An Engineered IL-2 Enhances T Cell Proliferation without Affecting Exhaustion or Memory Phenotype

Introduction

The process of using immune cells for cell therapy requires the consistent and robust growth of highly functional cells. To achieve this, T cells from patients or donors need to be activated and cultured outside of the body in the presence of anti-CD3, anti-CD28 antibodies and cytokines. The challenge is to balance the number of cells with their functionality, which scientists have been earnestly working on. There are currently no commercially available cytokines for *ex vivo* use that have improved function beyond the naturally occurring proteins. To address this issue, Bio-Techne has engineered a recombinant human IL-2 protein that supports improved expansion of immune cells for workflows heavily reliant on IL-2. One concern is that improving T cell expansion can result in increased numbers of terminally differentiated and exhausted T cells with reduced anti-tumor activity. However, our new IL-2 has been shown to greatly improve T cell expansion, without altering T cell phenotype or exhaustion marker expression.

Key Takeaways

- Engineered IL-2 provides increased and sustained growth of $CD8^+/CD4^+$ T cells when compared with the standard IL-2 protein, making it applicable to many T/NK/TIL cell workflows reliant on IL-2 for proliferation.
- While providing increased proliferation capacity, engineered IL-2 does not yield increased exhaustion or more terminally differentiated T cells than the standard IL-2 protein used in this study.

Experimental Workflow

T cells were isolated from whole blood, plated, and activated with anti-CD3, anti-CD28 antibodies conjugated onto Dynabeads. Cells were maintained at a cell density of $0.25x10^6$ or $0.5x10^6$ after day 15 with 50 ng/mL of either engineered or standard IL-2. IL-2 was refreshed every 2-3 days.

Note: The standard IL-2 protein used in this study is R&D Systems™ Animal-free Recombinant Human IL-2 (Catalog # BT-002-AFL), which is the commonly used Aldesleukin version of IL-2 containing a C145S amino acid substitution.

Results

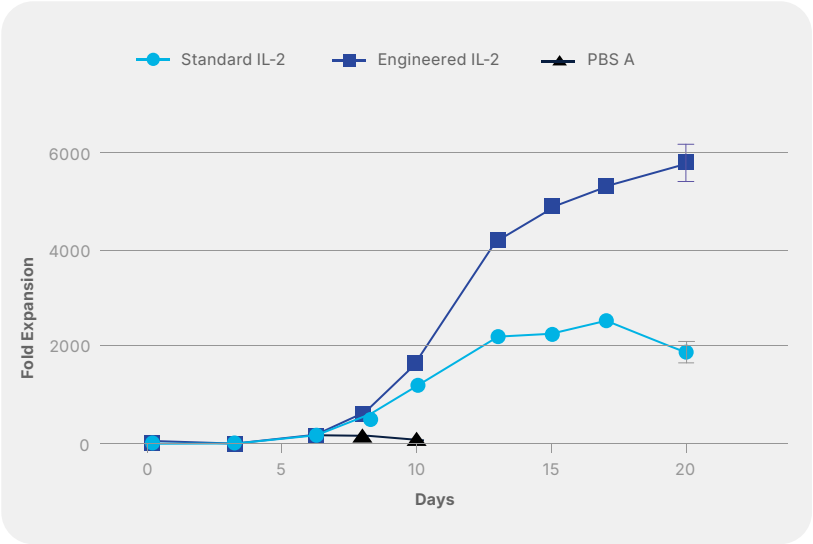

Figure 1. Engineered IL-2 Provides Increased and Sustained CD4⁺/CD8⁺ T Cell Proliferation in Side-by-Side Testing with the Standard IL-2 Protein. CD4⁺ and CD8⁺ T cells from two different donors were activated with anti-CD3⁻, anti-CD28-conjugated beads, and grown for 21 days in media containing 50 ng/mL Animal-free Recombinant Human IL-2 (R&D Systems, Catalog # BT-002-AFL) as the standard IL-2 protein or Engineered Recombinant Human IL-2, and refreshed every 2-3 days. The average fold expansion of the cells was determined every 2-3 days and demonstrated that the engineered IL-2 protein supports greater and more continuous CD4⁺/CD8⁺ T cell growth than the standard IL-2 protein.

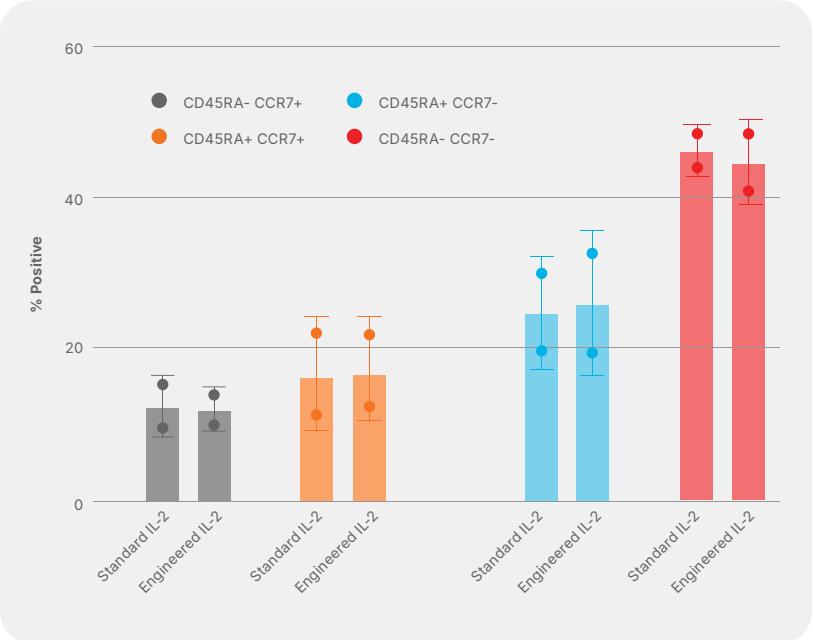

Figure 2. T Cells Expanded with Standard or Engineered IL-2 Display Similar Phenotypes. CD4⁺ and CD8⁺ T cells from two different donors were activated with anti-CD3-, anti-CD28-conjugated beads, and grown in media containing 50 ng/mL Animal-free Recombinant Human IL-2 (R&D Systems, Catalog # BT-002-AFL) as the standard IL-2 protein or Engineered Recombinant Human IL-2, and refreshed every 2-3 days. After 21 days, the phenotypes of the cells were compared by flow cytometry using a mFluor Violet 610 SE-conjugated Mouse Anti-Human CD45RA Monoclonal Antibody (R&D Systems, Catalog # FAB11444MFV610) and a PE-Cy7-conjugated mouse anti-human CCR7 monoclonal antibody to determine the number of CD4⁺ and CD8⁺ naive (CD45RA⁺ CCR7⁺), central memory (CD45RA⁻ CCR7⁺), terminal effector memory RA⁺ (CD45RA⁺ CCR7⁻), and effector memory (CD45RA⁻ CCR7⁻) T cells. The percentages of each of these cell populations were found to be similar whether the cells were expanded with the standard or engineered Recombinant Human IL-2 protein, leading to the conclusion that engineered IL-2 promotes increased T cell expansion, without changing the memory phenotype of the cells.

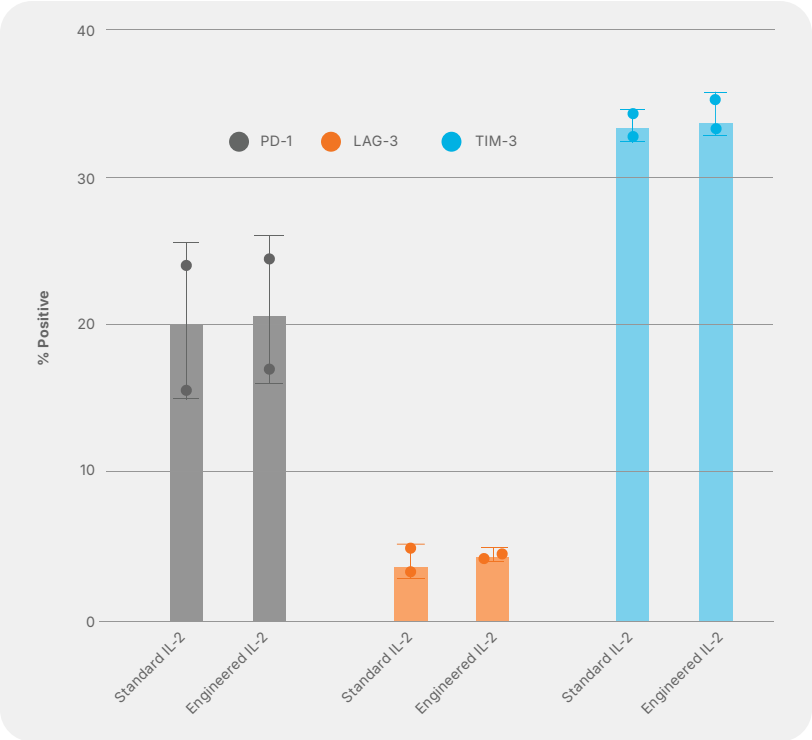

Figure 3. T Cells Expanded with Standard or Engineered IL-2 Express Similar Levels of Cell Exhaustion Markers. $CD4^+$ and $CD8^+$ T cells from two different donors were activated with anti-CD3-, anti-CD28-conjugated beads, and grown in media containing 50 ng/mL Animal-free Recombinant Human IL-2 (R&D Systems, Catalog # BT-002-AFL) as the standard IL-2 protein or Engineered Recombinant Human IL-2, and refreshed every 2-3 days, for 21 days. The number of cells expressing the T cell exhaustion markers, PD-1, LAG-3, and TIM-3 was determined by flow cytometry using a PE-Cy7-conjugated rabbit anti-human PD-1 monoclonal antibody, a mFluor Violet 610 SE-conjugated Mouse Anti-Human LAG-3 Monoclonal Antibody (R&D Systems, Catalog # FAB23193MFV610), and an Alexa Fluor® 647-conjugated Rat Anti-Human TIM-3 Monoclonal Antibody (R&D Systems, Catalog # FAB2365R). The results showed that the percentages of $CD4^+/CD8^+$ T cells that were also PD-1, LAG-3, or TIM-3 positive were similar whether the cells were expanded with the standard or engineered Recombinant Human IL-2 protein, supporting the conclusion that while engineered IL-2 promotes increased T cell expansion, it does not increase the exhaustion profile of the final expanded product.

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Conclusions

To meet the critical clinical need of maximizing the number of functional immune cells generated by expansion protocols for cell therapy workflows, the scientists at Bio-Techne have engineered a human IL-2 protein that supports the robust proliferation and survival of T cells. While there are many workflows available for T/NK/TIL cell therapies that influence proliferation and memory phenotype, a side-by-side comparison of the engineered IL-2 protein with a standard IL-2 protein in a simple T cell expansion protocol has demonstrated clear improvements in the ability of the engineered protein to support increased immune cell expansion, without altering the memory or exhaustion phenotype of the cells. As a result, incorporation of this reagent into any autologous or allogeneic cell therapy workflow should yield significant benefits.