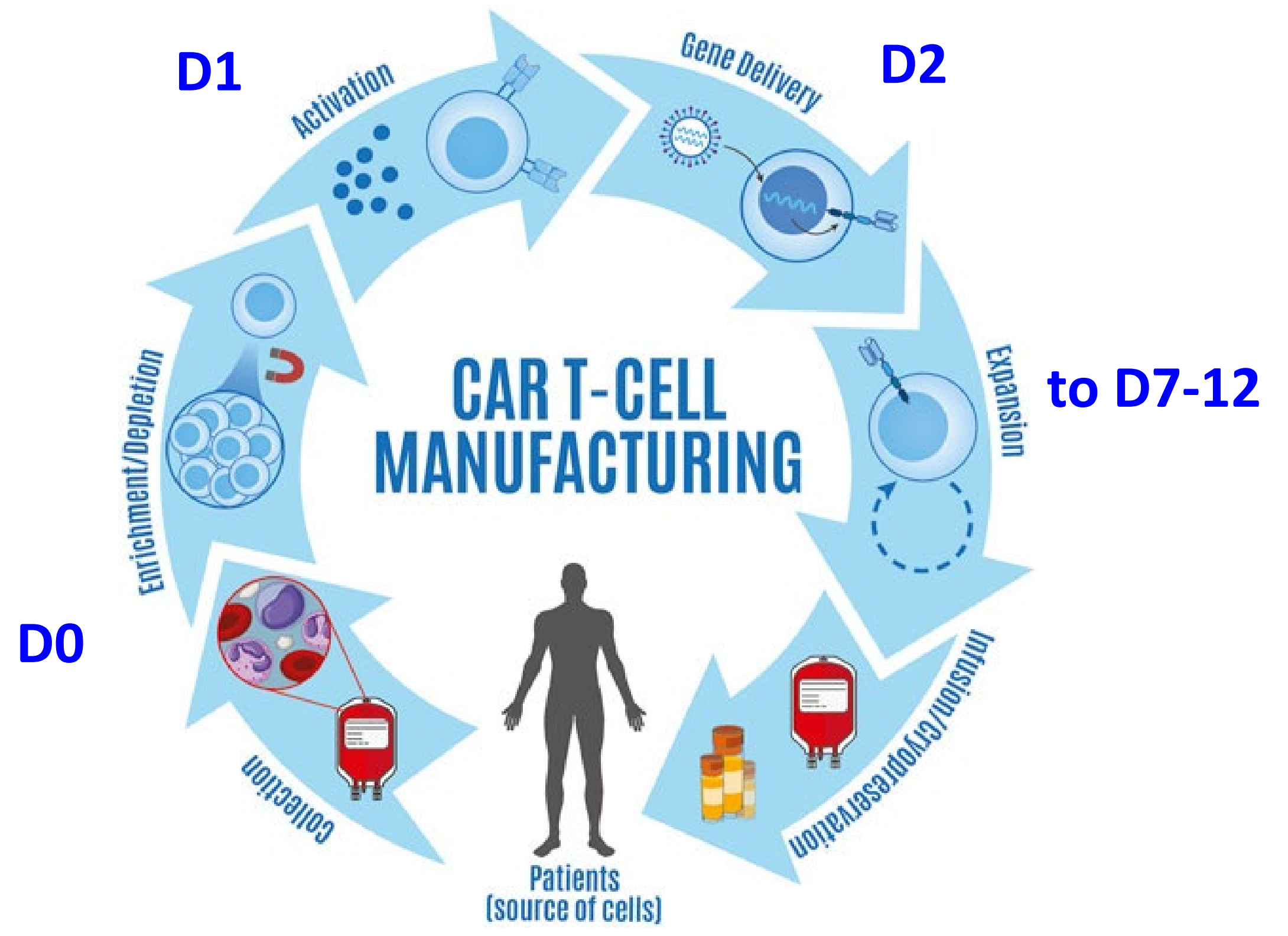
A Rapid and Cost-Effective Solution for CAR- T Cell Manufacturing

Huiyu Wang, PhD., Silin Sa and Liping Yu, PhD.
Applied Cells, Inc. Santa Clara, California, USA

Introduction

Conventional Process: 1-2 weeks



GoFast™ Process: less than 3 days

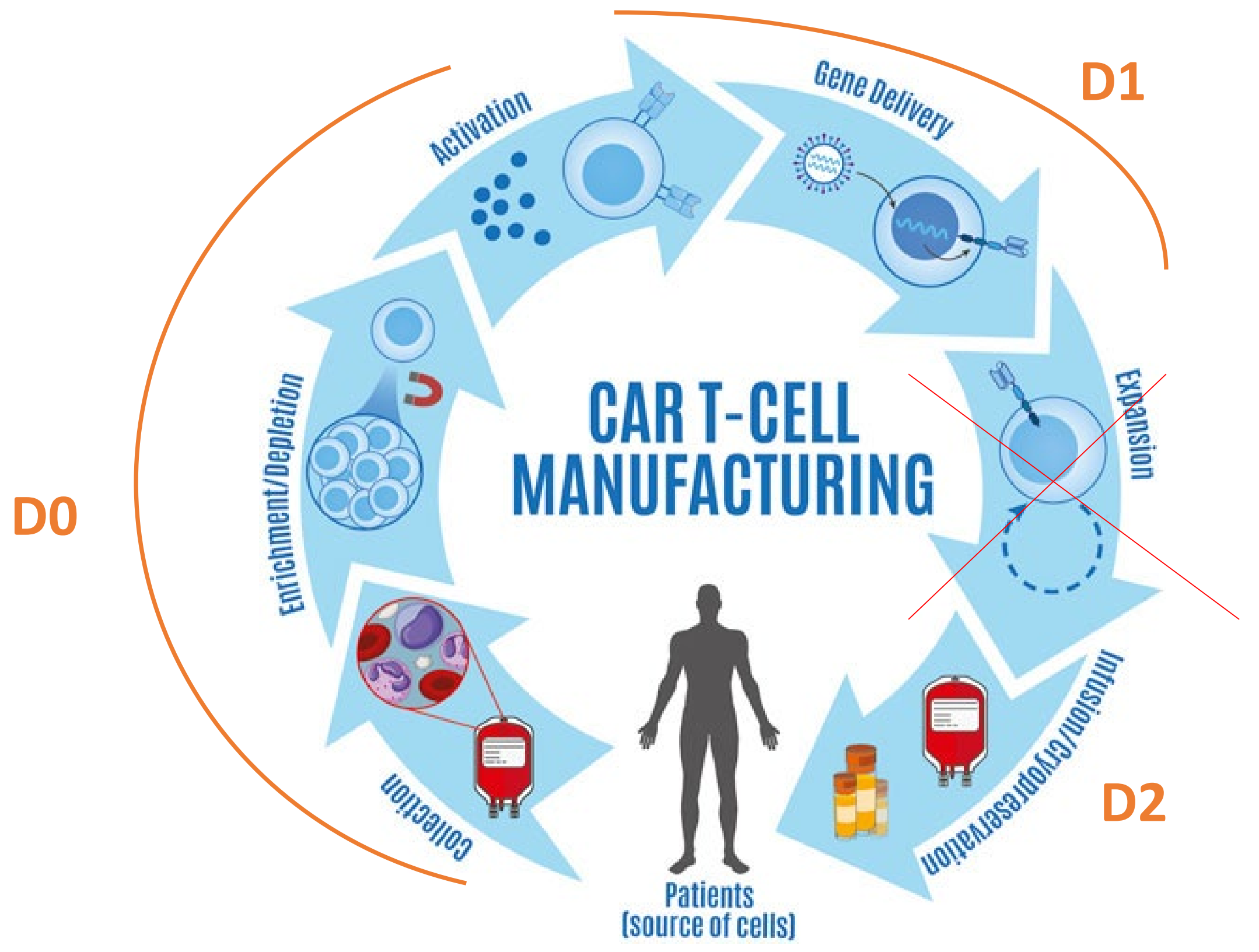
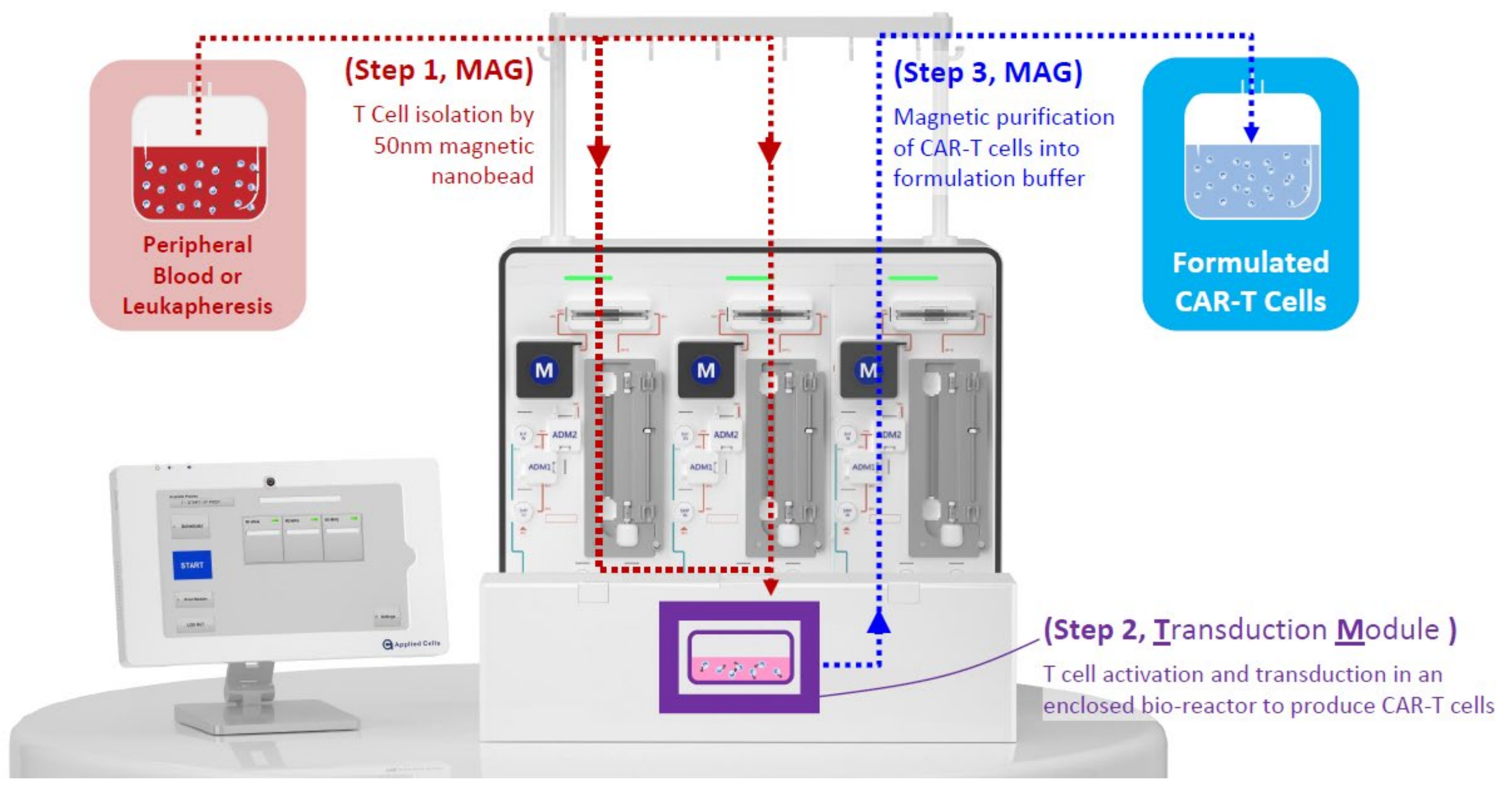


Image is from https://doi.org/10.3389/fmmed.2024.1310002

Figure1. The development of rapid and low-cost cell therapy products represents a significant advancement in the field of cell therapy. Compared to the conventional CAR-T cell manufacturing process which takes up to 14 days The rapid, so called "GoFast" process eliminates the expansion step and completes within 72 hours. This process minimizes cell differentiation and exhaustion, thus produces cells with higher potency and higher anti-tumor activity.

Methods



ISO 13485:2016

Figure 2. **Illustration** of an integrated platform -MARS® Atlas and how the GoFast CAR-T cell manufacturing process is done on MARS® Atlas .

MARS® Atlas is built with three column-free magnetic cell separation modules (M1, M2 and M3) and one temperature-controlled CO2 controlled "Transduction' module. The system comes with automation and single-use closed fluidic lines.

In the GoFast process there are three steps.

Step 1, T cells are isolated from peripheral blood or leukopak using CD4/CD8 50nm magnetic beads through M1 and M2 .

Step 2, T cells are activated with CD3/CD28 activation reagent for 24 hours and transduced with CD19 CAR lentivirus for another 24 hours in the Transduction module.

Step 3, CAR-T cells are purified through M3 and harvested in saline or media.

Results

D0 – T cell enrichment with CD4 &CD8 Nanomagnetic beads

	Total cell count	CD3+% (in CD45+%)	CD4+% (in CD3+%)	CD8+% (in CD3+%)	T cell recovery
frozen lp before T cell selection	1.20E+09	43.70%	62.80%	36.70%	
frozen lp after T cell selection	2.70E+08	95%	53.80%	38.60%	50.00%
fresh lp before T cell selection	5.67E+08	60.00%	40.70%	48.40%	
fresh lp after T cell selection	2.08E+08	96.20%	44.50%	53.40%	59%

D0 – T cell activation with CD3/CD28 nanobeads

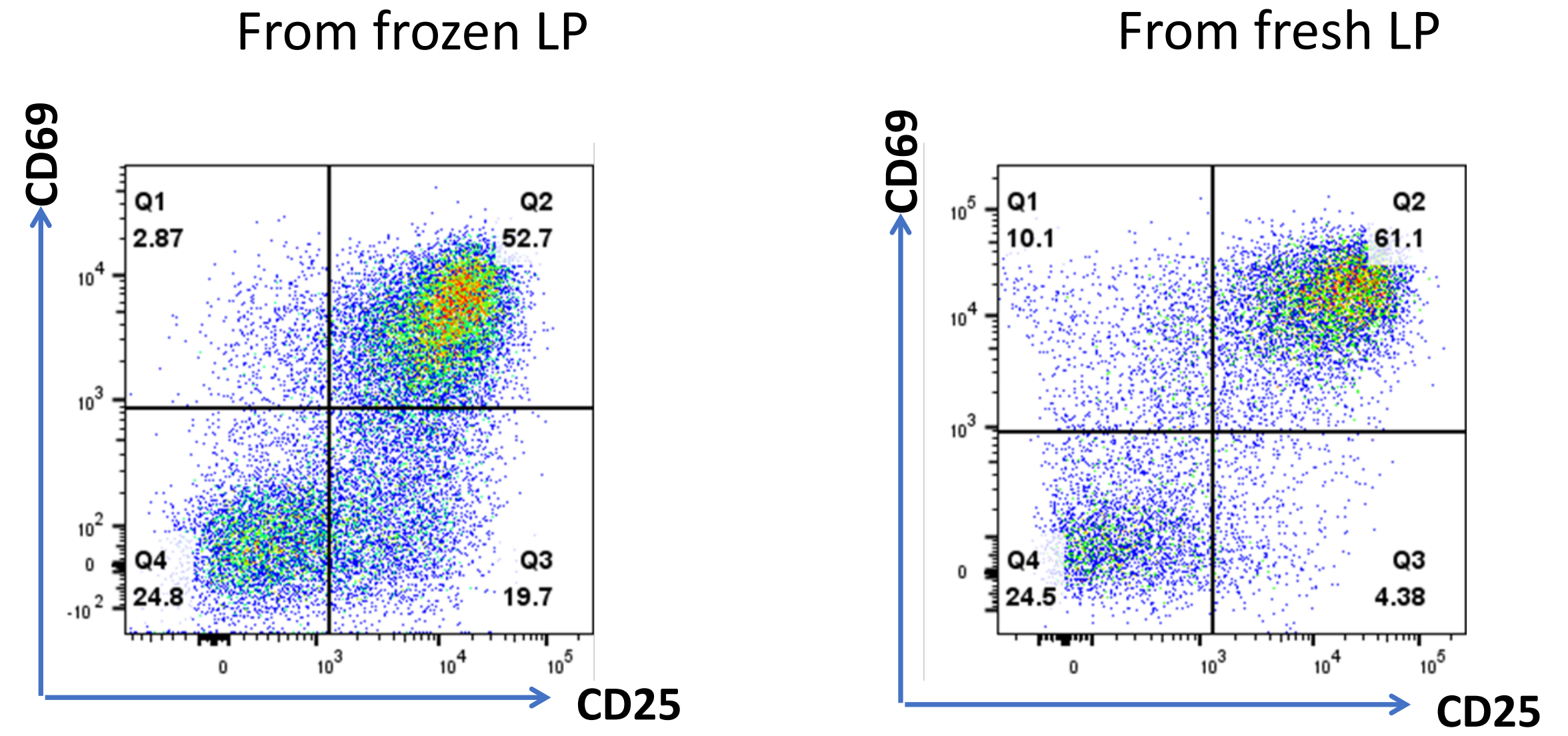

From frozen LP
From fresh LP

Figure 3. After 24-hour activation, T cell activation was assessed by CD25 and CD69 expression

D1 – T cell transduction with CD19 Lentivirus

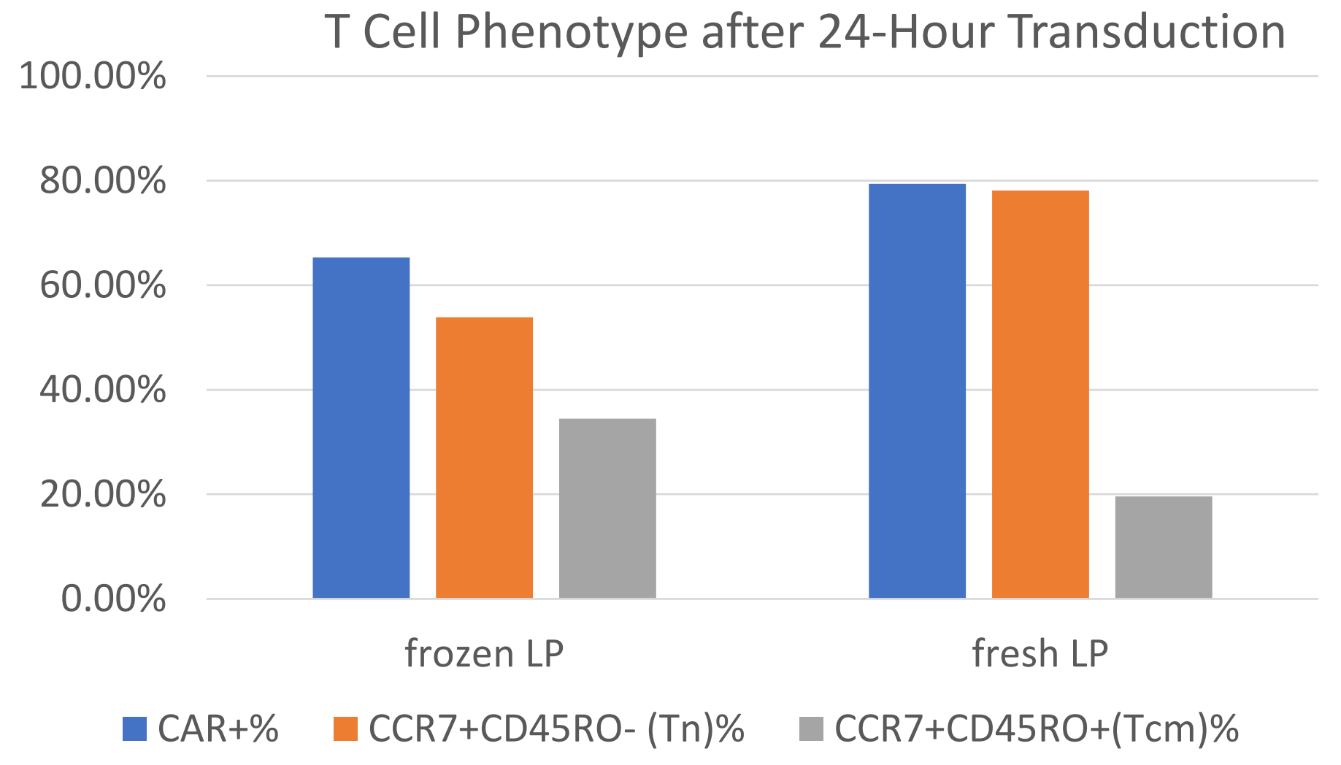
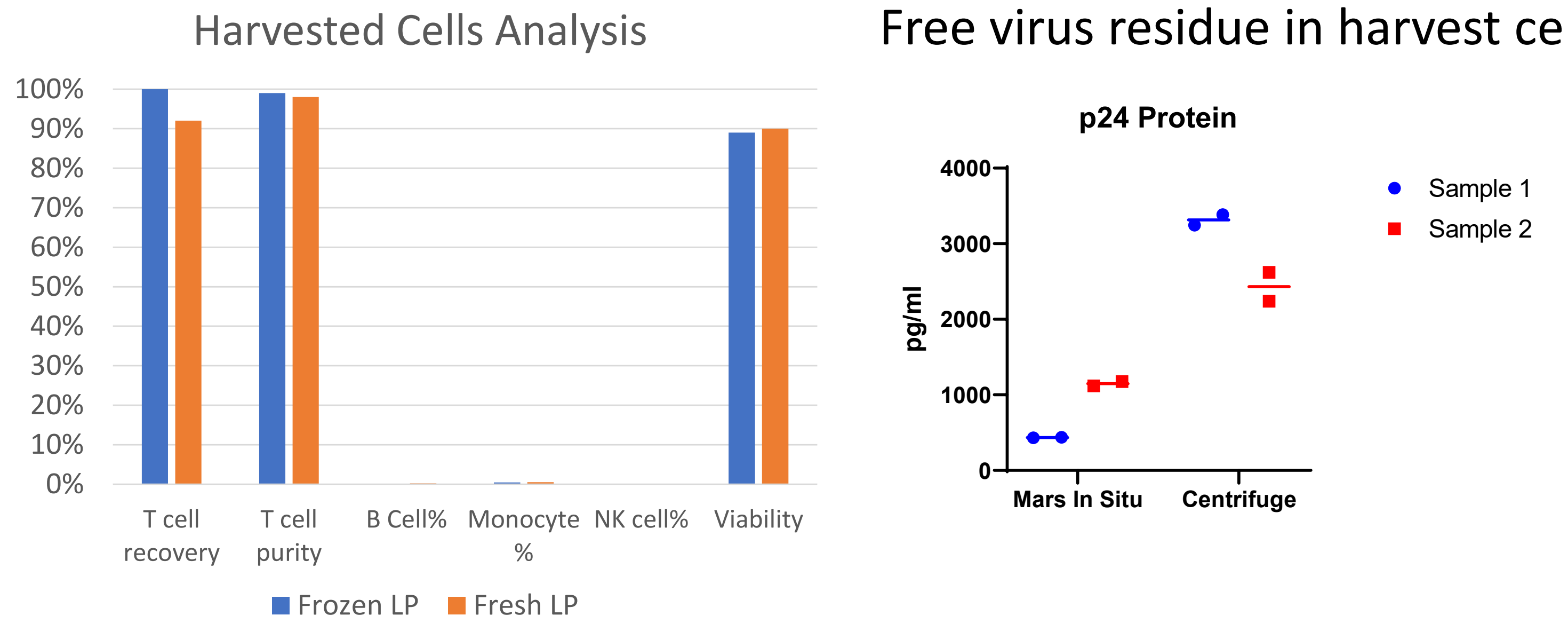

T Cell Phenotype after 24-Hour Transduction

Figure 4. After 24-hour activation, CD19 lentivirus was added to the flask inside the "transduction module" and incubated for another 24 hours. T cells were analyzed for their phenotypes and CAR+ expression

D2 – Harvest


Harvested Cells Analysis

Free virus residue in harvest cells

Figure 5. T cell recovery in the harvest step, T cell purity and viability were analyzed. Two harvest methods were compared by virus residue.

CAR-T cell function assessment

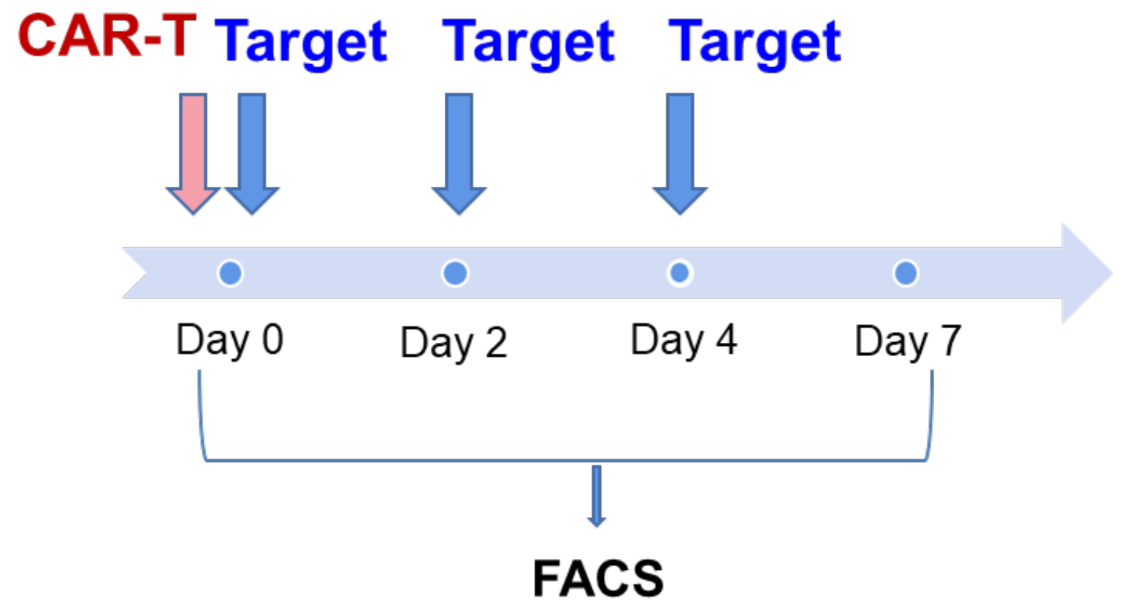
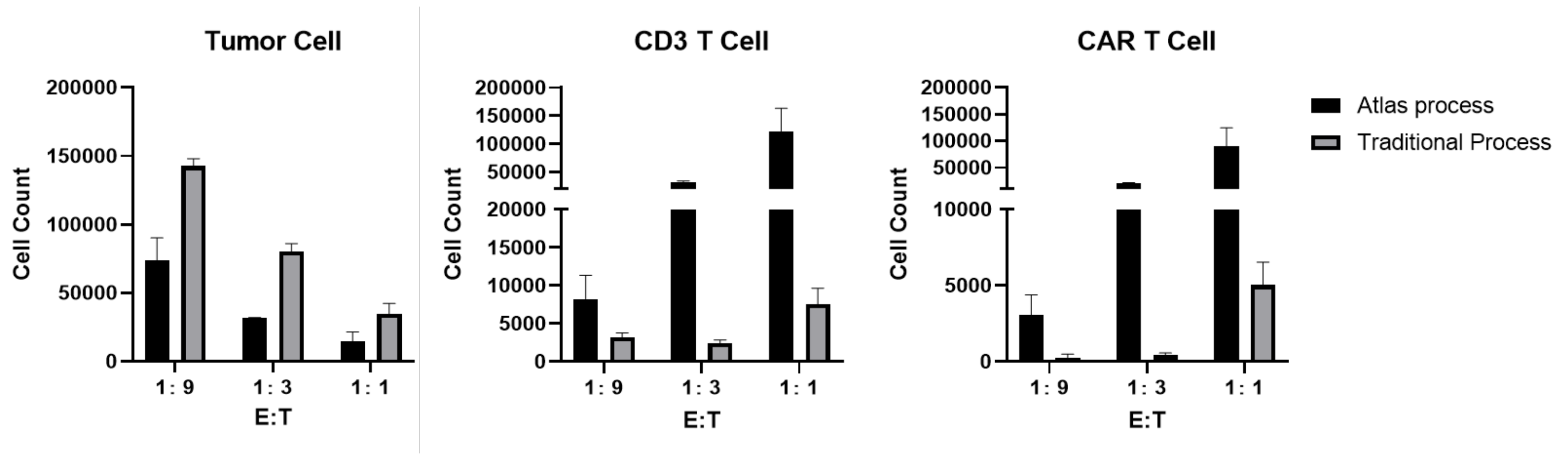
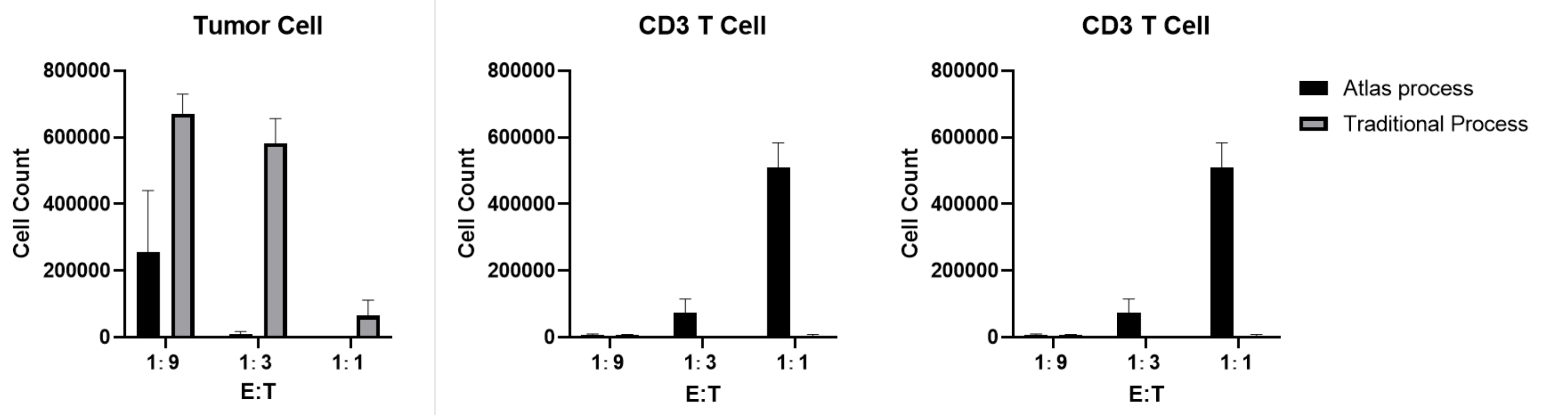


Figure 6. Function of manufactured CAR-T cell was assessed by killing assay– CAR-T cells were mixed with Nalm6 cells at different ratio and cocultured for 7 days. Tumor cells and CAR-T cells were counted on Day 4 and day 7.

D 4



D 7



Conclusion

- We have demonstrated the successful manufacturing of functional CAR-T cells on a single automated closed platform – MARS Atlas within 72 hours.
- The MARS Atlas will be the cost-effective solution for "point-of-care" CAR-T cell therapy soon.
- See MARS Atlas at booth 1018

info@appliedcells.com - www.appliedcells.com – Applied Cells Inc. , 3350 Scott Blvd Bldg 6, Santa Clara, CA 95054, Products are for research use only not intended for diagnostic or therapeutic uses