

# Improved Control of Activation and Expansion of Tregs for use in Cell Therapy Applications

Kerstin Bernström, Nora Lieske, Dorthea Gjølborg, Ida Caroline Schrøder, Grethe Økern, Hui Zhang, Tuva Holt Hereng, Ulrike Rehn and Øystein Åmellem. Cellular Medicine, Research and Development, Thermo Fisher Scientific, Ullernchausséen 52, 0379 Oslo, Norway

**ThermoFisher**  
SCIENTIFIC

Bioprocessing

## ABSTRACT

Our studies demonstrate that our new CTS™ Dynabeads™ Treg Xpander results in superior regulatory T cell (Treg) expansion with maintenance of high %FOXP3 expression after 12-14 days. The expanded Tregs have suppressive activity and display the characteristic Treg cytokine profile and phenotype. The stability of the expanded Tregs is high, based on analysis of the Treg-specific demethylated region.

Depending on the media, non-serum supplement and growth factors as well as the bead to cell ratio, the protocol can be altered to give a controllable drug of Tregs. The beads are easily removed by CTS DynaMag.

CTS Dynabeads Treg Xpander support consistent and scalable<sup>1</sup> Treg manufacturing for immunotherapies, supplementing our Cell Therapy System (CTS) portfolio.

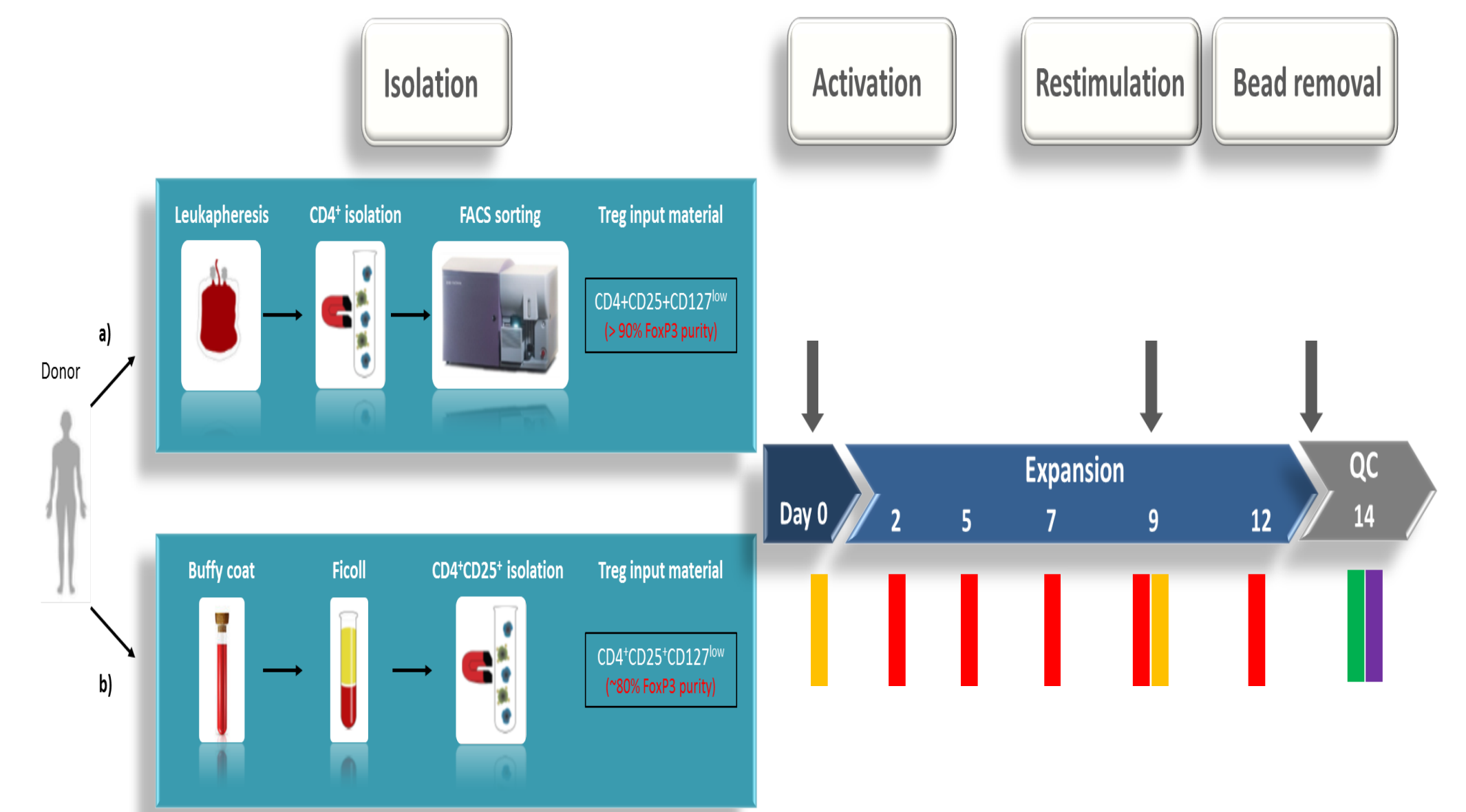
## INTRODUCTION

CTS Dynabeads CD3/CD28 is a platform technology applied for *ex vivo* manufacture of polyclonal T cells for use in various adoptive cell therapies. Here we present a new bead, CTS Dynabeads Treg Xpander, which is a modification of this platform to specifically activate and expand regulatory T cells (Tregs).

Tregs (CD4+CD25+FOXP3+) are a suppressive subset of CD4+ T helper cells important for the regulation of immune responses. Tregs are proven highly effective in preventing graft versus host disease (GvHD) and autoimmunity in murine models. These findings, together with the recent successes in adoptive T-cell therapy to treat severe blood cancers, have increased the interest of using Tregs for autologous and allogeneic therapies.<sup>2</sup>

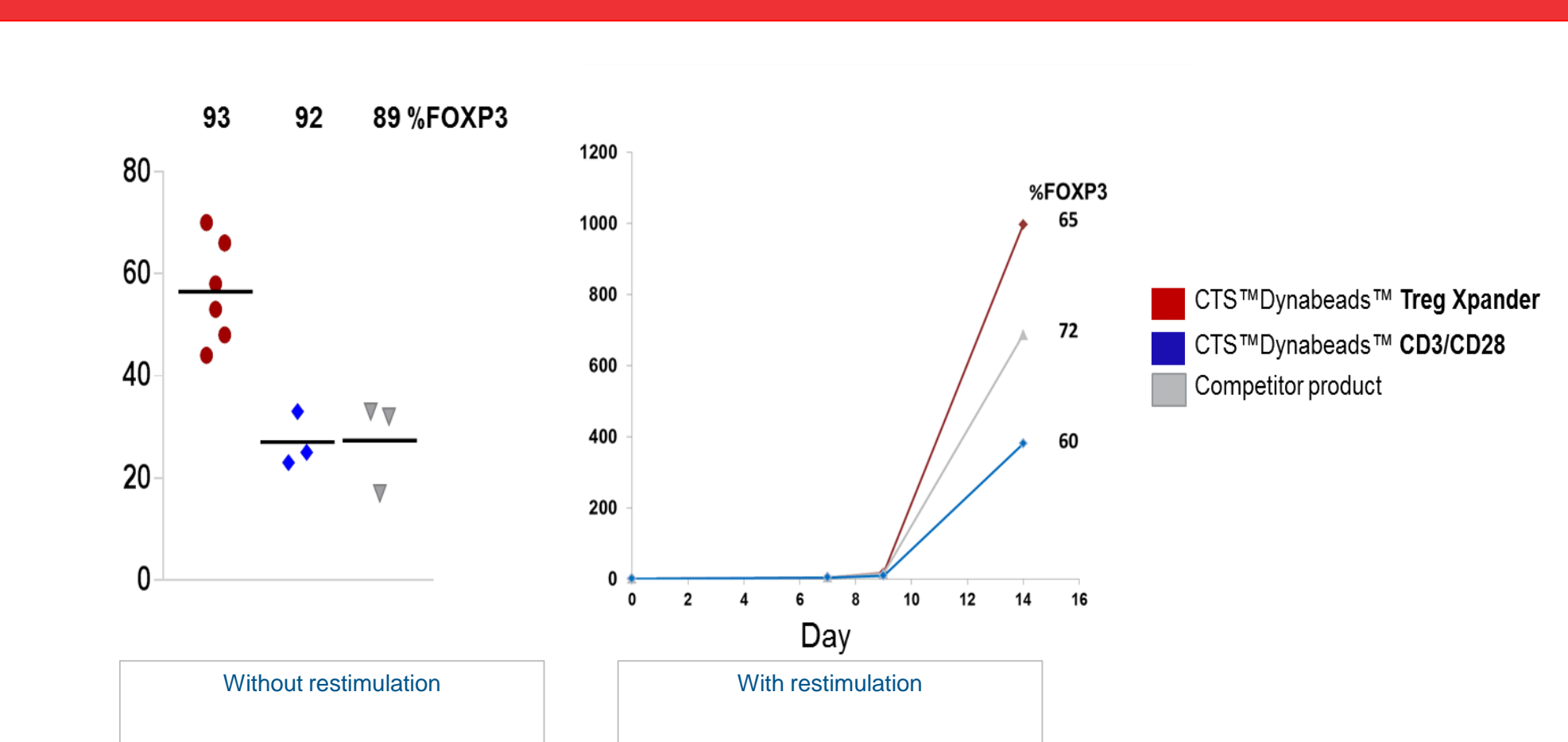
As the relative frequency of Tregs in peripheral blood is approximately 1-2% of the total lymphocyte population, *ex vivo* expansion of Tregs prior to adoptive transfer will be necessary for most clinical applications.

## MATERIALS AND METHODS



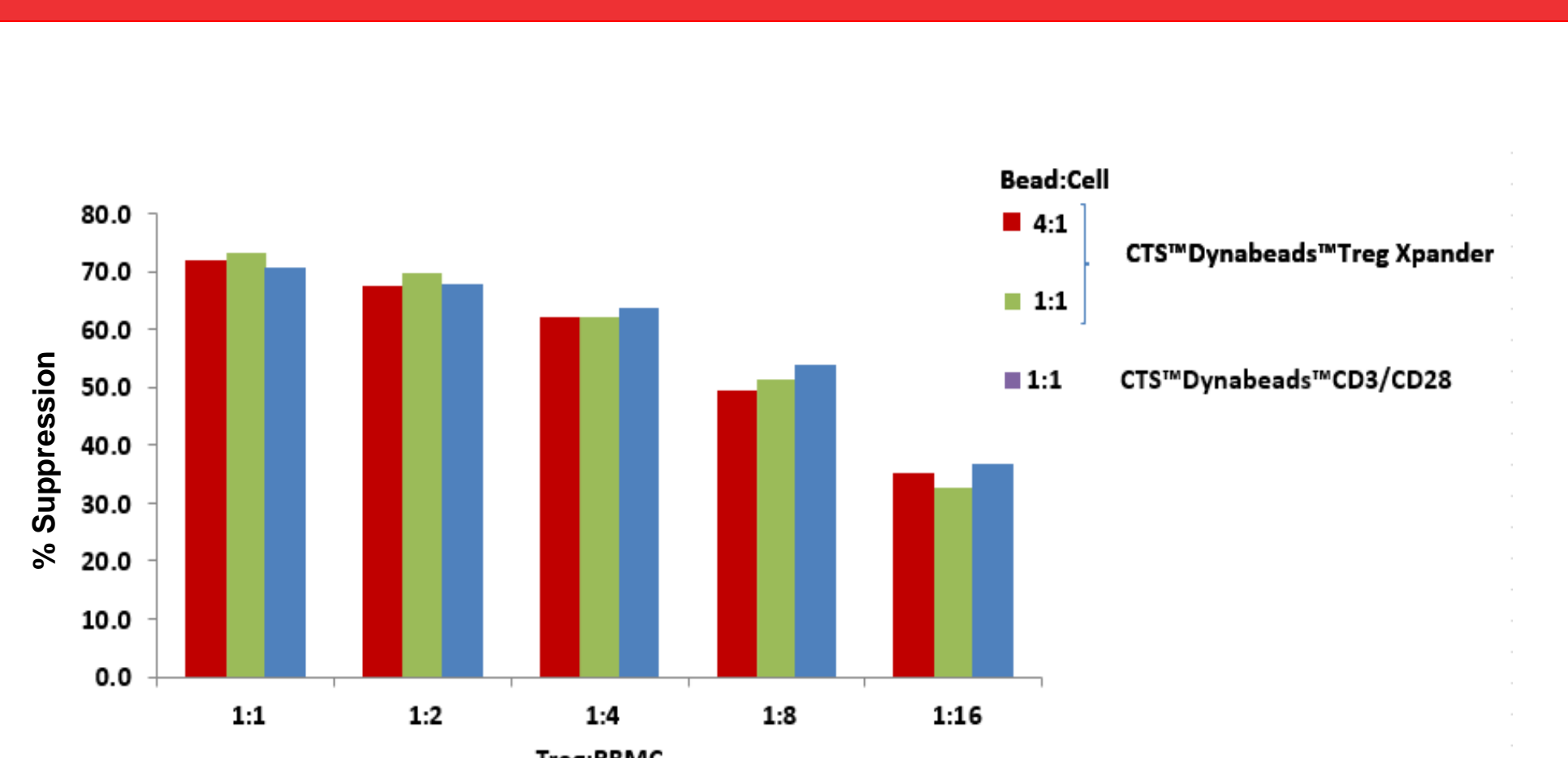
## RESULTS

### IMPROVED EXPANSION YIELDS NUMBERS NEEDED FOR CLINICAL TRIALS



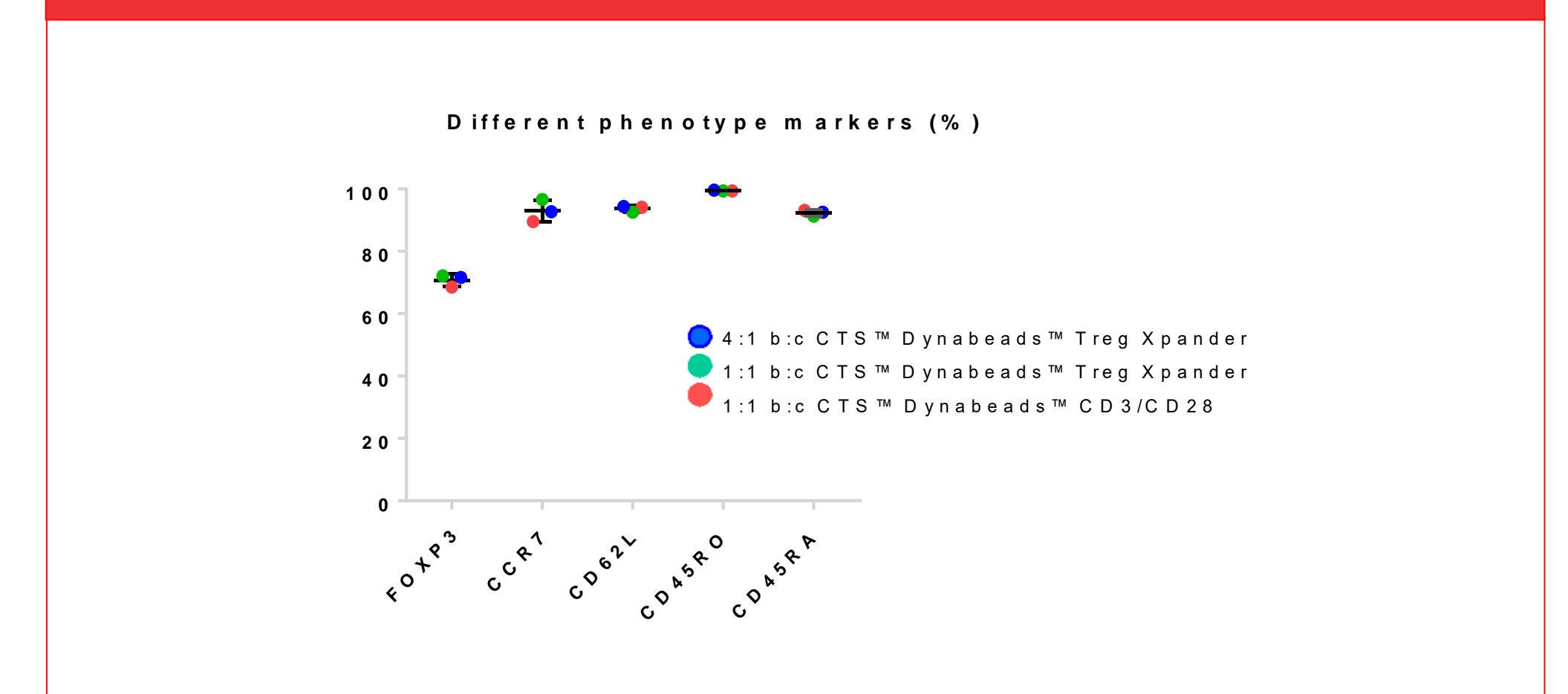
Expansion of magnetic isolated Treg cells (left) or FACS sorted Treg cells (right). Tregs were expanded with CTS Dynabeads Treg Xpander, CTS Dynabeads CD3/CD28, or competitor technology for 14 days with (right) and without (left) restimulation at day 9.

### HIGH SUPPRESSIVE CAPACITY OF EXPANDED TREG CELLS



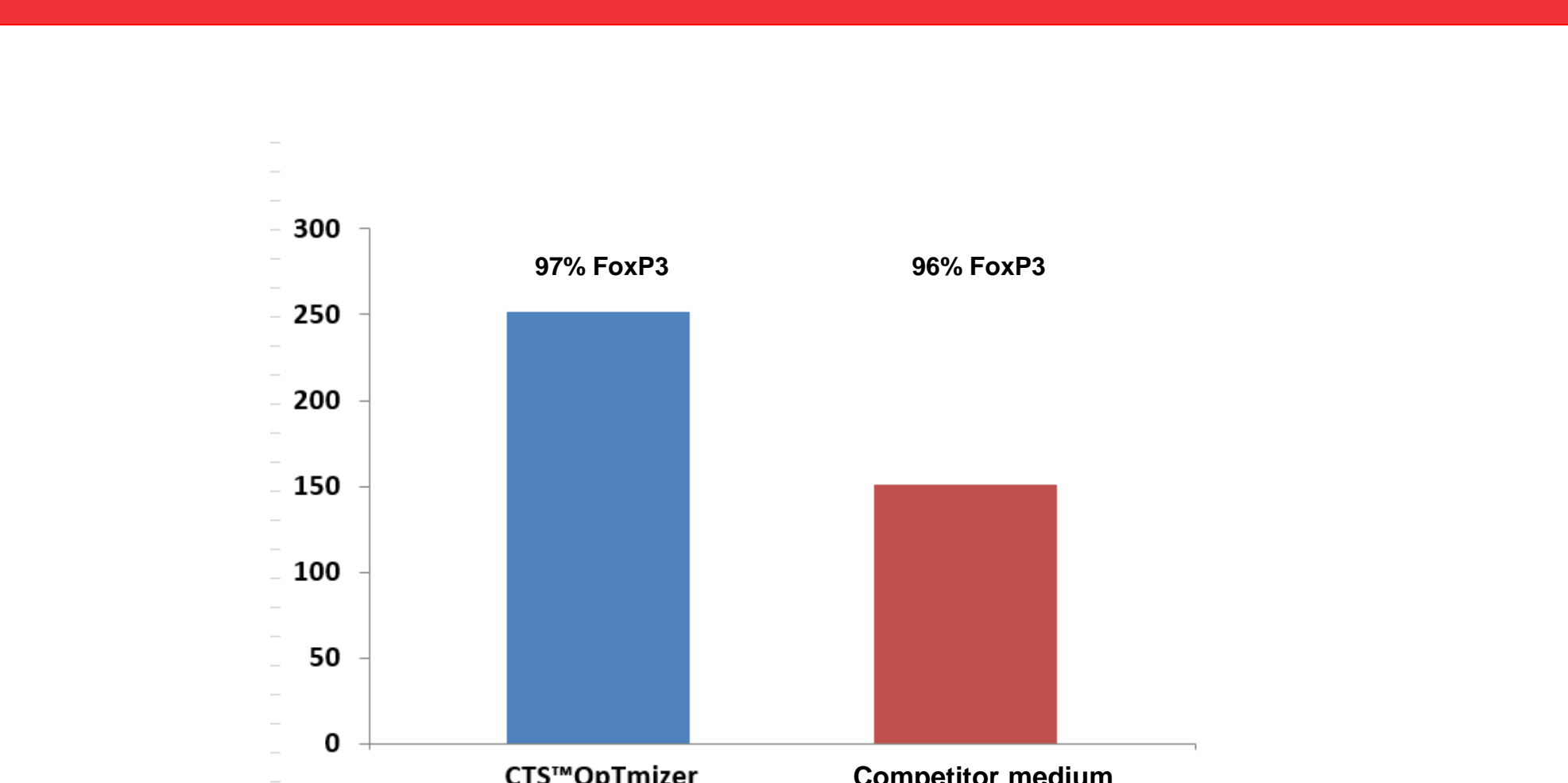
Percentage suppression of PBMC from expanded FACS sorted Treg cells in different ratios. Treg cells expanded with CTS Dynabeads Treg Xpander (4:1 or 1:1 bead:cell) and CTS Dynabeads CD3/CD28 (1:1 bead:cell). Each point = average of 3 blood donors

### PHENOTYPE OF EXPANDED CELLS



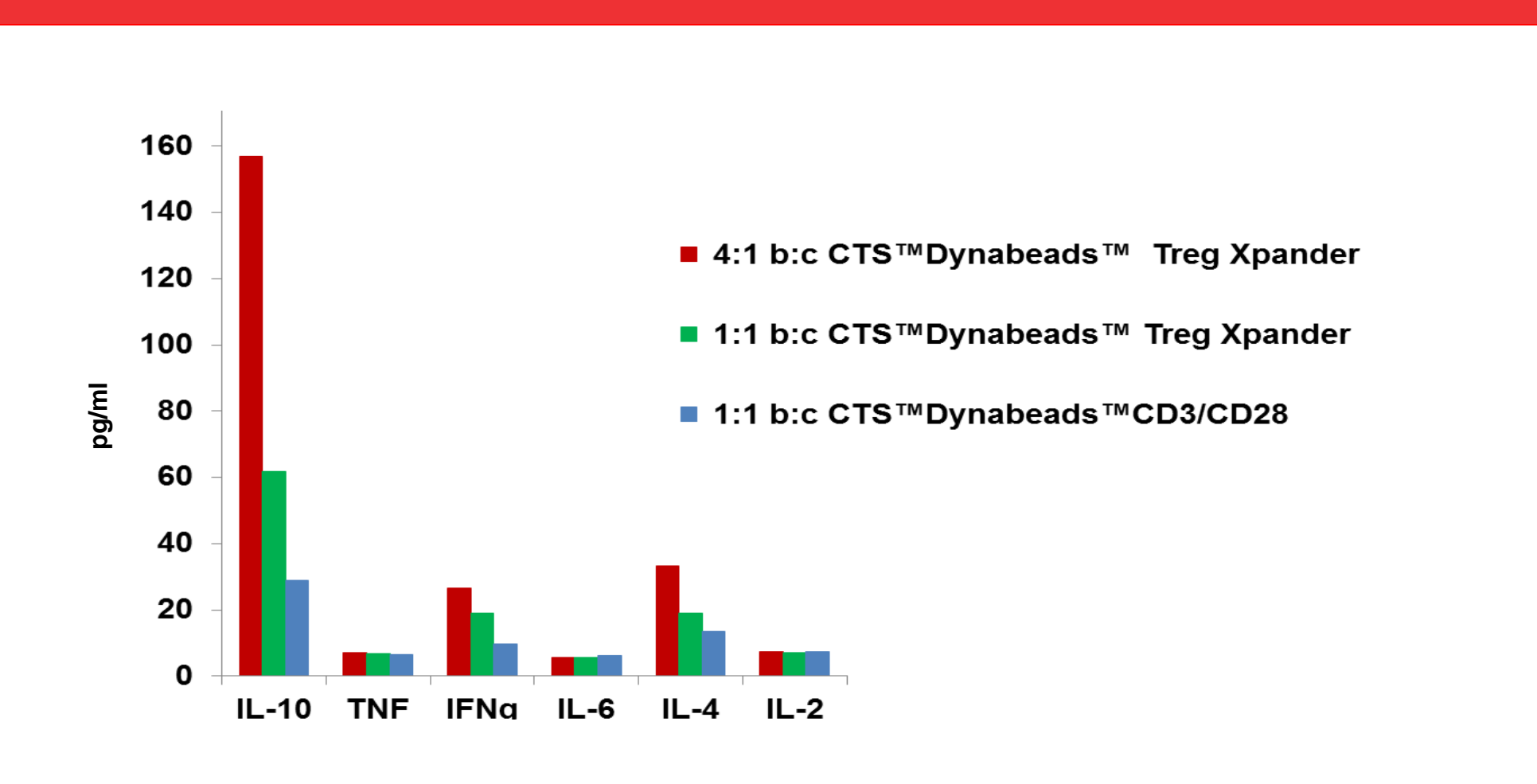
Tregs expanded (14 days) with CTS Dynabeads Treg Xpander express high percentage of FOXP3, CCR7, CD62L, CD45RO and CD45RA, characteristic for a Treg and central memory phenotype. Treg cells expanded with CTS Dynabeads Treg Xpander (4:1 or 1:1 bead:cell) and CTS Dynabeads CD3/CD28 (1:1 bead:cell). Each point = average of 3 blood donors

### CTS OPTIMIZER RESULTS IN SUPERIOR TREG EXPANSION



Total expansion of magnetic isolated Treg cells in CTS OpTmizer or competitor medium for 12 days with CTS Dynabeads Treg Xpander. Both media contained 5% CTS Immune Cell Serum Replacement. Purity (FoxP3%) is presented above.

### HIGH EXPRESSION OF IL-10 CYTOKINE



Tregs expanded with Dynabeads Treg Xpander express IL-10, which is a Treg associated cytokine. 4 beads to 1 cell give highest IL-10.

### EXPANDED TREGS EXHIBIT A STABLE PHENOTYPE

CTS Dynabeads	%FoxP3	%Demethylation <sup>3</sup>
Treg Xpander	89	92.8
CD3/CD28	92	74.9

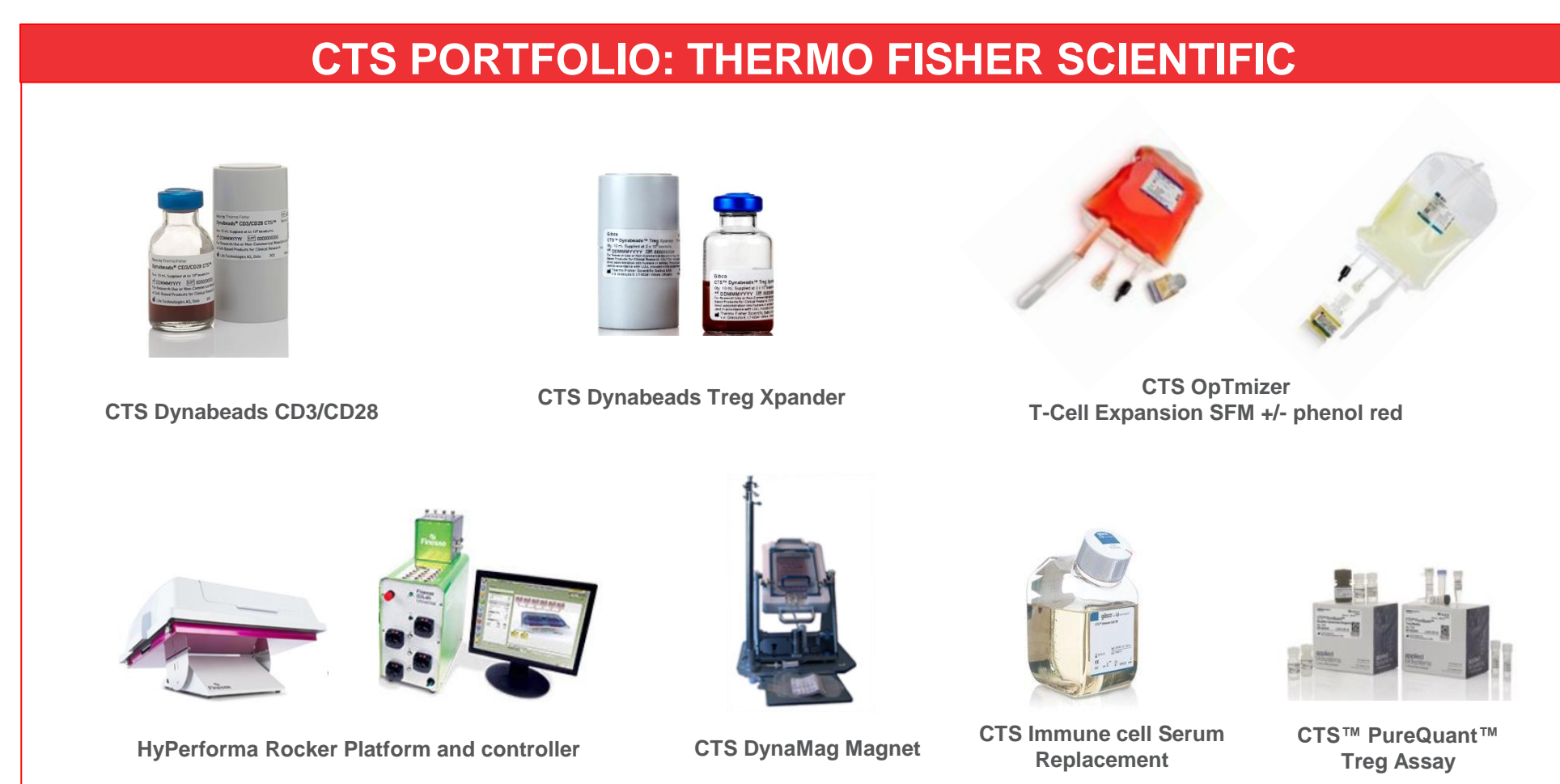
Demethylation status of the Treg-specific demethylation region (TSDR) after expansion of Tregs with CTS Dynabeads Treg Xpander and CTS Dynabeads CD3/CD28.

## CONCLUSIONS

CTS Dynabeads Treg Xpander shows improved performance compared to existing technologies:

- >800-fold expansion at day 14 when restimulating cells at day 9
- Maintained purity (FoxP3 expression), Treg -and central memory phenotype markers
- Suppressive capacity and cytokine profile with high IL-10 secretion
- Stable Treg phenotype proven by high % TSDR demethylation

CTS Dynabeads Treg Xpander will supplement Thermo Fishers Cell Therapy System (CTS) portfolio with reagents manufactured by aseptic processing under Good Manufacturing Practice (GMP) conditions.



## REFERENCES

- Katherine MacDonald et al (2018); Expansion of thymus-derived regulatory T cells using GMP-compatible reagents. Poster at BioCanRx Summit for Cancer Immunotherapy (un-published).
- Putnam et al. 2009, Diabetes, 58, 652-662.
- The analysis and results for Treg cell demethylation status were kindly provided by Uma Lakshmiathy, Thermo Fisher Scientific, Carlsbad.

## TRADEMARKS/LICENSING

©2019 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. This information is not intended to encourage use of these products in any manner that might infringe the intellectual property rights of others.

For Research Use or Non-Commercial Manufacturing of Cell-Based Products for Clinical Research. Caution: Not intended for direct administration into humans or animals.