Enhancing Flow Cytometry Assay Performance with Cell Mimics Controls for Immune Cell Profiling and Quality Control

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ABSTRACT

Flow cytometry is essential for immune profiling, but a lack of standardized controls limits reproducibility and assay reliability. Cell mimics expressing single, defined biomarkers offer a consistent, flexible alternative to biological controls. These mimics integrate into diverse flow cytometry assays, including Lymphocyte Subset, T-cell activation, exhaustion, CAR-T detection, and post-infusion biomarker analysis. Their stability, reproducibility, and compatibility with multicolor panels enable improved assay calibration, gating validation, and immune monitoring. To evaluate performance, mimics bearing single activation markers (CD25, CD69, CD278, CD38) and single exhaustion markers (PD-1, CTLA-4, TIM-3) were spiked into PBMCs and subjected to standard staining workflows. Resulting percent-positive signals closely aligned with those from activated PBMCs, confirming the mimics' utility as reliable, reproducible assay controls. This platform enables more accurate, standardized T-cell analysis for applications in immunotherapy, vaccine development, and immune monitoring.

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 for Flow Cytometry. By addressing the limitations of biological controls, cell mimics offer a scalable, reproducible solution to accelerate and improve the development of next-generation immunotherapies.

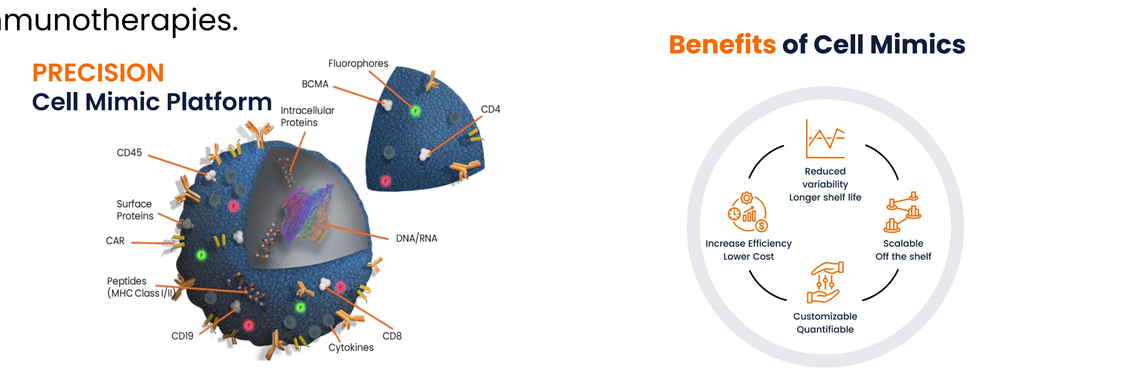

Figure 1. Schematic of Slingshot technology capability to match biological cells and benefits for standardized assay development

Seamless Integration of Slingshot Bioscience's Cell Mimic Controls into your workflow

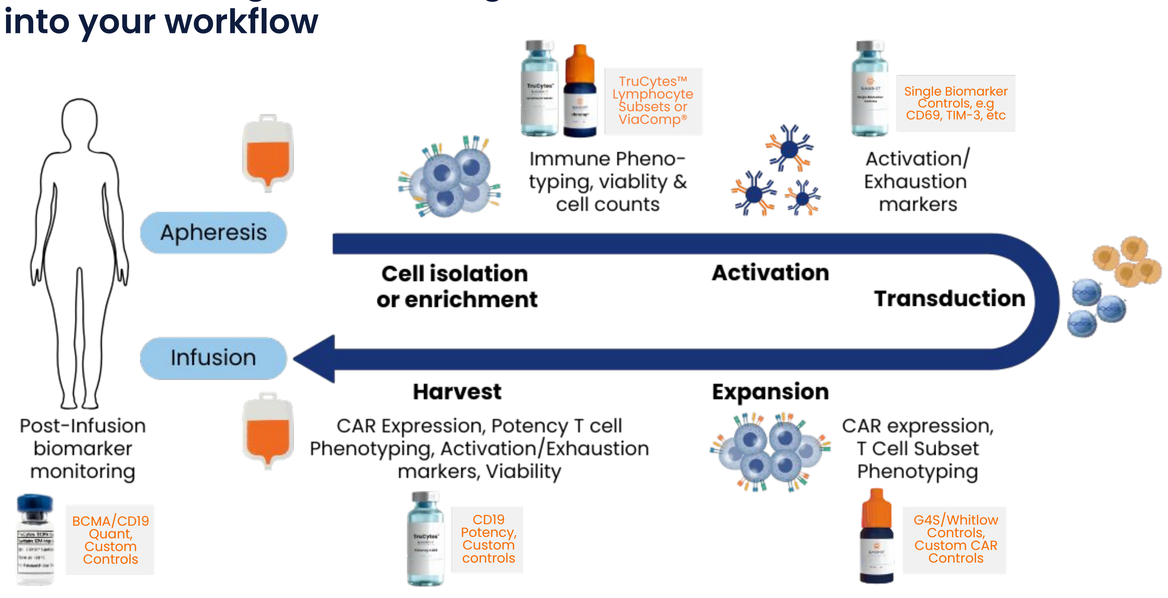

Figure 2. Schematic of Slingshot Bioscience's Cell mimics into Cell therapy workflow

Advantages:

- ***Clone Compatibility***: Fully compatible with commonly used antibody clones across immune profiling panels.
- ***Workflow Integration***: Seamlessly integrate into standard staining protocols and multicolor flow cytometry panels.
- ***Gating Accuracy***: Match PBMCs in size and internal complexity, supporting existing gating strategies without adjustment.
- ***Enhanced Assay Performance***: Improve assay calibration, gating validation, and instrument performance monitoring.

Single Biomarker TruCytes Cell Mimic Controls Can Be Spiked Into Peripheral Blood Mononuclear Cells

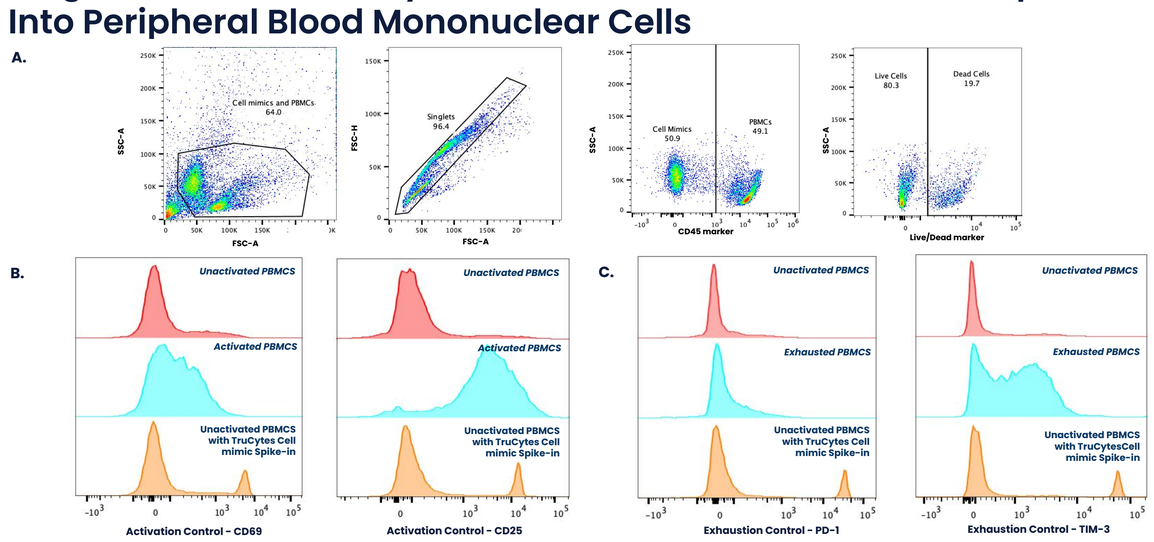

Figure 3. Single Biomarker Cell Mimic Controls Spiked Into PBMCs to Represent Exhausted or Activated States

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. Representation of Unactivated PBMCs, Activated PBMCs (activated with Dynabeads for 1 week), Unactivated PBMCs were spiked with single biomarker cell mimics expressing CD69 or CD25 to represent activated cells.
C. Representation of Unactivated PBMCs, Activated PBMCs (activated with Dynabeads for 1 week),Unactivated PBMCs were spiked with single biomarker cell mimics expressing PD-1 or TIM-3 to represent exhausted cells.

Development Of Multi-Biomarker LymphoCyte Subset Cell Mimics Demonstrating T, B and NK Cell Populations

Cell Population	Lyophilized PBMCs	Cryopreserved PBMCs	Lyophilized Lymphocytes	TruCytes Cell Mimics
Granulocyte $CD45^+$	N/A	N/A	N/A	2.5%
Monocyte $CD45^+$	13.9%	34.5%	N/A	4.4%
Lymphocyte $CD45^+$	10.2%	15.2%	0.9%	0.6%
T Cell $CD3^+$	12.1%	1.2%	0.5%	1.3%
B Cell $CD19^+$	36.6%	19.8%	6.5%	5.7%
$CD4^+$ T cell	15.2%	17.4%	0.8%	1.2%
$CD8^+$ T Cell	18.7%	18.7%	4.3%	2.3%
NK Cell $CD16^+CD56^+$	17.7%	3.3%	3.9%	0.3%
$CD14^+$ Monocytes	1.6%	8.3%	N/A	0.1%

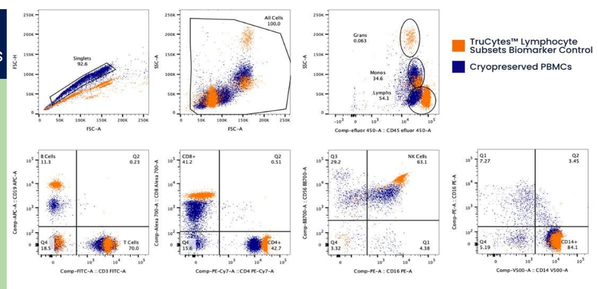

Figure 4. Schematic of Slingshot Biosciences' capability to match biological cells and representative flow plots for flow cytometry data for TruCytes Lymphocytes Subsets cell mimics compared to cryopreserved PBMCs. All samples underwent identical staining protocols using labeled monoclonal antibodies to CD45, CD3, CD19, CD4, CD8, CD14, CD16, and CD56, were collected on a Cytek Aurora spectral flow cytometer and analyzed via FlowJo.

Pre Approved Biomarkers for Custom Solutions*

Activation Markers	CD25, CD69, CD71 (Transferrin Receptor), CD134 (OX40), CD137, CD278 (ICOS), CD80, CD86
Exhaustion Markers	CD152 (CTLA-4), CD279 (PD-1), CD274 (PD-L1), CD223 (LAG-3), TIM-3, TIGIT
Chimeric Antigen Receptors	G4S, Whitlow, Custom CARs
T Cell Markers	CD2, CD3, CD4, CD5, CD7, CD8, CD25, CD27, CD28, CD45, CD154 (CD40L)
T Cell Memory Subset Phenotype markers	CD62L, CCR7, CD45RA, CD45RO, CD27, CD28, CD95
Tumor/Proliferation Markers	Ki-67 (MKI67), CD71 (Transferrin Receptor), CD99, CD227 (MUC1), CD340 (HER2/neu), LRRC15
NK Cell Markers	CD16, CD56, CD335 (NKp46)
Transcription Factors	FOXP3, ROR1
Cytokines & Cytokine Receptors	IL-2, IL-4, IL-21, TNFa, TGFbR2, CD120b (TNF-RII), CD122 (IL-2Rβ), CD124 (IL-4R), CD126 (IL-6R), CD127 (IL-7Rα), CD213a1 (IL-13Rα1), CD213a2 (IL-13Rα2), CD215 (IL-15Rα), CD217 (IL-17R), CD218a (IL-18Rα), CD218b (IL-18Rβ)
B Cell Markers	CD19, CD20, CD22, CD23, CD24, CD38, CD79b, CD86, CD267 (TACI), CD268 (BAFF-R), CD269 (BCMA)
Myeloid Markers	CD14, CD16, CD33, CD64, CD66, CD68, CD163, CD172a (SIRPα)
Stem/Progenitor Markers	CD34, CD117 (c-Kit), CD123 (IL-3Rα), CD135 (FLT3)
Adhesion Molecules	CD54 (ICAM-1), CD58 (LFA-3), CD102 (ICAM-2), CD166 (ALCAM)
Other Functional Markers	EGFR, CD36, CD39, CD40, CD47, CD52, CD59, CD93, CD109, CD112, CD147, CD150, CD155, CD162, CD203c, CD221, CD229, CD252 (OX40L), CD254 (RANKL), CD257 (APRIL), CD304 (Neuropilin-1), CD314 (NKG2D), CD319 (SLAMF7), CD328 (Siglec-7), CD329 (Siglec-9)

*Other markers can be evaluated

Table 1. Slingshot evaluated biomarkers for custom projects. These markers can be added to Slingshot cell mimics to develop customizable controls to meet unique assay needs.

CONCLUSIONS

Slingshot Biosciences has developed precision-engineered cell mimics serve as scalable, reproducible quality controls for flow cytometry, offering several key advantages:

- Bright, specific expression of defined biomarkers with phenotypic and optical profiles that closely resemble activated or exhausted T cells, enabling reliable performance when spiked into PBMC samples.
- Improved assay consistency and reproducibility across a wide range of immune profiling workflows.