Automated *ex vivo* isolation, transduction and expansion of CD137-positive tumor-reactive T cells on the CliniMACS Prodigy®

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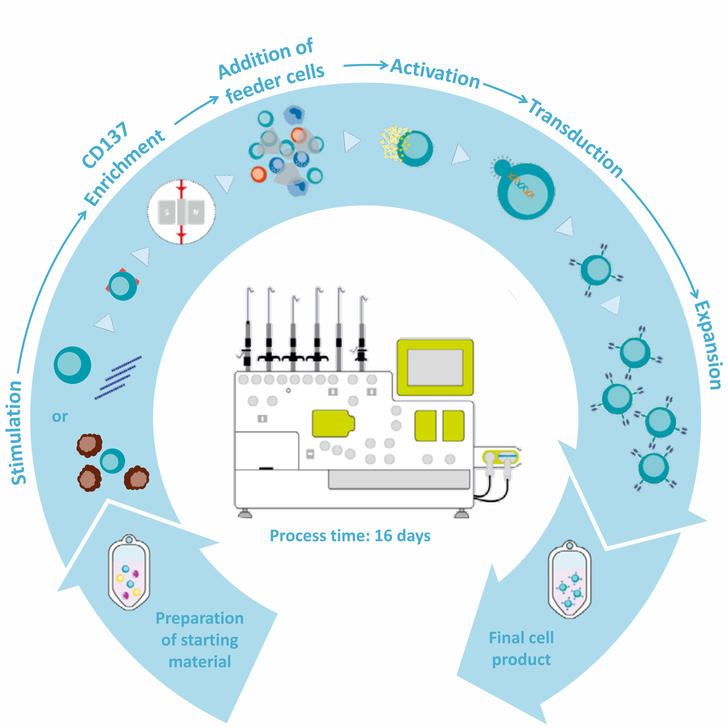
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Introduction

Tumor-infiltrating lymphocyte (TIL) therapy has been an efficacious and safe treatment for solid tumors for many years in early stage clinical trials. However widespread application of TILs is challenged by lack of method standartization, laborious and complicated manufacturing process and low numbers of tumor-reactive cells within the final cellular product. We have addressed these challenges by developing the CliniMACS Prodigy® Tumor Reactive T cell (TRT) System. The CliniMACS Prodigy® TRT Sytem has automated methodologies for T cell stimulation, activation-based selection, expansion with an optional transduction and the final formulation of the patient-ready product. Additionally, we also demonstrate the alternative uses of the TRT System for the isolation and culture of virus-specific T cells (VSTs) for other potential applications.

Methods

Figure 1

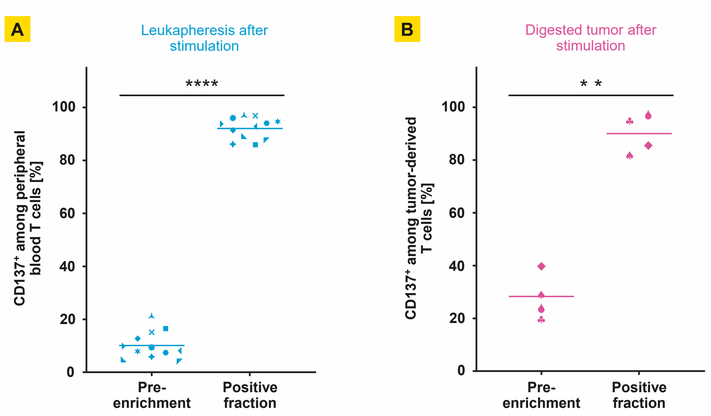


Tumor digest or blood product can be applied as a starting material to the CliniMACS Prodigy® TRT Process. After installation and priming of a single-use sterile Tubing Set, necessary materials are attached and the process begins with a stimulation culture in presence of autologous tumor cells or viral peptides of interest to trigger the activation of tumor-specific or antigen-specific T cells. A suitable stimulation time and culture conditions can be selected and optimized by the user. Following stimulation, T cells expressing CD137 activation marker are enriched and expanded in presence of anti-CD3 pure OKT3 antibody, 3000 IU/mL IL-2 and irradiated feeder cells for 13-14 days. Culture conditions including cytokine composition and medium exchanges can be adjusted. Optionally cells can be genetically modified by viral transduction. If desired, TILs can also be directly cultured within the TRT System without enrichment.

Results

1 CD137 enrichment on the CliniMACS Prodigy® yields a pure CD137 positive population

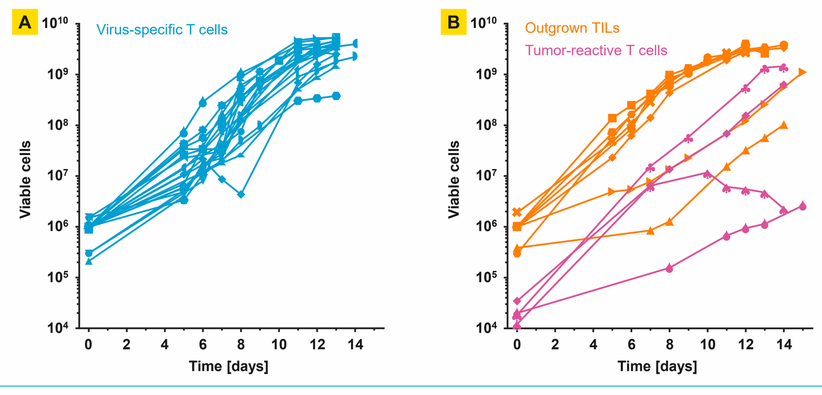
Figure 2



Flow cytometry analysis of the cell fractions before and after automated CD137 enrichment on the CliniMACS Prodigy®. (A) Leukapheresis from peripheral blood of a healthy donor was stimulated with PepTivator pools derived from AdV, CMV and EBV during stimulation culture for 16-18 hours to produce virus-specific T cells. On average, approximately 8 % of T cells expressed CD137 in the pre-enrichment fraction compared to 92.3 % in the enriched positive fraction. (B) Tumor-derived T cells within digested melanoma tumor material after overnight stimulation culture. Average frequency of CD137+ T cells was 28.27 % in the pre-enriched and 90.06 % in the positive fraction. Different symbols indicate samples from different donors.

2 Successful expansion of VSTs, TRTs and outgrown TILs with the TRT Process

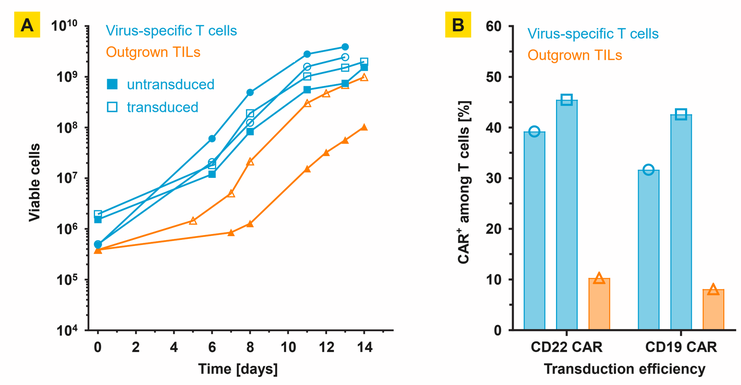
Figure 3



T cell expansion rates for automated culturing on the CliniMACS Prodigy®: (A) Virus-specific T cells had an expansion rate of 380-16,865 fold. (B) Tumor-reactive T cells had an expansion rate of 130-20,309 fold and outgrown TILs had an expansion rate of 120-78,804 fold. Each line represents a separate TRT run. Starting cell numbers from 10^4-10^6 T cells were sufficient to produce clinically relevant numbers of reactive T cells.

3 Automated transduction of VSTs and TILs with the TRT Process

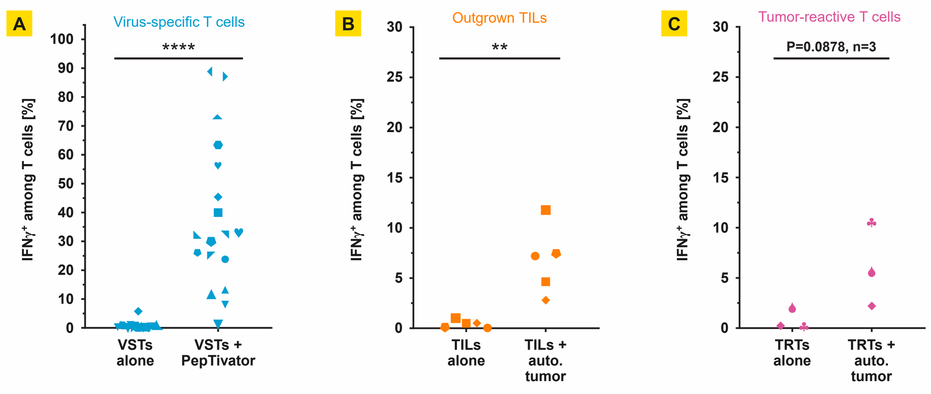
Figure 4



Virus-specific T cells or outgrown TILs were expanded with the CliniMACS Prodigy® TRT Process and transduced with lentiviral anti-CD22-19 tandem CAR. (A) High expansion rates could be preserved in transduced VSTs and TILs and were similar or higher compared to non-transduced controls. (B) VSTs were transduced with MOI 20 and TILs with MOI 13.

4 VSTs, TRTs and TILs expanded on the CliniMACS Prodigy® are reactive against the initial stimulus

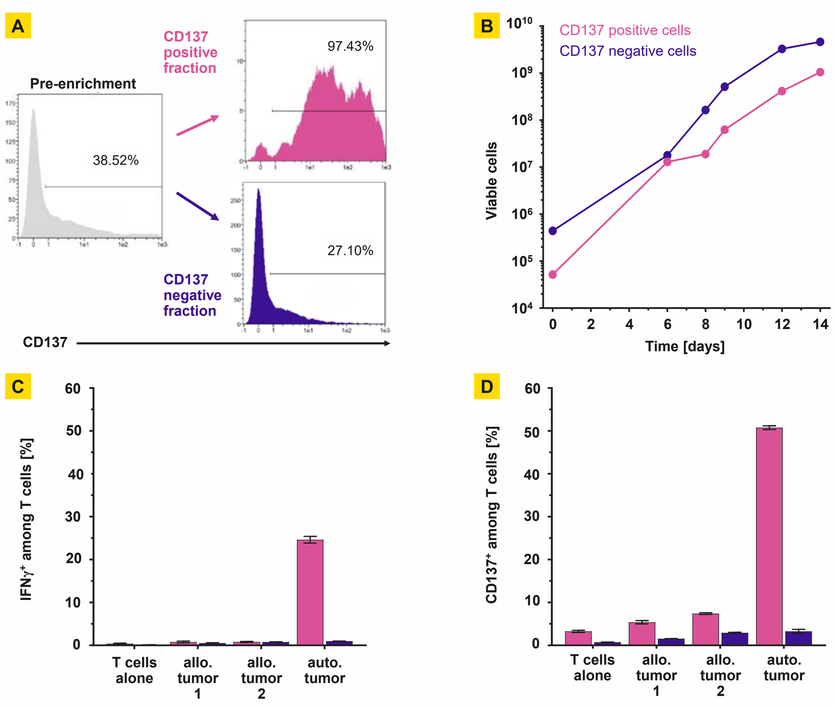
Figure 5



T cells harvested after REP on the CliniMACS Prodigy® were re-stimulated with virus PepTivator pools or autologous tumor cells. In order to access IFN-γ production, intracellular cytokine staining was performed and subsequently analyzed by flow cytometry. Different symbols indicate samples from different donors.

5 Generation of functional Tumor-reactive T cells: proof of concept

Figure 6



Melanoma tumor digest was stimulated overnight in the CliniMACS Prodigy®. (A) After the CD137 enrichment step both positive and negative fractions were expanded, reaching high expansion rates of 20,309 fold in the positive and 10,545 fold in the negative fraction (B). Harvested cells were re-stimulated with either allogeneic or autologous tumor cells overnight and reactivity was assessed by intracellular IFN-γ (C) or CD137 (D) expression. Data is presented as mean ± SD.

Conclusions and outlook

- The CliniMACS Prodigy® Tumor Reactive T cell System is suitable for manufacturing of tumor-reactive T cells, virus-specific T cells and for the expansion of outgrown TILs.
- Highly pure CD137-positive cell populations can be achieved by the automated magnetic enrichment, following stimulation on the CliniMACS Prodigy®.
- The TRT Process allows high rates of expansion of the enriched reactive T cells as well as outgrown TILs.
- Tumor-reactive T cells stimulated and enriched for CD137 have increased reactivity against the autologous tumor cells relative to CD137 depleted fractions.

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