High effective cell isolation to generate specific T-cells using CGX10 Cell Isolation System

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INTRODUCTION

Today, the main challenges of cell therapies development are cost and turnaround time, hindering its democratization despite impressive efficacy.
In this context, we developed a GMP compatible process with a reduced cost and limited open manual steps, to generate T cells specific to peptides presented by autologous monocytes (Ag-specific T cells).

METHODS

Isolated PBMC were stained with a GMP-compatible flow cytometry panel including CD4, CD8, CD45RA and CD62L, before being sorted on the Sony CGX10 Cell Isolation System (**Figure 1**). The CGX10 is a fully closed, bag-to-bag operated cell isolation system for GMP-compliant cell production and cell sorting applications. Thanks to its mixed sorting ratio control feature, the CGX10 allows the sorting of multiple target populations at fixed ratios. Using this function, naive CD4 cells, naive CD8 cells, and monocytes were simultaneously sorted in purity mode, in a predefined ratio designed to maximize the yield of CD8 isolation, under completely closed conditions.

Fig. 1: Process overview

RESULTS

Cell sorting strategy

Considering the limited availability of GMP-grade fluorochrome conjugated antibodies on the market and impact on manufacturing costs, we have established a cytometry panel based on four markers to isolate both naïve CD4 and CD8 T cells as well as monocytes. To distinguish monocytes ($CD4^{low}$) from CD4 T lymphocytes ($CD4^{hi}$) and CD8 T lymphocytes ($CD4^{neg}$) based on CD4 expression, we selected CD4-APC, available in GMP grade and enabling optimal separation (**Figure 2**). The other antibodies in the panel are CD8-PercP, CD45RA-FITC, and CD62L-PE (Sony Biotechnology).

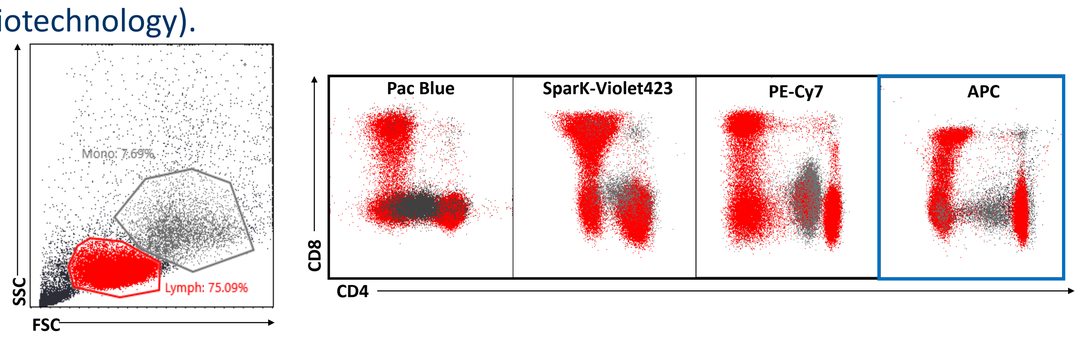


Fig. 2: Evaluation of CD4 antibodies coupled with different fluorochromes. Monocytes appear in gray.

Monocytes (SSC^{hi}, $CD4^{low}$), naive CD4 cells ($CD4^+CD45RA^+CD62L^+$), and naive CD8 cells ($CD8^+CD45RA^+CD62L^+$) were sorted in purity mode. Purity was assessed directly on the CGX10 post-sort, showing an impurity rate of less than 4% for all three populations (**Figure 3**, 1 representative experiment of 4).

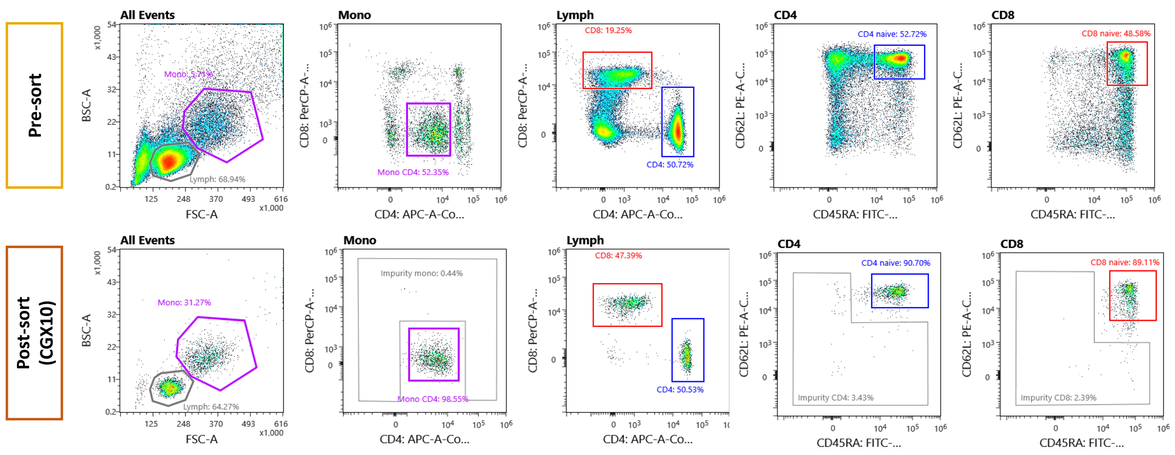


Fig. 3: Gating strategy and purity post-sort evaluated on the CGX10.

The CGX10 can be configured to sort in Purity mode up to 15,000 events/sec (**Table 1**) and to output fixed ratios of up to four target subpopulations, as shown in **Figure 4**. The post-sort viability was assessed using the LUNA-FX7™ Automated Cell Counter (Logos Biosystems) and was higher than 98% for the four donors.

Table I: Sort performance (n=4)

Donor	Sort Mode	Total cells sorted	Average event rate	Sort Time	% pre-sort Mono/CD4 Naïve/CD8 Naïve	Cells recovered
#1	Purity	1.00E+08	7,748/s	3h27min	10.15% / 9.29% / 3.84%	9.67E+06
#2	Purity	1.95E+08	12,995/s	4h01min	19.15% / 7.31% / 2.75%	1.37E+07
#3	Purity	1.50E+08	13,095/s	2h59min	9.36% / 10.40% / 2.65%	1.10E+07
#4	Purity	1.34E+08	13,670/s	3h16min	2.69% / 18.25% / 6.41%	4.53E+06

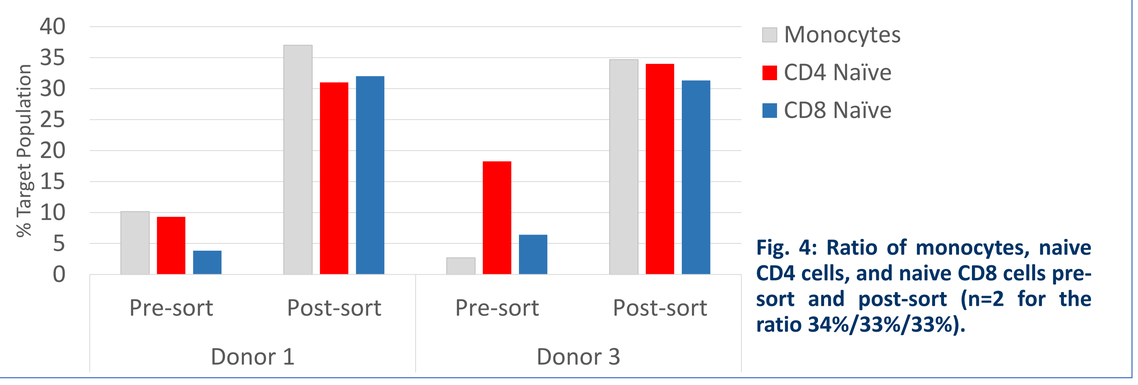


Fig. 4: Ratio of monocytes, naive CD4 cells, and naive CD8 cells pre-sort and post-sort (n=2 for the ratio 34%/33%/33%).

Quality assessment of T-cells : preliminary results

Cells were sorted directly into TexMACS (Miltenyi Biotech) culture medium with the aim of seeding them directly into G-Rex bioreactor (Wilson Wolf Manufacturing) using monocytes as antigen-presenting cells and specific peptides (**Figure 5**).
Cell proliferation was monitored by measuring glucose depletion and lactate production over time (**Figure 6A**). At the end of the process (D24), i*n vitro* activation was assessed by measuring CD69 and CD25 expression on the membrane of T-cells (**Figure 6B**).

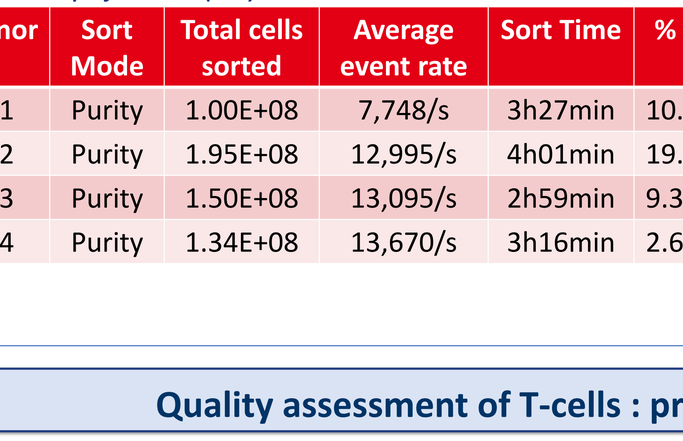
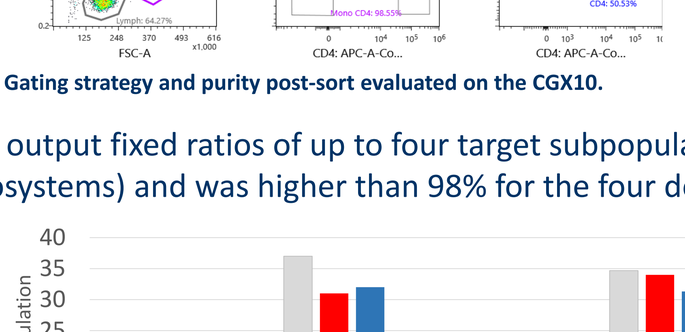


Fig. 5: Sterile connexion between CGX10 and Grex

A



B

Fig. 6: A/ Monitoring of cell proliferation by measurement of glucose and lactate concentration. Medium exchange is marked by the arrow

B/ Expression of the activation markers CD25 and CD69 on $CD3^+$, $CD4^+$ and $CD8^+$ T-cells

As glucose and lactate measurement show, and confirmed by activation marker expression, cells correctly expanded over 24 days.

CONCLUSION

Using this process, we demonstrated the feasibility of simultaneously sorting three distinct target cell populations at the pre-defined ratios, achieving high purity and viability. The sorted cells were able to proliferate following CEF presentation by monocytes. Although activation was assessed only at the end of the expansion phase, expression of CD25 and CD69 confirmed T cell activation. This work provides a first proof of concept for a rapid and scalable method to generate antigen-specific T cells. Further optimization is needed to close the process and reduce manual handling by integrating the various modular instruments.

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