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CTS™ Dynabeads™ CD3/CD28 enables simultaneous one-step naïve and early memory T cell isolation and activation

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ABSTRACT

CTS™ Dynabeads™ CD3/CD28 has been used successfully in advancing T cell therapies through >88 clinical trials and lately also in commercial T cell drug manufacturing.

CTS™ Dynabeads™ CD3/CD28 requires no upstream T cell selection as this technology simultaneously isolates and activates naïve and early memory T cells based on CD3 and CD28 co-expression. T cells are isolated with high recovery (>90%) and purity (>95%) and uniformly stimulated (>95% $CD25^+$ day 3 post-activation). Activated and expanded T cells preserve a young phenotype ($CD28^+$ $CD62L^+$) with low PD-1 expression day 7-10, as well as demonstrate increased clonal diversity compared to starting T cells.

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

MATERIALS AND METHODS

- T cells from healthy donor peripheral blood mononuclear cells (PBMCs) were isolated and activated using CTS™ Dynabeads™ CD3/CD28 (bead: cell ratio 3:1) (Thermo Fisher Scientific) in Permalife cell culture bags (Origen) with CTS™ OpTmizer™ T Cell Expansion SFM supplemented with CTS™ Immune Cell Serum Replacement, L-Glutamine and Gentamicin (incomplete media) (all Thermo Fisher Scientific).
- Post bead removal T cells were expanded in G-rex plates (Wilson Wolf) or Permalife cell culture bags (Origen)
- Isolated and activated T cells were expanded in complete media (incomplete media + 100 U/mL IL-2) for 7-10 days. Dynabeads were magnetically removed at day 3 post stimulation or day 2 and 5 as described (Figure 6).
- T cells were transduced with γ-retrovirus encoding EGFP (BionTech AGH) in retronectin (TaKaRa) coated corning microplates (Sigma-Aldrich) post bead removal.
- Flow cytometry analysis of T cells were was performed on a BD Fortessa (BD bioscience) instrument using FlowJo
- RNA from T cells was isolated using Dynabeads™ mRNA DIRECT™ Purification Kit (Thermo Fisher Scientific) and analyzed with AmpliSeq™ TCRβ sequencing (Thermo Fisher Scientific)

TYPICAL T CELL MANUFACTURING WORKFLOW

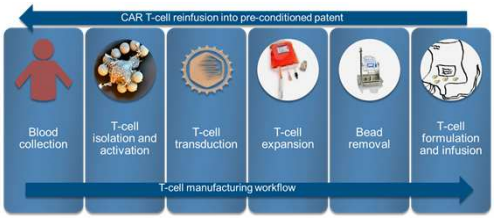

Figure 1: Example of manufacturing and delivery pipeline of CAR T cell therapies (modified from ref 1). PBMCs are harvested from the patient (or a T cell donor) and transferred to a GMP facility where the T cells are isolated and activated in the presence of CTS™ Dynabeads™ CD3/CD28 and genetically engineered by viral transduction to express the CAR. The activated T cells are expanded in CTS™ OpTmizer™ T Cell Expansion SFM supplemented with CTS™ Immune Cell Serum Replacement for a period, typically 7-10 days, to reach a therapeutic relevant number before magnetic bead removal and drug formulation, either for freezing or for adoptive transfer.

RESULTS

Nearly 100% recovery of naïve and early memory T cells with one-step simultaneous T cell isolation and activation

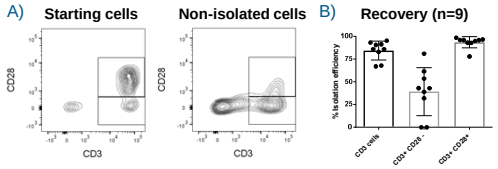

Figure 2. CTS™Dynabeads™CD3/CD28 preferentially isolates $CD3^+$ $CD28^+$ positive cells. PMBCs were incubated for 30 min with CTS™ Dynabeads™ CD3/CD28 at a ratio of 3:1 beads:cells and the negative (non isolated) fraction was analyzed and used to calculate isolation efficiency. Representative cytogram demonstrating efficiency of $CD3^+CD28^+$ T cells isolation (A). Recovery (n=9) experiments (B)

One-step isolation and activation yields pure and uniformly activated T cells

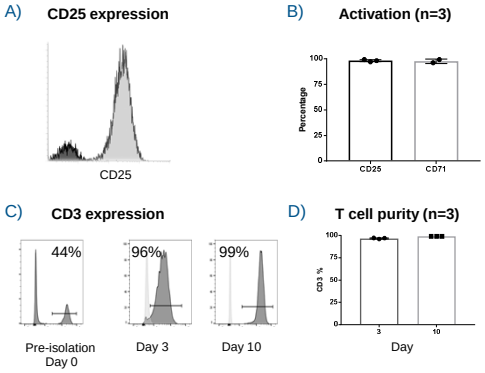

Figure 3. CTS™Dynabeads™CD3/CD28 isolated T cells are pure and uniformly stimulated. Isolated T cells were expanded for 3 days before analyzing activation markers (A,B). Overlay histogram of activated T cells and a non-activated control (A). T cell purity was analyzed at days 0 (starting material),3 and 10 post-activation (C, D).

CTS™Dynabeads™CD3/CD28 expanded CD4+ and CD8+ T cells maintains an early memory phenotype (day 10)

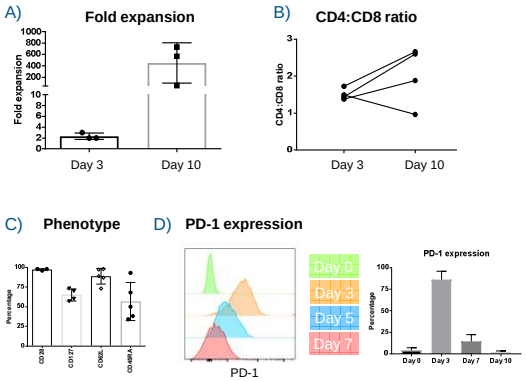

Figure 4. Activated T cells typically expands >100 fold in 10 days. Isolated and activated T cells were expanded for up to 10 days (A) and both CD4 and CD8 T cells proliferate (B). Day 10 post-activation, cells were mainly $CD45RA^{+/-}CD127^{+/-}CD28^+CD62L^+$ (C) with low PD-1 expression (D).

Dynabeads expanded T cells have increased clonal diversity

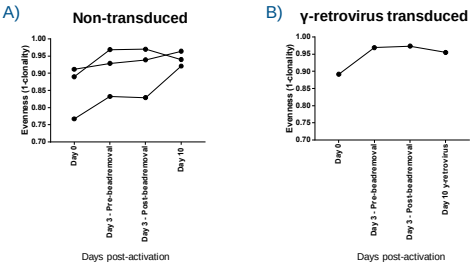

Figure 5. Increased clonal diversity after T cell expansion. T cells were isolated and stimulated with CTS™ Dynabeads™ CD3/CD28 (3 donors). At days 0, 3 and 10, samples were harvested for TCR-sequencing (A). In one experiment, T cells were also transduced with γ-retrovirus day 3 post stimulation (B).

Early bead removal is feasible and day 2 transduction improves γ-retroviral transduction

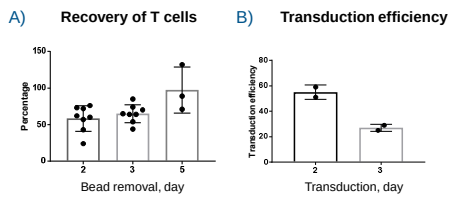

Figure 6. Shorter stimulated of T cells improves γ-retrovirus transduction efficiency. T cells were isolated with CTS™ Dynabeads™ CD3/CD28 in Origen cell culturing bags and stimulated for 2 or 3 days. The beads were magnetically removed day 2 or 3 before transducing the cells with γ-retrovirus encoding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

CONCLUSIONS

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

REFERENCE

(1) Modified from Journal of Immunology Research (2016), CAR T Cell Therapy: A Game Changer in Cancer Treatment. Almåsbak, Aarvak, and Vemuri

TRADEMARKS/LICENSING

ThermoFisher SCIENTIFIC