



Feasibility of Automated GMP Manufacturing of Tumor-Reactive T Cells from Antigen-Naïve Healthy Donors and Allogeneic Transplant Recipients

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Background & Aim

Tumor-reactive T cells (TRT) have demonstrated clinical efficacy in hematologic malignancies and solid tumors, but conventional manufacturing relies on labor-intensive, manual processes performed in high-grade GMP facilities. Automated closed GMP-compliant platforms may improve scalability and inter-institutional adoption. We evaluated the feasibility of producing clinical-grade TRT for relapse prevention in patients with acute myeloid leukemia (AML) after hematopoietic stem cell transplant (HSCT) using the CliniMACS Prodigy[®] system, with optional expansion in a closed GREX-CS[®] device to maximize throughput and minimize clean-room dependence.

Methods

For full-scale GMP validation, leukapheresis products from three healthy antigen-naïve donors ($n = 3$, one run per donor) $0.6\text{--}1.3 \times 10^9$ mononuclear cells [MNC] were stimulated for 36–40 h with PepTivator[®] peptide pools targeting PRAME, Survivin, and WT1 in the presence of CD28 costimulation. Activated (CD137⁺) T cells were enriched by immunomagnetic selection and expanded using irradiated feeder cells, high-dose IL-2, and CD3 stimulation (figure 1). To assess feasibility using engrafted T cells and to validate GREX-CS[®] expansion, 60–100 mL of peripheral blood from two recent pediatric transplant recipients (11 yo girl 6-month s/p allogeneic umbilical cord blood transplant and 13 yo boy 5-month s/p MUD bone marrow transplant) were processed at $\sim 1/10^{\text{th}}$ of full scale ($0.5\text{--}1 \times 10^8$ starting MNC) using manual enrichment and closed-system GREX 10M-CS[®] expansion. Open steps were performed in ISO 5 biosafety cabinet, while closed systems were operated in an unrated laboratory.

Results

Initial stimulation activated 2.41–10.58% viable CD3⁺ cells (vCD3). Prodigy[®]-based CD137⁺ enrichment (figure 2) yielded $0.32\text{--}6.01 \times 10^6$ CD137⁺ vCD3 ($0.6\text{--}8.41 \times 10^6$ vCD3, 21.2–79.5% selection efficiency), which expanded to $81.9\text{--}1,963 \times 10^6$ vCD3 within 10–12 days. Manual enrichment from 10^8 MNC yielded $0.18\text{--}0.29 \times 10^6$ CD137⁺ vCD3 ($1.0\text{--}2.8 \times 10^6$ vCD3, 92–94% recovery of CD137⁺ cells) which were expanded in GREX to $20.4\text{--}49.5 \times 10^6$ vCD3 over 14 days. All final products met predefined release criteria, including sterility, endotoxin, and mycoplasma testing, and demonstrated antigen-specific functional activity. Cytokine secretion and activation marker expression exceeded background controls for both CD4⁺ and CD8⁺ subsets: CD4⁺ T cells showed IFN- γ (2.46–7.79 vs 0.16–0.19%, $p < 0.000001$), TNF- α (9.47–28.57 vs 0.43–1.74%, $p < 0.000001$), CD137 (8.32–25.03 vs 0.41–0.54%, $p < 0.000001$), and CD154 (6.95–28.36 vs 0.97–1.90%, $p = 0.000016$), while CD8⁺ T cells demonstrated IFN- γ (1.18–23.58 vs 0.06–0.24%, $p = 0.000002$), TNF- α (0.65–26.12 vs 0.39–1.55%, $p = 0.000001$), and CD137 (11.89–43.62 vs 3.29–18.71%, $p = 0.000001$), figures 3–4 show results of a representative run. Notably, TRT products manufactured by this method were also enriched for $\gamma\delta$ T cells (31.59 – 42.11% of CD3⁺ cells), figure 5.

Conclusions

Clinical-grade TRT can be reproducibly manufactured from both antigen-naïve donors and allogeneic transplant recipients using CliniMACS Prodigy[®] platform with optional GREX[®] expansion in unrated laboratory space, supporting scalable and decentralized manufacturing. The TRT product demonstrates robust antigen-specific reactivity and high content of gamma-delta T cells, which further increases its therapeutic potential and reduces the risk of GVHD.

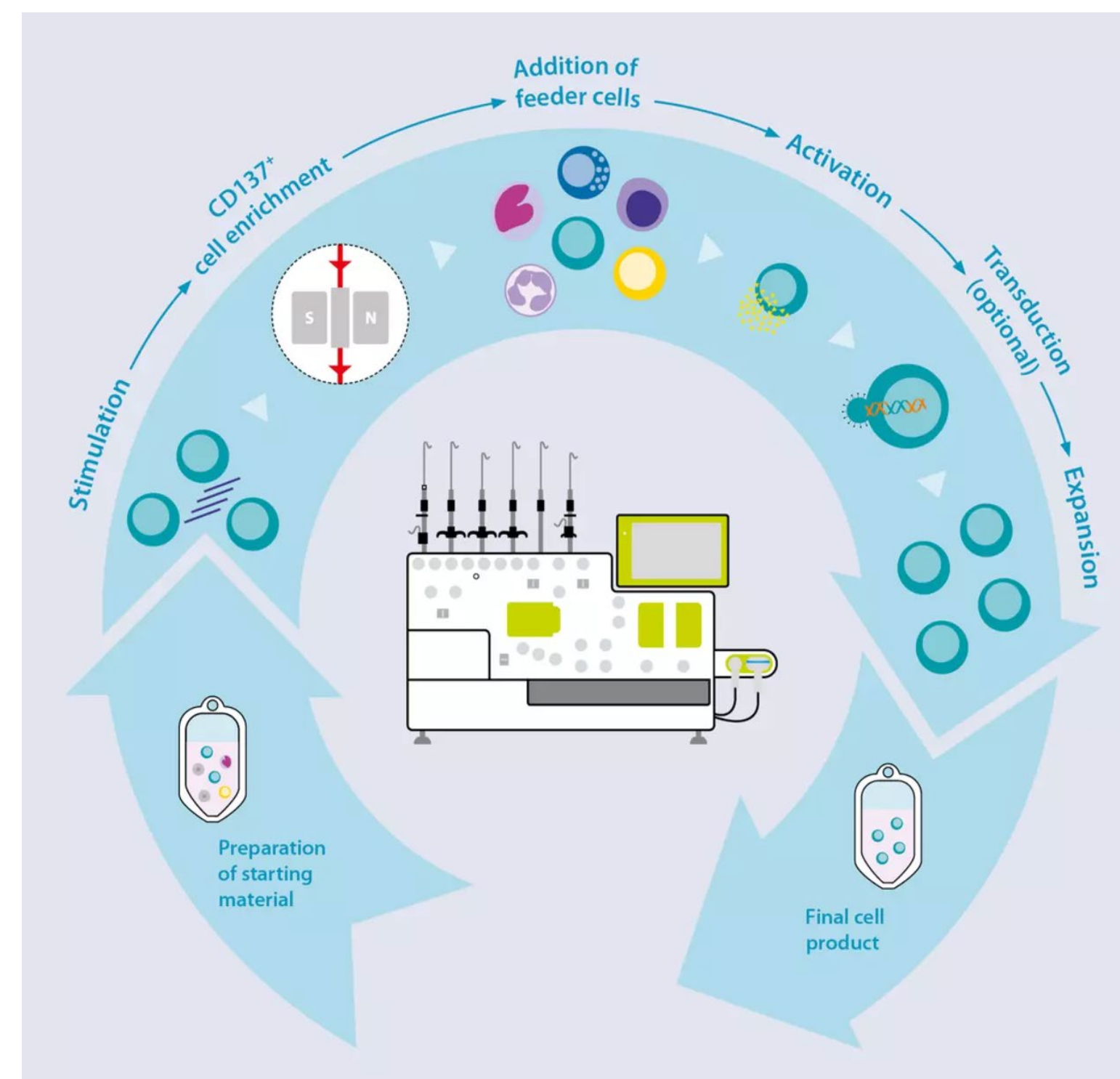


Figure 1. TRT Manufacturing workflow on CliniMACS Prodigy[®].

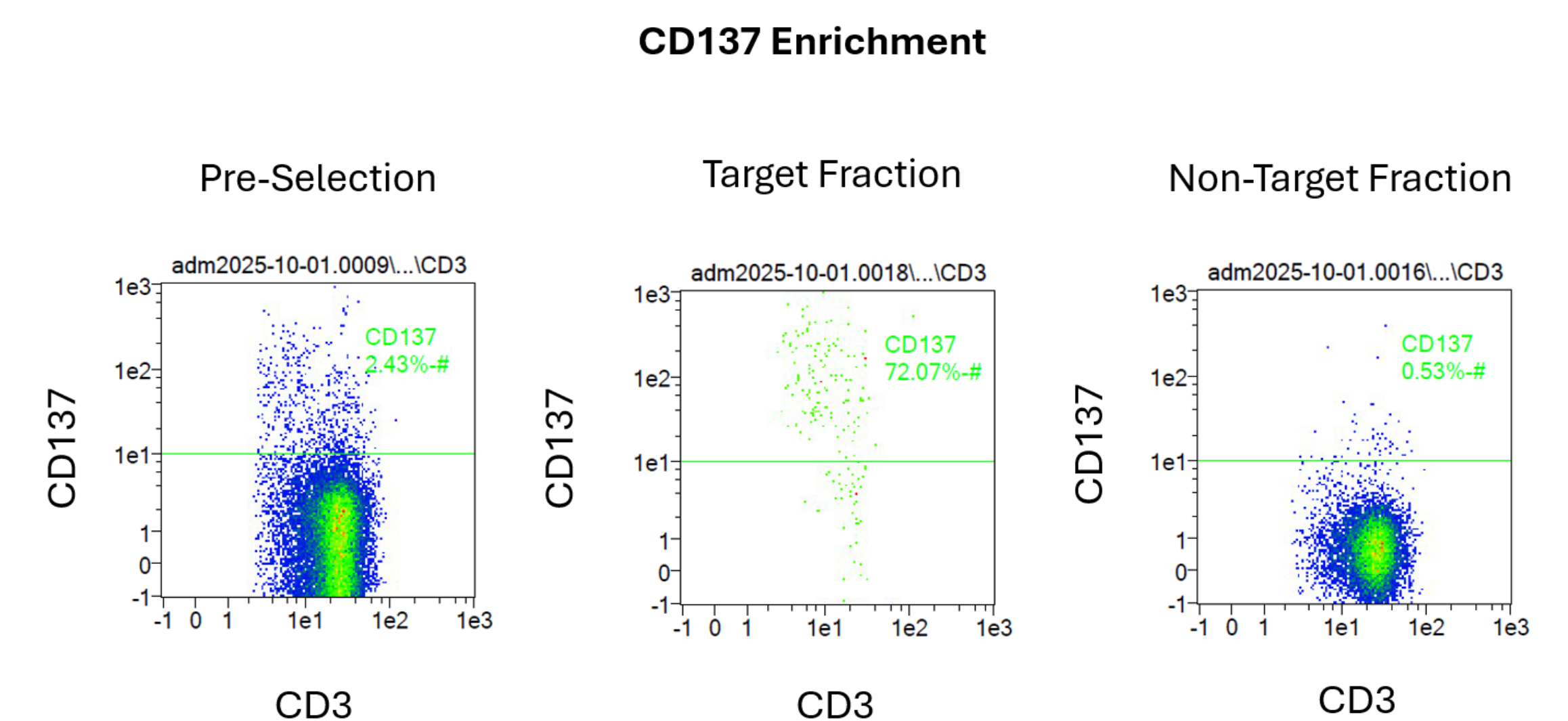


Figure 2. CD137 Enrichment. Following MNC stimulation with mixed tumor-associated peptide pools (GMP-grade PepTivators[®]: PRAME, Survivin, WT1), CD137⁺ cells underwent immunomagnetic selection on CliniMACS Prodigy[®]. Left: stimulated MNC prior to selection. Middle: target fraction. Right: non-target fraction. Representative run shown.

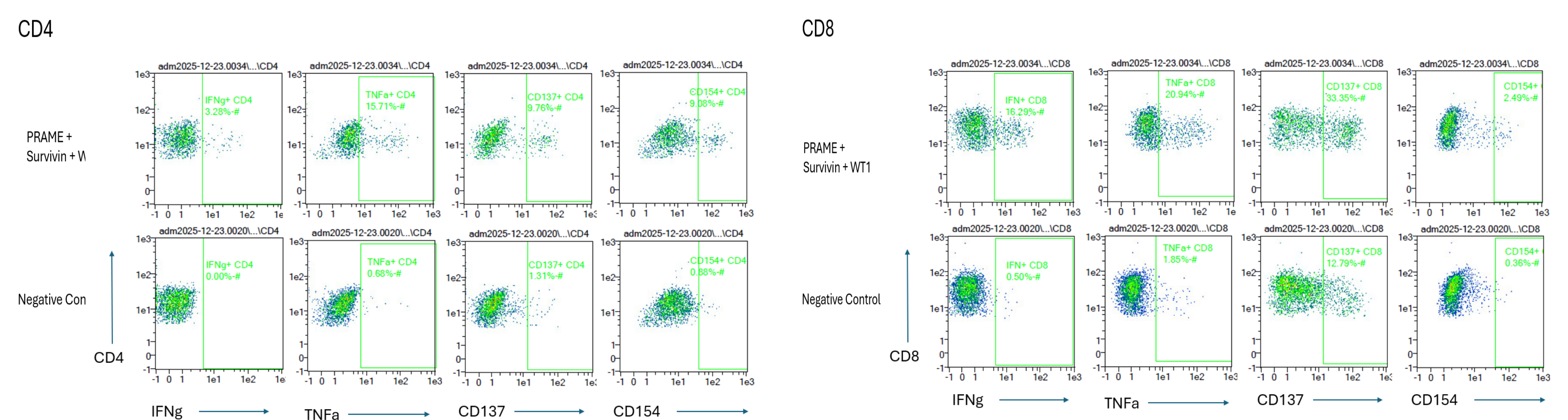


Figure 3. Expression of activation markers (IFN γ , TNF α , CD137 and CD154) in TRT re-stimulated with mixed pools of tumor-associated peptides (PRAME, Survivin, WT1 PepTivators[®]) vs negative control (media only, no PepTivators[®] added). Flow cytometry plots of CD4 and CD8 populations from one representative run shown.

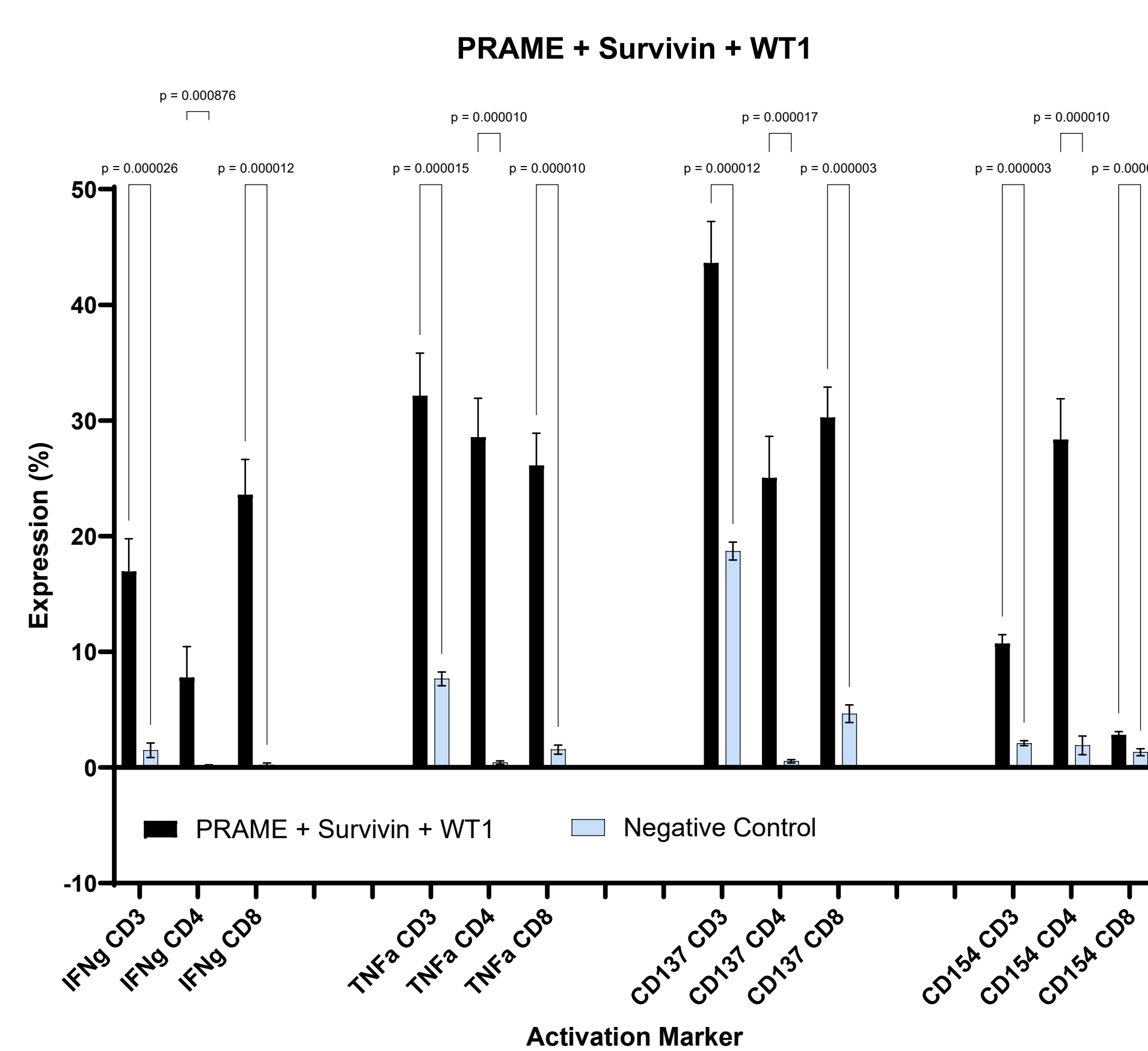


Figure 4. Expression of activation markers (IFN γ , TNF α , CD137, CD154) in CD3, CD4 and CD8 populations of expanded TRT following in-vitro re-stimulation with mixed tumor-associated peptide pools (PRAME, Survivin, WT1 PepTivators[®]). Negative control was performed with media only (no PepTivators[®] added). Data shown reflects testing of the final product of one representative TRT manufacturing run, performed in 9 replicates. Unpaired t test with Welch correction, bar graphs denote the mean +/- SD.

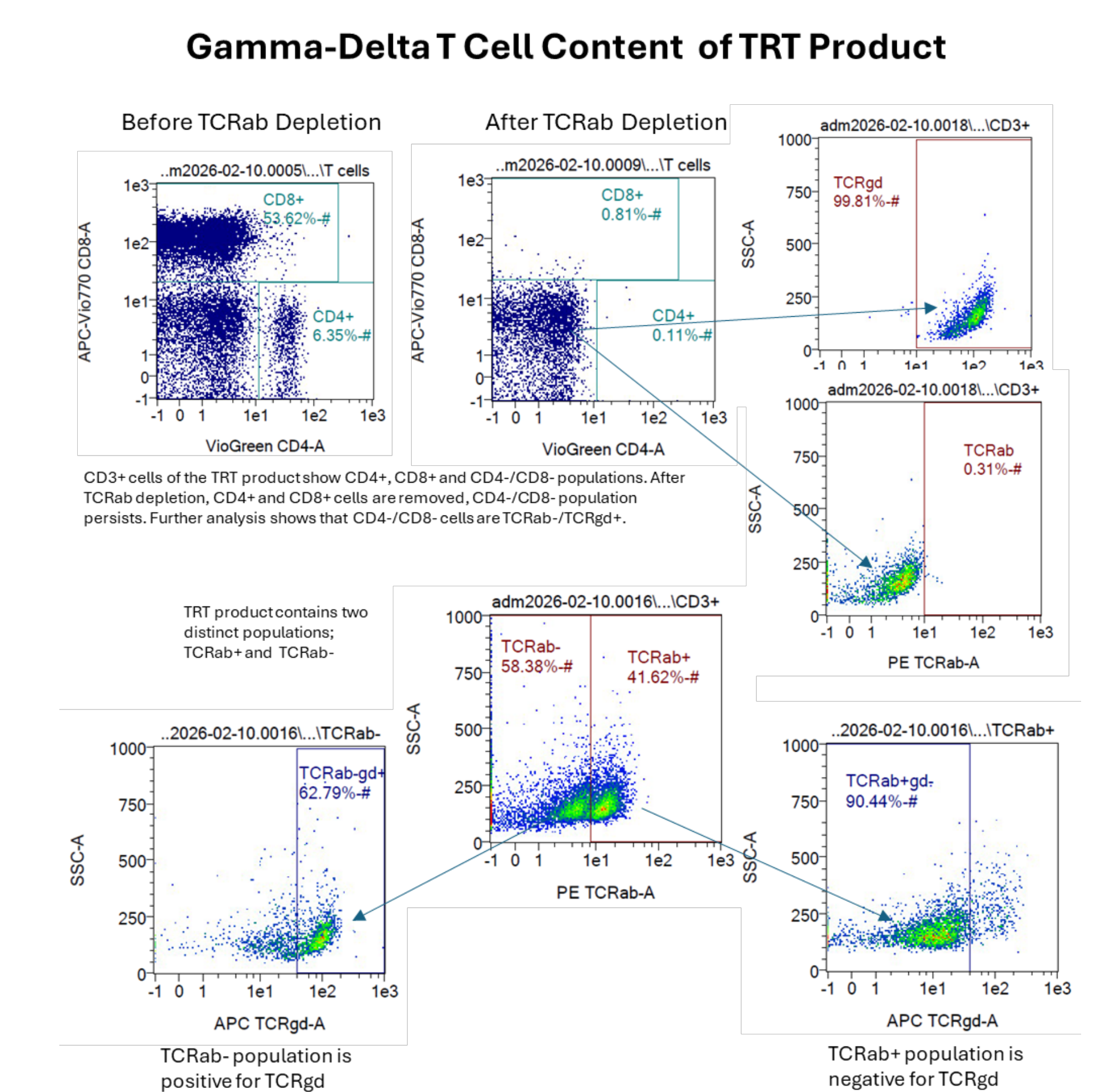


Figure 5. Gamma-delta T cell composition of TRT product. TRT products manufactured on CliniMACS Prodigy[®] contain a significant population of CD3⁺, CD4⁻, CD8⁻, TCRab⁻, TCRgd⁺ cells consistent with gamma-delta T cells. Representative run shown.

Disclosures

Significant portion of GMP manufacturing supplies and reagents for CliniMACS Prodigy[®] runs, including PepTivator[®] peptide pools, were generously provided by Miltenyi Biotec. GREX-CS[®] devices were kindly provided by G-Rex[®] Grant Program.