The 6-chromanol Derivative SUL-238 Extends The Shelf Life of CAR-T Cells During Hypothermic Storage

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Introduction

Point-of-care (PoC) manufactured chimeric antigen receptor (CAR)-T cells represent a promising approach to overcoming various challenges associated with centralized CAR-T, such as lengthy lead times that may result in missed therapeutic windows.

Notably, CAR-T cells can also be given to patients freshly formulated, eliminating the need for cryopreservation, freeze-thaw cycles, and may help optimize the vein-to-vein time. For fresh formulation, CAR-T cells are stored in hypothermic preservation formulations (HPFs) at 2–8°C until administration. However, fresh CAR-T cell formulations can only be stored for ≤2 days due to limited stability, which may limit fresh CAR-T implementation.

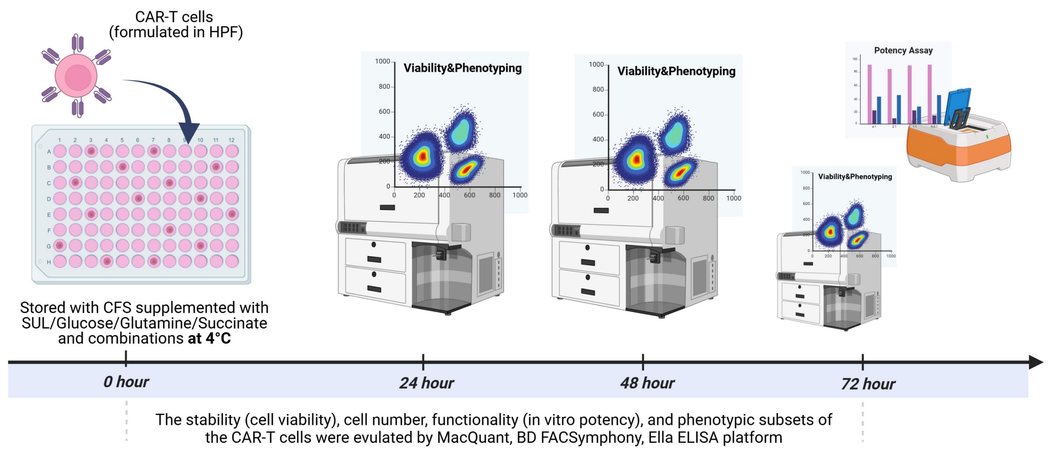
Here, we aimed to develop and characterize a novel HPF for the extended storage of PoC and fresh CAR-T cells, SUL -238, which is available in GMP-grade, enhances hypothermic preservation by maintaining mitochondrial integrity and sustaining cellular ATP production.

Objective(s)

The primary objective of this research was to develop a novel HPF (2–8°C) to enable the extended storage (at least 3 days) of PoC manufactured CAR-T cells.

- Assessing the stability-enhancing effects of the SUL-238 compound during a 3-day storage period.
- Investigating the functionality (in vitro potency assay) and phenotypic subsets of CAR-T cells stored during a 3-day storage period.

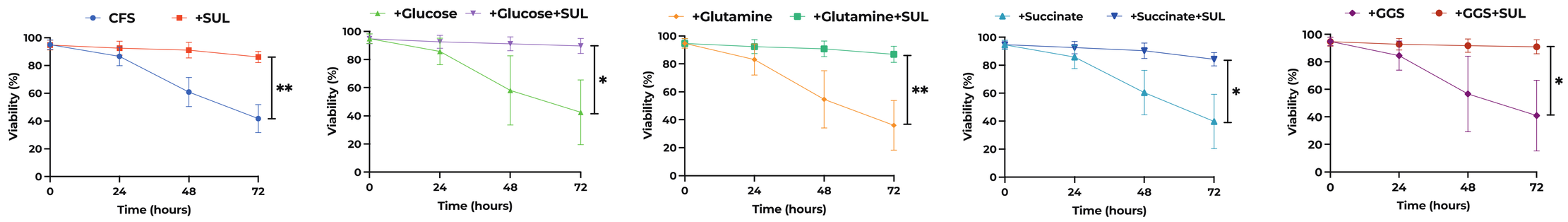
Method(s)



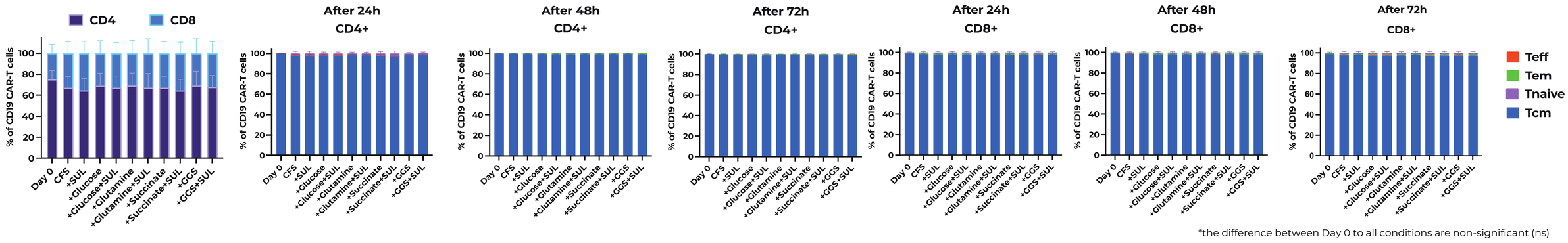
CAR: Chimeric Antigen Receptor; CFS: CliniMACS Formulation Solution PoC: Point-of-care

Result(s)

SUL-238 Enhances CAR-T Cell Survival During Hypothermic Storage at 4°C

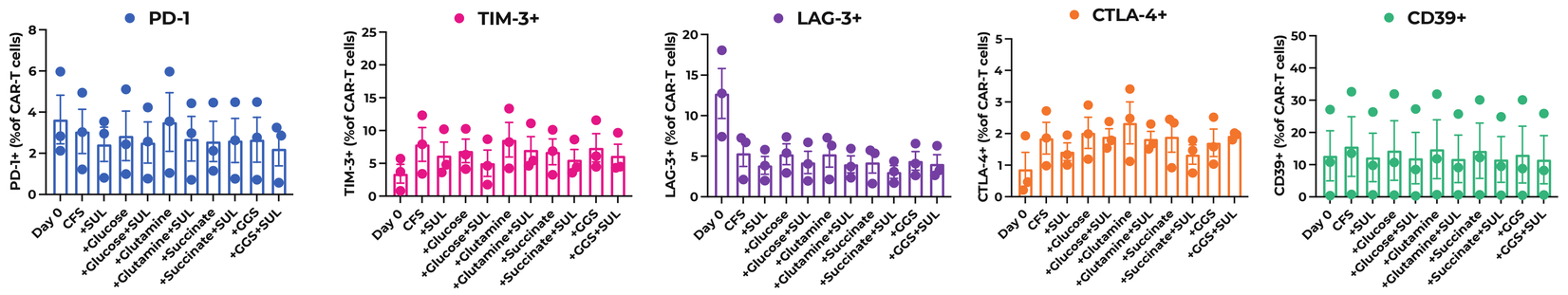


No Significant Phenotypic Shifts Observed in CAR-T Cell Subsets During Hypothermic Storage



*the difference between Day 0 to all conditions are non-significant (ns)

No Significant Expression of Exhaustion Markers in CAR-T Cells Stored Under Hypothermic Conditions



*the difference between Day 0 to all conditions are non-significant (ns)

Figure 1. Effects of Hypothermic Formulation Solutions Supplemented with SUL-238 on CAR-T Cell Stability and Phenotypic Characteristics During 3-Day Storage. **(A)** CAR-T cell viability was assessed every 24 hours using flow cytometry (n=3). **(B,C)** Phenotypic analysis of CAR-T cells was performed before and after 3 days of hypothermic storage using flow cytometry. Results are expressed as mean ± SD; statistical significance was determined using a unpaired t-test (*p < 0.05).

Conclusion(s)

- Storage for 3 days without SUL-238 resulted in a 50% reduction in cell viability, whereas the addition of SUL-238 preserved cell viability above 80%, preventing cell death.
- Neither the addition of other substrates/nutrients nor SUL-238 impacted the percentages of central memory/effector memory/naïve/ effector cells of the CAR-T cell population.
- CAR-T cells stored with SUL-238 exhibited lower exhaustion marker expression compared to the control group.
- These findings suggest that the SUL-238-containing formulation has the potential to extend the shelf life of CAR-T cells, making life-saving CAR-T therapy more accessible to patients.

References

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