

# NanoSpark® STEM-T T Cell Activator Enhances Stem-like CD8+ T Cell Populations

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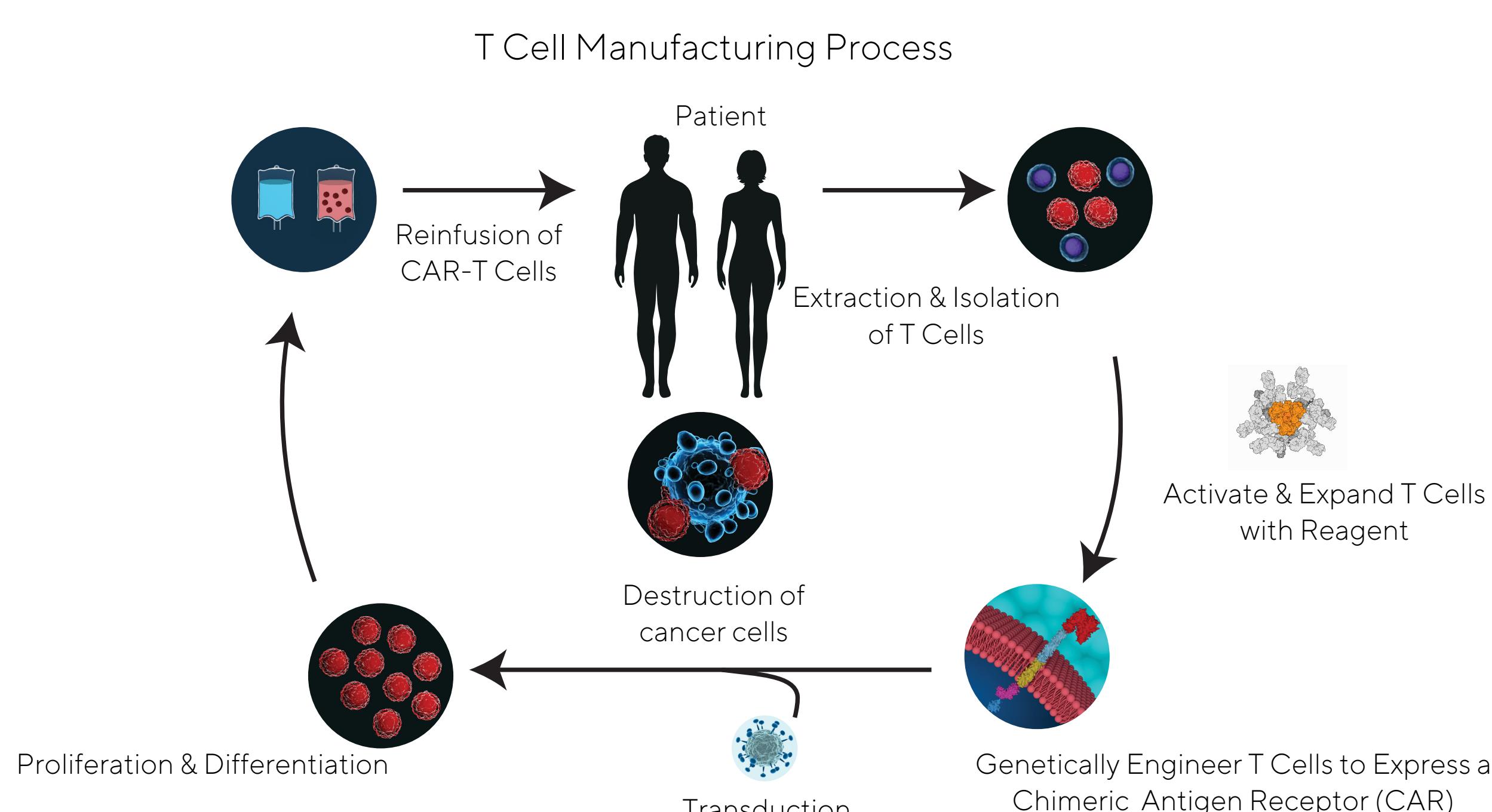
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## Introduction

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.



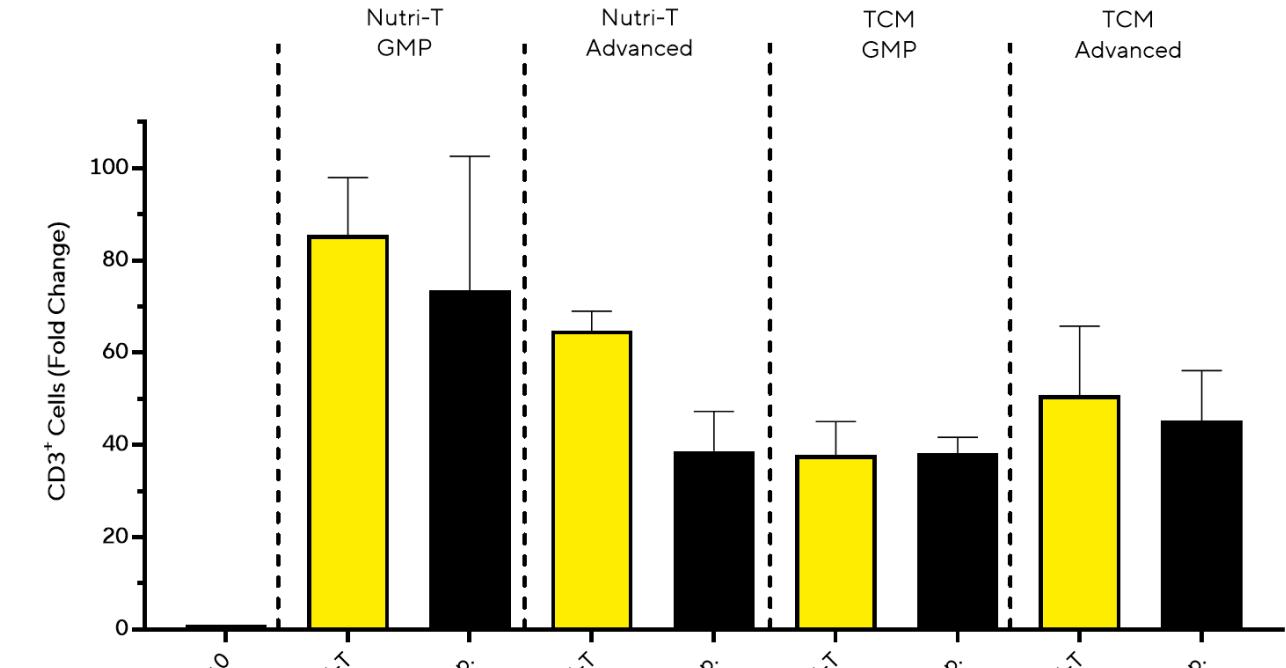
- Key challenge of CAR-T therapeutics is the manufacture of large numbers of long-lived, self-renewing CAR-T cells with increased efficacy that avoid patient relapse
- Degradable, soluble, bead-free manufacturing reagents are ideal for scalable production of T cell products

## 1. Experimental Approach

Cryopreserved CD3+ T cells (3 donors) were seeded in 24-well plates ( $3 \times 10^5$  cells/well) and activated with NanoSpark® STEM-T (8  $\mu$ L/mL) and a competitor activator per manufacturer's instructions. Cells were expanded in xeno-free media (4Cell® Nutri-T GMP, CellGenix® GMP TCM) and 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), IL-15 (15 ng/mL) without serum. On day 6, cells were transferred to G-Rex® 24 and cultured until day 10. Phenotyping (anti-CD4, CD8, CD45RA, CCR7, CD95), activation (anti-CD25, CD69), and exhaustion (anti-LAG-3, PD-1) markers were assessed by flow cytometry on days 0, 2, 3, 7, 10; phenotyping on days 0, 7, 10.

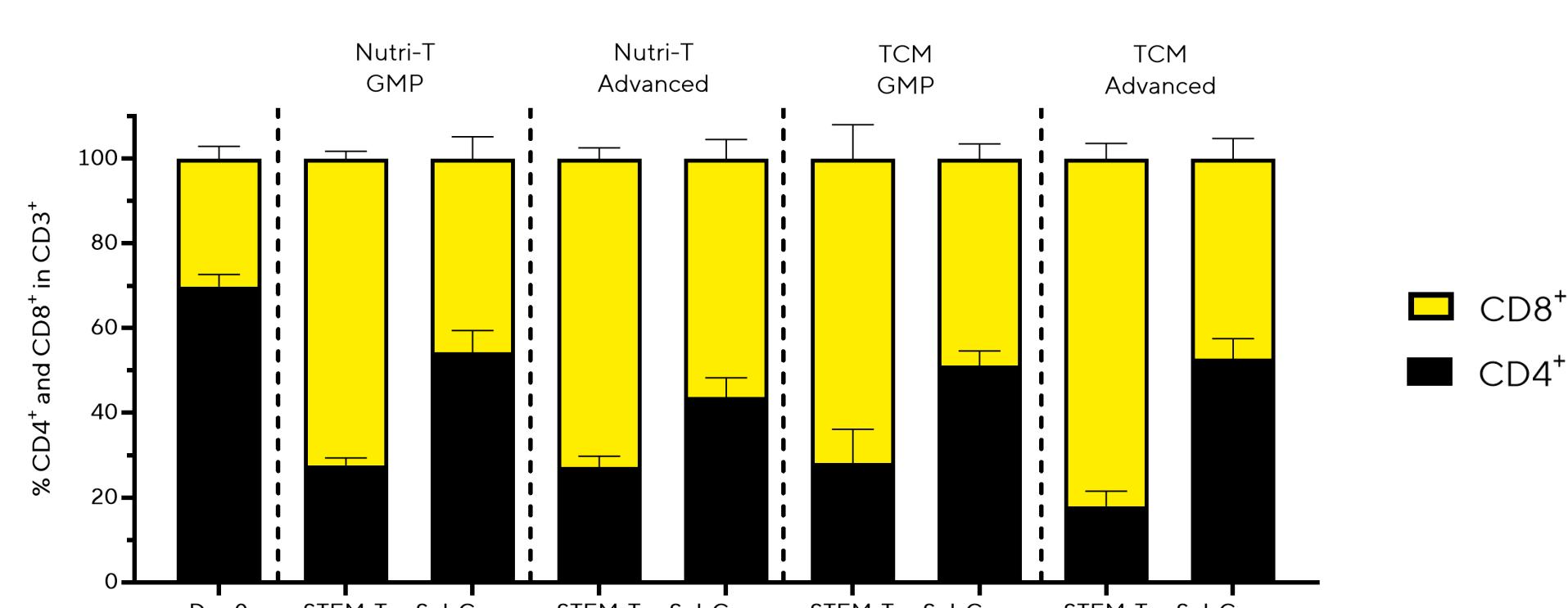


## 2. Results – Fold Expansion



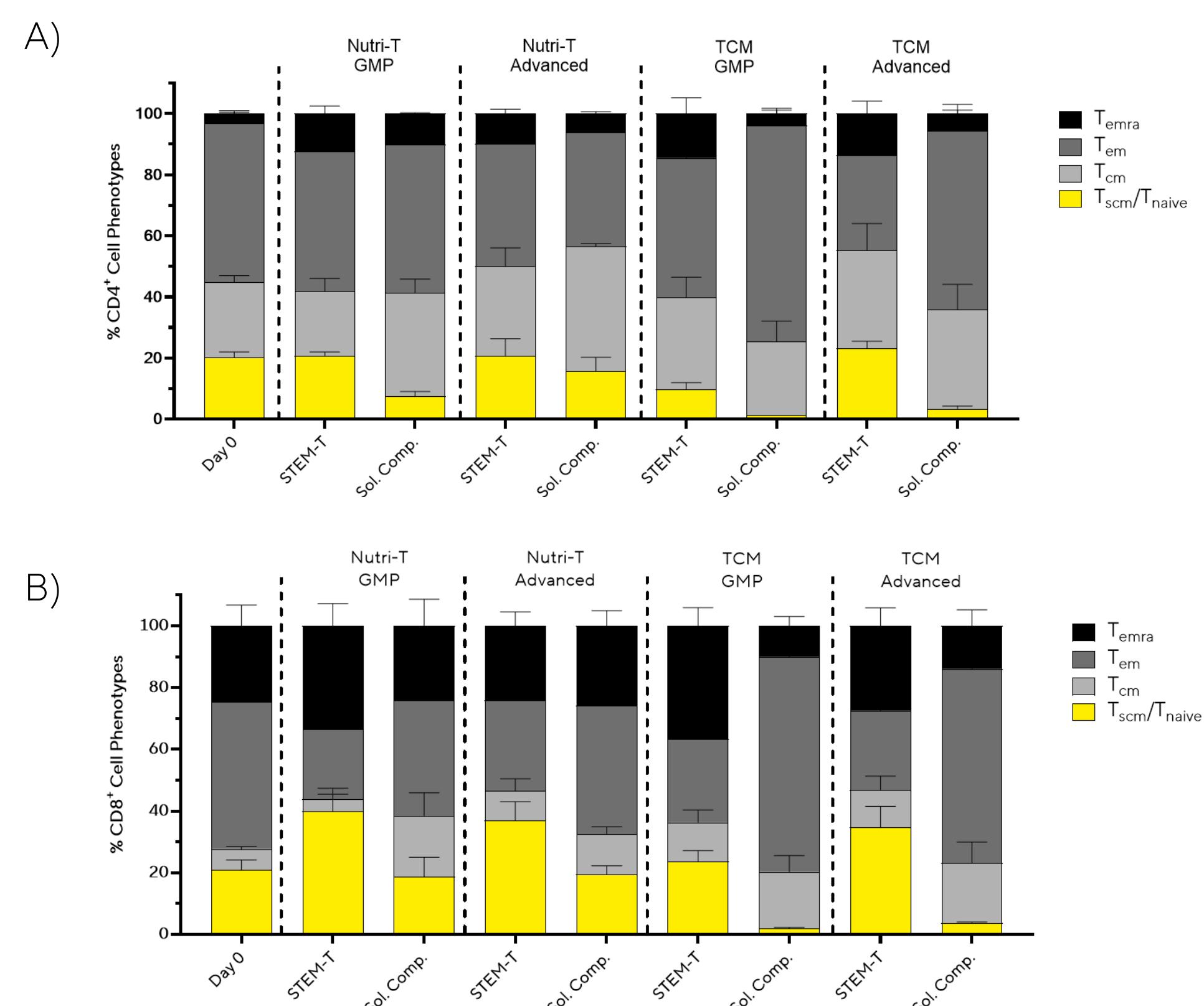
- NanoSpark STEM-T matches CD3+ T cell expansion of soluble competitor manufacturing reagent across all media tested in G-Rex® culture

## 3. Results – CD4/CD8 Ratio



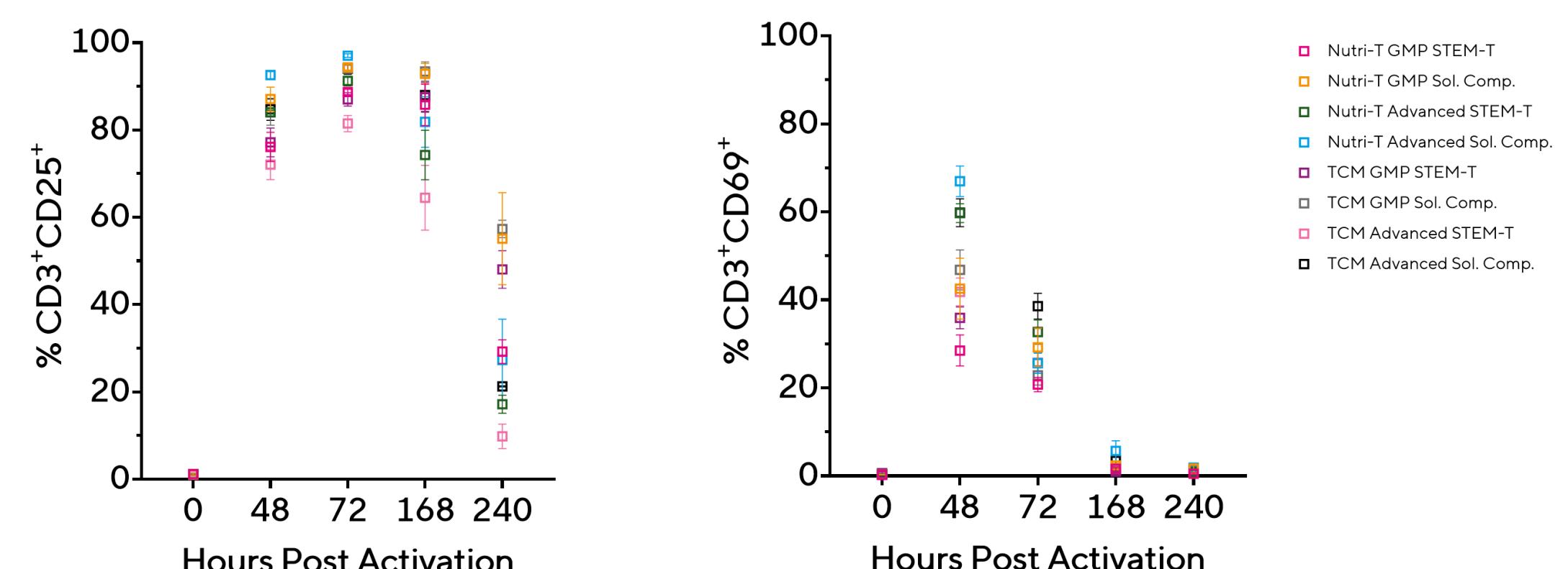
- NanoSpark STEM-T stimulates robust expansion of CD8+ T cells in G-Rex® culture across all media

## 4. Results – T Cell Phenotypes

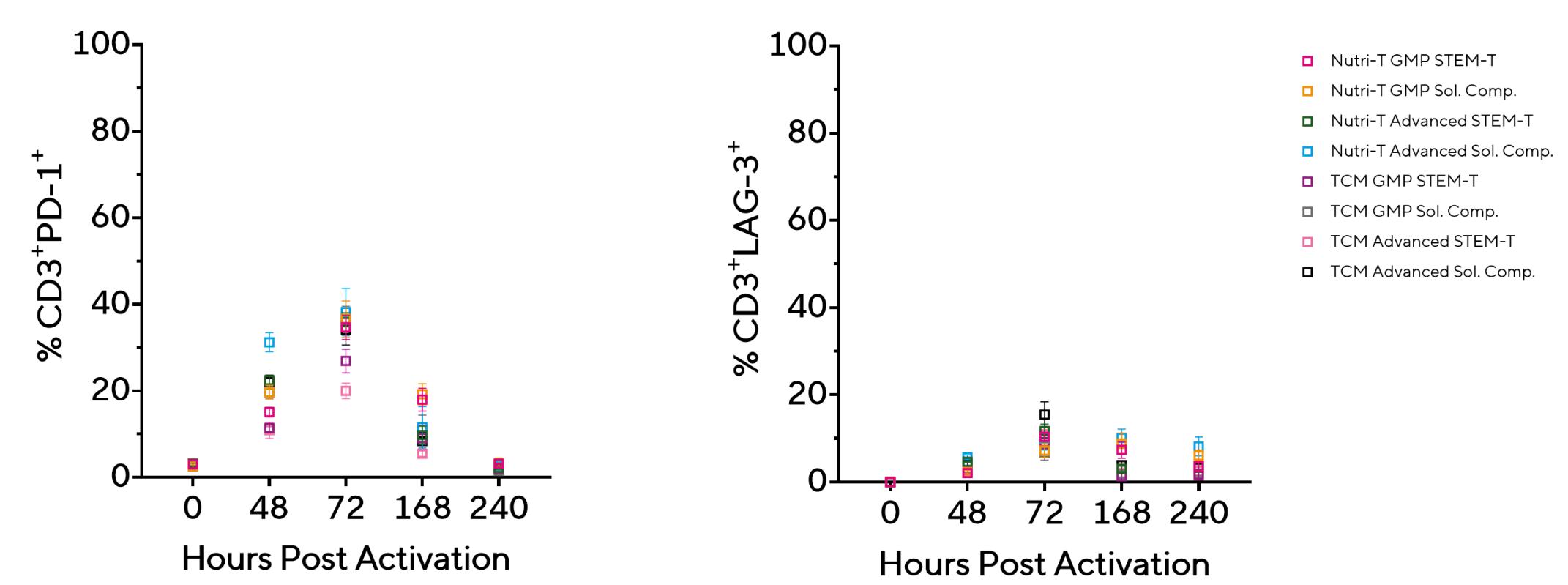


- NanoSpark® STEM-T preserves the therapeutically favorable, self-renewing  $T_{scm}$  phenotype in CD4+ and CD8+ on day 10 of expansion in all of Sartorius' suite of media

## 5. Results – Activation and Exhaustion Markers



- NanoSpark® STEM-T promotes gentler activation than soluble competitor as indicated by slightly lower expression of CD25 and CD69
- We hypothesize that this gentle activation is why  $T_{scm}$  phenotypes are more prevalent in NanoSpark® STEM-T activated cells on day 10 of expansion



- NanoSpark® STEM-T exhibits lower expression of exhaustion markers PD-1 and LAG-3 in comparison to the soluble competitor
- We believe this lower expression is also due to gentler activation of T cells by NanoSpark® STEM-T

## 6. Conclusion

- NanoSpark® STEM-T generates more CD8+ T cells than the soluble competitor across multiple donors and cell culture media
- Cells activated by NanoSpark® STEM-T have more stem-like T cells and lower exhaustion markers expressed in comparison to another soluble T cell activator
- NanoSpark® STEM-T has gentler activation of T cells in comparison to the soluble competitor as measured by CD25 and CD69 expression
- NanoSpark® STEM-T has comparable total cell expansion to the soluble competitor in all media tested

Explore more about NanoSpark® immune cell activators

