SARTORIUS

Simplifying Progress

Driving CAR-T Cell Research and Manufacturing with Next-Generation Chemically Defined T Cell Media and Single Use Bioreactors

Ursula Schultz[1], Mandy Rödiger[1], Kerstin Barth[1], Julia Hengst[2], Pedro S.Couto[3], Qasim Rafiq[3], Manuel Effenberger[1]

[1]Sartorius CellGenix GmbH, Freiburg, Germany. [2]Sartorius Stedim Biotech GmbH, Göttingen, NDS, Germany. [3]Department of Biochemical Engineering, University College London, London, United Kingdom

Introduction

Raw materials including cell culture media, cytokines, growth factors and cultivation systems can have a significant impact on the CAR T cell manufacturing process and the final cell product. Media supplements traditionally been used to culture primary cells like serum have several disadvantages, as these may contain adventitious agents and contribute to lot-to lot variability. In the recent years, CAR T cells have transitioned from academic research to industrial application, resulting in seven FDA-approved products. Consequently, there is a shift towards GMP-compliant raw materials and the adoption of closed and automated systems to ensure safety, reproducibility and availability for an increasing number of patients in need. In addition, different applications such as autologous therapies, based on patient-derived T cells and allogenic therapies, based on T cells from healthy donors, require different cell yields and therefore highly flexible manufacturing processes. The aim of this work was to test the compatibility of complete, chemically defined T cell media free of animal-derived components with static and stirred cultivation systems.

1. Experimental Approach

The impact of chemically defined cell culture media on expansion of primary T cells was investigated in different static (96 well, G-Rex 24M) and stirred (Applicon Mini Bio, PBS Mini Vertical Wheel® Bioreactor) cultivation systems. All experiments were performed with cryopreserved CD3+ T cells from healthy donors. Cells were thawed and activated with Dynabeads Human T-Activator CD3/CD28. On day 1 or 2 they were transduced with a Lentivirus and subsequently expanded for 7-10 days in the respective media supplemented with either IL-2 or IL-7 and IL-15. Upon expansion, CAR-T cells were phenotypically characterized by flow cytometry, and transduction efficiency was assessed using a CAR-specific monoclonal antibody. Co-cultures of T cells and CD19-expressing NALM6 cells were used to measure CAR-specific cytotoxicity.

2. Results

Advanced T cell medium for efficient CAR-T cell Generation

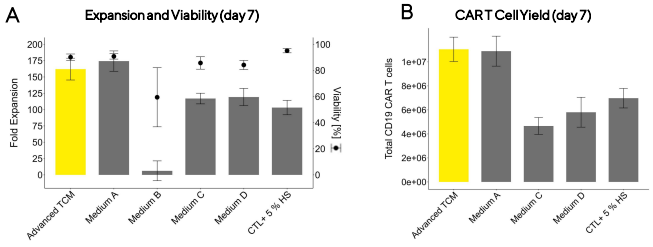

Fig. 1 Fold expansion and viability of T cells after 7 days of expansion (A) , Total CAR T cell yields achieved after lentiviral transduction of 10^5 T cells (B) Number was calculated based on the number of viable cells and the CD19 CAR expression

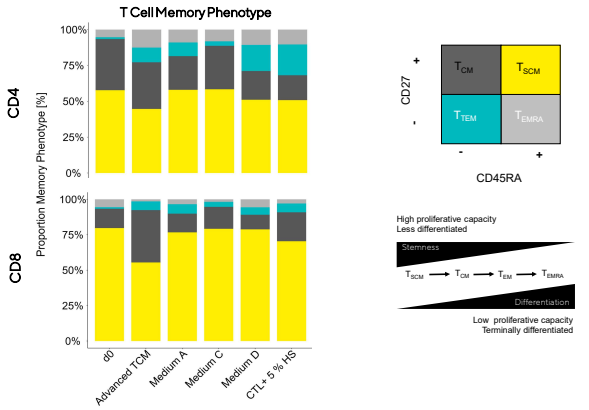

Fig.2 Representation of the different CD4 and CD8 memory subsets before and after 7 days of expansion (mean of 4 donors is shown)

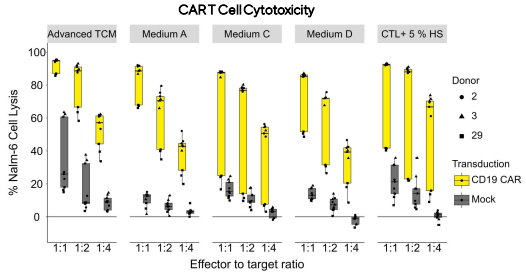

Fig.3 CD19 CAR T cell-mediated cytotoxicity on Nalm-6 cells at different effector to target ratios . (shown for 3 donors with 3 technical replicates)

These results show that CellGenix® Advanced TCM efficiently supports the generation of CAR T cells by promoting the expansion and allowing high transduction efficacy without need of any transduction enhancers. The generated CAR T cells maintain a favorable less differentiated phenotype and are highly cytotoxic. These data further indicate that the 96-well format is a suitable format for testing multiple conditions in parallel.

T cell Expansion in Static and Stirred Bioreactor Systems

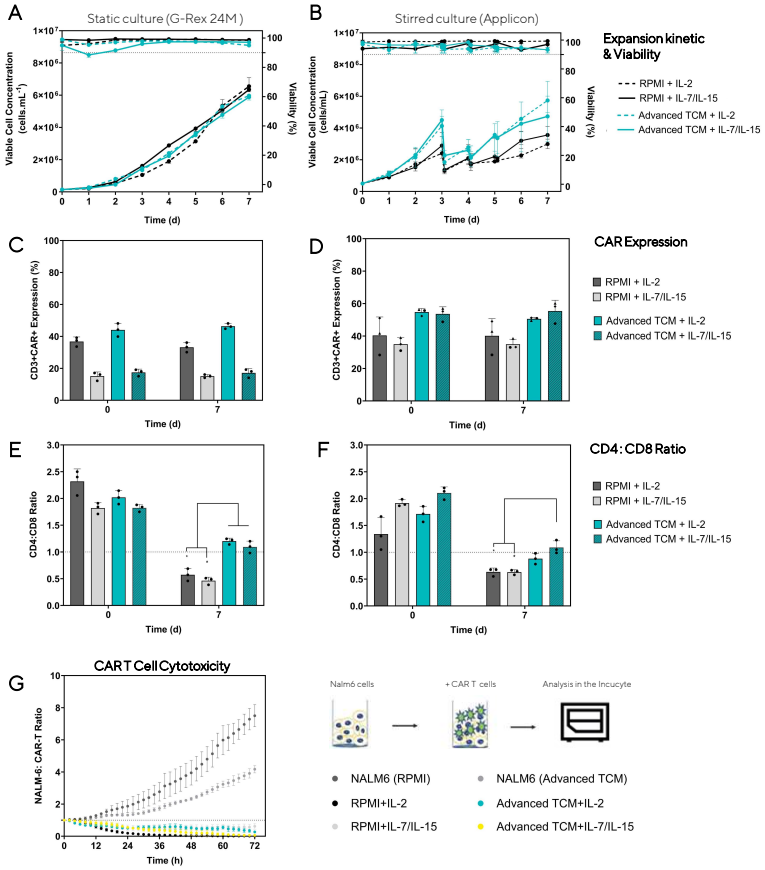

Fig. 4 Cell count and viability of T cells in a (A) static, (B) stirred bioreactor , CAR T cell expression (C) static, (D) stirred, CD4 to CD8 ratio before and after expansion (E) static), (F) stirred. CD19 CAR T cell mediated cytotoxicity on Nalm-6 cells analyzed by quantitative live-cell imaging using the Incucyte® (G). Cells were initially seeded in flasks, activated with Dynabeads Human T-Activator CD3/CD28 on day 1, transduced on day 2 and transferred to the bioreactors on day 5.

The chemically defined CellGenix® Advanced TCM shows in both formats comparable or better expansion of T cells and higher transduction efficiencies compared to a serum-supplemented medium. The expression of the CAR transgene is robust over time and CAR T cells generated under all conditions are highly cytotoxic. However, T cells expanded in CellGenix® Advanced TCM show a more balanced CD4 : CD8 ratio.

Compatibility of media with stirred culture processes

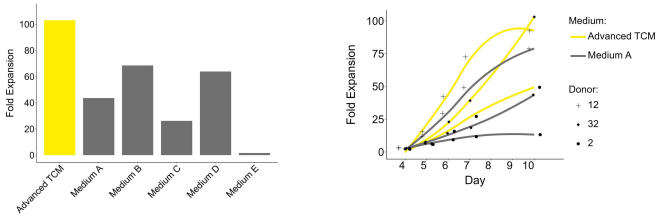

Fig. 5 Cell expansion of T cells cultivated for 7 days in different chemically defined media in the PBS Mini Vertical Wheel® Bioreactor. Cells were initially seeded in G-Rex 6 well. They were activated with Dynabeads Human T-Activator CD3/CD28 and cultured for 3 days in the presence of IL-7 and IL-15 before they were transferred the PBS Mini Vertical Wheel® Bioreactor

T cells cultivated in CellGenix® Advanced TCM show a consistently higher proliferation rate than T cells cultivated in other chemically defined media, indicating that it offers an advantage over other media in stirred cultures.

3. Conclusion

- **CellGenix® Advanced TCM is broadly applicable for CAR T cells manufacturing in both static and stirred culture systems while competitor media show strong dependence on the culture format**
- **CAR T cells generated in CellGenix® Advanced TCM exhibit a robust expression of the CAR over time**
- **The CAR T cells are functional as they kill target cells specifically whereby the majority of T cells display a less differentiated phenotype**
- **This work shows the compatibility of CellGenix® Advanced TCM with different bioreactor systems and emphasizes its benefit for stirred bioreactor systems compared to other chemically defined media**

Explore our solutions for immune cell process development and manufacturing