Utility of the CGX10 Cell Isolation System in Closed CAR-T Cell Therapy Manufacturing

CCRM Commercializing Living Therapies

A study in collaboration with CCRM.

Objective

Satisfy need for a GMP compliant closed cell sorting system for isolation of specific T-cell subtypes for closed CAR-T manufacturing

Process Developed

Integrated the CGX10 in closed T-cell manufacturing pipeline for isolation of defined T-cell subtypes before CAR-T cell engineering

Outcomes

The CGX10 Cell Isolation System from Sony Biotechnology can be integrated into closed manufacturing processes and enables single step multiparametric isolation of rare cell types

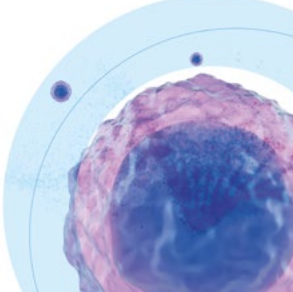
A GMP ready cell sorter for closed cell and gene therapy manufacturing

CAR-T therapies are life-saving revolutionary technologies that are rapidly evolving. Ex vivo isolation and manufacturing of T-cells results in complex mixtures of T-cell subtypes, which could lead to variable clinical outcomes. A current challenge in the field is the optimization of the manufacturing process; specifically obtaining well defined subsets of T-cells to enhance therapeutic outcomes as well as safety. Young, undifferentiated T-cells are a rare population of T-cells, thought to enhance therapeutic outcome, which are characterized by a combination of transiently expressed cell surface markers. There are currently no GMP ready scalable solutions to isolate such T-Naïve (T^{N}), T-Stem Cell Memory (T^{SCM}), or T-Central Memory (T^{CM}) T-cells. In this case study, we studied the utility of the CGX10, a GMP ready, closed cell sorting system from Sony, to isolate T-Naïve cells using multi-parametric selection, and incorporated the sorter into the CAR-T manufacturing process.

Methods and results

	Input	Process	Equipment and reagents	Output
Day -1	Fresh apheresis	Ficoll enrichment	Sepax	
		CD14/19 bead binding	Sepax	
		CD14/19 bead depletion	CliniMACS® Plus	
Day 0		Antibody staining	Sepax/ Open CD4/CD8/CD45RA/CD62L	
		Cell sorting	**CGX10 (Purity mode)**	**CD4 Naïve T cells**
		Seeding and activation	Plates/ closed reactor CTS-Optimizer + IL7 + IL15 + Immunocult	
Day 2		Transduction	Plates/ closed reactor	
Day 3-9		T-cell expansion	Plates/ closed reactor CTS-Optimizer + IL7 + IL15	Transduced & expanded T cells

Figure 1: Method 1 - Process flowchart for utilizing CGX10 Purity mode to isolate Naïve T-cell subsets in a T-cell manufacturing process.



Processing time and yield estimations from the CGX10					
	Sort time	**Total events sorted**	**% CD4 Naïve target pre-sort**	**# CD4 Naïve target recovered**	**% target recovery**
Donor 1	4.5 h	2.32E+08	8.6 %	1.30E+07	67.08 %
Donor 2	4.0 h	2.39E+08	8.9 %	1.40E+07	66.29 %

Table 1. Summary of the sorting data from the CGX10 for Method 1

In Method 1, post-Ficoll enrichment of PBMCs and CliniMACS depletion of monocytes and B cells, T-cells were stained with a combination of antibodies specific for the Naïve phenotype and subjected to cell sorting using CGX10 "Purity mode," gating for CD4+ CD62L+ CD45RA+. The CGX10 can be utilized for sorting a large number of input cells, thereby enriching rare populations with high % Target recovery in a single step, for manufacturing (Table 1).

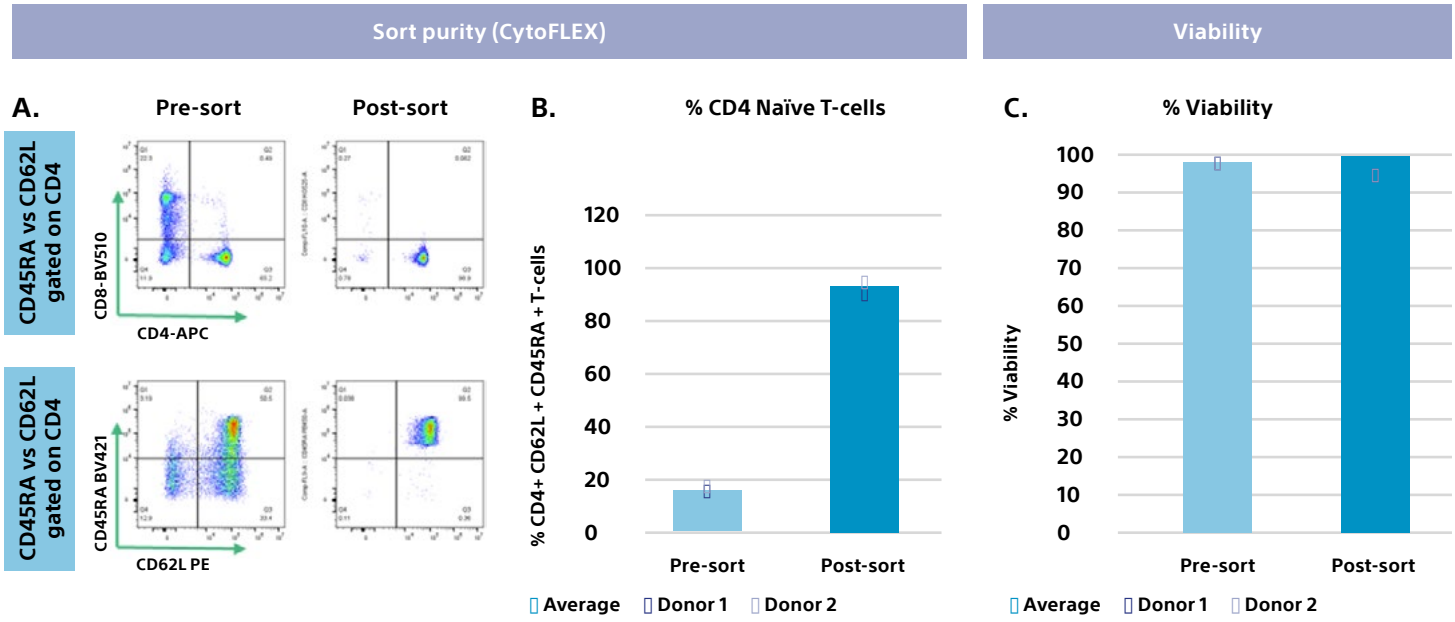


Figure 2. Analysis of post-sort purity (A and B) and viability (C) for Method 1

Flow cytometry assessment (CytoFLEX) of cell populations pre- and post-sorting indicated that CGX10 cell sorting in "Purity mode" resulted in significant enrichment of the rare CD4+ Naïve T target cells with >95% purity (Figure 2, A and B). Sorting by the CGX10 for ~4 hours did not impact the viability of the cells (n = 2) (Figure 2, C). NOTE: % Target cell estimations vary between the CGX10 and CytoFLEX due to different instrument cutoffs and gating.

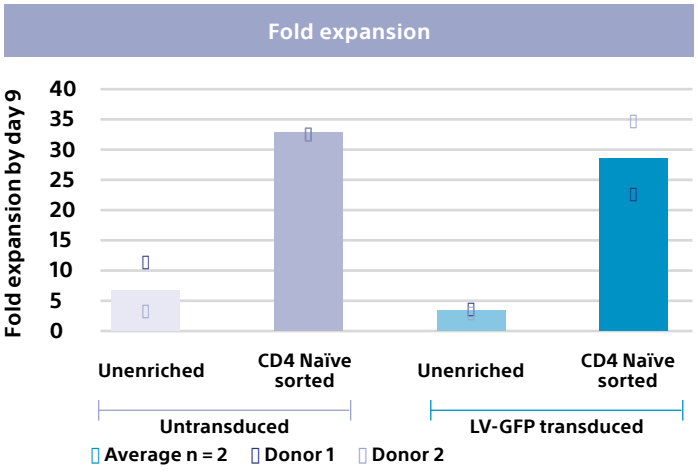


Figure 3. Analysis of fold expansion of unenriched vs CGX10-sorted cells

After sorting, cells were transduced with LV-GFP to mimic the CAR-T process. Cells were expanded in Naïve T-cell media for 9 days. Sorted CD4+ Naïve T-cells had the ability to be transduced and showed a significantly higher fold expansion in Naïve T-cell media compared to unenriched cells that have a smaller and variable percentage of Naïve T-cells (Figure 3). The CGX10 can also be deployed post-transduction, to enrich for transduced cells for expansion (data not shown).

Additionally, the CGX10 can be configured to sort and output fixed ratios of multiple target sub-populations (e.g: CD4 Naïve (25%), CD4 Central Memory (25%), CD8 Naïve (25%), and CD8 Central Memory (25%)) in Purity mode with no impact on cell viability (data not shown).

	Input	Process	Equipment and reagents	Output
Day -1	Fresh apheresis	Wash cells/RBC Lysis	Sepax/Open	
Day 0		Ab staining	Sepax/ Open CD4/CD8/CD45RA/CD62L	
		Cell sorting round 1	**CGX10 (Enrichment mode)**	**CD8 Naïve T-cells (low purity)**
		Cell sorting round 2	**CGX10 (Purity mode)**	**CD8 Naïve T-cells (high purity)**
		Seeding and activation	Plates/ closed reactor CTS-Optimizer + IL7 + IL15 + Immunocult	
Day 2		Transduction	Plates/ closed reactor	
Day 3-9		T-cell expansion	Plates/ closed reactor CTS-Optimizer + IL7 + IL15	Transduced & expanded T cells

NOTE: Italicized process steps are downstream steps that were not performed in this experiment.

Figure 4: Method 2 - Flowchart for a simplified manufacturing pipeline utilizing multi-round sorting by the CGX10 to isolate Naïve T cells.

Standard CAR-T cell manufacturing involves multiple pre-process steps to isolate T-cells from apheresis units. Since the CGX10 can perform multi-parametric, high throughput enrichment of cells, Method 2 aimed to bypass the multiple process steps by utilizing the CGX10 alone. In Method 2, after RBC lysis, apheresis units were stained with the combination of markers to isolate Naïve CD8+ T-cells.

A first round of sorting was performed using "Enrichment mode," where cells are sorted at a high speed with less stringency for purity. Output cells from the first round were sorted a second time using the more stringent Purity mode to obtain a pure population of target cells.

Processing time and yield estimations from the CGX10				
	Sort time	**Total no. of events sorted**	**% TGT pre-sort**	**No. of target cells recovered**
Round 1 (Enrichment mode)	1.7 h	5.42E+08	1.3 %	2.80E+07
Round 2 (Purity mode)	1.0 h	1.66E+07	13.9 %	2.00E+06

Table 2. Summary of the sorting data from the CGX10 for Method 2 (Donor 1)

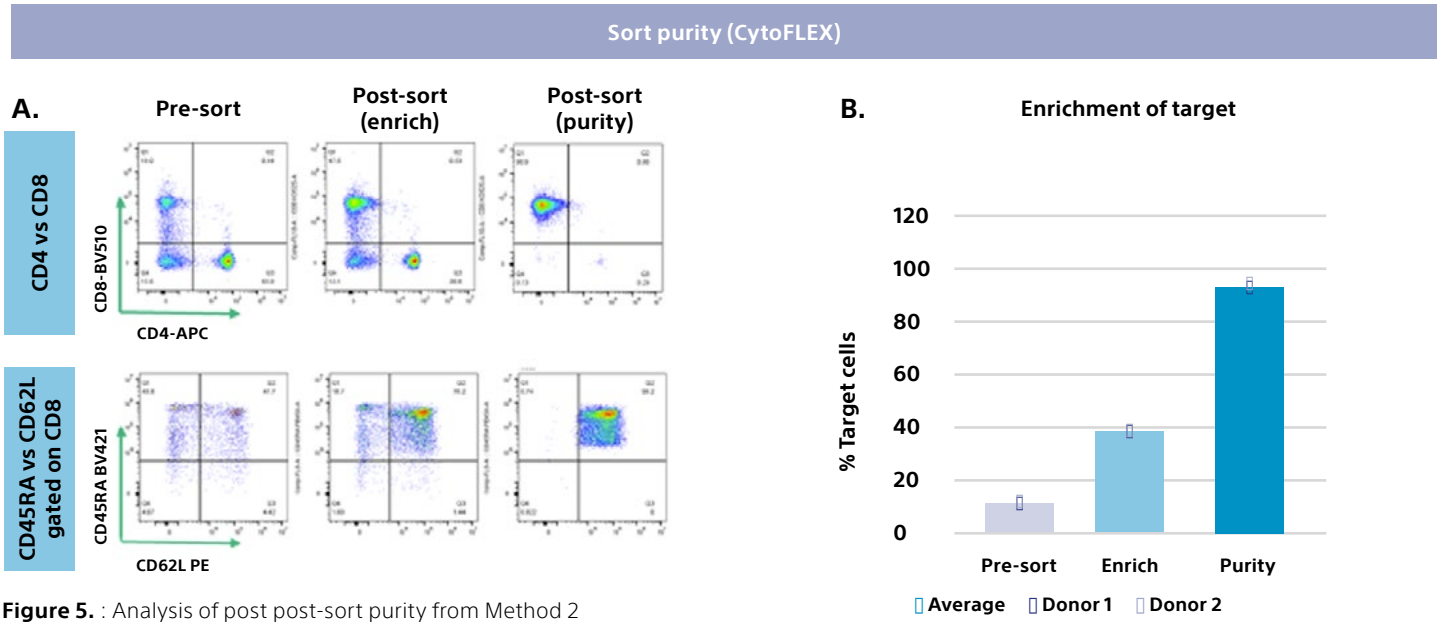


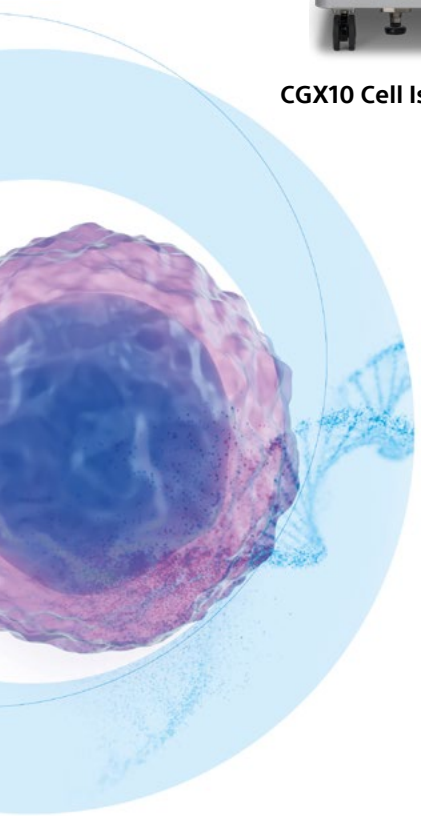
Figure 5. : Analysis of post post-sort purity from Method 2

CGX10 Cell Isolation System

In this proof-of-concept experiment, the first round of sorting in "Enrichment mode" removed a bulk population of the non-target cells and enriched the target cells to ~40%. The second round of sorting in "Purity mode" significantly enriched the CD8+ Naïve target cells resulting in >95% purity and 87% estimated target recovery. No other instruments or reagents were needed in this method, resulting in process simplification. Further optimization will enable high throughput sorting to achieve manufacturing scale output cell numbers.

Conclusion

- The CGX10 cell isolation system is a unique "closed" cell sorting instrument with customized single unit disposables and a GMP compatible user interface.
- The CGX10 can be easily integrated with closed upstream and downstream CAR-T process steps.
- The CGX10 has the ability to sort rare populations with no existing separation strategies using customized markers.
- The CGX10 has options for Purity vs Enrichment modes to cater to diverse purposes and process simplification.
- The CGX10 cell isolation system has the potential to be integrated into varied immunotherapy and cell and gene therapy manufacturing pipelines.



Customer support

Sony provides the highest quality of customer service, scientific support, and field service resources to ensure the best customer experience.

North America/International
1730 North First Street
San Jose, CA 95112 U.S.A.
Voice: +1 800-275-5963
FAX: +1 408-352-4130
sales@sonybiotechnology.com
https://www.sonybiotechnology.com

Japan
5-1-1, Minato Mirai, Nishi-ku,
Yokohama-shi, Kanagawa,
220-8750 Japan
Tel: +81 120-677-010
Fax:+81 120-388-060
sales_Japan@sonybiotechnology.com
https://www.sony.co.jp/LS

Europe
The Heights, Brooklands,
Weybridge, Surrey, KT13 OXW, UK
sales_EU@sonybiotechnology.com

The CGX10 Cell Isolation System and related products are intended for use by trained laboratory technicians in research, process development or manufacturing environments all related to ATMP/regenerative medicine, including cell and gene therapy. The CGX10 instrument and related products are for *ex vivo* cell separation processing only, and are not intended for therapeutic, diagnostic, or human *in vivo* applications. Any clinical application of the cells is exclusively within the responsibility of the user of the CGX10 instrument and related products. For the manufacturing and use of cells in humans, regulations must be followed. The CGX10 Cell Isolation System and related products are not sold as medical devices.

©2023 Sony Biotechnology Inc. All rights reserved. Sony and the Sony logo are trademarks of Sony Corporation. CliniMACS is a registered trademark of Miltenyi Biotec. CTS and OpTmizer are trademarks of Thermo Fisher Scientific. CytoFLEX is a registered trademark of Beckman Coulter, Inc. Ficoll and Sepax are registered trademarks of Cytiva. Immunocult is a trademark of STEMCELL Technologies. All other trademarks are property of their respective owners. The CGX10 Cell Isolation System is classified as a Class 1 laser product.

11.03.110922.0