Cell Mimics as High Precision Controls For CGT Analytics and Potency Assays

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BACKGROUND AND AIMS

Quality control (QC) testing of cell-based products requires controls that reflect relevant product characteristics. However, biological controls such as cell lines and primary cells have major limitations. Cell lines are costly to grow and bank, and vary in antigen expression over time in culture, while primary cells are inherently variable and present sourcing problems. Slingshot Biosciences has developed polymer-based cell mimics decorated with cell surface molecules identical to those associated with specific cell types. These mimics can be used as purity, identity, and potency testing controls. Coefficient of variation is a metric for assay precision. Lower %CV reflects higher precision and thus greater ability to resolve different assay results.

INTRODUCTION

Slingshot Biosciences leverages the principles of biochemistry, high-precision manufacturing, and polymer chemistry to engineer TruCytes™, cell mimics that match the features of biological cells, including optical, fluorescence and biochemical properties. Using this technology, TruCytes are customizable and reproducibly manufactured to express relevant surface antigens with controlled antigen density, making them ideal surrogates for biological controls that are prone to variability.

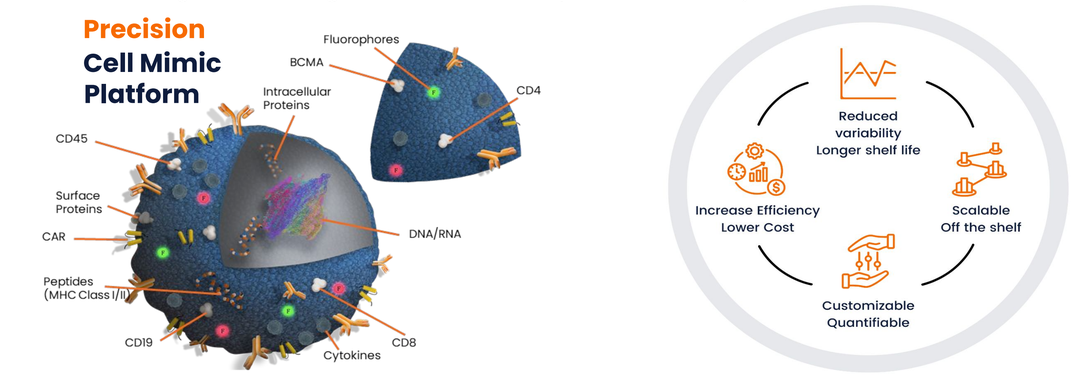


Fig 1. Schematic of Slingshot Biosciences' capability to match biological cells and benefits for standardized drug development

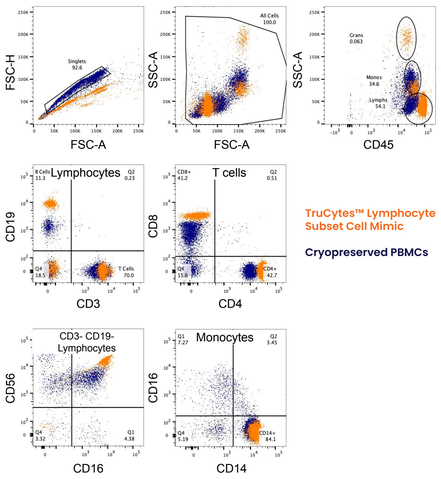
TruCytes™ Lymphocytes Subsets: Cell mimics to standardize PBMC flow cytometry

METHODOLOGY

- The relevant precision (%CV) of TruCytes Lymphocytes Subsets cell mimics (T, B, NK cells, monocytes, and granulocytes) vs. commercial cryopreserved/lyophilized controls was assessed via flow cytometry
- All samples underwent identical staining protocols using labeled monoclonal antibodies to CD45, CD3, CD19, CD4, CD8, CD14, CD16, and CD56
- All experiments were performed on a Cytek Aurora spectral flow cytometer and analyzed via FlowJo

RESULTS

TruCytes Lymphocytes Subsets Control demonstrates superior lot-to-lot consistency vs. commercial biological products



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

Figure 2. Representative flow plots for one experiment and %CVs for flow cytometry data for TruCytes Lymphocytes Subsets cell mimics compared to commercially-available cell controls (Table 1).

TruCytes Cell Mimics have High Lot-to-Lot Reproducibility

Population Type	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5	Lot 6	Lot 7	Lot 8	Lot 9	Lot 10	Lot 11	CV%
Grans CD45+	5	4	5.8	4.8	4.7	4.8	4.5	5	5.3	5	4.8	8.81%
Monos CD45+	15.5	15.4	15.5	14.2	14.7	14.4	14.6	14	15.8	13.8	14.1	4.57%
Lymphs CD45+	78.7	79.1	77	80	79.6	80	79	80.3	77.3	79.8	79.7	1.32%
T cells CD3+	77.3	76.2	69.6	74.8	73.6	74.3	72.9	74.6	74	72.5	73.4	2.58%
B cells CD19+	12.5	13.7	16.5	14.5	15.7	14.8	16.2	14.7	15.1	16	14.4	7.51%
T Helper CD4+	67	65.4	65.3	68.2	66.1	66.5	65.4	66	66.2	66	67.9	1.40%
T Cytotoxic CD8+	33	34.6	34.6	31.7	33.8	33.5	34.6	33.8	33.8	33.9	32.1	2.76%
NK cells CD16+CD56+	94.2	99.8	98.8	99.8	99.9	98.8	99	99.7	98.8	98.8	98.6	1.53%
Classical Monos CD14+	93.6	99.8	98.6	99.3	98.6	94.1	98.4	97.7	99.5	99.3	99.5	2.10%

Table 2. TruCytes Lymphocytes Subsets cell mimics composition across 11 lots from scale-up to full manufacturing and CV% across 11 lots demonstrate high reproducibility of production lots at full commercial scale.

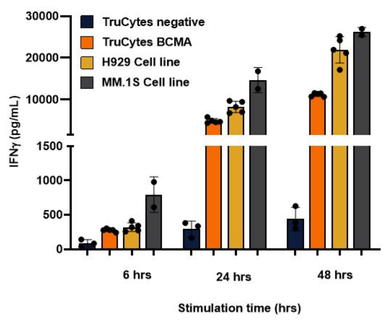
TruCytes™ Potency: Cell mimics to evaluate CAR-T function

METHODOLOGY

- Anti-BCMA CAR-T or anti-CD19 CAR-T and donor-matched untransduced cells were co-cultured with TruCytes Potency cell mimics or cell lines as indicated. Supernatant was collected at indicated time point and stored at -20 °C until cytokine assessment.
- IFNγ secretion in supernatant was quantified by ELISA or CBA.

RESULTS

TruCytes Potency BCMA induce reproducible IFNγ release with higher inter-day assay reproducibility than cell lines



% CV for IFNγ release in CAR-T Cell Potency Assays			
Culture Duration	TruCyte Cell Mimics (n=5)	H929 (n=5)	MM.1S (n=2)
6h	7.99%	19.2%	32.6%
24h	11.6%	16.5%	20.5%
48h	3.95%	14.4%	4.19%

Figure 3: BCMA CAR T-cell Potency Assay.

Negative control cell mimics, BCMA-labeled cell mimics, H929 cells and MM.1S cells cultured with anti-BCMA CAR T cells at 1:5 E:T ratio and evaluated for IFNγ secretion at 6, 24, and 48h. Symbols represent average IFNγ of two technical replicates of same lot of cryopreserved CAR-T in 5 separate experiments.

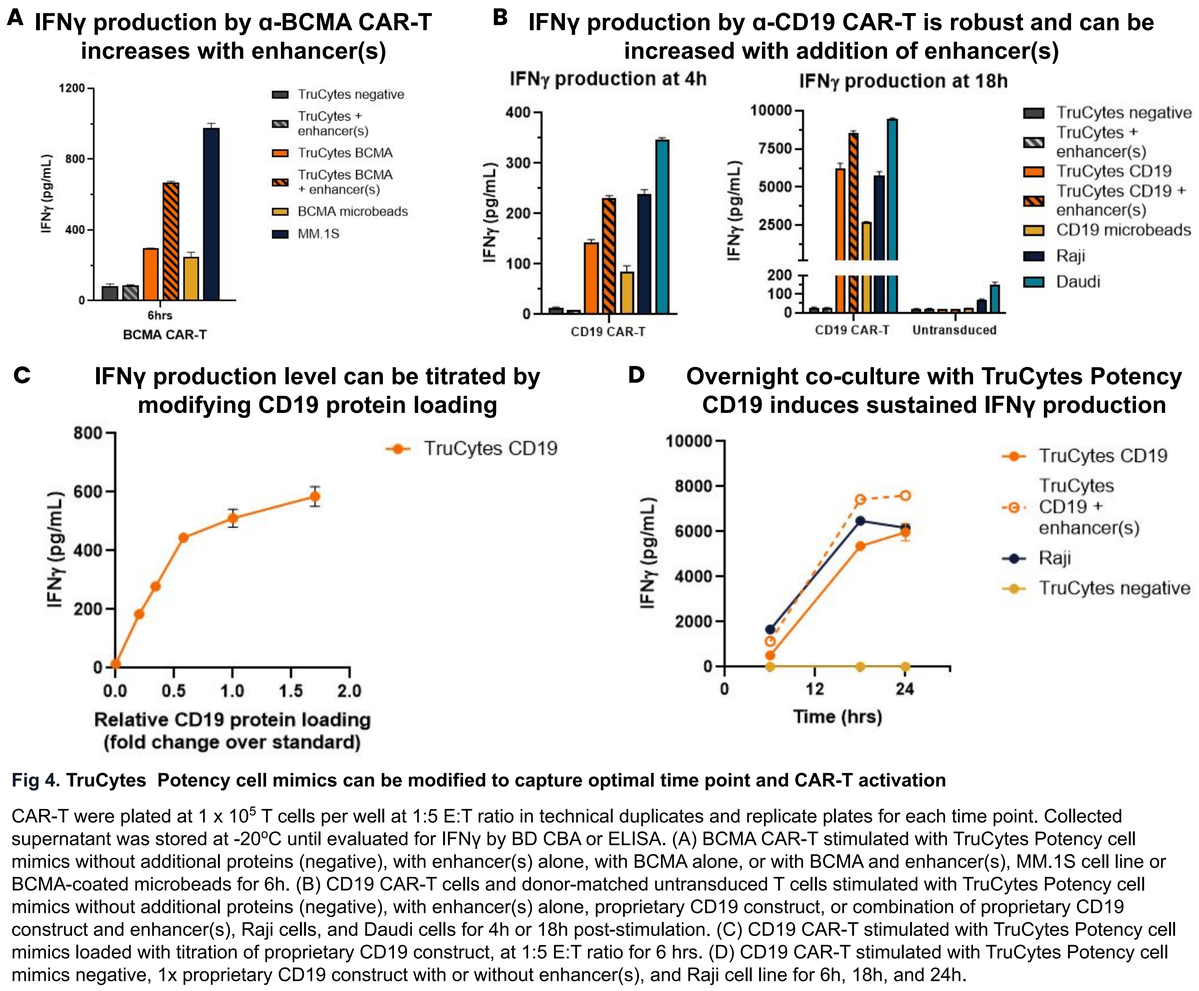


Fig 4. TruCytes Potency cell mimics can be modified to capture optimal time point and CAR-T activation

CAR-T were plated at 1 x 10^5 T cells per well at 1:5 E:T ratio in technical duplicates and replicate plates for each time point. Collected supernatant was stored at -20°C until evaluated for IFNγ by BD CBA or ELISA. (A) BCMA CAR-T stimulated with TruCytes Potency cell mimics without additional proteins (negative), with enhancer(s) alone, with BCMA alone, or with BCMA and enhancer(s), MM.1S cell line or BCMA-coated microbeads for 6h. (B) CD19 CAR-T cells and donor-matched untransduced T cells stimulated with TruCytes Potency cell mimics without additional proteins (negative), with enhancer(s) alone, proprietary CD19 construct, or combination of proprietary CD19 construct and enhancer(s), Raji cells, and Daudi cells for 4h or 18h post-stimulation. (C) CD19 CAR-T stimulated with TruCytes Potency cell mimics loaded with titration of proprietary CD19 construct, at 1:5 E:T ratio for 6 hrs. (D) CD19 CAR-T stimulated with TruCytes Potency cell mimics negative, 1x proprietary CD19 construct with or without enhancer(s), and Raji cell line for 6h, 18h, and 24h.

CONCLUSIONS

Slingshot Biosciences has developed precision-engineered cell mimics, made by controlled manufacturing, that are well defined, highly consistent, easily used standards for QC testing of cell-based products.

These cell mimics offer several key advantages:

- **Consistent manufacturing:** Cell mimics are manufactured with tight control to ensure reliable, standardized QC testing.
- **Superior reproducibility:** TruCytes Lymphocyte Subsets Control showed CVs of 0.1–5.7%, outperforming PBMC controls (CVs of 1.6–36.6%).
- **Enhanced precision:** In IFN-γ release assays, Trucytes Potency cell mimics delivered lower %CVs than cell lines when used as stimulators.
- **First-of-its-kind potency assessment solution:** Demonstrated biologically relevant activation with tunable antigen density— outperforming microbeads and comparable to cell lines.

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