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# Expansion of specific lymphocyte populations with 4Cell® Nutri-T xeno-free, serum-free medium

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## Abstract

The success of autologous CAR-T therapy requires robust expansion of specific subpopulations of immune cells derived from the patient. Successful expansion relies on the use of media designed for this specific purpose, preferably without serum which introduces variability which in turn leads to a lack of reproducibility and increases risk to the patient. In this application note, we describe the benefits of a xeno- and serum-free medium for selective expansion of cell subpopulations without the addition of serum. This medium enables robust manufacturing processes by facilitating process control while delivering exceptional performance in terms of fold expansion.

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## Introduction

Adoptive cell therapy based on chimeric antigen receptor (CAR) T therapy is a remarkable advancement in the fight against cancer. Landmark approvals of Kymriah and Yescarta in 2017 set the stage for rapid growth of this modality, and just a few short years later, hundreds of clinical trials are evaluating adoptive cellular therapies for cancer.<sup>1</sup>

Production of CAR-modified T-cells begins with isolation of peripheral blood mononuclear cells (PBMCs) from the patient, followed by *ex vivo* genetic modification and expansion of cells with the preferred phenotypes for infusion back into the patient. Use of media containing serum of human origin is problematic for cell expansion for several reasons. Serum introduces variability into the process and, as such, it can lead to a lack of reproducibility thus limiting the accumulation of process knowledge. As a result, development of a consistent, robust manufacturing protocol that can be applied across a large patient population becomes challenging. The use of serum also increases the risk of exposing patients to pathogens. At the same time, refrigerated storage of reserved serum lots increases facility costs, which further drives up cost of goods (COGs). Finally, the price and availability of serum can complicate operations as both can vary due to supply limitations.

In contrast, the use of chemically defined, xeno-free and serum-free medium formulations for cell expansion overcomes these challenges. Serum-free media enables robust manufacturing processes by facilitating process control while delivering exceptional performance in terms of fold expansion.

In this study, we demonstrate the robust performance of the xeno- and serum-free 4Cell® Nutri-T medium for the expansion of lymphocytes in comparison to serum-containing and other serum-free medium formulations. 4Cell® Nutri-T, which was developed using patient cells and has been optimized for the cultivation of CAR-T cells, tumor infiltrating T lymphocytes (TILs) and peripheral blood mononuclear cells (PBMCs), delivered superior results for fold expansion and cell viability compared to other medium formulations.

## Evaluation of Fold Expansion and Cell Viability

Fig. 1 shows the differences in fold expansion and cell viability of PBMCs from 3 healthy donors cultured in either 4Cell® Nutri-T, a competitor medium A with 5% human AB serum (HS) or in competitor medium B (serum-free). A total of  $2 \times 10^6$  PBMCs from healthy donors were seeded in 24-well plates (2 mL media/well). After an overnight rest, cells

were activated via CD3/CD28 using TransAct™ (Miltenyi) 1:100 and reseeded at  $1 \times 10^6$  cells/mL in the respective media supplemented with 600 IU/mL IL-2. 24 hrs after activation, cells were transduced with a CD19-CAR lentiviral vector. After transduction, cells were further expanded by splitting and reseeded cells at a density of  $0.2 \times 10^6$  cells/mL every 2-3 days; fold expansion and cell viability were measured on day 11.

This study demonstrated that 4Cell® Nutri-T medium was superior for expansion of healthy donor transduced T-cells.

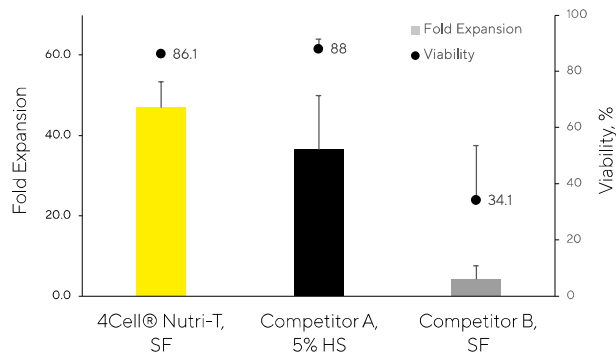


Fig. 1: Fold expansion and cell viability of healthy donor transduced CAR-T cells grown in different media formulations. SF, serum-free. HS, supplemented with human serum. SC, serum-containing.

Fold expansion and the efficiency of transduction were also measured for PBMCs isolated from the blood of a patient with lymphoma and grown in six serum-containing or serum-free media (Fig. 2). Media were supplemented with 50 ng/mL of anti-CD3 OKT3 and 300 IU/mL IL-2. At day 2 post seeding,  $2-3 \times 10^6$  cells were transduced with a CD19-CAR lentiviral vector in 6-well plates pre-coated with RetroNectin(R) (Takara Bio). Post transduction, the cells were collected and reseeded. At day 4, 4 mL of fresh medium and IL-2 were added; at day 6, 50% of the medium was replaced with fresh medium and IL-2. Transduction efficiency was evaluated at day 9 and fold expansion was measured at day 10.

The 4Cell® Nutri-T medium provided the best combination of both fold expansion and transduction efficiency.

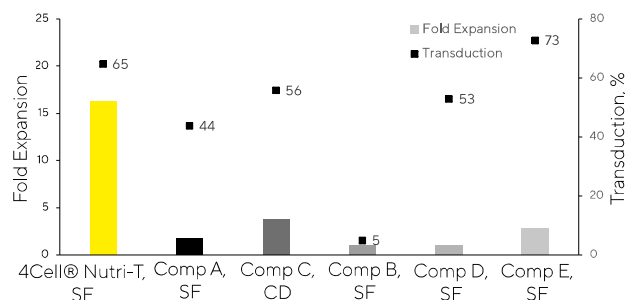


Fig. 2: Fold expansion and transduction efficiency of patient-derived T-cells transduced with CD19-CAR and grown in different medium formulations. SF, serum-free. CD, chemically defined.

# Evaluation of Cell Phenotype

The state of differentiation of anti-tumor T-cells, often referred to as their “stemness”, can impact the efficacy of immunotherapy.<sup>2</sup> The ratio of CD4+ and CD8+ cells, along with the presence of memory markers, is typically assessed for selection of optimal T-cell subpopulations for more effective cellular immunotherapy.<sup>3</sup> The medium in which T-cells are cultured and expanded can impact the ratio and phenotype of cells and as such, it is important to understand the influence of different medium formulations.<sup>4</sup>

A study conducted at the Ella Lemelbaum Institute for Immuno-Oncology at the Sheba Medical Center in Israel compared 4Cell® Nutri-T medium and a medium supplemented with 5% human serum for the culture and expansion of patient-derived CAR-T cells. Fluorescence activated cell sorting (FACS) analysis was used to assess the presence and ratios of T-cell subsets.<sup>5</sup>

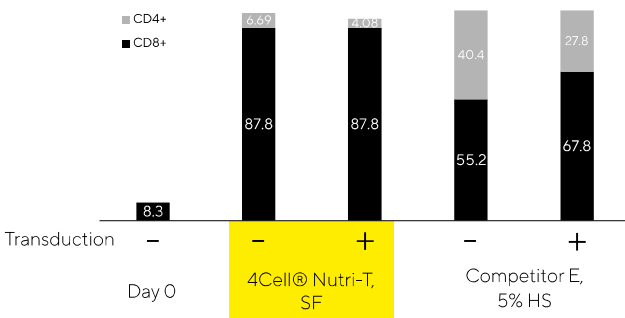


Fig. 3: Percentage of CD8+ and CD4+ cells at day 10 of culture in serum-free versus serum-containing media formulations. SF, serum-free. HS, supplemented with human serum.

As shown in Fig. 3, culture of CAR-T cells in 4Cell® Nutri-T medium led to a higher percentage of CD8+ cells as compared to the medium supplemented with human serum for both untransduced and transduced cells.

|                 | CD45RA | CCR7 |
|-----------------|--------|------|
| $T_N + T_{SCM}$ | +      | +    |
| $T_{CM}$        | -      | +    |
| $T_{EM}$        | -      | -    |
| $T_{EFF}$       | +      | -    |

Fig. 4: T-cell subpopulation markers.  $T_N$ , naïve T-cell.  $T_{SCM}$ , stem cell memory T-cell.  $T_{CM}$ , central memory T-cell.  $T_{EM}$ , effector memory T-cell.  $T_{EFF}$ , effector T-cell.

A similar study determined the percentages of four T-cell subpopulations (combined naïve and stem cell memory, central memory, effector memory, and effector (Fig. 4)) following 10 days of culture in the serum-free and serum-containing media (Fig. 5). While both media supported a high percentage of central memory cells ( $T_{CM}$ ), the percentage of naïve and stem cell memory cells ( $T_N + T_{SCM}$ ) was significantly higher in the untransduced and transduced cultures grown in 4Cell® Nutri-T medium.

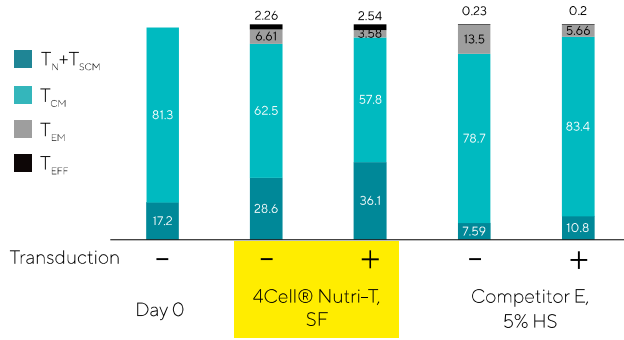


Fig. 5: Percentage of T-cell subsets cultured in 4Cell® Nutri-T serum-free medium or a serum-containing medium. SF, serum-free. HS, supplemented with human serum.

Fig. 6 shows the percentages of T-cell subpopulations achieved following a 10-day static expansion in the serum-free and serum-containing media. While both the serum-free and serum-containing media supported a robust percentage of  $T_{CM}$  in the small- and large-scale systems, the combined percentage of  $T_N$  and  $T_{SCM}$  cells was higher when the CAR-T cells were grown in the 4Cell® Nutri-T xeno- and serum-free medium.

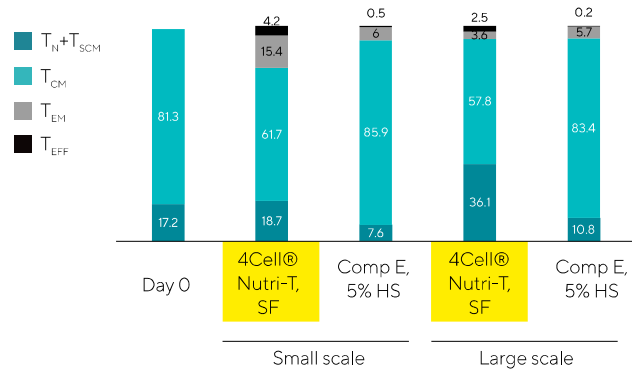


Fig. 6: Percentage of T-cell subsets cultured in 4Cell® Nutri-T xeno- and serum-free medium or a serum-containing medium in large- and small-volume static expansion. SF, serum-free. HS, supplemented with human serum.

## Discussion

Adoptive cell therapies such as CAR-T have shown remarkable results for cancer patients who have exhausted available treatment options. A growing population of patients will benefit from this novel modality as the industry further elucidates the underlying biology and optimizes manufacturing processes, including the culture and expansion of CAR-T cells and other types of genetically modified immune cells.

The efficacy of CAR-T therapy relies on a robust, consistent and preferential expansion of a subpopulation of T-cells that retain a degree of stemness. Incorporation of a serum-free medium into culture and expansion processes offers several benefits for this process:

- Delivers improved fold expansion of cells and supports robust cell viability and transduction efficiency
- Eliminates the risk of introducing pathogens present in human serum
- Improves reproducibility of protocols by minimizing variability
- Enables robust manufacturing processes and process control
- Reduces COGs through greater efficiency of cell expansion and by eliminating operational costs associated with serum
- Eliminates reliance on unpredictable serum supply chains with volatile pricing

In this application note, we highlight the performance of the 4Cell® Nutri-T xeno- and serum-free medium for the expansion of T-cells isolated from PBMCs and of CAR T-cells with desirable phenotypes. In all cases, the 4Cell® Nutri-T medium out-performed both serum-containing media and other serum-free formulations.

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## Ordering information

| Description               | Part Number   |
|---------------------------|---------------|
| 4Cell® Nutri-T Medium, 1L | 05-11F2001-1K |

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For further contacts, visit  
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