Simple automated manufacturing of gene-engineered T cells from melanoma and lymphoma patient material for adoptive T cell therapy

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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 the current clinical manufacturing methods. Conventionally, the preparation of autologous gene-modified T cells comprises many (open) handling steps, is labor intensive, and is not adapted for the treatment of large numbers of patients or for commercial manufacturing. Moreover, the cell-manufacturing process requires extensive training of personnel as well as a dedicated infrastructure, which restricts these clinical procedures to very few institutions worldwide.

We have developed a robust and reproducible automated manufacturing process for lentiviral gene modification and expansion of selected T cells. The CliniMACS Prodigy® TCT (T Cell Transduction) Process software allows purification and polyclonal T cell stimulation followed by gene-modification and expansion of T cells in a single-use closed tubing set.
The TCT process is capable of dealing with highly diverse cell sources while yielding a consistent cellular product. Such a novel manufacturing method should significantly facilitate manufacturing of gene-modified T cells and thereby support clinical application of adoptive cell transfer therapy.

Methods

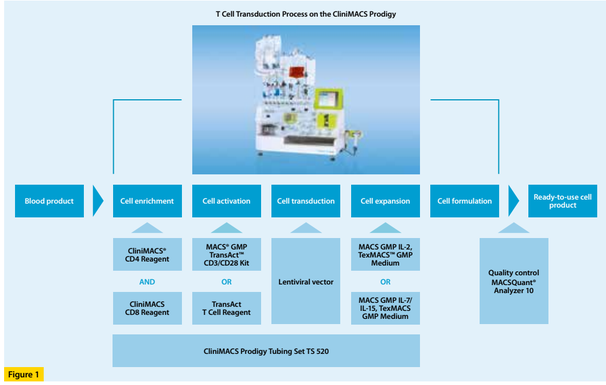

Figure 1

Results

1 Automated T cell selection process

(A) CD4⁺ and CD8⁺ cells from leukapheresis (LP) products obtained from a healthy donor or a patient were analyzed by flow cytometry. (B) Examples of cellular composition before and after CD4⁺/CD8⁺ cell enrichment from whole blood (WB) obtained from a melanoma patient or LP obtained from a lymphoma patient are shown. Average purities of enriched CD4⁺/CD8⁺ T cells were 84% for samples from healthy donors and 63% for patient samples (not shown). (C) The percentage of viable enriched cells among all CD45⁺ cells are depicted for samples from healthy donors or patients.

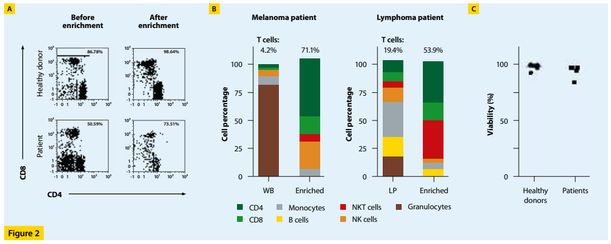

Figure 2

2 T cell activation

CD4⁺/CD8⁺ T cells from a healthy donor (upper row) or melanoma patient (lower row) were enriched and cultured in TexMACS™ GMP Medium supplemented with IL-7 and IL-15 and activated using the TransAct™ T Cell Reagent. Pictures were taken with the integrated microscope camera of the CliniMACS Prodigy® 24 and 72 hours after stimulation.

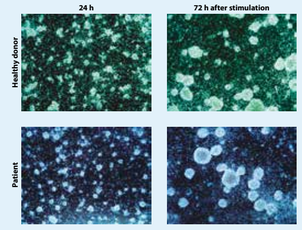

Figure 3

3 T cell transduction

Enriched CD4⁺ and CD8⁺ T cells stimulated using the TransAct T Cell Reagent were transduced on day 1 with a lentiviral vector encoding CD20 CAR. (A) Lentivirally transduced T cells derived from material obtained from a healthy donor or a lymphoma patient were analyzed by flow cytometry, and (B) transduction efficiencies of CD20 CAR-modified T cells were determined (healthy donors: n = 9; patients: n = 5).

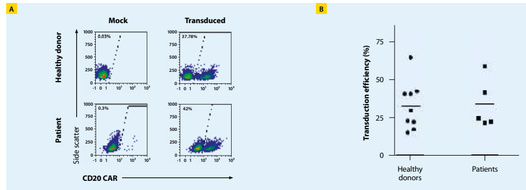

Figure 4

4 T cell expansion

Enriched CD4⁺/CD8⁺ T cells were automatically expanded in the CliniMACS Prodigy after selection, polyclonal stimulation, and transduction. Material from either healthy donors (n = 12) or patients (n = 6) was used. The T cell culture was monitored at different time points to determine cell density (A) and viability (B). The absolute cell count of viable T cells was calculated afterwards (C and table 1).

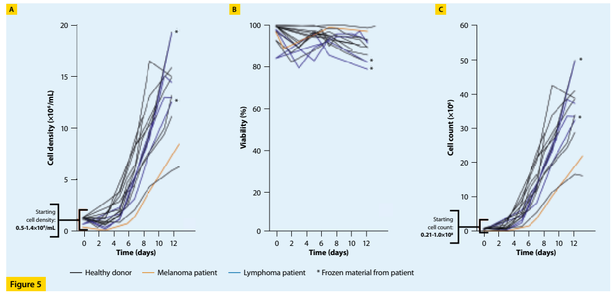

Figure 5

T cell expansion

Healthy donor material	T cell count for culture (mean)	Final T cell count (mean)	T cell expansion (mean)	**Final CAR⁺ T cell count (mean)**
Non-transduced (n = 5)	0.87×10^8 (±0.2)	2.80×10^8 (±1.3)	36-fold (±23)	–
Transduced (n = 7)	1.00×10^8 (±0.0)	3.06×10^8 (±0.9)	32-fold (±12)	**9.07×10^8 (±5.4)**

Patient material	T cell count for culture	Final T cell count	T cell expansion	**Final CAR⁺ T cell count**
Melanoma (M35-WB)	0.21×10^8	2.35×10^8	112-fold	**1.38×10^8**
Lymphoma (L42-LP)	1.00×10^8	3.68×10^8	37-fold	**1.54×10^8**
Lymphoma (L43-LP)	1.00×10^8	3.31×10^8	33-fold	–
Lymphoma (L55-LP)*	0.55×10^8	4.90×10^8	89-fold	**1.09×10^9**
Lymphoma (L56-LP)*	0.55×10^8	3.20×10^8	58-fold	**0.72×10^9**
Mean	**0.66×10^8 (±0.33)**	**2.89×10^8 (±0.7)**	**66-fold (±34)**	**1.18×10^9 (±3.6)**

* frozen start material

Table 1

5 Quality control – cellular composition

The cellular composition of enriched and cultured CD4⁺/CD8⁺ cells derived from material obtained from healthy donors (n = 10) or patients (n = 5) was analyzed by flow cytometry. Frequencies of T cells among viable CD45⁺ cells were determined for the enriched population and the final product generated in the CliniMACS Prodigy. All analyses were done with the MACSQuant® Analyzer 10.

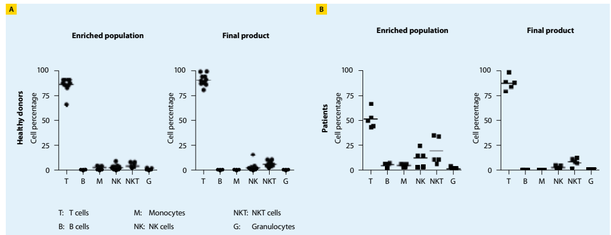

Figure 6

6 Quality control – functionality of CD20 CAR⁺ T cells

The functionality of gene-engineered CD20 CAR⁺ T cells was assessed based on cytokine secretion and cell killing assays. (A–B) Cytokine secretion of CD20 CAR⁺ T cells after co-culture with JeKo-1 as the target cell line (effector:target = 1:1) was measured with the MACSPlex Cytokine 12 Kit. CD20 CAR⁺ T cells were derived from healthy donor (A; n = 5) or patient (B; n = 4) samples. All cells were manufactured in the CliniMACS Prodigy. C: Specific CD20⁺ target cell killing was determined by co-culture of CD20 CAR⁺ T cells, which were either derived from healthy (n = 8) or patient material (n = 4), with the CD20⁺ target cell line, JeKo-1, at the indicated effector: target cell (E:T) ratios for 24 hours.

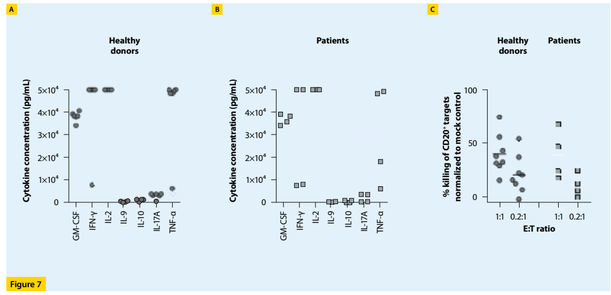

Figure 7

7 Quality control – T cell phenotype

The frequency of naive (T_{NC} CD62L⁺CD45RO⁻), effector memory (T_{EM} CD62L⁻CD45RO⁺), and central memory (T_{CM} CD62L⁺CD45RO⁺) T cells was analyzed by flow cytometry. Percentages of CD4⁺ and CD8⁺ T cells in the enriched population vs. the final product manufactured in the CliniMACS Prodigy were determined (healthy donors: n = 13; patients: n = 5).

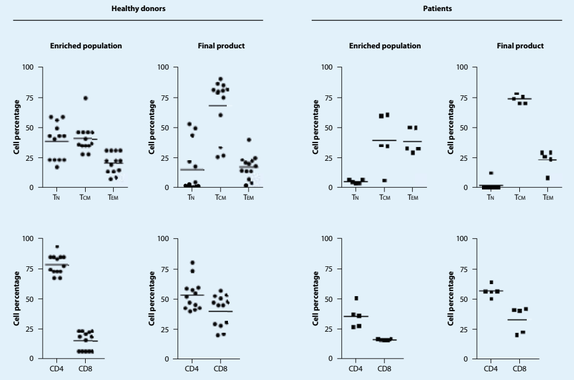

Figure 8

Conclusion

Here we show that the automated process on the CliniMACS Prodigy enables consistent manufacturing of T cells expressing a CAR against CD20, based on material obtained from healthy donors or lymphoma or melanoma patients.

- On average, 1×10^9 CAR-modified T cells could be obtained, both from healthy donor and patient material.
- No difference in cell growth was observed regardless of whether T cells were genetically engineered or not.
- All manufactured T cells mainly had a central memory phenotype.
- All lentivirally modified T cells showed *in vitro* functionality.

The automated process requires only 4 liters of medium, as little as 60 mL of human AB serum, and takes advantage of a humanized recombinant activation reagent for which no "bead removal" step is required.

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