



Characterization and Expansion of T-Cells Cultured in Xeno-Serum Free Media

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Introduction

Antigen specific suppressive immunotherapy such as Tregs therapy and highly selective targeted immunotherapy such as chimeric antigen receptor (CAR) and T-cell receptor (TCR) therapy have shown great benefits and therapeutic value to the patient population. These immunotherapy applications require ex vivo expansion of T-cells to high cell densities without loss of T-cell phenotypes and functionality for successful adoptive transfer.

Irvine Scientific (IS) has developed a xeno-free, serum free (XFSM) T-cell expansion medium that can be used to activate and expand naïve T-cells. In this study, we demonstrated this media's high growth expansion and its ability to support differentiation into other T-cell subsets. We also demonstrated the scalability of Prime XV T-cell expansion XFSM to expand the T-cells on a large scale platform.

Methods

T-cell Activation/Expansion:

Plates were coated overnight at 2-8°C with 1µg/mL of anti-human CD3 (clone UCHT1) and anti-human CD28 (clone CD28.2). CD3+ T-cells were seeded at 5x10⁵ cell/ml. Cells were fed every 2-3 days. All media were supplemented with IL-2 at 50µg/mL.

T-cell Differentiation:

The following cytokines were added one day after seeding:

- For Th1, 10ng/mL of IL-12 with 5µg/mL of anti-hIL-4.
- For Th2, 10ng/mL of IL-4 and 5µg/mL anti-IFNγ.
- For Tregs, 50ng/mL of TGF-β1 and 6ng/mL retinoic acid.

Static vs Spinner Flask Expansion:

CD4+ T-cells were initially expanded and exposed to cytokines for Tregs differentiation. Once expanded, 2.5x10⁷ cells were seeded into 125mL spinner flask and T-125 flask (Corning) with 75mL of Prime XV T-cell expansion XFSM. Half media exchange was performed every 2-3 days. The T-cells were reactivated every 10-12 days using the method above.

Assessment of the T-cells Subsets

The cells were stained and identified with flow cytometry:

- Th1: CD4+/hIFNγ+
- Th2: CD4+/hIL-4+
- Tregs: CD4+/CD25+/hFoxP3+

Results

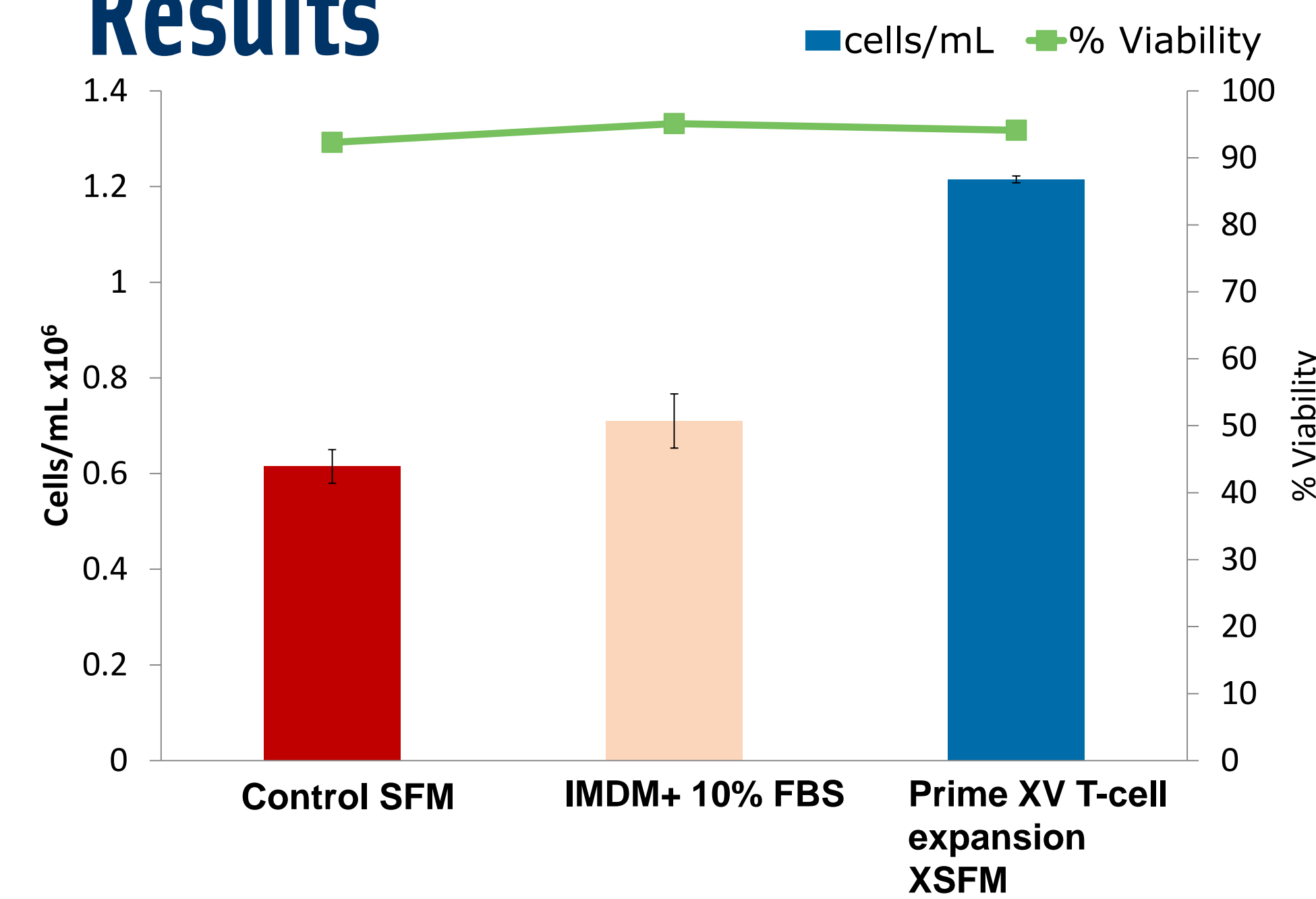


Figure 1: Proliferation of T-cells under different media.

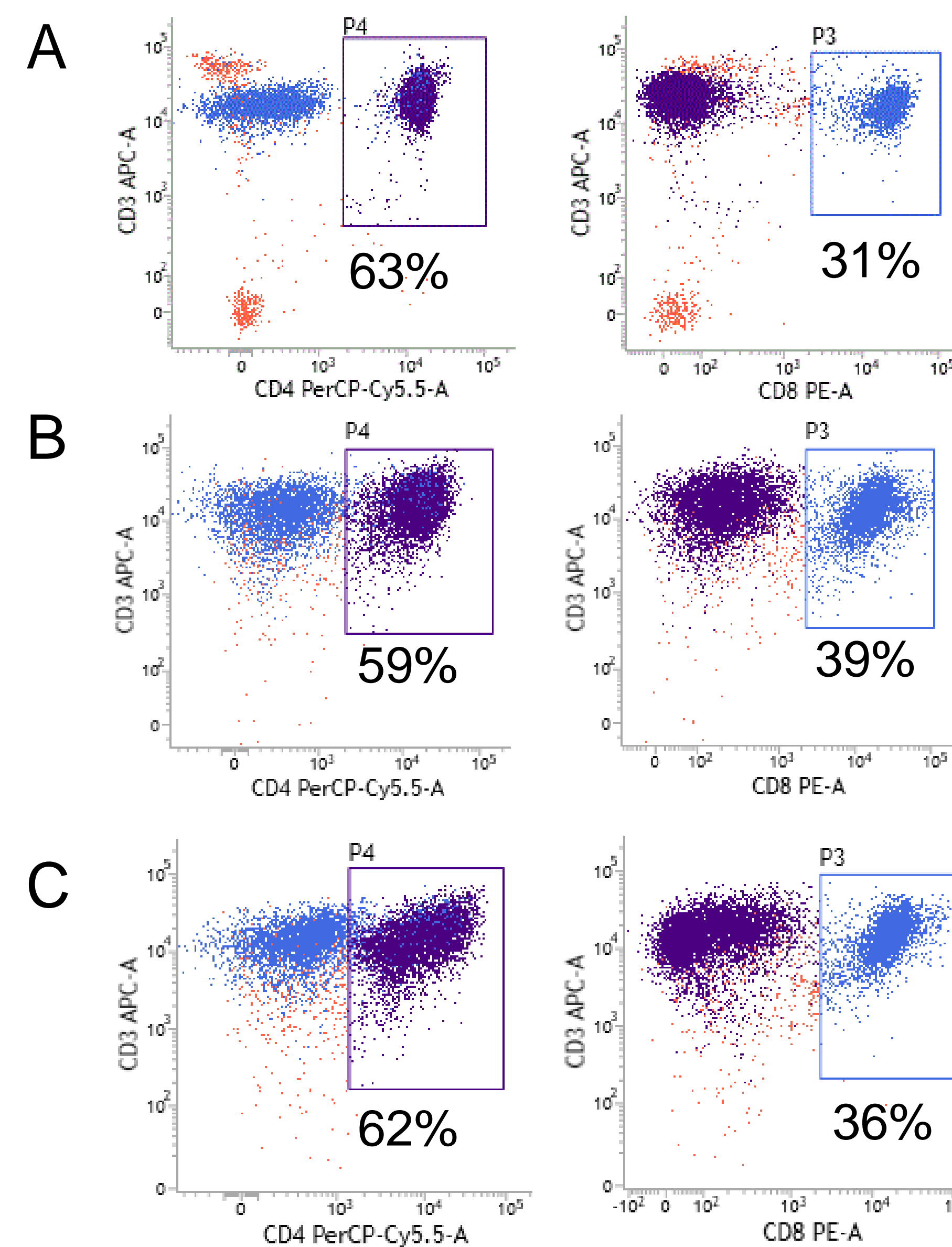


Figure 4A: CD4/CD8 ratios of CD3+ T-cells before activation and expansion with SFM. B. CD4/CD8 ratio of T cells after 5 days of expansion with Prime XV T-cell expansion XFSM. C. CD4/CD8 ratio of T-cells after 5 days of expansion with a control SFM.

Conclusion

This study found that Prime XV T-cell expansion XFSM supports a higher rate of growth expansion as compared to serum containing media and existing commercially available serum free media while maintaining their CD4/CD8 ratio. The T-cells in this media differentiated into the desired T-cell subsets. The use of the media for large scale expansion of Tregs was also demonstrated. Overall, the newly developed Prime XV T-cell expansion XFSM provides the quality and performance improvement required for a successful T-cell based immunotherapy application.

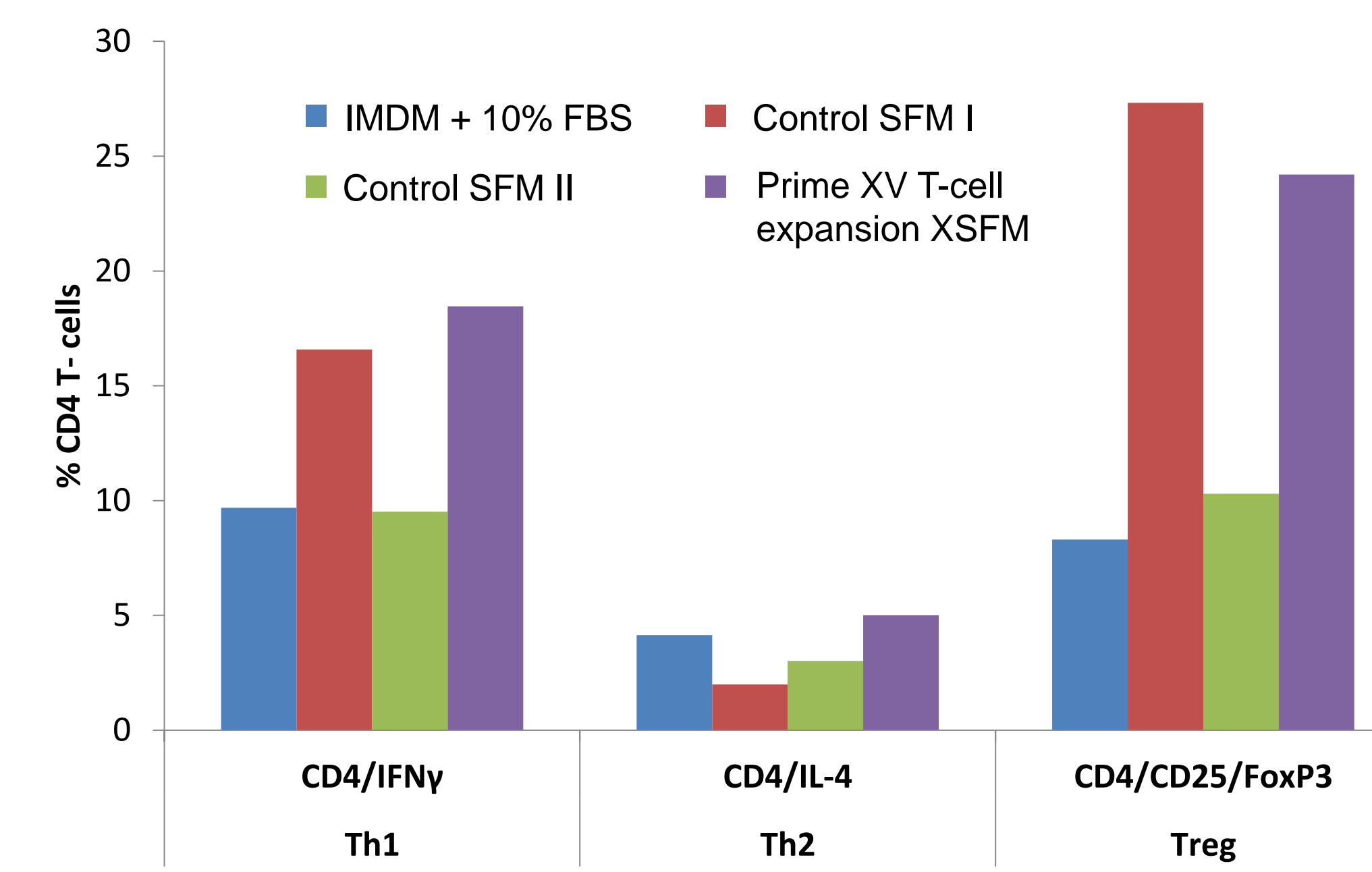


Figure 2: T-cells differentiation under different media.

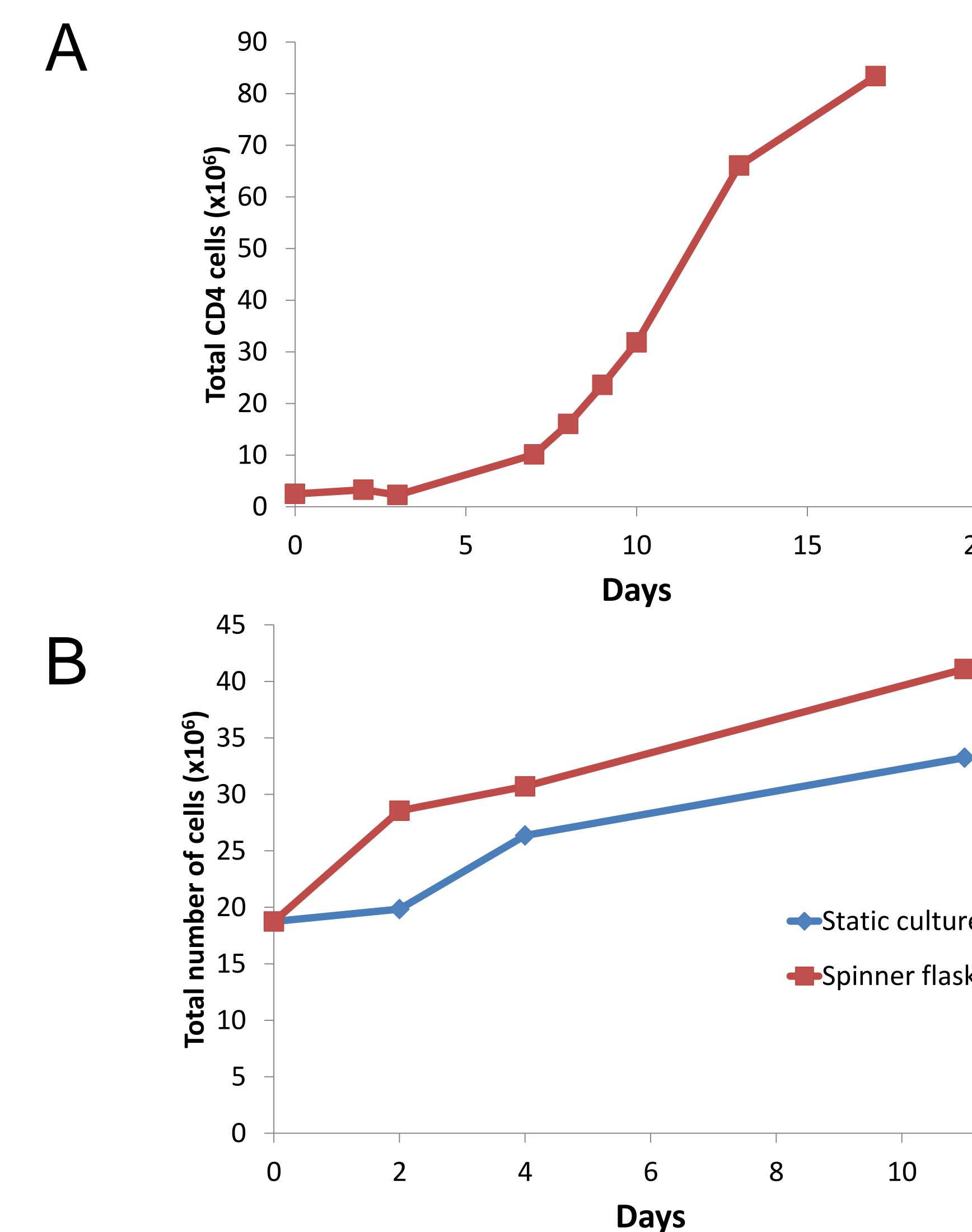


Figure 5A: Expansion of CD4+ T-cells in PRIME XV T-cell expansion XFSM with cytokines for Tregs differentiation. B. Static vs Spinner culture. Once expanded, T-cells were transferred to proliferate in T125 and 125mL spinner flasks. C. Flow cytometry of T cells from the spinner flask. The T-cells in the spinner flask were gated for CD25+ before gating for CD4+ and FoxP3+. D. Treg population in the spinner flask.

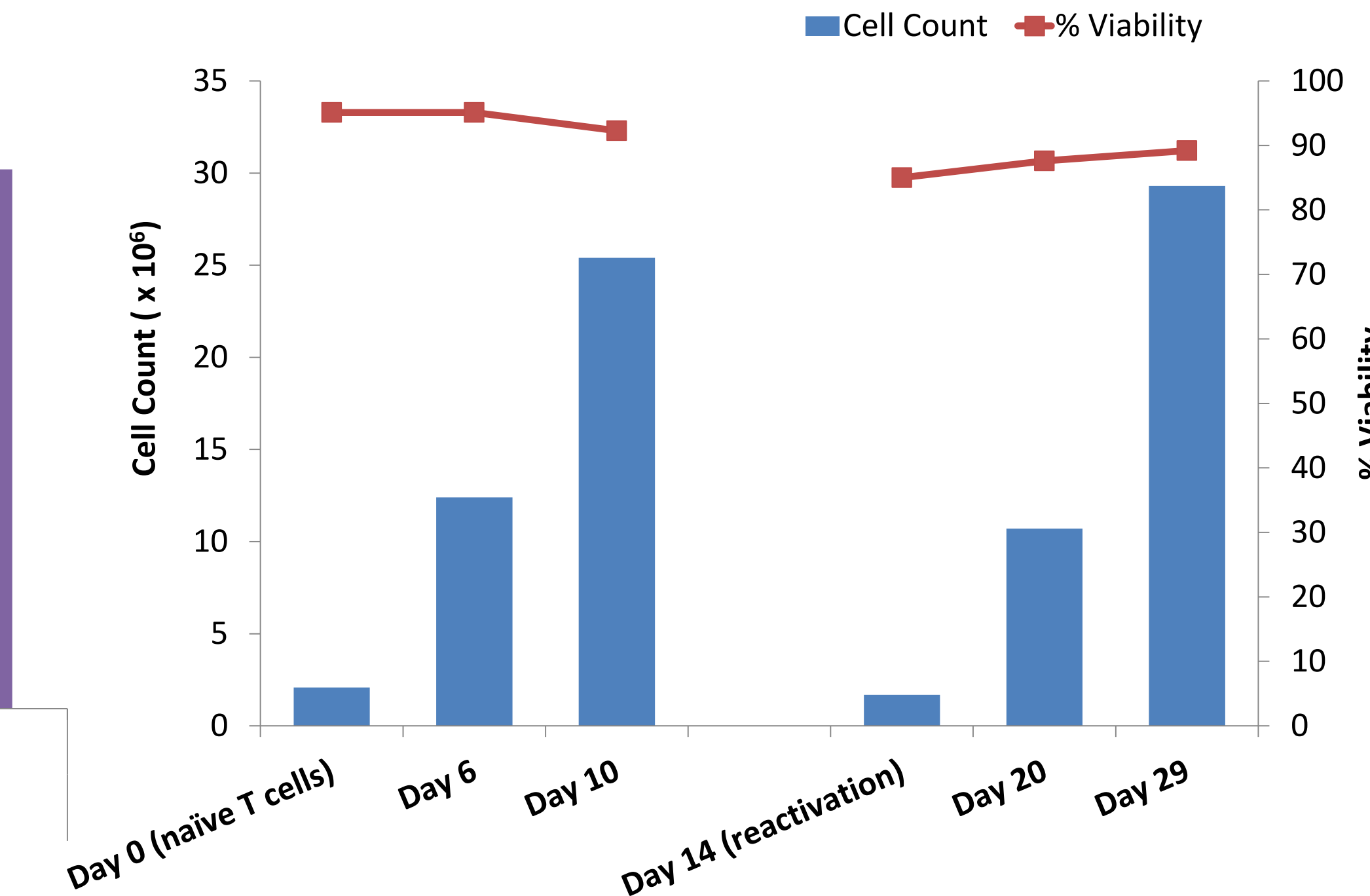


Figure 3: T-cells expansion and reactivation with Prime XV T-cell expansion.

