In Process Assessment of Critical Process Parameters Impacting CAR T Cell Potency, Metabolic Fitness and Phenotype

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CAR-TCR summit POSTER #

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Introduction

Chimeric Antigen Receptor (CAR) T cell therapy has emerged as a promising therapeutic approach for several cancers. Currently, there are six US FDA-approved therapies for hematological malignancies, with many ongoing clinical trials in solid cancers. Despite the growing interest, the widespread use of CAR T cell therapies is restricted due to their high cost and manufacturing challenges. Developing sensitive and reliable analytic tools to characterize the quality attributes (QAs) of the CAR T products and tailor the cell expansion process to achieve these QAs can lead to a more consistent manufacturing process.

Here, we demonstrated consistent production of CAR T cells using the functionally closed and automated cell manufacturing Lonza Cocoon platform, using a novel combination of cell analysis parameters during process development and for in-process analysis. Cell products were sampled during expansion or at the end of production and analyzed using the combination of immunophenotypic characterization by flow cytometry, real-time live-cell metabolic assay using an Agilent Seahorse XF analyzer, and a real-time immune cell killing potency assay using the impedance-based Agilent xCELLigence RTCA system.

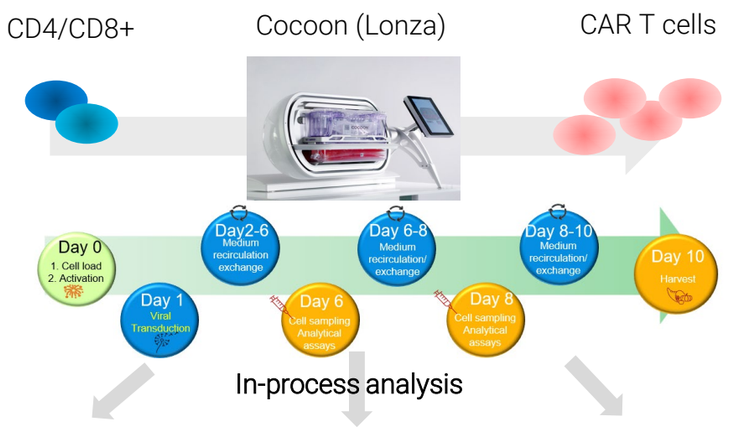
Experimental

Cells:

T-47D cells: breast cancer cell line expressing EpCAM antigens on the surface. They were purchased from ATCC.

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

Workflow:



Results and Discussion

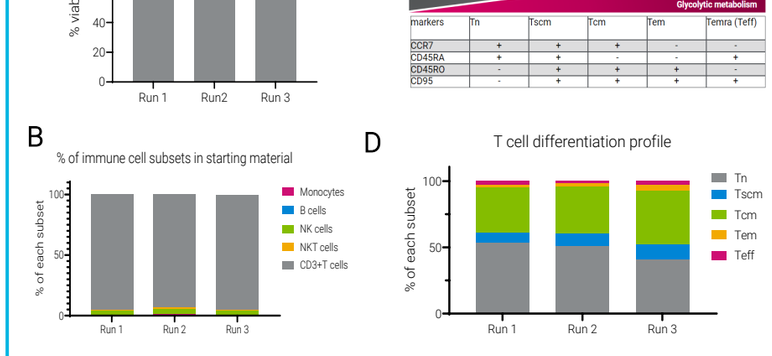


Figure 1. Characterization of the starting material for CAR T-cell manufacturing across 3 productions using Agilent NovoCyte Quanteon flow cytometer. A. Viability B. Immune cell subsets C. Key biomarkers for each subpopulation of T cells. D. Differentiation profile

Results and Discussion

Impacts of expansion media on CD19 CAR T-cell products

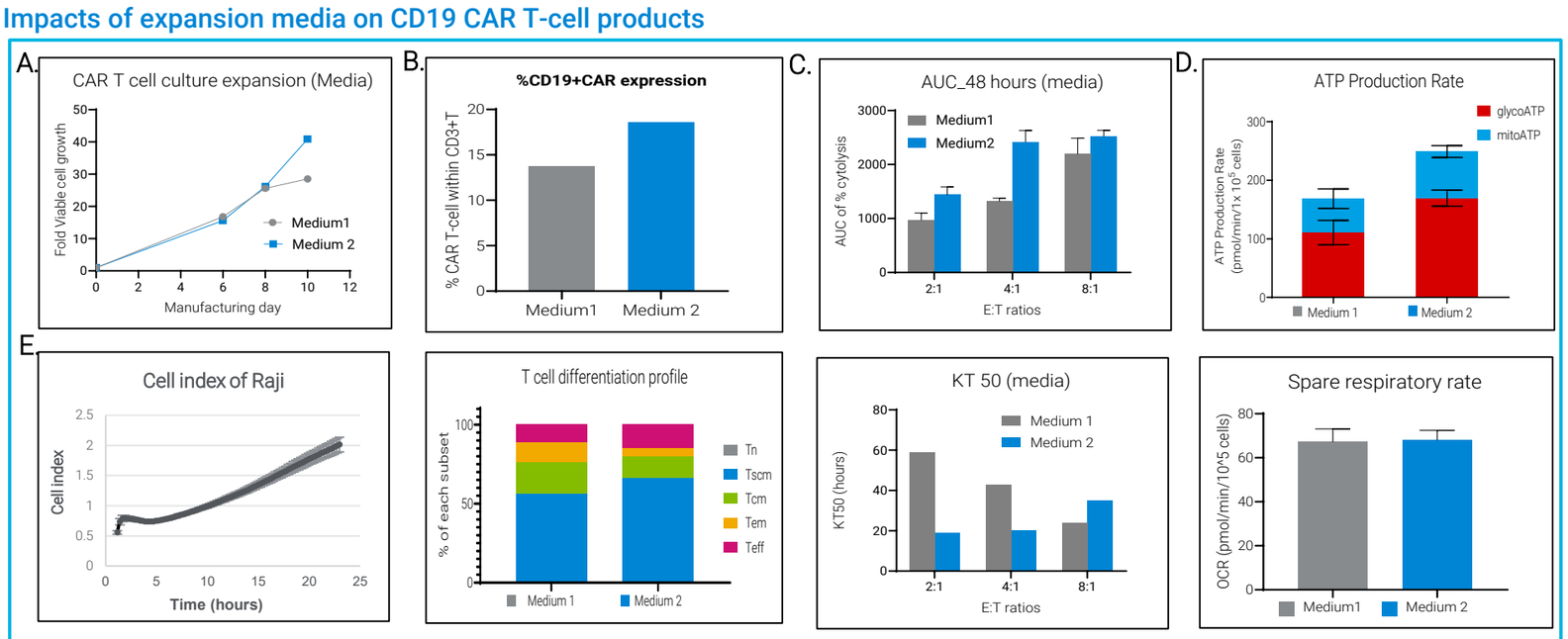


Figure 2. Characterization of CD19 CAR T cells production in Cocoon system using two different expansion media. A. Cell proliferation B. Percent CAR T and differentiation profile using flow cytometer; C. Functional killing potency using RTCA system; D. Metabolic fitness using XF Pro system; E. Robust Cell Index of suspension target cells, Raji, after they were tethered to the E-Plate 96 using Agilent immunotherapy (IMT) kit.

Impacts of cytokines on the EpCAM CAR T-cell products

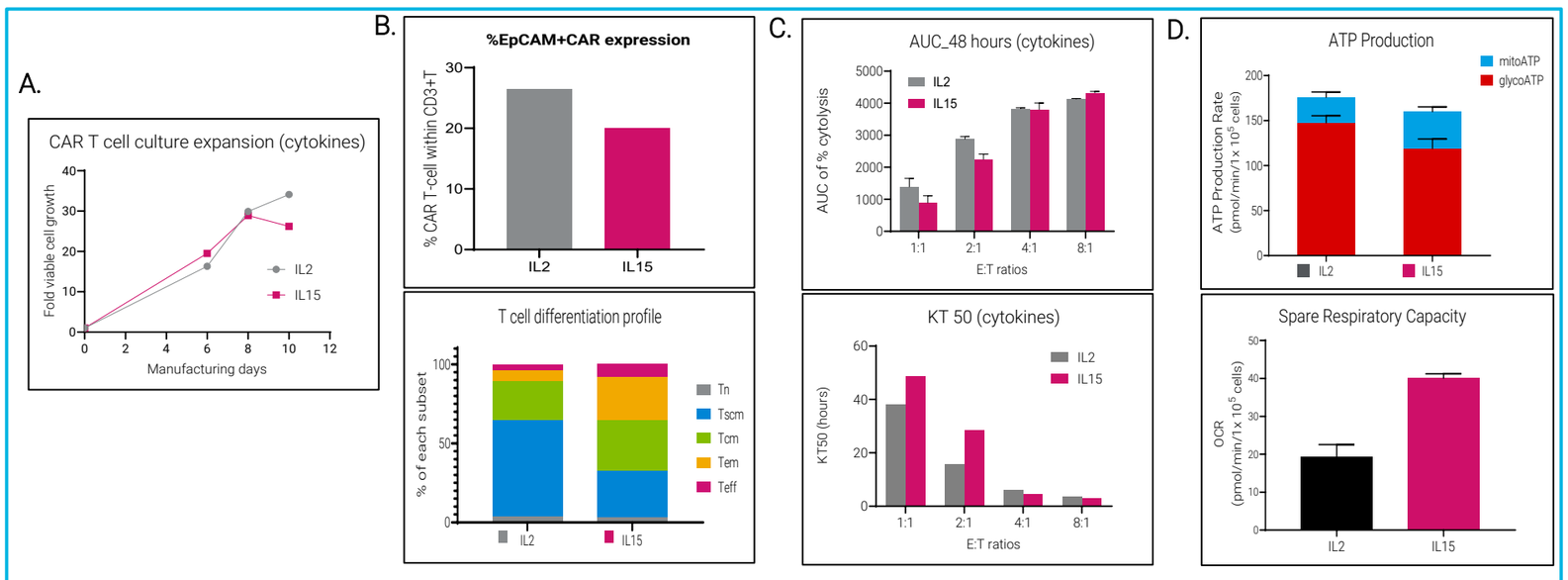


Figure 3. Characterization of EpCAM CAR T cells production in Cocoon system using IL2 and IL15 as growth supplements, respectively. A. Cell proliferation B. Percent CAR T and differentiation profile using flow cytometer; C. Functional killing potency using RTCA system, D. Metabolic fitness using XF Pro system.

In-process analysis during cell manufacture

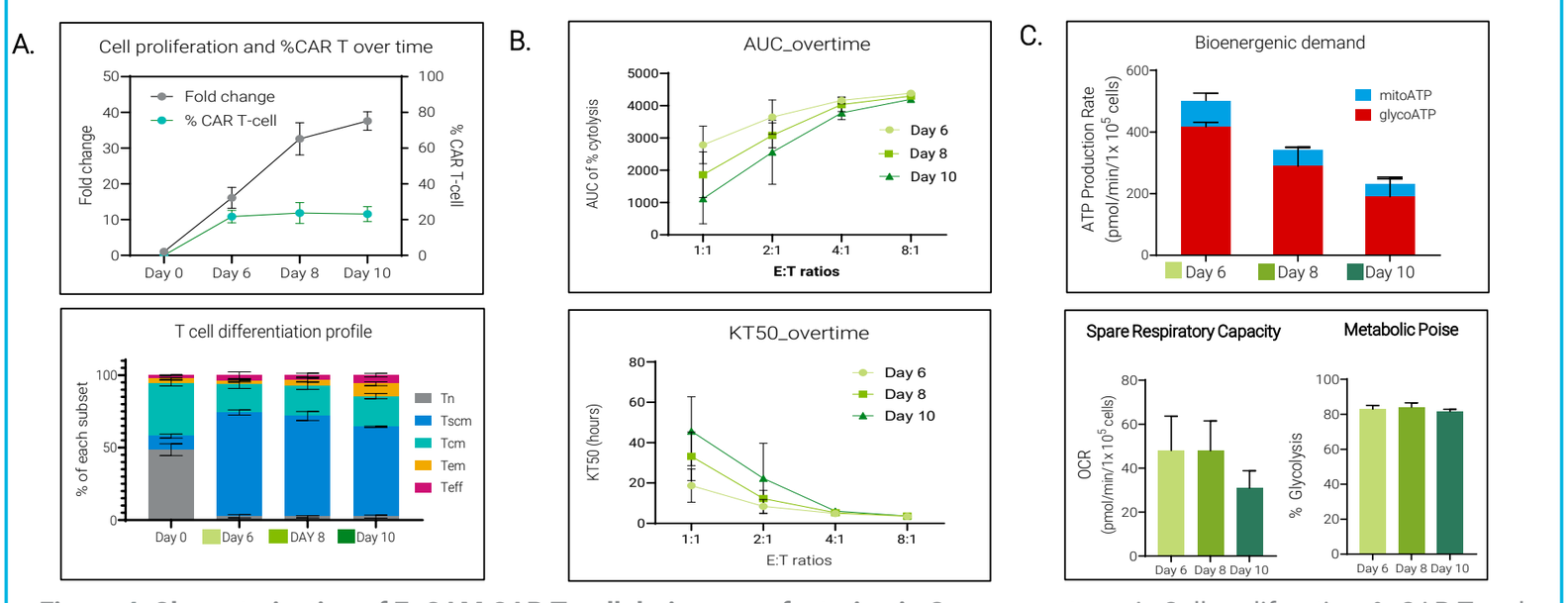


Figure 4. Characterization of EpCAM CAR T-cell during manufacturing in Cocoon system. A. Cell proliferation, % CAR T and differentiation profile using flow cytometer; B. functional killing potency using RTCA system, C. metabolic fitness using XF Pro system.

Conclusions

- High quality and consistent CAR T cell products were manufactured in the Lonza Cocoon platform.
- Agilent NovoCyte, xCELLigence RTCA, and XF Seahorse instruments provided precise, reproducible, sensitive, and reliable results, enabling the evaluation and identification of key factors that influence the quality of cell products during process development.
- The integration of these analytical systems offers complementary solutions, enabling a comprehensive characterization of CAR T cells' immunophenotype, functional potency, and metabolic profile during the CAR T product life cycle.
- In-process analysis during CAR T cell manufacturing is pivotal for gaining valuable insights into the optimal harvest time of the CAR T cell products.