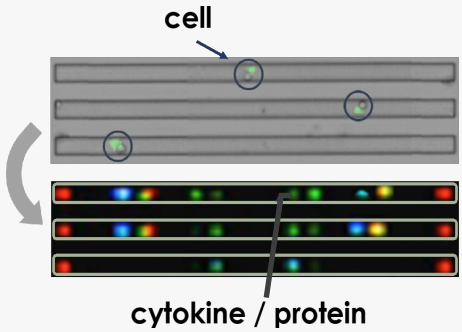
- the percentage of highly polyfunctional cells in a sample, and
- the multiplexed signal intensity of all profiled secreted cytokines.

Polyfunctional cells are recognized as key effector cells contributing to the development of potent and durable cellular immunity against viral infection, cancer, and other diseases [6, 7, 8]. While the percentage of polyfunctional

PSI™ is measured using Bruker's systems



IsoCode single-cell chip:
Single T cells in high throughput



Complete function of each cell:
30+ plex secreted protein ELISA per cell

Figure 4 | Panel of simultaneously measured 32 secreted proteins from single cells. 32-plex barcoded ELISA array categorized into different functional groups: • Effector: Granzyme B, IFN-γ, MIP-1α, Perforin, TNF- α, TNF-β • Stimulatory: GM-CSF, IL-2, IL-5, IL-7, IL-8, IL-9, IL-12, IL-15, IL-21 • Regulatory: IL-4, IL-10, IL-13, IL-22, sCD40L, sCD137, TGFβ1 • Inflammatory: IL-1β, IL-6, IL-17A, IL-17F, MCP-1, MCP-4 • Chemoattractive: CCL-11, IP-10, MIP-1β, RANTES.

32-plex panel for functional proteins secreted from single cells

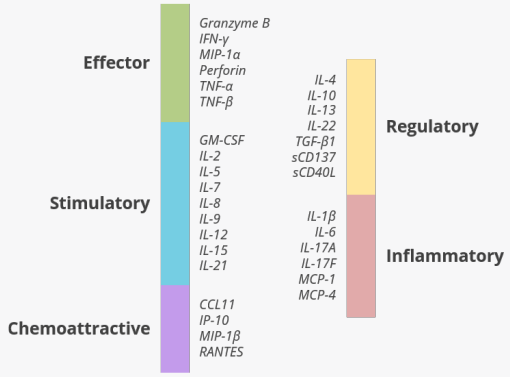


Figure 5 | Bruker's 32-plex single-cell polyfunctional strength panel. The ability to capture the range of relevant cytokines from each immune cell represents a unique secreted protein multiplexing capability.

cells on its own is a meaningful indicator of potency, Bruker's unique ability to capture the range of relevant functional secretions from each immune cell that orchestrate the immune response, for the first time, represents a unique secreted protein multiplexing capability. See Figure 5 for the Bruker Single-Cell Polyfunctional Strength Panel. Additionally, the Bruker system quantitates the intensity of the cytokines secreted by these highly polyfunctional cells. Having both of these key factors in tandem has helped capture the potency of important and highly functional T-cell and other immune cell subsets, which has correlated with the potency of cell therapy products [5] and in-vivo response [2] (Figure 6).

The unique potency correlates that PSI provides in CAR T has been extended to multiple other areas, with cited and published studies (Figure 10).

How is PSI visualized?

To visualize PSI, a color-coded system is used to indicate the relative contribution by each profiled functional group of cytokines (effector, stimulatory, regulatory, inflammatory, and chemoattractive).

To determine which cytokines are driving the polyfunctional response, each cytokine's contribution to the PSI value can be calculated. This is the fraction of the total PSI coming from a specific cytokine; it is found by calculating the percentage of each cell's signal corresponding to that cytokine, averaging it across all cells, and multiplying this percentage by the total PSI. See Figure 5 for the breakdown of which cytokines in the Single-Cell Polyfunctional Strength Panel correspond to the individual cytokine functional group. Then see Figure 6 for how these cytokines have been visualized in a real-world setting, with data published in Blood [2] (Figure 6,7).

CD4+ CAR T PSI™ as a predictive biomarker

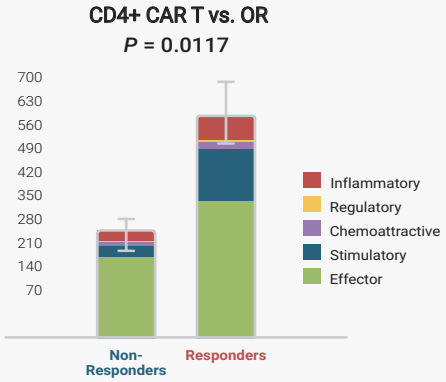


Figure 6 | Major cytokines driving polyfunctional product CD4+ T cells by CD19 stimulation that distinguish responders to the therapy from nonresponders. Single-cell proteomic analysis of a panel of 32 secreted cytokines, chemokines, and cytotoxic molecules was performed on product T cells from 20 patients treated with CAR T cells.

Pre-infusion CAR T product PSI™ correlates with clinical outcome to CD19 CAR T therapy

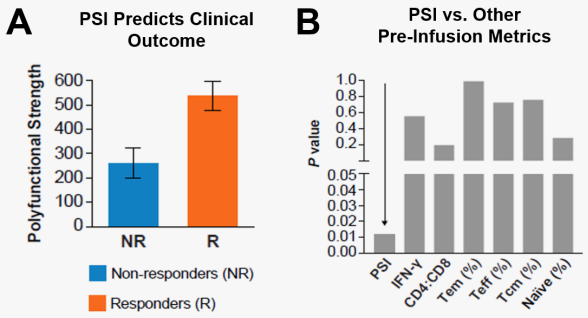


Figure 7 | PSI is shown to be superior over other pre-infusion CAR T product potency metrics in associating with objective response. In a study with Kite Pharma and the NCI, the pre-infusion CAR T cell product PSI outperformed other analyzed pre-infusion metrics, incl. IFN-γ co-culture cytokine intensities, $CD4^+$:$CD8^+$ T cell ratio, and various T cell phenotype frequencies, which did not show statistically significant associations with clinical response [2].

How should PSI be interpreted?

PSI benchmarking comparisons are most effective in the context of a specific study with a specified control population included, and/or in studies with the same setup where different conditions are being compared. Utility of having the PSI to differentiate potency can be effective in a variety of research contexts, including discovery, development and in biomarker discovery as well. We outline ways to delve deeper into the context of the Polyfunctional strength contributions as well below.

While PSI has proven valuable for decision making and for revealing choice in discovery and development, like any technology, comparing readouts that are based on different therapies interacting with a target, or comparing the same therapy in different indications can yield different baseline PSI measures. Thus, comparing in an absolute sense without context of disease type and therapy type may have its limitations.

IsoSpeak software enables unique single-cell cell readouts that capture the most potent subsets of cytokine producing cells

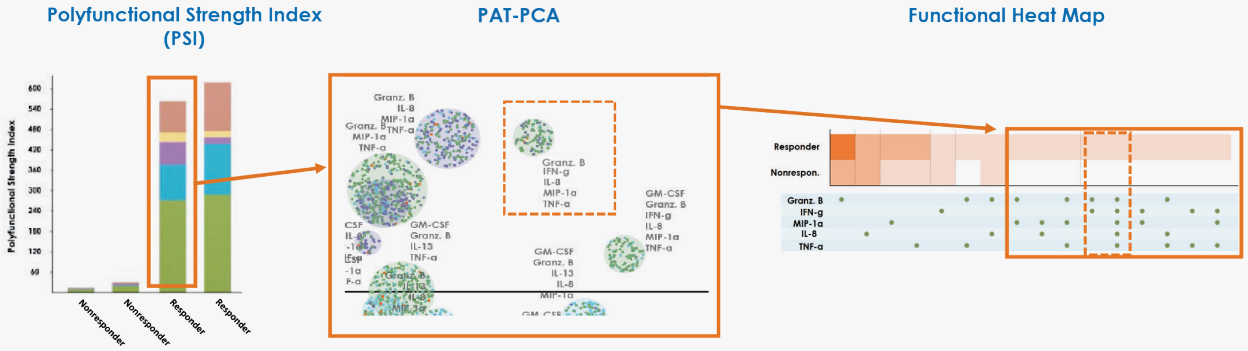


Figure 8 | IsoSpeak Software captures the key differences driving correlates. IsoSpeak's end-to-end informatics and statistics enables comparisons and answers from one piece of software. Our visualization workflow can take you from start to finish to find the key differences in your cell subsets.

Throughout published studies, having both stimulatory and effector contributions to cell therapy product PSI is key, as opposed to just one or the other. $CD4^+$ product PSI has been more informative than $CD8^+$ product PSI in the context of clinical biomarker studies as well in some cases, which illustrates the power not only on the killing and effector side, but also the recruitment and helping side of T-cell response [1-3].

Are there other visualizations of PSI that help further explain mechanism & cytokine contributions?

The PSI is a powerful summary metric that can be decomposed into its contributing cytokine parts (Figure 9). It has been demonstrated that multiple non-redundant cytokines contribute to both CD4 and CD8 polyfunctionality as well, which can be visualized in terms of component contributions (Figure 9). This figure illustrates that the overall PSIs were driven by multiple effector, inflammatory and stimulatory secretions. More importantly, the relative contribution of each individual secretion can be clearly visualized on this PSI composition analysis, which helps us understand the underlying mechanisms. More visualization tools of Single-Cell Polyfunctional Strength can be found in the IsoSpeak Software available with the IsoLight system (Figure 8). With IsoSpeak Software, you can:

- View how the PSI can summarize key polyfunctional and signal strength data from the single cell subsets that make a difference
- Dive further into the data by comparing multiple samples according to their polyfunctional strength
- Dissect the sources of their polyfunctional strength,with advanced contribution graphics, PCA visualizations, functional heat maps, and more (Figure 8).

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Decomposing total PSI™ to analyze cytokine drivers

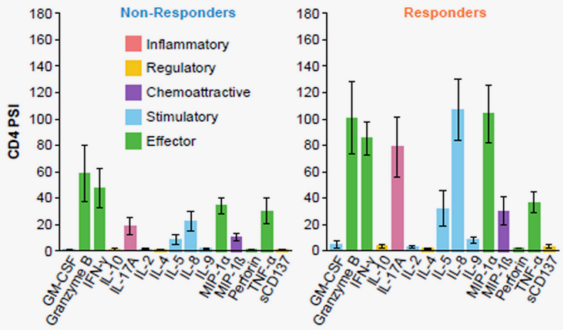

Figure 9 | Major cytokines driving polyfunctional product CD4⁺ T cells by CD19 stimulation. Product CD4⁺ T-cell PSI profiles were broken down per cytokine, between patient groups with no response and OR to CAR T-cell therapy. Only cytokines that were upregulated relative to mock stimulation are shown. Each cytokine PSI level reflects its average secretion intensity in polyfunctional single cells. The diagram shows the cytokines that contribute to the polyfunctionality index in the CD8⁺ and CD4⁺ T-cell populations.

Applications of PSI throughout discover-optimize-predict immunotherapy development continuum:

Many uses of PSI have been employed to find critical differences that can help reveal choice, and powerful correlates as well (Figure 10) in multiple disease areas and development strategies including use as:

Discover:

- Preclinical cell therapy product evaluation metric with TCR-T therapies combined with Pegylated IL-2 [9]

Optimize:

- Dissect subtle differences in bioprocessing methods [10]
- Vaccine efficacy evaluation and benchmarking metric in vaccination induced T cell response [8]

Predict:

- Clinical outcome biomarker in CD19 CAR T Therapy [2]
- Clinical post-infusion therapy monitoring tool [7]

PSI™ demonstrates the power to correlate with response or progression across a range of research areas and indications

RESEARCH AREA	METRIC	PREDICTIVE IN: INDICATION
Combination Immuno-therapies	PSI	Melanoma response (ipi/nivo).[5] **P =0.029**
	PSI	AML response in checkpoint. [11] **P =0.0288**
	PSR	Pancreatic cancer survival. [2] **P =0.001**
	PSI	Pre-clinical NP cancer vaccine efficacy. [4] **P =0.003**
Cell Therapies	PSI	Pre-infusion CAR-T in DLBCL.[1] **P = 0.012**
	PSI	Pre-clinical correlates with in-vivo mouse studies in TCR & NK cell Therapy
AutoImmune, CNS & Inflammation	PSI	MS progression.[5] **P = 0.035**
	PolyF	PV vs. ET (myelofibrosis).[6] **P = 0.018**
	PII	Neurotoxicity in cell therapy. [7] **P = 0.037**

Figure 10 | Polyfunctional cells range from CD4 to CD8 T cells, and NK cells, Macrophages, and others.

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Takeaways

- PSI has been shown to be a powerful indicator of immunotherapy potency and durability, in a manner that correlates with in vivo outcome in patients
- PSI captures two critically relevant parameters of a sample secretion profile: polyfunctionality, and the intensity of each cell's secreted cytokines, and aggregates them into a simple to use metric
- Combining the percentage of polyfunctional cells in a sample with single-cell secretion intensities creates a metric that has outperformed other pre-infusion metrics in CAR T therapy, and has shown unique correlates in side by side studies in a variety of other immunotherapy areas
- PSI is being used throughout the development of immunotherapies, from Discovery to Bioprocessing to Predicting Response

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