Automated isolation and expansion of CD137 positive tumor-reactive T cells on the CliniMACS Prodigy®

Christina Völzke, Lisa Böttcher, Elvira Criado-Moronati, Peter Maul, Andrew Kaiser, Mario Assenmacher, Andrzej Dzionek, and Bianca Heemskerk
Miltenyi Biotec GmbH, Bergisch Gladbach, Germany

Introduction

Despite the remarkable success of adoptive cell transfer (ACT) of tumor-infiltrating lymphocytes (TILs) for cancer therapy, conventional TIL expansion methods constitute a laborious process which hampers a widespread application. The anti-tumor response of this therapy usually relies on a small fraction of tumor-specific T cells within the TILs. As tumor samples are not always available, the use of blood as a source for tumor-specific T cells represents a promising approach. Consequently, the enrichment of tumor-reactive T cells from tumor or peripheral blood by isolating the CD137 positive T cells after stimulation prior to their *ex vivo* expansion and the automation of the process may improve the efficacy and usability of this therapy. Therefore, we developed a fully automated procedure for the CD137 isolation and subsequent expansion of tumor-reactive T cells on the CliniMACS Prodigy (Tumor-Reactive Lymphocyte - TRL process).

Methods

Figure 1

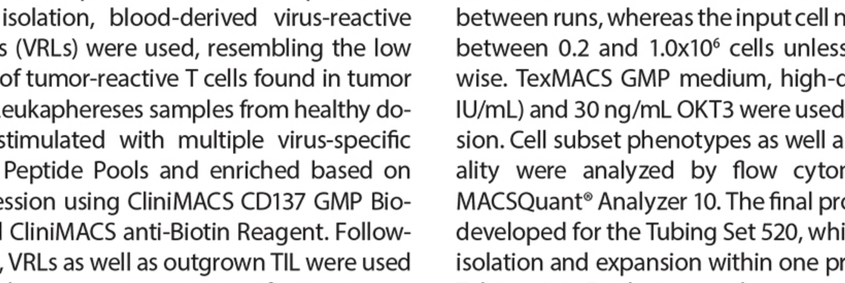


As a proof of concept and for the development for the CD137 isolation, blood-derived virus-reactive lymphocytes (VRLs) were used, resembling the low frequencies of tumor-reactive T cells found in tumor and blood. Leukaphereses samples from healthy donors were stimulated with multiple virus-specific PepTivator® Peptide Pools and enriched based on CD137 expression using CliniMACS CD137 GMP Biotin GMP and CliniMACS anti-Biotin Reagent. Following isolation, VRLs as well as outgrown TIL were used to develop the expansion process of virus- or tumor-reactive lymphocytes, respectively. TILs and isolated CD137$^+$ VRLs were expanded based on a rapid expansion protocol (REP) for 13–14 days in the fully automated CliniMACS Prodigy, using a cultivation matrix. The feeder cell number (4×10^8) was constant between runs, whereas the input cell number ranged between 0.2 and 1.0×10^6 cells unless stated otherwise. TexMACS GMP medium, high-dose IL-2 (3000 IU/mL) and 30 ng/mL OKT3 were used for the expansion. Cell subset phenotypes as well as cell functionality were analyzed by flow cytometry on the MACSQuant® Analyzer 10. The final process has been developed for the Tubing Set 520, which allows both isolation and expansion within one process and one Tubing Set. Furthermore, the process is flexible in that it can also be used for expansion of low TIL numbers only (in case CD137 isolation is not desired). Finally, in proof-of-concept runs frozen digests (melanoma) were used for isolation and expansion of TRLs.

Results

1 CD137 enrichment on the CliniMACS Prodigy®

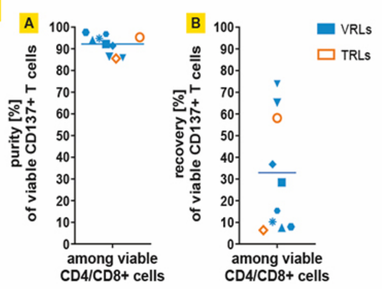
Figure 2



An analysis example is shown (fig. 2) of an automated virus-reactive lymphocyte (VRL) separation based on CD137 in the CliniMACS Prodigy using the TRL process. A leukapheresis (LP) sample of a healthy donor was stimulated with PepTivator Pools for AdV, CMV and EBV and cultured for 18 hours. A small sample was stained for CD137 expression (stimulated sample) and the remainder of the cells was automatically labeled and separated for CD137 positive cells on the CliniMACS Prodigy. The labeled cells as well as negative fraction and positive fraction were analyzed using the Labeling Check Reagent as no counterstain for CD137 after labeling with the isolation reagents is available.

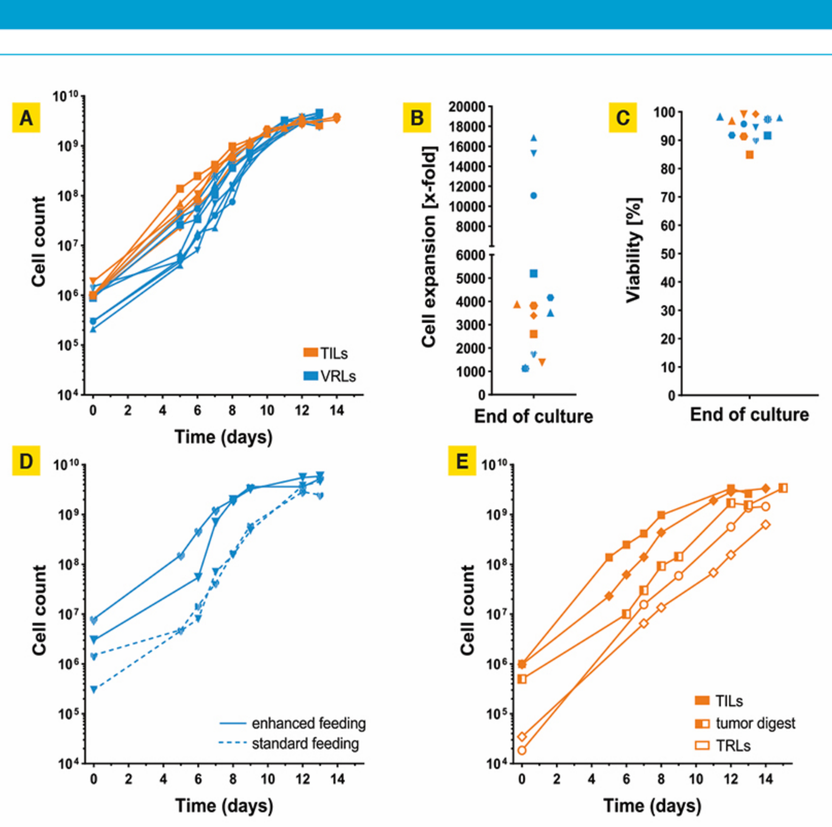
Multiple VRL (blue) from LP as well as some proof-of-concept tumor-reactive lymphocytes (TRL - orange) from frozen digests were isolated using the TRL process (fig. 3). The average purity of CD137 positive T cells was 92% (A) and the recovery of CD137 positive cells within the positive fraction was 33% on average (B). However, a large variability can be observed which was mainly donor-dependent. Different symbols indicate samples from different donors.

Figure 3



2 Expansion of outgrown TILs, VRLs and TRLs

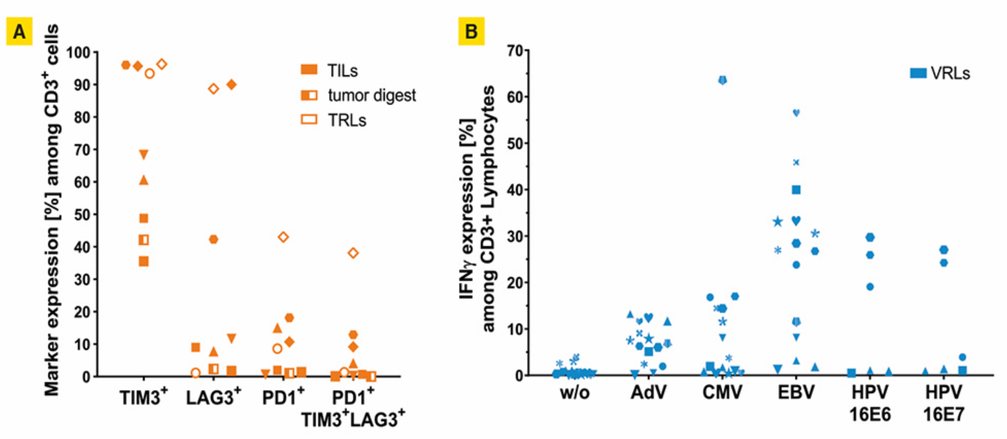
Figure 4



Low input numbers (2×10^5-1×10^6) of outgrown TILs (orange) and VRLs (blue) could successfully be expanded to more than 4×10^9 cells after 13–14 days of T cell culture on the CliniMACS Prodigy (A), reflecting a 1,000 to 17,000-fold expansion (B). The viability at the end of culture was consistently high (C) and the final products were >95% T cells with variable percentages of CD8$^+$ T cells and CD4$^+$ T cells (data not shown). Higher input numbers (up to 9×10^6 cells VRL – solid line) could be expanded by implementing a medium exchange on d3 and an increased feeding schedule (D). For comparison, the expansion of low input cell numbers is shown (dotted line). Finally, in first proof-of-concept runs, TRL were isolated from small samples of frozen tumor digest and expanded using the TRL process (E, open symbols). When available, outgrown TIL from the same patient were expanded in parallel (closed symbols). In one instance, total digest (checkered symbol) was expanded and compared to outgrown TIL.

3 T cell phenotype and *in vitro* functionality of expanded TRL and VRL

Figure 5



Phenotypically, expanded cells were predominantly T_{CM} and T_{EM} (data not shown). The exhaustion state of expanded T cells was defined based on TIM3, LAG3 and PD1 (co-)expression. The functionality of expanded VRLs was assessed *in vitro* by analyzing the production of IFN-γ among CD3$^+$ T cells in the final product. To this end, VRLs were stimulated with the virus-specific PepTivator Peptide Pools for AdV, CMV and EBV (and HPV in some runs) and assessed for intracellular IFN-γ expression. VRLs alone were used as negative control (w/o). Different symbols indicate samples from different donors.

Conclusions and outlook

- Following activation, CD137 positive cells could be enriched to high purities using the developed TRL process on the CliniMACS Prodigy.
- Large numbers of TILs and VRLs were obtained by rapid expansion of low cell numbers in an automated manner on the CliniMACS Prodigy.
- T_{CM} and T_{EM} predominated the final product (data not shown), with high frequencies of TIM3$^+$ and LAG3$^+$ TILs and TIM3$^+$ VRLs.
- Expanded VRLs were functional as assessed by analysis of cytokine production upon antigen recognition.
- These results serve as proof of concept for the isolation and expansion of tumor-reactive T cells with the TRL process established on the CliniMACS Prodigy.

We thank Prof. John Haanen/ Prof. Ton Schumacher from the Netherlands Cancer Institute and Dr. Steven Rosenberg from the NCI for their support in supplying melanoma digest samples for testing.

Unless otherwise specifically indicated, Miltenyi Biotec products and services are for research use only and not for therapeutic or diagnostic use. MACS® GMP Products are for research use and *ex vivo* cell culture processing only, and are not intended for human *in vivo* applications. For regulatory status in the USA, please contact your local representative. MACS GMP Products are manufactured and tested under a quality system certified to ISO 13485 and are in compliance with relevant GMP guidelines. They are designed following the recommendations of USP <1043> on ancillary materials. The CliniMACS® System components, including Reagents, Tubing Sets, Instruments, and PBS/EDTA Buffer, are designed, manufactured and tested under a quality system certified to ISO 13485. In the EU, the CliniMACS System components are available as CE-marked medical devices for their respective intended use, unless otherwise stated. The CliniMACS Reagents and Biotin Conjugates are intended for *in vitro* use only and are not designated for therapeutic use or direct infusion into patients. The CliniMACS Reagents in combination with the CliniMACS System are intended to separate human cells. Miltenyi Biotec as the manufacturer of the CliniMACS System does not give any recommendations regarding the use of separated cells for therapeutic purposes and does not make any claims regarding a clinical benefit. For the manufacturing and use of target cells in humans the national legislation and regulations – e.g. for the EU the Directive 2004/23/EC ("human tissues and cells"), or the Directive 2002/98/EC ("human blood and blood components") – must be followed. Thus, any clinical application of the target cells is exclusively within the responsibility of the user of a CliniMACS System. In the US, except for the CliniMACS CD34 Reagent System, all other products of the CliniMACS Product Line are available for use only under an approved Investigational New Drug (IND) application or Investigational Device Exemption (IDE). CliniMACS, CliniMACS Prodigy, MACS, the MACS logo, MACSQuant, PepTivator, and TexMACS are registered trademarks or trademarks of Miltenyi Biotec GmbH and/or its affiliates in various countries worldwide. Copyright © 2019 Miltenyi Biotec GmbH and/or its affiliates. All rights reserved.