Single Biomarker Cell Mimics for Standardized Monitoring of T-Cell Activation and Exhaustion in Antibody Drug Development

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ABSTRACT

Monitoring T-cell activation and exhaustion is critical across antibody drug development, from early discovery through clinical trial assay validation. Traditional biological controls such as activated PBMCs or transduced cell lines are limited by variability, preparation time, and lack of long-term stability, making it difficult to standardize immune profiling assays.

Slingshot Biosciences has developed single biomarker cell mimics expressing key activation (CD25, CD69, CD278/ICOS, CD38) and exhaustion (PD-1, CTLA-4, TIM-3) markers. These engineered particles can be directly spiked into non-activated PBMC samples and processed using standard staining protocols. Flow cytometry analysis demonstrates marker-specific positivity with signal levels comparable to biologically activated PBMCs.

These novel reagents offer a robust, reproducible alternative to biological controls and are ideally suited for applications such as high-throughput screening of immune-targeting antibodies, assay development and validation, pharmacodynamic biomarker analysis, and lot-to-lot quality control. By enabling consistent and standardized measurement of immune markers, these controls enhance assay reliability for checkpoint inhibitor research, CAR-T therapy development, and vaccine immunogenicity studies.

Cell mimics address a longstanding need for stable, easy-to-use positive controls in immunophenotyping workflows, ultimately supporting faster and more reliable development of next-generation immunotherapies.

INTRODUCTION

Slingshot leverages the principles of biochemistry, high-precision manufacturing, and polymer chemistry to engineer cell mimics that match the features of biological cells, including optical, fluorescence and biochemical properties. By addressing the limitations of biological controls, cell mimics offer a scalable, reproducible solution to accelerate and improve the development of next-generation immunotherapies.

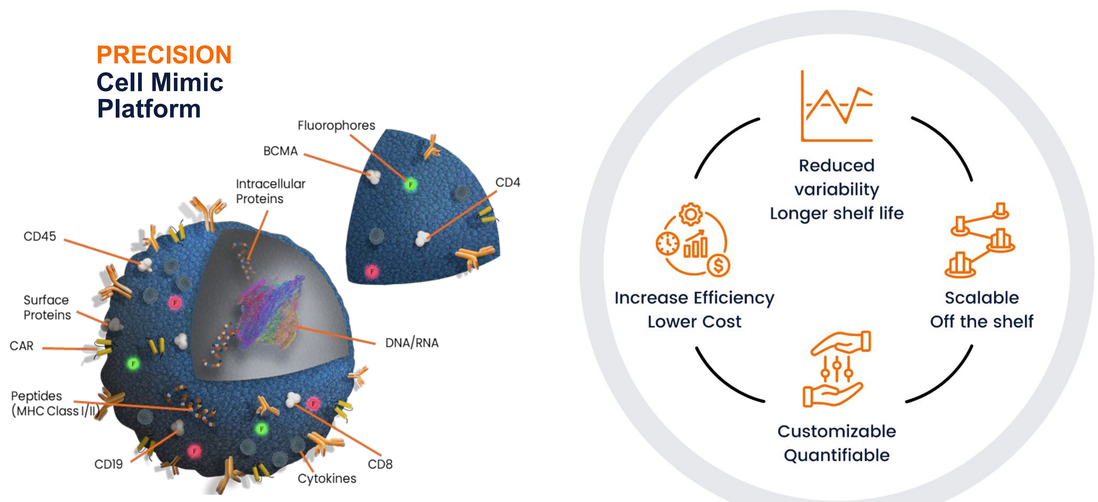

Fig 1. Schematic of Slingshot technology capability to match biological cells and benefits for standardized drug development

METHODOLOGY

- Commercially available cryopreserved PBMCs were thawed and split into three groups for treatment as follows: 1. activation, 2. single biomarker cell mimic spike in, or 3. remain untreated.
- Group 1 PBMCs were activated using ImmunoCult™ Human CD3/CD28 T Cell Activator
- Group 2 PBMCs were mixed with reconstituted single biomarker cell mimics expressing CD25, CD69, CD278 (ICOS), CD38, PD-1, TIM-3, and CTLA-4. In each tube, 8.3 x 10^4 cell mimics were mixed with 1 x 10^5 PBMCs
- Samples were stained with fluorophore conjugated antibodies to the respective activation or exhaustion markers as well as CD45, CD3, CD4, CD19, CD8, CD14, CD56 and CD16.
- All experiments were performed on a Cytek Aurora flow cytometer and analyzed using Flowjo software

RESULTS

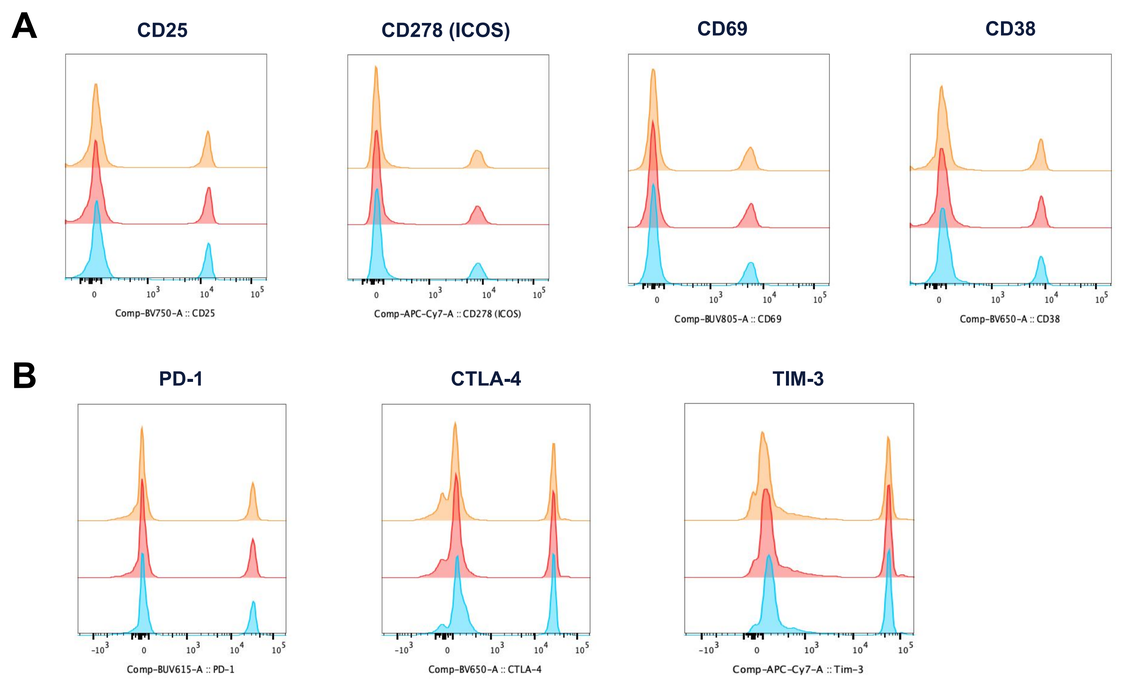

Fig 2. Single Biomarker Cell Mimics Demonstrate Positive Expression of Activation And Exhaustion Markers. Histograms of positive expression of **A**. Activation and **B**. Exhaustion Markers.

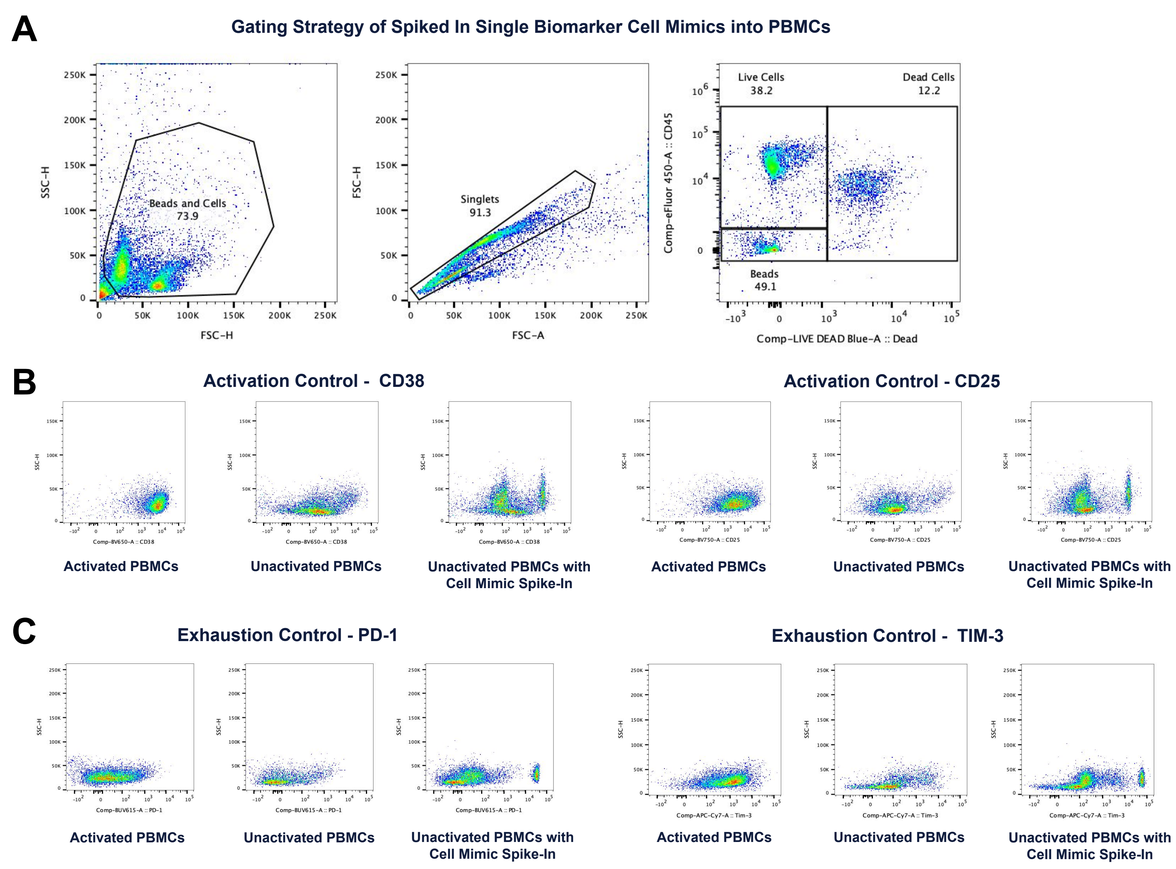

Fig 3. Single Biomarker Cell Mimic Controls Can Be Spiked Into PBMCs to Represent Exhausted or Activated States

A. Gating scheme showing how cell mimics are represented based on optical parameters on a flow cytometer and gated out from live vs. dead cells.

B. Unactivated PBMCs were spiked with single biomarker cell mimics expressing CD38 or CD25 to represent activated cells.

C. Unactivated PBMCs were spiked with single biomarker cell mimics expressing PD-1 or TIM-3 to represent exhausted cells.

* Similar results compared to activated PBMCs were also observed with CD69, CD278/ICOS, CTLA-4 single biomarker cell mimics compared to activated PBMCs.

CONCLUSIONS

Slingshot Biosciences has developed precision-engineered cell mimics expressing key activation and exhaustion biomarkers, providing scalable, reproducible quality controls for antibody drug development. These cell mimics offer several key advantages:

- Specific biomarker expression detectable by flow cytometry with bright signal intensity
- When spiked into PBMC samples, closely replicate the optical and phenotypic profiles of activated or exhausted T cells
- Match PBMCs in granularity, ensuring compatibility with established gating schemes in immune cell analysis workflows
- Seamlessly integrate into standard antibody staining protocols