ion torrent

Evaluation of therapeutic T cell manufacture using long amplicon TCRβ immune repertoire sequencing

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INTRODUCTION

Following the demonstration of the tremendous potential of T cell therapies in blood cancers, the field is evolving rapidly with focus on commercial, cost-effective manufacture to offer therapies for larger patient groups. There exists a need for ***streamlined and quality-controlled manufacturing processes, with closed-system operations and simplified workflows***. We demonstrate the utility of long-amplicon T cell receptor beta (TCRβ) sequencing to sample repertoire features of therapeutic T cells at various timepoints during the manufacturing process.

T cell populations from multiple donors are tracked in each step of the therapeutic T cell manufacture process, beginning with baseline repertoire measurement after isolation from the donor and expansion using CTS™Dynabeads™CD3/CD28 in CTS™ OpTmizer™ T Cell Expansion Serum Free Media with 5% CTS™ Immune Cell Serum Replacement. The repertoire is then examined after the viral transduction process, and final T cell product. We survey the TCRβ repertoire of therapeutic T cell populations using the Oncomine™ TCR Beta – LR Assay, sequencing on the Ion Torrent S5 system, and repertoire analysis using Ion Reporter immune repertoire analysis software.

Donor PBMC-derived T cells were isolated with high recovery (>90%) and purity (>95%) and uniformly stimulated (>95% $CD25^+$day 3 post-activation). Activated T cells were expanded and preserved a young phenotype ($CD28^+CD62L^+$) at day 7-10. TCRβ sequencing was used to measure the initial pre-isolation T cell population revealing a diverse polyclonal repertoire (evenness = 0.76-0.88). Importantly, this clonal diversity persists and often increases post-isolation, through activation, expansion, bead removal, transduction, and in the final product (evenness = 0.90-0.96). The consistent increase in evenness with cell culture time suggests that the manufacturing process used here promotes a polyclonal (unbiased) T cell expansion.

Measurement of a therapeutic T cell repertoire provides a sequence level understanding of the diversity within a cell product. We demonstrate that repertoire sequencing can ensure a diverse repertoire of T cells are maintained during manufacture. A 48h turnaround time, from sample to analysis result, allows this testing to occur at multiple timepoints in the manufacturing process using both the richness and evenness of the repertoire to track therapeutic T cell populations longitudinally through manufacture. In addition, TCRβ repertoire sequencing of a therapeutic T cell product provides a rich baseline for further monitoring of the T cell repertoire after administration.

MATERIALS AND METHODS

T cells from peripheral blood mononuclear cells (PBMCs) (healthy donors) 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).

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. T cells were transduced with y-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).

The **Ion Oncomine™ TCR Beta-LR Assay** leverages Ion AmpliSeq™ technology to profile the TCR repertoire through the enrichment of the TCR beta gene, including both the highly diverse CDR3 region as well as the CDR1-2 regions of the germline encoded variable gene. The 350bp amplicon containing all three CDR regions allows for high accuracy clonotyping for determination of diversity in quality control applications involving T cell culture.

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RESULTS

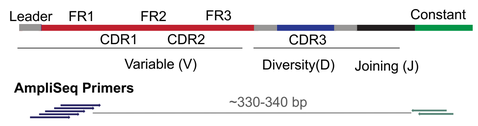


Figure 1. Ion Oncomine TCRB – LR Assay. Multiplex AmpliSeq primers target the framework region 1 (FR1) and constant (C) regions of the TCRβ producing a ~330bp amplicon which covers the entire variable gene and the CDR3 region.

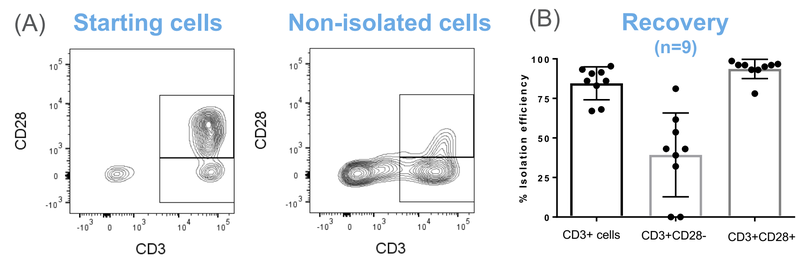


Figure 2. CTS™Dynabeads™CD3/CD28 preferentially isolates $CD3^+$ $CD28^+$ positive cells. PMBCs were incubated for 30 min with CTS™ Dynabeads™ CD3/CD28 at a ratio of 3:1 beads:cells and the negative (non-isolated) fraction was analyzed and used to calculate isolation efficiency. (A) Representative cytogram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

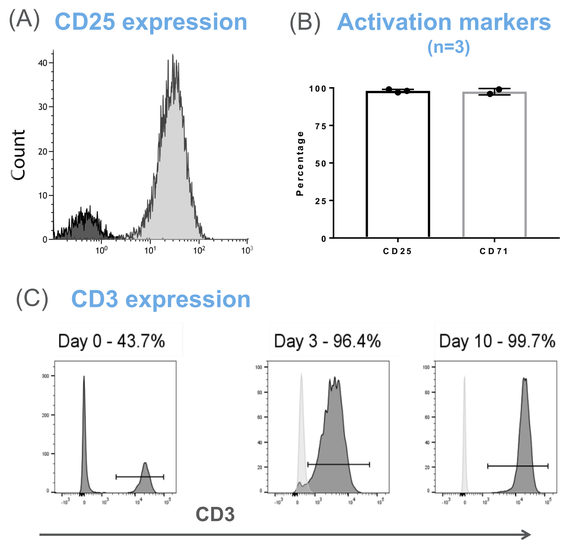


Figure 3. CTS™Dynabeads™CD3/CD28 isolated T cells are pure and uniformly stimulated in a single step process. Isolated T cells were expanded for 3 days before analyzing activation markers (A, B). T cell purity were analyzed at days 0 (starting material), 3, and 10 post-activation (C, D).

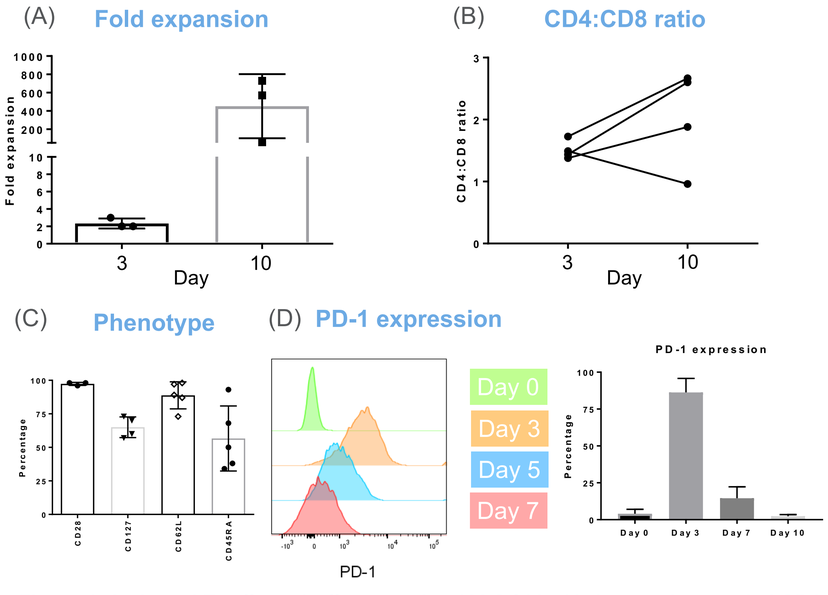
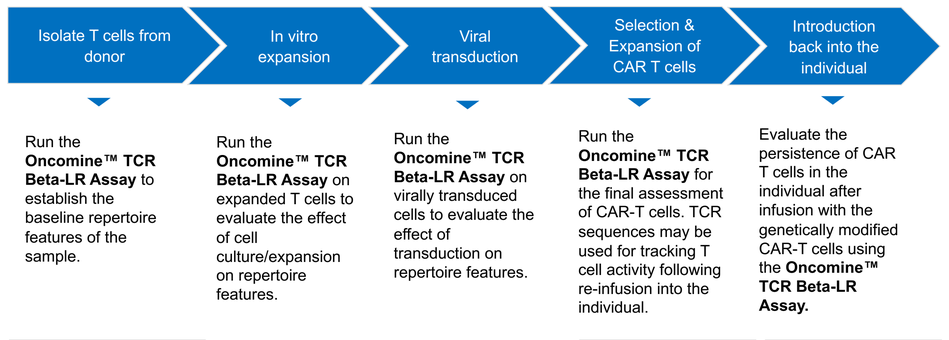


Figure 4. Activated T cells typically expands >100 fold in 10 days and expanded CD4+ and CD8+ T cells maintain an early memory phenotype at day 10. Isolated and activated T cells were expanded for up to 10 days (A) and both CD4 and CD8 T cells proliferate (B). Day 10 post-activation, cells were mainly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

Repertoire Analysis using TCRβ sequencing



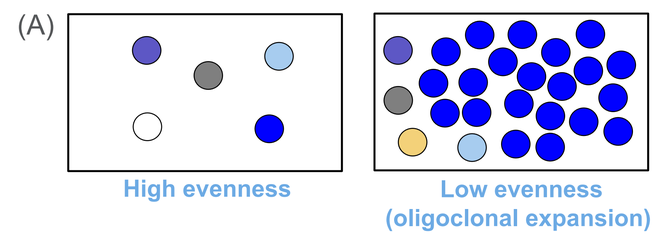
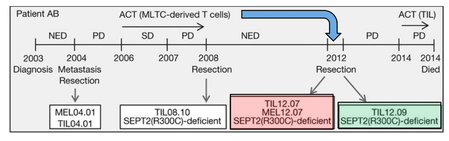
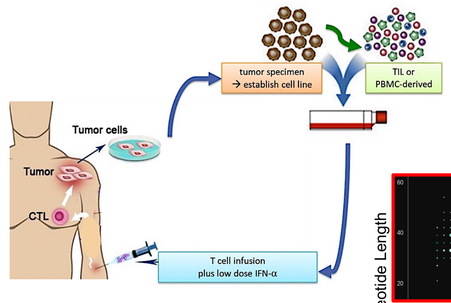


Figure 5. Increased clonal diversity after T cell expansion. (A) Overview of evenness, illustrating the difference in clonal expansion between samples with high evenness (left) and low evenness (right). T cells were isolated and stimulated with CTS™Dynabeads™ CD3/CD28. At days 0, 3 and 10, samples were harvested for TCR-sequencing (B). In one experiment, T cells were also transduced with **γ**-retrovirus day 3 post stimulation (C).

Case Study from Dr. Noel Miranda
Principal Investigator at Leiden University Medical Center

Leiden University Medical Center





- Stage IV melanoma patient received adoptive T cell transfer (ACT) therapy and lived for 11 years following diagnosis.
- In 2012 the cancer came back and they had to decide one of two ACT products to give the patient.
- At this time they did not have the technology to perform TCR sequencing on the ACT product.
- TCR sequencing analysis of the TIL 12.07 and TIL 12.09 ACT products show the presence of large expanded clones in the TIL 12.07 product.

- TCR sequencing analysis of ACT products may identify products with characteristics that may be more beneficial (higher evenness/lack of expanded clones).