Transact or Dynabead-mediated T cell expansion in a controlled, volume-expandable Osilaris™ bioreactor

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INTRODUCTION

Engineered Chimeric Antigen Receptor T cell (CAR-T) therapy has revolutionized the immunotherapy field, showing clinical efficacy in several haematological malignancies. Automation, standardization and increase of the production scale are necessary to develop a reproducible, cost-effective and robust process. Particularly for autologous products, a GMP-ready and closed production workflow is highly needed to support personalized interventions. Here we demonstrate that, following T cell activation and seeding into the Osilaris single-use culture bag (Figure 1), the Osilaris bioreactor can support a >100-fold cell expansion.

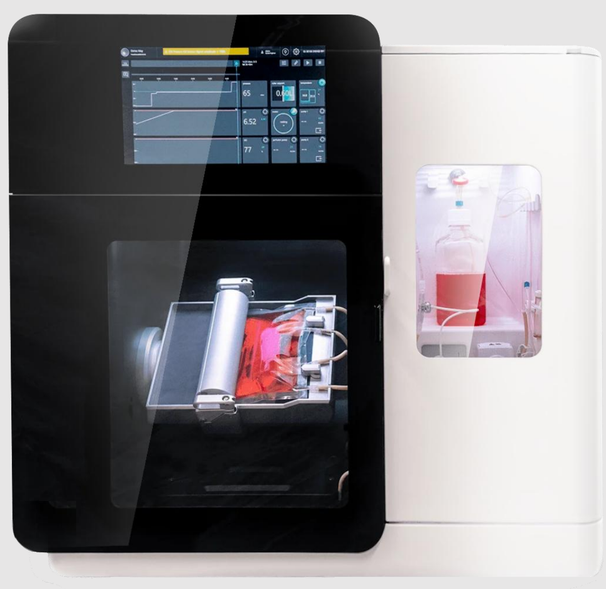

Figure 1: The Osilaris™ bioreactor, a closed bioreactor for cell therapy production.

MATERIALS AND METHODS

T cell isolation and activation using Dynabeads or TransAct

Approximately 70 million cryopreserved CD3+ T cells were activated with either 1:3 Dynabeads (Thermofisher/Gibco™) or 1:100 TransAct (Miltenyi) and pre-cultured in a 5% CO2 incubator for 72 hours in a T75. Three days after starting activation, the activation reagent was exchanged with fresh X-Vivo 15 medium (Lonza). The cells were then diluted to 200,000 viable cells/ml and transferred into the Osilaris bioreactor (see Figure 2). Parameters were set to 75% dissolved oxygen and a pH of 7.3

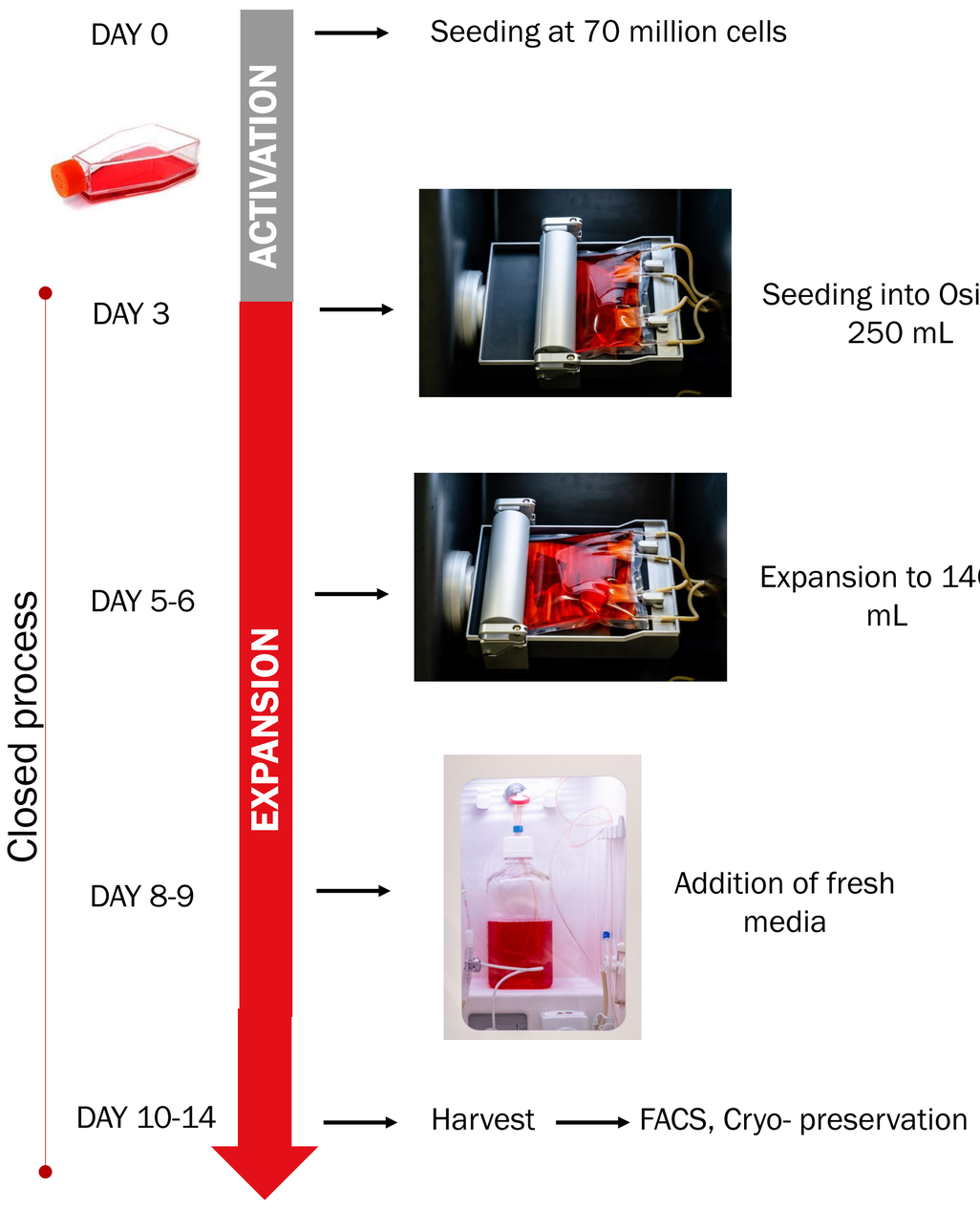

Figure 2: Osilairs mediated T cells expansion workflow.

RESULTS

Cell yield

All four donors yielded over 7 billion cells at the end of culture, which is a 100-fold cell expansion. (Figure 3). Transact activated donors exhibited the highest yield with a maximum of 17 billion CD3+ cells on day 14.

These results show the Osilaris' capacity to support high cell densities (>7 million cells/mL) while maintaining environmental setpoints for oxygen tension and pH.

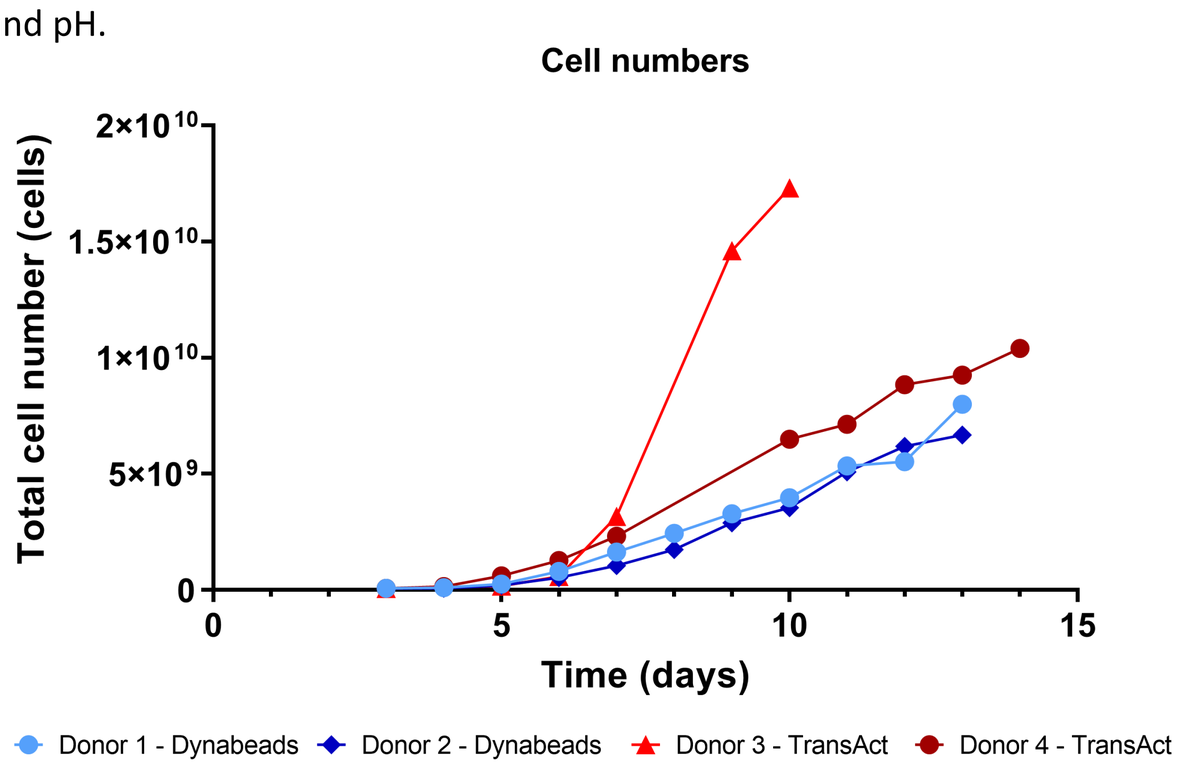

Figure 3: Expansion of activated T cells in the Osilaris bioreactor. Total yields of over 7 billion cells were obtained for Dynabeads activated donors, and even a maximum yield of 17 billion viable cells for Transact activated donors.

Phenotyping

At harvest, flow cytometry was performed to detect the presence of cell surface markers CD3, CD4, CD8, CD25, PD-1, and CD45RA and CD45RO. Phenotype was compared to control cultures in static T-flasks. No difference was observed between the Osilaris-generated cells and the control cultures (Figure 4). For all donors, the ratio of CD4+ and CD8+ was also not significantly different, compared to control cultures.

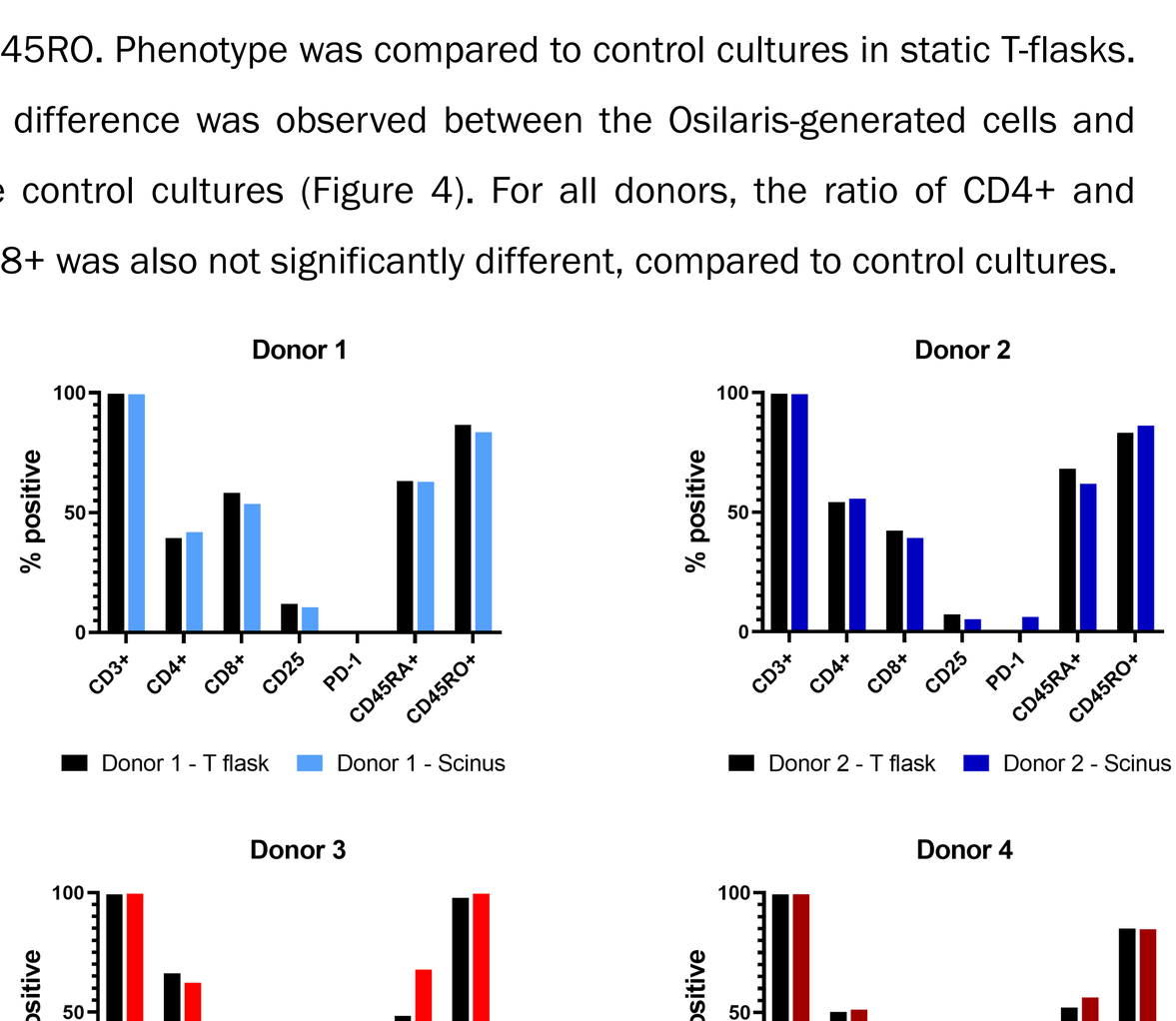

Figure 4: Marker expression and CD4/CD8 ratios for all donors.

CONCLUDING REMARKS

- The Osilaris bioreactor, and its single-use bioreactor bag, is a suitable culture vessel for T cell expansion for e.g. CAR-T cell therapy applications.
- From a starting inoculum of 70 million total viable cells, we reproducibly generated over 7 billion total viable cells in four different donors.
- High culture densities (>7 million cells/ml) were obtained with more efficient use of cell culture medium.
- Osilaris mediated cell expansion produces cells with a highly functional phenotype.

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