

NanoSpark® STEM-T T Cell Activator Enhances Stem-like CD8⁺ T Cell Populations in G-Rex®6M Bioreactor

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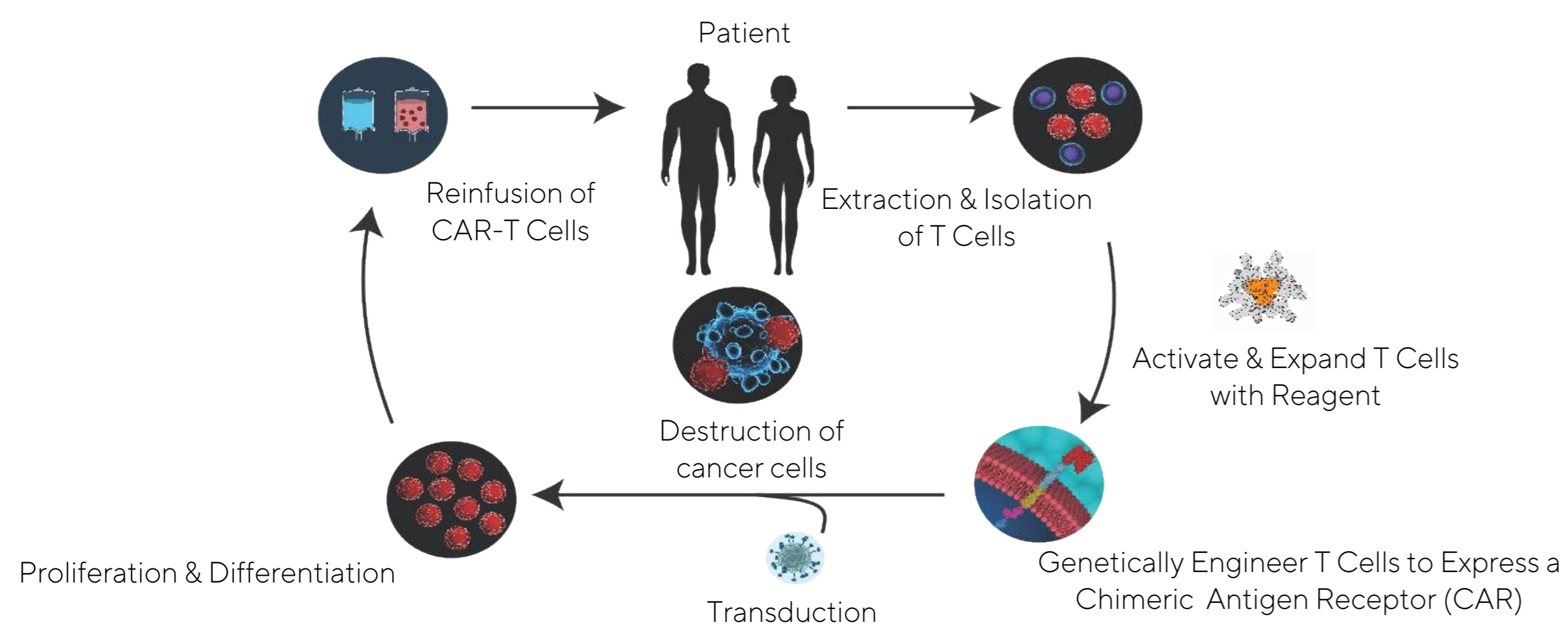
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Introduction

Cellular therapies are the cancer treatment of the future. Chimeric antigen receptor (CAR) T cell therapy has been a revolutionary advancement in cell and gene therapy and is currently, the most clinically and commercially advanced cell therapy. Despite their effectiveness, these therapies have encountered some obstacles including manufacturing failures, long manufacturing times, and short-lived or poor treatment outcomes. One solution to these challenges is optimizing the activation and expansion step of the manufacturing process. Nanotein's novel platform technology NanoSpark®, armed with anti-CD3 and anti-CD28 antibodies, is a soluble reagent that promotes the growth of stem-like, less differentiated T cells with a bias towards clinically relevant CD8⁺ stem cell-like memory T cells (T_{scm}) during 7 to 10-day expansions of donor T cells. These cells are long-lived, with high proliferative potential, and are capable of further differentiation and self-renewal, which can produce more durable responses in patients.

T Cell Manufacturing Process



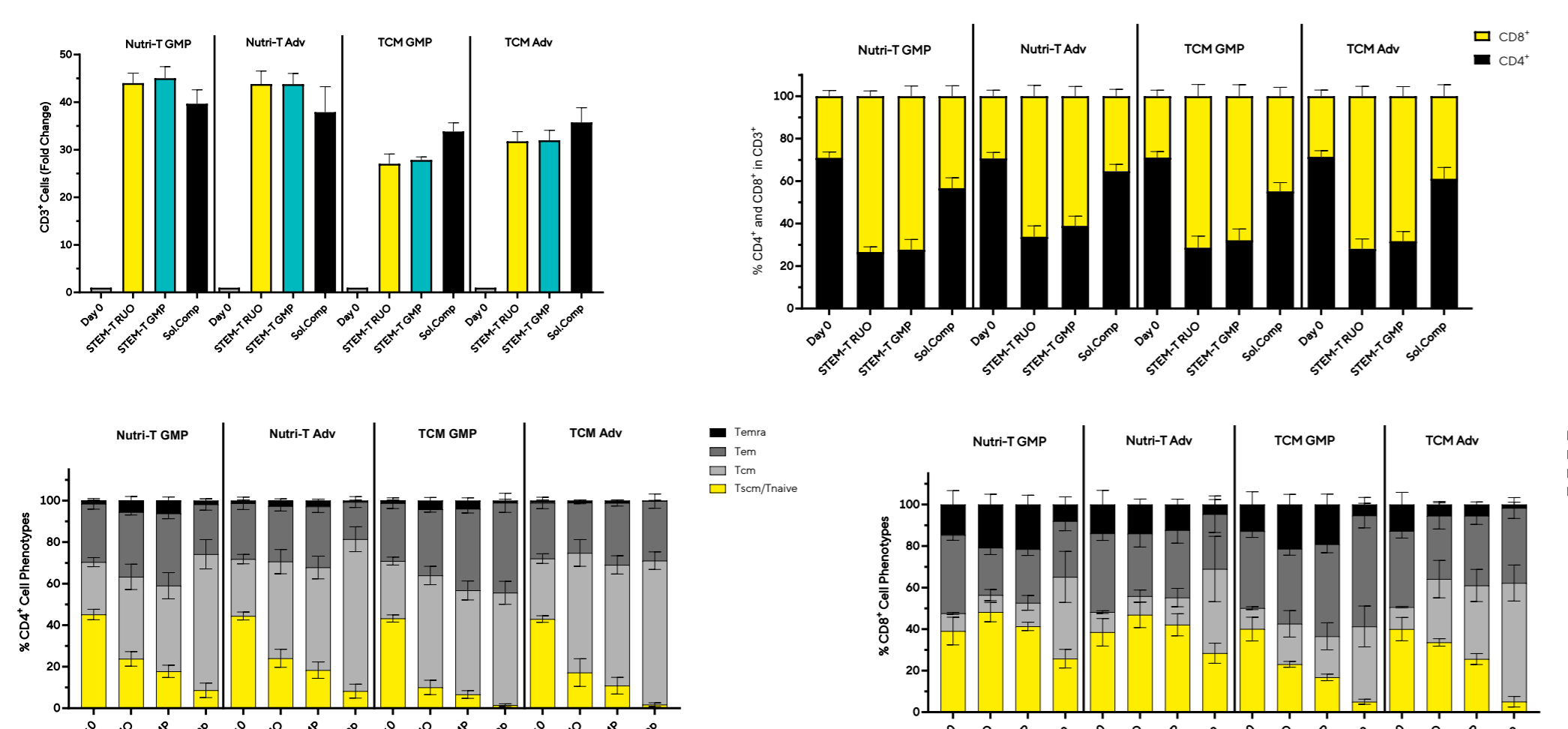
- Key challenge of CAR-T therapeutics is the manufacture of large numbers of long-lived, self-renewing CAR-T cells with increased efficacy that avoid patient relapse.
- Degradable, soluble, bead-free manufacturing reagents are ideal for scalable production of T cell products.

1. Experimental Approach

Cryopreserved CD3⁺ T cells (3 donors) were seeded at 1–1.5 × 10⁶ cells/well in G-Rex® 24 plates and 13–17 × 10⁶ cells/well in G-Rex® 6M plates, then activated with RUO or GMP versions of NanoSpark® STEM-T (8 μL/mL) or a soluble competitor according to the manufacturers' instructions. Cells were expanded in xeno-free media (4Cell® Nutri-T GMP, CellGenix® GMP TCM) or animal component-free media (4Cell® Nutri-T GMP Advanced, CellGenix® GMP Advanced TCM), supplemented with IL-2 (5 ng/mL), IL-7 (10 ng/mL), and IL-15 (15 ng/mL), without serum. T cells were transduced with lentiviral vector at MOI = 3, 24 hours after activation, with a scFv-41BB-CD3ζ, FMC63 construct. Phenotypic markers (CD4, CD8, CD45RA, CCR7, CD95), activation markers (CD25, CD69), and exhaustion markers (LAG-3, PD-1) were assessed by flow cytometry on days 0, 3, 5, 7, and 10. Transduction efficiency and total CAR⁺ T-cell counts were measured on day 10.

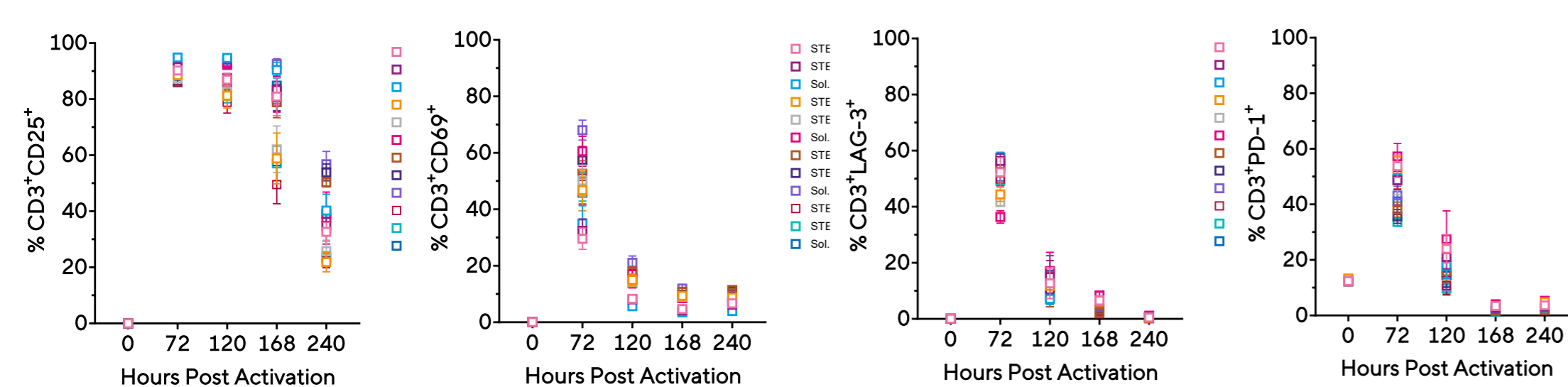


2. Results – Fold Expansion and Phenotypes in G-Rex®24



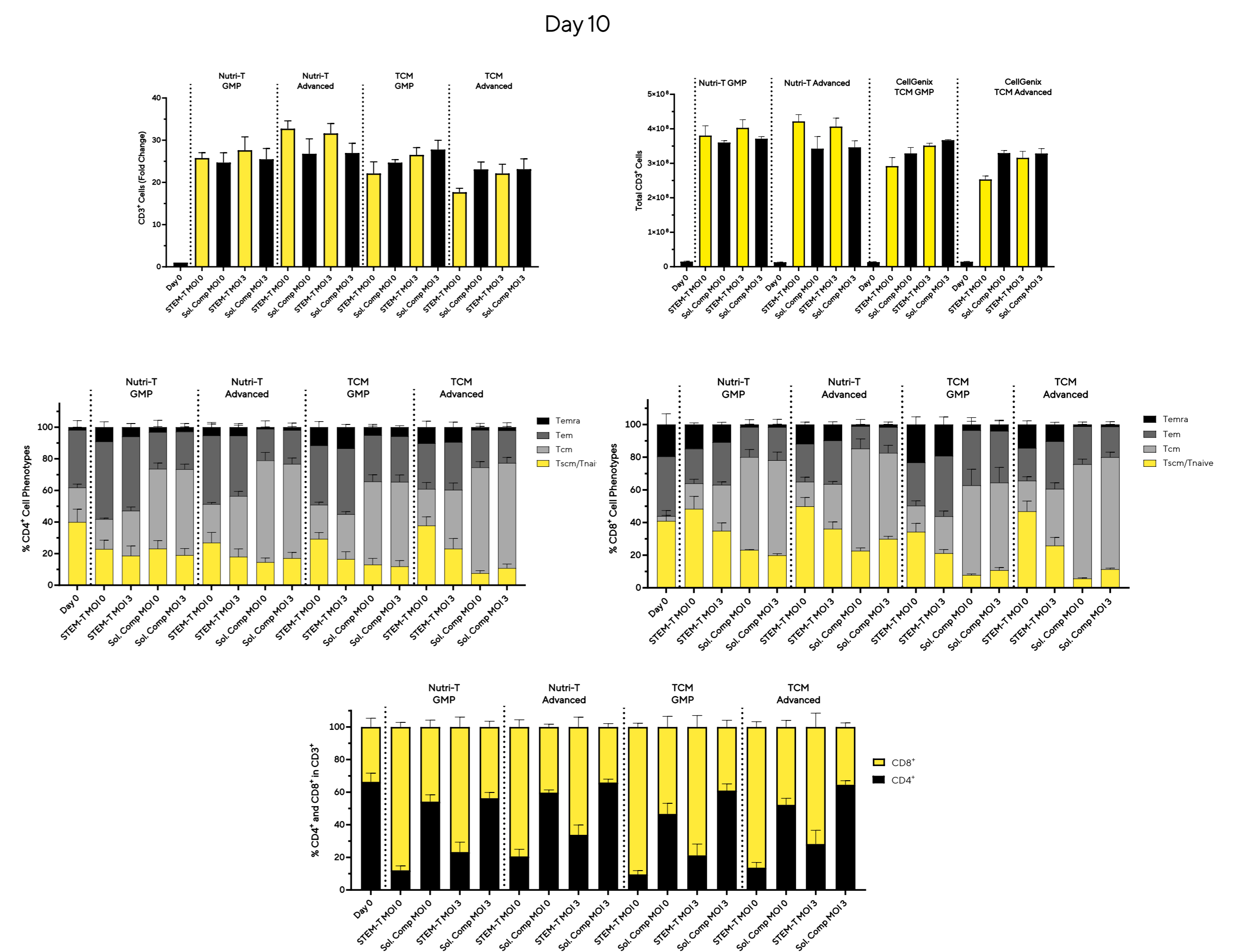
- NanoSpark® STEM-T RUO performs comparably to NanoSpark® STEM-T GMP, matching the CD3⁺ T-cell expansion achieved with the soluble competitor and driving robust stem cell-like CD8⁺ T-cell expansion across all media tested in G-Rex® 24-well plates.

3. Results – Activation and Exhaustion Markers in G-Rex®24



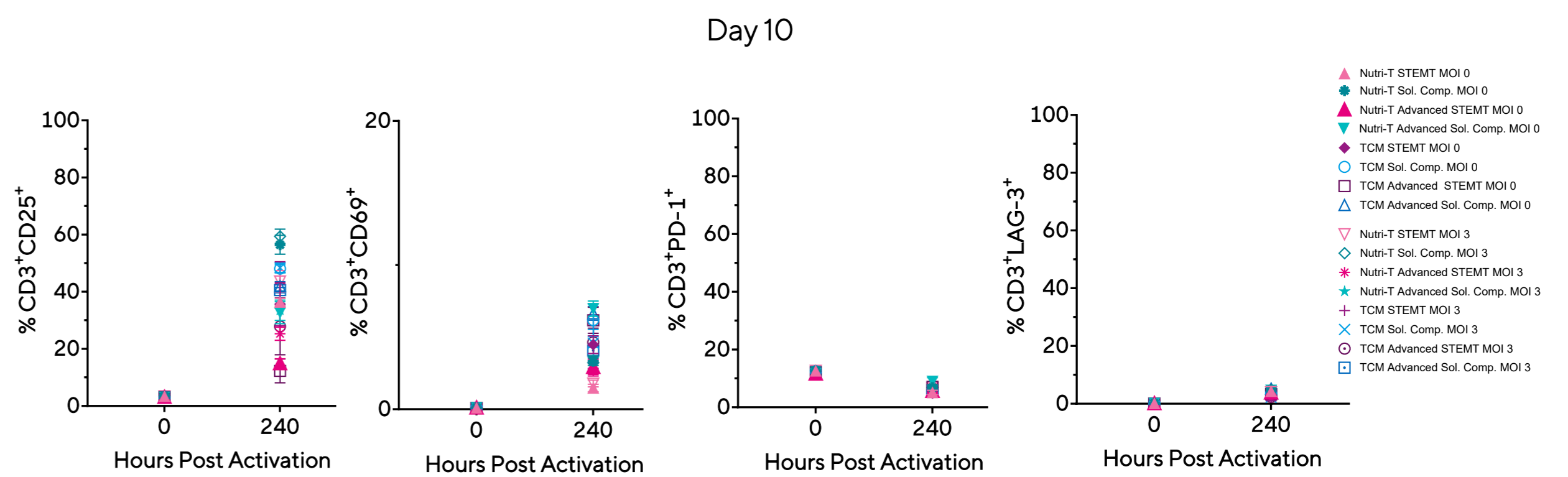
- NanoSpark® STEM-T GMP boosts the expression of activation markers CD25 and CD69 in T cells, with peak levels observed at 48–72 hours post-activation, while maintaining low levels of exhaustion markers PD-1 and LAG-3 in CD3⁺ T cells throughout the activation timeline—mirroring the performance of the RUO version across all media tested in G-Rex® 24-well plates.

4. Results – Fold Expansion and Phenotypes in G-Rex®6M



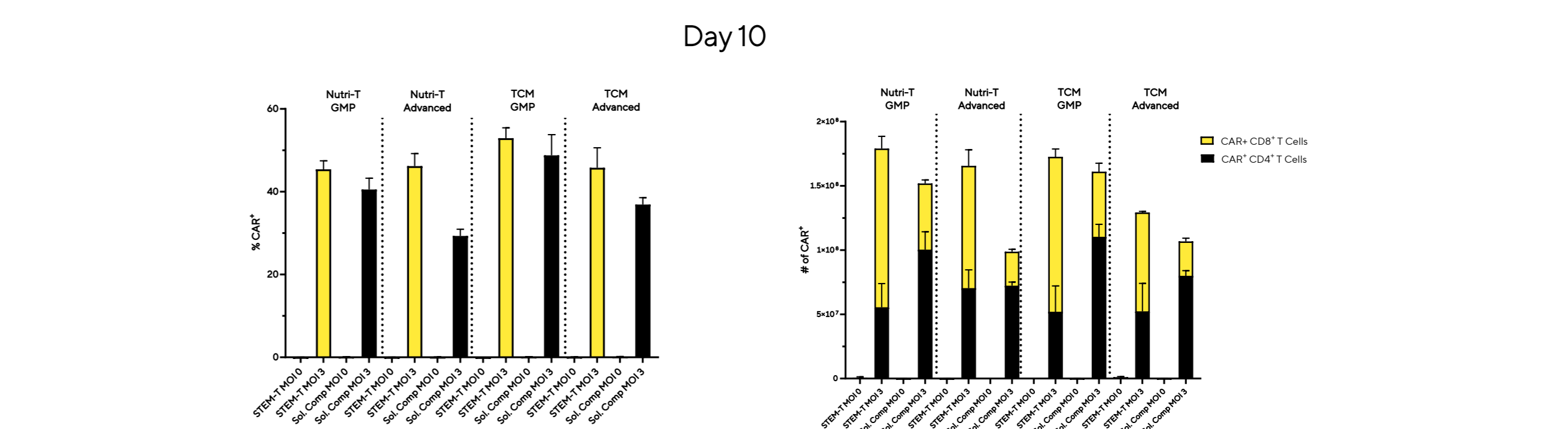
- NanoSpark® STEM-T preserves the therapeutically favorable, self-renewing T_{scm} phenotype in both CD4⁺ and CD8⁺, while effectively promoting robust CD8⁺ T-cell expansion across all Sartorius media in G-Rex® 6M plates.

5. Results – Activation and Exhaustion Markers in G-Rex®6M



- NanoSpark® STEM-T gently activates CD3⁺ T cells compared to the soluble competitor, while maintaining low levels of exhaustion markers PD-1 and LAG-3 in G-Rex® 6M plates.

6. Results – Transduction Efficiency and Total CAR⁺ Cells in G-Rex®6M



- NanoSpark® STEM-T exhibited the highest transduction efficiency in 4Cell® Nutri-T GMP, 4Cell® Nutri-T GMP Advanced, CellGenix® GMP TCM and CellGenix® GMP Advanced TCM without the need for Polybrene or spinfection, and consistently generated a greater number of CD8⁺ CAR⁺ T cells than the soluble competitor across all media tested.

7. Conclusion

- NanoSpark® STEM-T RUO performs comparably to NanoSpark® STEM-T GMP, supporting strong CD3⁺ T-cell expansion, promoting stem-like CD8⁺ T-cell phenotypes, and maintaining gentler activation with low exhaustion marker expression in G-Rex® 24-well plates.
- Findings from G-Rex® 24-well plates were successfully replicated in G-Rex® 6M plates, demonstrating scalability across formats.
- NanoSpark® STEM-T enabled higher lentiviral transduction efficiency and produced more CD8⁺ CAR⁺ T cells than the soluble competitor across all Sartorius media tested.

Explore more about NanoSpark® immune cell activators

