Superior Expansion of γδ T-cell Subsets for Solid Tumor T-cell Therapy Using a Chemically Defined Non-animal Origin Cell Culture Medium

Lonza
Cell & Gene

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Abstract

One limitation of current adoptive T-cell therapy is that engineered αβ T cells cannot sufficiently penetrate solid tumors to yield a significant clinical response. On the other hand, γδ T cells have extraordinary properties including the enhanced ability to infiltrate solid tumors and to directly recognize and kill transformed cells independently of HLA-antigen presentation. However, it remains a challenge to achieve sufficient expansion of γδ T cells to unleash their potential for clinical solid tumor applications.

We previously reported the development of a fully chemically defined (CD) non-animal origin (NAO) serum-free culture medium, containing only recombinant proteins, which supports superior expansion of αβ T cells through anti-CD3 and anti-CD28 co-stimulation. Additionally, this culture medium supports more than 4,000-fold expansion of γδ T cells (including both Vδ1 and Vδ2 subsets) out of peripheral blood in two weeks using an *ex vivo* γδ T cell expansion process specifically tailored to exclude the expansion of contaminating αβ T cells. Compared to other approaches, this process only requires a low amount (100 IU/mL) recombinant human IL-2 and supports more consistent and scalable expansion of engineered T-cell subsets that target solid tumors in adoptive T-cell therapy.

Methods

Medium preparation: Recombinant human IL-2 (R&D Systems) is added to the T-cell culture medium at 100IU/mL in all experiments. Human AB serum (Gemini) is used at 5% where indicated.

T-cell activation with T Cell TransAct™: Cryopreserved PBMCs or CD3+ T cells from healthy donors are thawed and seeded in 24-well plates (1.0 x 10^6 PBMC or 0.5 x 10^6 CD3+ T cells) in 1 mL medium. T Cell TransAct™ (Miltenyi Biotec) is used to activate T cells (10 µL/mL medium). On day 3, T Cell TransAct™ is removed by centrifugation where indicated.

γδ **T-cell expansion using zoledronic acid:** Cryopreserved PBMCs from healthy donors are thawed and seeded in 24-well plates (2.0 x 10^6 cells) in 1 mL medium. Zoledronic acid (McKesson) is added at 6 µM.

γδ **T-cell isolation:** Cryopreserved PBMCs from healthy donors are thawed and rested in T cell culture medium overnight (without cytokine). γδ T cells are isolated using the TCRγ/δ+ T Cell Isolation Kit (Miltenyi Biotec).

T-cell expansion in T-flask: The cells are counted every 2 – 3 days. Fresh medium (with 100 IU/mL IL-2) is added to adjust the cell density back to 0.5-1.0 x 10^6 cells/mL at the time of medium addition.

Flow cytometry: The cells are stained and analyzed on BD FACSCelesta™.

T-cell expansion in G-Rex® and Xuri™: See details in each section.

Bench-scale T-cell Expansion in T-Flask or G-Rex®

T-cell expansion in T-flask using various medium with or without human AB serum

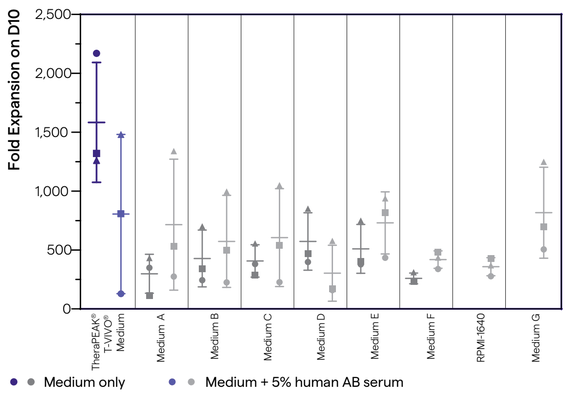
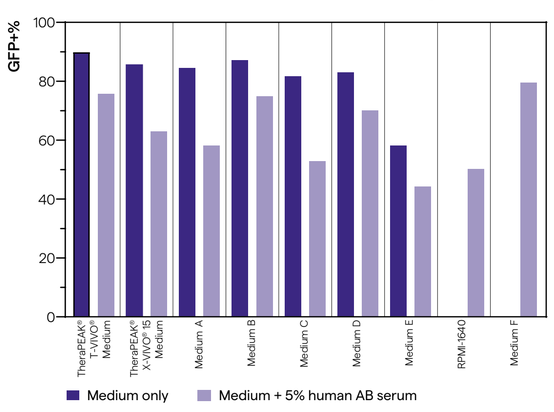


Figure 1. TheraPEAK® T-VIVO® Medium outperforms various commercially available T-cell culture media when measuring T cell fold expansion using T-flasks on day 10, after activation with anti-CD3 and anti-CD28 co-stimulation (Mean±SD; n=3 donors, each donor is represented by the ●■▲ symbols).

Lentivirus transduction efficiency



T cells composition (CD4% and CD8%)

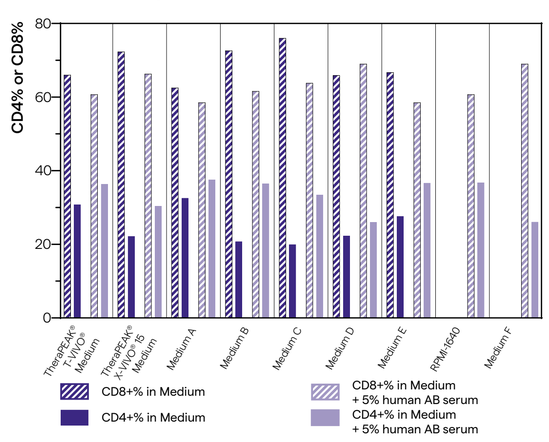


Figure 2. Lentivirus transduction efficiency and cellular phenotype is analyzed on day 9 after culturing with TheraPEAK® T-VIVO® Medium or other media in G-Rex® (Wilson Wolf, 10cm²). T cells are transduced with a lentivirus expressing GFP at MOI = 10 and analyzed via flow cytometry. Most of the cells are αβ T cells.

Expansion of Vδ2 T Cells Out of PBMCs With Zoledronic Acid

γδ T-cell expansion from PBMCs using zoledronic acid

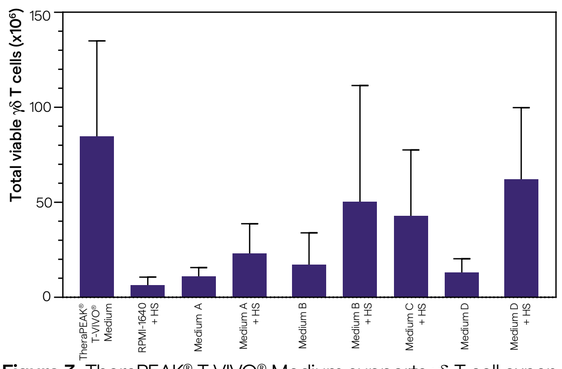


Figure 3. TheraPEAK® T-VIVO® Medium supports γδ T cell expansion out of PBMCs (2.0 x 10^6 cells) using zoledronic acid (6 µM), without the supplementation of human serum (HS). Recombinant human IL-2 (100 IU/mL) is used in all media. Graph shows the total viable γδ T cells on day 14 (Mean±SD; n=5 donors).

Cell composition by flow cytometry

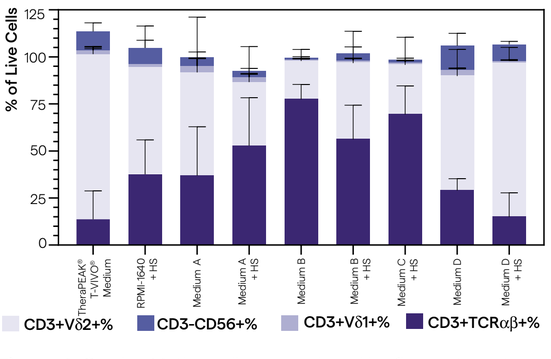
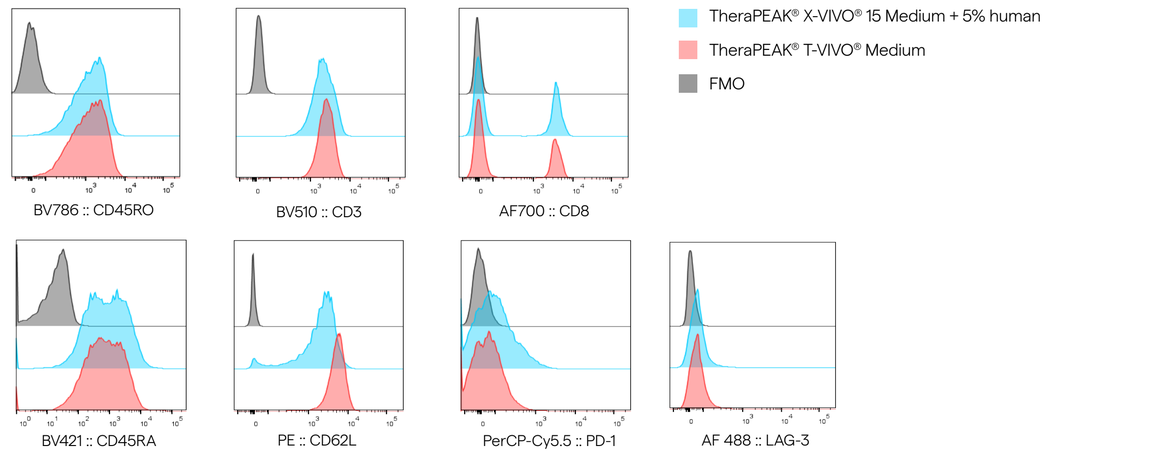


Figure 4. On day 14, the cells are analyzed by flow cytometry using various antibodies for specific surface markers. Each cell type is presented as the percentage of viable cells (Mean±SD; n=5 donors). Most viable cells in TheraPEAK® T-VIVO® Medium are Vδ2 T cells, with few Vδ1 T cells.

Large-scale T-cell Expansion in Xuri™ W25

Day	T-cell expansion in Xuri™ Cellbag™
0	Inoculate cells into Xuri™ 2 L Cellbag™ @ 0.5 x 10^6 cells/mL
1	Add fresh medium to dilute back to 0.5 x 10^6 cells/mL
2	Add fresh medium to 1 L max volume
3–7	Perfusion



T-cell expansion in Xuri™ 2L Cellbag™

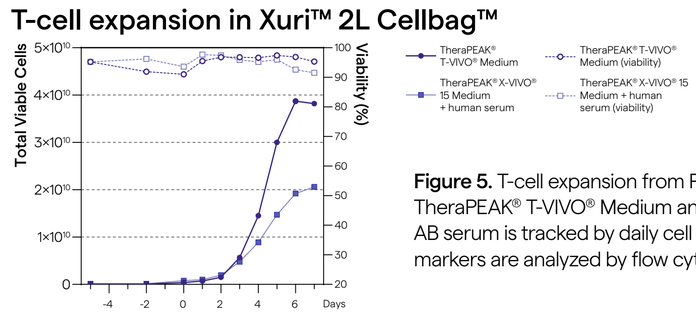


Figure 5. T-cell expansion from PBMCs (110 x 10^6 cells) by T Cell TransAct™ using TheraPEAK® T-VIVO® Medium and TheraPEAK® X-VIVO® 15 Medium plus 5% human AB serum is tracked by daily cell count. On day 10, the expression of various cellular markers are analyzed by flow cytometry. Most of the cells are αβ T cells.

Virus Transduction in PBMCs Activated With Zoledronic Acid

Donor	Lv-GFP MOI	GFP+%	CD3+%	γδT%	GFP+ γδT%
Donor A	–	0.0	86.1	96.1	0.0
	10	18.3	88.0	95.7	19.5
	20	24.7	83.5	94.8	27.8
	40	23.4	89.8	90.6	25.0
Donor B	–	0.0	80.8	94.4	0.0
	10	21.9	73.6	92.0	26.1
	20	32.4	74.2	85.7	36.9
	40	36.4	79.6	91.9	38.8

Figure 6. A GFP lentivirus is used to transduce cells at different MOIs on day 8 after expansion initiated with zoledronic acid (6µM). On day 16, the cell are analyzed by flow cytometry to assess the lentivirus transduction efficiency and cell composition. Both $CD3^+CD56^-$ and $CD3^+CD56^+$ cells are pooled for the assessment of αβTCR and γδTCR expression.

Expansion of Both Vδ1 and Vδ2 T Cells from PBMCs

Day	γδ T cell		Non- γδ T cell	
	Total viable cells	Viability %	Total viable cells	Viability %
0	0.24 x 10^6	86.4%	0.24 x 10^6	86.4%
7	8.0 x 10^6	85.9%	9.0 x 10^6	88.0%
9	28.8 x 10^6	87.9%	38.6 x 10^6	93.4%
12	207.8 x 10^6	93.1%	274.4 x 10^6	97.5%
19	6,000 x $10^{6(*)}$	93.4%	1,500 x $10^{6(*)}$	91.9%

** calculated based on fold-expansion from day 12-19*

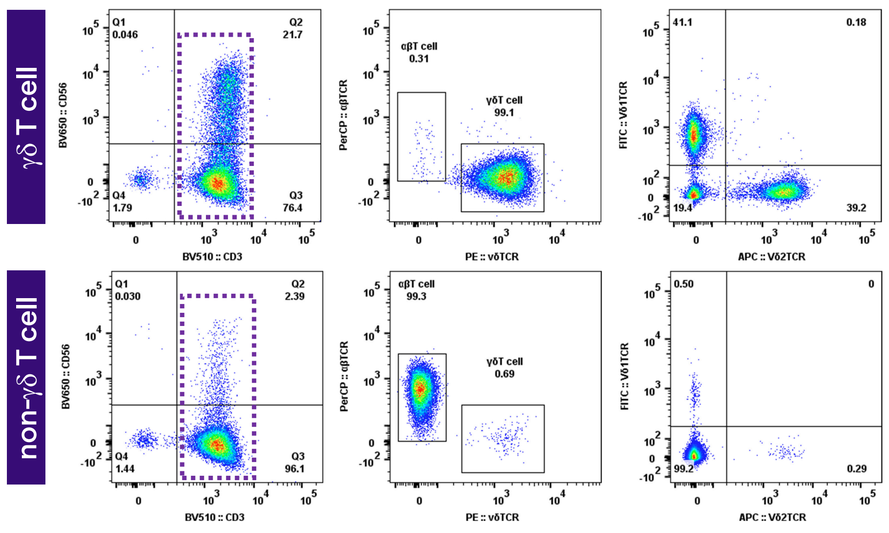


Figure 7. Expansion of both Vδ1 and Vδ2 T cells from PBMCs with TheraPEAK® T-VIVO® Medium. MACS-isolated γδ T cells and non-γδ T cells are activated with T Cell TransAct™ and expanded for 19 days in TheraPEAK® T-VIVO® Medium with recombinant human IL-2 (100 IU/mL). On day 12, cell samples are analyzed by flow cytometry. Both $CD3^+CD56^-$ and $CD3^+CD56^-$ cells are pooled for the assessment of αβTCR and γδTCR expression, or Vδ1TCR and Vδ2TCR expression.

Discussion

Summary: TheraPEAK® T-VIVO® Medium supports superior expansion of T cells through anti-CD3 and anti-CD28 co-stimulation. Moreover, this medium supports robust expansion of γδ T cells (including both Vδ1 and Vδ2 subsets) out of peripheral blood mononuclear cells using *ex vivo* expansion processes specifically tailored to exclude the expansion of αβ T cells. Compared to other approaches, this process only requires a low amount (100 IU/mL) recombinant human IL-2. TheraPEAK® T-VIVO® Medium thus enables the development of adoptive T-cell therapies that target solid tumors using γδ T cells. The chemically defined nature of this medium present lower risk to patients and remove the variability associated with human-sourced components used in the manufacturing process.

Learn more:

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