Safety assessment of MACS® GMP T Cell TransAct™, a robust and potent polyclonal T cell activation and expansion tool for clinical-scale manufacturing of gene-modified T cells

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Introduction

Automated manufacturing of gene-modified T cells for adoptive T cell therapy requires robust and reproducible processes using materials and reagents that fulfill requirements for safe application of the cellular products. Aiming to streamline the safe and robust clinical manufacturing of gene-engineered T cells, we have developed a cGMP-compliant stimulation reagent, MACS® GMP T Cell TransAct™, which allows potent polyclonal T cell activation prior to gene modification (lenti- and retroviral) without the need for feeder cells, and which can be easily integrated into a closed manufacturing process.
MACS GMP T Cell TransAct is a polymeric nanomatrix conjugated to humanized CD3 and CD28 agonists, which is soluble and can be sterile filtered. In contrast to the large beads previously used in the field, MACS GMP T Cell TransAct presents several advantages: No "de-beading" steps are required, resulting in simplification of the manufacturing process. As MACS GMP T Cell TransAct is soluble, there is no more critical dependence on a bead-to-cell ratio, thus enabling more reliable stimulation of T cells over a large range of T cell densities.
However, using immunomodulating substances for the manufacturing of cellular products for, e.g., advanced T cell therapies, a safety assessment of these reagents is essential to identify potential risks associated to their use.

Results

1 Dose-response curve of MACS® GMP T Cell TransAct™

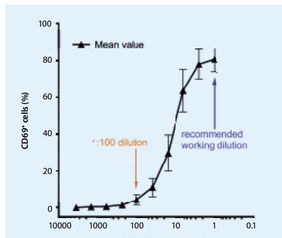

Figure 1

The dose-response curve of MACS® GMP T Cell TransAct™ shows the concentration-dependent activation of T cells in TexMACS™ GMP Medium. With a dilution higher than 1:100 typically no activation of T cells was observed. Figure 1 shows short-time stimulation (24 h) of PBMCs from 12 donors (each in duplicate with defined distribution of age and sex) with MACS GMP T Cell TransAct. The early activation marker CD69 was detected by flow cytometry on the MACSQuant® Analyzer.

2 Typical dilution of MACS® GMP T Cell TransAct™ during a standard CliniMACS Prodigy® TCT run

Residual amounts of MACS® GMP T Cell TransAct™ in the culture were calculated based on the typical dilution factors resulting from a standard manufacturing process on the CliniMACS Prodigy®. After day 6, MACS GMP T Cell TransAct has been diluted below its lowest active concentration based on the dose-response curve (table 1). The reagent was diluted over one million (10^6)–fold, which represents a safety assurance level (SAL) of 4 logs.

Dilution of MACS GMP T Cell TransAct during a TCT run

Day	Step performed	Note	Volume added (mL)	Volume removed (mL)	Total volume (mL)	Dilution factor process step (1:x)	Dilution factor (1:x)
0	Stimulation	Initial dilution of TransAct: 1:17.5	70	-	70	1	1
1	Transduction	Add virus	30	-	100	1.43	1.43
3	Culture wash	Step 1: Remove supernatant	-	60	40	-	
		Step 2: Wash cells	260	-	300	7.5	10.7
		Step 3: remove supernatant	-	260	40	-	
		Step 4: resuspend cells for culture	160	-	200	5	53.6
5	Feed	Add fresh medium and increase total volume	50	-	250	1.25	67
6	Feed	Replacement of medium	125	125	250	2	134
7	Feed	Replacement of medium with fresh one	150	150	250	2.5	335
8	Feed	Replacement of medium with fresh one	150	150	250	2.5	837.5
9	Feed	Replacement of medium with fresh one	180	180	250	3.57	2,989.9
10	Feed	Replacement of medium with fresh one	180	180	250	3.57	10,673.9
11	Feed	Replacement of medium with fresh one	180	180	250	2	38,105.7
12	Final harvest	Step 1: Remove supernatant	-	180	70	-	-
		Step 2: Wash cells	230	-	300	4.29	163,473.3
		Step 3: Remove supernatant	-	230	70	-	-
		Step 4: Wash cells	230	-	300	4.29	701,300.3
		Step 5: Remove supernatant	-	230	70	-	-
		Step 6: Resuspend cells in a total volume of 100 mL	30	-	100	1.43	1,002,859.5
Table 1		Final dilution of MACS GMP T Cell TransAct (for standard process)					1,002,859.5

3 Functionality in T cell culture supernatant

Culture wash is an efficient approach to reduce the amount of MACS GMP T Cell TransAct in the culture. Functional testing of MACS GMP T Cell TransAct in the supernatant of cultures by its capacity to stimulate unstimulated T cells is shown in figure 1. No stimulation via MACS GMP T Cell TransAct in the supernatant was observed. Frequencies of CD69⁺ T cells (A) and CD25⁺CD69⁺ T cells (B) after stimulation with the supernatant of previous T cell stimulation and expansion cultures (obtained from the culture at indicated time points) are depicted. Bars represent mean values of samples from 4 donors (each in triplicate). The stimulation capacity of the supernatant was analyzed after 48 h using the MACSQuant Analyzer.

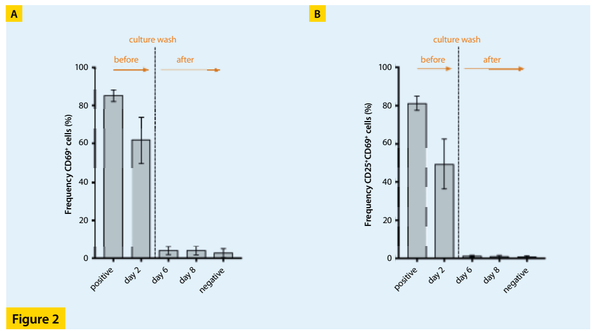

Figure 2

4 Stimulated T cells do not have cross-stimulating capacity

T cells cultured and stimulated for 7 days with MACS GMP T Cell TransAct (with or without wash) were not capable of cross-stimulating other T cells. Figure 3 shows the frequency of CFSE-positive T cells expressing activation markers 48 h after coculture with MACS GMP T Cell TransAct–stimulated T cells cultured for 7 days and washed on the indicated days. Results shown represent data of two experiments (each with samples from two donors in duplicate). Non-stimulated T cells served as negative control. The positive control corresponds to the CFSE-positive cells stimulated for 48 h with MACS GMP T Cell TransAct (standard working dose).

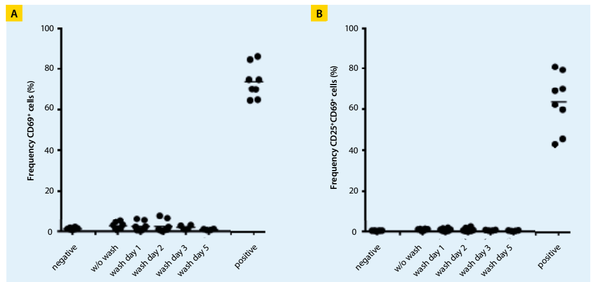

Figure 3

5 No superagonistic behavior of immobilized CD28 agonist

The CD28 agonist covalently linked to the nanomatrix did not show superagonistic behavior. Induction of T cell proliferation (fig. 4) or cytokine secretion from T cells (fig. 5) by monoclonal antibodies or immobilized recombinant humanized agonist was performed using the RESTORE assay[1]. Proliferation was analyzed via CFSE dilution 5 days after initial stimulation (fig. 4). CD3 mAB (OKT3) served as positive control, and CD28 mAB (15E3) as negative control. Lines represent the mean values from 4 donors (each in duplicate). Cytokine secretion was analyzed via MACSplex Cytokine Assay 24 hours after stimulation (fig. 5).

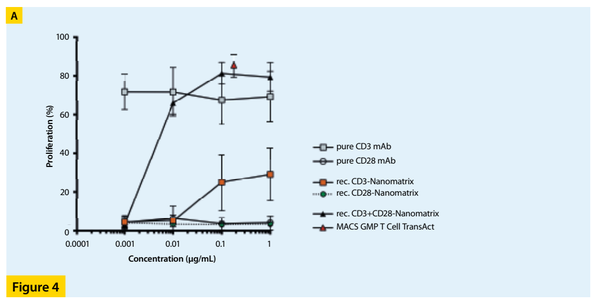

Figure 4

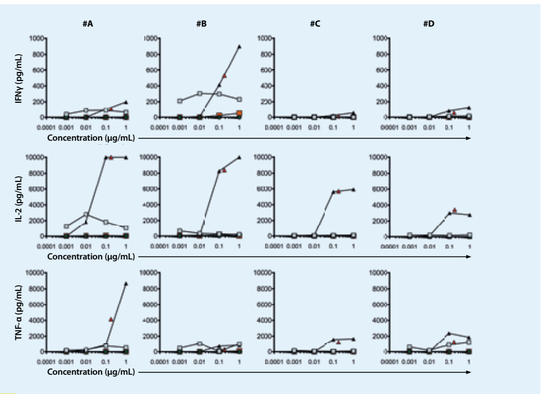

Figure 5

Conclusion

Here we demonstrate the safe use of MACS GMP T Cell TransAct on the automated CliniMACS Prodigy Platform using the TCT application software.

- A dilution higher than 1:100 of the recommended working dilution does not activate T cells.
- After day 6, MACS GMP T Cell TransAct has been diluted below its lowest active concentration based on dose-response curve.
- At the end of the process MACS GMP T Cell TransAct is diluted over one million (10^6)–fold, which represents a safety assurance level (SAL) of 4 logs.
- Culture wash is an efficient approach to reduce the amount of functional MACS GMP T Cell TransAct .
- No stimulation via released MACS GMP T Cell TransAct can be seen.
- T cells stimulated with MACS GMP T Cell TransAct are not capable of cross-stimulating other T cells.
- The immobilized CD28 agonist does not show superagonistic behavior.

Reference

1. Römer *et al.* (2011) Blood 118: 6772–6782.

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