Two Distinct Activation Methods Yield Clinical-Scale Expansion of Peripheral Blood Derived γδ T Cells

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Abstract

Gamma delta (γδ) T cells have inherent ability to infiltrate solid tumors and can directly recognize and kill transformed cells independently of HLA-antigen presentation. Moreover, γδ T cells do not cause graft-versus-host disease and provide a promising platform for the development of T-cell therapies targeting solid tumors. However, due to the low prevalence of γδ T cells in peripheral blood, it remains a challenge to generate sufficient numbers of γδ T cells *ex vivo* to produce a clinical dose.

In this study, we demonstrate that billions of γδ T cells can be generated from peripheral blood mononuclear cells (PBMCs) with two distinct activation methods, with TheraPEAK® T-VIVO® Medium – a chemically defined, non-animal origin, serum-free medium, in a rocking motion platform. The first method uses zoledronic acid as the activating agent and more than 1 x 10^9 Vδ2+ T cells are produced within 14 days starting from 0.5 x 10^9 PBMCs. In the second approach either αβ T cells are specifically depleted from PBMCs or γδ T cells are isolated via negative selection prior to anti-CD3 and anti-CD28 co-stimulation, and billions of γδ T cells – including both Vδ1+ and Vδ2+ T cell subsets – are produced from PBMCs source. The expanded γδ T cells exhibit innate cytotoxicity towards the K562 cell line and produce cytokines including IFN-γ and TNF-α *in vitro*. Both activation methods and expansion protocols may be utilized to generate enough γδ T cells to support clinical applications.

Summary: In this study, we first demonstrate robust expansion of billions of γδ T cells (Vδ2+) from cryopreserved PBMCs with zoledronic acid using the TheraPEAK® T-VIVO® Medium supplemented with low amount (100 IU/mL) of IL-2, without the use of human serum. Moreover, TheraPEAK® T-VIVO® Medium supports the expansion of γδ T cells (including Vδ1+ and Vδ2+ T-cell subsets) by anti-CD3/anti-CD28 co-stimulation, following simple isolation process that depletes αβ T cells and other cells from PBMCs. Importantly, the anti-CD3/anti-CD28 approach supports high lentivirus transduction efficiency. Collectively, the data indicate that TheraPEAK® T-VIVO® Medium benefits expansion of γδ T cells for the development of T-cell therapies.

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Vδ2+ T-Cell Expansion Using Zoledronic Acid

Medium preparation:

- T-225: Add IL-2 (100 IU/mL) to TheraPEAK® T-VIVO® Medium in 1 L bottle (BP12-970Q), and store at 4°C. Use until day 7.
- Xuri™: Add IL-2 (100 IU/mL) to TheraPEAK® T-VIVO® Medium in 1 L bag (BP08-970Y) or 5 L bag (BP08-970H), store at room tempature (protect from light).
- Medium usage: 1 L bottle + 1 L bag + 5 L bag = 7L

Figure 1. Workflow of expanding Vδ2+ T cells out of PBMCs using zoledronic acid. Cryopreserved PBMCs are thawed and activated with 6 μM zoledronic acid in T-225 flasks. On day 7, 500 x 10^6 viable cells are inoculated into Xuri™ 2 L Cellbag™ (Cytiva) for further expansion until day 16.

Expand γδ T Cells Using anti-CD3/anti-CD28 Co-Stimulation

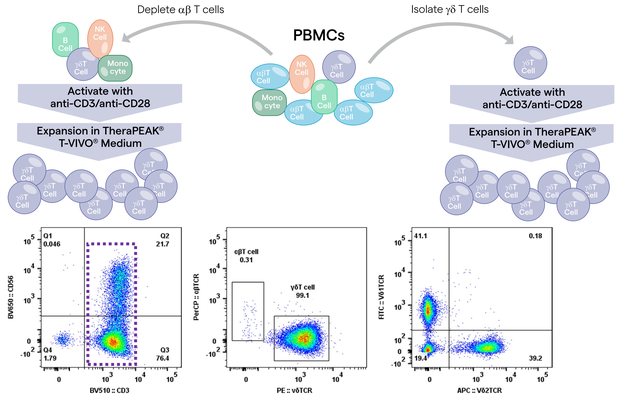

Figure 4. Workflow of expanding γδ T cells using anti-CD3/anti-CD28 co-stimulation. Depleting αβ T cells from PBMCs or isolating γδ T cells out of PBMCs permits the expansion of γδ T cells, including both Vδ1+ and Vδ2+ T cells. Both CD3+CD56- and CD3+CD56+ cells are included for the assessment of pan-αβTCR, pan-γδTCR, Vδ1TCR and Vδ2TCR expression.

Generating Billions of Vδ2+ T Cells from PBMCs Using the TheraPEAK® T-VIVO® Medium

	Day	Cell viability	Total viable cells	% of total cells				% of CD3+ T cells				Total γδ T cells
				CD3-CD56+	CD3+CD56-	CD3+CD56+	CD3-CD56-	αβT %	γδT %	Vδ2+%	Vδ1+%	
Donor #1	0	89.6%	497 x 10^6	6.1%	51.8%	0.7%	41.4%	97.6%	0.5%	0.12%	0.4%	**1.3 x 10^6**
	7	74.3%	519 x 10^6	33.2%	42.0%	11.8%	13.1%	20.6%	77.2%	69.3%	4.9%	216 x 10^6
	10	79.5%	2,840 x 10^6	43.0%	28.3%	26.2%	2.5%	7.16%	92.0%	81.6%	7.1%	1,424 x 10^6
	16	76.0%	8,530 x 10^6	39.4%	22.9%	36.8%	0.9%	11.1%	87.5%	76.0%	10.1%	**4,456 x 10^6**
Donor #2	0	94.2%	719 x 10^6	9.2%	59.3%	4.9%	26.5%	95.1%	4.7%	4.6%	0.1%	**21.2 x 10^6**
	7	75.6%	1,250 x 10^6 (*)	5.2%	29.7%	58.7%	6.4%	2.7%	95.3%	89.4%	0.7%	1,053 x 10^6
	10	82.1%	1,300 x 10^6	1.2%	26.2%	72.1%	0.5%	1.4%	98.2%	94.6%	0.4%	1,253 x 10^6
	14	94.4%	16,700 x 10^6	0.7%	36.3%	62.7%	0.3%	1.7%	98.1%	95.0%	0.3%	16,219 x 10^6
	16	91.9%	18,700 x 10^6	0.5%	35.3%	63.9%	0.3%	2.2%	97.6%	94.8%	0.2%	**18,105 x 10^6**
Donor #3	0	87.4%	535 x 10^6	14.5%	27.2%	0.8%	57.5%	87.9%	11.1%	10.8%	1.8%	**16.6 x 10^6**
	7	87.7%	1,340 x 10^6 (*)	1.9%	78.5%	15.9%	3.7%	2.0%	97.5%	95.2%	0.2%	1,233 x 10^6
	9	89.4%	2,540 x 10^6	1.3%	59.3%	39.2%	0.2%	1.4%	98.5%	95.9%	0.2%	2,464 x 10^6
	11	96.0%	11,300 x 10^6	0.7%	66.9%	32.3%	0.2%	0.9%	99.0%	97.3%	0.1%	11,098 x 10^6
	15	94.9%	31,300 x 10^6	0.9%	61.1%	37.9%	0.1%	0.7%	99.2%	97.5%	0.1%	**30,739 x 10^6**

Figure 2. Expansion of Vδ2+ T cells from PBMCs with TheraPEAK® T-VIVO® Medium using zoledronic acid. For donor #2 and #3, only 500 x 10^6 cells are inoculated into 2 L Cellbag™ on day 7 for further expansion. Cell samples are analyzed by flow cytometry (BD FACSCelesta™). Both CD3+CD56- and CD3+CD56+ cells are included for the assessment of pan-αβTCR, pan-γδTCR, Vδ1TCR and Vδ2TCR expression.

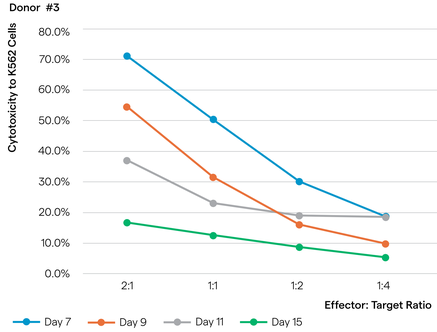

Figure 3. Cytotoxicity of the expanded T cells towards K562 cells are assessed after 24-hour co-cultivation at various Effector : Target ratios with the K562-luc2 cell line (ATCC). The killing of target K562 cells is measured by luciferase activity using the One-Glo™ Luciferase Assay System (Promega).

Expansion of Both Vδ1+ and Vδ2+ T Cells

		PBMC Donor #2			PBMC Donor #4	
Process overview		Deplete αβ T cells using anti-TCRα/β antibodies; Expand in Xuri™ for up to 16 days			Isolate γδ T cells by negative selection; Expand in T-flask for 10 days	
Days		Day 0	Day 10	Day 16	Day 0	Day 10
Cell viability		90.5%	89.5%	93.7%	92.0%	88.2%
Total viable cells		135 x 10^6	3,450 x 10^6	38,400 x 10^6	6.9 x 10^6	1,112 x 10^6
% of total cells	CD3-CD56+	10.7%	0.4%	0.4%	1.0%	0.2%
	CD3+CD56-	**4.2%**	**51.2%**	**56.6%**	**63.9%**	**63.9%**
	CD3+CD56+	**0.2%**	**47.6%**	**42.2%**	**31.4%**	**35.6%**
	CD3-CD56-	86.9%	0.8%	0.9%	3.7%	0.3%
% of CD3+ T cells	αβT %	2.5%	2.3%	3.6%	0.1%	0.1%
	γδT %	96.1%	88.3%	90.6%	100.0%	98.6%
	Vδ2+%	85.4%	57.1%	68.1%	86.5%	81.4%
	Vδ1+%	2.9%	18.6%	12.9%	9.0%	10.7%
Total γδ T cells		**5.7 x 10^6**	**3,040 x 10^6**	**33,800 x 10^6**	**6.6 x 10^6**	**1,091 x 10^6**

Figure 5. Expansion of γδ T cells using the anti-CD3/anti-CD28 co-stimulation approach. For donor #2, αβ T cells are depleted from PBMCs by anti-TCRα/β antibodies. The remaining cells are then activated with T Cell TransAct™ (Miltenyi) and expanded for up to 16 days in Xuri™ 2 L Cellbag™. For donor #4, γδ T cells are isolated by negative selection before activation with T Cell TransAct™ and expanded in T-flasks for 10 days. Cell samples are analyzed by flow cytometry (BD FACSCelesta™) at indicated days. Both CD3+CD56- and CD3+CD56+ cells are included for the assessment of pan-αβTCR, pan-γδTCR, Vδ1TCR and Vδ2TCR expression.

Lentivirus Transduction in γδ T Cells

Starting Materials		PBMCs	Isolated γδ T cells	Isolated αβ T cells
Activation Reagent		Zoledronic acid	TransAct™	TransAct™
Transduction on day		8	1	1
Day 13 Flow Cytometry	CD3+%	89.9%	99.5%	99.5%
	αβTCR+%	9.3%	0.3%	99.3%
	γδTCR+%	90.7%	99.3%	0.7%
	Vδ1 TCR+%	0.6%	55.6%	0%
	Vδ2 TCR+%	91.7%	21.6%	0.4%

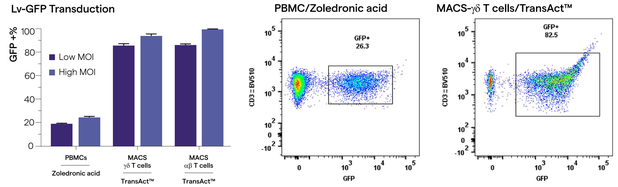

Figure 6. T cells are transduced with a GFP lentivirus under different conditions. For PBMCs activated with zoledronic acid, the cells are transduced with the GFP lentivirus on day 8 when γδ T cells become majority based on flow cytometry, at Low (10) or High (40) MOIs. For MACS-isolated γδ T cells or αβ T cells that are activated using T Cell TransAct™, the lentivirus is added on day 1 after activation at Low (10) or High (100) MOIs. On day 13, the cells are analyzed for lentivirus transduction efficiency based on GFP expression and the cell composition using antibodies targeting CD3, CD56, pan-αβTCR, pan-γδTCR, Vδ1TCR and Vδ2TCR.