Polyclonal T cell activation and expansion tool for clinical-scale manufacturing of gene-modified T cells

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Introduction

The clinical success of adoptive T cell transfer therapy is resulting in growing enthusiasm as indicated by the ever-increasing number of clinical trials and the involvement of large pharmaceutical companies.
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™. This reagent enables potent polyclonal T cell activation prior to gene modification (lenti- and retroviral) in the absence of feeder cells and can be easily integrated into a closed manufacturing process such as the TCT Process on the CliniMACS Prodigy®. MACS GMP T Cell TransAct is a soluble polymeric nanomatrix conjugated to humanized CD3 and CD28 agonists. It can be sterile-filtered and is biodegradable. In contrast to the large beads previously used in the field, MACS GMP T Cell TransAct presents several advantages: "de-beading" steps are no longer required resulting in simplification of the manufacturing process. Moreover, as the reagent is soluble, there is no more critical dependence on a bead-to-cell ratio, thus enabling more reliable and flexible conditions for the stimulation of T cells over a large range of T cell densities.

Results

1 TransAct™ T Cell Reagent enables potent T cell stimulation, proliferation, and expansion in research settings

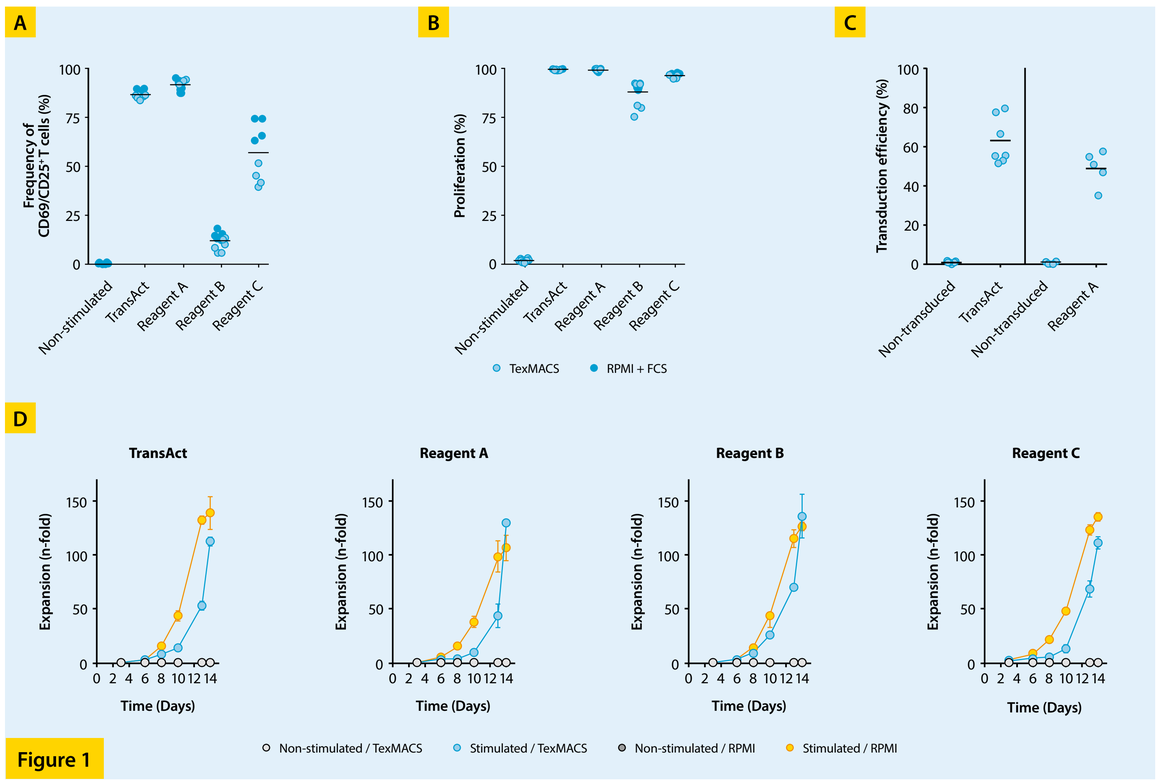

Figure 1

Pan T cells were stimulated with TransAct T Cell Reagent or three different products from other manufacturers (reagents A, B, C). Activation was assessed by measuring expression of the activation markers CD25 and CD69 on day 2 (fig. 1A). On day 6, proliferation was analyzed by CFSE dilution assay (fig. 1B). Lentiviral gene expression, i.e., transduction efficiency, after polyclonal stimulation was evaluated on day 7 (fig. 1C). Expansion was analyzed at the indicated time points for T cells cultured in serum-free conditions (TexMACS™ Medium) or RPMI + 10% FCS (fig. 1D). All cultivation steps took place in 96-well culture dishes. Data represent the average from two experiments, each in triplicate, with T cells from two donors.

2 Clinical-scale manufacturing of gene-modified T cells

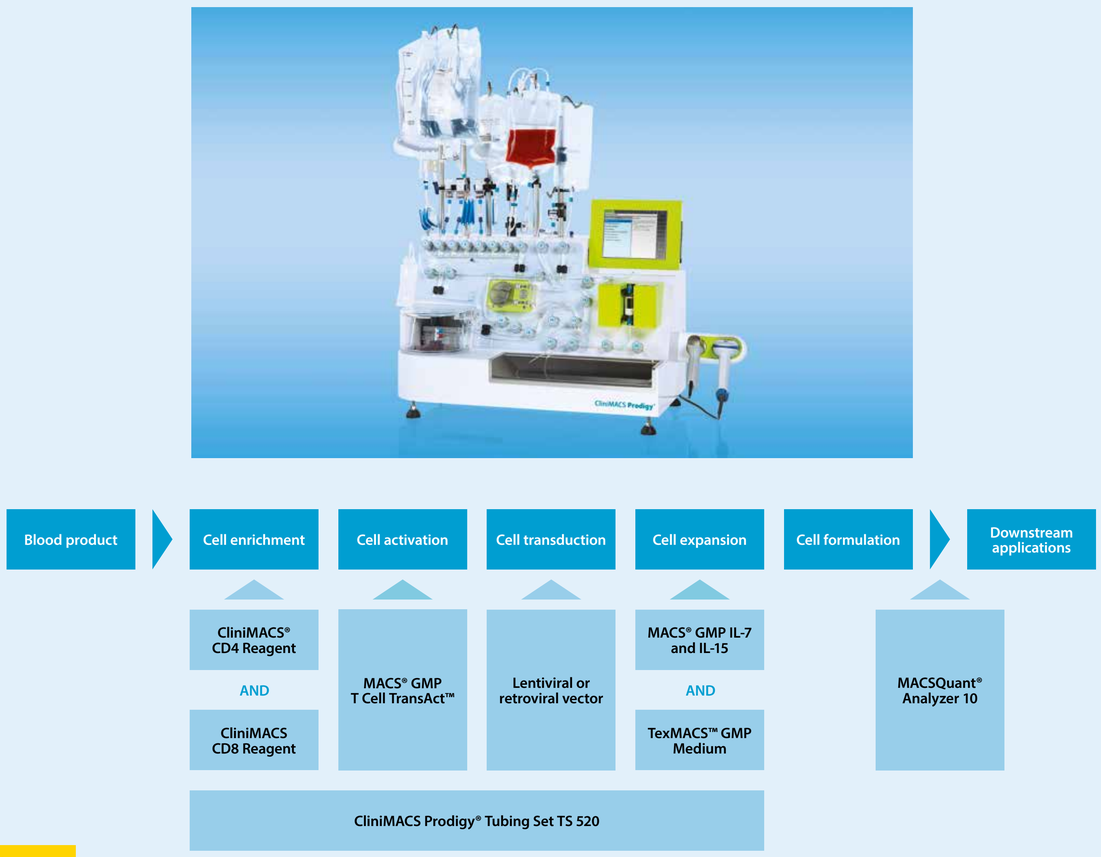

Figure 2

Automated polyclonal T cell stimulation
For evaluation of clinical-scale manufacturing, $CD4^+/CD8^+$ T cells were enriched from a blood sample, subsequently cultured in TexMACS GMP Medium supplemented with IL-7 and IL-15, and activated using MACS GMP T Cell TransAct. The entire process was performed automatically in the CliniMACS Prodigy (fig. 2). Pictures were taken with the integrated microscope camera of the CliniMACS Prodigy, 24, 48, and 72 hours after stimulation (fig. 3).

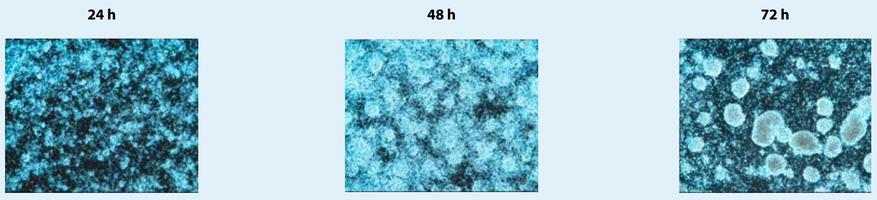

Figure 3

Generic manufacturing of CAR T cells
Enriched $CD4^+/CD8^+$ T cells were subjected to polyclonal stimulation with MACS GMP T Cell TransAct, transduced with lentiviral vectors, and expanded in TexMACS GMP Medium supplemented with IL-7 and IL-15 (n = 11). All steps were performed using the standard TCT Process (fig. 2) on the CliniMACS Prodigy. The T cell culture was monitored at different time points to determine cell viability (fig. 4A). The absolute cell count of viable cells over time was calculated (fig. 4B).

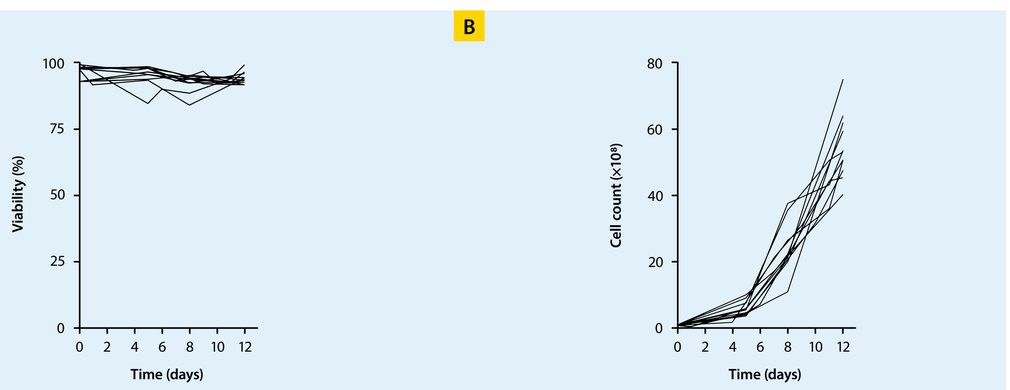

Figure 4

Lenti- or retroviral modification after polyclonal activation
T cells were enriched using CliniMACS CD4 and CD8 Reagents and stimulated with MACS GMP T Cell TransAct. Polyclonally activated T cells were modified using lentiviral or retroviral constructs encoding GFP or CAR. The complete process to generate genetically engineered T cells was performed in a single closed platform, the CliniMACS Prodigy and Tubing Set TS 520. The transduction efficiency was determined six days after transduction (fig. 5).

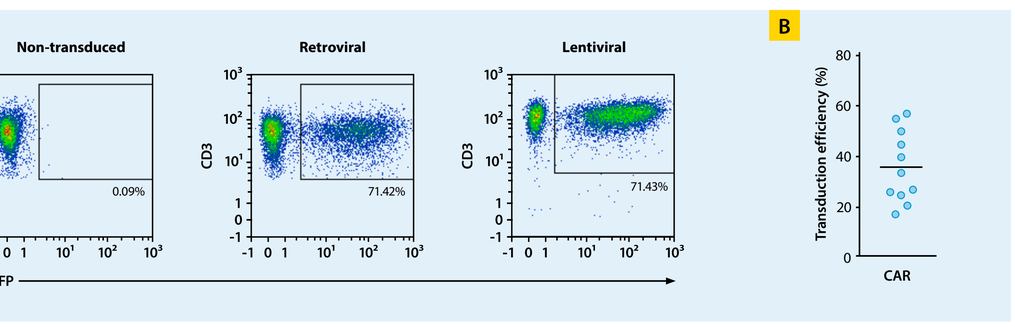

Figure 5

3 High-efficiency transfection of human T cells using electroporation

T cells were enriched using CliniMACS CD4 and CD8 Reagents, stimulated with TransAct T Cell Reagent, and transfected with plasmid DNA or RNA, both encoding GFP, three days after activation. Transfection was performed using the electroporation platform of the CliniMACS Prodigy. The transfection efficiency was assessed by flow cytometry (MACSQuant® Analyzer 10) 24 hours after electroporation.

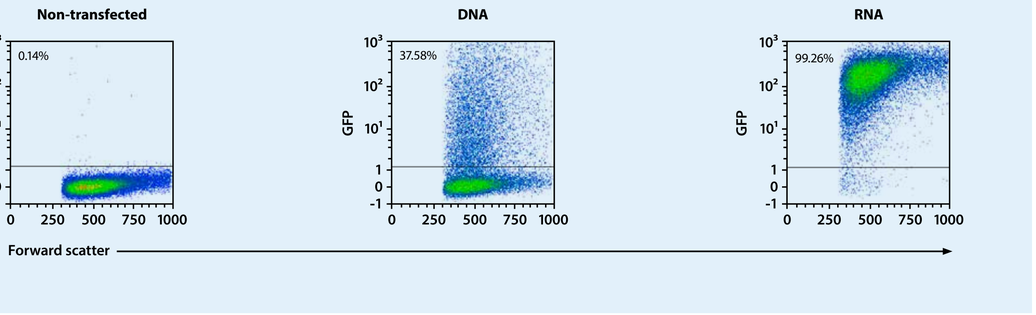

Figure 6

Conclusion

- MACS GMP T Cell TransAct enables potent and robust T cell activation.
- The reagent can be used in combination with the commercially available TCT Process on the CliniMACS Prodigy.
- The automated process for manufacturing gene-modified T cells yields a consistent cell product for the development of adoptive T cell therapies.

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