Simplifying Progress

Accelerating T Cell Manufacturing Immunotherapy With Nutri-T ACF Medium

Sharon Daniliuc[1], Mira Genser-Nir[1], Marina Teverovsky[1] , Adi Aisenshtat[1], David Fiorentini[1]

[1] [1]Research and development, Advanced Therapy Solution (ATS) Unit, Sartorius, Beit Haemek, Israel.

Introduction

The development of T cell-based immunotherapies like chimeric antigen receptor (CAR) T cells and tumor-infiltrating lymphocytes (TILs) has shown great promise in cancer treatment. However, robust manufacturing of T cell products faces challenges including variability of cellular starting material and the use of human serum-containing media. This study present 4Cell® Nutri-T Advanced Medium, a novel Animal Component-Free (ACF) medium, tailored for the expansion of T cells from healthy donors and cancer patients.

4Cell® Nutri-T Advanced Medium consists of recombinant proteins and other defined pharmaceutical-grade components optimized to promote rapid T cell proliferation. The exclusion of components originating from human and animals ensures ethical procurement and augments patient safety. The medium underwent systematic examination utilizing diverse culture systems, including conventional plastic culture ware, gas-permeable systems (G-Rex), spinners, and small-scale bioreactors. T cells from both healthy donors and cancer patients were expanded in 4Cell® Nutri-T Advanced Medium in comparison to other ACF formulations as well as conventional serum-containing media.

Results indicate the outstanding performance of 4Cell® Nutri-T Advanced Medium, demonstrating high fold expansion for both healthy and patient T cells. Importantly, it maintained a naive and cytotoxic T cell phenotype ideal for cell therapy. In terms of functionality, T cells expanded in 4Cell® Nutri-T Advanced Medium exhibited potent target cell killing and enabled efficient CAR transduction and expansion of the engineered T cells, all accomplished within a regulatory-friendly environment.

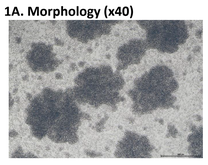
In summary, 4Cell® Nutri-T Advanced Medium exhibits optimal performance specially tailored for T cell manufacture. By enabling a reliable and scalable expansion of T cells, this advanced ACF medium holds the potential to significantly streamline the efficient production of quality-assured T cells for immunotherapy, ultimately enhancing patient outcomes.

1. Healthy donors' PBMCs expansion using 4Cell® Nutri-T Advanced Medium

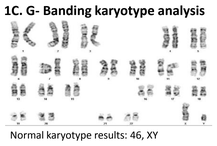
PBMCs from 11 healthy donors were seeded at 0.3x10^6 cells per well in a 24-well plate (2 ml medium per well). Cells were activated with TransAct at a 1:100 ratio and 300 IU/ml IL-2, and cultured for 7 days with a split on days 3 and 5.

The results show high cell viability and proliferation of healthy donors PBMCs using 4Cell® Nutri-T Advanced Medium in a small-scale culture. Expanded cells maintain stable karyotype.

1A. Morphology (x40)



1C. G- Banding karyotype analysis



Normal karyotype results: 46, XY

1B. Proliferation (avg. of 11 donors)

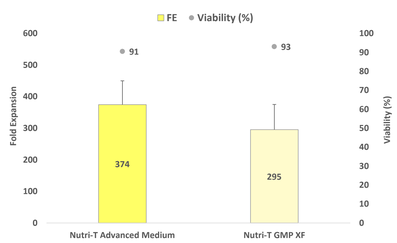


Figure 1: (A) Representative image of culture state at day 7. (B) Fold expansion (FE) and % of viable cells at day 7 of 11 healthy donors. (C) G-banding karyotype analysis of healthy donor's PBMCs after 7 days of culture in Nutri-T Advanced Medium.

2. Compatibility with different culture systems

Healthy donor PBMCs were thawed and allowed to recover for 24 hours. They were then activated with TransAct at a ratio of 1:100 and supplemented with 300 IU/ml of IL-2. Subsequently, they were seeded into 100ml of fresh ACF medium, 4Cell® Nutri-T Advanced Medium, along with fresh IL-2 in various culture systems and expanded for an additional 7 days.

In disposable spinner flasks, 60x10^6 cells were seeded per vessel and agitated at 30rpm. Cell counts were conducted on days 2, 4, and 5, and fresh medium was provided accordingly. Similarly, in the PBS-Mini Vertical Wheel® Bioreactor, 5x10^6 cells were seeded per vessel and rotated at 20rpm. Cell counts and medium replenishment occurred on days 2, 4, and 5. In G-Rex6M, 5x10^6 cells were seeded per well, and fresh IL-2 was added on day 3.

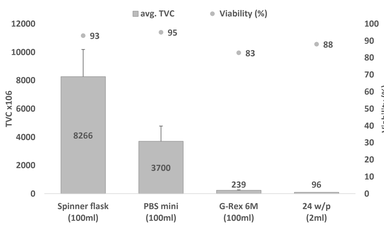
For the 24-well plate setup, 0.3x10^6 cells were seeded per well in each of the 24 wells, with 2 ml of medium per well. On days 3 and 5, cells were split.

At the end of the 7-day culture period, cells from each culture system were characterized for T cell phenotype and IFN secretion using ELISA (BioLegend).

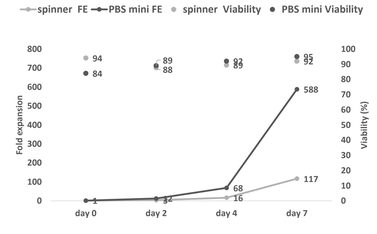
The results show that 4Cell® Nutri-T Advanced Medium efficiently promotes the expansion of healthy donor PBMCs, as well as high cytotoxic CD8+ levels and IFNγ secretion in diverse culture systems: static G-Rex, dynamic spinner flasks, and PBS Minin bioreactor.

2A. Proliferation

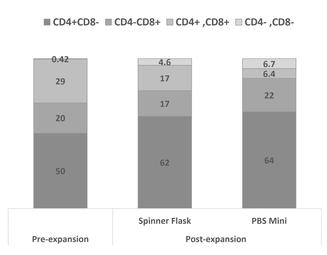
TVC and viability



Fold expansion and viability



2B. T cell phenotype (FACS)



2C. IFN secretion

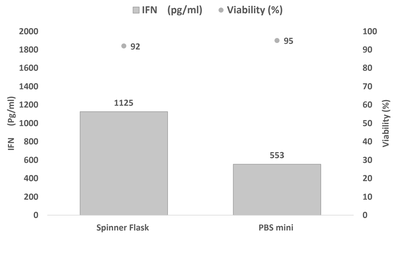


Figure 2: (A) Total Viable Cells (TVC) and % of viable cells at day 7 (left), fold expansion and % of viable cells for 7 days (right). (B) CD8+/CD4+ expression at day 7. (C) IFN secretion at day 7.

3. Patient's TILs and CAR-T cells expansion using 4Cell® Nutri-T Advanced Medium in a large-scale clinical procedure

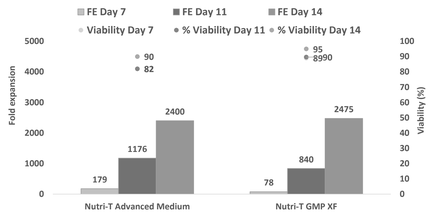
Hematological disease patients' PBMCs were cultured in either 4Cell® Nutri-T Advanced Medium or 4Cell® Nutri-T GMP XF. The culture was initiated with the addition of OKT3 (50 ng/ml) and IL-2 (300 IU/ml). Transduction of CD19-CAR was performed on day 2, followed by re-seeding of 60x10^6 cells per G-Rex100 in 450ml and cultured up to day 10.

TILs cells were isolated from surgically resected metastatic lesions using fragmentation, enzymatic digestion, and cell remnants technique. TILs cultures were established, and ex- vivo expanded. Roughly 5 × 10^6 TILs were seeded per G-Rex100 and expanded to treatment levels in a large-scale expansion procedure. The expansion process utilized 4Cell® Nutri-T Advanced Medium, and a XF commercial medium supplemented with 5% human serum (HS), anti-CD3 antibody (0.2mg/ml), rhIL-2 (3000 IU/ml), and irradiated feeder cells from healthy donors (1:100 ratio) in 450ml for 14 days.

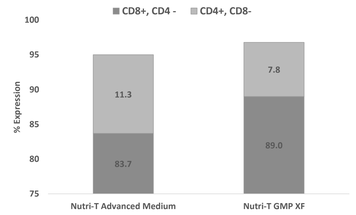
The results show that 4Cell® Nutri-T Advanced Medium efficiently promotes the expansion of both patients' TILs and CAR-T cells, with high cytotoxic CD8+ levels.

Patient's TILs cells

3A. Fold expansion and viability

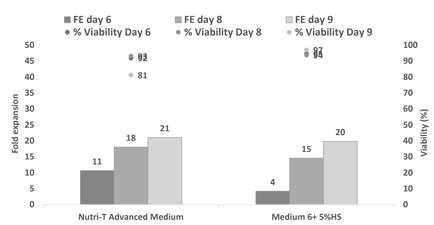


3B. T cell phenotype (FACS)



Patient's CAR-T CD19 Cells

3C. Fold expansion and viability



3D. T cell phenotype (FACS)

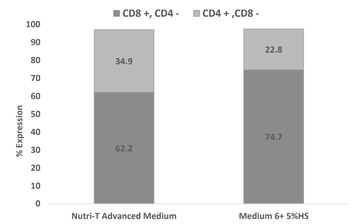


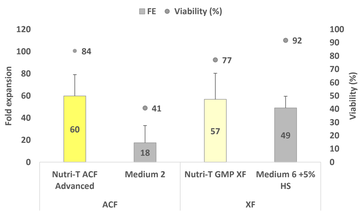
Figure 3: Patients'- derived TILs: (A) fold expansion and % of viable cells for 14 days. (B) % expression of CD8+/CD4+ at day 14.
Patients'- derived CAR-T CD19: (C) FE and % of viable cells for 9 days. (D) % expression of CD8+/CD4+ at day 8.

4. Patient's CAR-T cells expansion using 4Cell® Nutri-T Advanced Medium in small scale G-Rex

Patients'- derived PBMCs were cultured in either 4Cell® Nutri-T Advanced Medium, 4Cell® Nutri-T GMP XF, or commercial media. The culture was initiated with the addition of OKT3 (50 ng/ml) and IL-2 (300 IU/ml). Transduction of either CD19-CAR or BCMA-CAR was performed on day 2, followed by re-seeding of 5x10^6 cells per G-Rex6M in 100ml of each medium and cultured up to day 10.

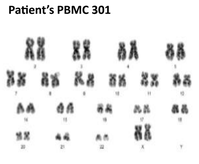
The results show that 4Cell® Nutri-T Advanced Medium efficiently promotes the expansion of patients' CAR-T cells from multiple donors with a normal karyotype, as well as high CAR-T BMCA cell expansion, high transduction efficiency, and high cytotoxic CD8+ levels.

4A. Avg. fold expansion and viability of 7 patients' cells



4B. Genomic stability

Patient's PBMC 301

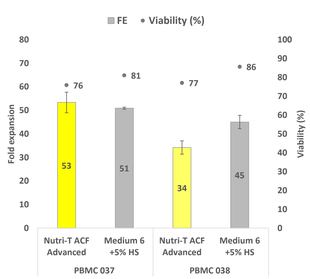


Normal karyotype, 46, XX

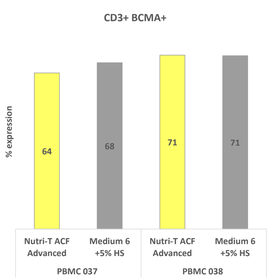
Patient's PBMC 302

Normal karyotype, 46, XY

4C. CAR-T BCMA fold expansion and viability



4D.CAR-T BCMA transduction efficacy



4E. T cell phenotype (FACS)

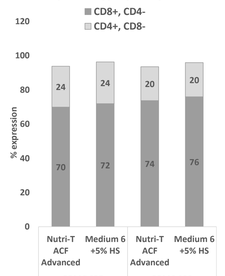


Figure 4: Patients'- derived CAR-T (A) Fold expansion (FE) and % of viable cells at day 10. Average results of 7 patients' cells (B) G-banding karyotype analysis of 2 patients' PBMCs.
(C) Patients'- derived CAR-T BCMA: FE and % of viable cells at day 10. (D) CD3+ BCMA transduction efficiency (E) % expression of CD8+/CD4+ at day 10.

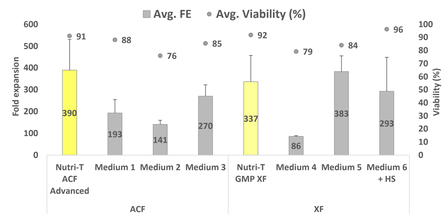
5. Comparing performance to other culture media

Patients'- derived PBMCs were cultured in either 4Cell® Nutri-T Advanced Medium, 4Cell® Nutri-T GMP XF, or commercial media, with the addition of OKT3 (50 ng/ml) and IL2 (300 IU/ml). Transduction of either CD19-CAR or BCMA-CAR was performed on day 2, followed by re-seeding of 5x10^6 cells per G-Rex6M in 100ml of each medium. A 50% medium change was conducted at day 7, and the cells were cultured up to day 10.

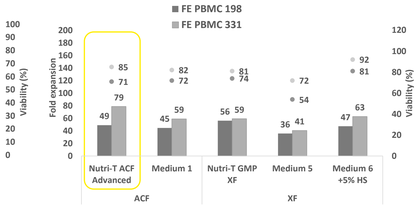
Healthy donor PBMCs from 11 donors were seeded at 0.3x10^6 cells per well in a 24-well plate (2 ml/well of each medium). Cells were activated with TransAct at a ratio of 1:100 and supplemented with 300 IU/ml of IL-2. The cells were cultured for 7 days with a split on days 3 and 5.

The results show superior performance of 4Cell® Nutri-T Advanced Medium using both healthy and patient-derived CAR-CD19 cells, exhibiting high transduction efficiency and cytotoxic CD8+ levels.

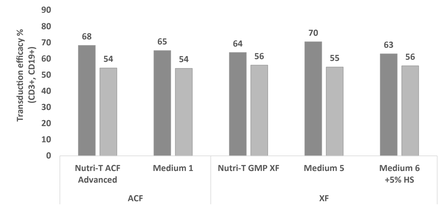
5A. Fold expansion and viability
Healthy donors PBMCs (avg. of multiple donors)



5B. Fold expansion and viability
Patients'- derived PBMCs



5C. Transduction efficiency (CD3+, CD19)



5D. T Cell phenotype (CD8+, CD4+)

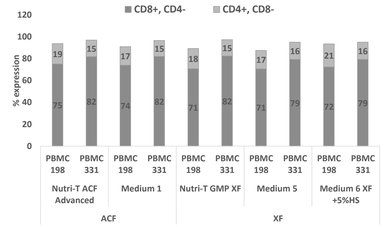


Figure 5: (A) Fold expansion (FE) and % of viable cells at day 7 of multiple healthy donors expanded in different ACF and XF media. (B). Patients'- derive CAR-T fold expansion (FE) and % of viable cells at day 10 of expansion in different ACF and XF media. (C) CD3+ CAR-CD19 transduction efficiency at day 10 (D) % expression of CD8+/CD4+ at day 10.

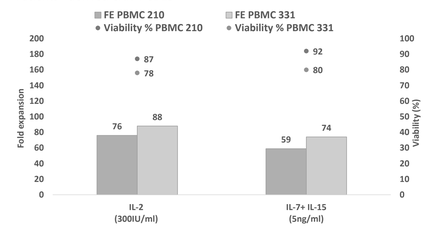
6. Compatibility of Nutri-T Advance Medium with various interleukins

Healthy donor PBMCs from 11 donors were seeded at a density of 0.3x10^6 cells per well in a 24-well plate (2ml/well). The cells were then activated using TransAct at a ratio of 1:100 and supplemented with 300 IU/ml of IL-2, or with 5 ng/ml of each IL-7 and IL-15. They were cultured for 7 days with a split on days 3 and 5.

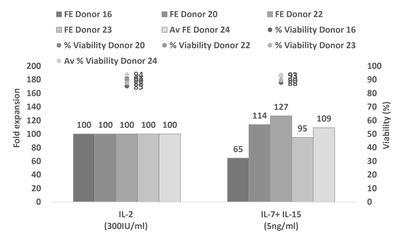
4Cell® Nutri-T Advanced Medium is compatible with different interleukins: IL-2 and IL-7+IL-15. Both IL-2 and IL-7+IL-15 support high cell expansion and viability. The main difference lies in donor-to-donor variation.

6A. Fold expansion and viability

Patients'- derived CAR-T CD19

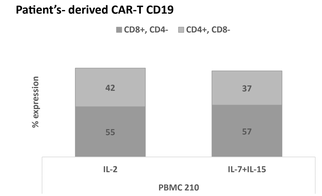


Healthy PBMC



6B. T Cell phenotype (CD8+, CD4+)

Patient's- derived CAR-T CD19



Healthy PBMC

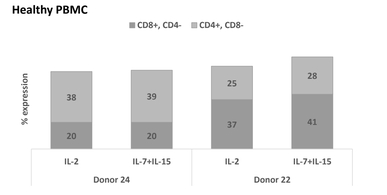


Figure 6: Expansion of patients'- derived CAR-T CD19 and healthy donors PBMCs in the presence of IL-2 or IL-7+IL-15. (A) Fold expansion (FE) and % of viable cells at day 10 or 7 respectively. (B) % expression of CD8+/CD4+ at day 10 or 7 respectively.

Summary

The data presented indicates that 4Cell® Nutri-T Advanced Medium is a versatile animal component-free medium that efficiently supports the expansion of T cell derived from healthy donors as well as patients'- derived TILs and CAR-T cells.

Features of the 4Cell® Nutri-T Advanced Medium:

- **Compatible with various static and dynamic cell culture systems; G-Rex, spinner flasks, and PBS Mini bioreactor**
- **Compatible with diverse interleukin supplements, including IL-2 or the combined cytokine pair of IL-7 and IL-15**
- **Supports high cell's viability and proliferation rates**
- **Promotes stable CAR-expression by expanded cells, high levels of CD8+ T cells and normal karyotype**