A Novel Automated Platform for Rapid Manufacturing of CAR-T Cells

Silin Sa and Liping Yu, Applied Cell, Inc. Santa Clara, California, USA

OFFICIAL EXHIBITOR AACR American Association for Cancer Research ANNUAL MEETING 2024 • SAN DIEGO

Abstract 6331

Introduction

Autologous cell therapy using T cells that are genetically modified to express a chimeric antigen receptor (CAR) has yielded durable responses in patients with cancers. However, globally only a small fraction of eligible patients are privileged to receive the treatment. High cost and long manufacturing time contribute to the key limitations prohibiting a broad adoption of the therapy. Researchers have discovered that reduction of ex vivo culture improves the anti-tumor activity of CAR-T cells[1] and recently demonstrated the process of generating functional CAR-T cells within 24 hours[2]. This rapid process provides great promise in reducing the cost of CAR-T therapy and making it possible to have cells manufactured in close-to-patient settings.

The CAR-T cell manufacturing process involves: (1) collection of peripheral blood; (2) isolation of T cells; (3) performing transduction via viral vector; (4) washing CAR-T cells to remove viral vector, and (5) harvesting CAR-T cells in IV fluid. Conventional process uses magnetic separation and serial centrifugation technologies on multiple platforms with manual operations. Here we demonstrate the feasibility of rapid manufacturing CAR-T cells on an automated platform with closed fluidics and without any centrifugation step. The platform, **MARS® Atlas**, incorporates innovative technologies including column-free immuno-magnetic cell isolation, acoustic cell washing, and modular workflow.

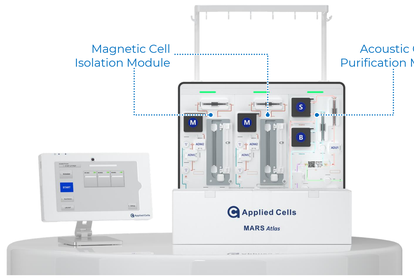
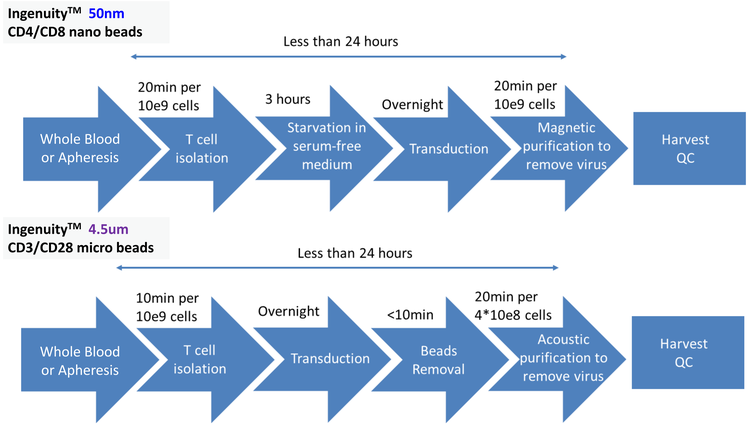


Figure 1. The MARS® Atlas platform – an automated system for completing CAR-T cell manufacturing within 24 hours. The modular system is built with 2x column-free magnetic cell isolation modules, 1x acoustic cell purification module, as well as 1x integrated cell transduction module. The system is equipped with single-use sterile fluidic kit to ensure closed operation. Cell isolation, cell transduction, cell purification processes are controlled by software designed for 21CFR11.

MARS® Atlas Workflows



Workflow and Results
50nm Nano Magnetic Beads

Figure 2. Schematic illustration of the workflow of rapid CAR-T cell manufacturing on MARS® Atlas using 50nm CD4/CD8 magnetic beads.

T Cell Isolation

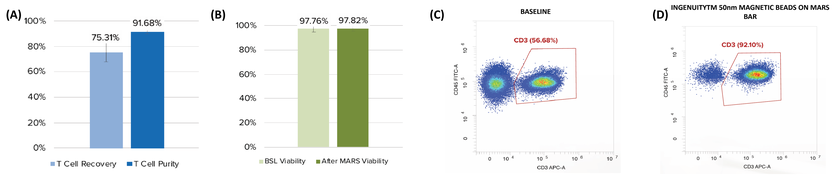


Figure 3. Efficient isolation of T cells from PBMC using the MARS® Bar platform, with no compromise in viability. (A) T cell positive selection using Ingenuity™ 50nm CD4 and CD8 beads on MARS® Bar; average recovery of 75.31% T cells with an average purity of 91.68% from an initial average T cell purity of 49.92%. n= 12 PBMC samples. (B) Viability was not significantly affected before and after positive selection with Ingenuity™ 50nm CD4 and CD8 beads on MARS® Bar (P<0.5). (C) purity of human T cells in PBMC prior to separation, (D) purity T cells enriched Ingenuity™ 50nm beads, reflect the median performance shown in Figure 1(A).

Cell Purification

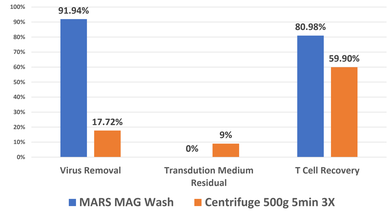


Figure 4. MARS mag wash VS. centrifuge after transduction. 24hr after transduction start, MARS mag wash (one time) and centrifuge 500g 5min (three times) were used to remove free virus and polybrene. Flow cytometry were used to measure virus removal and T cell recovery. Fluorescent plate reader was used to measure transduction medium residual. MARS mag wash resulted in 91.94% virus removal and 80.98% T cell recovery with no medium left.

Viral Transduction and Expression

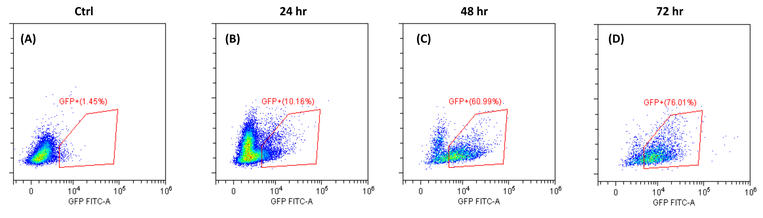


Figure 5. Lentivirus transduction in non-activated T cells. Freshly isolated human T cells were culture in IL-7 and IL-15 and transduced with GFP-lentivirus. The gene transduction efficiency was measured by percentage of cells expressed GFP signal. Representative flow cytometry plots of (A) GFP expression from control cells without transduction, (B) 24hr after transduction and purification, (C) 48hr after transduction and purification, and (D) 72hr after transduction and purification, are shown.

Workflow and Results
4.5um Micro Magnetic Beads

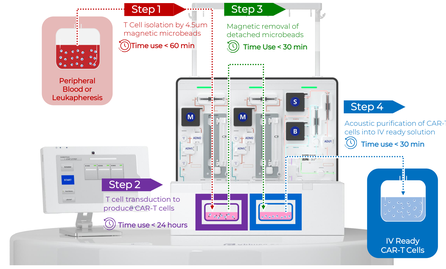


Figure 6. Schematic illustration of the workflow of rapid CAR-T cell manufacturing on MARS® Atlas using 4.5um CD3/CD28 magnetic beads. 4.5um beads dissociate from T cells after transduction in **Step 2**, free beads and remaining cells bound with beads are 100% removed during **Step 3**.

T Cell Isolation

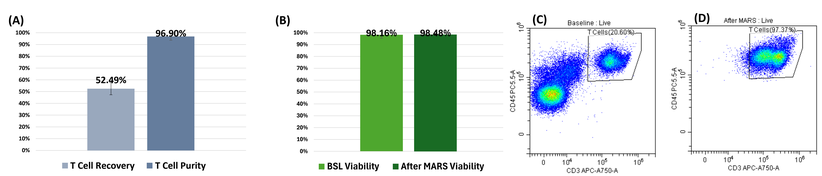
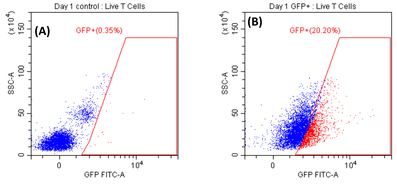


Figure 7. Efficient isolation of T cells from whole blood using the MARS® Bar platform, with no compromise in viability. (A) T cell positive selection using Ingenuity™ CD3/CD28 microbeads at a 1:1 bead to T cell ratio on MARS® Bar; average recovery of 52.49% T cells with an average purity of 96.90% from an initial average T cell purity of 25.10%. n= 3 healthy donor samples. (B) Viability was not significantly affected before and after positive selection with Ingenuity™ CD3/CD28 microbeads on MARS® Bar (P<0.5). Purity of human T cells isolated using the MARS® Bar platform. (C) whole blood prior to separation, and (D) T cells enriched using Ingenuity™ CD3/CD28 microbeads.

Viral Transduction



Cell Purification

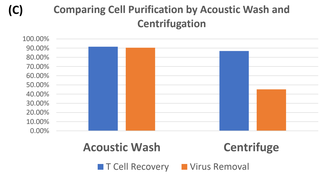


Figure 8. (A) and (B) are 24 hour transduction results using the same protocol described in Figure 4. After 24 hour incubation 20% T cells became GFP positive. (C) Efficient removal of free virus. Using acoustic module to purify T cells after de-beads results in 90% T cell recovery and 90% virus removal by running through the acoustic chip once. In comparison, 500g 3 times centrifuge wash results in 80% cell recovery and 50% virus removal. (Free beads were removed 100% through beads removal process. Dana not shown)

Summary

- MARS® Atlas platform has demonstrated the capability of completing CAR-T cell manufacturing process on one platform within 24 hours
- MARS® Atlas provides the solution to automation of rapid CAR-T production and are suited for point-of-care facilities
- The MARS® Atlas approach contributes to the global needs of low- cost manufacturing process for cell therapy

Applied Cells

info@appliedcells.com

WWW.APPLIEDCELLS.COM