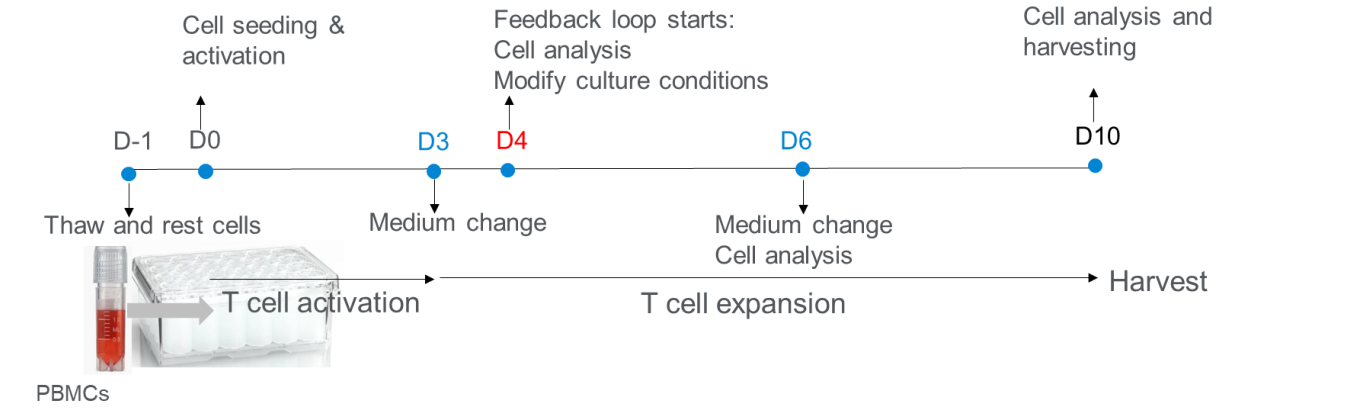


Introduction

Chimeric Antigen Receptor (CAR) T cell immunotherapies show astonishing promise as a treatment for cancer and other diseases. However, many challenges remain including unaffordable and lengthy manufacturing processes, short term persistence of cells, and poor cell expansion. Manufacturing CAR T cell products involves ex vivo expansion of T cells to obtain enough viable cells for infusion into subjects. Evidence has suggested that modifying certain Critical Process Parameters (CPPs), such as cytokines or metabolites can successfully manipulate T cell expansion and differentiation state in ex vivo cultures. However, deciphering how media composition can be adjusted in suboptimal conditions to improve the efficiency of the T cell product, in a time-saving manner, remains unresolved. Hence, it is imperative to develop advanced process analytical tools and methods that can automate and integrate the processes of T cell production, cell sampling, sample monitoring, and cell analysis, such that feedback based on these real-time culture analytics can efficiently guide and improve cell production to enhance the quality and speed-up the production processes for the therapeutic cells. In this study, we developed integrated cell analysis methods to investigate cell expansion and T cell differentiation states in ex vivo culture, and based on the feedback control from those analytics, we successfully demonstrated that adjustment of the cell culture media composition can significantly improve the critical quality attributes in T cell product manufacturing. Such methodology can be applied to large scale cell therapy product manufacturing to address the challenges in current practices.

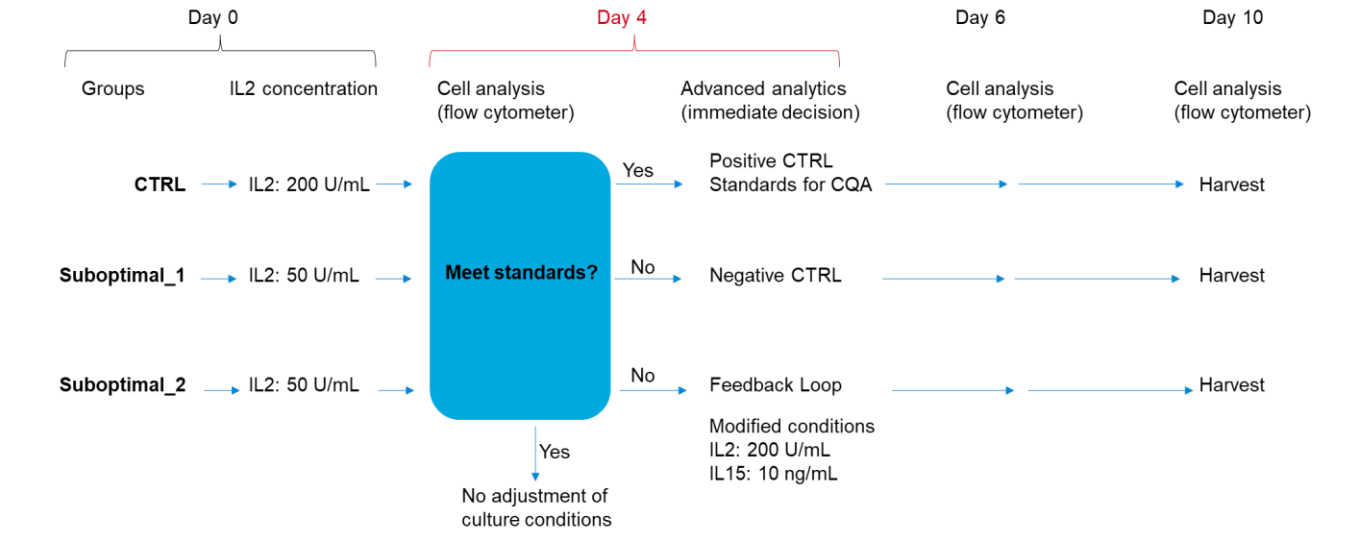
Experimental Method

Cells:
PBMCs: healthy donor PBMCs were activated with human CD3/CD28 T cell activator and expanded in basic RPMI 1640 media supplemented with various IL2 concentration and cultured in gas-permeable rapid expansion G-Rex (ScaleReady) platform for 10 days.



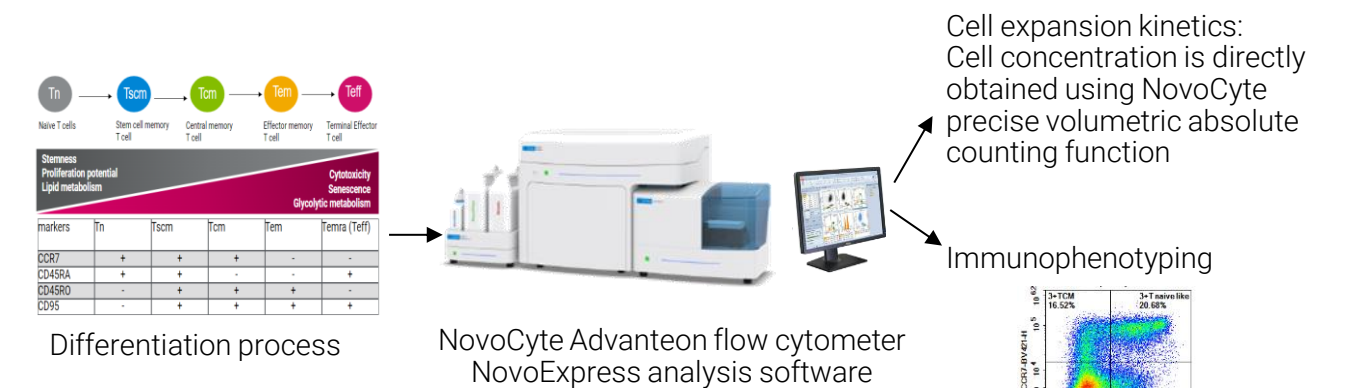
Workflow of T-cell production process with a feedback loop:

To mimic variations in cell performance and cell phenotype caused by donor differences, PBMCs from the same donor were activated and expanded in the RPMI 1640 supplemented with varying concentrations of IL2 at day 0. The cells cultured with 200 U/mL IL2 were used as the standard control (CTRL), while those expanded with IL2 50 U/mL were designated as the suboptimal group. The first cell analysis was performed at Day 4 using flow cytometry. If the cells in the suboptimal group do not meet the CTRL-determined critical quality attributes (CQAs) for the cell viability, cell purity, total cell number, frequency of stem cell memory T cells (%Tscm), and the number of Tscm cells, the manufacturing conditions for the suboptimal group were adjusted immediately. A 20% deviation threshold for CQAs from the CTRL values was set to initiating culture condition adjustments. All groups were cultured for a total of 10 days with a cell analysis check at Day 6 and Day 10.



Analytical platform and material:

We established integrated cell analysis processes that are suitable to characterize immune cells subpopulations and T cells differentiation state during expansion. Agilent NovoCyte Advanteon flow cytometer possess a precise volumetric for accurate absolute count without the need for counting beads. 8-colors/10 parameters flow cytometry panel: viability dye and markers to study T cell viability, T cell differentiation and T cell subsets.



Results and Discussion

Cell expansion number and %Tscm are key attributes to guide the initiation of feedback loop

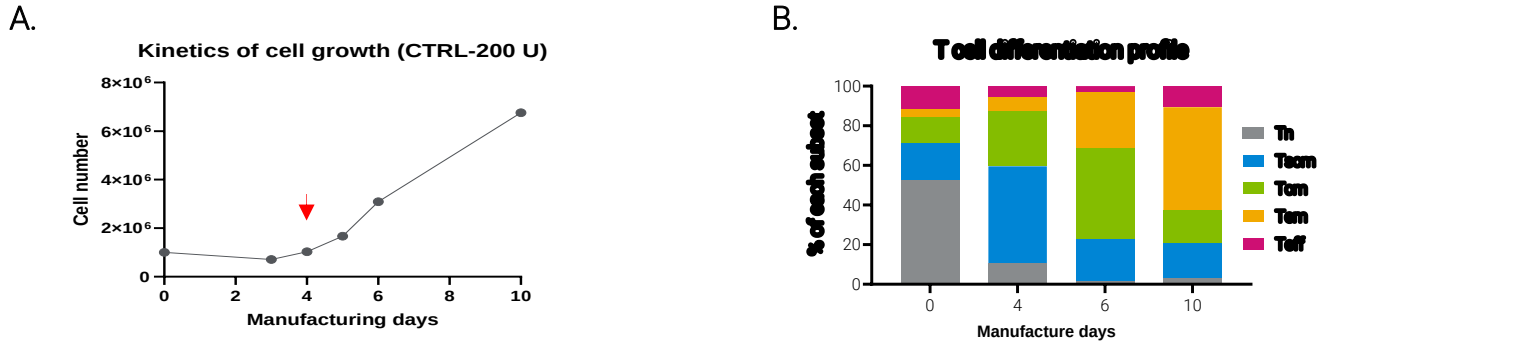


Figure 1. Kinetic of cells growth and differentiation profile of the reference control group. A. Kinetics of cell expansion; **B.** Kinetics of differentiation profile. Day four appears as most suitable starting point for the adjustment of the cell culture conditions as analysis revealed cells in the reference control group at day four start to recover from activation-induced cell death and display very high %Tscm.

Suboptimal groups display less total cell number, less %Tscm and less Tscm cell number at day four

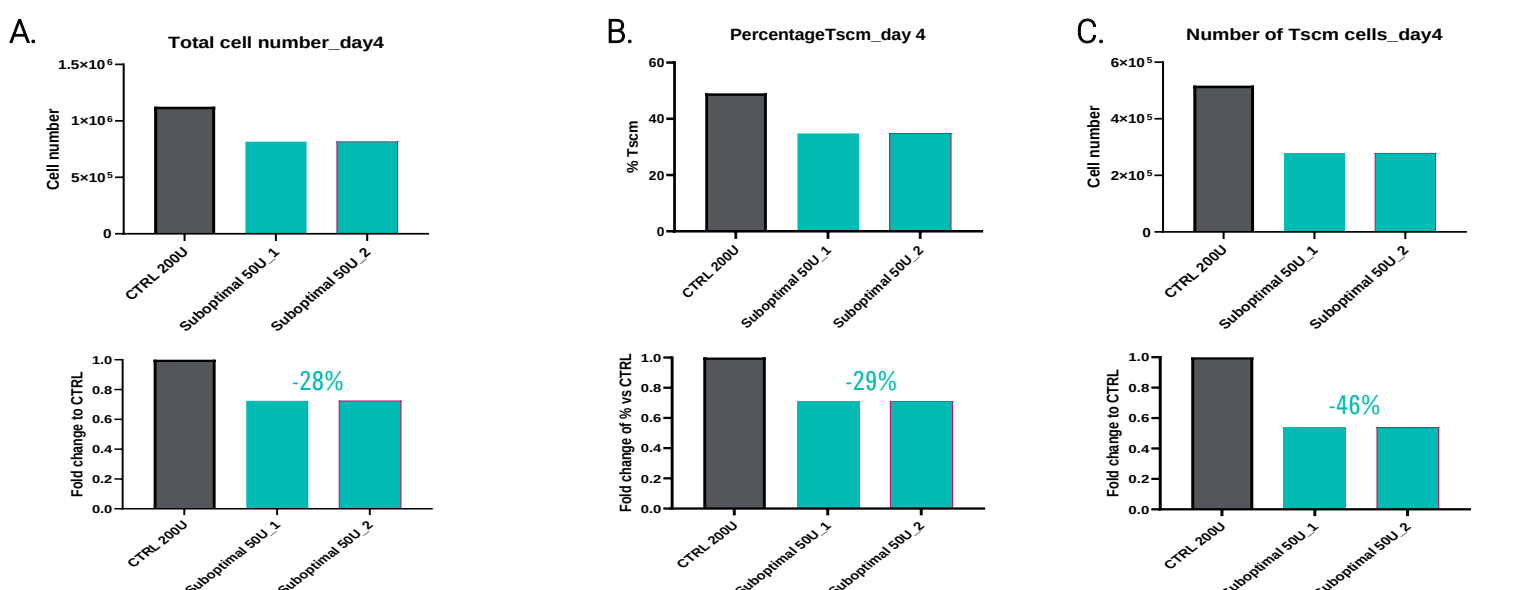


Figure 2. Total cell number, % Tscm and Tscm cell number on day four in control group and suboptimal group. A. Total cell number and fold change; **B.** % Tscm and fold change %Tscm; **C.** Tscm cell number and fold change. The selected key quality attributes in the suboptimal groups are below the benchmark threshold, requiring adjustment of the cell culture conditions (IL2, IL15, glucose) to hopefully improve the production of sufficient-dose efficacious T cells by the end of the process.

Adjusting IL-2 and IL-15 concentrations successfully reconstitute the CQAs of the final T cell products

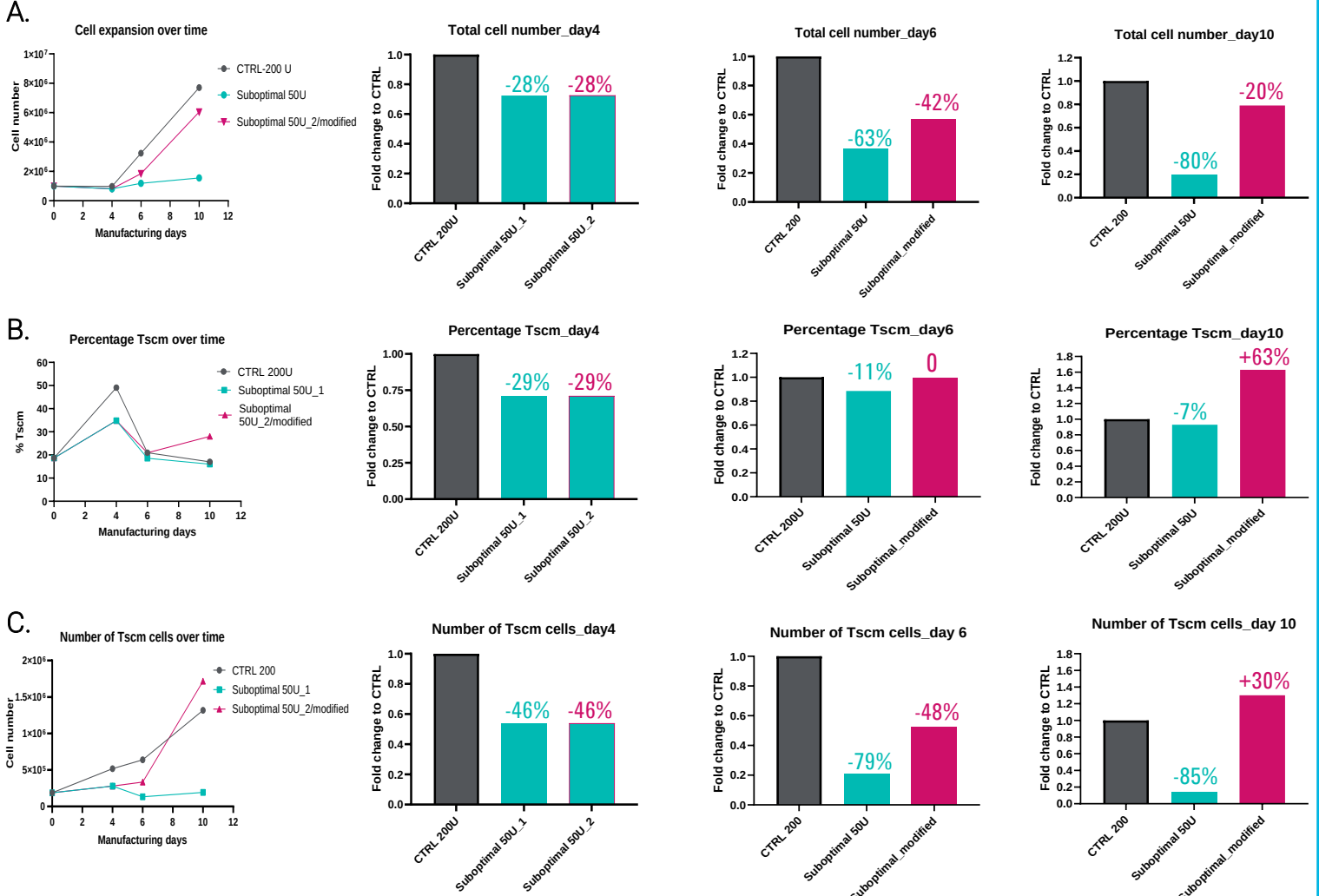


Figure 3. Effectiveness of the modified culture conditions in feedback loop and its delayed impact on final T cell product quality. A. Kinetics of cell expansion number overtime in all groups; **B.** Kinetics of % Tscm overtime in all groups; **C.** Kinetics of Tscm cell number overtime in all groups.

Conclusion

- The quality of the starting cells for the manufacture of adoptive immunotherapy products can vary depending on the individual's health conditions and status. Manufacture failures often result from insufficient dosage (not enough cell number) and improper cell attributes (highly differentiated cells) in the final T cell product.
- Adjusting cell culture factors such as growth factors, pH, dissolved O₂, amino acids or temperature can manipulate T cell expansion and the differentiation profile in ex vivo cultures.
- Applying integrated cell analysis methods to characterize T cell subsets, T cell expansion and T cell differentiation state throughout the production process shows immense potential as it can detect any deviation from critical quality attributes and guide the initiation of cell culture adjustment in a feedback loop, which could restore the desired CQAs in the final product.
- Our findings highlight the crucial role of using flow cytometry to monitor T cell CQAs. We also demonstrate how adjusting IL2 and IL15 concentrations in failing conditions can successfully restore desired CQAs (dose, differentiation phenotype) for final T cell product.
- These data indicate a prominent breakthrough, which could limit manufacture failures, ensuring development of sufficient-dose efficacious T cell products regardless of the status of the starting material, which can promote the widespread adoption of immunotherapies.