

Expansion of human T cells using TheraPEAK® AmpliCell® Cytokines

Lonza
Specialized Modalities

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Abstract

The cell and gene therapy landscape has seen significant advancements, as evidenced by recent approvals of novel therapeutics and a robust pipeline of developmental programs targeting various indications. While chimeric antigen receptor (CAR) T cells targeted against B cell antigens (CD19, BCMA, and CD22) continue to dominate the hematologic cancer pipeline, novel technologies are focusing on strategies to fight solid tumors. The development of clinical-grade products for cell and gene therapy heavily relies on the availability of cGMP-compliant high-quality raw materials used during the manufacturing process, to allow consistent manufacturing of robust drug products that are safe for patient use.

To address this need, we are introducing Lonza's TheraPEAK® AmpliCell® Cytokines, which are GMP-grade, animal-component free, xeno-free products that are produced in human cell lines thus enabling these proteins to express the appropriate post-translational modifications characteristic of human cytokines. The TheraPEAK® AmpliCell® Cytokines are manufactured in accordance to USP, EP, and WHO standards, supporting maximum safety, consistency, and reproducibility. To demonstrate the expansion and downstream application of TheraPEAK® AmpliCell® Cytokines, we cultured healthy human peripheral blood mononuclear cells (PBMC) or enriched T cells in either serum-containing conditions using X-VIVO® 15 Medium or in serum-free culture supported by TheraPEAK® T-VIVO® Medium, supplemented with either IL-2 alone or IL-7 and IL-15 cytokines. T cell activation was induced using a soluble CD3/CD28-targeting reagent, and cells were cultured in either polystyrene tissue culture vessels or a bioreactor for a period of 9 – 10 days. To demonstrate the effector function of the cells, we transduced the cells using a Lonza-manufactured lentivirus vector to deliver a CD19-CAR construct and assessed transduction efficiency as well as cytotoxicity against NALM6 tumor cells.

We demonstrate that TheraPEAK® AmpliCell® Cytokines support the expansion of highly viable T cells, exhibiting preferred stem-like T_{SCM} or central T_{CM} characteristics. Transduction efficiency was similar for the different cytokine conditions, and CAR-T cells demonstrated robust cytotoxicity upon co-culture with tumor cells. Notably, during the co-culture with NALM6 cells, we observed the enrichment of CD19-CAR T cells demonstrating preferential expansion of antigen-specific tumor-killing T cells. Altogether, our data demonstrate that TheraPEAK® AmpliCell® Cytokines are able to support robust expansion of genetically modified T-cell products that exhibit strong phenotype and function.

In conclusion, Lonza's TheraPEAK® Portfolio of products comprising T-VIVO® Medium, X-VIVO® 15 Medium, and AmpliCell® Cytokines provide solutions to support cGMP-compliant manufacture of robust cell and gene products that exhibit strong quality attributes.

Study Design

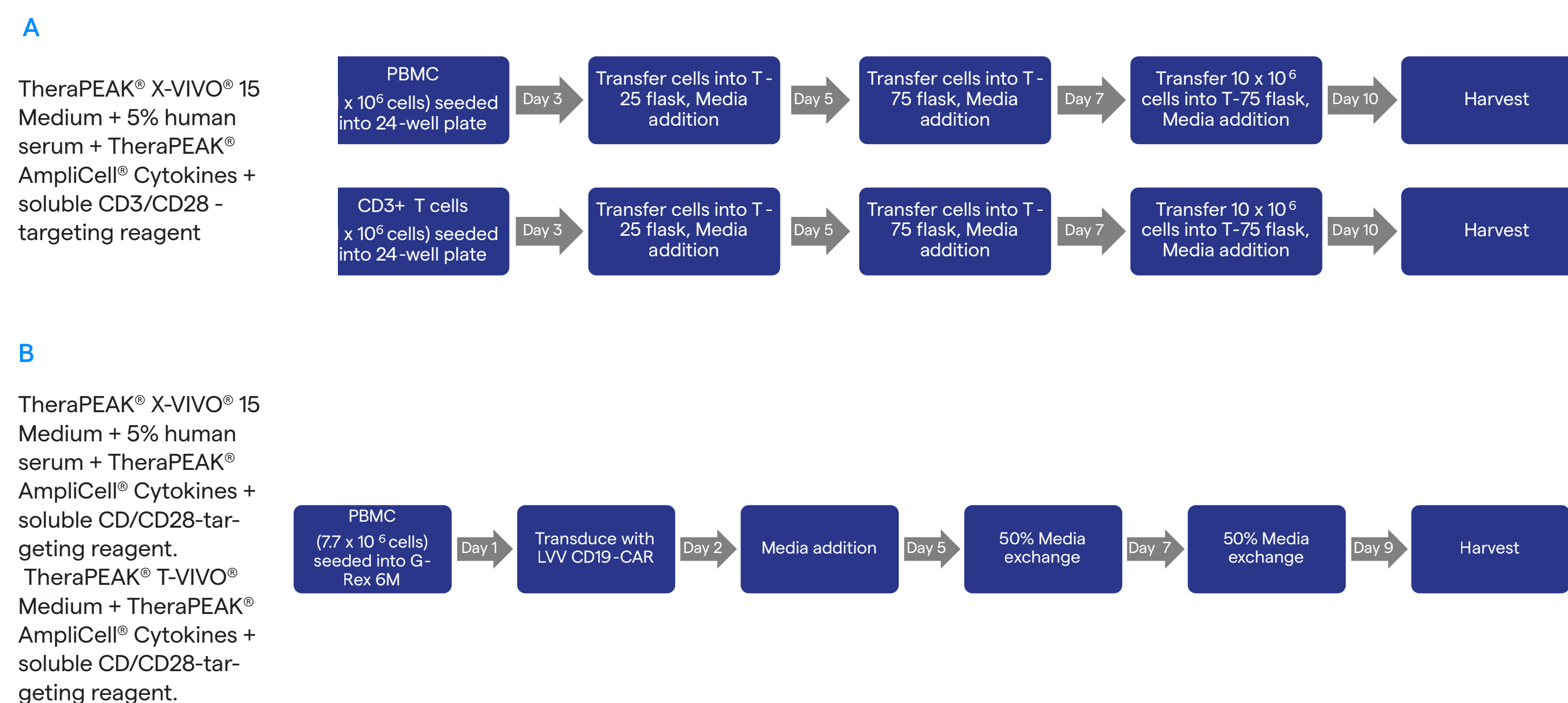


Figure 1: Process overview of cells expanded with TheraPEAK® AmpliCell® Cytokines. (A) PBMC input material or selected CD3+ T cells, each from 3 healthy donor (HD) donors, were seeded in TheraPEAK® X-VIVO® 15 Medium supplemented with 5% human AB serum and TheraPEAK® AmpliCell® IL-2 at 100 IU/mL, or TheraPEAK® AmpliCell® IL-7 at 10 ng/mL and TheraPEAK® AmpliCell® IL-15 at 10 ng/mL. Cells were activated using a soluble CD3/CD28-targeting reagent at day 0 and cultured in polystyrene vessels. At specific timepoints, cells were transferred to larger T-flasks and supplemented with more complete media, with harvest occurring at day 10 of process. (B) PBMC input material were seeded in either TheraPEAK® X-VIVO® 15 and 5% human AB serum or TheraPEAK® T-VIVO® Medium both supplemented with TheraPEAK® AmpliCell® IL-2 at 100 IU/mL, or TheraPEAK® AmpliCell® IL-7 at 10 ng/mL and TheraPEAK® AmpliCell® IL-15 at 10 ng/mL. Cells were activated with a soluble CD3/CD28-targeting reagent at day 0 and cultured in G-Rex® 6M plates. For one healthy donor, the cells were not transduced, however for the second healthy donor, cells were transduced with a LVV CD19-CAR at day 1. Following a series of media additions throughout the process, the cells were harvested on day 9 of the process.

Results

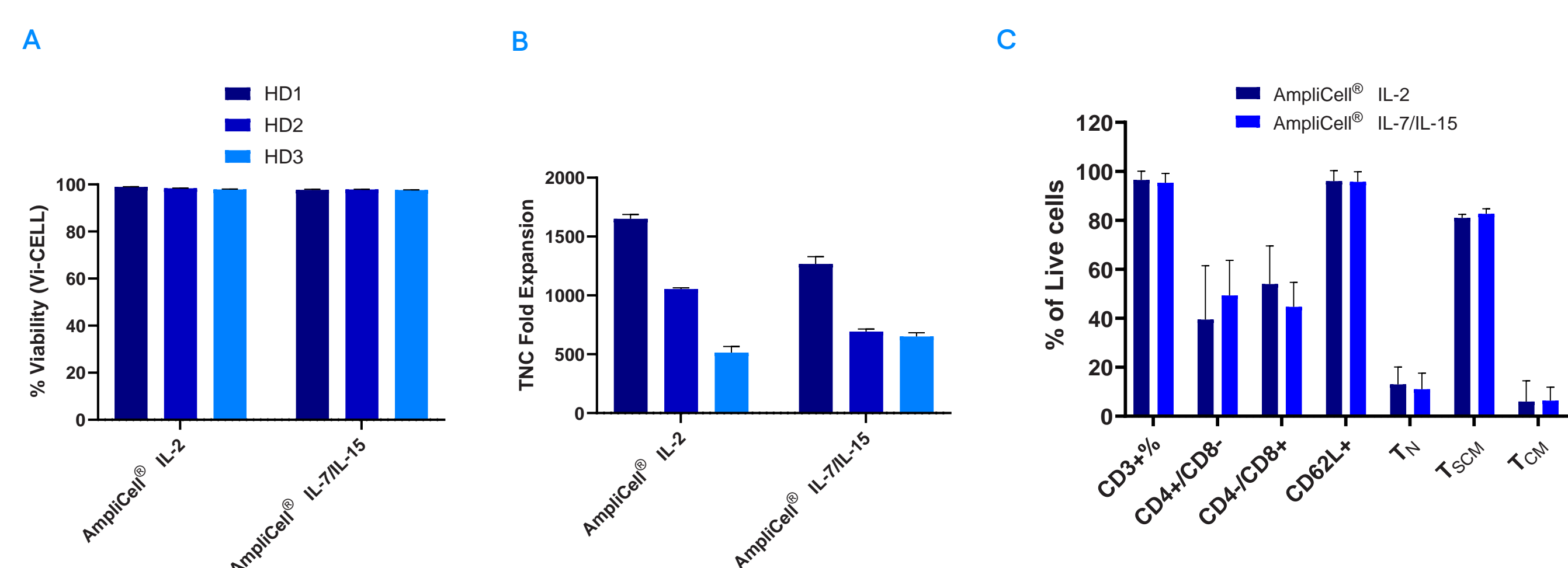


Figure 2: PBMC expansion in T-flasks with complete TheraPEAK® X-VIVO® 15 Medium Supplement with TheraPEAK® AmpliCell® Cytokines. (A) Cell viabilities were assessed at the end of the process (day 10) for three healthy donors (HD), demonstrating maintenance of highly viable, healthy cells throughout culture. (B) High fold expansion of cells is achieved, with high frequency of T cells exhibiting stem-like memory (T_{SCM}) phenotype. (C) Comparable frequencies of T-cell specific markers were observed when cells were cultured with both AmpliCell® IL-2 and AmpliCell® IL-7/IL-15 Cytokines. CD45RO, and CD45RA were used to define memory phenotypes.

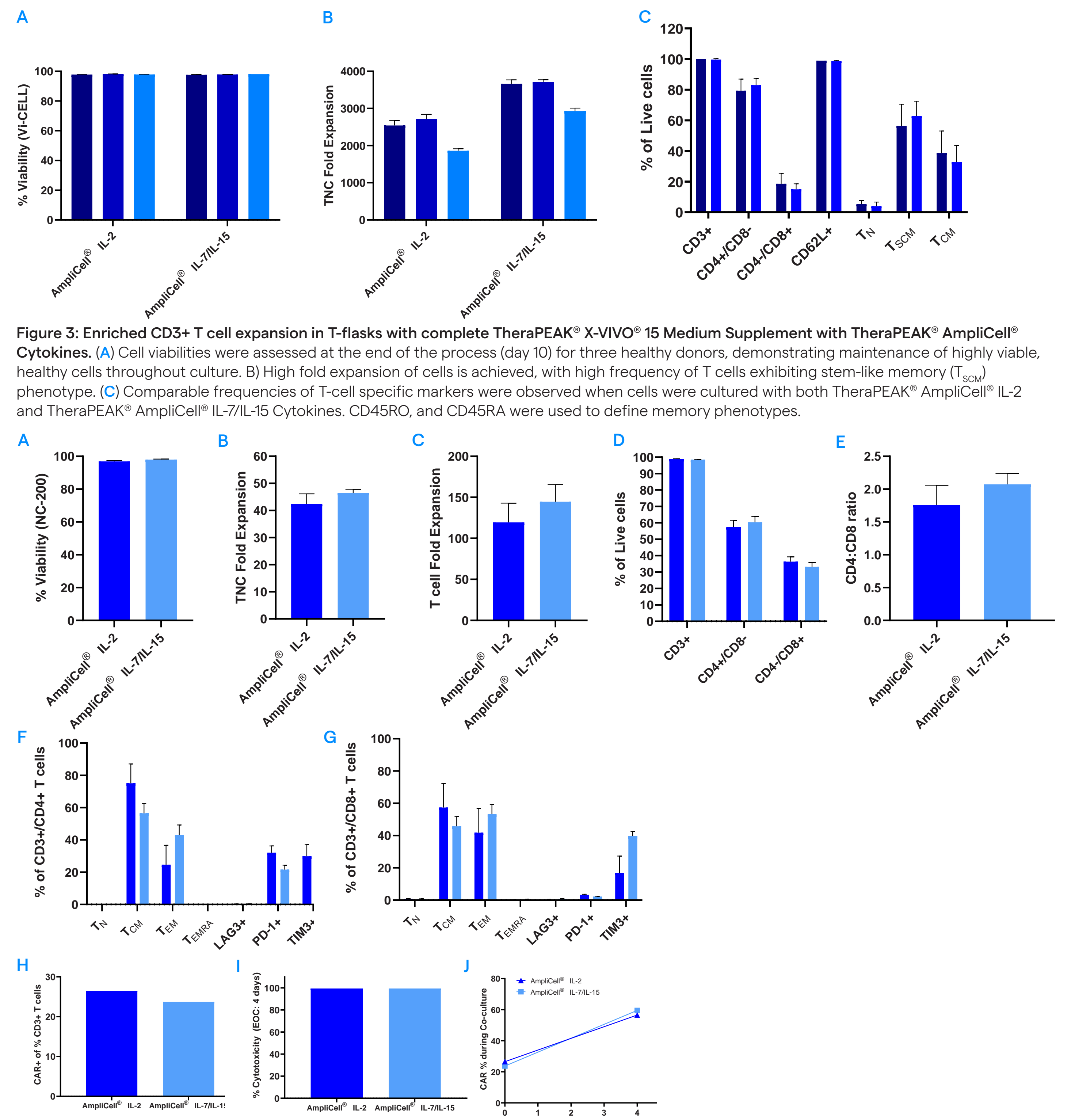


Figure 3: Enriched CD3+ T cell expansion in T-flasks with complete TheraPEAK® X-VIVO® 15 Medium Supplement with TheraPEAK® AmpliCell® Cytokines. (A) Cell viabilities were assessed at the end of the process (day 10) for three healthy donors, demonstrating maintenance of highly viable, healthy cells throughout culture. (B) High fold expansion of total cells is achieved with both AmpliCell® Cytokines, with (C) high fold expansion of T cells as indicated by a (D) >99% frequency of T cells at the end of culture. (E) Comparable frequencies of CD4:CD8 ratios were observed for TheraPEAK® AmpliCell® IL-2 and TheraPEAK® AmpliCell® IL-7/IL-15 Cytokines. (F) CD4+ T cells expanded in TheraPEAK® AmpliCell® IL-2 exhibit higher frequencies of central memory (T_{CM}) phenotype, and lower exhaustion marker expression, as compared to AmpliCell® IL-7/IL-15 expanded cells. (G) CD8+ T cells expanded in both TheraPEAK® AmpliCell® IL-2 and TheraPEAK® AmpliCell® IL-7/IL-15 exhibit comparable frequency of T_{CM} and effector (T_{EM}) phenotype, with low exhaustion marker expression. (H) CAR-T transduction efficiencies were similar for both cytokine groups. (I) Cytotoxicity of CAR-T products with NALM-6 tumor cells demonstrated potent CAR-mediated anti-tumor response, with an increase in CAR-T frequency during culture. (J) CD62L and CD45RA were used to determine memory phenotypes. EOC: End of Challenge.

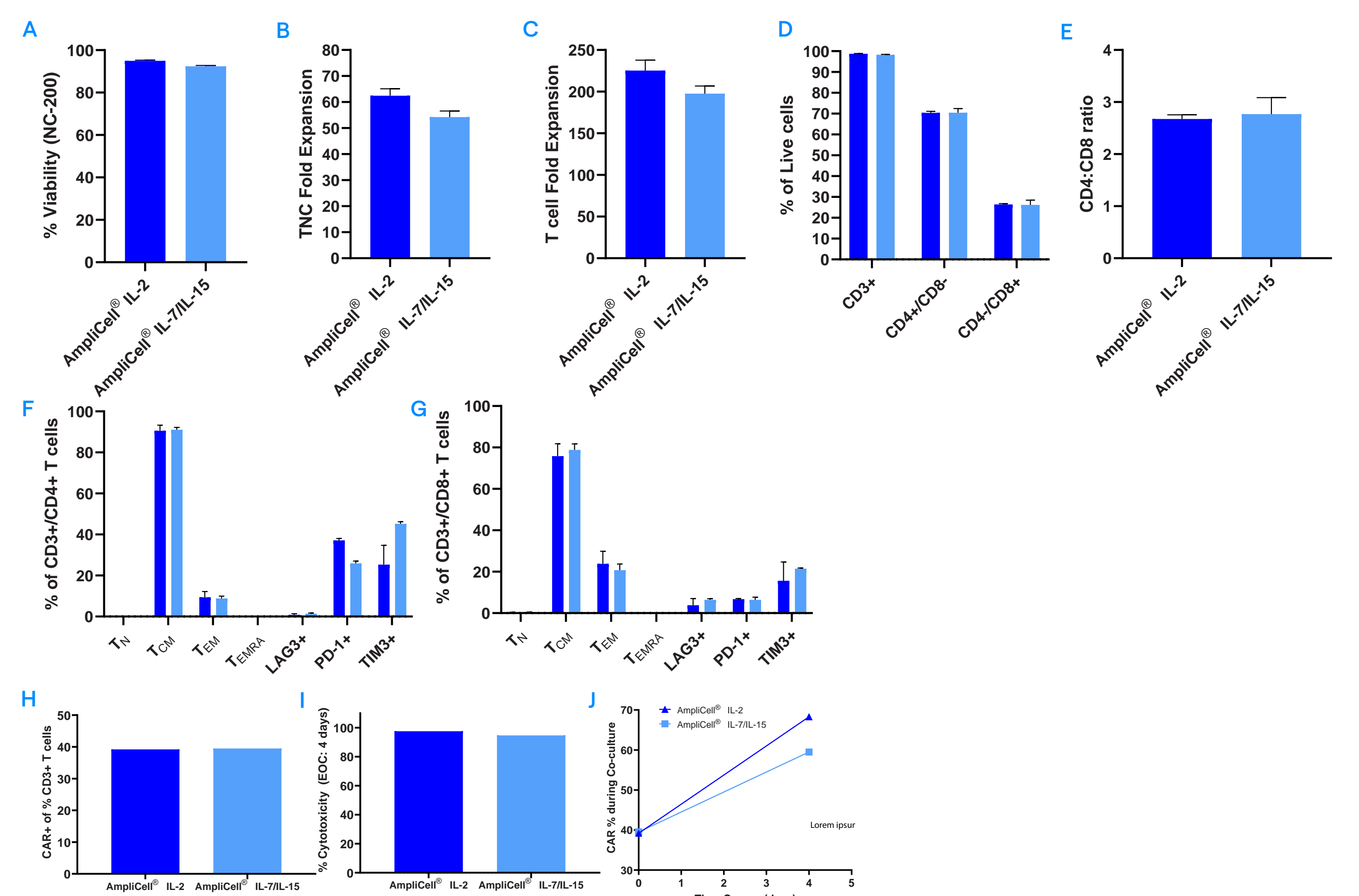


Figure 4: PBMC expansion in G-Rex® 6M plates with complete TheraPEAK® X-VIVO® 15 Medium Supplement with TheraPEAK® AmpliCell® Cytokines. (A) Cell viabilities were assessed at the end of the process (day 9) for two healthy donors, demonstrating maintenance of highly viable, healthy cells throughout culture. (B) High fold expansion of total cells is achieved with both TheraPEAK® AmpliCell® Cytokines, with (C) high fold expansion of T cells as indicated by a (D) >99% frequency of T cells at the end of culture. (E) Comparable frequencies of CD4:CD8 ratios were observed for both TheraPEAK® AmpliCell® IL-2 and TheraPEAK® AmpliCell® IL-7/IL-15 Cytokines. (F) CD4+ T cells and (G) CD8+ T cells expanded in both TheraPEAK® AmpliCell® IL-2 and TheraPEAK® AmpliCell® IL-7/IL-15 exhibit comparably higher frequency of T_{CM} than effector T_{EM} phenotype, with low expression of exhaustion markers. (H) CAR-T transduction efficiencies were similar for both cytokines. (I) Cytotoxicity of CAR-T products with NALM-6 tumor cells demonstrated potent CAR-mediated anti-tumor response, with an increase in CAR-T frequencies during culture. (J) CD45RO, and CD45RA were used to define memory phenotypes. EOC: End of Challenge.

Conclusions

Lonza's TheraPEAK® Portfolio of products comprising TheraPEAK® T-VIVO® Medium, TheraPEAK® X-VIVO® 15 Medium, and TheraPEAK® AmpliCell® Cytokines provide solutions to support cGMP-compliant manufacture of robust cell and gene products that exhibit strong quality attributes.

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