

Introduction

Cell-mediated Chimeric Antigen Receptor (CAR) T cell immunotherapies have revolutionized cancer research with currently seven FDA-approved CAR T therapies against blood cancers for hematological malignancies and with many ongoing clinical trials in solid cancers and autoimmune disorders. Despite this growing interest, the widespread implementation of CAR T cell therapies has been restricted mainly due to CAR T cell inflated cost and production challenges when compared with traditional methods for manufacturing and product QC. Evidence often correlates the therapeutic efficacy of CAR T cell therapies with T cell key quality attributes. Other studies have suggested that modifications in certain Critical Process Parameters (CPPs) such as cytokines or culture vessel, can impact T cell phenotype and function in *ex vivo* cultures. However, elucidating how changes in manufacturing conditions can influence the final CAR T cell product's Critical Quality Attributes (CQAs) is not fully understood. In this study, we investigate how alteration in specific CPPs affect the final CAR T cell product CQAs using multidimensional analytics including multiparametric flow cytometry, label-free Agilent Seahorse metabolic assays and xCELLigence RTCA real-time killing potency assays.

Experimental Method

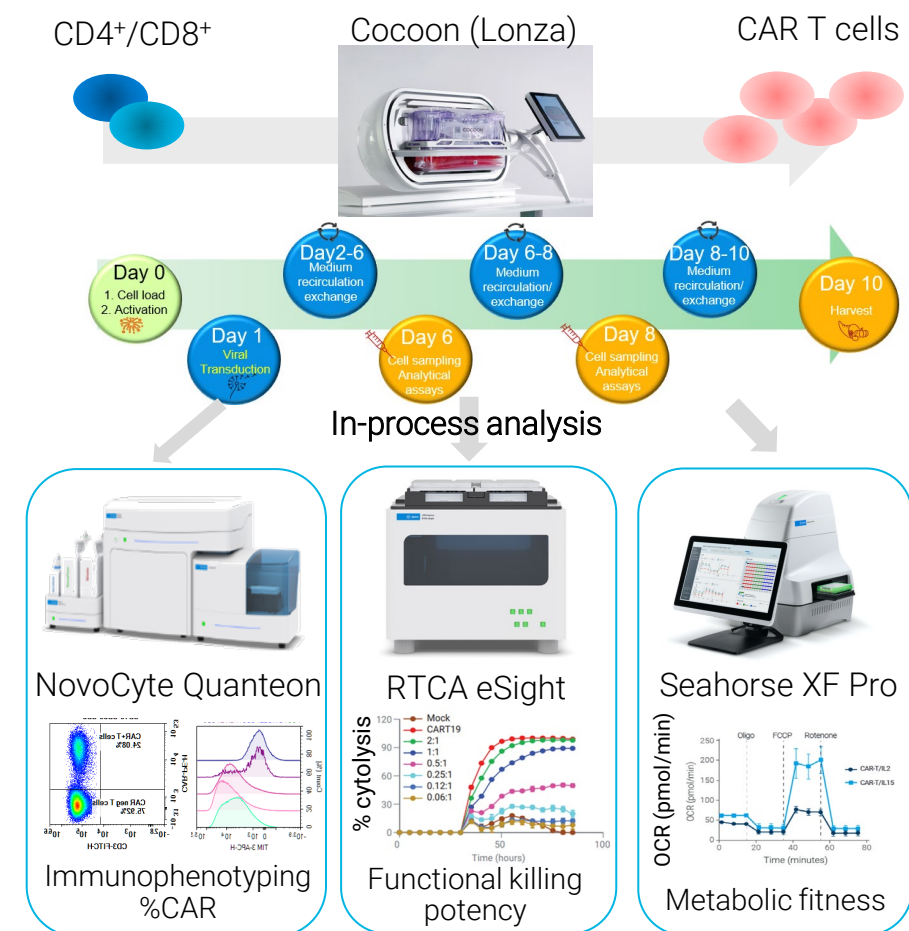
Cells:

T-47D cells: breast cancer cell line expressing EpCAM antigens on the surface (ATCC).

Raji cells: B lymphocyte cancer cell line expressing CD19 antigens on the surface (ATCC).

CAR T cells: produced from CD4⁺/CD8⁺ enriched T cells, which were activated, transduced, and expanded in the Lonza Cocoon cell therapy manufacture platform for 10 days.

Workflow:



Results and Discussion

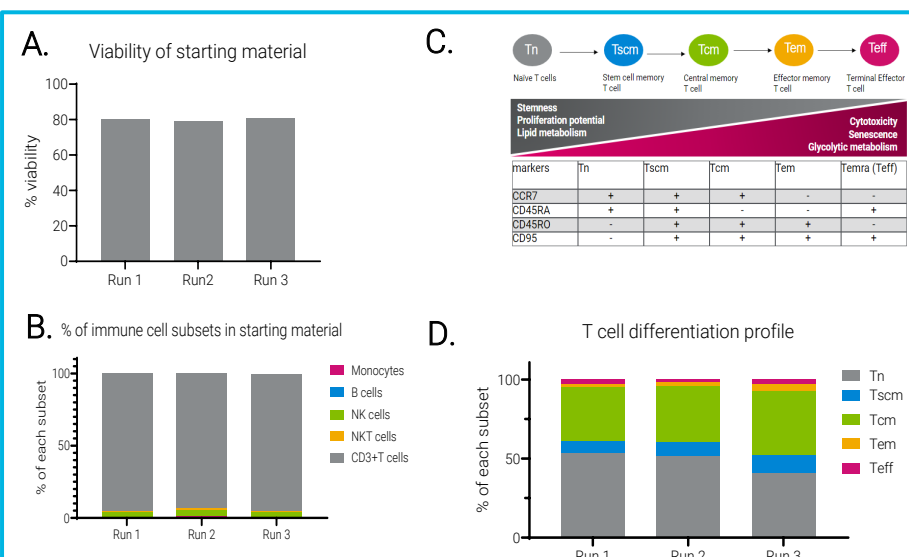


Figure 1. Characterization of the starting material for CAR T cell manufacturing across three productions using the Agilent NovoCyte Quanteon flow cytometer. A. Viability; B. Immune cell subsets; C. Key biomarkers and metabolic profile of T cells differentiation; D. Differentiation profile of starting material.

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Results and Discussion

Changes in expansion media impacts CD19 CAR T cell final products

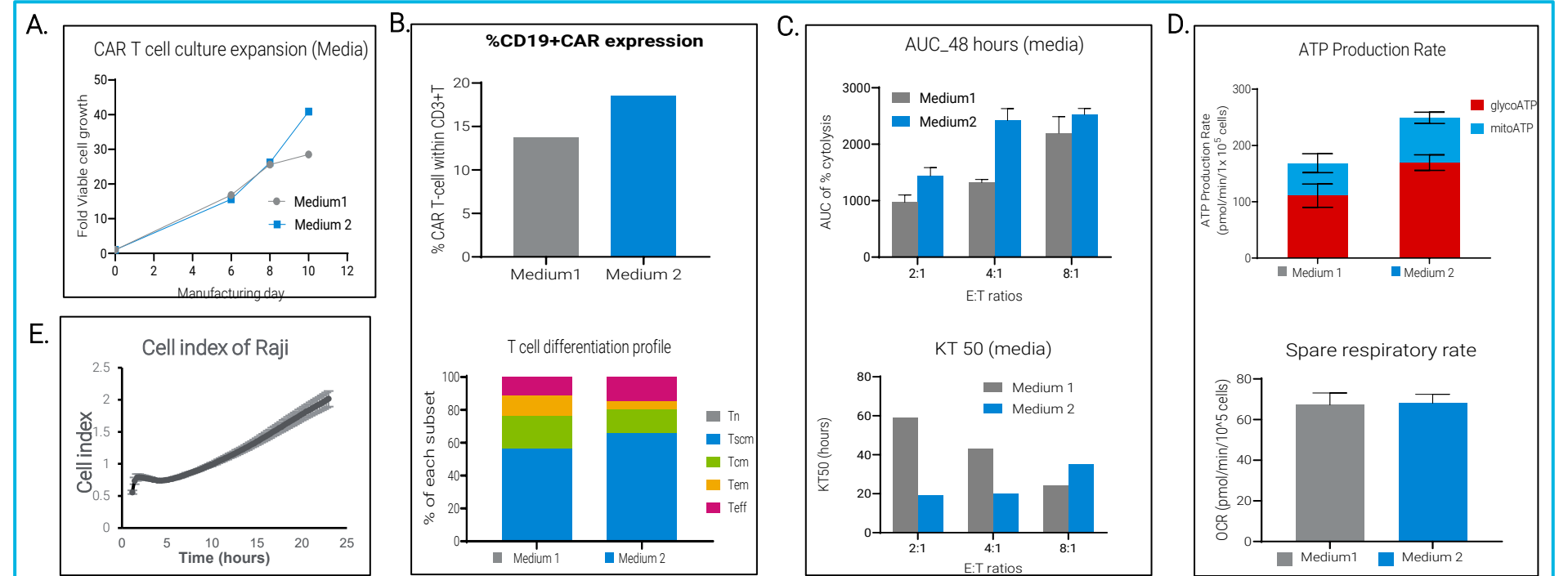


Figure 2. Characterization of CD19 CAR T cells production in Cocoon system using two commonly used T cell culture media (X-VIVO15 Serum-free Hematopoietic Cell Medium/ Medium1 and ImmunoCult XF T Cell expansion medium/ Medium 2). A. Cell proliferation; B. Percent CAR expression and differentiation profile using the Agilent NovoCyte Quanteon flow cytometer; C. Functional killing potency using the Agilent xCELLigence RTCA eSight; D. Metabolic fitness using Agilent Seahorse XF Pro system; E. Robust Cell Index of suspension target cells, Raji, after they were tethered to the E-Plate 96 using Agilent immunotherapy (IMT) kit.

Changes in cytokine growth supplement impacts EpCAM CAR T cell final products

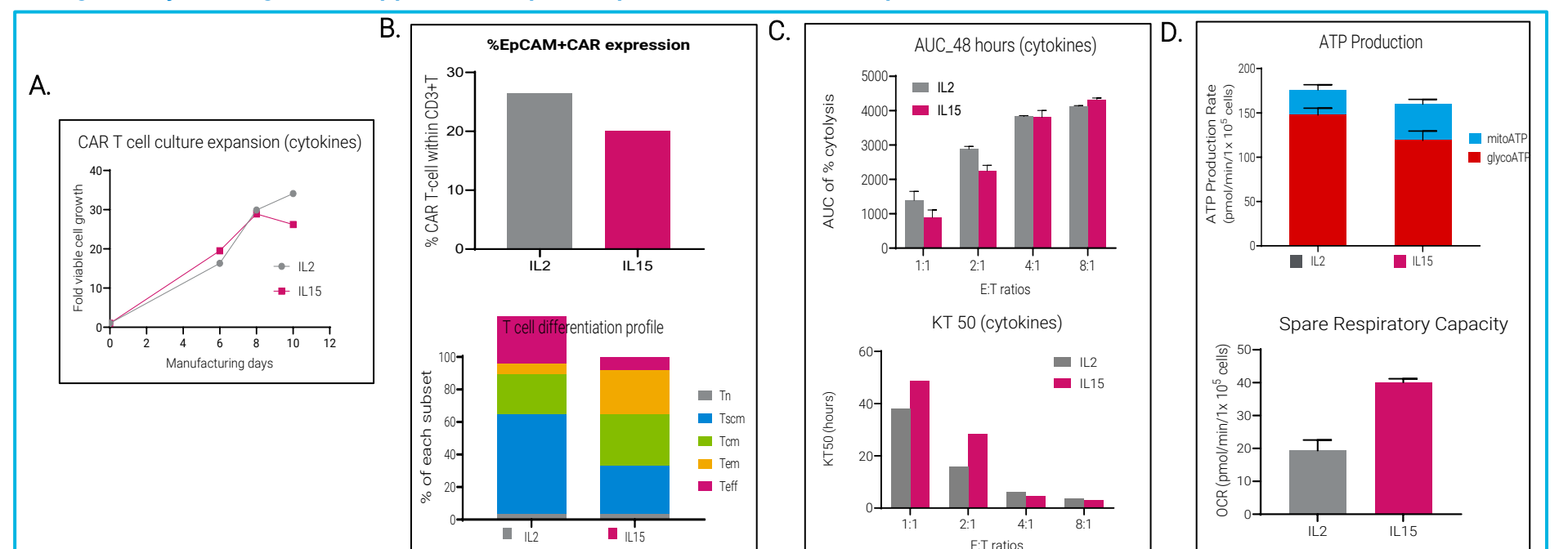


Figure 3. Characterization of EpCAM CAR T cells production in Cocoon system using two commonly used T cell growth cytokines (IL2 and IL15). A. Cell proliferation; B. Percent CAR expression and differentiation profile using NovoCyte Quanteon flow cytometer; C. Functional killing potency using xCELLigence RTCA eSight; D. Metabolic fitness using Seahorse XF Pro system.

Changes in bioreactor impacts EpCAM CAR T cell final products

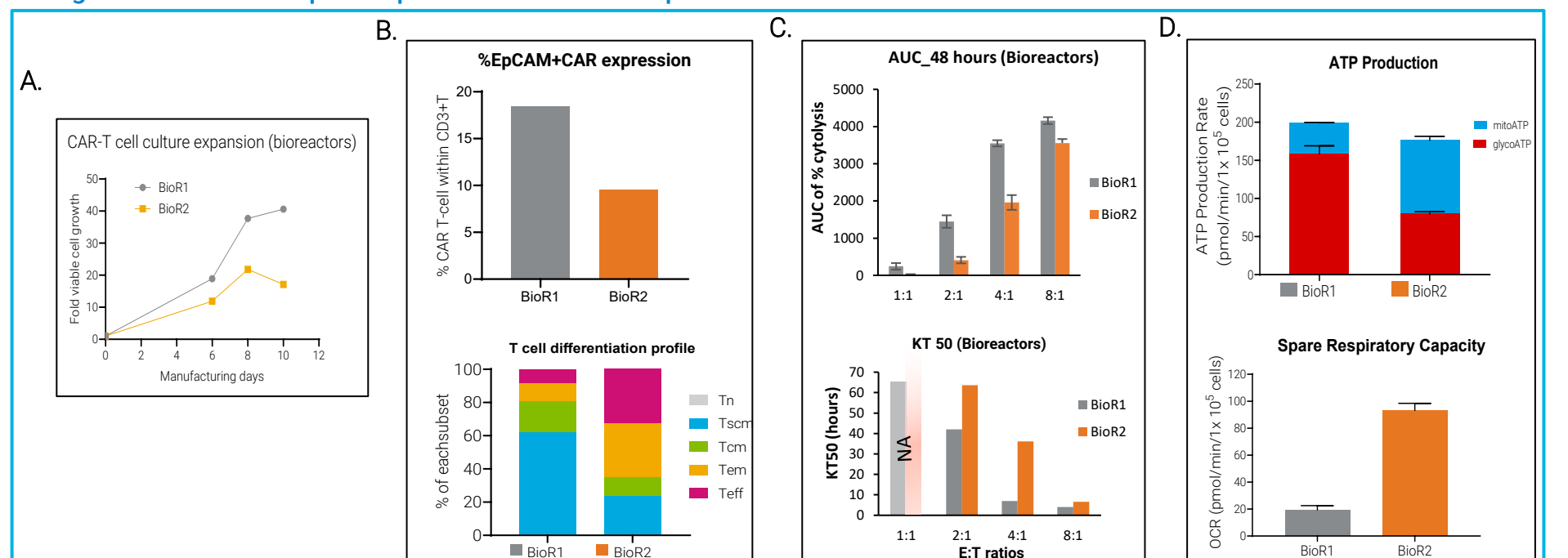


Figure 4. Characterization of EpCAM CAR T cells production using two different Bioreactors (Lonza Cocoon/ BioR1 and G-Rex platform/ BioR2). A. Cell proliferation; B. Percentage CAR expression and differentiation profile using NovoCyte Quanteon flow cytometer; C. Functional killing potency using the xCELLigence RTCA eSight; D. Metabolic fitness using Seahorse XF Pro system.

Conclusion

- The selection of adequate cell culture conditions for CAR T cell manufacture is paramount as changes in media, cytokine growth factor or cell culture bioreactor yield to distinct cell expansion, differentiation state and potency of the resulting CAR T cells products.
- Agilent NovoCyte Quanteon flow cytometer, xCELLigence RTCA eSight and Seahorse instruments provided precise, reproducible, sensitive, and reliable results, enabling the evaluation and identification of key factors that influence the quality of CAR T cell products during process development.
- We established a powerful workflow using Agilent enhanced analytics, such as Novocyt Quanteon flow cytometer, Seahorse and xCELLigence RTCA eSight. We performed in-process analysis of cells during CAR T cell manufacturing. These analytics offered valuable insights informing on the optimal harvest time of the CAR T cell products.
- Our findings highlighted the crucial role of combining multiparametric flow cytometry with other complementary cell analysis solutions which enable greater insight of CAR T cells' immunophenotype, functional potency, and metabolic profile, which can expedite the manufacture of more efficient adoptive immunotherapy products.
- The combination of complementary cell analysis solutions, including flow cytometry, during CAR T cell product life cycle emerges as decisive and can possibly shorten CAR T production timelines and reduce associated costs.

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