

# Activation and expansion of MART-1-specific CD8+ T cells in PBMCs from healthy individuals

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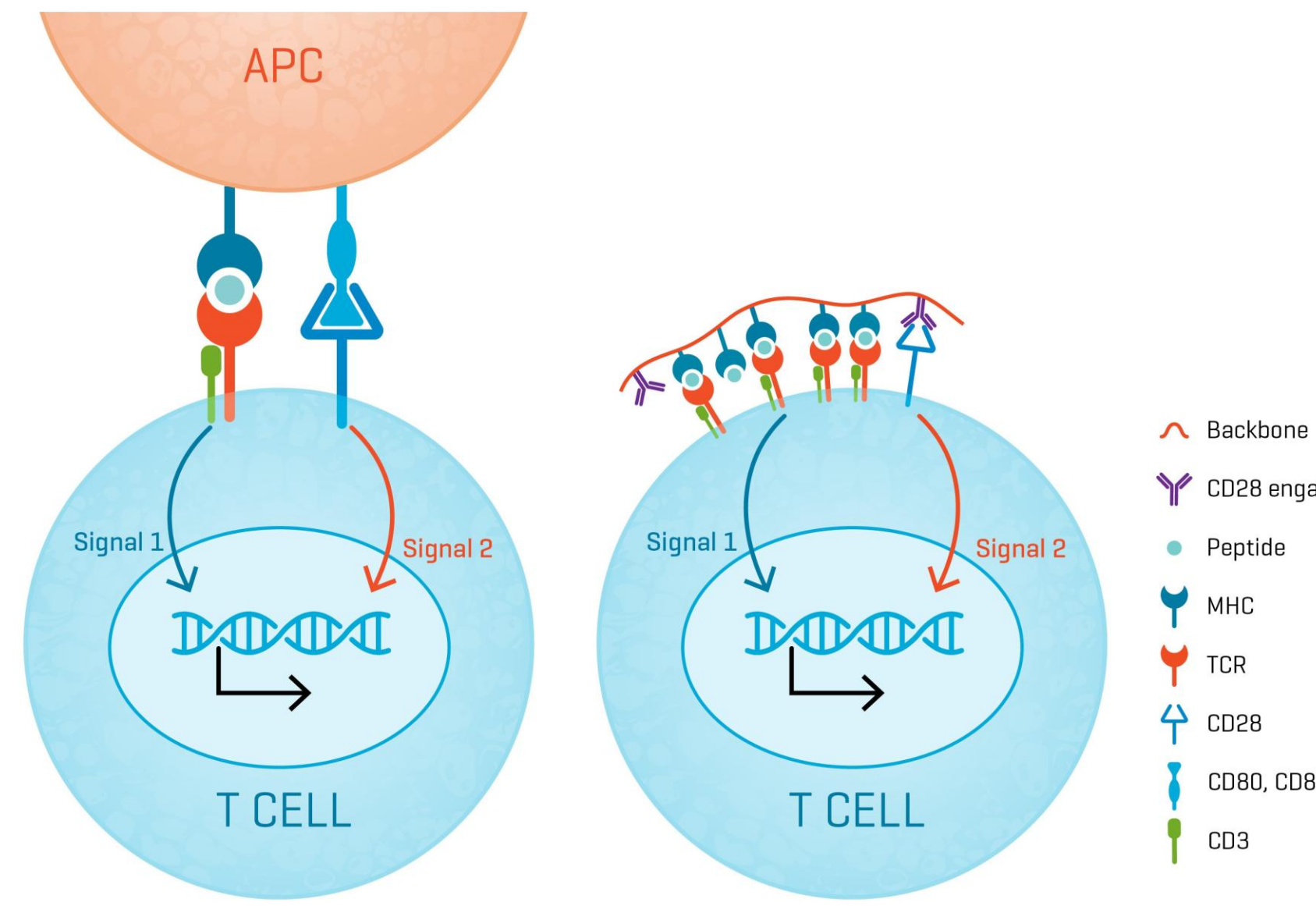
## Artificial antigen presenting cell scaffold

### Introduction

Antigen-specific T cells play a central role in adaptive immunity and are key targets in immunotherapy and immune monitoring. However, their low frequency in peripheral blood mononuclear cells (PBMCs) presents a challenge for detection and analysis.

To overcome this, robust methods for in vitro stimulation and expansion are required to selectively enrich these rare populations, enabling functional characterization and downstream applications such as flow cytometry, cytotoxicity assays, and TCR sequencing.

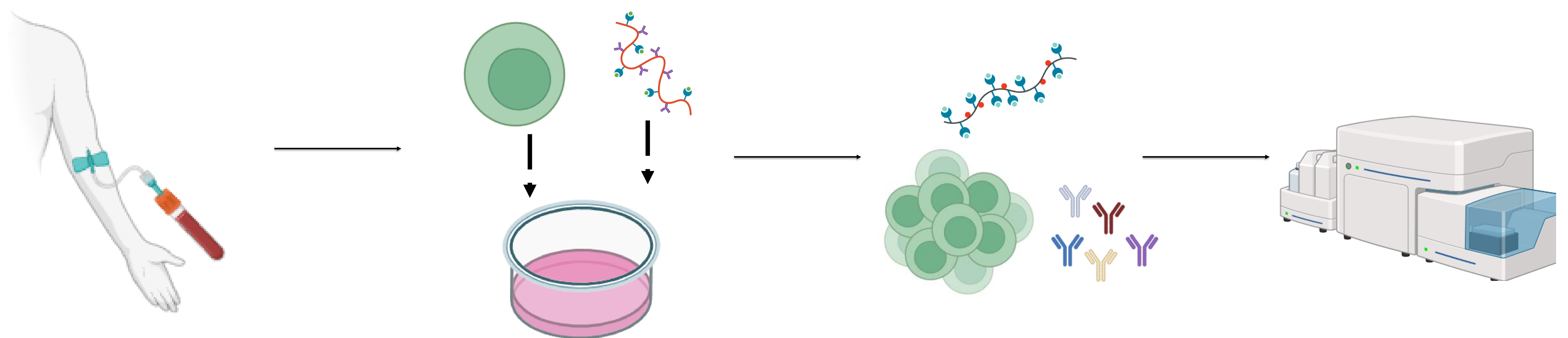
In this study, we demonstrated how Xynapse™-T can expand naïve antigen-specific CD8+ T cells without negatively affecting their differential and functional status, using MART-1-specific T cells as a model system as these cells are known to display a naïve phenotype [1]. The differential and functional status was compared with peptide-expanded cells.



### What is Xynapse™-T?

Xynapse™-T is an artificial scaffold comprising an optimized number of MHCp monomers and CD28-engaging molecules, providing both TCR-specific and co-stimulatory signals to T cells.

## Expansion of MART-1 specific CD8+ T cells from healthy donor PBMCs with Xynapse™-T

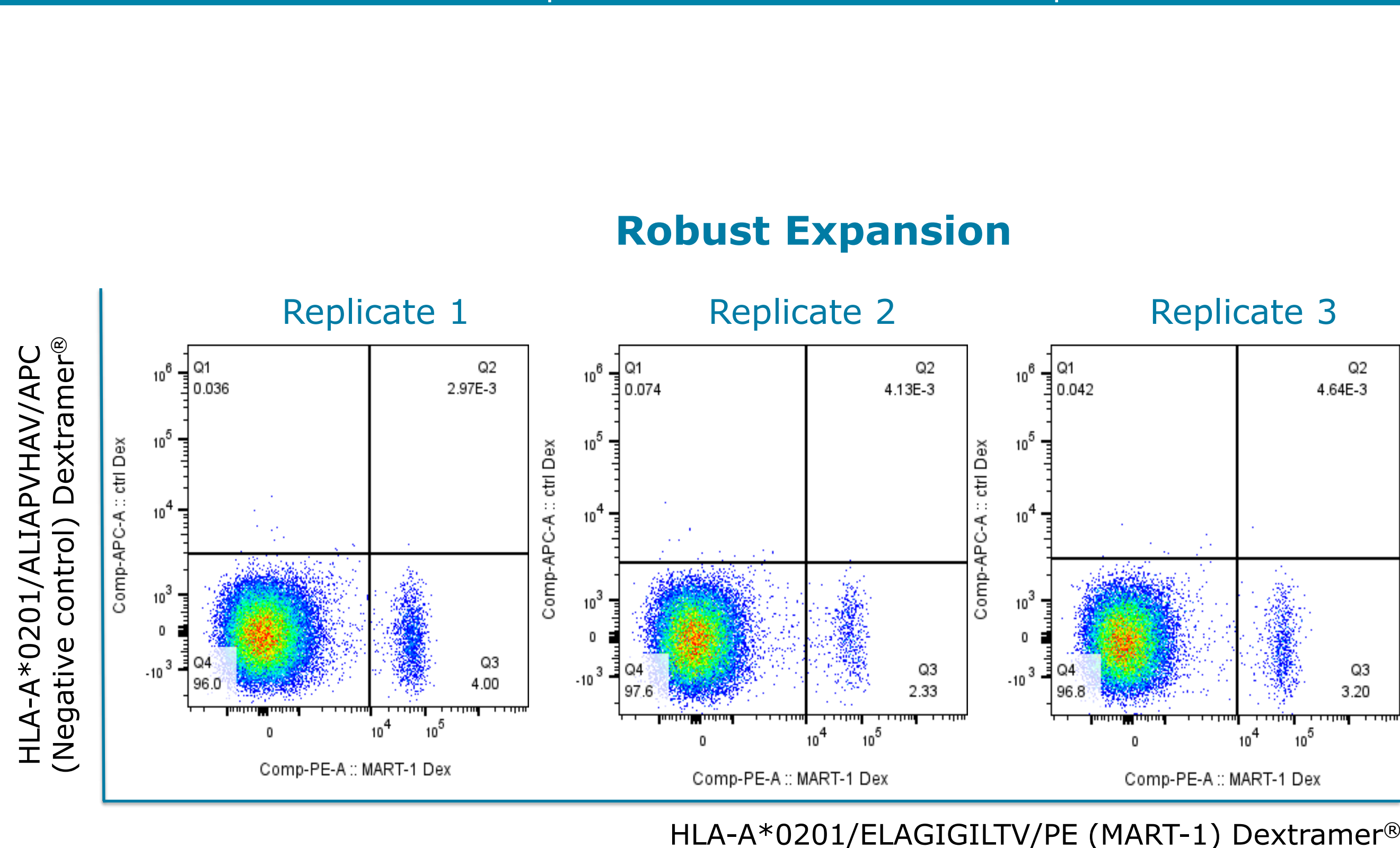


Human PBMCs were isolated from a healthy HLA-A\*02:01 donor. CD3+ T cells were then enriched from PBMCs using negative selection.

CD3+ T cells were stimulated with MART-1-specific Xynapse™-T reagent and also added a negative control Xynapse™-T reagent. The cells were cultivated for 14 days at 37°C in 5% CO<sub>2</sub> with medium refreshed every 2–3 days.

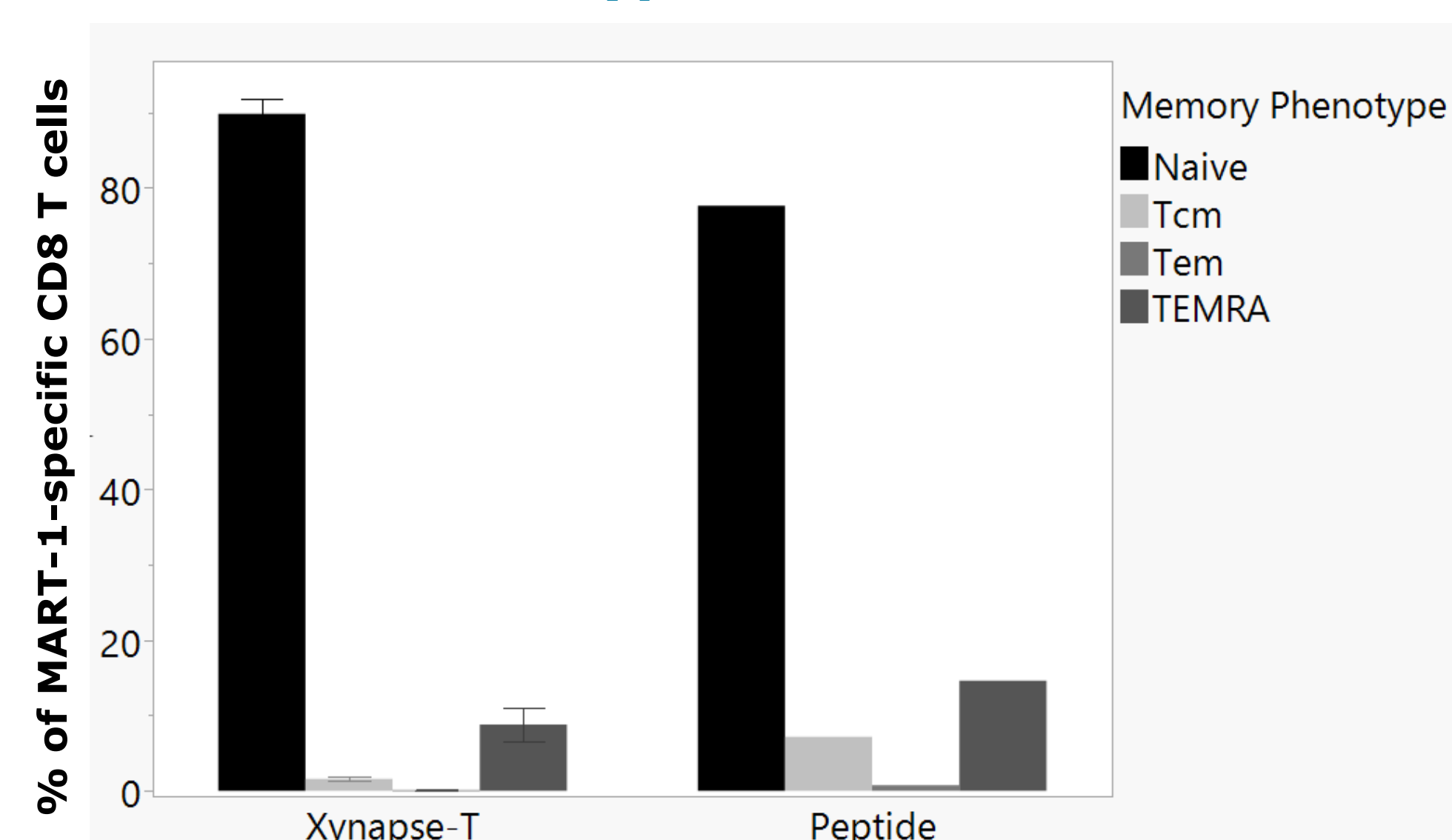
After 14 days of culture, cells were harvested and expanded cells analysed by flow cytometry assessing frequency and phenotypic characteristics. MART-1-specific CD8+ T cells were detected using HLA-A\*0201/MART-1 Dextramer®. A surface marker antibody panel consisting of CD45RA, CCR7, CD28, CD27, LAG-3, TIM-3 and PD-1 antibodies was used to investigate memory phenotype, activation status and exhaustion profile.

## Expansion of MART-1 specific CD8+ T cells from healthy donor PBMCs with Xynapse™-T



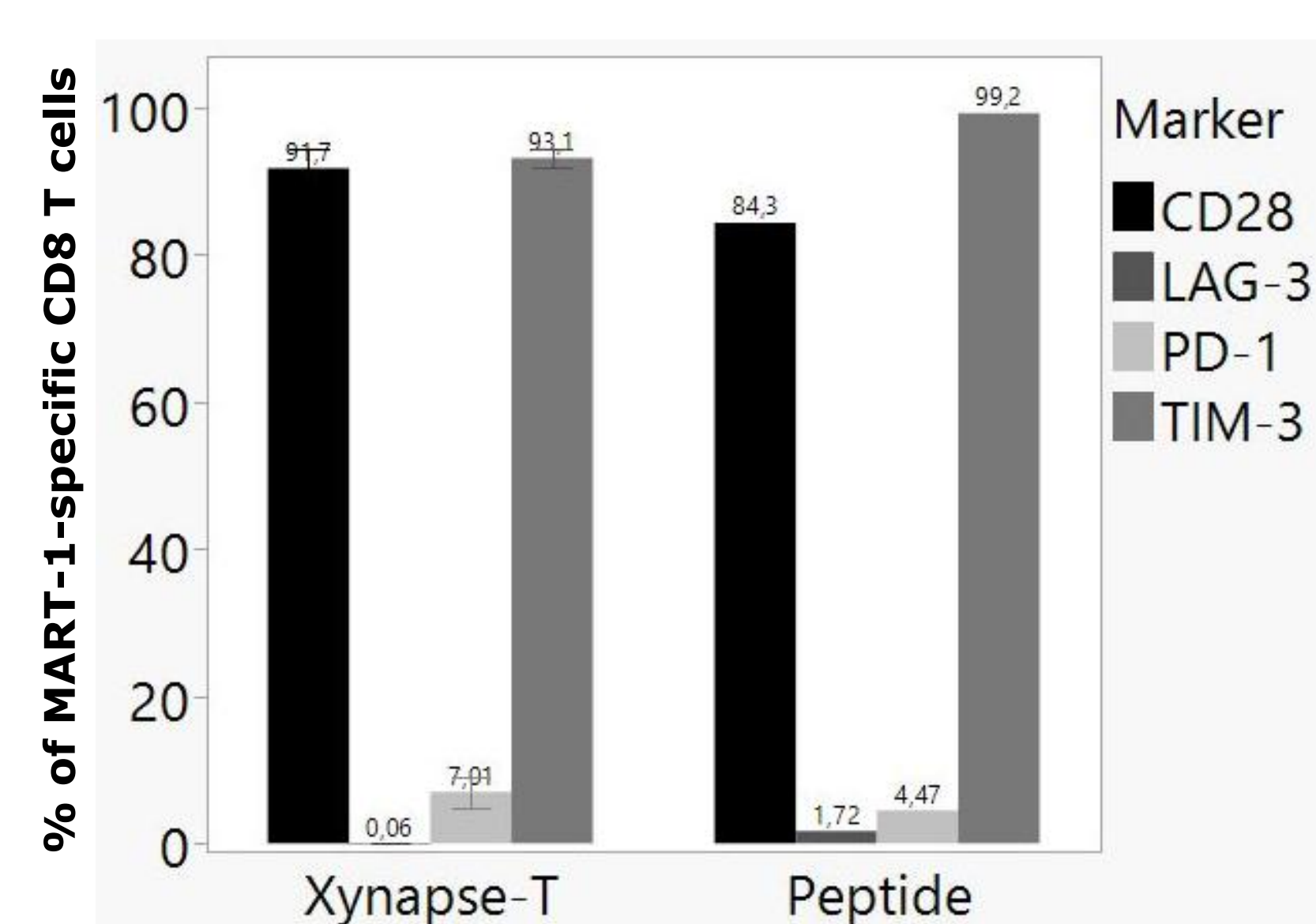
**Figure 1.** Xynapse™-T expanded MART-1-specific CD8+ T cells in peripheral blood from a healthy donor, starting from a barely undetectable baseline level. The findings were consistently reproducible across three independent replicates.

### Naïve Phenotype Preservation



**Figure 2.** The HLA-A\*0201/MART-1 Dextramer® CD8+ T cells exhibit a naïve phenotype with ~90% of cells being CD45RA<sup>+</sup>/CCR7<sup>+</sup>. The findings are comparable with peptide-expanded cells.

### Favorable Functional Profile



**Figure 3.** The majority of the expanded MART-1-specific cells (92%) express the activation marker CD28. Only a minor subset of cells (<1% and 7%) express the T cell exhaustion markers LAG-3 and PD-1, respectively. The early activation marker TIM-3 is expressed by 93% of the cells.

The functional profile of Xynapse™-T-expanded cells is comparable with peptide-expanded cells.

## Conclusion

### Robust Expansion

- Xynapse™-T expanded MART-1-specific CD8+ T cells 64-fold compared to baseline.

### Naïve Phenotype Preservation

- A high proportion of expanded cells remained naïve (CD45RA<sup>+</sup>/CCR7<sup>+</sup>) (90%).

### Favorable Functional Profile

- Xynapse™-T primed T cells expressed CD28, which mediates co-stimulation necessary for activation and survival. The cells also displayed a TIM3<sup>+</sup>/PD1<sup>-</sup>/LAG3<sup>-</sup> phenotype not consistent with exhaustion. High TIM3 expression levels alongside expression of other checkpoint receptors (such as PD1 and LAG3) is a characteristic exhausted or dysfunctional T cells. However, our flow data does not measure expression levels. We suggest that the CCR7<sup>+</sup>/CD45RA<sup>+</sup>/TIM3<sup>+</sup>/PD1<sup>-</sup>/LAG3<sup>-</sup> phenotype of the Xynapse™-T primed cells indicates that they are transitioning from naïve to early memory differentiation.

### Overall Implication

- Xynapse™-T generates MART-1-specific CD8+ T cells with strong proliferative capacity, a predominantly naïve phenotype, and a non-exhausted functional profile. The memory phenotype and the functional profile is comparable to peptide stimulation.



[1] Pittet, M. J. et al. (1999). High frequencies of naïve Melan-A/MART-1-specific CD8(+) T cells in a large proportion of human histocompatibility leukocyte antigen (HLA)-A2 individuals. *J Exp Med*, 190(5), 705-715. doi:10.1084/jem.190.5.705.

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