Sustained T Cell Proliferation and Early Memory Retention with OptiLeukin™ 2 Recombinant IL-2

Application Note

Mark Stathos, PhD, Product Applications Scientist, InVitria, Inc.

Andrew Hamann, PhD, Product Applications Scientist, InVitria, Inc.

Key Points

- InVitria's OptiLeukin™ 2, a high-purity (≥ 98%), GMP-grade, recombinant human interleukin-2 (IL-2) enables robust expansion of primary T cells.
- OptiLeukin 2 supports the generation of T cells with a favorable memory phenotype, essential for long-term therapeutic persistence.
- OptiLeukin 2 matches the performance of leading GMP IL-2 products, enabling a seamless like-kind replacement.
- OptiLeukin 2 is manufactured in a system free from serum, microbial fermentation, and mammalian cells minimizing endotoxins and host cell protein contaminants.

Introduction

Critical Role of IL-2 in Cell Therapy Manufacturing

Generating a large quantity of cells with a desirable phenotype efficiently and consistently is a major challenge in cell therapy manufacturing and is critical for attaining positive clinical outcomes. The exogenous addition of cytokines is indispensable for this process as these proteins act as potent signals for cells to proliferate and differentiate but are present at very low levels in serum. The most widely used cytokine in cell therapy applications is interleukin-2 (Watanabe, 2022). IL-2 plays a

critical role in the expansion and function of numerous clinically relevant immune cell types, including conventional T cells, regulatory T Cells (Tregs), natural killer (NK) cells, and tumor infiltrating lymphocytes (TILs) and γδ T cells (Raeber, 2023).

However, the importance of cytokines such as IL-2 imposes several hurdles for cell therapy production. Regulatory frameworks (e.g., USP Chapter 1043, ISO 20399:2022, Ph. Eur. General Chapter 5.2.12) strongly recommend that ancillary materials, including cytokines used in cell therapy manufacturing should be produced using animal-origin-free (AOF) processes to reduce safety risks (Tanaka 2023). Supply chain constraints are also a major concern as the cell therapy market is expected to grow significantly in the coming years with a compound annual growth rate of 40% from 2021 to 2026 (Piñel-Neparidze, 2024) and IL-2 supply is limited. Finally, consistent performance is critical in this application as it directly affects outcomes for patients.

To address these issues, InVitria has developed OptiLeukin 2, a GMP recombinant human IL-2. Like all of InVitria's products, including Optibumin 25 recombinant human albumin and ITSE+A serum replacement supplement, OptiLeukin 2 is produced in a dedicated AOF facility using a process that is animal component-free at the tertiary level. Moreover, InVitria's expression system is highly amenable to scale up, ensuring a robust and reliable inventory of recombinant protein products and eliminating supply chain concerns. OptiLeukin 2's bioactivity is measured relative to the NIBSC 1st International Standard for IL-2, providing confidence in reproducibility across research and clinical workflows. This study demonstrates that OptiLeukin 2 supports robust and reproducible T cell expansion with favorable memory phenotype profiles, comparable to leading AOF GMP IL-2 products on the market.

Results and Discussion

OptiLeukin 2 Drives Robust and Dose-Responsive T Cell Proliferation

Primary human T cells from two healthy donors were thawed and cultured in microplates in media containing 5 ng/mL, 20 ng/mL, or 60 ng/mL (equivalent to 50 IU/mL, 200 IU/mL, or 600 IU/mL of NIBSC 1st international standard IL-2 respectively) (Gearing, 1988) of either OptiLeukin 2 (one of two lots) or competitor IL-2 or no IL-2. A CD3/CD28 antibody cocktail was added to induce activation, and the cells were then left undisturbed for 3 days. After the activation period, cells were counted well by well and split volumetrically on Day 3, Day 6, Day 8, and Day 10 and cumulative fold expansion was determined (**Figure 1**).

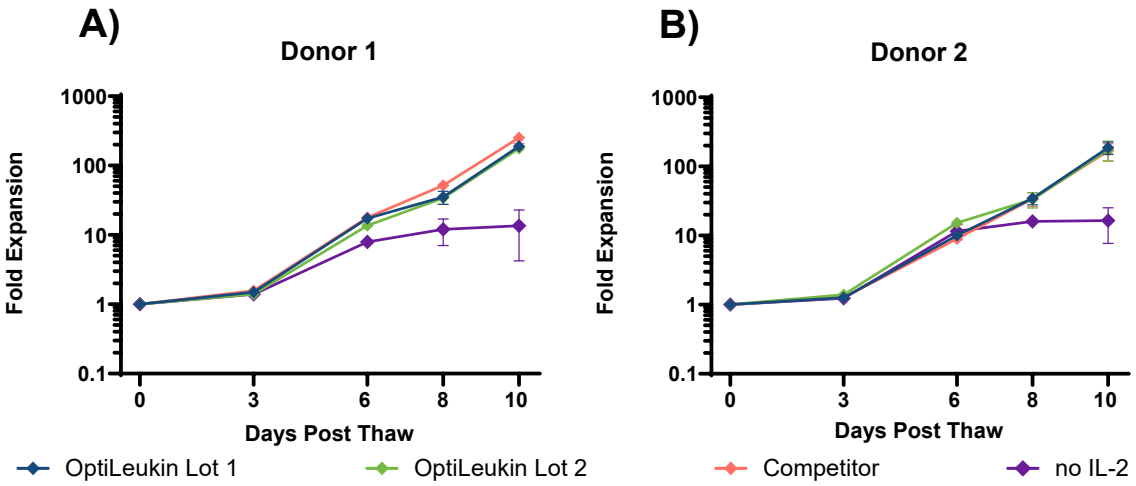


Figure 1: Cumulative fold expansion over 10 days of primary T cells from A) Donor 1 or B) Donor 2 treated with 20 ng/mL of one of either two different lots of OptiLeukin 2 or GMP competitor IL-2 or no IL-2. Expansion is plotted on a log10 scale. No significant differences were found in Day 10 cumulative fold expansion among IL-2 treated groups but all groups exhibited significantly more growth than the no IL-2 control. (Brown-Forsythe test $p < 0.05$).

Comparable expansion with or without IL-2 was observed until day 6 which can be attributed to inherently slow initial growth during T cell activation as cells undergo a prolonged G1 phase (Lewis, 2021) and endogenous production of IL-2 by T cells after activation (Bachmann, 2007).

However, each IL-2 treatment group exhibited a significantly higher fold expansion by Day 10 compared to the corresponding zero IL-2 control group (Figure 1)

highlighting the critical need for IL-2 supplementation in T cell therapy manufacturing. A 20 ng/mL dose of IL-2 yielded an average of 206-fold for Donor 1 (Fig. 1A) and 176-fold for Donor 2 (Fig. 1B). IL-2 doses of 5 ng/mL and 60 ng/mL were also evaluated to cover the concentration range typically used in T cell culture (data not shown). A mild IL-2 dose dependence was observed but Day 10 cumulative expansion was not significantly different between each lot of OptiLeukin 2 and competitor IL-2 at any dose. Expansion kinetics also followed similar trends within a given donor and IL-2 dose with both lots of OptilLeukin-2 sustaining log phase growth from day 3 through day 10.

This data demonstrates that OptiLeukin 2 promotes robust and consistent expansion of primary T cells for the duration of a typical CAR-T manufacturing process with no discernable loss in performance compared to the GMP competitor product. These results support the use of OptiLeukin 2 in T cell manufacturing workflows requiring recombinant IL-2 supplementation.

Early Memory Phenotype Retention Observed in T Cells Cultured with OptiLeukin 2

As part of the same experiment used to characterize T cell expansion, memory phenotype was also assessed using flow cytometry. The persistence and efficacy of CAR-T therapies heavily depend on maintaining early T cell memory phenotypes, such as stem cell memory (Tscm) and central memory (Tcm) cells, which exhibit superior proliferation, persistence, and anti-tumor activity (Fazeli, 2023; Meyran, 2023). In contrast, more differentiated phenotypes, such as effector memory (Tem) and terminal effector memory (Temra) cells, are less desirable due to their reduced proliferative potential and limited persistence (Farietta, 2018). These phenotypes translate to differences in clinical outcomes for CAR-T patients (Lin, 2023).

The effects of OptiLeukin 2 on memory phenotype of both donors were assessed on Day 4, Day 7, (data not shown) and Day 9 (Fig. 2A-B). Phenotypes were defined by CCR7 and CD45RA expression patterns as in Tian et al. 2017. Phenotypes for all groups were very similar and mostly comprised Tscm cells on day 4. Phenotypes of all

groups progressed similarly throughout the experiment. By day 9, cells in all groups comprised a roughly equal split of Tscm, Tcm, Tem, and Temra cells. This temporal phenotypic progression is natural and expected for primary T cells cultured in the presence of IL-2. Additionally, cells treated with higher levels of IL-2 expressed a more differentiated phenotype than those treated with lower levels which is also in line with expectations given that additional IL-2 effected increased proliferation from these groups (Moynihan, 2024). Importantly, within a given donor, timepoint, and IL-2 dose, little difference in phenotype was observed indicating that OptiLeukin 2 has no deleterious effects on this critical readout compared to the competitor IL-2.

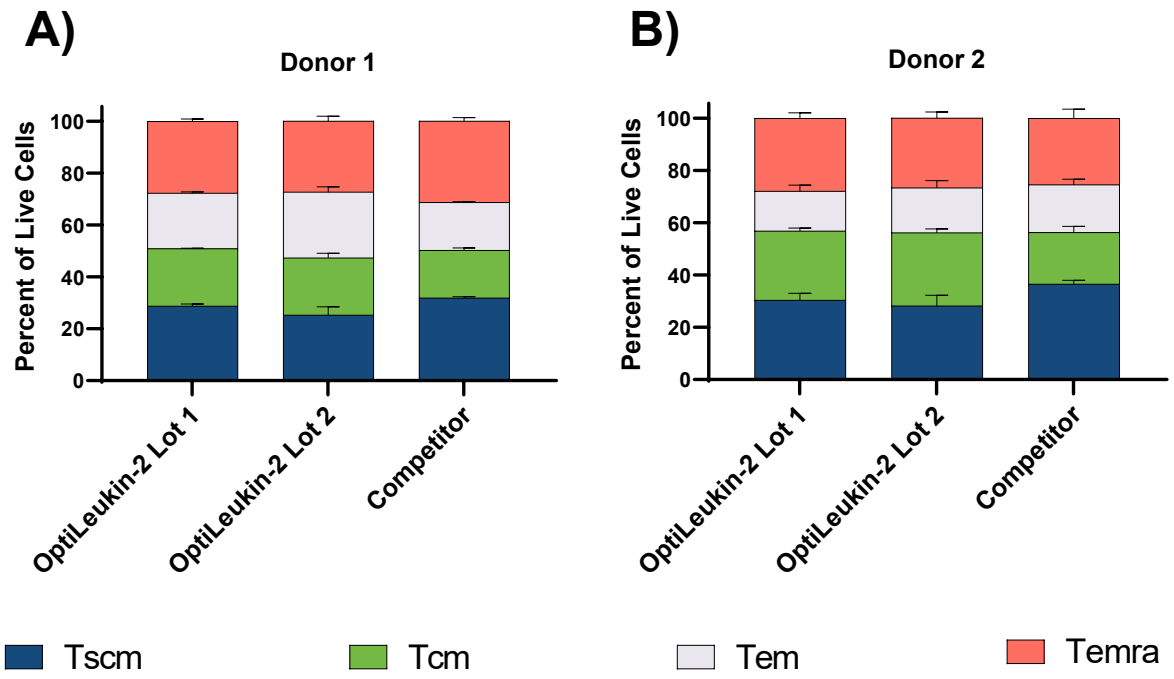


Figure 2: Proportions of stem cell memory (Tscm, blue), central memory (Tcm, green), effector memory (Tem, gray) and terminal effector memory expressing CD45RA (Temra, coral) measured on Day 9 in T cells from Donor 1 A) or Donor 2 B) treated 20 IL-2 from one of two lots of OptiLeukin 2 or a GMP competitor.

Conclusion

OptiLeukin 2 was shown to perform comparably to on market GMP competitor IL-2 driving rapid proliferation of T cells populations with a favorable phenotype in the 10-day culture and expansion of primary human T cells from two healthy donors. Additionally, OptiLeukin 2 is GMP and animal origin free facilitating regulatory acceptance. Furthermore, OptiLeukin 2 is derived from a highly scalable expression system offering supply chain and cost benefits. Collectively, the data demonstrates that OptiLeukin 2 provides a reliable, animal-origin-free recombinant IL-2 solution for T cell expansion, with performance comparable to current market leaders and added advantages in supply scalability and cost efficiency.

Materials and Methods

IL-2 Preparation:

A vial of 100 µg of OptiLeukin 2 was reconstituted by adding 1 mL of sterile cell culture grade water to the lyophilized powder. The sample was visually inspected to confirm complete reconstitution, aliquoted, and stored at -20°C for future use. A fresh aliquot of OptiLeukin 2 was thawed prior to each cell passage and diluted into fresh cell culture media to ensure maximal activity. Used aliquots were kept at 4°C for up to 1 week for other applications or discarded rather than being refrozen. The competitor IL-2 product was reconstituted and stored according to the manufacturer's instructions and a fresh aliquot was used to supplement media prior to each cell passage.

T Cell Culture and Activation:

Primary T cells were isolated from leukopaks derived from 2 healthy, non-smoker donors aged between 18 and 65 years with a BMI <30. Isolation was performed using a negative selection kit following the manufacturer's protocol. The isolated cells were cryopreserved in liquid nitrogen using a formulation containing 10% DMSO.

Before the experiment cells were thawed and adjusted to a density of 1.0×10^6 cells/mL in serum-free T cell media. The media was supplemented with the indicated concentrations of one of two lots of OptiLeukin 2 or a GMP competitor IL-2 immediately after reconstitution of the protein. Activation was initiated by adding a CD3/CD28 antibody cocktail to the cells and incubating them for three days.

T Cell Expansion:

After three days of activation, cells were counted in triplicate using an NC-200 NucleoCounter (ChemoMetec, Allerod, Denmark) and split 1:10 volumetrically into aliquots of media with freshly added IL-2. Duplicate samples were prepared to enable flow cytometry analysis the next day. Splitting was repeated after counting on day 6 and day 8. A final count was then taken on day 10 and cumulative fold expansion determined based on the initial seeding of one million cells, the split ratios and the prior counts.

T Cell Phenotyping by Flow Cytometry:

On day 4, day 7 and day 9 post-thaw, cells were harvested for flow cytometry. Cell samples were transferred to a 96-well V-bottom plate (as an example of references other products, we could put the product name it's part number and a hyperlink to get it), centrifuged, washed with PBS, and stained with an amine reactive fixable viability dye. After incubation with the viability dye, cells were washed and then stained with an optimized antibody cocktail with antibodies against CCR7, CD45RA, CD4, and CD8. Finally, the cells were fixed in 4% paraformaldehyde in PBS and transferred to cell staining buffer before being analyzed on the flow cytometer.

Flow cytometry analysis began with gating for cell population based on forward scatter (FSC) versus side scatter (SSC) plots. Single cells were identified from FSC area versus FSC height plots. Dead cells were excluded based on high amine dye staining.

Cells were then classified as either CD4+ or CD8+ populations using a 2D plot with a quadrant gate. Memory phenotypes were further defined based on CD45RA and CCR7 expression, as described by Tian et al (2017):

- **Stem Cell Memory (Tscm):** CCR7+, and CD45RA+
- **Central Memory (Tcm):** CCR7+ CD45RA-
- **Effector Memory (Tem):** CCR7- CD45RA-
- **Terminal Effector Memory (Temra):** CCR7- CD45RA+

References

1. Watanabe, N., Mo, F., & McKenna, M. K. (2022). Impact of manufacturing procedures on CAR T cell functionality. *Frontiers in Immunology*, *13*. https://doi.org/10.3389/fimmu.2022.876339
2. Raeber, M. E., et al. (2023). A systematic review of interleukin-2-based immunotherapies in clinical trials for cancer and autoimmune diseases. *eBioMedicine*, *90*, 104539. https://doi.org/10.1016/j.ebiom.2023.104539
3. Tanaka, T., Yoshimura, K., Chang, R., Choi, B., Gai, Y., Gupta, P. K., Kolkundkar, U., Lee, S. M., Lee, S., Liao, W., Zhao, X., & Takakura, K. (2023). Comparison of guidelines for biological ancillary materials used for the manufacture of gene and cellular therapy products in Asia. *Cytotherapy*, *25*(2), 220-228. https://doi.org/10.1016/j.jcyt.2022.11.002
4. Piñel-Neparidze, C., Bickerstaffe, H., Shah, S., & Versnel, J. (2024). The importance of a go-to-market strategy in the commercialisation of cellular immunotherapies. *Drug Discovery Today*, *29*(7), 104028. https://doi.org/10.1016/j.drudis.2024.104028
5. Gearing, A. J. H., & Thorpe, R. (1988). The international standard for human interleukin-2: Calibration by international collaborative study. *Journal of Immunological Methods*, *114*(1-2), 3-9. https://doi.org/10.1016/0022-1759(88)90145-7
6. Lewis, D. A., & Ly, T. (2021). Cell cycle entry control in naïve and memory CD8+ T cells. *Frontiers in Cell and Developmental Biology*, *9*, 727441. https://doi.org/10.3389/fcell.2021.727441
7. Bachmann, M. F., & Oxenius, A. (2007). Interleukin 2: From immunostimulation to immunoregulation and back again. *EMBO Reports*, *8*(12), 1142-1148. https://doi.org/10.1038/sj.embor.7401099
8. Fazeli, P., Kalani, M., & Hosseini, M. (2023). T memory stem cell characteristics in autoimmune diseases and their promising therapeutic values. *Frontiers in Immunology*, *14*, 1204231. https://doi.org/10.3389/fimmu.2023.1204231
9. Meyran, D., et al. (2023). TSTEM-like CAR-T cells exhibit improved persistence and tumor control compared with conventional CAR-T cells in preclinical models. *Science Translational Medicine*, *15*(690), eabk1900. https://doi.org/10.1126/scitranslmed.abk1900
10. Fraietta, J. A., Lacey, S. F., Orlando, E. J., Pruteanu-Malinici, I., Gohil, M., Lundh, S., Boesteanu, A. C., Wang, Y., O'Connor, R. S., Hwang, W. T., Pequignot, E., Ambrose, D. E., Zhang, C., Wilcox, N., Bedoya, F., Dorfmeier, C., Chen, F., Tian, L., Parakandi, H., Gupta, M., Young, R. M., Johnson, F. B., Kulikovskaya, I., Liu, L., Xu, J., Kassim, S. H., Davis, M. M., Levine, B. L., Frey, N. V., Siegel, D. L., Huang, A. C., Wherry, E. J., Bitter, H., Brogdon, J. L., Porter, D. L., June, C. H., & Melenhorst, J. J. (2018). Determinants of response

and resistance to CD19 chimeric antigen receptor (CAR) T cell therapy of chronic lymphocytic leukemia. *Nature Medicine*, *24*(5), 563–571. https://doi.org/10.1038/s41591-018-0010-1

11. Lin, Y., Raje, N. S., Berdeja, J. G., Siegel, D. S., Jagannath, S., Madduri, D., et al. (2023). Idecabtagene vicleucel for relapsed and refractory multiple myeloma: Post hoc 18-month follow-up of a phase 1 trial. *Nature Medicine*, *29*, 2286–2294. https://doi.org/10.1038/s41591-023-02496-0

12. Tian, Y., Babor, M., Lane, J., Schulten, V., Patil, V. S., Seumois, G., Rosales, S. L., Fu, Z., Picarda, G., Burel, J., Zapardiel-Gonzalo, J., Tennekoon, R. N., De Silva, A. D., Premawansa, S., Premawansa, G., Wijewickrama, A., Greenbaum, J. A., Vijayanand, P., Weiskopf, D., Sette, A., & Peters, B. (2017). Unique phenotypes and clonal expansions of human CD4 effector memory T cells re-expressing CD45RA. *Nature Communications*, *8*(1), 1473. https://doi.org/10.1038/s41467-017-01728-5

13. Moynihan, K. D., Kumar, M. P., Sultan, H., Pappas, D. C., Park, T., Chin, S. M., Bessette, P., Lan, R. Y., Nguyen, H. C., Mathewson, N. D., Ni, I., Chen, W., Lee, Y., Liao-Chan, S., Chen, J., Schumacher, T. N. M., Schreiber, R. D., Yeung, Y. A., Djuretic, I. M. (2024). IL2 targeted to CD8+ T cells promotes robust effector T-cell responses and potent antitumor immunity. *Cancer Discovery*, *14*(7), 1206–1225. https://doi.org/10.1158/2159-8290.CD-23-1266

For additional product or technical information, please contact us at

2718 Industrial Drive
Junction City, KS 66441

Phone: 1.800.916.8311
Email: Info@InVitria.com

Copyright © 2025 InVitria, Inc. All rights reserved.
Reproduction or distribution of any InVitria materials, in whole or in part, is prohibited without prior written consent. All logos, names, designs, and marks displayed, including InVitria®, the InVitria® brand design and OptiLeukin 2 (TM), are trademarks or service marks owned or licensed by InVitria, Inc., Kansas, USA, unless otherwise noted. NucleoCounter® is a registered trademark of ChemoMetec A/S, Allerod, Denmark For details on InVitria's registered intellectual property, patents, and additional terms and conditions, please visit www.InVitria.com/terms.