Miltenyi Biotec

Translatability of T Cell TransAct™ for T cell activation process development

Introduction

Process development is riddled with challenges — and one major obstacle involves finding reagents that can achieve an equivalent physiological response when moving from a research setting to commercial manufacturing. A critical aspect is the ability to take a protocol developed to activate T cells, such as one optimized in the laboratory, and then translate it into a process intended for commercial manufacturing for clinical applications.

Herein, we provide protocol guidance that demonstrates how to best attain equivalent results when using two different T Cell TransAct formats. This study illustrates the equivalent physiological response of T cell subsets to our activation-reagent T Cell TransAct. To demonstrate this equivalent physiological response, both formats of the reagent - research-use T Cell TransAct and MACS® GMP T Cell TransAct™ —were used.

We employed two parallel sets of experiments using a non-automated method that reflects a laboratory setting, while making pertinent observations of activation and exhaustion markers based on flow cytometry data throughout 10 days of cultivation.

In summary, the data show that T Cell TransAct can yield consistent outcomes with respect to exhaustion and activation markers, irrespective of product format.

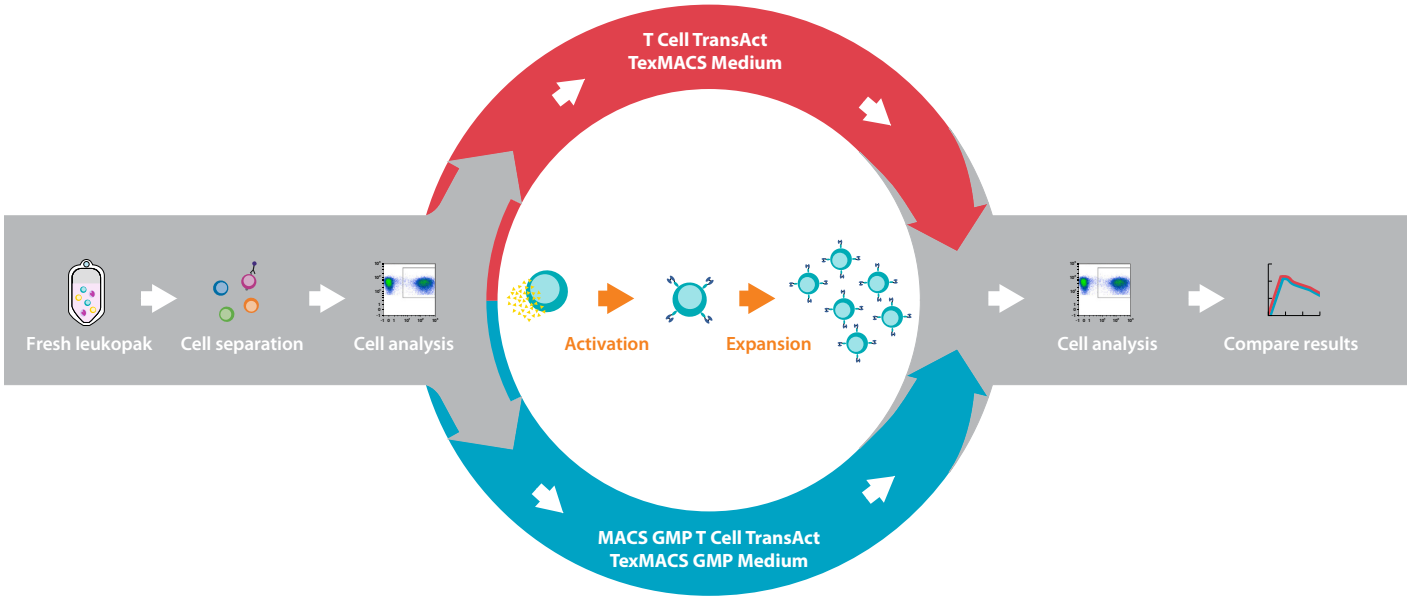

Figure A: This study employs two parallel sets of experiments starting with the same single-donor leukopak and cell-separation method. Subsequent to activation and expansion of T cells, using either research-use (top path) or GMP-compliant (bottom path) media and activation reagents, a final cell analysis step is performed to ascertain the expression of activation and exhaustion markers for both $CD4^+$ and $CD8^+$ cell populations.

Methods

Cell separation

We performed the isolation of CD4+ and CD8+ T cells from a single leukopak which was received within 24 hours post-draw. The sample originated from a single healthy male donor < 50 year of age. We separated cells using the StraightFrom® leukopak CD4+/CD8+ MicroBead Kit for human cells and the MultiMACS™ Cell24 Separator Plus. In order to achieve better clinical relevance, we replaced bovine serum albumin (BSA) with human serum albumin (HSA) in buffer solution to reduce the amount of animal components from the study.

Cell culture and analysis

After washing cells to remove ethylenediaminetetraacetic acid (EDTA) and HSA, cells were resuspended in a master mix solution comprised of TexMACS™ Media and MACS Premium-Grade Cytokines (both sets of parallel experiments employed MACS Premium-Grade Cytokines). The amount of each cytokine used is as follows:

- Human IL-7, premium grade: 1,600 U/mL
- Human IL-15, premium grade: 150 U/mL

In order to achieve equal concentrations between the two parallel experiments, T Cell TransAct was added to the culture at the following final dilutions:

- T Cell TransAct: 1:100
- MACS GMP T Cell TransAct : 1:17.5

Cells were allowed to activate and expand in T Cell TransAct-supplemented media for three days. They were then washed and resuspended in regular TexMACS Media. Cells from triplicate samples were then harvested and analyzed every other day for 10 days.

Using flow cytometry, an analysis of CD4+ and CD8+ was performed where we measured the expression of PD-1, LAG-3, and TIM-3 - all markers for exhausted T cells. We also measured the proportion of cells with double-positive CD69+/CD25+ expression, which is a dual marker for activated T cells.

Results

Positive fraction

After the cell separation process, and before the administration of T Cell TransAct, an assessment of the positive fraction collected from the leukopak showed that at least 500,000 cells were recovered with 99.1% viability. The separation yielded over 90% CD4+ and CD8+ T cells, while a low proportion consisted of other types of immune cells.

Total cells	500,000
% live	99.1%
% CD4+	53.9%
% CD8+	36.6%

Table 1: Breakdown of the positive fraction collected on Day 0.

Marker expression analysis via flow cytometry

Here, we demonstrate the close resemblance of exhaustion and activation marker-expression profiles between CD4+ and CD8+ cells in the two parallel experiments. We used R-squared (R^2) values to quantify the physiological-response similarity between T Cell TransAct and MACS GMP T Cell TransAct. Triplicate samples provide a standard deviation for each data point.

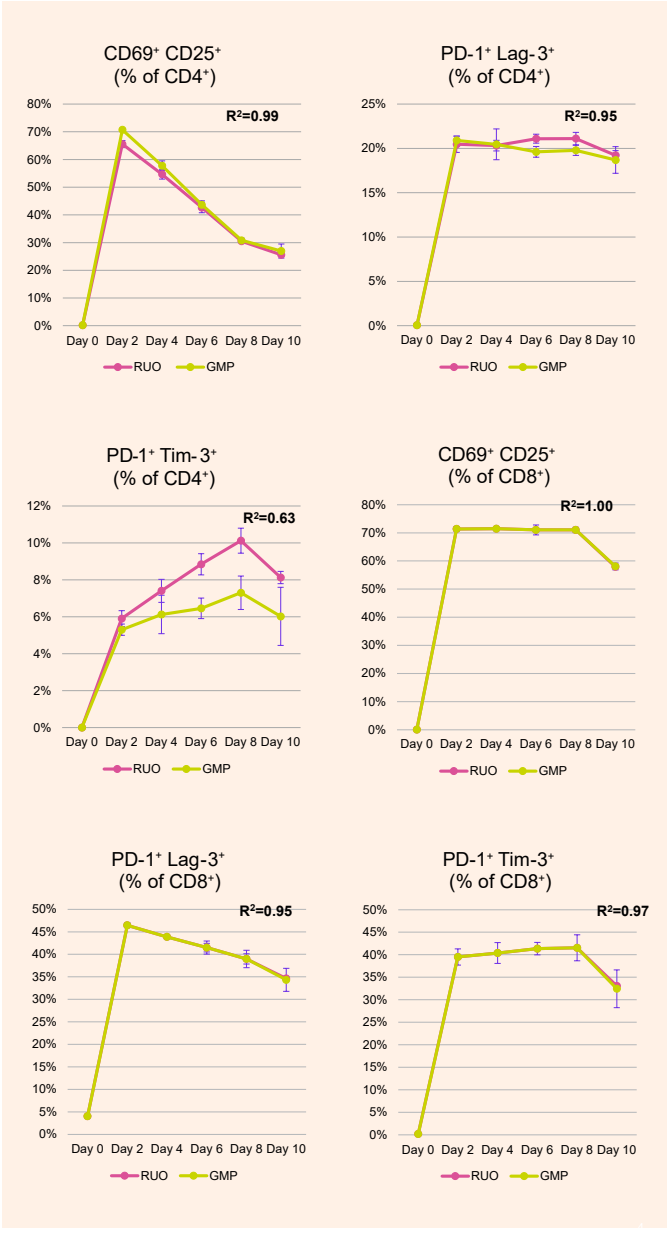

Figure B: Expression of activation (CD69+/CD25+) and exhaustion markers (PD-1+/LAG-3+ and PD-1+/TIM-3+) in CD4+ and CD8+ populations treated with T Cell TransAct for research-use versus GMP-compliant MACS GMP T Cell TransAct reagent.

Conclusion

Our study showed a similar expression of T cell activation and exhaustion markers between cell cultures that utilized T Cell TransAct for research-use or the GMP-compliant MACS GMP T Cell TransAct. An analysis of the data shows strong reproducibility, achieving R^2 values >0.95 for 5 of the 6 analysis.

This was particularly true for $CD8^+$ cells, where all analysis were nearly identical between the two formats for the same T cell subtype. For the one analysis that showed an R^2=0.63, we saw $CD4^+$ cells with a relatively low frequency of PD-1^+/Tim-3^+ expression that resulted in a more exaggerated variance compared to the $CD8^+$ subset analyzed for the same markers.

Ultimately, we discovered that it's critical to establish the day-10 endpoint for each of the markers tested. For 5 of the 6 analyses shown, we found that activation and exhaustion marker expression was lower in frequency at day 10 compared to day 2 with minimal variance, as is expected of T cells after activation.

Based on our protocol guidance and the corresponding data shown, we conclude that T Cell TransAct is an effective reagent for activating T cells without a sustaining a high expression of activation and exhaustion markers throughout 10 days of cell expansion.

Nonetheless, an expanded study including more donors and markers could provide deeper insight of similarities and differences in marker expression of $CD4^+$ and $CD8^+$ T cell populations upon stimulation with T Cell TransAct.

Due to the equivalent results upon stimulation with T Cell TransAct compared to MACS® GMP T Cell TransAct, the products are suited for process development efforts aimed at translating protocols from a laboratory setting to commercial manufacturing processes.

Materials

The Miltenyi Biotec reagents and instruments used in this study include:

Cell separation

Product name	Part number
StraightFrom® leukopak CD4/CD8 MicroBead Kit, human	130-122-352
MultiMACS™ Cell24 Separator Plus	130-098-637

Cell analysis

Product name	Part number
CD366 (TIM-3) Antibody, anti-human, REAfinity™, PE-Vio 770	130-121-334
CD223 (LAG-3) Antibody, anti-human, REAfinity™, Vio Bright V423	130-126-616
CD4 Antibody, anti-human, REAfinity™, Vio Bright B515	130-114-535
CD8 Antibody, anti-human, REAfinity™, VioGreen	130-110-684
CD14 Antibody, anti-human, REAfinity™, VioBlue	130-110-524
CD279 (PD-1) Antibody, anti-human APC	130-120-383
CD25 Antibody, anti-human APC-Vio 770	130-123-469
CD69 Antibody, anti-human PE	130-112-613
7-AAD Staining Solution	130-111-568

Cell culture

Product name	Part number
Human IL-7, premium grade	130-095-362
Human IL-15, premium grade	130-095-764
TexMACS™ Medium, 500 mL	130-097-196
TexMACS™ GMP Medium (Phenol Red)	170-076-309
T Cell TransAct™, human	130-128-758
MACS® GMP T Cell TransAct™	170-076-156

References

1. Zhang, W. *et al.* (2018) Characterization of clinical grade CD19 chimeric antigen receptor T cells produced using automated CliniMACS Prodigy system. Drug. Des. Devel. Ther. 12:3343-3356. PMID: 30323566; PMCID: PMC6181073. doi: 10.2147/DDDT.S175113
2. Jin, H.T. (2010) Cooperation of Tim-3 and PD-1 in CD8 T-cell exhaustion during chronic viral infection. PNAS 107(33):14733-14738. doi.org/10.1073/pnas.1009731107
3. Osuch, S. *et al.* (2020) Decrease of T-cells exhaustion markers programmed cell death-1 and T-cell immunoglobulin and mucin domain-containing protein 3 and plasma IL-10 levels after successful treatment of chronic hepatitis, C. Sci. Rep. 10:16060. doi.org/10.1038/s41598-020-73137-6
4. Yang, Z.Z., *et al.* (2017) Expression of LAG-3 defines exhaustion of intratumoral PD-1^+ T cells and correlates with poor outcome in follicular lymphoma. Oncotarget. 8:61425-61439. doi:10.18632/oncotarget.18251

Miltenyi Biotec, Inc | 6125 Cornerstone Court East | San Diego, CA 92121, USA | Phone 800 FOR MACS | macs@miltenyibiotec.com | **www.miltenyibiotec.com**

Miltenyi Biotec provides products and services worldwide. Visit **www.miltenyibiotec.com/local** to find your nearest Miltenyi Biotec contact.

US421