Employing PSI™ to Identify Optimal Bi-Specific Antibody Candidates

Revealing functional immune-tumor interaction and mechanism of therapy to advice lead choice

- **In this Application Highlight we:**
- Highlight the use of IsoPlexis' Polyfunctional Strength Index, PSI™, a single-cell cytokine-based metric of cellular potency, in the context of pre-clinical assessment of bispecific antibodies
- Identify subtle differences in quality of novel bispecific antibody constructs relative to controls, utilizing the PSI of the potent T cell subsets engaged, and
- Demonstrate that PSI can improve the choice of lead bispecific immunotherapeutic products by identifying key differences in the therapies' cytokine-based mechanisms at the single-cell level.

Application of bispecific antibody constructs to improve immunotherapy success

Recent clinical progress in immuno-oncology significantly improved clinical outcomes for many patients with hard-to-treat malignant tumors, leading to partial and complete remission and long-term survival in a fraction of patients. However, a large number of patients do not respond to these treatments, creating an urgent need for novel strategies to enhance the efficacy of immunotherapies.

Targeted engagement of T cells using bispecific antibody constructs that directly connect T cells with tumor-associated surface antigens (TAAs)

isoplexis

is a promising strategy. Bispecific antibody constructs (bispecifics) have two distinct binding specificities in their variable regions. By binding to both T cells and tumor cells, bispecifics can help endogenous T cells recognize and selectively kill these cancer cells. CD19/CD3 bispecific T cell engager antibody constructs have been shown to induce high response rates and durable complete remission in patients with minimal residual disease in r/r B-ALL [1]. In this case, the bispecific antibody engages CD3 positive T cells directly with CD19 positive malignant B-cells and attacks the tumor cells [5].

Despite clinical success in treating hematologic malignancies and promising outcomes in solid tumors, it remains challenging to precisely dissect response differences of patients with various disease indications, post introduction of bispecifics, and therefore to improve treatment efficacy and minimizing side effects [2].

Since this T cell engagement strategy shares many similarities with CAR-T therapies in terms of tumor targeting, polyclonal T cell activation, killing mechanisms through perforin and granzyme, and cytokine-based toxicities like cytokine release syndrome, better tools and markers, which measure protein secretions in a more sensitive fashion may help reveal the underlying mechanisms of both types of therapies [3].

IsoPlexis has previously revealed cytokine-based biomarkers based on the Polyfunctional Strength Index (PSI), which can objectively evaluate the quality of anti-tumor activity of CAR-T response in patients pre-therapy and predict clinical outcome of patients with diffuse large B-cell lymphoma. IsoPlexis' systems were able to correlate polyfunctional cytokine secreting CAR-T cells to patient outcome [4]. Utilizing the PSI may help researchers better understand how T cells functionally respond to bispecifics engagement as well. In the future, it may also provide predictive biomarkers and mechanistic insights, which can significantly improve the procedure for choosing bispecifics for introduction into the clinic.

Understanding bispecific mechanism in the context of the T cell and the tumor microenvironment

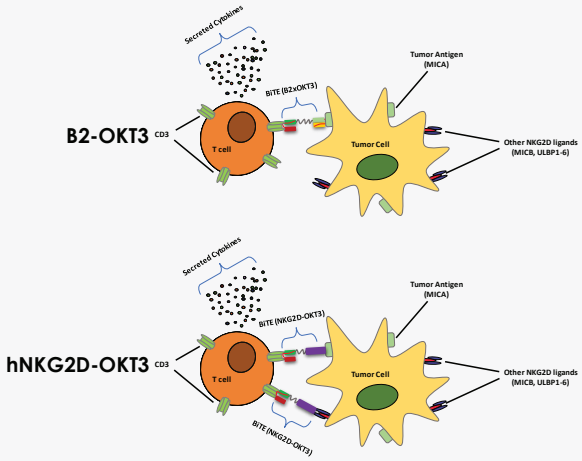

Figure 1 | Understanding bispecific cytokine-based mechanism in the context of the T cell and the tumor microenvironment. In bispecifics one of the scFvs binds to T cells via the CD3 receptor, and the other to a tumor cell via a tumor specific molecule. The upper panel shows B2-OKT3 bispecific construct which recognizes both CD3 on T cells and MICA on tumor cells. The lower panel shows hNKG2D-OKT3 bispecific construct which recognizes both CD3 on T cells and NKG2D ligands, including MICA on tumor cells. The hNKG2D part is the extracellular part of the NKG2D molecule which binds to all NKG2D ligands [5].

A novel bispecific candidate

Here, researchers explored whether natural killer group 2, member D (NKG2D) ligands (MICA, MICB, ULBP1-6) are appealing targets for cancer therapy and whether targeting these antigens can enhance anti-tumor T cell responses with novel bispecific therapies. NKG2D ligands are expressed on more than 90% of human tumors but very little on normal tissues suggesting an important role. To explore whether targeting these antigens can enhance anti-tumor T cell responses, researchers engineered bispecific T cell engagers for MICA and NKG2D: B2-OKT3 (MICA x CD3) and hNKG2D-OKT3 (NKG2D ligands x CD3, Figure 1) and investigated the polyfunctional profile of T cells with these two bispecifics treatments using the IsoPlexis system [5].

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

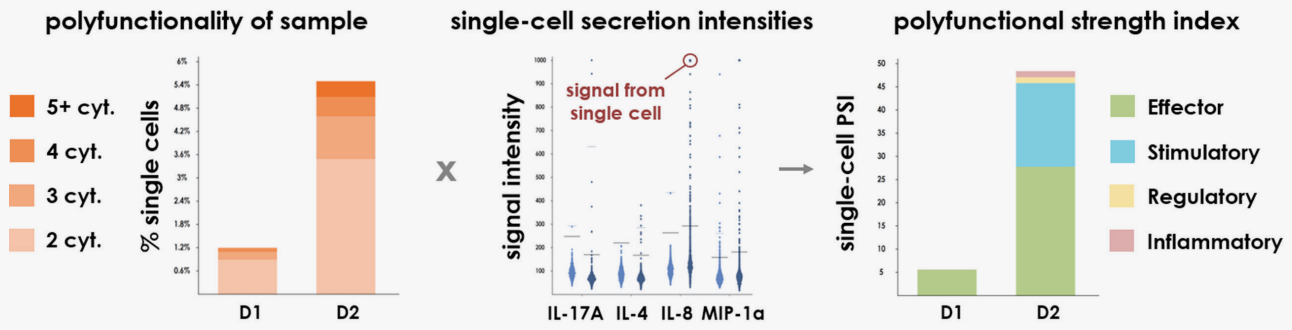

Figure 2 | PSI (Polyfunctional Strength Index) is defined as the percentage of polyfunctional single-cells (secreting 2 or more proteins, i.e. left panel) in a sample, multiplied by the average signal intensity of the secreted proteins from individual functional groups (middle panel) from each cell. 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.

Using IsoPSI to evaluate the quality of bi-specifics

To test whether the engineered bi-specific constructs can induce potent antigen-specific T cell response, we evaluated the T cell polyfunctional strength across five functional groups - effector, stimulatory, regulatory, inflammatory and chemoattractive cytokines - on the IsoPlexis system.
PSI (Polyfunctional Strength Index), developed by IsoPlexis, provides a unique measurement of cancer immunotherapy product potency by analyzing cytokine secretions on a single cell level. 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 2) [5].

The experiments revealed a significant polyfunctional response induced by both bispecifics in CD4+ and CD8+ T cells. By comparison, the control sample stimulated by Tz47-2C11 showed very little response. Between the two bispecifics, hNKG2D-OKT3 induced a more potent polyfunctional T cell response than the B2-OKT3 construct in both CD4+ and CD8+ T cells (Figure 3) [5]. It is known that NKG2D can recognize multiple ligands, such as MICA, MICB and ULBP1-6, while B2 only binds MICA [4]. These results suggest that bispecifics capable of binding multiple tumor antigens may yield better therapeutic effect.

PSI demonstrates key quality and potency differences of a novel bispecific relative to control

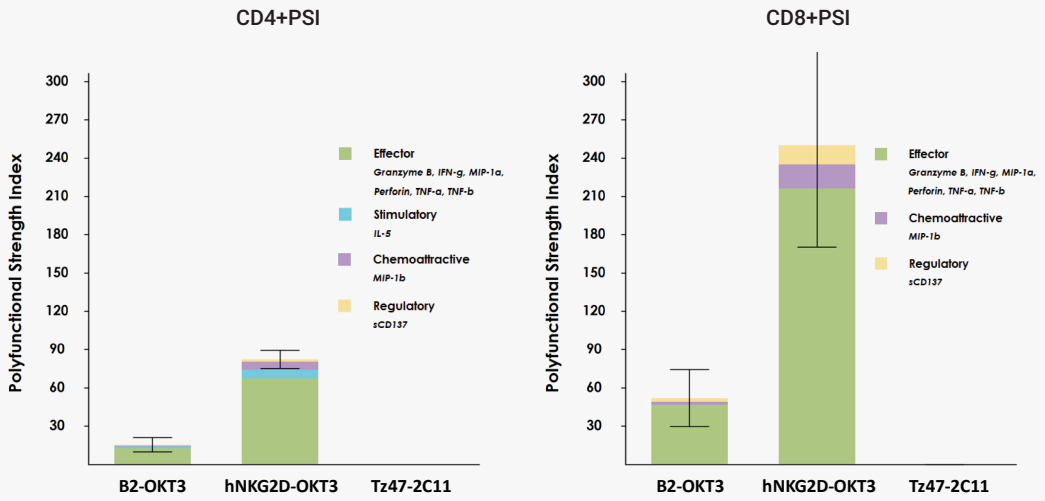

Figure 3 | PSI identified T cell potency upregulation triggered by novel bispecifics and revealed unique T cell cytokine mechanisms in response to tumor target. PSI was computed for CD4+ and CD8+ T cells; the profiles of both B2-OKT3 and hNKG2D-OKT3 showed significant polyfunctional upregulation relative to the Tz47-2C11 control sample, indicating their effectiveness in triggering a potent T cell response. Both bispecific profiles are dominated by effector cytokines. hNKG2G-OKT3 stimulation is more potent in terms of polyfunctional strength index (PSI) than B2-OKT3, and sources of upregulation are revealed through the differential cytokine profile (i.e. chemoattractive and effector cytokines). CD4+ and CD8+ cells show the same trend and almost identical cytokine secretion with the exception of IL-5.

Visualizing critical cellular subsets to determine optimal bispecific candidates

To further analyze the polyfunctional subsets within each treatment group and dissect the population architecture among different groups, we used a novel modified principal component analysis (PCA) visualization tool called Polyfunctional Activation Topology PAT PCA. We used this to better understand differences in T cell response when

stimulated with K562 cells in the presence of hNKG2D-OKT3 versus B2-OKT3.

PAT PCA revealed a complex landscape of polyfunctional subsets secreted by the CD4+ T cells (see Figure 4). The scatterplot focuses on combinatorial differences, rather than intensity differences; each color-coded circle represents a unique polyfunctional subset, with the color identifying the sample which secretes this subset most frequently. The size of the circles corresponds to the frequency of the group, and the cytokines listed along the axes reflect their increased presence as one moves in that direction on the plot [5].

Overall, CD4+ T cells from the hNKG2D-OKT3 group secreted polyfunctional effector proteins more frequently than those cultured in the presence of the B2-OKT3 bispecific. The hNKG2D-OKT3 group had a notably more diverse response, with many cells secreting combinations of 2-5+ effector cytokines, such as Granzyme B, IFN-g, MIP-1a, MIP-1b and TNF-b, along with MIP-1b and sCD137. While the B2-OKT3 CD4+ group had a more active response than the control, it was notably less potent than the hNKG2D-OKT3 CD4+ group. CD8+ cells showed similar differences across the two bispecific groups [5].

IsoPlexis' Polyfunctional PAT PCA visualization elucidates differences in cellular cytokine behavior of two bispecifics

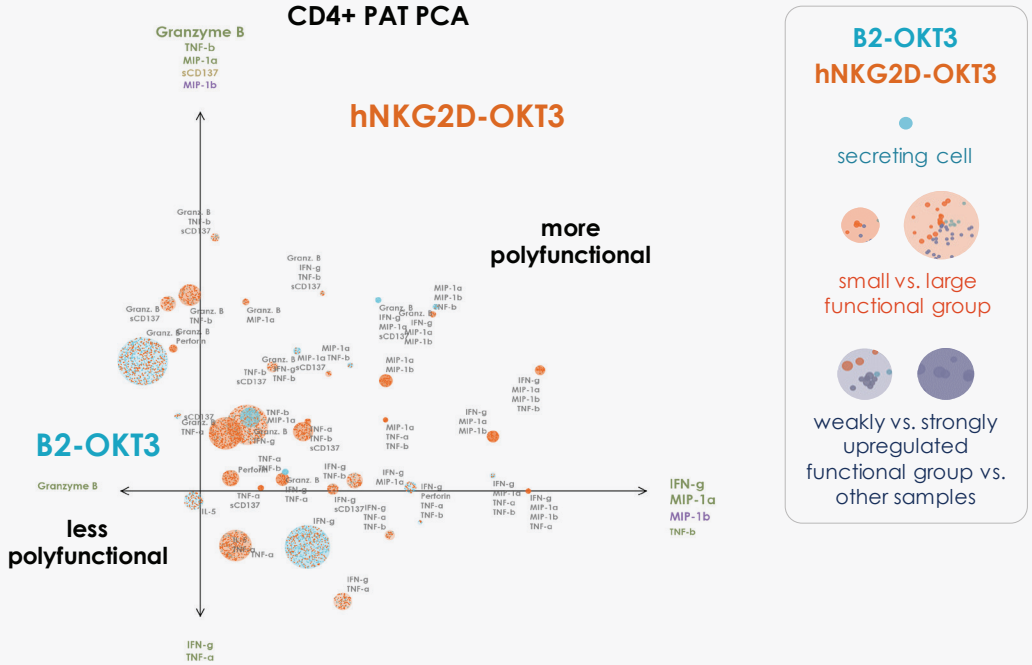

Figure 4 | PAT PCA visualization illustrates the differential T cell upregulation triggered by novel hNKG2D-OKT3 and B2-OKT3 bispecifics, by identifying highly functional cell subsets. CD4+ T cells from the hNKG2D-OKT3 group (orange) secreted polyfunctional effector proteins more frequently than those cultured in the presence tumor cells and the B2-OKT3 bispecific (blue). This polyfunctionality includes diverse combinations of secretions, whereas B2-OKT3 overall elicited fewer polyfunctional and more monofunctional secretions. The increased polyfunctional response and T cell potency driven by hNKG2D-OKT3 indicates its potential superiority over B2-OKT3.

Conclusions

Using the PSI metric for single cell profiling and potency evaluation in bispecifics:

- Allows improved evaluation of the quality of bispecific constructs by dissecting the T cell specific mechanisms of the therapy,
- Highlights critical differences between novel and control candidates in response to tumor targets, and
- Can connect discovery to mechanisms that are important to later stages in the development process.

References

1. Baeuerle PA, et al. Bispecific T-cell engaging antibodies for cancer therapy. Cancer Res 69, 4941-4944 (2009).
2. Thakur A, et al. Bispecific antibody based therapeutics: Strengths and challenges. Blood Rev 32, 339-347 (2018).
3. Slaney CY, et al. CARs versus BiTEs: A Comparison between T Cell-Redirection Strategies for Cancer Treatment. Cancer Discov 8, 924-934 (2018).
4. Rossi J, et al. Preinfusion polyfunctional anti-CD19 chimeric antigen receptor T cells are associated with clinical outcomes in NHL. Blood 132, 804-814 (2018).
5. Mackay S, et al. Single-cell proteomic analysis of T cells stimulated by Bi-specific T-cell Engagers shows robust and unique polyfunctional secretion profile. Journal for Immunotherapy of Cancer 6, Suppl 1, 114. (2018).