Automated manufacturing of gene-engineered T cells under serum-free conditions

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Introduction

Automated manufacturing of gene-modified T cells for adoptive T cell therapy requires robust and reproducible processes that are based on materials and reagents that must fulfill strict safety requirements. However, it can be difficult to obtain these materials and reagents in large enough quantities. GMP-compliant human AB serum is one of such reagents, which is, in most cases, used for potent T cell expansion in culture systems. Therefore, improving methods to generate sufficient numbers of gene-engineered T cells suitable for clinical use, independent of human AB serum, is essential for the commercial scalability of automated cell manufacturing processes.

Methods

1 T Cell Transduction Process on the CliniMACS Prodigy®

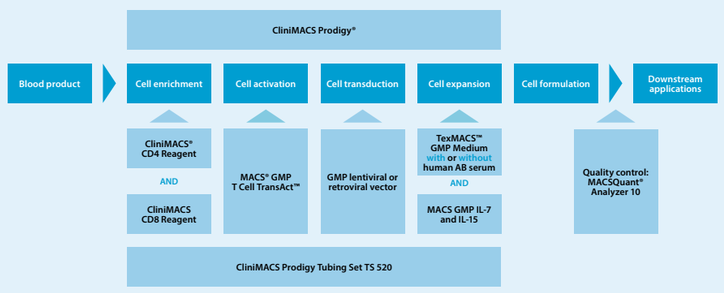

Figure 1

Results

1 Fully automated manufacturing of gene-modified T cells

$CD4^+/CD8^+$ T cells were automatically enriched, stimulated, and expanded in the CliniMACS Prodigy® (fig. 1). Either the standard TCT Process with 3% human AB serum (n = 11) or a serum-free TCT Process (n = 11) was performed. The T cell cultures were monitored at different time points to determine cell count (fig. 2A) and viability (fig. 2B). The absolute expansion of viable T cells was calculated afterwards (fig. 2C).

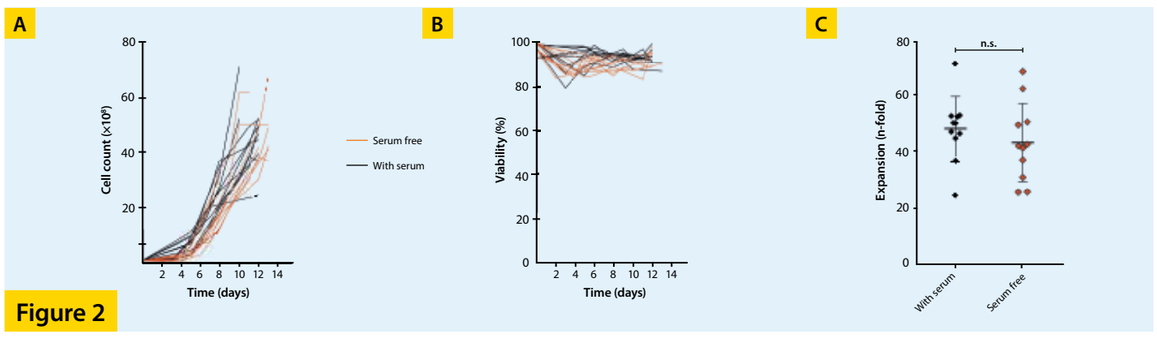

Figure 2

2 Quality control

Immunophenotype

The cellular composition of enriched $CD4^+/CD8^+$ cells manufactured in the CliniMACS Prodigy with 3% human AB serum (n = 11) or serum-free condition (n = 11) was analyzed by flow cytometry using the MACSQuant® Analyzer 10. Frequencies of different cell types among viable $CD45^+$ cells were determined for the enriched population (fig. 3A) or the final product automatically generated in the CliniMACS Prodigy (fig. 3B).

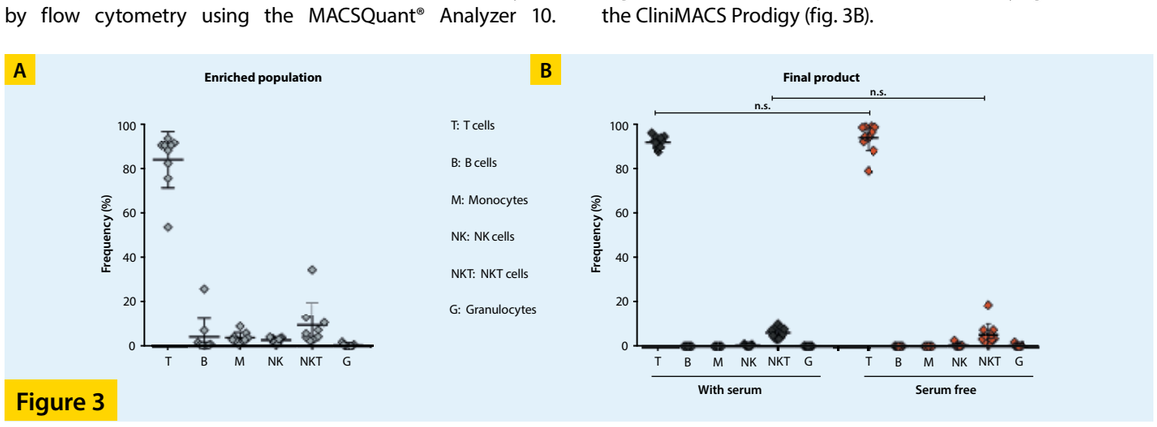

Figure 3

T cell phenotype

Frequencies of naive (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. 4A). The percentages of $CD4^+$ and $CD8^+$ T cells were analyzed in the enriched population and the final product (fig. 4B).

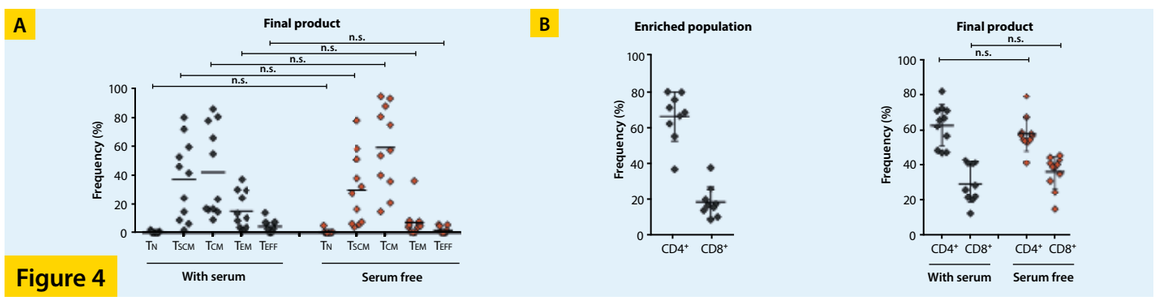

Figure 4

Transduction efficiency and CAR T cell counts

CAR T cell frequencies in the final product were analyzed for the automatically manufactured CAR T cells (CliniMACS Prodigy) in the presence of 3% human AB serum (n = 8) or under serum-free conditions (n = 8) (fig. 5A). The absolute numbers of CAR T cells were calculated based on the number of viable T cells of the respective manufacturing run (fig. 5B).

Figure 5

3 *In vitro* functionality of CAR T cells

The functionality of gene-engineered CAR T cells was assessed based on a cell killing assay. Specific cell lysis was determined by coculture of target cells with CAR T cells at the indicated effector-to-target cell (E:T) ratios for 24 hours (fig. 6). CAR T cells were automatically manufactured (CliniMACS Prodigy) either in the presence of 3% human AB serum (n = 6) or under serum-free conditions (n = 6).

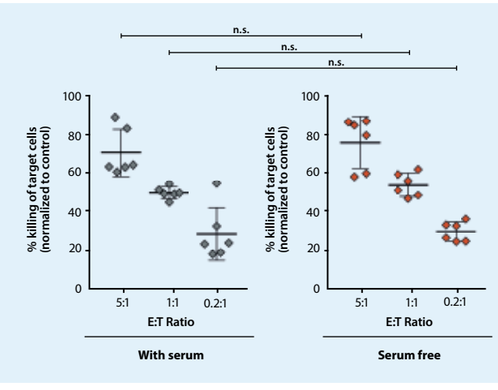

Figure 6

4 *In vivo* functionality of CAR T cells

Anti-tumor activity of automatically manufactured CAR T cells (CliniMACS Prodigy) either in the presence of 3% human AB serum or under serum-free conditions was analyzed. NSG mice received 5×10^5 target cells (i.v.). After tumor engraftment (7 days) 1×10^6 CAR T cells or mock T cells were injected and tumor growth was measured at the indicated time points (fig. 7).

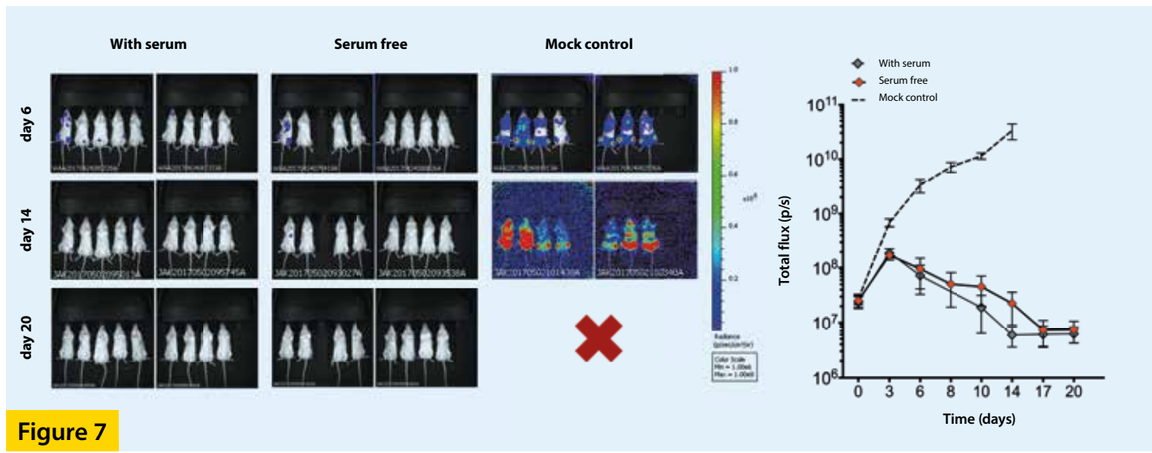

Figure 7

Conclusion

- The T Cell Transduction (TCT) process developed on the CliniMACS Prodigy platform enables robust manufacturing of gene-modified T cells without the need for serum supplementation.
- T cell numbers, viability, and phenotype were comparable between the serum-free TCT process and a standard process which relies on 3% human AB serum.
- Lentiviral transduction in the absence of serum led to an increased efficiency of gene modification resulting in larger CAR T cell numbers. CAR T cells were fully functional *in vitro* and *in vivo*.

These improvements are another step towards simplified, fully automated manufacture of CAR T cells, designed for the treatment of a large number of patients, at a commercial scale.

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