Cell therapy

Optimization of the CTS Detachable Dynabeads CD3/CD28 magnetic beads workflow

Abstract

To expand the reach of adoptive T cell immunotherapy, dynamic tools that are both flexible and reliable are needed to manage technical advances, commercialization demands, and regulatory requirements associated with cell-based drug production. Gibco™ CTS™ Detachable Dynabeads™ CD3/CD28 magnetic beads were developed to enable T cell isolation and activation with one step. This simplifies the workflow and yields T cell products that exhibit high viability, robust proliferation, and optimal phenotype for strong clinical performance. In this study, we evaluated the performance of CTS Detachable Dynabeads CD3/CD28 beads for T cell isolation and activation using apheresis units from three healthy donors. Bead-to-cell ratios of 1:1, 3:1, and 5:1 were assessed along with bead release times ranging from 1 to 3 days. Our data show that CTS Detachable Dynabeads CD3/CD28 beads can support shorter T cell manufacturing workflows without compromising quality.

Introduction

Autologous CAR T cell production typically involves multiple steps, including isolation, activation, genetic modification, and cell expansion. This process can take from 7 to 14 days, with *ex vivo* expansion accounting for the longest phase [1].
The T cell drug therapy pipeline can be delayed by slow manufacturing and suboptimal expansion, which can impact treatment accessibility for patients. Early validation and process optimization are thus critical to advancing these treatments from the laboratory to the clinic [2]. Over 200 CAR T cell therapies are currently in clinical trials. To advance these therapies to commercialization, manufacturing protocols must be adaptable and efficient at generating high-quality T cell products.

Gibco™ CTS™ Dynabeads™ magnetic beads have led the market in advancing cell therapies from small-scale manufacturing to clinical use. Designed for use with the CGMP-compliant Gibco™ CTS™ DynaCellect™ Magnetic Separation System, CTS Detachable Dynabeads CD3/CD28 beads integrate into a closed, automated platform for flexible manufacturing suited for clinical and commercial needs.

This study explored the use of CTS Detachable Dynabeads CD3/CD28 magnetic beads at 1:1, 3:1, and 5:1 bead-to-target cell ratios in a one-step T cell isolation and activation protocol with bead release on day 1, day 2, or day 3. Key metrics assessed included T cell purity, activation marker expression, transfection efficiency, recovery, viability, and memory phenotype.

By evaluating different bead-to-cell ratios and bead release timing, CTS Detachable Dynabeads CD3/CD28 magnetic beads can be more effectively optimized for *ex vivo* T cell expansion. Their flexibility, consistent performance, and ability to preserve key T cell quality attributes make CTS Detachable Dynabeads CD3/CD28 magnetic beads well suited for automated, large-scale manufacturing.

Materials and methods

Medium

Gibco™ CTS™ OpTmizer™ T-Cell Expansion SFM (serum-free medium) without phenol red (Cat. No. A3705001) was supplemented with 2.5% (v/v) Gibco™ CTS™ Immune Cell Serum Replacement (Cat. No. A2596101) and 4 mM L-glutamine (Cat. No. 25030081). Cultures were supplemented with 10 ng/mL Gibco™ Human IL-2 Recombinant Protein (Cat. No. PHC0023).

T cell isolation and activation

Frozen quarter leukopaks from three healthy donors were thawed at 37°C and diluted 1:1 with calcium- and magnesium-free D-PBS with 1% (w/v) human serum albumin (HSA) prior to isolation. A total of 4.0 x 10^8 $CD3^+$ T cells were isolated and activated with CTS Detachable Dynabeads CD3/CD28 magnetic beads (Cat. No. A56996) at a bead-to-cell ratio of 1:1, 3:1, or 5:1, using the CTS DynaCellect Magnetic Separation System (Cat. No. A55867) and the Gibco™ CTS™ DynaCellect™ Cell Isolation Kit (Cat. No. A52300).

After T cell isolation, an aliquot of each positive fraction was collected and evaluated for day 0 cell purity. The remaining positive fractions were transferred with medium to three 6-well 100 mL G-Rex™ 6M Well Plates (Wilson Wolf, Cat. No. 80660M), and the cell fractions remained in culture with the beads for the duration of activation. On day 1, day 2, or day 3 post-activation, T cells were harvested from the G-Rex plates. To release the beads, the pellets containing the T cells bound to the beads were incubated in Gibco™ CTS™ Detachable Dynabeads™ Release Buffer (Cat. No. A5588303) for 45 minutes at room temperature with rotation. The beads were then removed using the Invitrogen™ DynaMag™-50 Magnet (Cat. No. 12302D) prior to electroporation.

T cell transfection and expansion

Each T cell sample was resuspended in Gibco™ CTS™ Xenon™ Genome Editing Buffer (Cat. No. A4998001) and transfected with 4 µg of a pAAV-GFP (Green Fluorescent Protein) plasmid. The cells were electroporated using the Invitrogen™ Neon™ Transfection System 100 µL Kit (Cat. No. MPK10096) and the Invitrogen™ Neon™ NxT Electroporation System by applying five pulses per sample at 2,500 V with a 2 ms pulse width. After electroporation, the T cells were transferred to G-Rex™ 24-well plates (Wilson Wolf, Cat. No. 80192M) and maintained through day 10. Cell counts and viability were monitored throughout the expansion period using a NucleoCounter™ NC-250 cell counter (ChemoMetec).

Performance criteria

T cell purity, activation (CD69, CD25), and phenotype (CD3, CD62L, CCR7, CD45RA, CD45RO) were evaluated on days 1, 2, 3, and 10 of expansion using the Invitrogen™ Attune™ NxT Flow Cytometer. Recovery was calculated as:

Recovery (%) = [(average % $CD3^+$ cells in positive fraction) x (average count x volume)]/[average % $CD3^+$ input x (average count x volume)] x 100%

Statistical analysis

Data were expressed as a mean ± standard error of the mean (SEM), or by individual donor. Differences between two groups were tested for statistical significance using unpaired, two-tailed Student's *t*-tests.

Results

Impact of bead-to-cell ratio on T cell recovery and purity

To evaluate T cell purity and recovery post-isolation, we assessed $CD3^+$ cell enrichment and viability at different bead-to-cell ratios. Across all conditions, $CD3^+$ T cells comprised the majority of the isolated population (Figure 1). Non–T cell lymphocytes, including NK cells, B cells, and monocytes, were reduced to less than 3% of the population compared to ≥10% in the starting material. Comparable purity was observed after isolation at all three bead-to-cell ratios.

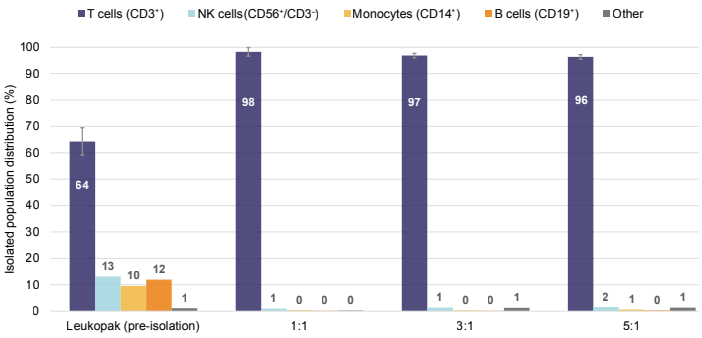

Figure 1. T cell purity and lymphocyte subset distribution following isolation with CTS Detachable Dynabeads CD3/CD28 beads on the CTS DynaCellect Magnetic Separation System. Lymphocyte subpopulations were assessed post-isolation using cells from three healthy donors (n = 3). Each dark blue bar represents the average percentage of T cells ± SEM.

Prior to expansion on day 0, the average recoveries of $CD3^+$ cells isolated at bead-to-cell ratios of 1:1, 3:1, and 5:1 were 59%, 86%, and 80%, respectively (Figure 2). Viability at the time of isolation exceeded 85% across all conditions. Based on these results, using a 3:1 or 5:1 ratio will yield comparable results and higher recoveries than can be achieved at a 1:1 ratio. These parameters can be adjusted according to process needs without compromising the quality of T cell isolation.

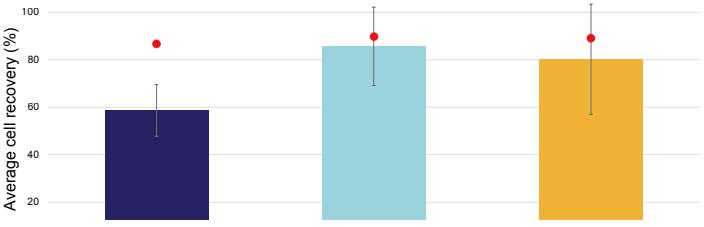

Figure 2. T cell recovery and viability following isolation with CTS Detachable Dynabeads CD3/CD28 beads on the CTS DynaCellect Magnetic Separation System. Recovery (bars) and viability (red dots) of T cells from three healthy donors (n = 3) were assessed after magnetic separation. Each bar represents the mean ± SEM.

Impact of bead-to-cell ratio on T cell activation, transfection, and viability

To evaluate the impact of bead release timing and bead-to-cell ratio on T cell function, activation kinetics and transfection efficiency were assessed. Activation marker expression followed the expected kinetics across all bead-to-cell ratios. CD69 expression peaked on release days 1 and 2, averaging 72–81%, then declined to 35–40% by day 3 (Figure 3A). CD25 expression increased over time, exceeding 90% by release day 2 and remaining high through day 3 (Figure 3B). These trends were consistent across bead-to-cell ratios.

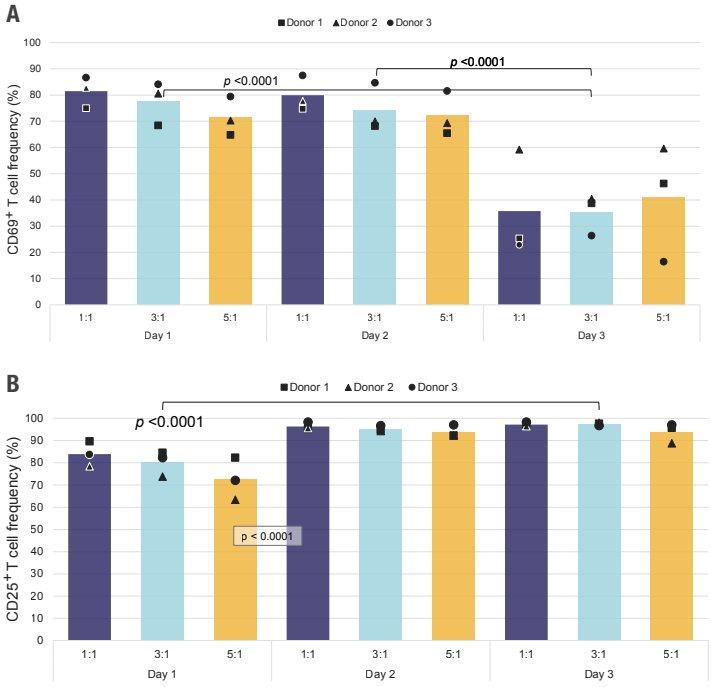

Figure 3. Activation marker expression across bead-to-cell ratios after release on day 1, day 2, or day 3. (A) Frequency of CD69⁺ T cells. **(B)** Frequency of CD25⁺ T cells. Each bar represents the mean, with individual donor data shown.

Transfection efficiency was assessed following bead release and electroporation with a pAAV-GFP plasmid. T cells released on day 1 showed the lowest transfection efficiency, while cells released on day 2 exhibited higher transfection efficiency and viability across all conditions (Figure 4). The post-transfection viability of cells activated at 1:1 and 3:1 bead-to-cell ratios remained high after release on day 1, 2, or 3. However, the viability of cells activated at the 5:1 bead-to-cell ratio was lower after release, particularly on day 3. Although the bead-to-cell ratio had minimal impact on transfection efficiency, it may have influenced post-transfection viability. These findings suggest that the bead-to-cell ratio and release day can be adapted to optimize for specific quality attributes based on individual process requirements.

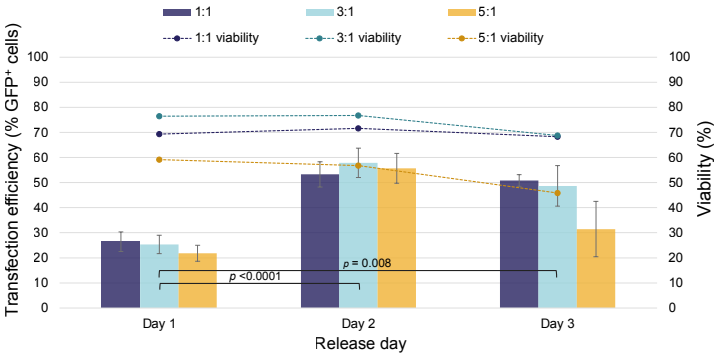

Figure 4. Transfection efficiency and cell viability 24 hours post-electroporation. Transfection efficiency and viability were assessed 24 hours after electroporation of T cells from three healthy donors. Each bar represents the mean ± SEM.

Impact of bead-to-cell ratio on T cell expansion and phenotype

T cell expansion and viability were monitored over a 10-day culture period in G-Rex 24-well plates. T cells released on day 2 after activation at bead-to-cell ratios of 1:1, 3:1, and 5:1 achieved the highest average growth with 1.15 x 10^8, 1.19 x 10^8, and 1.08 x 10^8 cells, respectively (Figure 5A). The average viability of cells released on day 1 or day 2 remained above 89% from day 6 to day 10, regardless of the bead-to-cell ratio. In contrast, T cells released on day 3 initially exhibited lower viability; however, viability increased to 86–89% by day 8 of expansion. While earlier bead release may have affected short-term viability, neither the release day nor the bead-to-cell ratio had a significant impact on overall viability by day 10 (Figure 5B).

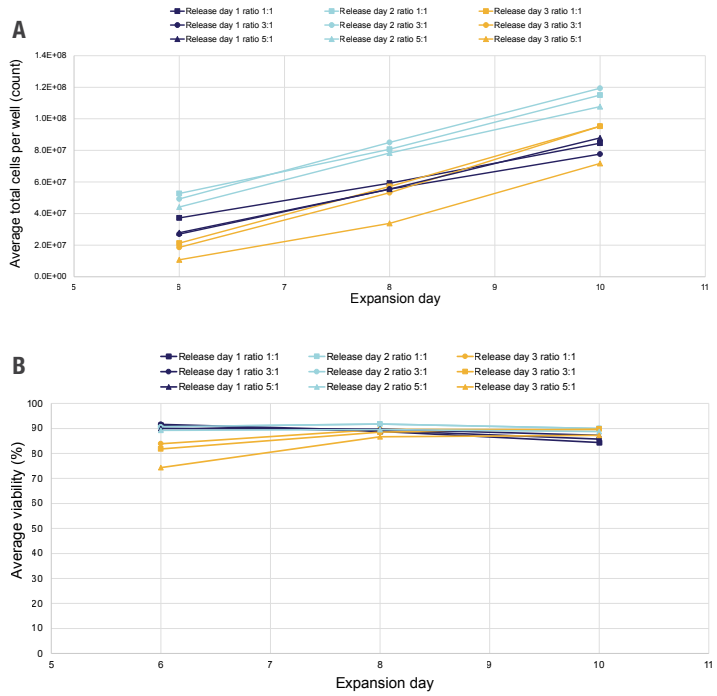

Figure 5. T cell expansion and viability over 10 days following bead release on day 1, 2, or 3. (A) Average cell growth and **(B)** viability were monitored over a 10-day culture period after bead release. Each data point represents the mean of T cells from three healthy donors.

T cell memory phenotype was assessed on day 10 of expansion. The highest average frequencies of CD62L^{+}/CCR7^{+} T cells were observed after release on day 2 and ranged from 70% to 88%. Frequencies of CD45RA^{+}/CD45RO^{-} T cells were comparable across all conditions, ranging from 48% to 57%. Although there was some variability between donors, the highest frequencies of CD45RA^{-}/CD45RO^{+} T cells were observed after release on day 1 across all conditions (Figure 6).

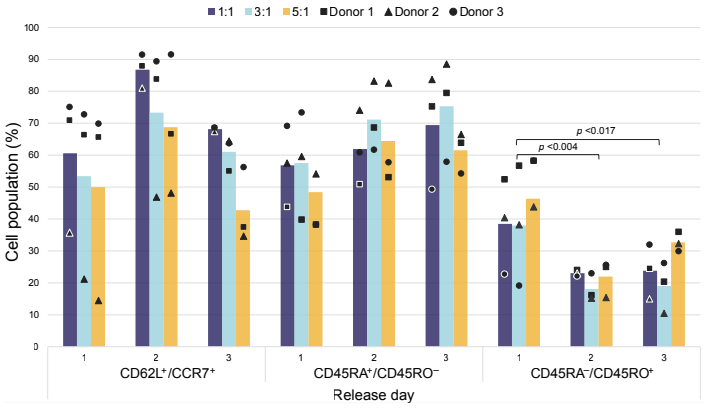

Figure 6. T cell memory phenotype after 10 days of expansion. CD45RA^{+} and CD45RO^{+} subsets were gated from the CD62L^{+}/CCR7^{+} population. Each bar represents the mean ± SEM of T cells from three healthy donors.

Conclusion

The results of this study demonstrate that CTS Detachable Dynabeads CD3/CD28 magnetic beads can enhance T cell therapy manufacturing by offering a flexible and adaptable platform that is compatible with automated, CGMP-compliant closed systems. Process-relevant findings included:

- High post-isolation purity (>95%) with negligible non–T cell contamination supported effective activation. We observed optimal outcomes with cells released on day 2, enabling a shorter and more efficient workflow.
- Bead release on day 2 consistently resulted in robust T cell growth and sustained viability over a 10-day culture period, indicating a healthy and proliferative cell population.
- Higher transfection efficiency and favorable purity, expansion, and viability were also observed with cells released on day 2.

The customizable nature of CTS Detachable Dynabeads CD3/CD28 beads allows users to tailor the bead-to-cell ratio and bead release timing to fine-tune process parameters. This enables cost efficiency, rapid scalability, and simplified manufacturing without compromising cell quality. With careful selection of process parameters, users can optimize T cell isolation, activation, expansion, and phenotype to support consistent and scalable T cell therapy production.

References

1. Tumaini B, Lee DW, Lin T et al. (2013) Simplified process for the production of anti–CD19-CAR–engineered T cells. *Cytotherapy* 15(11):1406–1415. **doi.org/10.1016/j.jcyt.2013.06.003**
2. Wang H, Tsao S-T, Gu M et al. (2022) A simple and effective method to purify and activate T cells for successful generation of chimeric antigen receptor T (CAR-T) cells from patients with high monocyte count. *J Transl Med* 20:608. **doi.org/10.1186/s12967-022-03833-6**

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