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Application Note

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Evaluating the Performance of CellGenix® GMP TCM and CellGenix® Cytokines in CAR-T Processes

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Abstract

Cell and gene therapies, including chimeric antigen receptor (CAR) T cell therapy, have become a promising avenue for treating various diseases. However, successfully manufacturing these therapies requires manufacturers to overcome operational challenges associated with new and emerging modalities.

This study explores the use of CellGenix® GMP T cell culture medium (TCM) and cytokines for generating functional CAR-T cells with an early differentiated memory T cell phenotype. The study examines the expansion, functionality, and phenotype of CAR-T cells cultured in CellGenix® GMP TCM and other commercially available T cell media. The findings shed light on the potential of CellGenix® GMP TCM to optimize CAR-T cell manufacturing.

Introduction

Cell and gene therapies continue to be the fastest-growing area of therapeutics, with several new therapies available to patients and hundreds more in development. One of the most prominent examples of cell therapy is the chimeric antigen receptor (CAR) T cell therapy, which relies on T cells that harbor a specific CAR to target and kill tumor cells more effectively.

Developing such innovative therapies requires biopharmaceutical companies to solve many operational challenges, particularly during manufacturing, scaling up, and scaling out. Sourcing GMP-grade raw materials that can be used in clinical manufacturing can be very challenging and should be considered early in the development of these therapies.

This study focuses on generating functional CAR-T cells with an early differentiated memory T cell phenotype using CellGenix® GMP TCM and CellGenix® cytokines. CellGenix® GMP TCM is a ready-to-use xeno-free medium that does not require the addition of glutamine or human serum. It can be used for various T cell applications when supplemented with CellGenix® GMP IL-7, CellGenix® GMP IL-15, and CellGenix® GMP IL-2, or for the generation of Tregs with CellGenix® GMP IL-2 and, CellGenix® GMP TGF-β1.

In this study, we investigated the functionality of CellGenix® GMP TCM for the generation of CD19 CAR-T cells. This application note provides an overview of a CAR production process, including CAR-T cell generation and functional characterization, such as CD19 CAR expression, killing capacity, and T cell phenotype. We used a 96-well scale-down model to compare CellGenix® GMP TCM with other commercially available T cell media.

Materials and Methods

CAR T Cell Generation and Expansion

$CD3^+$ T cells were purified from healthy donor peripheral blood mononuclear cells (PBMCs) isolated from leukocyte reduction chambers using a negative selection kit, aliquoted, and cryopreserved. After thawing, cells were activated with Dynabead Human T-Activator CD3 | CD28 (Thermo Fisher) at a bead-to-cell ratio of 1:1 in CellGenix® GMP TCM or competitor media in the presence of CellGenix® IL-7 and CellGenix® IL-15 (10 ng/mL). Control (CTL) media was prepared as published previously (50% advanced RPMI 1640 (Gibco), 45% Click's (Irvine Scientific), 2 mmol/L GlutaMAXTM (Gibco), and 5% Human AB Serum).[1]

The cells were seeded in triplicates in 100 µL medium into 96-well plates. Twenty-four hours after seeding, cells were transduced by adding 100 µl medium containing lenti-CD19 CAR (scFv-41BB-CD3 ζ) viral particles (Creative Biolabs) to achieve a final MOI of 3. Cells were passaged on days 3, 6, and 8. T cell count and viability were determined by flow cytometry using the Attune NXT (Thermo Fisher).

In Vitro Cytotoxicity Assay

NALM-6 cells were stained with CellTrace™ Violet (Invitrogen) and co-cultivated with transduced T cells at different effector-to-target (E:T) ratios for 18 hours in CellGenix® GMP TCM. Wells containing mock transfected T cells or only labeled NALM-6 cells served as controls. CAR-mediated cytotoxicity was determined by analyzing the residual alive CellTrace™ Violet target cells at each E:T ratio. Absolute cell counts were determined on an Attune NTX (Thermo Fisher).

Immunophenotyping of CAR-T Cells

Staining with CD19 CAR detection reagent (Miltenyi) and flow cytometric measurements were used to determine the percentage of CD19 CAR-expressing T cells. To assess the T cell memory phenotype, the expression of CD4, CD8, CD45RA, and CD27 were analyzed by flow cytometry. The memory T cell populations were defined as previously published.[2]

Results

High T Cell Expansion in CellGenix® GMP TCM

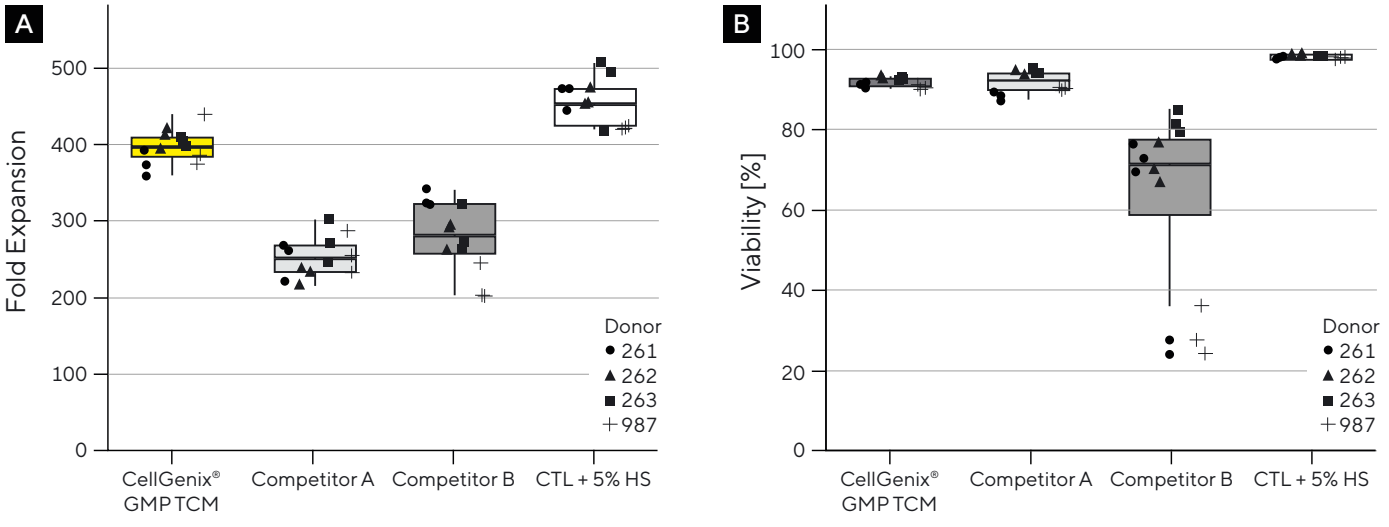
The generation of autologous CAR-T cells is a multi-step process performed *ex vivo*. In the case of autologous CAR-T cell therapies, patient cells are collected by leukapheresis, after which T cells are isolated. Those T cells are activated and then transduced predominantly with lentiviral vectors to introduce a gene for a CAR. To reach high numbers of CAR-T cells, they are further expanded before they are injected back into the patient. These cells should then be able to recognize and eliminate specific cancer cells, e.g., CD19-positive cancer cells.

In this study, we mimicked the CAR-T process to show the suitability of CellGenix® GMP TCM and the CellGenix® GMP cytokines IL-7 and IL-15 for this application. The first parameter we looked at was the expansion of CAR-T cells in the CellGenix® GMP TCM compared to other commercially available T cell media. Within 10 days of *ex vivo* cultivation, the T cells expanded 390-fold in CellGenix® GMP TCM (Figure 1A). The T cell expansion in CellGenix® GMP TCM is slightly lower compared to CTL media with 5% serum. However, it is still much higher than cultivation in competitor media A and B. The viability of all expanded CAR-T cells was above 90%, with the exception of competitor B (Figure 1B). Additionally, cells expanded in CellGenix® GMP TCM showed less donor variance than in competitor media.

Generation of High CD19 CAR-T Cell Yield in CellGenix® GMP TCM

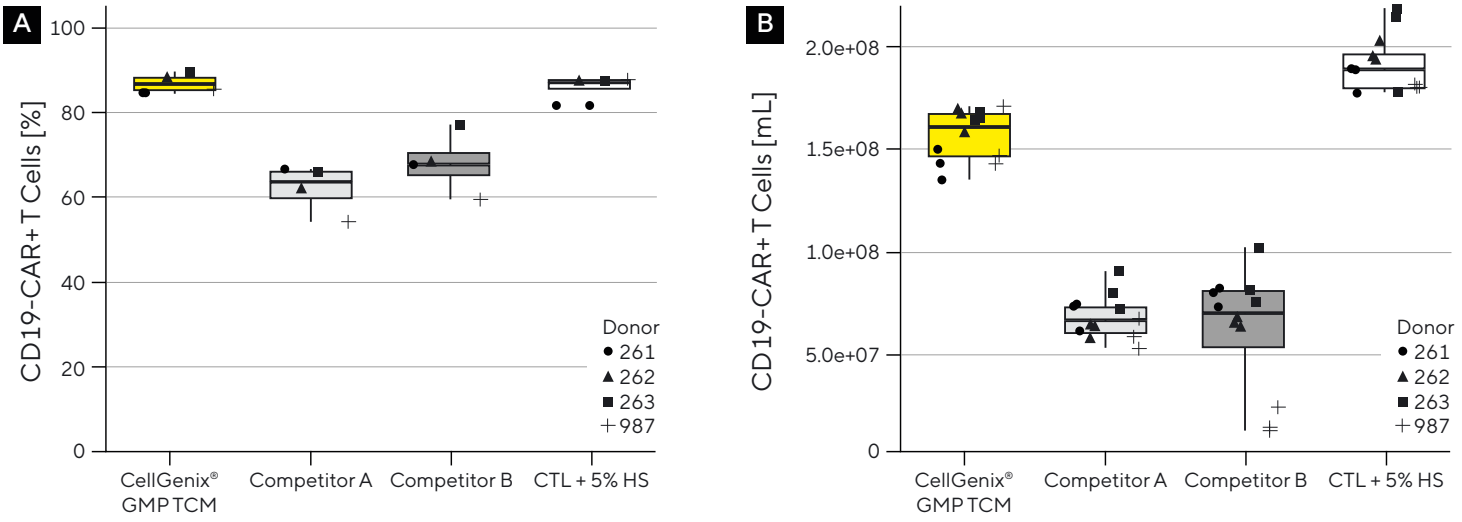
Besides the total yield of T cells at the end of cultivation, it is crucial that the majority of cells carry the CAR transgene. The expression of the inserted CD19 CAR gene was analyzed by flow cytometry. In both CellGenix® GMP TCM and CTL medium with 5% human serum, 88% of the T cells at the end of the cultivation period expressed the CD19 CAR gene on their surface. In contrast, competitor A reached only 64% CD19 CAR- T cells, and competitor B 68% (Figure 2A).
By combining the cell number, viability, and proportion of CD19 CAR-expressing cells, the final yield of CD19 CAR-T cells per mL of harvested cells can be calculated (Figure 2B). Based on this calculation, a CD19 CAR-T cell yield of 1.61×10^8 per mL could be reached through cultivation in CellGenix® GMP TCM.

Figure 1: *CellGenix® GMP TCM Supports Greater T Cell Expansion Than Other Serum-Free Competitor Media While Maintaining a High Level of Viability.*



Note. CD3⁺ T cells purified from healthy human donors (n=4) were cultured in triplicates for 10 days with CellGenix® GMP TCM or competitor media. T cell expansion (A) and viability (B) were determined by flow cytometry in technical triplicates.

Figure 2: *Ex Vivo Expansion of Lentivirus-Transduced T Cells Results in a High Proportion of CD19 CAR-T Cells in CellGenix® GMP TCM*



Note. Cultivated T cells were transduced on day 1 with lenti-CD19 CAR viral particles and cultivated for 10 days. Expression of CD19 CAR was analyzed on day 10 (A). Based on the number of viable cells and CD19 CAR expression, the yield of CD19 CAR-T cell per mL was calculated (B).

Functionality of Anti-CD19 CAR-T Cells

Following cognate antigen recognition, CAR-T cells should be able to kill the antigen-bearing tumor cell. To mimic this and to verify the functionality of the generated CD19 CAR-T cells, we used the NALM-6 cell line, which expresses the CD19 antigen.

The CellGenix® GMP TCM- expanded CD19 CAR-T cells eliminated 68% of the NALM-6 cells when co-cultivated in a 1:2 effector to-target ratio (Figure 3). Compared to CellGenix® GMP TCM, the CD19 CAR-T cells generated in competitor A medium showed a lower killing capacity at all ratios, and a higher donor-dependent variance. Thus, expansion with CellGenix® GMP TCM may contribute to the reduction of failed production runs.

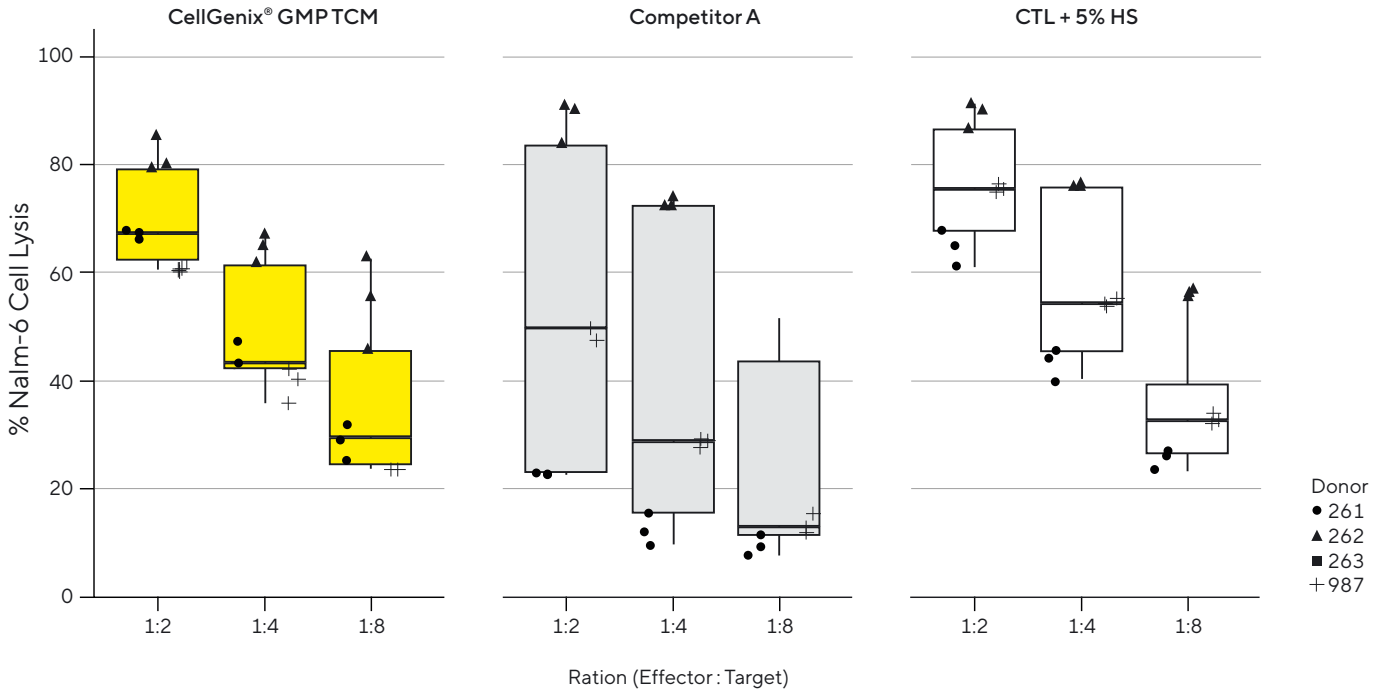
The CD19 CAR-T cells cultivated in the human serum containing CTL medium showed comparable specific killing as the CellGenix® GMP TCM CD19 CAR-T cells. Nevertheless, using human serum in clinical production carries uncontrollable risks such as potential viral contaminations, high lot-to-lot variations, and supply shortages.

High Proportion of Early Differentiated $CD8^+$ T Cells in CellGenix® GMP TCM

Another important quality attribute of the final CAR-T cell product is the phenotype of the T cells. After T cell activation, different memory T cell populations associated with different therapeutic potentials are formed. Stem cell-like memory T cells (T_{scm}, $CD45RA^+CD27^+$) and central memory T cells (T_{cm}, $CD45RA^-CD27^+$) are associated with longevity or lymphoid homing, whereas effector memory T cells (T_{em}, $CD45RA^-$ $CD27^-$) and terminal effector memory $CD45RA^+$ T cells (T_{emra}, $CD45RA^+CD27^-$) are associated with effector functions and peripheral homing.[2]

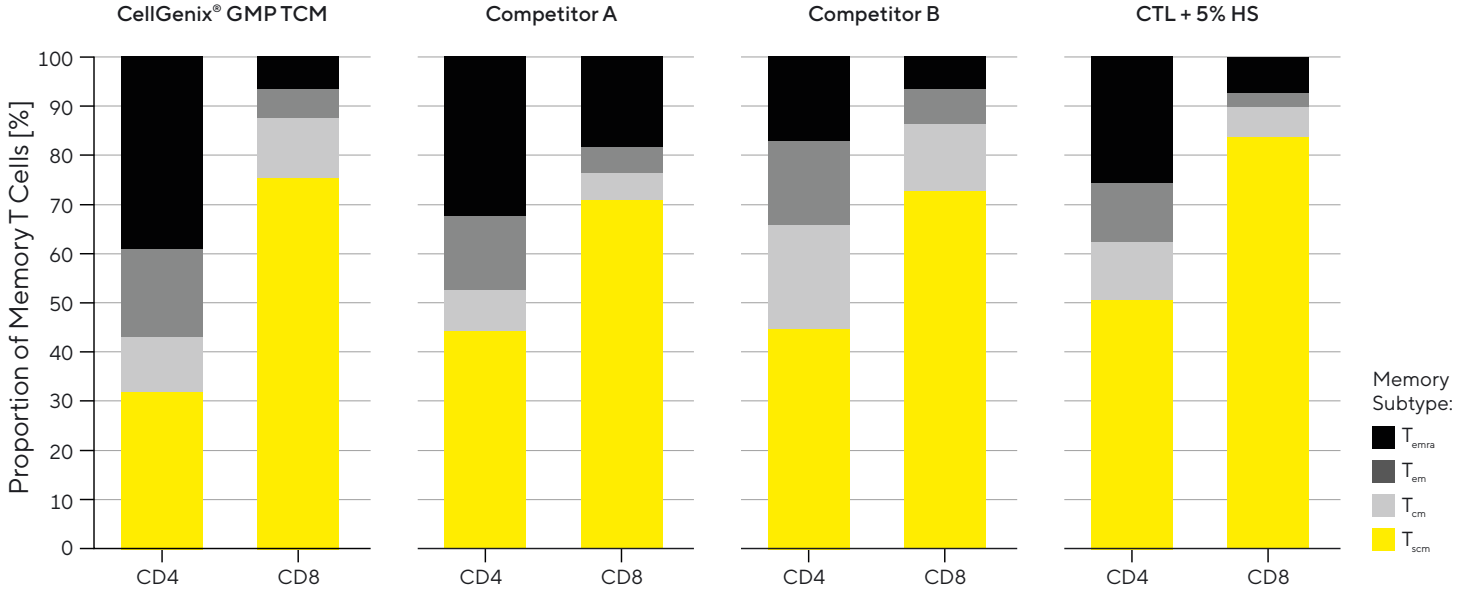
In each cultivation medium tested, the $CD8^+$ T cells had high proportions of T cells with T_{scm} and T_{cm} phenotype (Figure 4). The proportion of T_{scm} and T_{cm} in the $CD4^+$ T cell population is significantly lower. Culture conditions such as pH and dissolved oxygen are known to affect the differentiation state of T cells. These parameters cannot be controlled in a 96-well format. Culturing T cells in a bioreactor offers the possibility to optimize the culture conditions pH and dissolved oxygen which can further enhance the proportion of a less differentiated T cell phenotype.[3]

Figure 3: *Generated CAR-T Cells Specifically Target and Kill CD19⁺ NALM-6 Cells*



Note. On day 10, the CAR-T cells were co-cultivated with CTV-labeled NALM-6 cells in 1:2, 1:4, and 1:8 effector to-target ratios. After 18 hours, CTV⁺ Nalm-6 cells were counted. Specific lysis of NALM-6 cells was calculated as the percentage of NALM-6 cells remaining in the co-culture compared to the control culture.

Figure 4: *Early Differentiated T_{scm} and T_{cm} Phenotype of CAR-T Cells*



Note. On day 10, the CAR-T cells were co-cultivated with CTV-labeled NALM-6 cells in 1:2, 1:4, and 1:8 effector to-target ratios. After 18 hours, CTV⁺ Nalm-6 cells were counted. Specific lysis of NALM-6 cells was calculated as the percentage of NALM-6 cells remaining in the co-culture compared to the control culture.

Conclusion

Successful cell and gene therapy manufacturing depends on high-quality raw materials (ancillary materials, according to the United States Pharmacopeia). Results from this study indicate that CellGenix® GMP TCM supplemented with CellGenix® GMP IL-7 and CellGenix® GMP IL-15 provides a significant benefit over other commercially available serum-free T cell media, for the generation of CAR-T cells.

The CAR-T cells show robust expansion and maintain their functionality and a favorable phenotype. Previously it has been shown that CellGenix® GMP TCM, in combination with CellGenix® GMP cytokines IL-7 and IL-15, is also suitable for T cell cultivation in static bioreactor systems as well as in stirred systems. This suggests that CellGenix® GMP TCM with CellGenix® GMP IL-7 and IL-15 gives rise to a more favorable CAR T-cell therapy product and can be used in different manufacturing modalities.

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