One-step isolation and activation of naive and early memory T cells with CTS Dynabeads CD3/CD28

Introduction

Here we demonstrate nearly 100% recovery of naive and early memory T cells using Gibco™ CTS™ Dynabeads™ CD3/CD28 beads. This one-step process of simultaneous T cell isolation and activation yields pure and uniformly activated T cells. $CD4^+$ and $CD8^+$ T cells expanded with CTS Dynabeads CD3/CD28 maintain an early memory phenotype on day 10 and have increased clonal diversity. Early bead removal is feasible and improves transduction efficiency of a γ-retrovirus. CTS Dynabeads CD3/CD28 beads deliver reliable results and have been used in more than 80 clinical trials and in commercial T cell drug manufacturing.

During the manufacturing process (Figure 1) peripheral blood mononuclear cells (PBMCs) are harvested from the patient (or a T cell donor) and transferred to a GMP facility, where the T cells are isolated and activated in the presence of CTS Dynabeads CD3/CD28 and genetically engineered by viral transduction to express a chimeric antigen receptor (CAR). The activated T cells are expanded in Gibco™ CTS™ OpTmizer™ T Cell Expansion Serum-Free Medium (SFM) supplemented with Gibco™ CTS™ Immune Cell Serum Replacement for a period, typically 7–10 days, to reach a therapeutically relevant number. They are then harvested with magnetic bead removal, and formulated for either freezing or adoptive transfer.

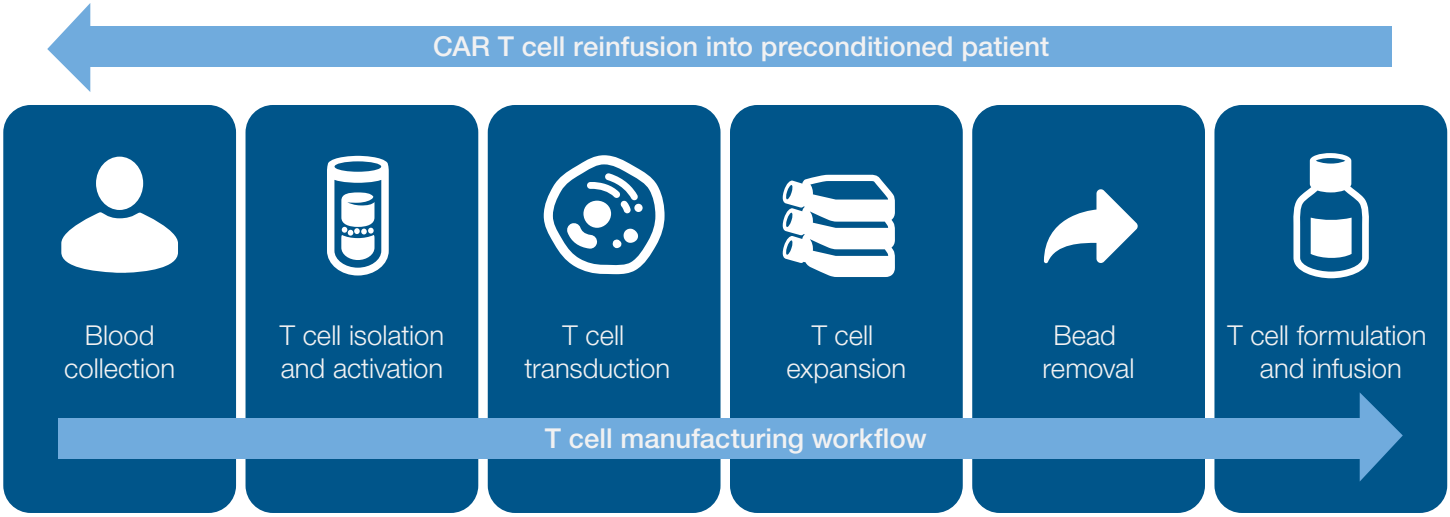

Figure 1. Example of a manufacturing and delivery pipeline for CAR T cell therapies (modified from ref. 1).

Materials and methods

1. T cells from PBMCs of healthy donors were isolated and activated using CTS Dynabeads CD3/CD28 (bead:cell ratio of 3:1) in PermaLife™ cell culture bags (OriGen) with CTS OpTmizer T Cell Expansion SFM supplemented with CTS Immune Cell Serum Replacement, Gibco™ L-Glutamine, and Gibco™ Gentamicin (incomplete medium).
2. After bead removal, T cells were expanded in G-Rex™ plates (Wilson Wolf) or PermaLife cell culture bags (OriGen).
3. Isolated and activated T cells were expanded in complete medium (incomplete medium plus 100 U/mL IL-2) for 7–10 days; CTS Dynabeads CD3/CD28 were magnetically removed at day 2, 3, or 5 post-stimulation.
4. T cells were transduced with a γ-retrovirus encoding EGFP (BioNTech AG) in Corning™ microplates coated with RetroNectin™ reagent (Takara Bio).
5. Flow cytometry analysis of T cells was performed on a BD LSRFortessa™ cell analyzer (BD Biosciences) using FlowJo™ software.
6. RNA from T cells was isolated using the Invitrogen™ Dynabeads™ mRNA DIRECT™ Purification Kit and analyzed by TCRβ sequencing using Ion AmpliSeq™ kits.

Results

CTS Dynabeads CD3/CD28 beads require no upstream T cell selection, as this technology simultaneously isolates and activates naive and early memory T cells based on CD3 and CD28 coexpression. Flow cytometry analysis shows that $CD3^+$ $CD28^+$ T cells are isolated with high (>90%) recovery (Figure 2). The cells are uniformly stimulated (>95% $CD25^+$) and highly pure (>95% $CD3^+$) at day 3 post-activation (Figure 3).

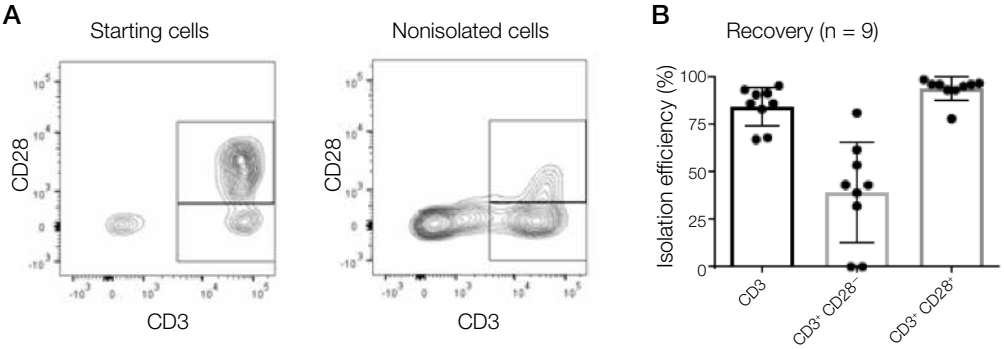

Figure 2. CTS Dynabeads CD3/CD28 preferentially isolate $CD3^+$ $CD28^+$ cells. PBMCs were incubated for 30 min with CTS Dynabeads CD3/CD28 at a ratio of 3:1 beads:cells, and the negative (nonisolated) fraction was analyzed and used to calculate isolation efficiency. **(A)** Representative cytograms demonstrating efficiency of $CD3^+$ $CD28^+$ T cell isolation. **(B)** Summary of recovery experiments (n = 9).

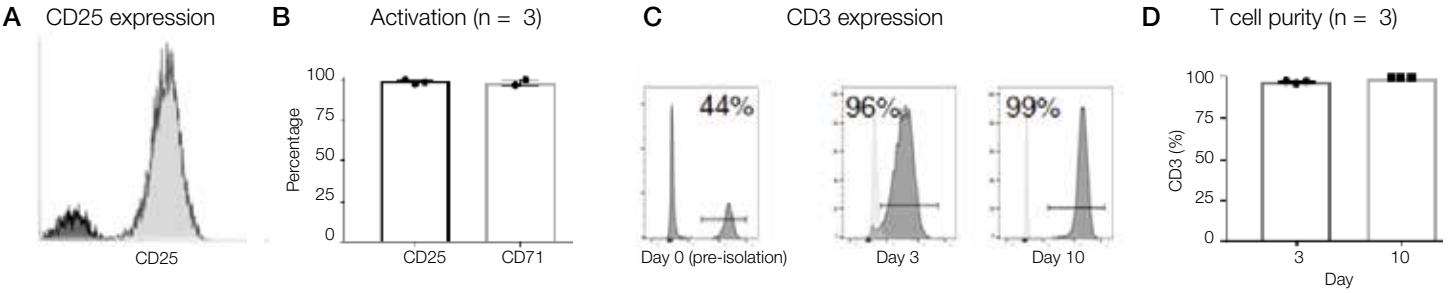

Figure 3. T cells isolated with CTS Dynabeads CD3/CD28 are uniformly stimulated and highly pure. (A, B) Isolated T cells were expanded for 3 days before analyzing activation markers. **(C, D)** T cell purity was analyzed at day 0 (starting material) and days 3 and 10 post-activation.

Activated T cells expand >100-fold in 10 days and preserve a young phenotype ($CD28^+$ $CD62L^+$) with low PD-1 expression on days 7–10 (Figure 4). The cells also demonstrate increased clonal diversity compared to starting T cells, as determined by TCRβ sequencing (Figure 5).

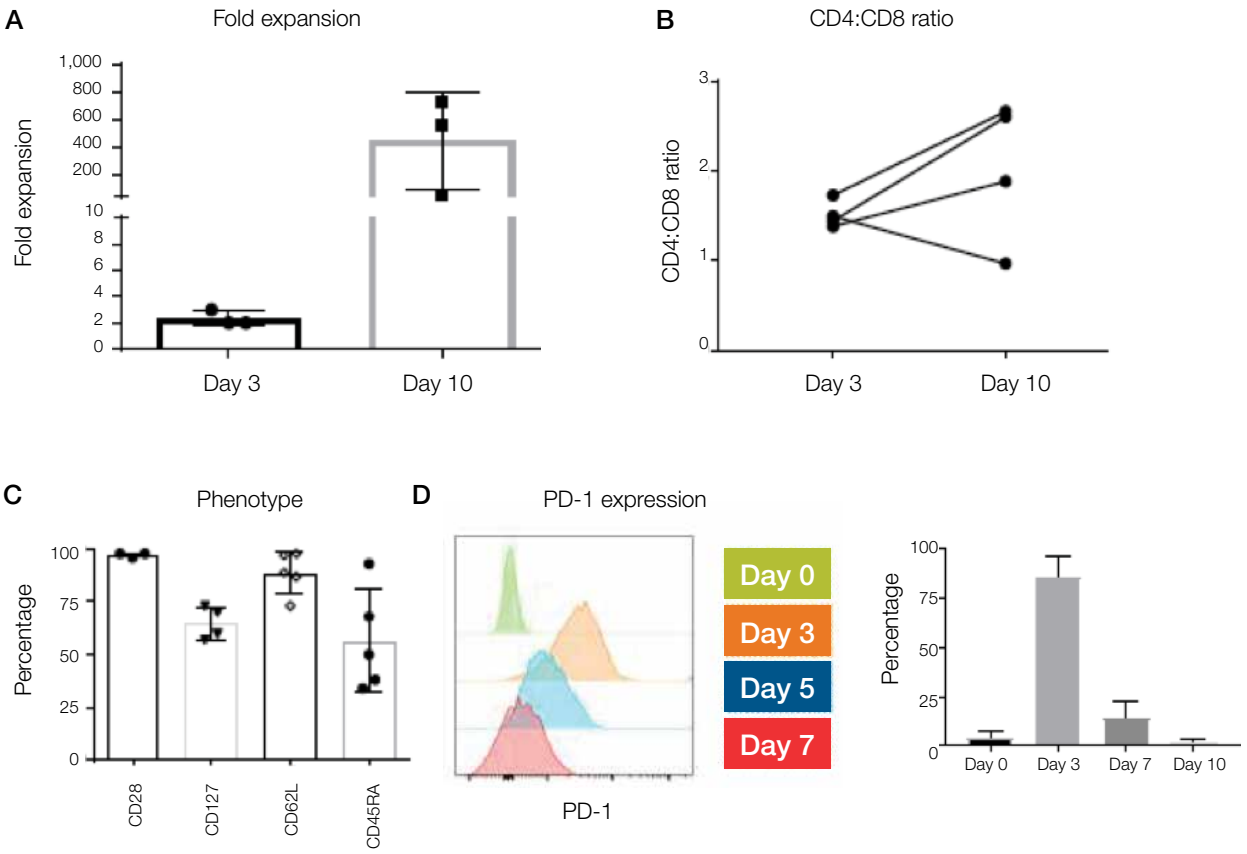

Figure 4. Activated T cells typically expand >100-fold in 10 days. (A, B) Isolated and activated T cells were expanded for up to 10 days, and both $CD4^+$ and $CD8^+$ T cells proliferated. **(C, D)** On day 10 post-activation, there were mainly $CD45RA^{+/-}$ $CD127^{+/-}$ $CD28^+$ $CD62L^+$ cells with low PD-1 expression.

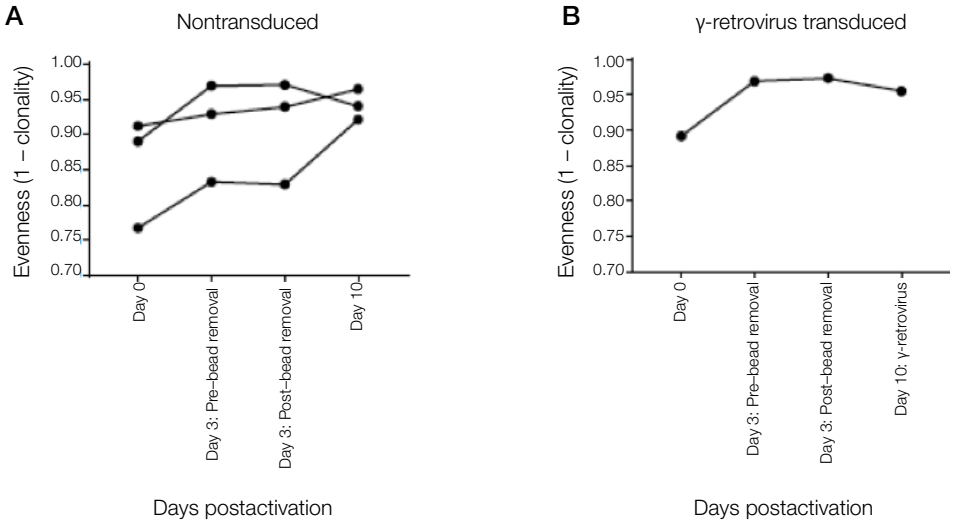

Figure 5. Increased clonal diversity after T cell expansion. T cells were isolated and activated with CTS Dynabeads CD3/CD28. **(A)** At days 0, 3, and 10, samples were harvested for TCRβ sequencing. **(B)** In one experiment, T cells were also transduced with a γ-retrovirus on day 3 post-activation.

gibco

Early removal of CTS Dynabeads CD3/CD28 (days 2, 3, and 5 post-activation) is feasible but comes with some cell loss on days 2 and 3 (T cell recovery 50–80%, Figure 6). Nearly all T cells are recovered following bead removal on day 5. Transduction efficiency is higher when beads are removed and cells are transduced on day 2 compared to day 3 post-activation.

Conclusions

- CTS Dynabeads CD3/CD28 isolate $CD3^+$ $CD28^+$ T cells with high efficiency and to high purity.
- Isolated and activated $CD4^+$ and $CD8^+$ T cells expand 100- to 800-fold in 10 days while retaining a young phenotype ($CD45RA^{+/-}$ $CD127^{+/-}$ $CD62L^+$ $PD\text{-}1^-$).
- Expanded T cells have increased clonal diversity based on TCRβ sequencing.
- Bead removal on day 2 improves transduction efficiency of a γ-retrovirus.

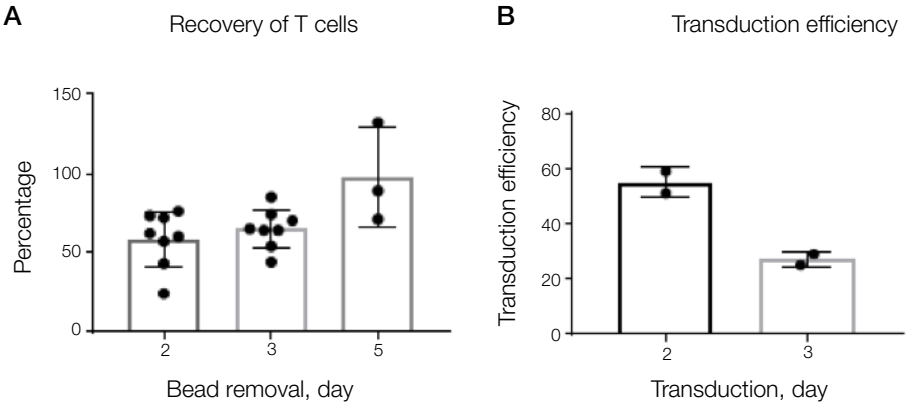

Figure 6. Shorter stimulation of T cells improves *γ*-retrovirus transduction efficiency. T cells were isolated with CTS Dynabeads CD3/CD28 in PermaLife cell culture bags and stimulated for 2 or 3 days. The beads were magnetically removed on day 2 or 3 before transducing the cells with a γ-retrovirus encoding an EGFP reporter in wells coated with RetroNectin reagent (2.5 MOI). **(A)** The recovery of T cells was lower after early bead removal compared to 5 days of stimulation. **(B)** Transduction efficiency was assessed 2 and 3 days post-activation.

Reference

1. Almåsbak H, Aarvak T, Vemuri MC (2016) CAR T cell therapy: A game changer in cancer treatment. *J Immunol Res* 2016:5474602.

Find out more at **thermofisher.com/cts**

ThermoFisher SCIENTIFIC