End-to-end manufacturing of autologous CAR T cell therapies with the new Sefia™ cell therapy manufacturing platform

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Introduction

Gene-modified T cell therapies have shown clinical efficacy in targeting cancer, with promising results in the treatment of other diseases. However, the commercialization of these breakthrough therapies remains a challenge due to complexity and high cost of manufacturing. To facilitate scalable, cost-effective solutions, we have developed the Sefia™ cell therapy manufacturing platform, a modular, functionally closed technology consisting of the Sefia Select™ system to automate T cell magnetic isolation, washing and final formulation steps, and the Sefia expansion system to automate activation, transduction, and expansion steps of chimeric antigen receptor (CAR) T cells.

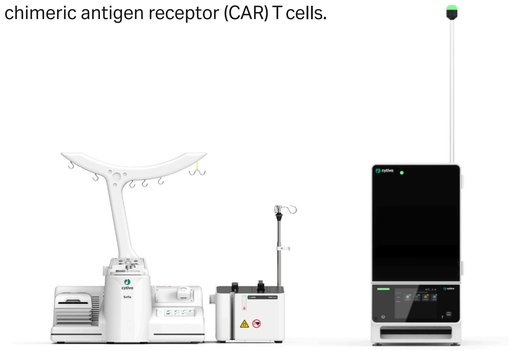


Fig 1. Sefia S-2000 with the Sefia Select module (left) used for T cell isolation, washing and formulation and Sefia expansion system (right) used for activation, transduction, and expansion.

Materials and methods

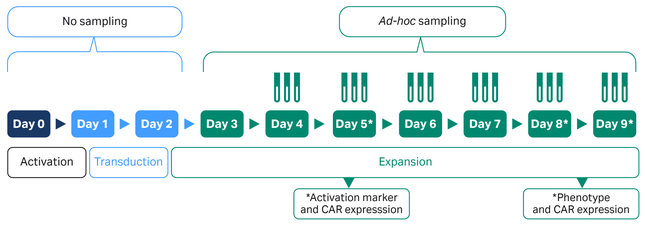
Sefia Select system, using MagnetSelect application software:

- Frozen (n = 3) and fresh (n = 3) leukopaks from healthy donors.
- Magnetic CD4/CD8 isolation.
- Wash out of excess beads and resuspension in serum-independent, xeno-free Akron ImmunoCell growth medium (Akron ICGM), supplemented with IL2.

Sefia expansion system, using Universal application software:

- Seed of isolated T cells (1×10^8 or 3×10^5 live cells/cm^2) in 150 mL of Akron ICGM.
- Activation with T Cell TransAct (10 µL/ 1 × 106 viable cells) on day 0 for 24 h.
- Transduction with CAR-lentiviral vector (LVV), MOI 2.5, on day 1 for 20 h.
- Expansion to 2×10^9 total viable cells.
- Analysis of cell recovery, viability, and phenotype on days 0, 5, and at harvest.

Final product formulation (3 cryobags) on Sefia Select system, using S-Wash and ReadySelect application software.



Sefia cell therapy manufacturing platform

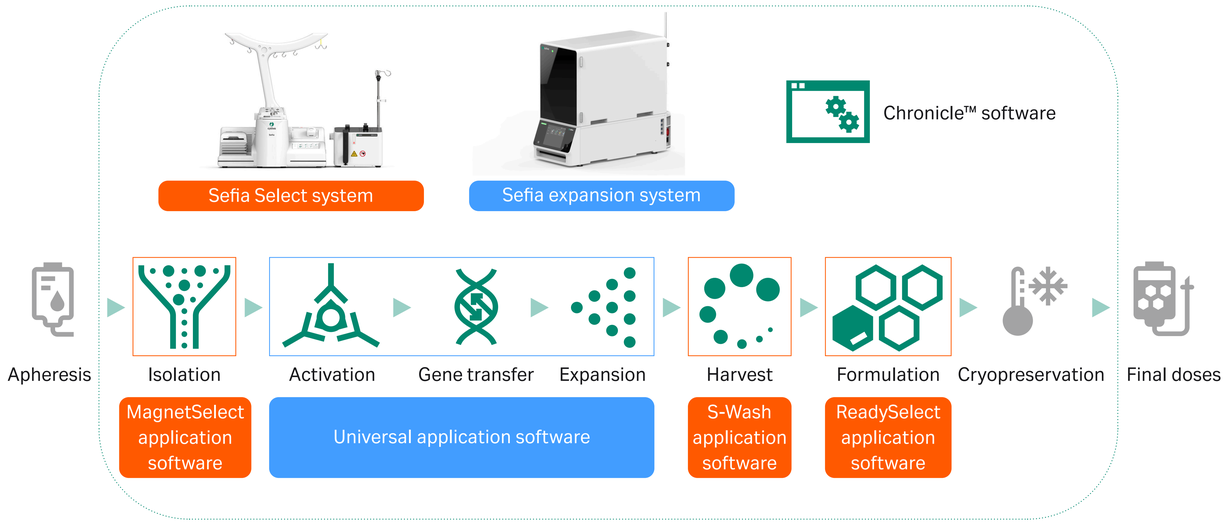


Fig 2. Cells are isolated with the Sefia Select system, and then transferred to the Sefia expansion system for activation, gene transfer, and expansion. Final wash and formulation are completed on the Sefia Select system. All the steps are orchestrated by dedicated and flexible applications. Parameter groups management is supported by Chronicle automation software.

Results

Table 1. Sefia Select system: magnetic isolation

MagnetSelect application software	Fresh apheresis healthy donor	Frozen apheresis healthy donor
Initial total white blood cells	$5.7 \times 10^9 \pm 4.6 \times 10^8$	$2.5 \times 10^9 \pm 2.2 \times 10^8$
Initial percentage of CD3	45.4% ± 11.6%	64.3% ± 4.3%
Average T cell recovery	66.3% ± 11.5%	54.8% ± 10.3%
Average T cell purity	88.0% ± 4.7%	92.0% ± 1.6%
Average platelet depletion	98.2% ± 1.2%	N/A
Average red blood cell depletion	93.5% ± 5.2%	N/A

Sefia expansion system: activation, transduction, and expansion

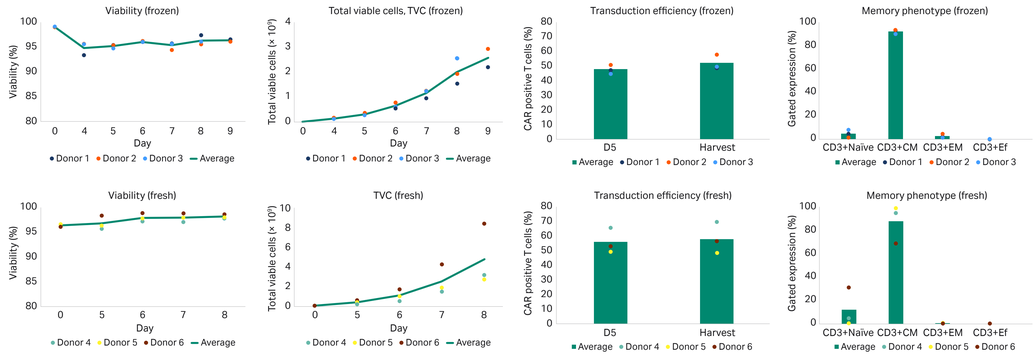


Fig 3. Biological performance of the Sefia expansion system. Cell viability was maintained high throughout the entire process. At day 5, flow cytometry analysis indicated a strong activation marker (CD25) expression: 84.2% ± 2.7% (frozen) and 99.3% ± 0.5% (fresh). At harvest (recovery from the culture vessels > 94%), the expanded T cells showed a central memory phenotype, with high levels of transduction efficiency.

Table 2. Sefia Select system: washing and formulation

S-Wash application software	Fresh apheresis healthy donor	Frozen apheresis healthy donor
Washout efficiency	99.9%	99.9%
Average cell recovery	90.9% ± 14.7%	92.5% ± 8.9%
Average cell viability loss	1.3% ± 1.1%	1.04% ± 0.2%
ReadySelect application software	**Fresh apheresis healthy donor**	**Frozen apheresis healthy donor**
Average cell recovery	106.1% ± 23.4%	99.8% ± 10.7%
Average cell viability loss	2.0%	2.2%

Conclusions

Here we present an end-to-end solution for autologous CAR T cell therapy manufacturing, using our new modular, and digitally integrated platform, that consists of two functionally closed systems:

- The Sefia Select system to magnetically isolate T cells from fresh or frozen apheresis, and to formulate the final product.
- The Sefia expansion system, which comprises application software (Universal App) and a single-use kit (Sefia expansion kit) to automate activation, transduction, and expansion steps for a a wide range of CAR T cell manufacturing workflows.

Both systems offer an application software with high flexibility to execute multiple workflows by enabling the selection of the desired user parameters, according to diverse reagents and input materials.

While the Sefia expansion system is a leading cell therapy solution with broad capabilities, Cytiva has not validated and verified all workflows or use cases. This poster describes one workflow.

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