

TheraPEAK[®] AmpliCell[®] Cytokines for *ex vivo* cell culture

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TheraPEAK[®] AmpliCell[®] Cytokines are lyophilized recombinant proteins that are animal-component free, xeno-free products, 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, validated in accordance with ISO 13485 quality management system and is compliant with GMP, thereby supporting maximum safety, consistency, traceability, and reproducibility.

Overview

The TheraPEAK® AmpliCell® Cytokines reviewed herein are: recombinant human IL-2, recombinant human IL-7, and recombinant human IL-15.

- Interleukin-2 (IL-2) is a cytokine secreted by activated T cells as well as natural killer (NK) cells. It exerts its effects by binding to the IL-2 receptor (IL-2R), a complex found on the surface of immune cells. Due to its importance in T cell and NK biology, research and clinical programs have used IL-2 in the production and differentiation of chimeric-antigen receptor (CAR) expressing immune cells that target cancer cells.
- Interleukin-7 (IL-7) is a cytokine that plays a crucial role in lymphocyte development, survival, and function, by promoting T cell activation, proliferation survival, and maintenance of T cells and NK cells. Due to its importance in T cell biology, several studies are assessing the capability of IL-7 in improving CAR-T cell survival, expansion, persistence, and anti-tumor activity.
- Interleukin-15 is a cytokine that plays a critical role in development, proliferation, and survival of T cells and NK cells. Upon receptor engagement, IL-15 can drive activation, lymphocyte homeostasis, and enhances anti-tumor responses of NK and T cells. Consequently, clinical trials are exploring the use of recombinant IL-15 treating various cancers, as well as using IL-15 alone or in combination with other molecules to produce CAR-T or CAR-NK cells.

TheraPEAK® AmpliCell® Cytokines have demonstrated performance when cultured with PBMC, serum-containing TheraPEAK® X-VIVO® 15 Medium or serum-free TheraPEAK® T-VIVO® Medium, in a G-Rex® bioreactor. For comparative assessments, media were supplemented with either TheraPEAK® AmpliCell® IL-2 alone or IL-7 and IL-15 cytokines or a leading competitor (Vendor A) cytokines.

Materials and methods

Cell culture

Cryopreserved PBMC (Lonza) were seeded starting at 7.7 x 10⁶ cells input and cultured in either TheraPEAK® X-VIVO® 15 Medium (Lonza) supplemented with 5% human AB serum (Sigma) or TheraPEAK® T-VIVO® Medium (Lonza) both supplemented with either IL-2 at 100 IU/mL, or IL-7

TheraPEAK® X-VIVO® 15 + 5% human serum + TheraPEAK® AmpliCell® cytokines + soluble CD3/CD28 -targeting reagent
TheraPEAK® T-VIVO® + TheraPEAK® AmpliCell® AmpliCell® cytokines + soluble CD3/CD28 -targeting reagent



Figure 1.

Process Overview. Polystyrene vessel cell culture process (A). G-Rex® bioreactor cell culture process (B).

Features and benefits

- Produced in human cell lines thereby enabling expression of appropriate post-translational modifications characteristic of human cytokines.
- Animal-component free, xeno-free products.
- Multiple size formats, including customizable sizes for flexibility of use.
- Manufactured in a GMP-certified facility with quality management system according to USP, WHO & ISO 13485 standards.
- Stringent final product release process.
- Flexibility of use, with demonstrated performance of cytokines when cultured with different cell inputs, serum-containing and serum-free media, and various culture vessels.

at 10 ng/mL and IL-15 at 10 ng/mL. Cytokines were either sourced from TheraPEAK® AmpliCell® (Lonza) or from a leading commercial organization (Vendor A). Cells were activated with a soluble CD3/CD28-targeting reagent at day 0 and cultured in G-Rex® 6M (Wilson Wolf) 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 at MOI of 1. Following a series of media additions throughout the process, the cells were harvested on day 9 of the process. Data from both donors were combined for data analysis, for all parameters except transduction and cytotoxicity function.

Analysis

Cells count and cell viability were assessed by NucleoCounter® NC-200 (Chemometec). Phenotype, CAR-T efficiencies, and CAR-T mediated cytotoxicity were evaluated using either the BD Celesta, BD LSRFortessa of BD FACSLyric, and analyzed using FlowJo software.

Results

Comparable viability and expansion between TheraPEAK® AmpliCell® and Vendor A cytokines in both TheraPEAK® Media Systems.

When PBMC were cultured in both serum-containing TheraPEAK® X-VIVO® 15 Medium and TheraPEAK® T-VIVO Medium, we report comparable performance between the TheraPEAK® AmpliCell® and a leading competitor's (Vendor A) cytokines, as determined by high final product viability (Figure 2A), expansion of total nucleated cells (TNC, Figure 2B), and robust expansion of T cells (Figure 2C). Demonstrating that healthy viable T cells expand when cells are cultured with media supplemented with TheraPEAK® AmpliCell® Cytokines.

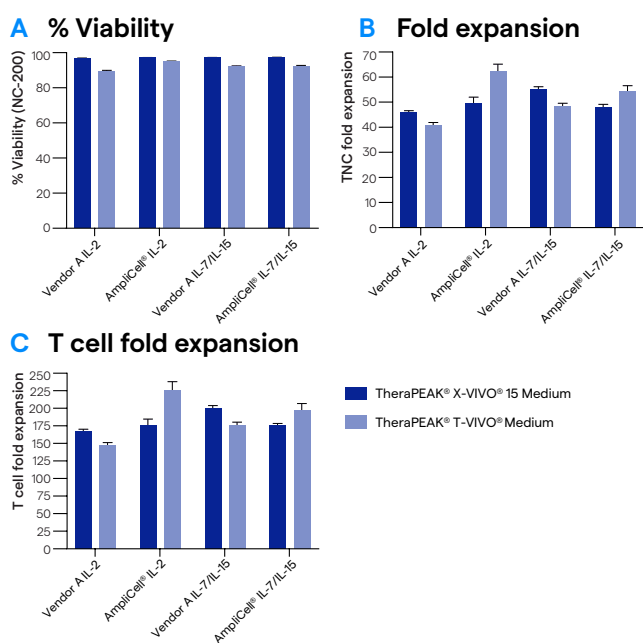


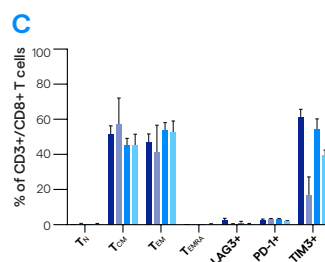
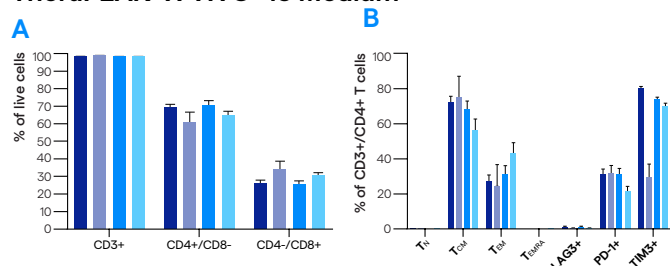
Figure 2. Comparable performance in viability (A), total nucleated cell (TNC) expansion (B), and T cell fold expansion (C) between TheraPEAK® AmpliCell® and Vendor A; average of two healthy donors.

Comparable T cell phenotypes between TheraPEAK® AmpliCell® and Vendor A cytokines in both TheraPEAK® Media Systems.

It is important to assess the T cell phenotypes following expansion, to determine whether the biological attributes were comparable when cell cultures were expanded with media supplemented with either TheraPEAK® AmpliCell® or Vendor A cytokines. We determined that irrespective of the TheraPEAK® Media System, both TheraPEAK® AmpliCell® and Vendor A final products yielded similarly high frequencies of CD3+ T cells at the end of the process, with comparable CD4:CD8 ratios (Figure 3A and 3D). The CD4+ cells (Figure 3B and 3E) and CD8+ T cells (Figure 3C and 3F) exhibited high levels of T central memory (TCM) phenotypes that were comparable between both TheraPEAK® AmpliCell® and Vendor A. Notably,

CD8+ T cells cultured in TheraPEAK® AmpliCell® Cytokines demonstrated lower levels of TIM3 exhaustion marker, when compared to Vendor A (Figure 3C and 3F). LAG3 and PD-1 expression levels were consistently low between the cytokine types. Altogether, these data demonstrate that supplementation of media with TheraPEAK® AmpliCell® Cytokines would maintain the phenotype of the T cells similar to competitor cytokines.

TheraPEAK® X-VIVO® 15 Medium



TheraPEAK® T-VIVO® Medium

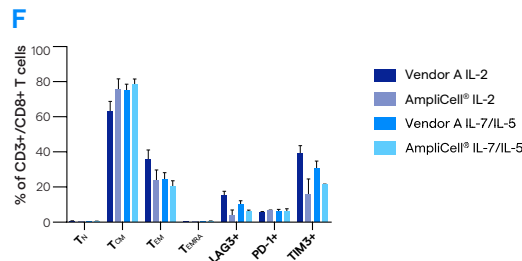
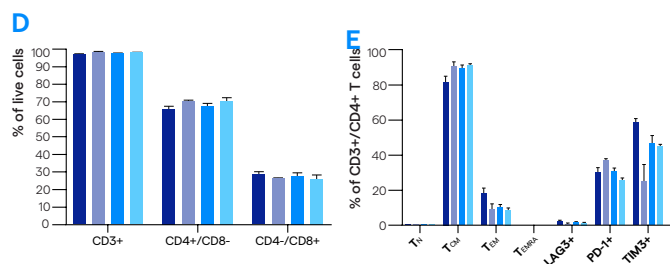


Figure 3. Comparable T cell phenotypes between TheraPEAK® AmpliCell® and Vendor A when cells are cultured in TheraPEAK® X-VIVO® 15 Medium (top panels) and TheraPEAK® T-VIVO® (bottom panels). CD3, CD4, CD8 frequencies (A and D), CD4+ T cell memory phenotype and exhaustion markers (B and E), and CD8+ T cell memory phenotype and exhaustion markers (B and E); average of two donors.

Equivalent transduction and robust cytotoxicity between TheraPEAK® AmpliCell® and Vendor A cytokines in both TheraPEAK® Media Systems.

Since CAR-T cells are a promising therapeutic for cancer treatment, we sought to assess whether there are differences in transduction efficiency and potency of the CAR+ cell products in the final products when media is supple-

mented with either TheraPEAK® AmpliCell® or Vendor A cytokines. At day 1 of the process, cells were transduced with a LVV CD19-CAR vector, and transduction efficiency evaluated at day 9. The final products were also assessed for cytotoxic function by co-culturing the T cells with CD19-expressing NALM6 tumor cells at a 1:2 CAR-T:tumor cell ratio for four days. We report that CAR-T transduction efficiencies were similar for both TheraPEAK® AmpliCell® and Vendor A cytokines, with notably enhanced transduction with serum-free TheraPEAK® T-VIVO® Medium (Figure 4A). When CAR-T products when cultured with NALM-6 tumor cells, we demonstrated that TheraPEAK® AmpliCell® and Vendor A cytokines yielded potent CAR-mediated anti-tumor response (Figure 4B). Assessment of the frequency of CAR-T+ cells at the end of the challenge, demonstrated that for TheraPEAK® X-VIVO® 15 supplemented media, the % CAR-T cells increased during culture for both TheraPEAK® AmpliCell® and Vendor A cytokines, with notably lower CAR-T cells in the Vendor A IL-2 condition (Figure 4C, left panel). For TheraPEAK® T-VIVO® supplement media, cultures with TheraPEAK® AmpliCell® Cytokines demonstrated higher frequency of CAR-T cells at the end of the co-culture period, as compared to Vendor A cytokines (Figure 4C, right panel). Altogether, these studies demonstrated that the TheraPEAK® AmpliCell® Cytokines when supplemented with serum-containing or serum-free TheraPEAK® Media can yield CAR-T cells with robust cytotoxic function, and which increase in frequency during co-culture with tumor cells, as compared to competitor media.

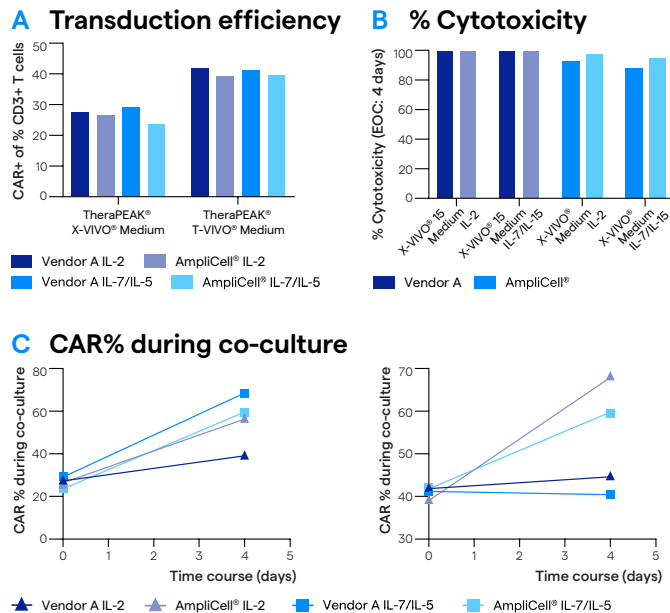


Figure 4. Equivalent transduction and cytotoxic function. CAR-T transduction efficiencies were similar for both cytokine vendors (A). Cytotoxicity of CAR-T products with NALM-6 tumor cells demonstrated potent CAR-mediated anti-tumor response (B), with an increase in CAR-T frequency during culture for AmpliCell® Cytokines (C).

Conclusion

TheraPEAK® AmpliCell® Cytokines enables the expansion of robust healthy T cells in both serum-containing and serum-free media systems. 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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