Automated Rapid CAR-T Cell Manufacturing Process On a Single Platform

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Outline

- MARS Atlas™ platform for small scale Rapid CAR-T manufacture in point-of-care settings
- MARS Atlas™ protocol step-wise performance:
  - Rapid CAR-T starting from T cell selection by nano-beads
  - Rapid CAR-T starting from T cell selection by micro-beads
- Summary

Conventional CAR-T Manufacturing Process

- **Autologous CAR-T has yielded durable responses in patients with cancers**
- **Broad adoption of the treatment – Not yet**
- **Centralized manufacture logistics** ← Limiting access
- **Long manufacture time : 15+ days** ← Limiting throughput
- **Large sample size : Leukopak of 10B total cells or more**
- **High cost of approved therapies : USD $300k+** ← Limiting adoption
- **Challenge for point-of-care implementation**
  - Overall cost-of-goods not providing desired cost reduction (hard to achieve <$30k final cost)

Rapid CAR-T Process

Rapid high potency CAR-T therapies manufacturing

Sample → **Immunomagnetic Cell isolation** → Genetic Engineering → Wash, concentration, media exchange → Dosage Ready

Penn, 2024 ASCO

7004 Oral Abstract Session

Safety and efficacy of armored huCART19-IL18 in patients with relapsed/refractory lymphomas that progressed after anti-CD19 CAR T cells.

Jakub Svoboda, Daniel J Landsburg, Sunita Dwivedy Nasta, Stefan K. Barta, Elise A. Chong, Michael J. Lariviere, Joanne Shea, Amanda Cervini, Elizabeth O. Hexner, Amy Marshall, Megan Four, Rachel M. Leskowitz, Megan M. Davis, Wei-Ting Hwang, Noelle V. Frey, Donald L. Siegel, Joseph A. Fraietta, David L. Porter, Stephen J. Schuster, Carl H. June; Lymphoma Program, Abramson Cancer Center, University of Pennsylvania, Philadelphia, PA; Center for Cellular Immunotherapies, Abramson Cancer Center, University of Pennsylvania, Philadelphia, PA; Department of Biostatistics, Epidemiology, and Informatics, University of Pennsylvania, Philadelphia, PA; Clinical Cell and Vaccine Production Facility, University of Pennsylvania, Philadelphia, PA

Responses and expansion by prior CAR product.

	CD28 (axi-cel, brexu-cel) N=10	4-1BB (tiso-cel, liso-cel) N=9	p-value
ORR	100%	56%	0.03*
CR	80%	22%	0.02*
Mean huCART19-IL18 expansion	26,326	5,479	0.01**

*) Fisher's exact t. **) Wilcoxon rank sum t.

ARTICLES
https://doi.org/10.1038/s41551-021-00842-6
nature biomedical engineering

Rapid manufacturing of non-activated potent CAR T cells

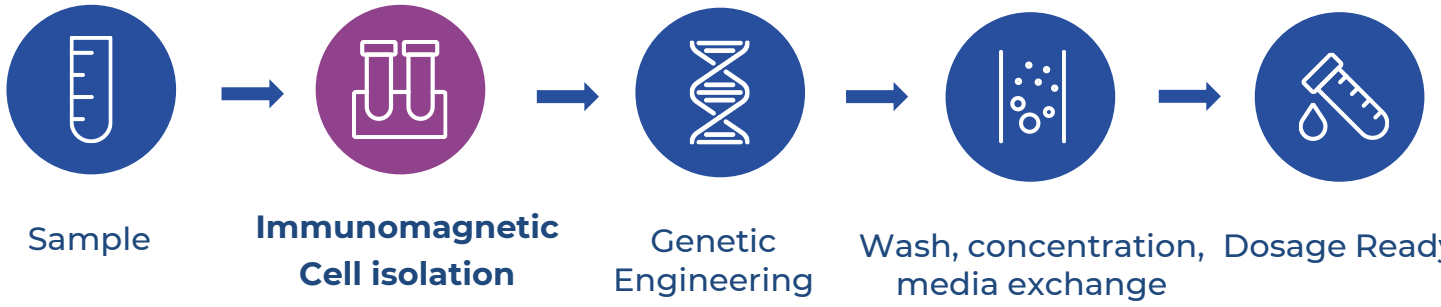
Saba Ghassemi[1,2], Joseph S. Durgin[1], Selene Nunez-Cruz[1,2], Jai Patel[1], John Leferovich[1,2], Marilia Pinzone[2], Feng Shen[1], Katherine D. Cummins[1], Gabriela Plesa[1], Vito Adrian Cantu[3], Shantan Reddy[3], Frederic D. Bushman[3], Saar I. Gill[1,4], Una O'Doherty[2], Roddy S. O'Connor[1,2] and Michael C. Milone[1,2]

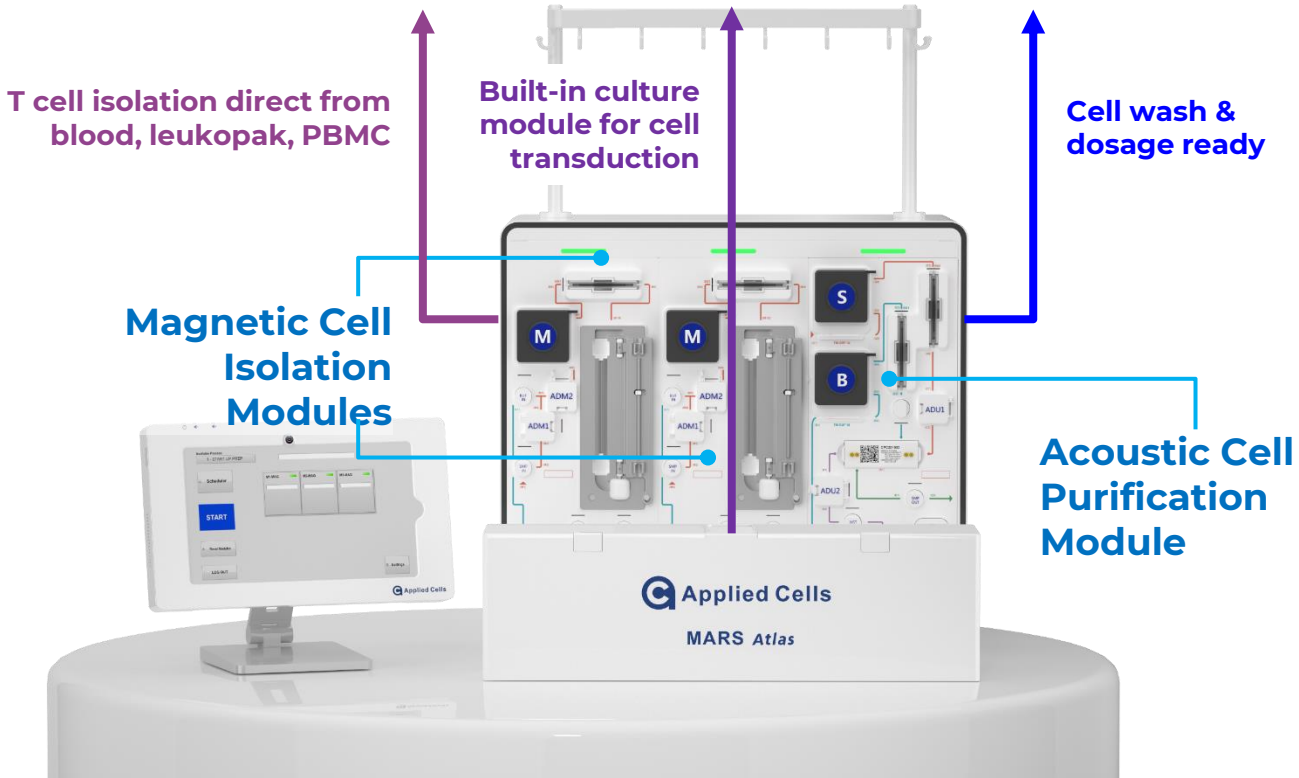
Key benefits of Rapid CAR-T process

i. No expansion, limited T cells exhaustion
ii. High potency : Reported **100x potent** CAR-T cells compared traditional CAR-T 4 wks after injection
iii. Simple & fast: **isolation → transduction → purification**
iv. Potentially very low cost :
- **3 million** dosed CAR-T cells are effective
- Potentially starting from ~50mL PB, 0.2B total cells vs 10B total cells of traditional CAR-T
- Complete with manufacture cost estimated achievable **< $15k**

MARS Atlas for Rapid CAR-T Process

Designed for Point-of-Care setting, Rapid Process, Directly from PB / Small LP / PBMC





MARS Atlas

- **3 STEP process** : MARS magnetic and acoustic technologies are uniquely positioned to complete the 3-step process
- Complete rapid small scale cell manufacture within 2~3 days
- Directly from peripheral blood, or small leukopak, or PBMC

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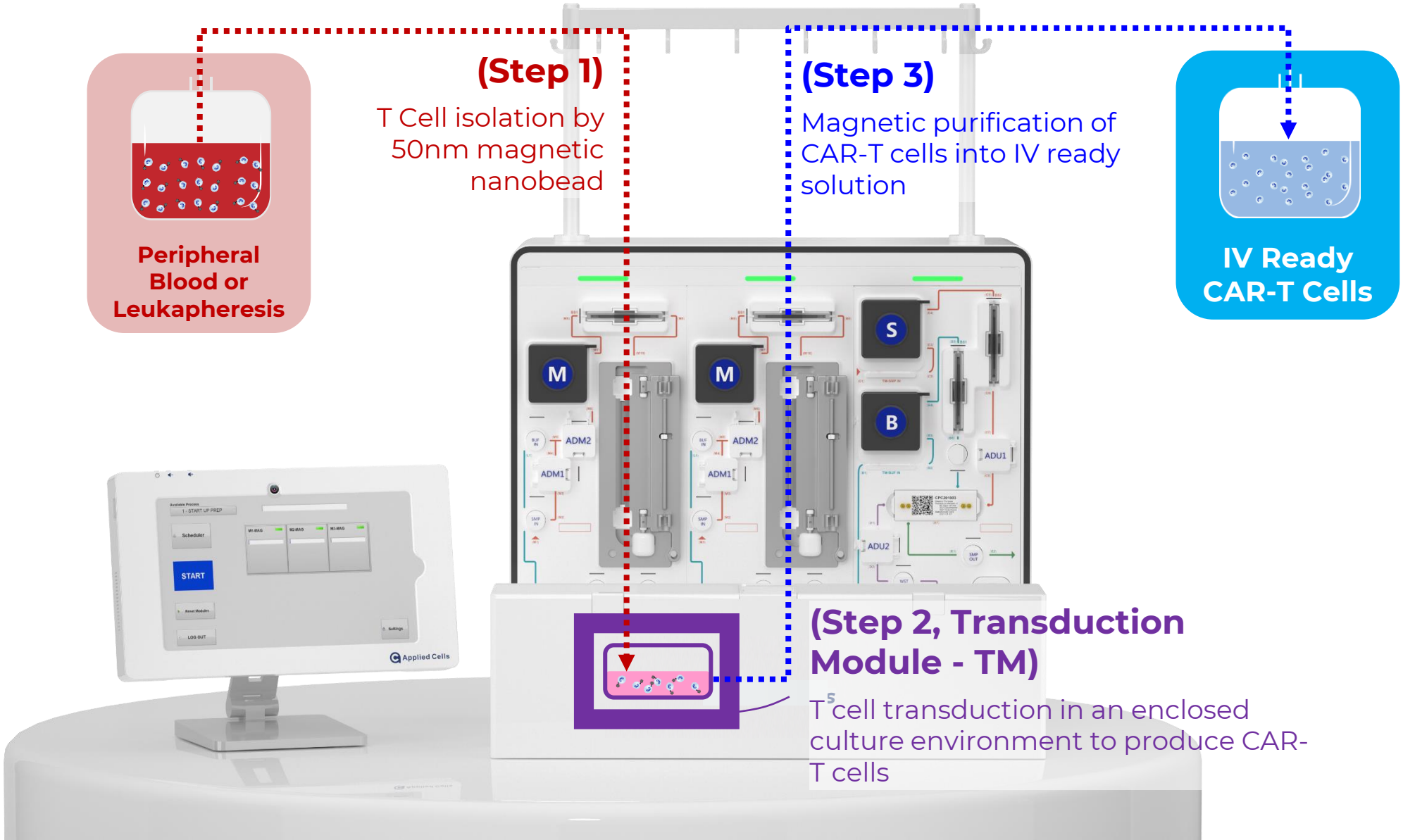
Rapid CAR-T Manufacture

Starting From

T Cell Isolation With 50nm Nano-Beads

Rapid CAR-T with 50nm Beads: MAG → TM → MAG



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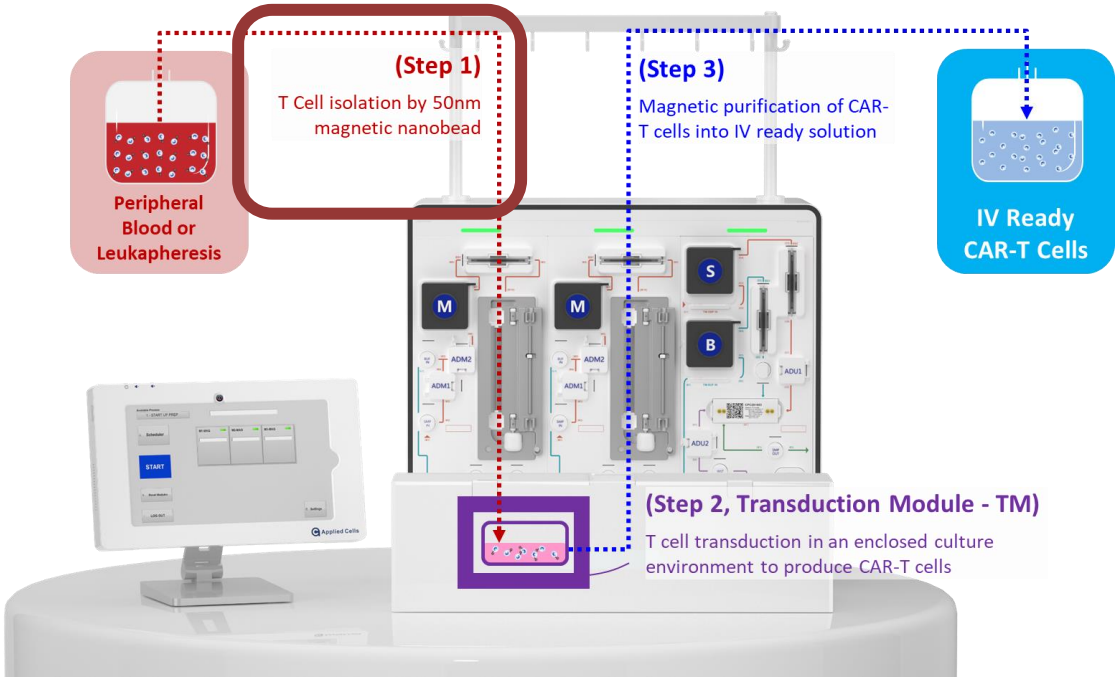
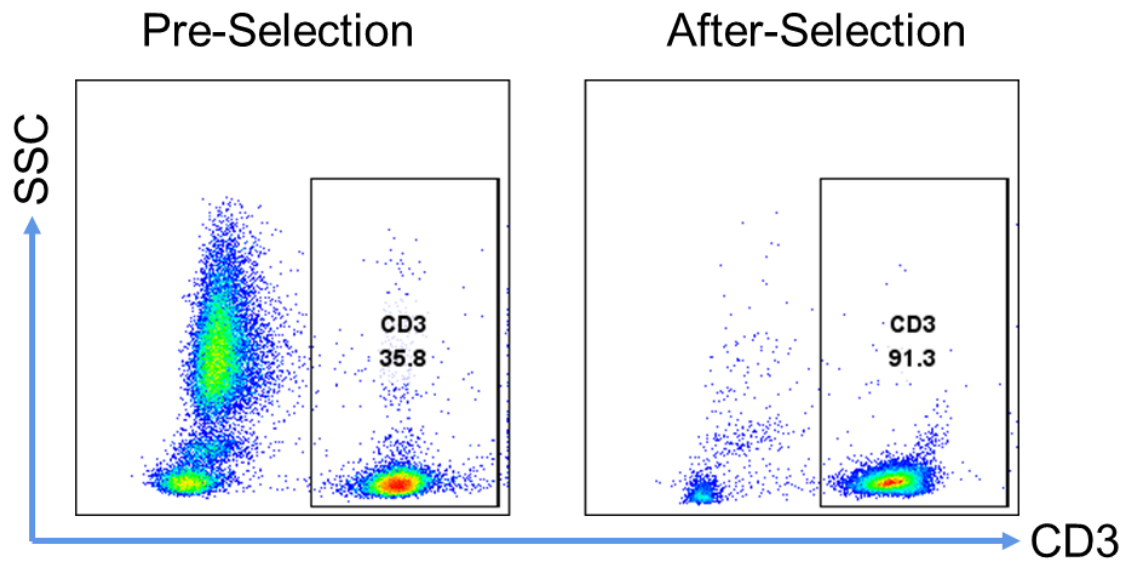
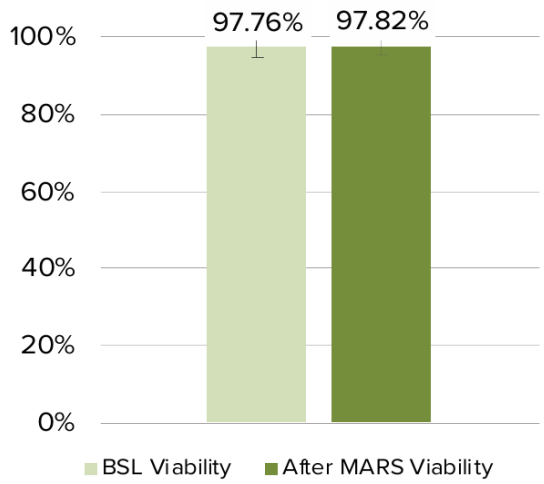
Rapid CAR-T with 50nm Beads: MAG → TM → MAG

(Step – 1) T cell selection by Applied Cells Ingenuity 50nm CD4 and CD8 GMP grade magnetic beads







Efficient isolation of T cells from peripheral whole blood with purity of >90% , and viability not affected after positive selection (P<0.5)

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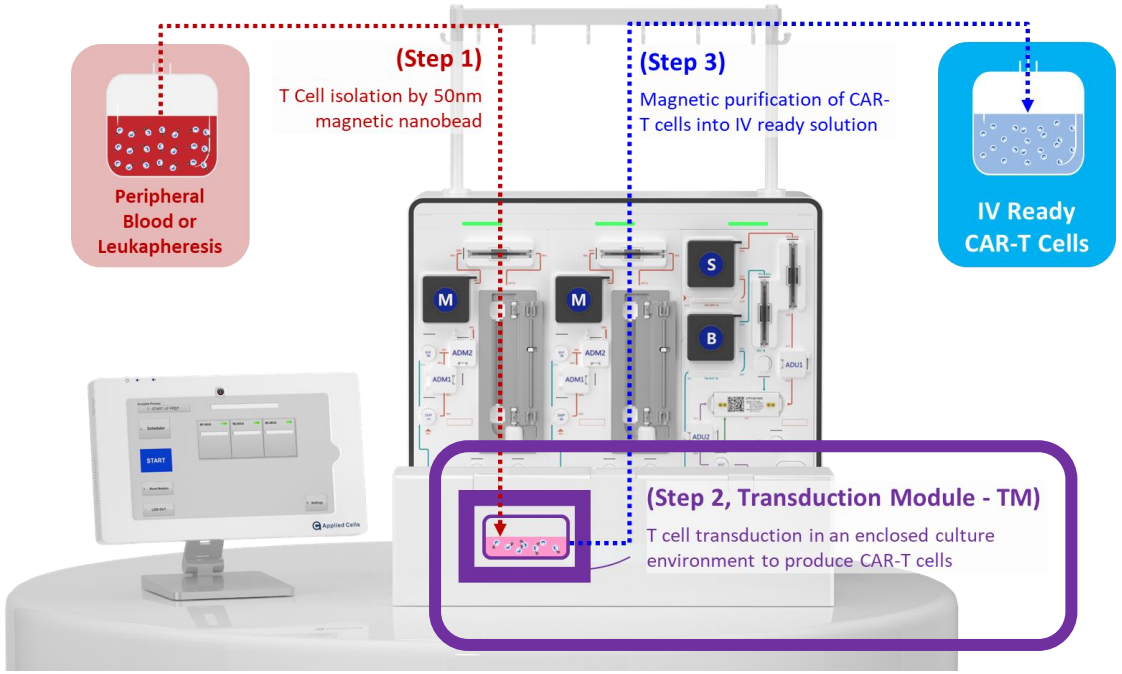
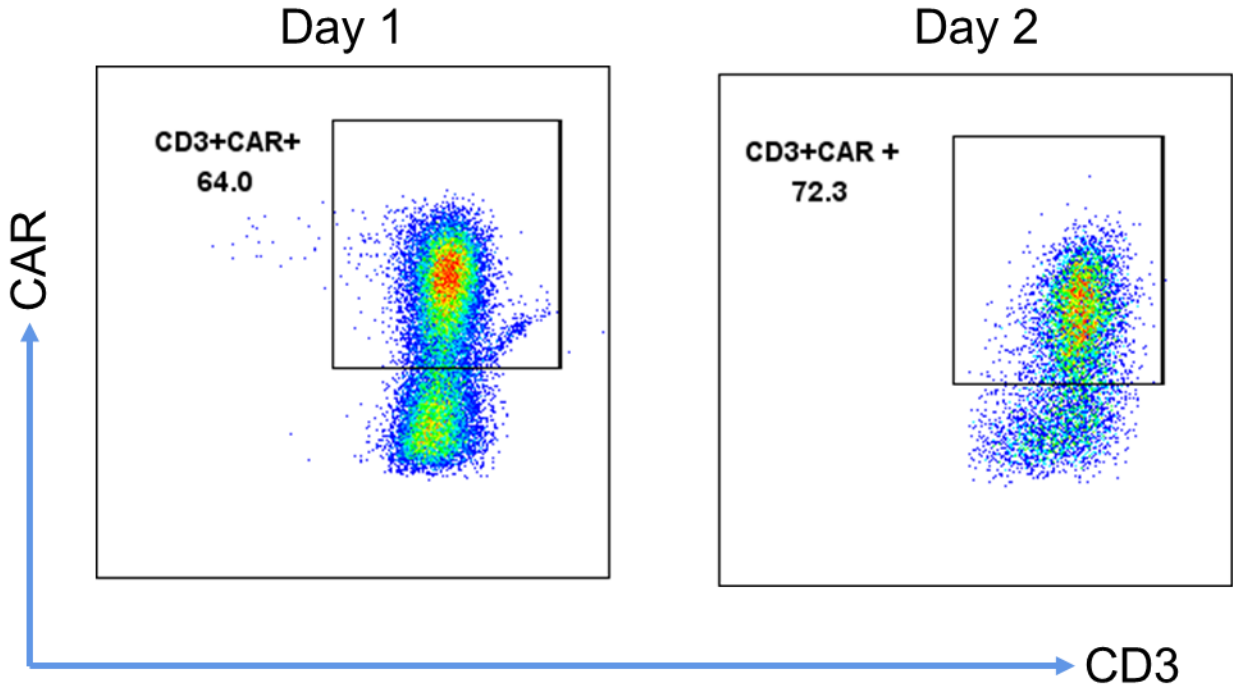
Rapid CAR-T with 50nm Beads: MAG → TM → MAG

(Step – 2) T cell transduction with CAR-19 lentivirus



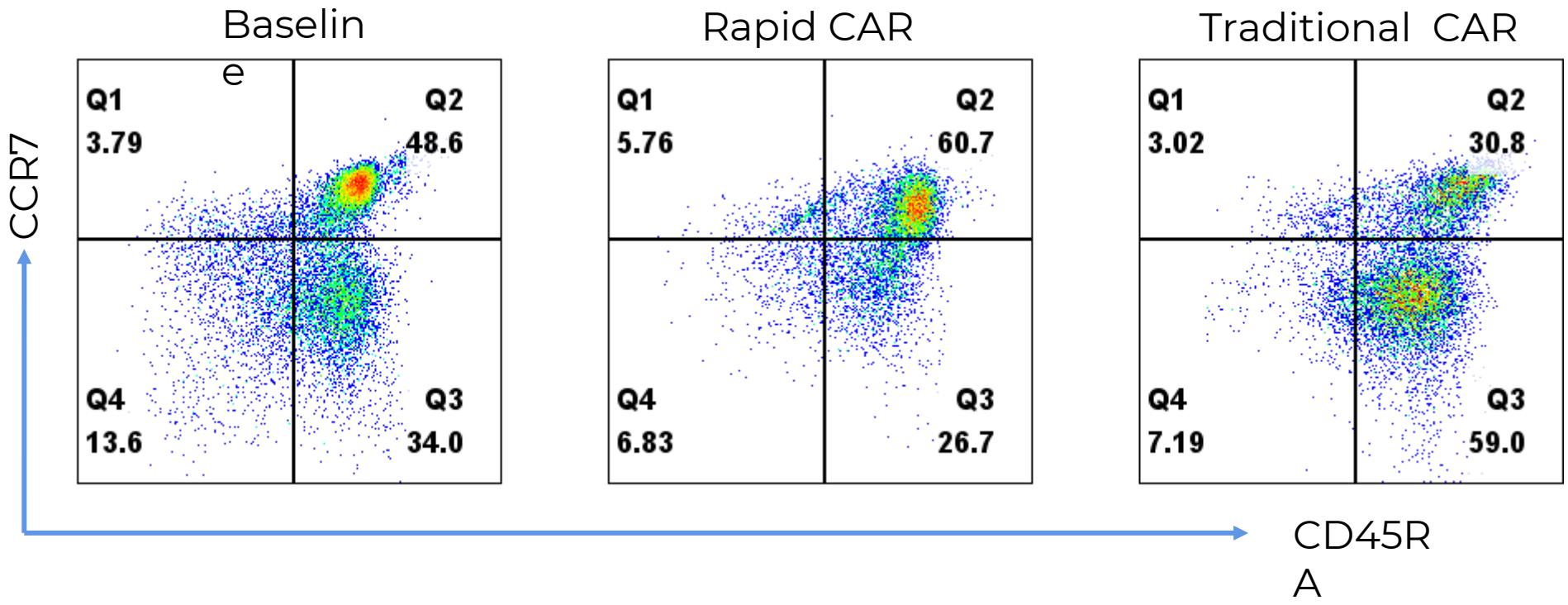


- **Transduction performed on isolated T cells with CAR-19 lentivirus for 2 days, with molecular activation**
- **72.3% CAR+ expression at Day 2**

Applied Cells

Rapid CAR-T with 50nm Beads: MAG → TM → MAG

(Step – 2) T cell transduction with CAR-19 lentivirus



Post transduction with Rapid CAR transduction process at Day 2, T cells have almost 2x Naive population than via traditional multiple-day transduction process

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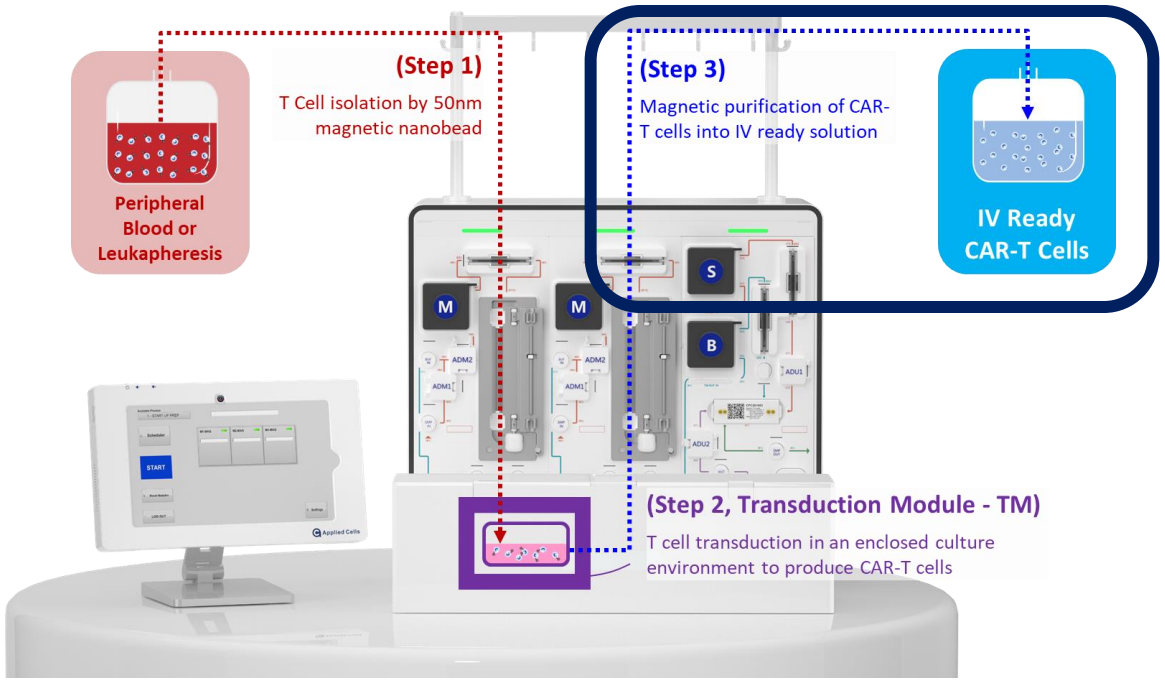
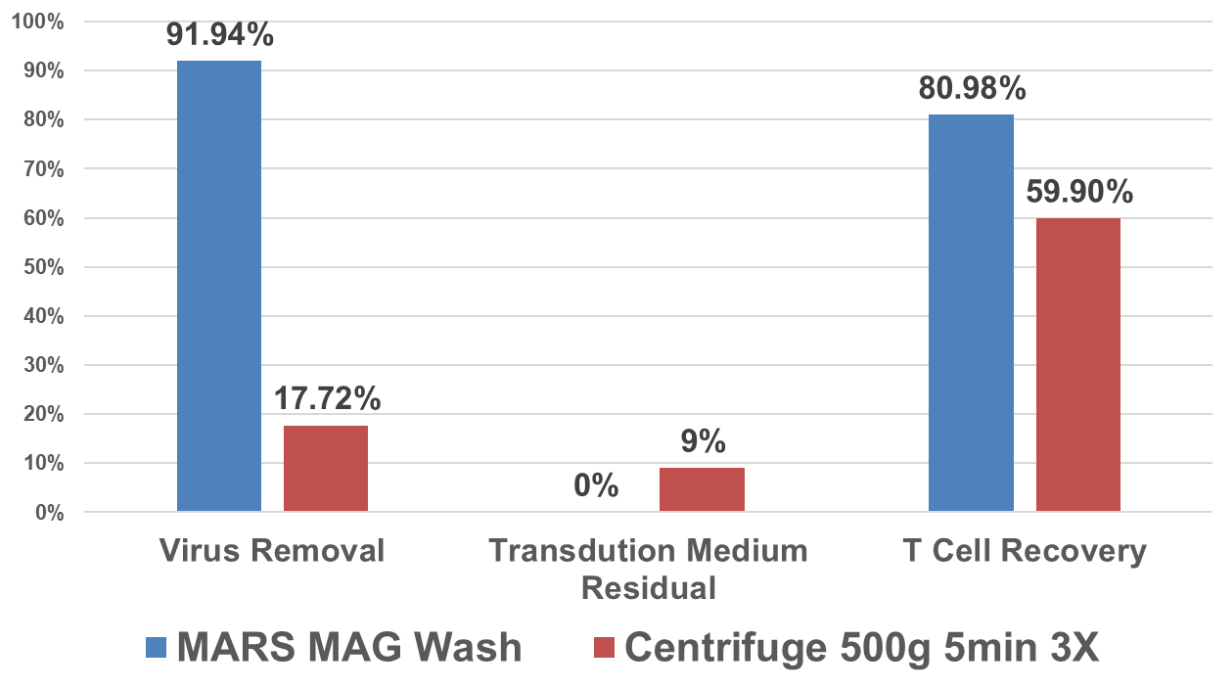
Rapid CAR-T with 50nm Beads: MAG → TM → MAG

(Step – 3) CAR-T cell purification with MAG module



Cell Purification



- **Using 50nm beads that are still on T cells after 48 hours to magnetically purify CAR-T cells**
- **Virus removal achieves >90% and T cells recovery > 80%**

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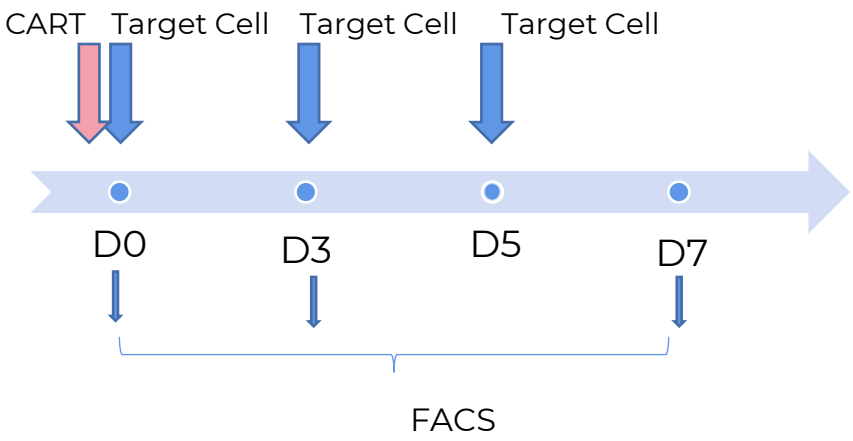
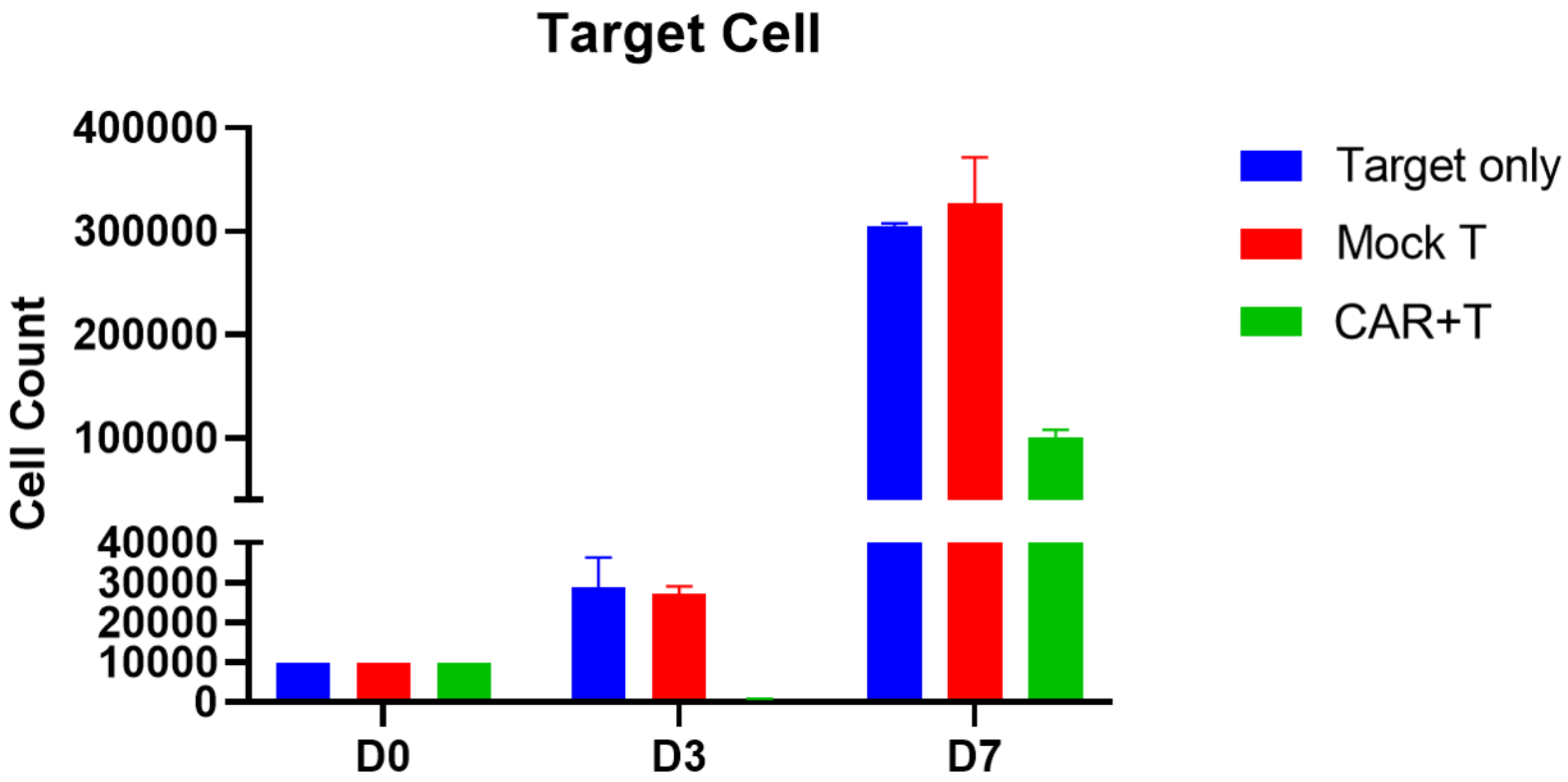
Rapid Process CAR-T Cells Killing Function Verification





Target Cells co-cultured with Rapid Process CAR-T cells show killing of Target Cells in comparison to controls

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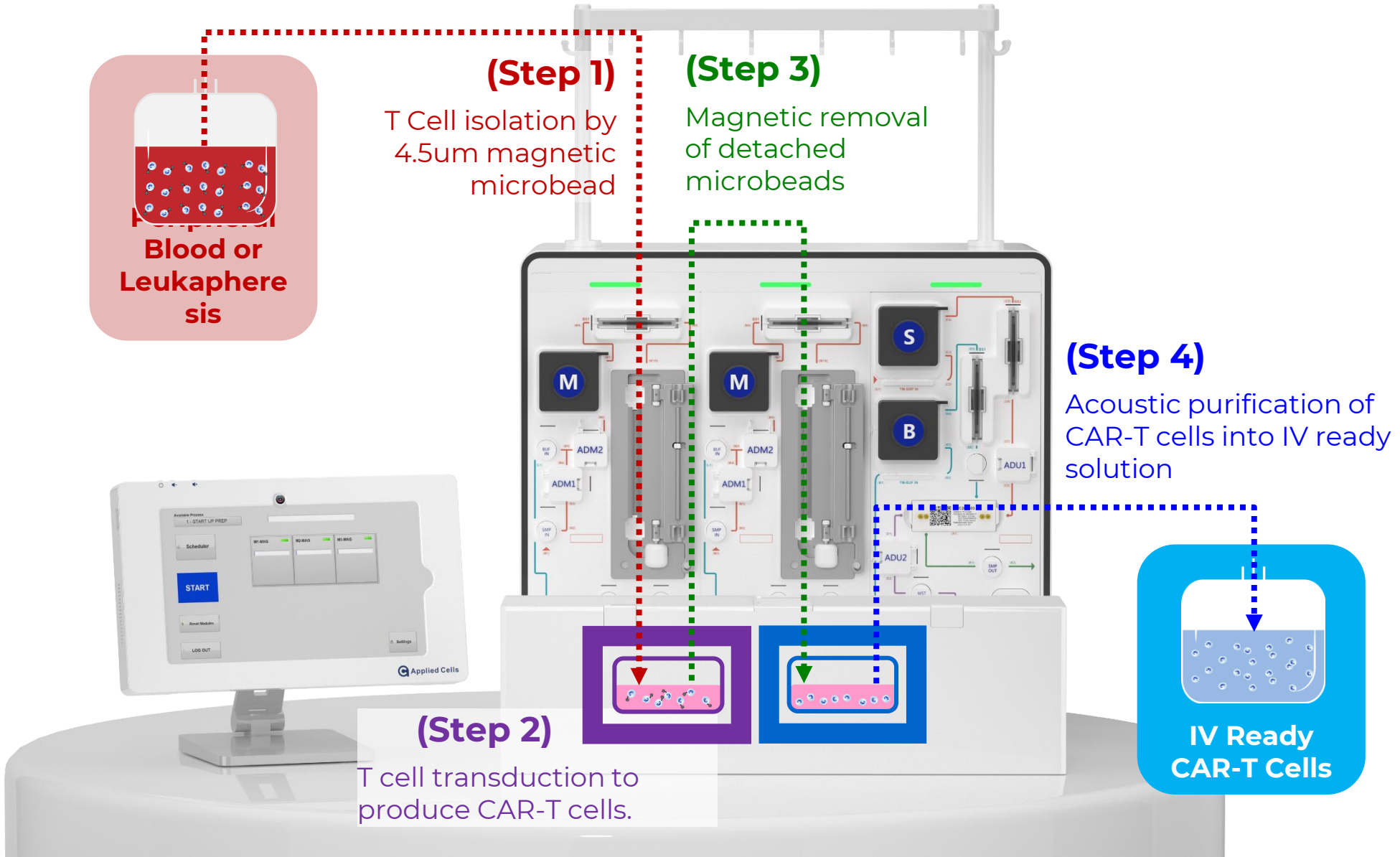
Rapid CAR-T Manufacture

Starting From

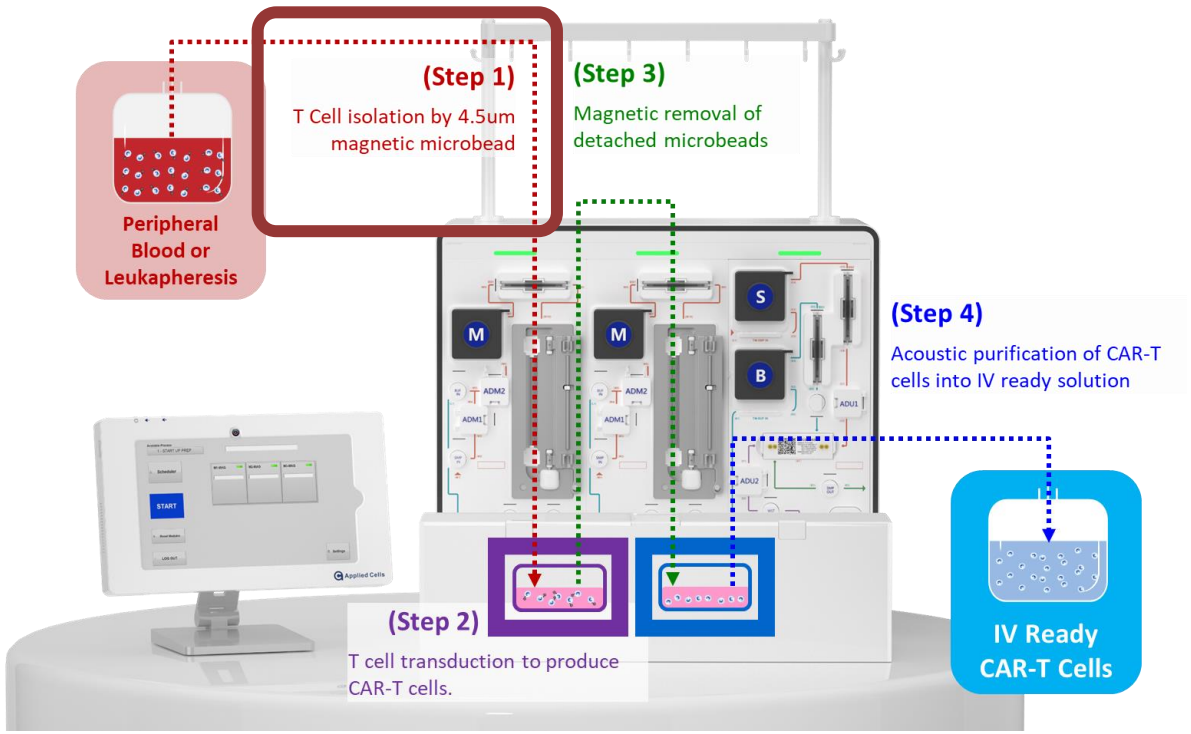
T Cell Isolation With 4.5um Micro-Beads

Rapid CAR-T with 4.5um Beads : MAG→TM→MAG→Acoustic Wash



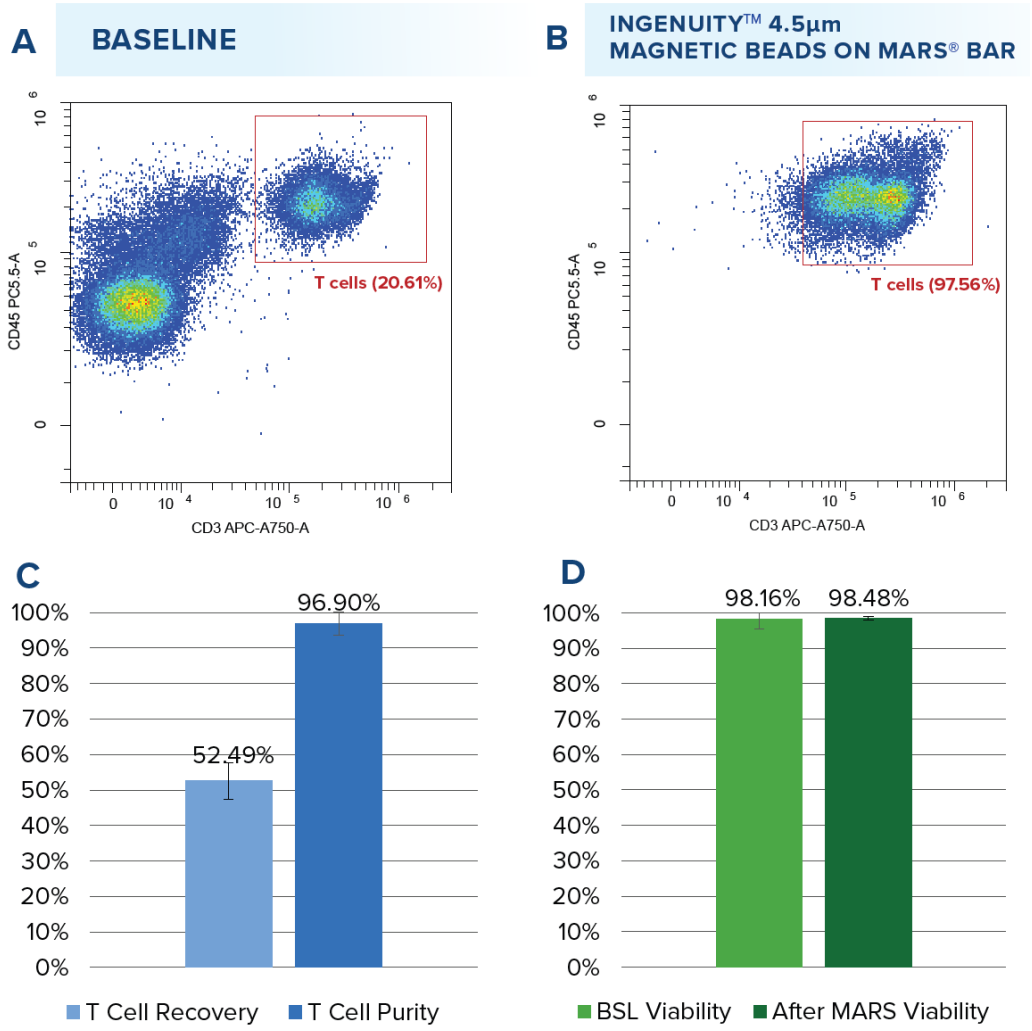
Applied Cells

Rapid CAR-T with 4.5um Beads : MAG→TM→MAG→Acoustic Wash



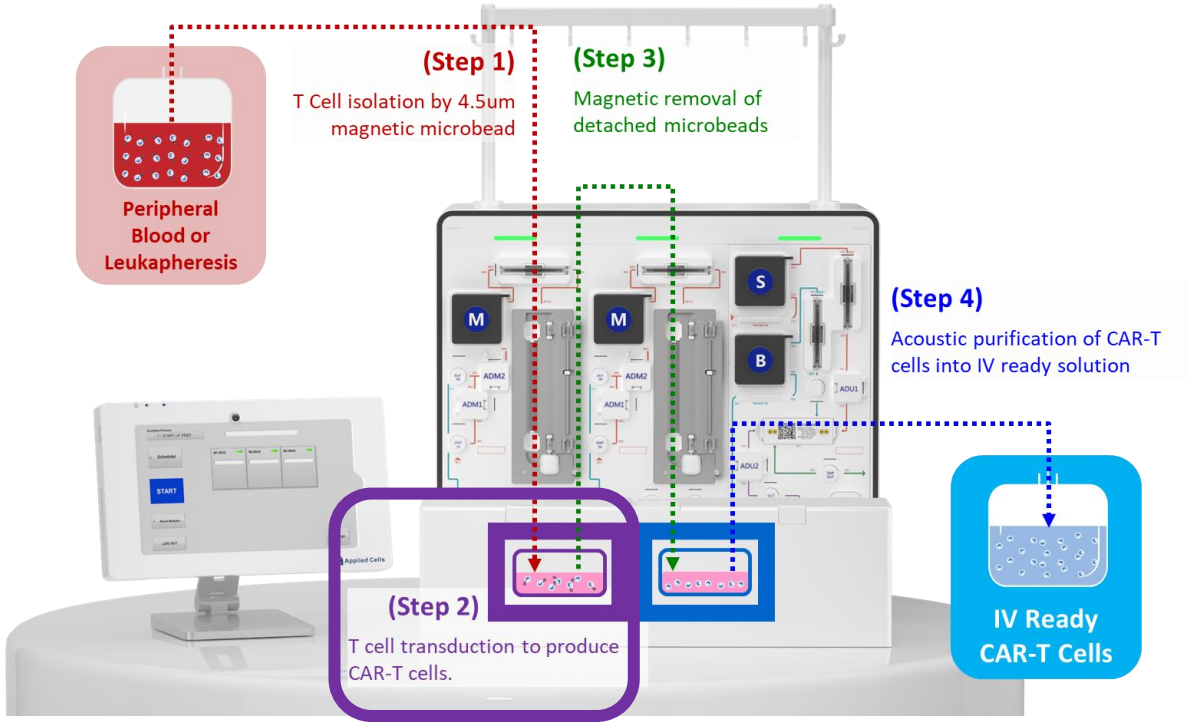
- **Direct T cell isolation from peripheral blood with CD3/28 beads**
- **Typical purity >95%, reasonable recovery >50%**

T Cell Isolation from Whole Blood



Applied Cells

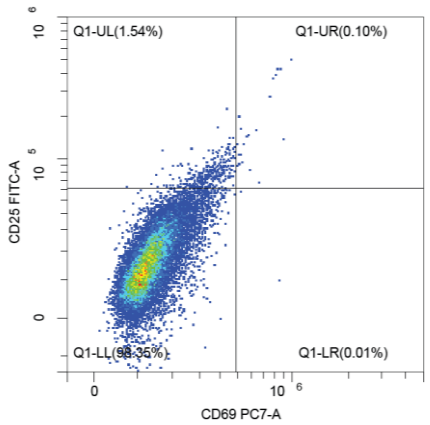
Rapid CAR-T with 4.5um Beads : MAG→TM→MAG→Acoustic Wash



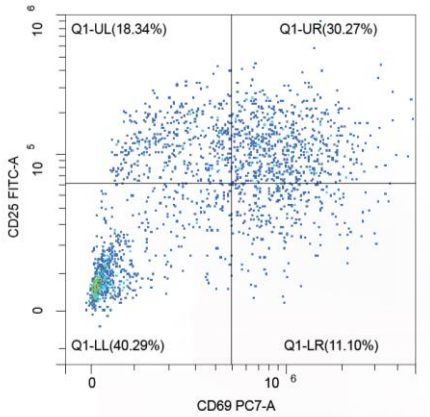
- T cell activation by CD3/28 beads show ~50% efficiency at 48 hours, and ~75% efficiency at 72 hours, after T cell isolation
- Transduction after CD3/28 activation is standard protocol

T Cell Activation

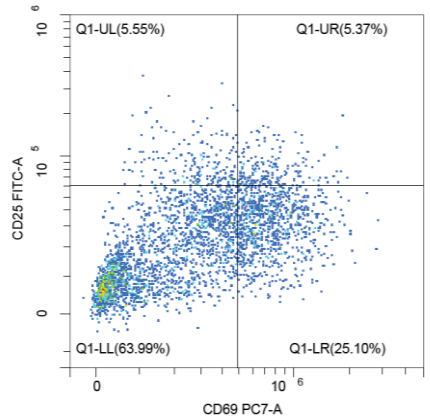
A - 0hr post enrichment



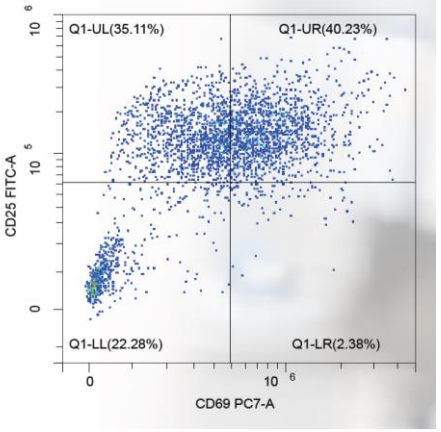
C - 48hr post enrichment



B - 24hr post enrichment



D - 72hr post enrichment



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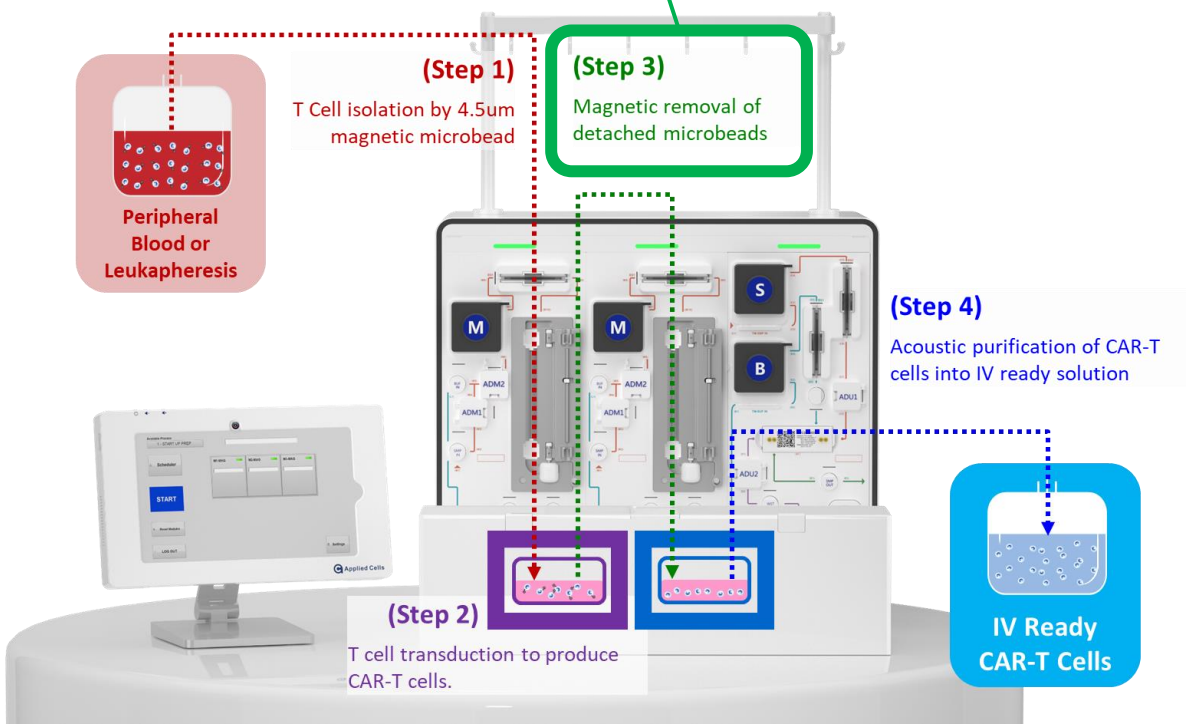
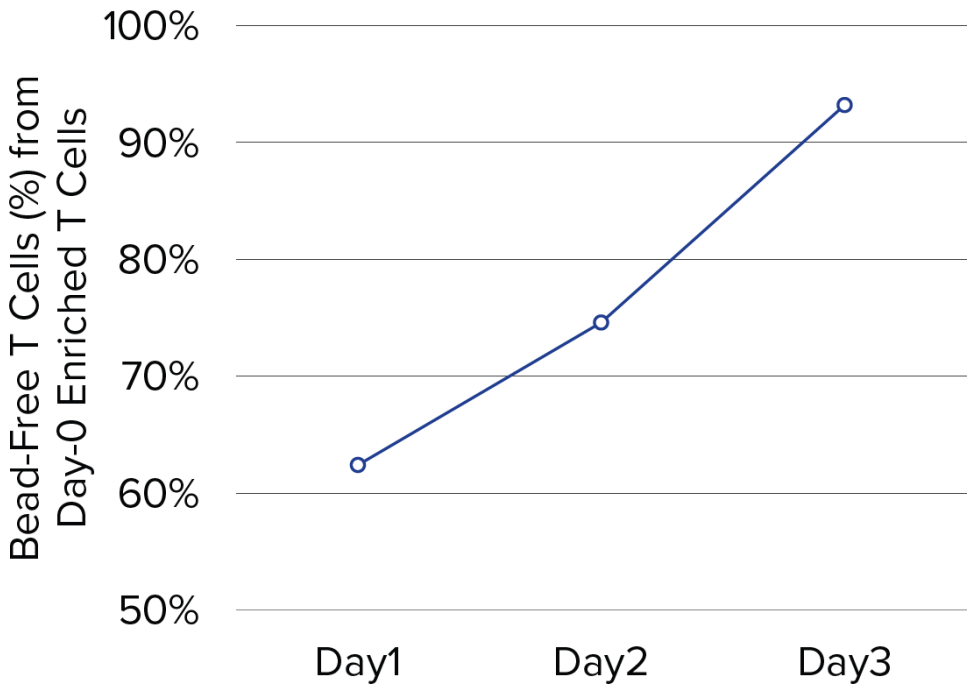
Rapid CAR-T with 4.5um Beads : MAG→TM→MAG→Acoustic Wash

Beads or cells bound with beads are 100% removed



- **Microbeads start detachment from T cells within 24 hours**
- **MAG removal of beads and cell bound with beads is 100%**

Microbeads Shedding



- Upon activation and culture of initially isolated T cells, at intervals of 24hr, 48hr and 72hr after start of culture, micro-bead are depleted by MAG module
- **Recoveries of 62.4%, 74.6%, and 93.2% of bead-free T cells from the initially isolated Day-0 T cells**

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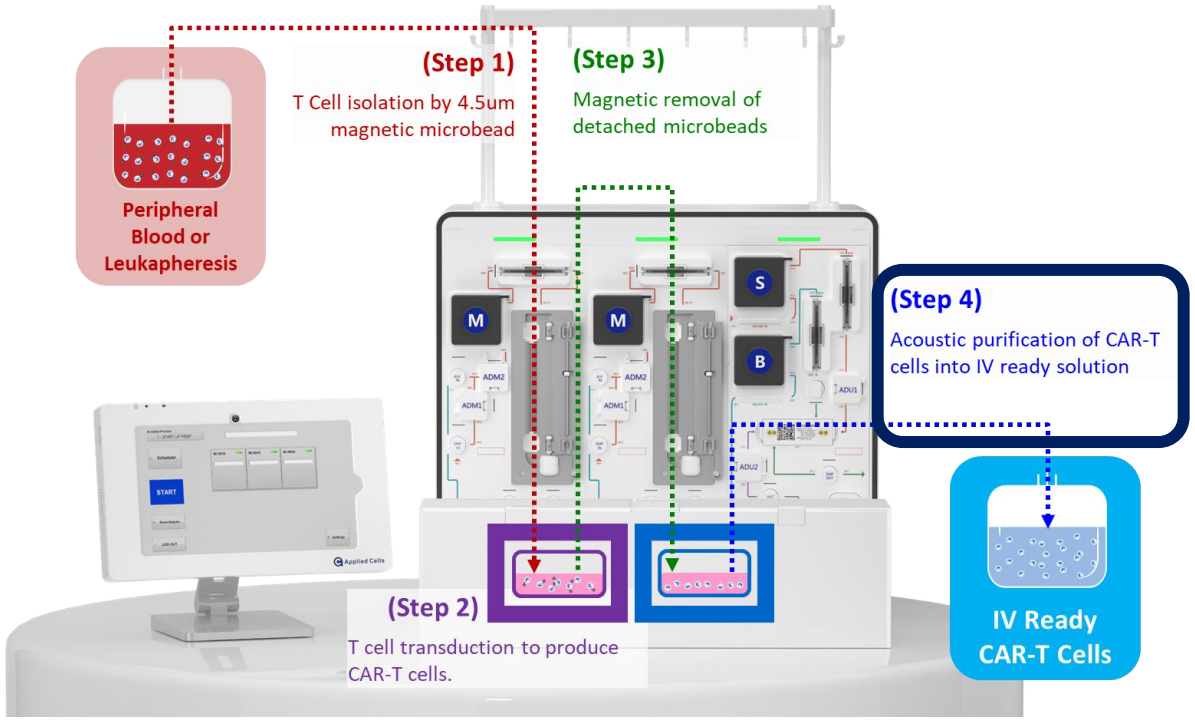
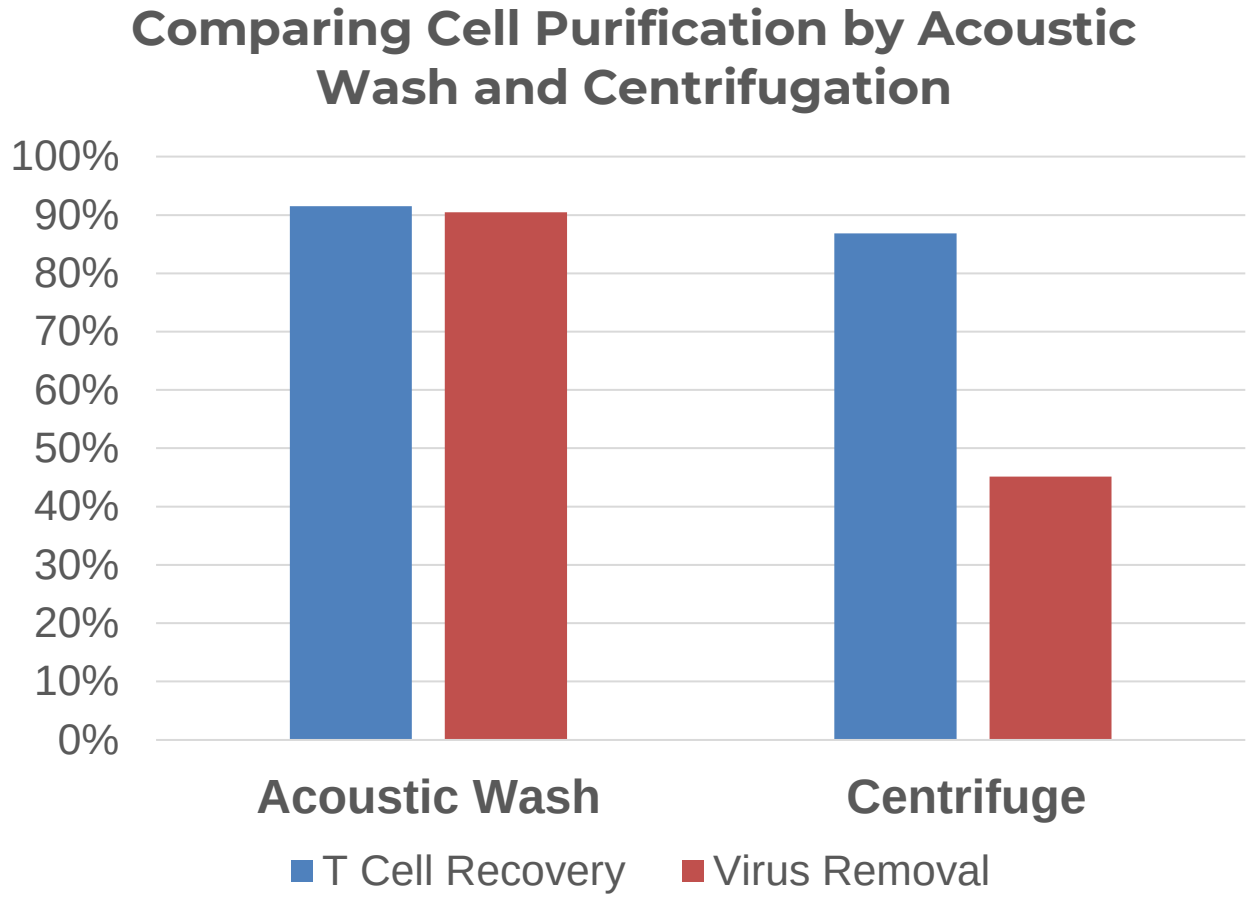
Rapid CAR-T with 4.5um Beads : MAG→TM→MAG→Acoustic Wash



Cell Purification



Acoustic purification of T cells shows better than centrifuge performance in virus removal

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Summary

- MARS Atlas protocol demonstrated the promise of high potency CAR-T cell manufacturing on single platform within 2~3 days
- MARS Atlas provides key solution to an automated rapid CAR-T production designed for point-of-care purpose
- MARS Atlas approach answers directly to the global need of accessible low-cost CAR-T therapies

Thank You