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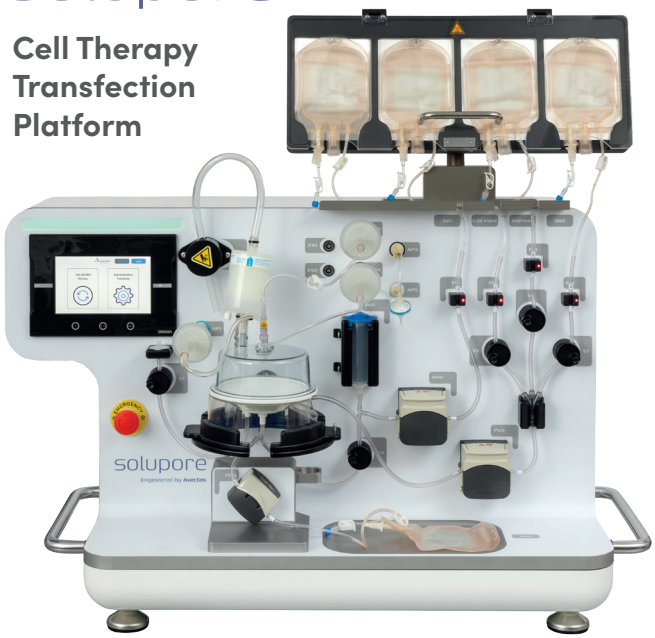
SONY

solupore®

Cell Therapy
Transfection
Platform



CGX10 Cell
Isolation System

Solupore® transfected cells isolated using Sony's CGX10 yield highly cytotoxic CAR-T cells

SCAN ME

APPLICATION NOTE

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Editing CAR-T cells to enhance their efficacy is an important development step in cell therapy. Sorting specific populations is another avenue that can be taken for the optimization of therapies and minimization of adverse effects. The heterogeneity within T cell populations results in different patients' cells not having equal therapeutic potential – stem cell memory T cells (T_{SCM} as defined by CD4/8+ CD45RA+ CD62L+ CCR7+ CD95+), exhibit superior persistence and anti-tumor activity compared to more differentiated, exhausted cells. By sorting for these specific subsets, we can potentially enhance the longevity and effectiveness of CAR-T therapy. Precise sorting is thus critical for refining CAR-T products, maximizing potency, and reducing risks like relapse or severe toxicity. In this study we have sorted on non-activated CD3+ T cells (objective 1, pages 2-3), and additionally sorted on naïve/unactivated T cells that were transfected by Solupore (objective 2, page 4).

✓ Objective 1

Assess cytotoxicity of purified T_{SCM} cells in specific ratios after transfection with Solupore.

✓ Summary

TRAC KO T cells generated from the Solupore process were originally, successfully Tscm cell sorted with the CGX10 Cell Isolation System from Sony. Sorted cells that were transduced performed better in a single cell targeted killing assay compared to unsorted cells.

Table 1: Process overview.
Purity refers to % Parent T_{SCM}

	Process	Phenotypic Analysis
Day -1	Apheresis, CD3 enrichment and cryopreservation.	Frozen
Day 0-3	Thaw cells Sort with the CGX10, stimulate with TransAct™	>95% Purity of sorted cells (CD4/8+ CD45RA+ CD62L+ CCR7+, subset of the fully defined T_{SCM}) Viability
Day 1	Transfect with Solupore (Cas9 RNP + TRAC gRNA)	
Day 2	Transduction Lentivirus CD19 CAR	
Day 3	Cryo-preservation	Pre/post thaw CAR+, T_{SCM}, TRAC KO
Day 4-6	CAR-T:CD19+ Raji co-culture Live cell imaging	Cytotoxicity (Incucyte single cell analysis)

Table 2: Sorted ratio's of CD4:CD8 T_{SCM} cells and their respective KO and cytotoxicity levels

Sorted population (CD4:CD8)	% Purity T_{SCM}	Viability	Solupore Process (KO %)	Relative Cytotoxicity
Unsorted Ratio 67:29	59.77	95.8	TRAC KO (71%)	0.92
Sorted Ratio (SR) 50:50	97.36	96.4	TRAC KO (78%)	5.11
Sorted Ratio (SR) 25:75	96.08	93.5	TRAC KO (81%)	5.28

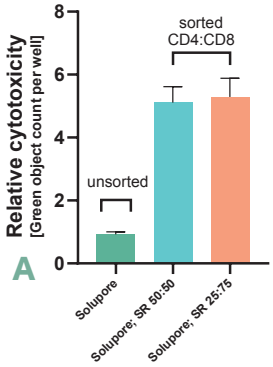


Figure 1A:
Samples were transduced with lentivirus for anti-CD19 CAR, cryopreserved, and thawed. Upon thawing, cells were rested for 24 hours prior to co-culture with CD19+ Raji cells, and the cytotoxicity of T cells was measured by single cell analysis (Cytotox green, Incucyte imaging system). Data is representative of n=3, 48 hours post-co-culture.

Figure 1B-C:
Subsequently, upon thaw, CAR-T cells were analyzed for Stem Cell Memory (T_{SCM}) markers (T_{SCM} CD4/CD8, B and C respectively).

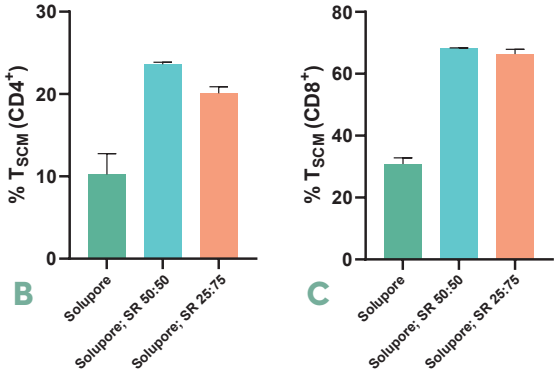
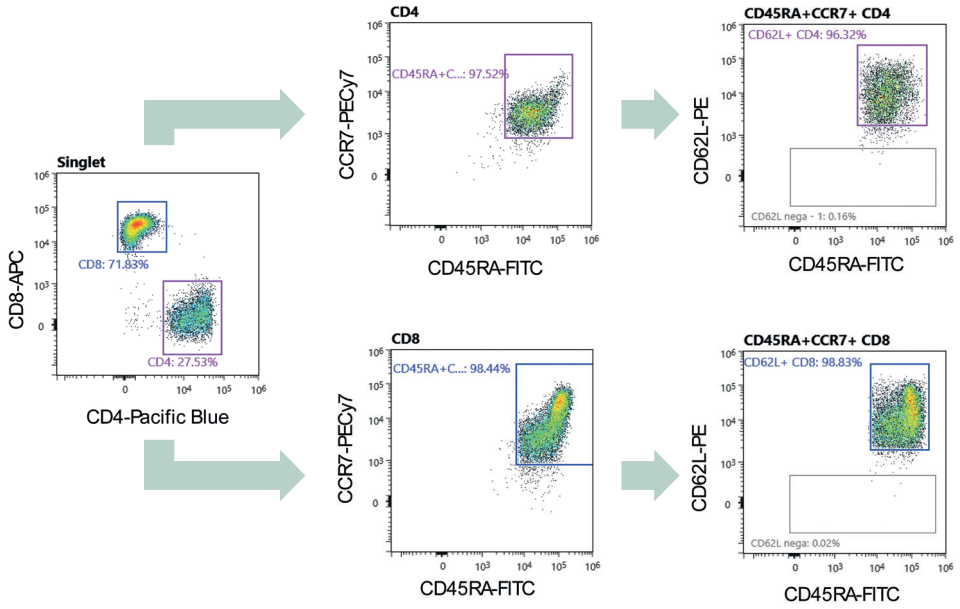


Figure 2:
Purity and gating of T_{SCM} (CD45RA+CD62L+CCR7+) CD4/CD8 T cell ratios (shown is CD4:CD8 = 25:75 ratio) sorted on the CGX10 cell isolation system.



Conclusion

The Solupore process generates healthier, more potent transfected T cells. When combined with the CGX10 cell isolation system, T_{SCM} (triple positive CD45RA, CD62L, CCR7) T cells can be enriched with high purity and viability. Sorted cells outperform non-sorted cells in a single cell image-based targeted killing assay.

Objective 2

Transfect PBMCs containing naïve T cells using Solupore process to knock-out TRAC, followed by sorting using CGX10 for TRAC negative naïve T cells.

Summary

PBMCs obtained from leukapheresis were transfected using Solupore flow through system (FTS) process to knock-out TRAC in a naïve T cell population (transfection from whole PBMC process). Cells were sorted using the CGX10 Cell Isolation System for TRAC negative naïve T cells. Cells were measured for activation status, purity, and cytotoxicity potential.

Figure 3:

A-B.
Early activation markers (CD25, CD69) following transfection with Solupore and sorting with the CGX10 in comparison to a non-transfected control.

C.
Samples were transduced with lentivirus for anti-CD19 CAR, cryopreserved, and thawed. Upon thaw, samples were co-cultured with CD19+ Raji cells (1:10 Ratio Raji:CAR-T) and cytotoxicity was measured.

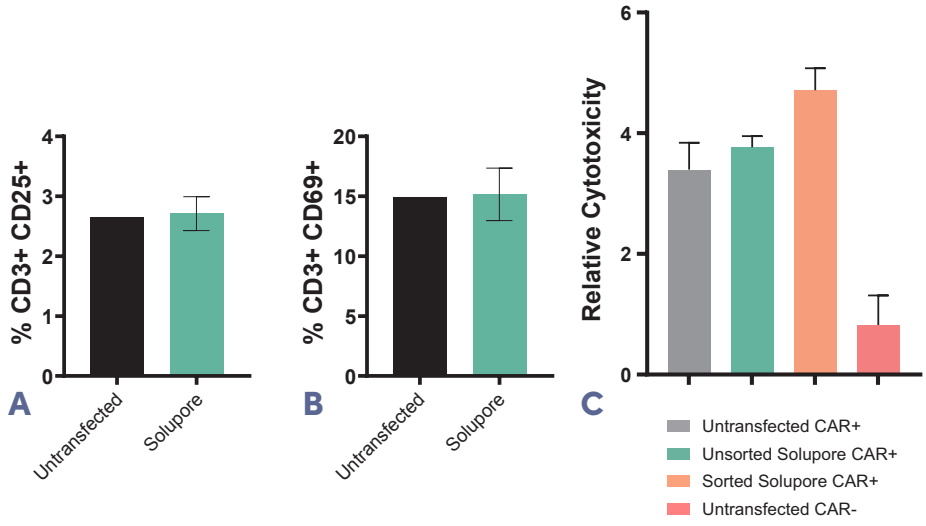


Table 3: Process overview.

	Process	Phenotypic Analysis
Day -1	Apheresis	
Day 0	Thaw cells Transfect with Solupore (Cas9 RNP + TRAC gRNA)	Viability
Day 1	Culture cells	Viability, CD25, CD69
Day 2	Transduction Lentivirus CD19 CAR	Viability
Day 3	Isolation TRAC KO, T naïve positive cells with Sony CGX10 Cryo-preservation	TRAC KO, T naïve Sort, Pre/post thaw CAR+, T naïve

Conclusion

Solupore transfection and CGX10 sorting maintained the Naïve state of T cells, with no increase in CD25 or CD69 expression. Solupore transfection preserves T cell health and function. Isolated cells from the CGX10 performed well in a cytotoxicity assay. Solupore, when paired with the CGX10 sorter for precise T cell population selection and purification, enhances the functional potency of cells.