Bringing automation to large-scale manufacturing of TCR modified T cells for adoptive cell therapy

Carola Barth, Daniela Gudert, Alexander Bohn, Sophia Lollies, Peter Maul, Marion Braun, Ulrike Abramowski-Mock, Ronald Janzen, Carolin Kolbe, Eiad Kabaha, Kai Schicktanz, Juliane Raasch, Burgund Kauling, Nadine Mockel-Tenbrinck, Andrew Kaiser
Miltenyi Biotec B.V. & CO. KG, Germany

Introduction

Adoptive immunotherapy using gene-modified T cells redirected against cancer has proven clinical efficacy and tremendous potential in several medical fields. However, such personalized medicine faces several challenges in the complexity associated with current clinical manufacturing methods. Conventionally, preparation of autologous gene-modified T cells comprises of many open handling steps, is labor intensive and is not adapted to automatically manufacture large numbers of gene-engineered T cells. Moreover, the cell manufacturing process requires extensive training of personnel as well as dedicated infrastructure, both restrict the widespread use of these clinical procedures.

Here, we demonstrate that our automated process enables the generation of large numbers of gene-engineered T cells for adoptive T cell therapy. On average $1.55x10^{10}$ T cells could be obtained, meaning a 2.3-fold higher number compared to our standard T Cell Transduction (TCT) process making it more suitable for applications where high doses of gene-engineered T cells are meant to be used such as in TCR-transduced T cells. Furthermore, this new process yields a cell product comparable in respect to cellular composition, T cell subset ratio and differentiation. In addition, *in vitro* functionality of the gene-modified T cells was demonstrated.

Methods

1 T Cell Transduction – Large Scale (TCT-LS)

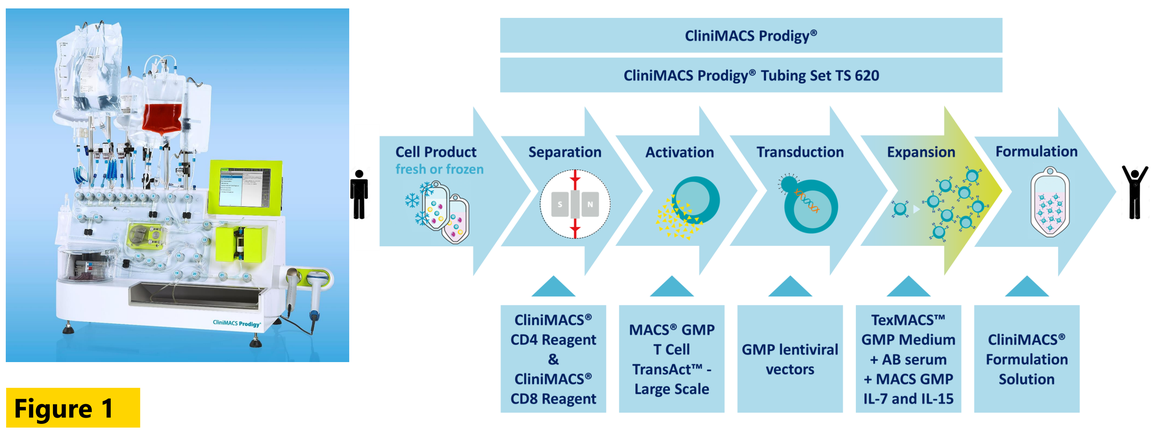

Figure 1

Results

1 Automated enrichment of T cells

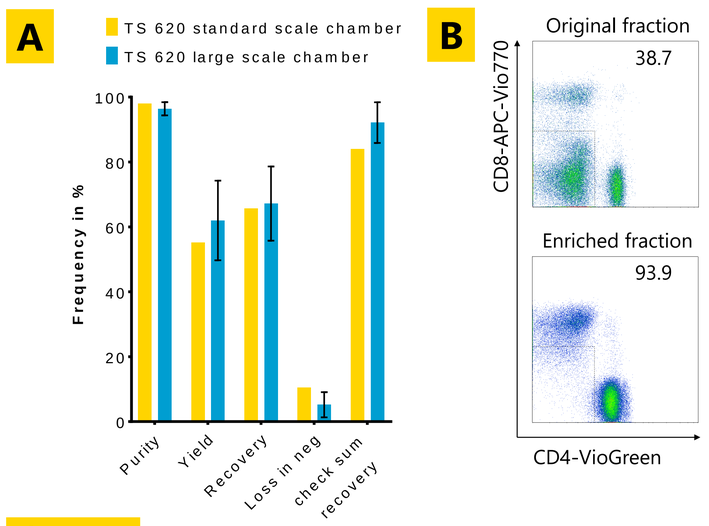

Figure 2

T cells were automatically enriched from an apheresis product (healthy donor) using the CliniMACS Prodigy® in combination with the TS 620, which contains either the standard chamber (n=1) or the large-scale chamber (n=4).
After purification of CD4+ and CD8+ cells selection performance was calculated. In Figure 1A mean average and standard deviation of all runs are shown. Enriched CD4+ and CD8+ cells were analyzed by flow cytometry on the MACSQuant® Analyzer 10 (Fig. 1B)

2 Automated manufacturing of T cells

Enriched $CD4^+/CD8^+$ T cells were automatically expanded in the CliniMACS Prodigy® after polyclonal stimulation with MACS GMP T Cell TransAct (standard or Large Scale). Either the standard scale chamber (n=4) or the large-scale chamber (LS, n=10) was used for culture. The T cell culture was monitored at different time points to determine the absolute cell count of viable T cells which was calculated afterwards (Fig. 3A), the cell density (Fig. 3B) and viability (Fig. 3C). On average, a total cell number of $1.55x10^{10}$ cells was reached using the large-scale chamber in comparison to $6.5x10^9$ cells expanded in the standard scale chamber.

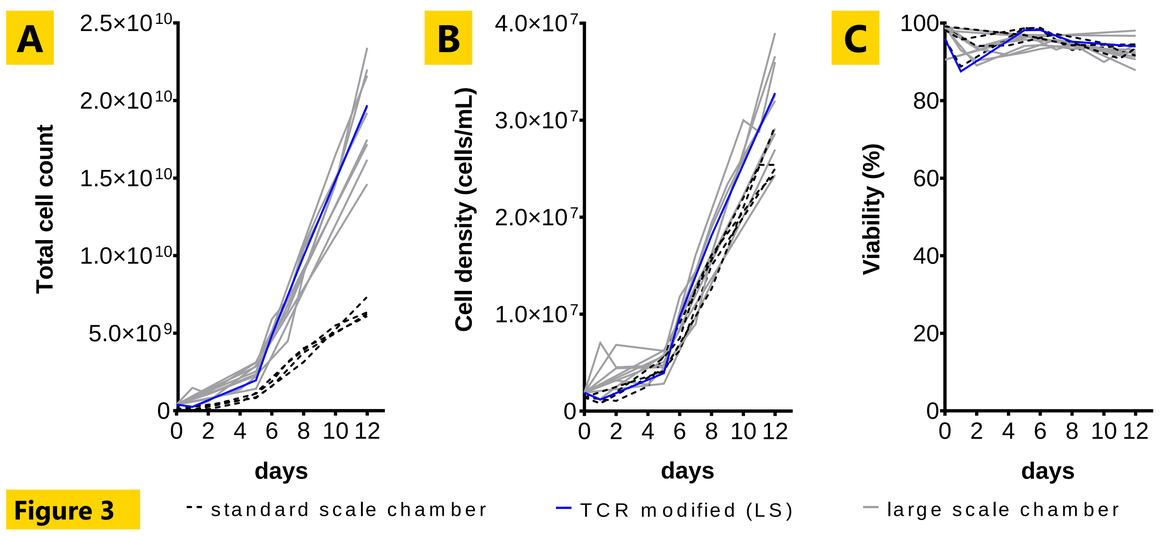

Figure 3

3 Quality Control

Immunophenotyping

The cellular composition of the enriched fraction either separated with the standard scale chamber (n=4) or the large-scale chamber (n=10) was analyzed by flow cytometry on the MACSQuant® Analyzer 10.

Frequencies of immune cell types among viable $CD45^+$ cells were determined for the enriched population (Fig. 4A), or the final product automatically generated in the CliniMACS Prodigy® (Fig. 4B).

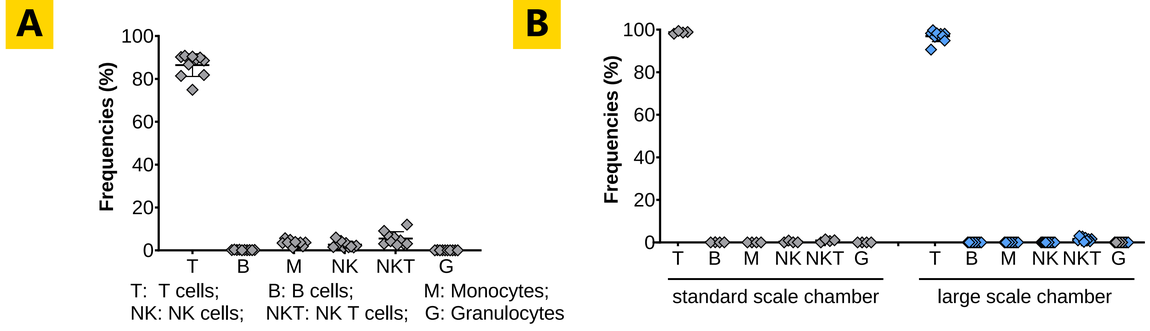

Figure 4

T cell phenotype

Frequencies of naïve (T_N), stem cell memory (T_{SCM}), central memory (T_{CM}), effector memory (T_{EM}) and effector T cells (T_{EFF}) were analyzed based on CD95, CD62L and CD45RO expression in the final product (Fig. 5A). Frequencies of $CD4^+$ and $CD8^+$ T cells were analyzed in the enriched fraction and the final product (Fig. 5B).

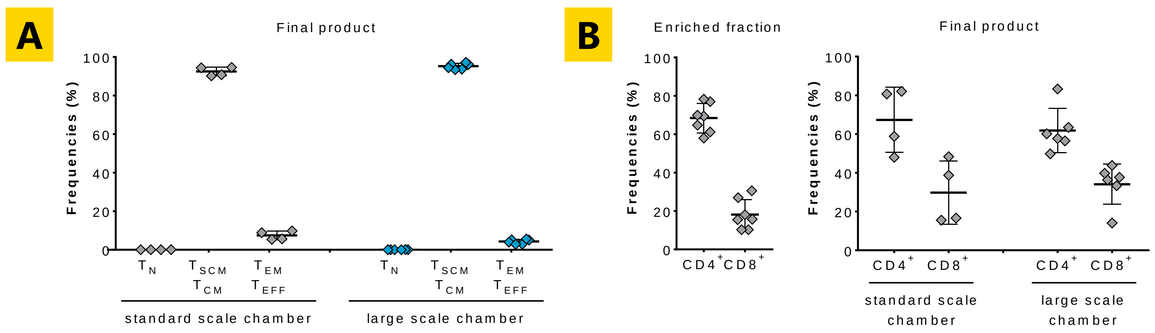

Figure 5

Transduction efficiency and numbers of gene-modified T cells

Enriched $CD4^+$ and $CD8^+$ T cells were stimulated for 24 hours before transduction using lentiviral vectors encoding different Chimeric Antigen Receptors (CAR, n=6) or a T Cell Receptor (TCR, n=1). All lentiviral transductions were performed using the TS 620 which contains the large-scale chamber. Efficiency of genetic modification was analyzed by flow cytometry (Fig. 6) followed by calculation of the cell count of gene-modified T cells (Tab. 1).

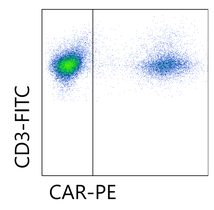

Figure 6

n=	Genetic modification	Transduction efficiency	T cell count	Cell count of gene-modified T cells
6	CAR	39% (± 14%)	$1.85x10^{10}$ ($\pm 3.15x10^9$)	$7.05x10^9$ ($\pm 1.95x10^9$)
1	TCR	28%	$1.97x10^{10}$	$5.52x10^9$

Table 1

4 *In vitro* functionality of CAR T cells

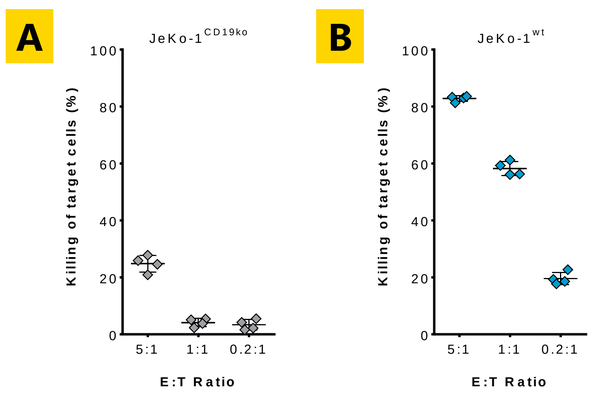

Figure 7

The functionality of gene-engineered CAR T cells, manufactured automatically using the CliniMACS Prodigy® TCT - LS (n=2), was assessed based on a cytotoxicity assay. Specific cell lysis was determined with JeKo-1^{CD19ko} control cells (Fig. 7A) or JeKo-1^{wt} target cells (Fig. 7B) co-cultured with CD19CAR T cells for 24 hours at the indicated effector-to-target (E:T) cell ratios (Fig. 7).

Conclusion

We have further extended capacity of the automated process for lentiviral gene-modification and expansion of selected T cells enabling the manufacturing of large amounts of T cells. The CliniMACS Prodigy® TCT – LS application allows purification of CD4 and CD8 positive cells , polyclonal T cell stimulation (using MACS GMP T Cell TransAct – Large Scale) followed by lentiviral gene-modification and expansion of T cells in a single-use closed tubing set (TS 620).

The large-scale application is an advancement of the CliniMACS Prodigy® TCT application and allows the cultivation and expansion of higher cell numbers by using a larger cell preparation and cultivation unit.
Taken together, the automated TCT-LS application on the CliniMACS Prodigy® is capable of yielding a cell dose compatible with the needs for higher infusion doses often applied clinically for TCR modified T cells.