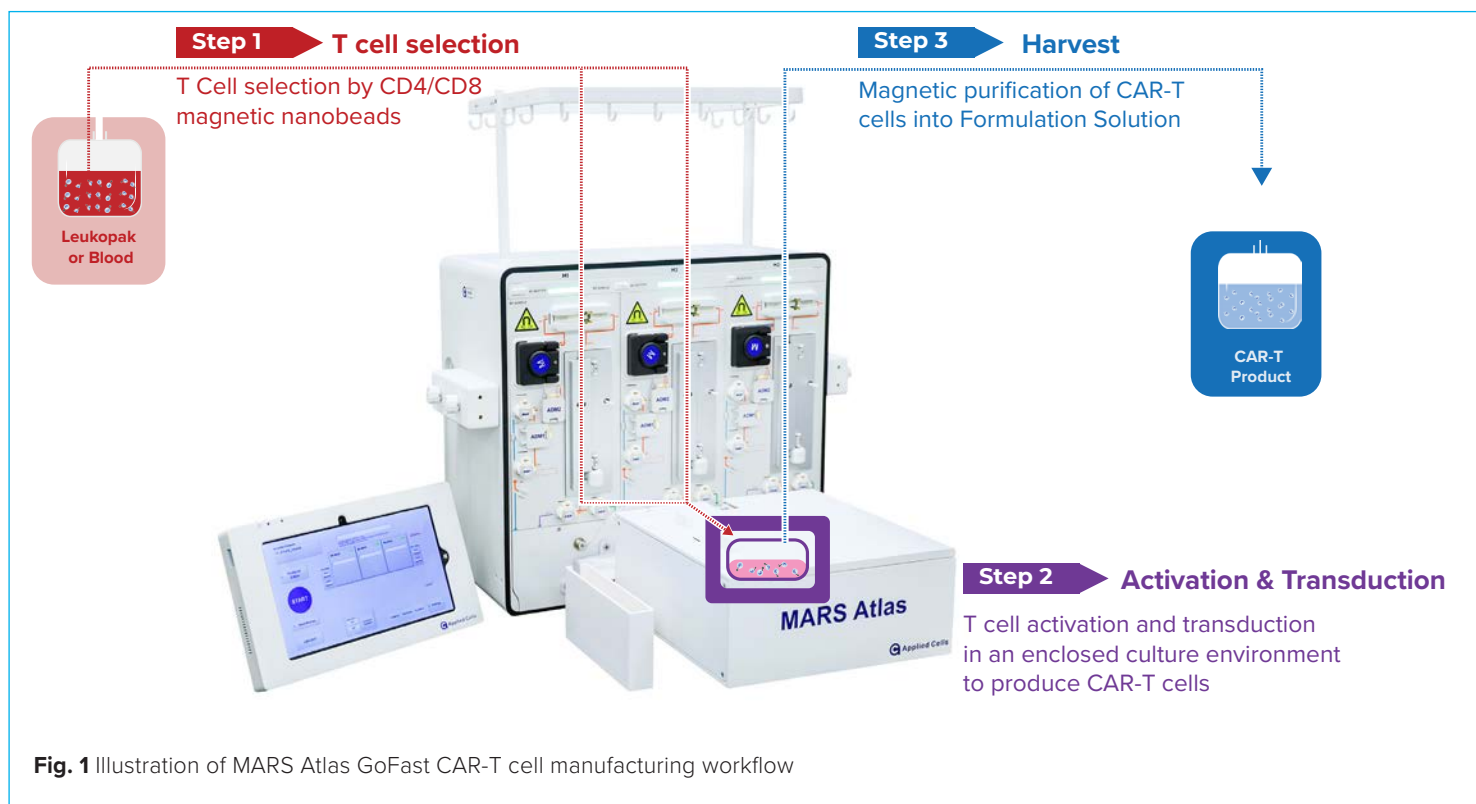


Automated GoFast™ CAR-T Manufacturing on the Integrated MARS Atlas® Platform with COGS Below \$10,000

INTRODUCTION

Autologous chimeric antigen receptor T-cell (CAR-T) therapy has significantly improved outcomes for patients with relapsed or refractory hematologic malignancies and has become an important treatment modality in modern oncology. However, the broader use of CAR-T therapy remains limited by the complexity of current manufacturing processes. Conventional production typically requires centralized manufacturing facilities, multiple manual processing steps, prolonged ex vivo cell expansion, and extensive quality control testing, resulting in vein-to-vein times that often extend from several weeks to more than a month. These delays may adversely affect patients with rapidly progressing disease and contribute substantially to the high cost and limited accessibility of treatment.

To address these challenges, rapid manufacturing approaches have emerged with the goal of reducing production time while maintaining product quality and therapeutic potency. The MARS Atlas® platform from Applied Cells, together with the GoFast™ workflow, is designed as a closed, automated, end-to-end manufacturing system that integrates T-cell isolation, activation and transduction, and harvest into a streamlined process reportedly capable of producing autologous CAR-T cells within <72 hr hours (Figure 1). By minimizing prolonged culture steps and reducing operational complexity, this approach has the potential to lower manufacturing costs, improve patient access, and enable decentralized or point-of-care production models.



CASE STUDY – A GOFAST CAR-T MANUFACTURING ON INTEGRATED PLATFORM MARS ATLAS

METHODS

To validate the MARS GoFast workflow on MARS Atlas we conducted a group of tests with 3 healthy donors and 2 Multiple myeloma patients' blood samples. The starting materials include fresh and frozen leukopak as well as frozen PBMC. On day 0, blood samples were incubated with CD4 and CD8 nanomagnetic beads (Ingenuity) in the sample bag and then T cells were isolated on MARS Atlas. The selected T cells were automatically released with cell culture media into the vessel contained in the built-in CO2 incubator on MARS Atlas. In average, 100 million T cells were seeded. CD3/CD28 nanobeads activator (AC) was added to the vessel through thermos sealer and incubate with cells at 37 degree C for 24 hours. on day 1, bispecific BCMA/CD19 lentiviral vector was added to the vessel at MOI=3. Cells were transduced for another 24 hours. On day 2, CAR-T cells were harvested by going through the third module on MARS Atlas to complete buffer exchange. Harvested cells were aliquoted for QC testing and infusion (Table 1).

T cell phenotype and viability before and after isolation, after activation and transduction was measured by flow cytometer. CD3, CD8 and CD4 were used to measure purity and recovery; CD25 and CD69 were used to monitor activation status and CCR7 and CD45RO were used to identify the stem and memory status of T cells. CAR+ was detected by antibody recognizing FCM63.

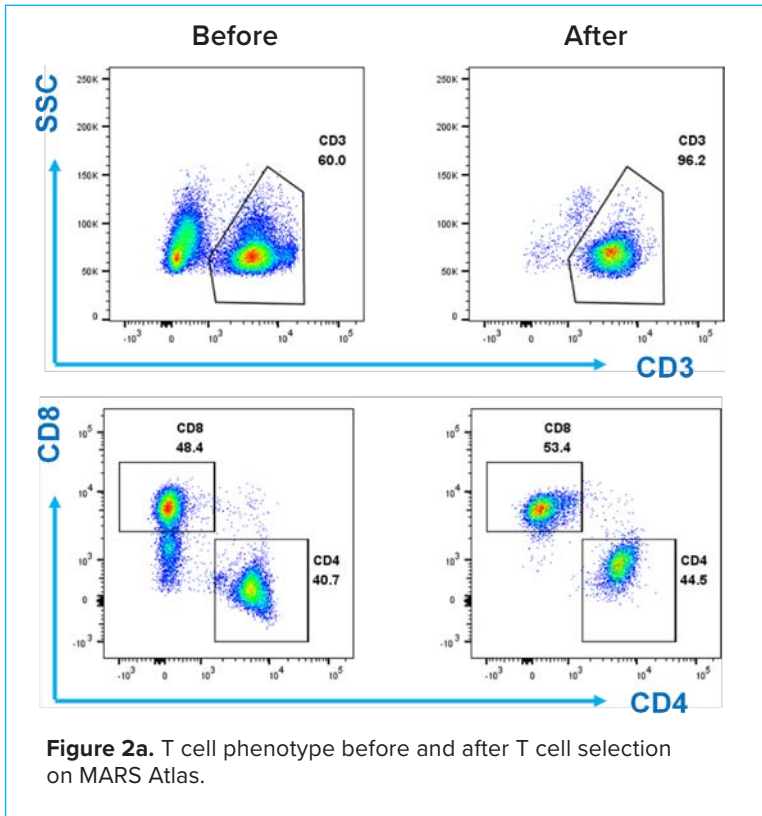
Ex vivo killing assays were performed to test GoFast CAR-T potency and killing persistency in comparison to CAR-T cells manufactured with conventional method (ex vivo expansion for 7 days after transduction). CAR-T cells were co-cultured with CD19+ NALM6 cells at E:T ratio at 1:1, 1:3 and 1:9. At day 4 and day 7 both CAR-T cells and Nalm-6 cells were counted. A serial killing assay by adding NALM-6 cells into coculture on day 0, day 2 and day4 was performed and cells were counted until day 7.

To further test the function of the GoFast CAR-T cells, cells were injected into mice with certain tumor burdens. Conventional CAR-T cells as a comparison were also injected.

To evaluate whether the in vitro cytotoxic properties of BCMA/CD19 CAR-T cells translate to in vivo anti-tumor activity, we utilized a xenograft model in NPG mice implanted with CD19-positive Nalm6 cells. Tumor-bearing mice received an adoptive transfer of either mock T cells or BCMA/CD19 CAR-T cells, and tumor burden was quantified over time using luciferase-based bioluminescent imaging. Using 2E6 as a standard dose, 1/5 of the standard dose and 1/25 of the standard dose were applied.

Table 1. MARS Atlas GoFast validation results.

Sample	Pre-selection total leukocytes	Preselection CD3%	Post-selection cell count in positive collection	Post selection positive collection CD3%	Transduction efficiency	Harvest recovery	CAR+ T cells
fresh leukopak (MM)	2.62E+08	60%	1.10E+08	97%	44.50%	78.90%	6.68E+06
frozen leukopak	4.50E+08	42.70%	1.50E+08	93%	49.20%	95.10%	2.56E+07
frozen leukopak	3.60E+08	44.80%	5.50E+07	95%	45.60%	78.80%	1.20E+07
frozen PBMC	5.70E+08	22.50%	9.60E+07	83%	40.10%	75.60%	1.00E+07
frozen leukopak (patient)	6.69E+08	50%	9.00E+07	88%	56.60%	100.00%	3.80E+07
fresh leukopak (MM)	2.62E+08	60%	1.10E+08	97%	44.50%	78.90%	6.68E+06
frozen leukopak	4.50E+08	42.70%	1.50E+08	93%	49.20%	95.10%	2.56E+07



RESULTS

In the validation runs, with starting materials as fresh and frozen leukopak from healthy donors and multiple myeloma patients, we demonstrated the consistent success of producing 6.6 million to 38 million CAR+T cells. (table1).

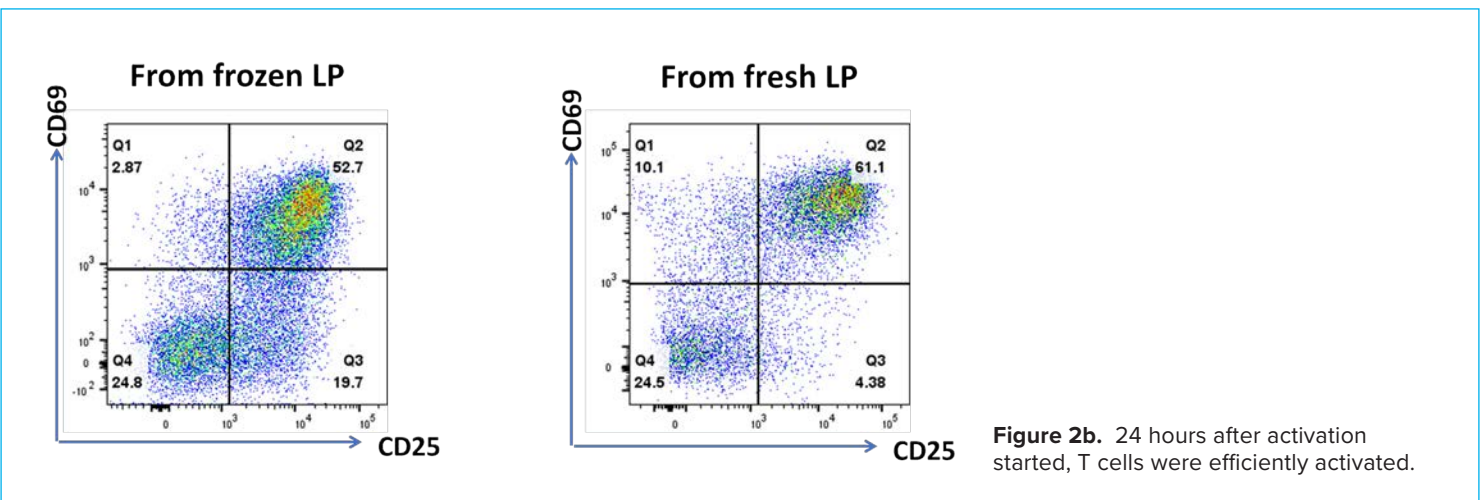
In the fresh leukopak sample, on day 0, the T cell selection process results in 96.2% purity and the ratio of CD4+ and CD8+ T cells remained before and after selection. On day1, after T cells being activated for 24 hours, T cells from both frozen and fresh leukopak had over 50% CD25+CD69+ population. On day 2, after 24 hours of transduction, T cells from frozen leukopak showed 65% CAR+ and T cells from fresh leukopak showed 80% CAR+. Both samples had higher percentage of CCR7+CD45RO-(Tn/Tscm) population than CCR+CD45RO+(Tcm) population. (Figure 2)

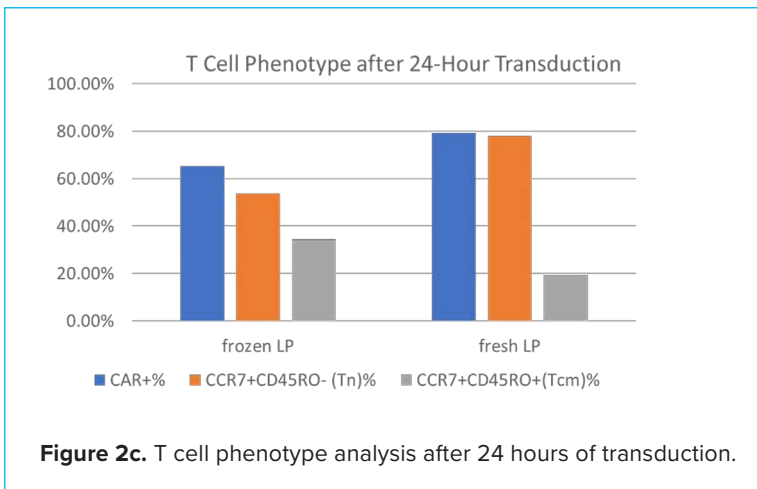
To evaluate the functional capacity of rapidly manufactured CAR-T cells, GoFast BCMA/CD19 CAR-T cells were compared with traditional (Tra) BCMA/CD19 CAR-T cells in a co-culture assay with Nalm-6 target cells across multiple effector-to-target (E:T) ratios.

At Day 4, GoFast CAR-T cells demonstrated enhanced expansion relative to Tra CAR-T cells, as reflected by higher CD3+ and CAR-T cell counts, particularly at 1:1 and 1:3 ratios. This increased proliferation correlated with improved tumor control, with Fast CAR-T cells effectively suppressing tumor growth across all E:T conditions. In contrast, Tra CAR-T cells exhibited weaker expansion and only partial tumor clearance, with reduced efficacy at higher tumor burden (1:9).

By Day 7, although CAR-T cell numbers decreased across groups, GoFast CAR-T cells maintained superior anti-tumor activity, achieving near-complete elimination of tumor cells even under high tumor burden conditions. Tra CAR-T cells, however, showed diminished persistence of function, with residual tumor cells remaining detectable.

Together, these results indicate that rapid manufacturing of BCMA/CD19 CAR-T cells enhances early expansion kinetics and sustains functional anti-tumor activity, particularly in settings of high tumor burden. (Figure 3 a).





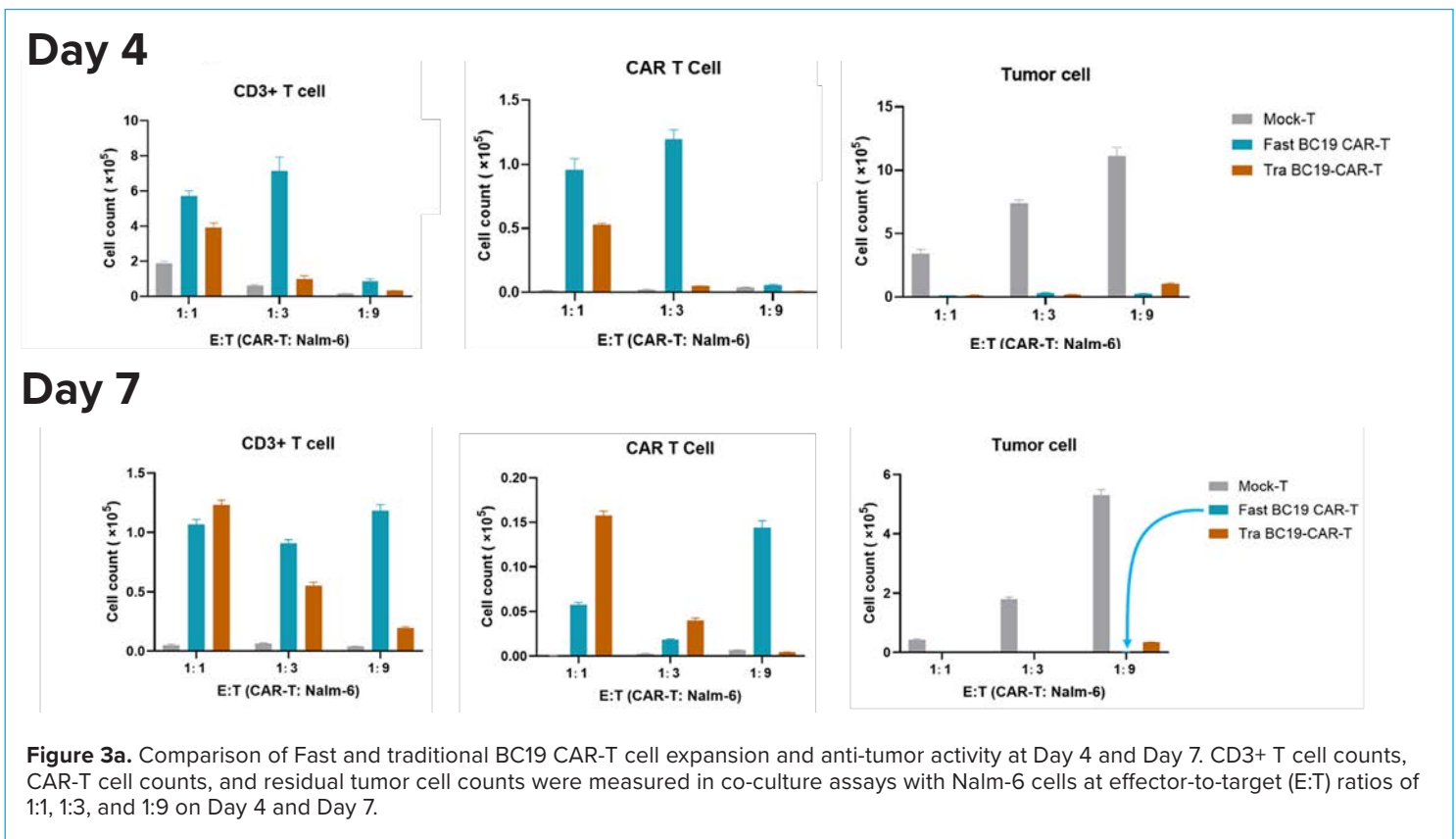
Fast-manufactured BCMA/CD19 CAR-T cells demonstrated superior expansion and persistence compared to traditionally manufactured CAR-T cells under repeated antigen stimulation.

At the CD3⁺ T cell level, GoFast CAR-T cells showed a rapid increase by day 2, followed by sustained expansion through day 7, whereas traditional CAR-T cells exhibited only modest expansion and declined over time, similar to Mock T cells.

This trend was more pronounced in the CAR-T compartment, where GoFast CAR-T cells maintained and further increased their numbers after an initial fluctuation, reaching the highest levels by day 7. In contrast, traditionally manufactured CAR-T cells

rapidly contracted after day 0 and remained at minimal levels throughout the culture period. These findings indicate that Fast manufacturing preserves proliferative capacity and enhances resistance to exhaustion or attrition during repeated antigen exposure. (Figure 3b).

In the NCG mouse model, *in vivo* imaging demonstrated differential tumor control across treatment groups over time. Bioluminescent signals increased progressively in control cohorts, indicating continued tumor growth, whereas mice treated with CAR-T cells showed varying degrees of signal suppression depending on the manufacturing approach. Notably, the Fast-manufactured CAR-T group exhibited more sustained control of tumor burden, with lower overall signal intensity and delayed progression compared to other CAR-T groups. The different dose levels show a clear dose–response relationship in antitumor activity, with higher CAR-T cell doses providing more effective tumor control *in vivo*. Mice treated with the highest dose (2×10^6 cells) exhibited the strongest suppression of bioluminescent signal over time, indicating robust and sustained tumor control.



CAR-T Target Target Target

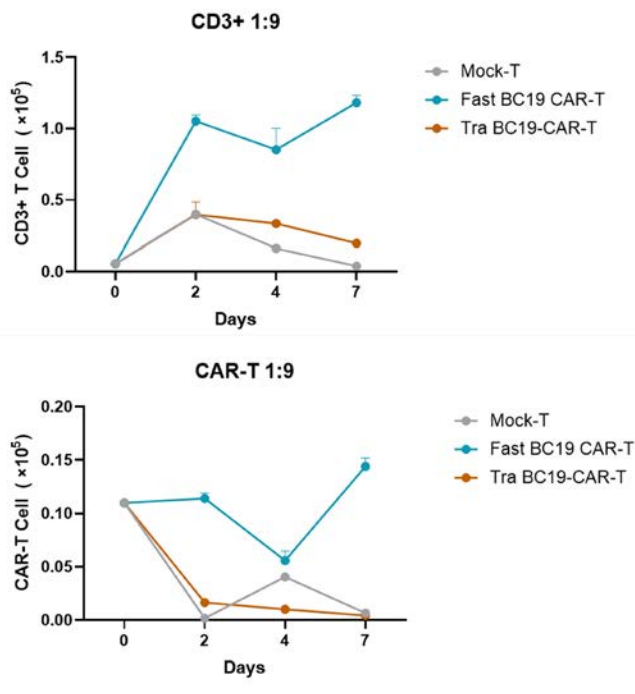
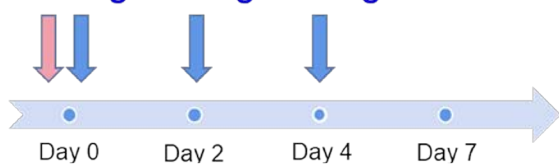


Figure 3a - continued: At Day 4, Fast BC19 CAR-T cells exhibited greater expansion compared to traditional (Tra) BC19 CAR-T cells, particularly at 1:1 and 1:3 ratios, accompanied by more effective tumor cell control across all conditions. In contrast, Tra CAR-T cells showed reduced expansion and partial tumor clearance, with diminished efficacy at higher tumor burden (1:9). Mock-T controls demonstrated progressive tumor growth.

By Day 7, Fast CAR-T cells maintained superior anti-tumor activity, achieving near-complete tumor elimination even at high tumor burden (1:9), whereas Tra CAR-T cells showed incomplete tumor control with detectable residual tumor cells. Although total CAR-T cell counts declined at Day 7, consistent with contraction following expansion, Fast CAR-T retained greater functional activity compared to Tra CAR-T.

The intermediate dose (4×10^5 cells) produced partial efficacy, slowing tumor progression but not achieving the same level or durability of control as the high-dose group, with signals gradually increasing at later time points.

In contrast, the lowest dose (8×10^4 cells) showed minimal therapeutic effect, with tumor growth kinetics resembling those of control groups and rapidly increasing signal intensity throughout the study. Notably, GoFast CAR-T at 1/5 of the dose level (4×10^5) of the traditional CAR-T (2×10^6), GoFast CAR-T can still suppress tumor while traditional CAR-T cannot even at 5x higher dose.

The results indicate that GoFast CAR-T cells have superior tumor suppressive capacity compared to traditional CAR-T. (Figure 4).

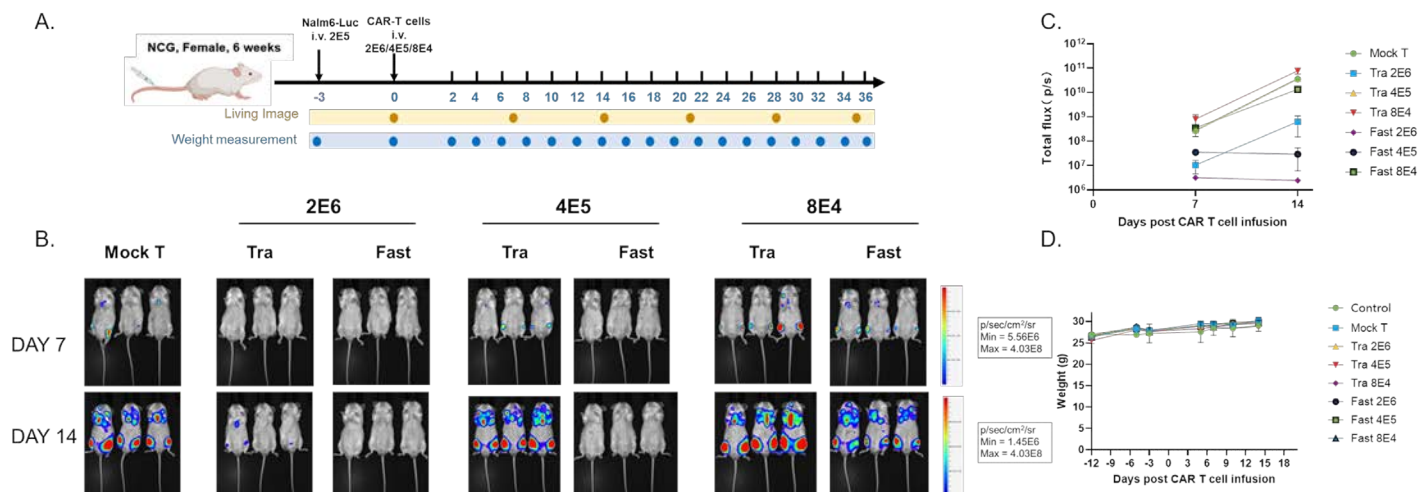


Figure 4. Female NCG mice aged 6–8 weeks were used. On Day (-3), each mouse was inoculated with 2×10^5 Nalm6 cells transduced with a luciferase reporter gene via tail vein injection to establish a CD19-positive xenograft tumor model. On Day (-3), each mouse also received an intraperitoneal injection of 15 mg/kg of the luciferase substrate D-luciferin. Tumor progression was monitored using a small animal in vivo imaging system under isoflurane anesthesia. On Day 0, the mice were randomly divided into 7 groups, and each group was treated via tail vein injection with the corresponding CAR-T cells, including a Mock T cell group (2×10^6 T cells, $n=3$), traditional BCMA/CD19 CAR-T groups (Tra), and GoFast CAR-T groups (Fast). The doses administered were 2×10^5 , 4×10^5 , and 8×10^4 CAR-T cells per mouse. Starting from the day after CAR-T treatment initiation, in vivo imaging was performed every 7 days for a total of 14 days. The results showed that the GoFast CAR-T (Fast) group at the 4×10^5 dose outperforms the traditional CAR-T group at the 2×10^6 dose, indicating GoFast CAR-T having a superior tumor suppressive capacity compared to traditional CAR-T.

DISCUSSION

Across in vitro and in vivo models, Fast-manufactured BCMA/CD19 CAR-T cells consistently demonstrated enhanced functional performance compared to traditionally manufactured products. In repeated antigen stimulation assays, GoFast CAR-T cells exhibited superior expansion and persistence, maintaining higher CD3+ and CAR-T cell counts over time, whereas traditionally manufactured cells showed early contraction and limited proliferative capacity. These findings suggest that the accelerated manufacturing process does not compromise, and may in fact better preserve T-cell fitness under chronic antigen exposure conditions.

In vivo, Fast CAR-T cells translated this functional advantage into improved anti-tumor activity in the NCG mouse model. Longitudinal bioluminescence imaging showed more effective and sustained tumor control in Fast CAR-T–treated mice compared to other groups, which displayed either partial response or progressive disease. Importantly, this enhanced efficacy was observed without measurable differences in systemic toxicity, as reflected by stable body weight across all cohorts.

Dose-escalation analysis further highlighted a clear dose-dependent response, with 2×10^6 CAR-T cells achieving the most robust tumor suppression, 4×10^5 cells producing intermediate control, and 8×10^4 cells showing minimal therapeutic benefit. Together, these data indicate that both manufacturing strategy and administered cell dose are critical determinants of in vivo efficacy. The superior expansion capacity observed in Fast CAR-T cells may contribute to improved in vivo persistence and tumor clearance, potentially enabling effective responses even at lower functional thresholds compared to conventional products.

Moreover, the CoGs of MARS GoFast have clear breakdown:

**Cost of Goods to produce GoFast CAR-T
MARS Atlas GoFast™ CAR-T Essential Kit
Per Patient <72 hr and <\$9,500 USD**

1. MARS Atlas fluidics kit including freeze-down kit (1 set)
2. GMP CD4, CD8 beads (1 set)
3. GMP T cell activation reagent (1 vial)
4. GMP complete media with ILs (200 mL)
5. GMP running buffer, formulation buffer, freezing media (50 mL each)
6. GMP LVV



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