Abstract #74

SERUM FREE OPTMIZER™ T CELL EXPANSION MEDIUM: A NEW cGMP MEDIUM SPECIFICALLY DESIGNED FOR EXPANSION OF T CELLS

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Abstract

We have developed OpTmizer™, a cGMP serum-free T cell culture medium, which supports the *in-vitro* expansion and culture of various T cell populations under different activation and expansion conditions, including gene modification protocols. As various T cell-based immunotherapy applications are tested in early phase clinical trials, there is a critical need to support the transition of these applications to later phase trials, and eventual commercialization by the development of reagents that meet regulatory requirements and help reduce the cost of goods to manufacture a cell product. Except for HSA content, OpTmizer™ is a protein-free, chemically defined medium, and is made under cGMP. The medium supports high density, high viability, and rapid expansion of T cells in the WAVE™ bioreactor in the absence of human serum (HS) or at low serum concentrations. Moreover, the medium supports different T cell gene-modification protocols, including moloney- and lentiviral-based transduction systems. Data comparing OpTmizer to other culture media will be presented, demonstrating a favorable profile for the culture, expansion, and function of different T cell populations, including peripheral blood lymphocytes, regulatory T cells, γδ T cells, as well as gene-modified T cells.

METHODS NOTE: Data shown in Figures 2-6 and 13-14 were generated in cultures of human T cells activated using cGMP clinical grade Dynabead® ClinExVivo™ CD3/CD28 beads.

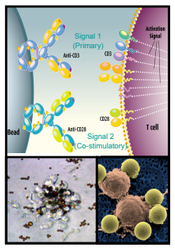
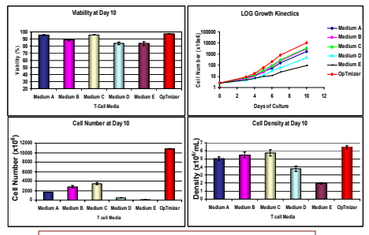


Figure 1. Dynabeads® ClinExVivo CD3/CD28 beads have antibodies directed against the T cell CD3/TCR receptor and the CD28 surface molecule covalently attached to their surface. Contacting human T cells with the bead leads to a strong activation and proliferation signal. Beads can be magnetically removed during culture or at the end of culture.

cGMP Dynabeads® *ClinExVivo™* CD3/CD28 Beads: *Activate T Cells via TCR/CD3 & CD28 Engagement*

The data shown here was generated internally at Invitrogen/Life Technologies, via contract manufacturing services at Progenitor Cell Therapies (WAVE expansions), and by several beta-testers evaluating OpTmizer in a number of T cell expansion protocols using gd T cells, TILs, moloney-based gene modified T cells, lentivirus-based gene modified CD4+ T cells from HIV+ donors, as well as CD4+CD25+FoxP3+ human regulatory T cells.

No Human Serum Added



Medium A: Kohjin 551 Medium C: Stemline Medium E: DCGM
Medium B: Kohjin 561 Medium D: X-Vivo 15 OpTmizer™

Figure 2. Viability, Cell Density, and Expansion Kinetics for Dynabead® ClinExVivo™ CD3/CD28 Activated T Cells Cultured in Various Media without Human Serum. T cells from cryopreserved PBMC were incubated with Dynabead® ClinExVivo™ CD3/CD28 beads, magnetically selected, and placed in culture for 10 days in various media containing IL-2 (200 IU/ml), but without the addition of exogenous human serum. Cells were fed fresh media when they reached a prescribed density, and split down to ~0.5x10e6 cells/ml when fed. Cells were last fed at day 7 of culture and allowed to grow to high density over the final 3 days of culture. The overall kinetics of cell expansion are displayed, as are cell viability, total cell yield, and final cell density at day 10 of culture.

Comparison +/- Serum

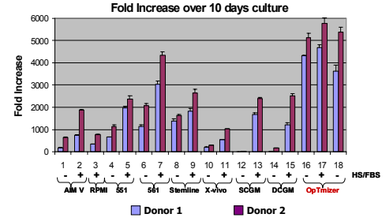
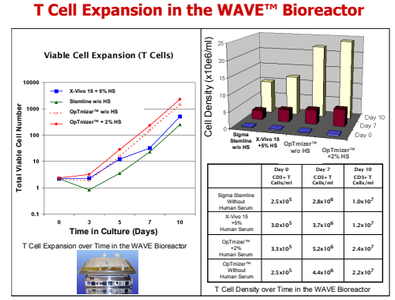


Figure 3. Comparison of total T cell expansion following activation and expansion with Dynabead® ClinExVivo™ CD3/CD28 bead in Various Media with or without the addition of human serum (HS) or FBS. Cells were activated and cultured as described in Figure 1. All media were tested either with no serum, or tested with the addition of 5% human serum, with the exception of OpTmizer, which had serum reduced to 2%, and the exception of RPMI, which was tested with 10% FBS.

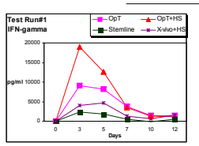
T Cell Expansion in the WAVE™ Bioreactor

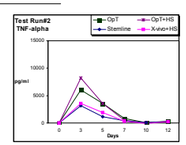


	Day 0 CD3+ T Cells/ml	Day 7 CD3+ T Cells/ml	Day 10 CD3+ T Cells/ml
Sigma Stemline Without Human Serum	2.5×10^5	2.8×10^6	1.0×10^7
X-Vivo 15 +5% Human Serum	3.0×10^5	3.7×10^6	1.2×10^7
OpTmizer™ +2% Human Serum	3.3×10^5	5.2×10^6	2.4×10^7
OpTmizer™ Without Human Serum	2.5×10^5	4.4×10^6	2.2×10^7

T Cell Density over Time in the WAVE Bioreactor

Figure 4. Comparison of total T cell expansion in the WAVE Bioreactor following activation and expansion with Dynabead® ClinExVivo™ CD3/CD28 bead in Various Media +/- Human Serum. Cells were activated and cultured as described in Figure 1 for the first 5 days in static culture bags. Cells were transferred to the WAVE on day 5 and maintained while rocking at 37ºC with feeding via perfusion. Graphs show expansion kinetics and cell density (per ml) over time. OpTmizer +/- 2% HS was compared to Sigma Sternline without serum and to X-Vivo 15 + 5% HS.





pg/ml	Days of Culture w/ WAVE					
Test Run#1	0	3	5	7	10	12
OpT	0	9143.28	8144.79	3799.74	1498.53	1481.50
OpT+HS	0	5934.8	12535.5	3533.31	1349.68	1135.44
Stemline	0	2392.89	1969.99	577.68	-189.36	657.76
X-vivo+HS	0	3883.16	4075.38	1353.72	641.58	1580.31

pg/ml	Days of Culture w/ WAVE					
Test Run#1	0	3	5	7	10	12
OpT	0	6010.25	3599.329	840.525	108.45	330
OpT+HS	0	8165.9	3558.825	536.85	86.325	200
Stemline	0	3119.95	1186.975	397.45	110	260
X-vivo+HS	0	3583.85	1941.275	316.875	-106.65	250

Figure 6. IFNγ and TNFα Secretion During T Cell Expansion in the WAVE Bioreactor in the presence or absence of human serum. T cells were cultured in complete medium containing IL-2 as described in Figures 1 and 4. Cells were cultured in either OpTmizer without serum, OpTmizer with 2% human serum, Sigma Stemline without serum, or X-Vivo 15 with 5% human serum. Supernatants were collected and evaluated by ELISA for cytokine content. Data for IFNγ and TNFα are shown.

Expansion and Function of γδ T Cells

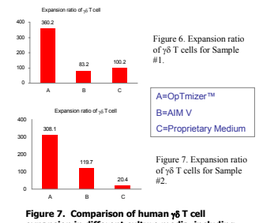


Figure 6. Expansion ratio of γδ T cells for Sample #1.

A=OpTmizer™
B=AIM V
C=Proprietary Medium

Figure 7. Expansion ratio of γδ T cells for Sample #2.

Figure 7. Comparison of human γδ T cell expansion in different culture media, including OpTmizer™. Mononuclear cells were isolated by ficoll from several healthy donors and cultured in several media containing IL-2 and 5% autologous blood plasma. Zometa "diphosphonic acid monohydrate" (NOVARTIS-Zoledronic Acid Hydrate) was added to culture (5ug/ml) to induce expansion of γδ T cells. Cells were cultured for 13 days in either OpTmizer™ medium (A), AIM-V medium (B), or a proprietary media (C). Total fold expansion of γδ T cells is shown.

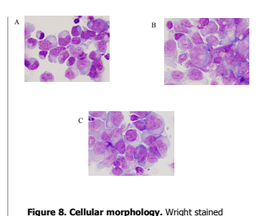


Figure 8. Cellular morphology. Wright stained preparations of cells cultured in medium (A)-OpTmizer™, (B)-AIM V, and (C)-proprietary medium. Original magnification 312 X.

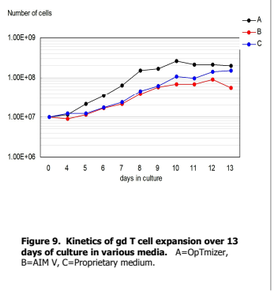


Figure 9. Kinetics of gd T cell expansion over 13 days of culture in various media. A=OpTmizer, B=AIM V, C=Proprietary medium.

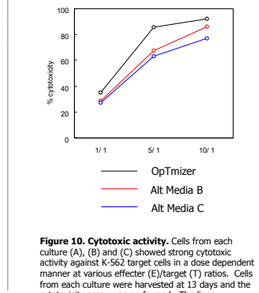


Figure 10. Cytotoxic activity. Cells from each culture (A), (B) and (C) showed strong cytotoxic activity against K-562 target cells in a dose dependent manner at various effecter (E)/target (T) ratios. Cells from each culture were harvested at 13 days and the cytotoxicity assay was performed. The lines represent the mean of triplicate analysis.

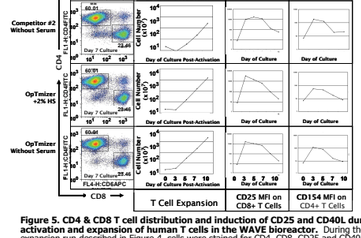
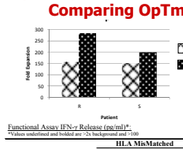


Figure 5. CD4 & CD8 T cell distribution and induction of CD25 and CD40L during activation and expansion of human T cells in the WAVE bioreactor. During the WAVE expansion run described in Figure 4, cells were stained for CD4, CD8, CD25 and CD40L at various timepoints by FACS. Similar CD4 & CD8 distribution patterns, as well as similar induction profiles for CD25 and CD40L were observed for all culture conditions.

Rapid Expansion Protocol for TIL using PBL Comparing OpTmizer vs. AIM-V

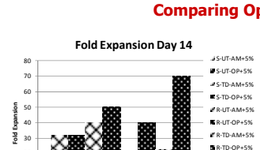


Functional Assay IFN-γ Release (pg/ml)*:
*Values underlined and bolded are >2x background and >100

	HLA MisMatched			HLA Matched		
	888	938	gp154	526	624	MART 1.0
R2-08						
S-Transduced AIM V+4.5%	72	47	839	2710	2680	69900
S-Transduced OP+2.5%	221	159	875	54800	6170	>79200
R2-08						
R-Transduced AIM V+4.5%	10	15	164	3770	6080	27100
R-Transduced OP+2.5%	8	11	168	4790	6570	31300

Figure 11. Comparing TIL expansion and function in OpTmizer + 2.5% HS to expansion in AIM-V + 4.5% HS. TIL were cultured in the presence of feeder PBL + OKT3 and IL-2 for 14 days. Fold expansion is shown in the bar graph. Expanded melanoma-specific TILs were cultured with HLA-matched or mismatched melanoma-antigen expressing cell lines and IFNγ secretion was assessed.

OKT3-Induced Expansion of Gene-Modifed T Cells: Comparing OpTmizer vs. AIM-V



Functional Assay IFN-γ Release (pg/ml)*:

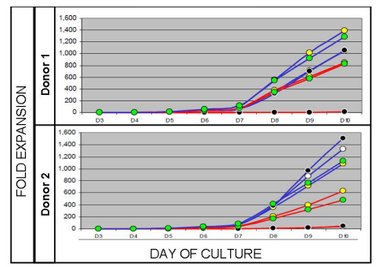
	HLA MisMatched			HLA Matched		
	888	938	gp154	526	624	MART 1.0
S1d10						
S-TD-OP+5%	81	108	364	4040	83600	9570
S-TD-AIMV+5%	49	143	147	4270	59300	3670
S-UN-OP+5%	112	115	150	81	89	147
S-UN-AIMV+5%	132	133	135	126	121	244
S1d10						
R-TD-OP+5%	37	127	134	4430	15300	3730
R-TD-AIMV+5%	75	128	156	3730	12840	5610
R-UN-OP+5%	169	123	161	89	109	257
R-UN-AIMV+5%	54	121	144	58	88	250

*Values underlined and bolded are >2x background and >100

Figure 12. Comparing expansion and functions of gene-modified (MLV) T cells expansion in OpTmizer + 2.5% HS to expansion in AIM-V + 4.5% HS. Gene-modified T cells were expanded as described in Figure 11 for 14 days. Fold expansion is shown in the bar graph. Expanded gene-modified T cells were cultured with HLA-matched or mismatched melanoma-antigen expressing cell lines and IFNγ secretion was assessed as a readout of specific reactivity.

Expansion and Lentiviral Transduction of CD4+ T Cell from HIV+ Donors

Figure 13.



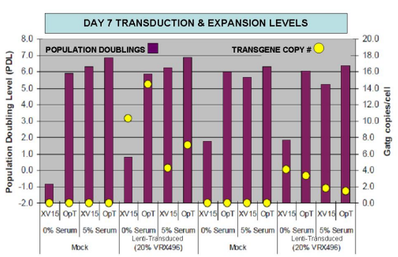


Figure 13. Expansion and Lentiviral Transduction of CD4+ T Cells from HIV+ Donors +/- Human Serum: Comparison between X-Vivo 15 and OpTmizer™. Purified CD4 T cell were obtained from two HIV+ donors. T cells were activated using Dynabead® ClinExVivo™ CD3/CD28 T cell expansion beads, transduced with a lentiviral vector, and cultured with feeding as needed over 10 days. Cells were analyzed for viability, fold expansion, and transduction levels. Test conditions are shown below:

Donor 1 (HIV-3):	
CD4 count = 477/ul	
Viral Load = 13,836	
95% CD3+CD4+	
Donor 2 (HIV-4):	
CD4 count = 288/ul	
Viral Load = 58,943	
94% CD3+CD4+	

Culture Condition								
Sample	1	2	3	4	5	6	7	8
Mock Transduced	+	+	+	+				
Lentivirus Transduced					+	+	+	+
0% Human AB Serum	+		+		+		+	
5% Human AB Serum		+		+		+		+
X-Vivo 15 + HEPES	+	+			+	+		
OpTmizer™			+	+			+	+

Summary: CD4+ T cells from HIV-infected donors were efficiently transduced using Lentiviral vectors in OpTmizer™ culture medium and exhibited optimal expansion over 10 days in media containing either no human serum or 5% human serum. Transduction levels appeared to be better when no serum was present during the transduction using either OpTmizer™ or X-Vivo 15 media.

Expansion of Regulatory T Cells

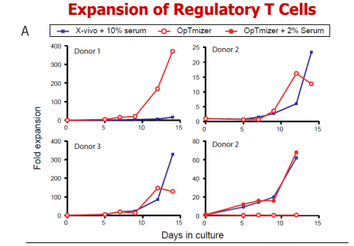


Figure 14. Comparison of X-vivo and OpTmizer™ in supporting the expansion of regulatory T cells. CD4+CD25+CD127- regulatory T cells were isolated from peripheral blood of healthy donors by FACS. The cells were then stimulated with cGMP Dynabeads® ClinExVivo™ anti-CD3/CD28 T cell expansion beads at a 1:1 ratio in medium indicated in the charts. X-Vivo 15 was supplemented with 10% human serum, while OpTmizer™ was either supplemented with 2% human serum or no serum. The cells were restimulated at day 9 by addition of ClinExVivo™ CD3/CD28 beads at 1:1 ratio. Cell expansions in various cultures are shown in A and Foxp3 and CD25 expression profiles of the cells at 14 days of culture are shown in B.

Conclusions

OpTmizer medium supports the rapid expansion of polyclonal human T cells, as well as a variety of subsets of human T cells using different activation and expansion protocols. T cell types successfully expanded in OpTmizer medium include polyclonal T cells, γδ T cells, TILs, gene-modified T cells and regulatory T cells. OpTmizer has also been demonstrated to expand antigen-specific T cells effectively (data not shown).

Polyclonal T cells derived from PBMC are rapidly expanded with high viability and to high densities using OpTmizer either in static or bioreactor cultures.

OpTmizer supports the expansion of T cells without the addition of exogenous human serum in a variety of applications, and effectively supports the expansion of T cells with the addition of as little as 2% human serum in other instances. In general, the data shows that OpTmizer without serum or with 2% serum supported equivalent or superior T cell expansion in comparison to a variety of other T cell expansion media either without serum or in the presence of 5-10% human serum.

Gene modification of T cells has been demonstrated using different vector systems during culture in OpTmizer medium.

Cells grown in OpTmizer appear to have a normal CD4 and CD8 phenotype, exhibit normal induction of key surface molecules, such as IL-2 R (CD25) and CD40L. Moreover, T cells grown in OpTmizer secrete high levels of key cytokines upon stimulation and exhibit potent response to target cells, either via the secretion of IFNγ or by direct killing of target cells.

As OpTmizer is manufactured under cGMP, supports T cell culture and expansion in the absence of serum, or in the presence of low amounts of serum, and is chemically defined/animal origin-free, with the exception of HSA, it may be an ideal medium for T cell expansion protocols moving toward clinical and eventual commercial applications.

Acknowledgements

We thank Dr. Mark Dudley of the NCI for kindly providing beta-test data on the expansion of TIL and gene modified T cells.