GMP-compliant flow cytometric cell sorting of antigen-specific T cells using MACS® GMP CD8-APC and MACS GMP CD137-PE Fluorescent Antibodies on the MACSQuant® Tyto® Cell Sorter

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Introduction

Adoptive T cell therapy has been shown to be a promising strategy for treatment of a variety of malignancies. Exploitation of this potent therapeutic approach increases the need for easy and effective isolation of antigen-specific T cells in compliance with GMP cell manufacturing requirements.
Antigen-specific T cells can be identified and purified after *ex vivo* stimulation using the activation marker CD137 (4-1BB). However, CD137 is also expressed in a number of other immune cells like B cells, dendritic cells, and monocytes, which hampers the isolation of antigen-specific T cells based solely on this marker. Moreover, the presence of CD137$^+$ regulatory T cells could inhibit an effective expansion of antigen-specific T cells after enrichment.
Here, we demonstrate the generation of large numbers of CD8$^+$CD137$^+$ antigen-specific T cells with high purity based on GMP-compatible reagents and sorting system. Sorted cells showed robust expansion as well as strong activation marker expression and the potential for cytotoxic activity. These results demonstrate a reliable process for isolation of highly pure antigen-specific T cells from heterogeneous human blood products within a GMP-compliant manufacturing environment.

Materials and methods

1 Workflow for isolation and expansion of antigen-specific T cells

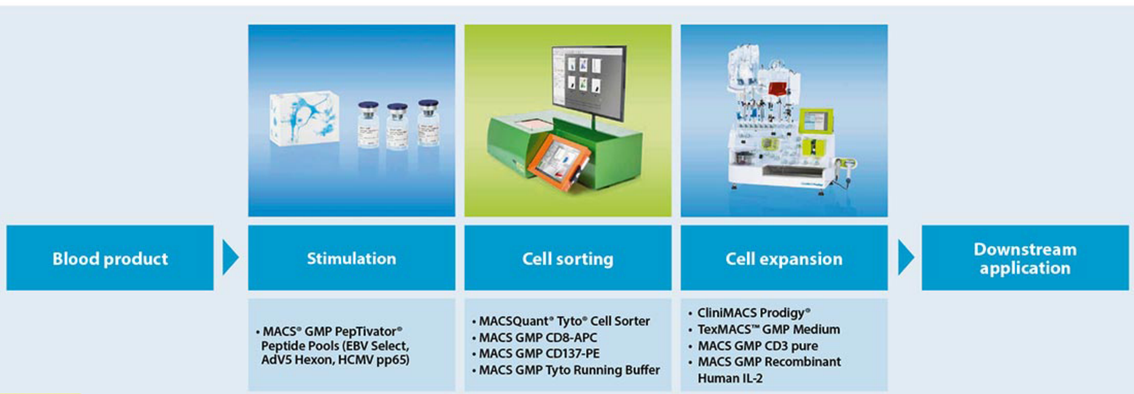


Figure 1

Leukapheresis products were collected from healthy donors, resuspended in RPMI 1640 medium supplemented with 5% human AB-serum and stimulated for 16–42 h with MACS® GMP PepTivator® Peptide Pools (EBV Select, AdV5 Hexon, and HCMV pp65). Subsequently, cells were stained with MACS® GMP CD8-APC and MACS GMP CD137-PE and resuspended in MACS GMP Tyto® Running Buffer (MACS GMP PBS/$MgCl_2$ buffer and MACS GMP Tytonase) for isolation on the MACSQuant® Tyto® Cell Sorter. The sorted cells were then transferred to the CliniMACS Prodigy® to allow for an 11-day expansion using the rapid expansion protocol. This process is under development and uses following reagents: TexMACS™ GMP Medium, MACS GMP CD3 pure (OKT3), MACS GMP Recombinant Human IL-2, and irradiated leukapheresis product as feeder cells. After expansion, cells were restimulated with the MACS GMP PepTivator Peptide Pools and assessed for activation marker expression and degranulation by flow cytometry.

2 Strategy for sorting of antigen-specific T cells

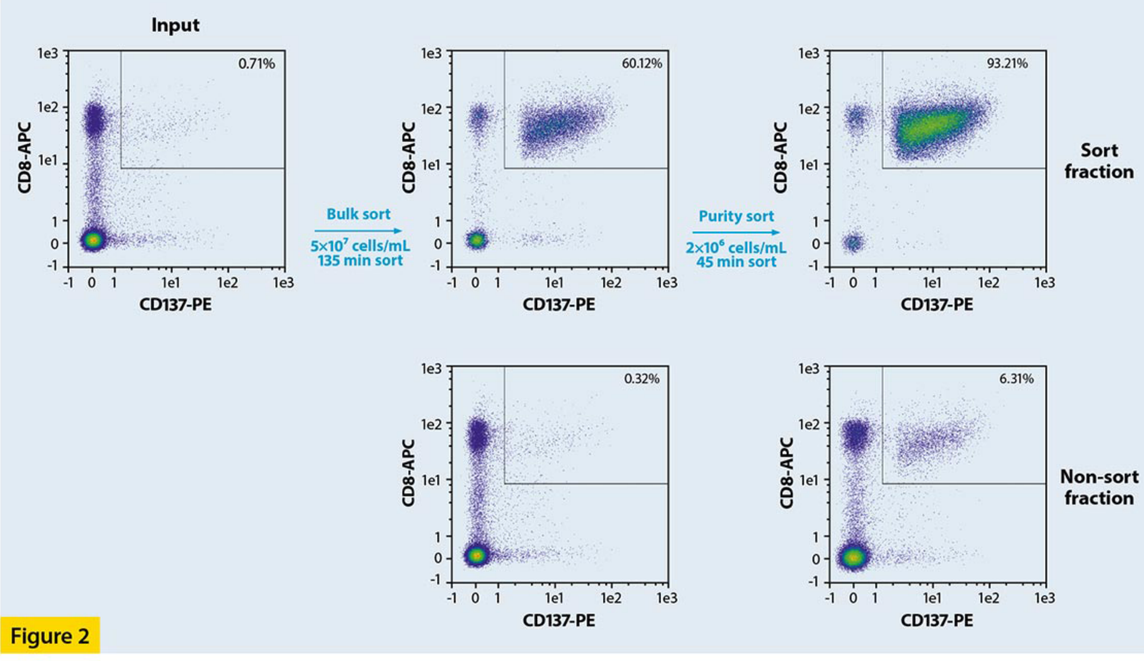


Figure 2

Two sort processes were performed sequentially: the first process was based on a high cell frequency option (bulk sort), and the second one used a low cell frequency option (purity sort). Samples were analyzed on the MACSQuant Analyzer 10 as in-process controls (IPC). Gating strategy for the IPC was as follows: i) gating on lymphocytes, ii) singlets, iii) live cells, using propidium iodide (PI) as exclusion marker, and iv) CD8-APC versus CD137-PE.

Results

1 Sort performance for the isolation of antigen-specific T cells

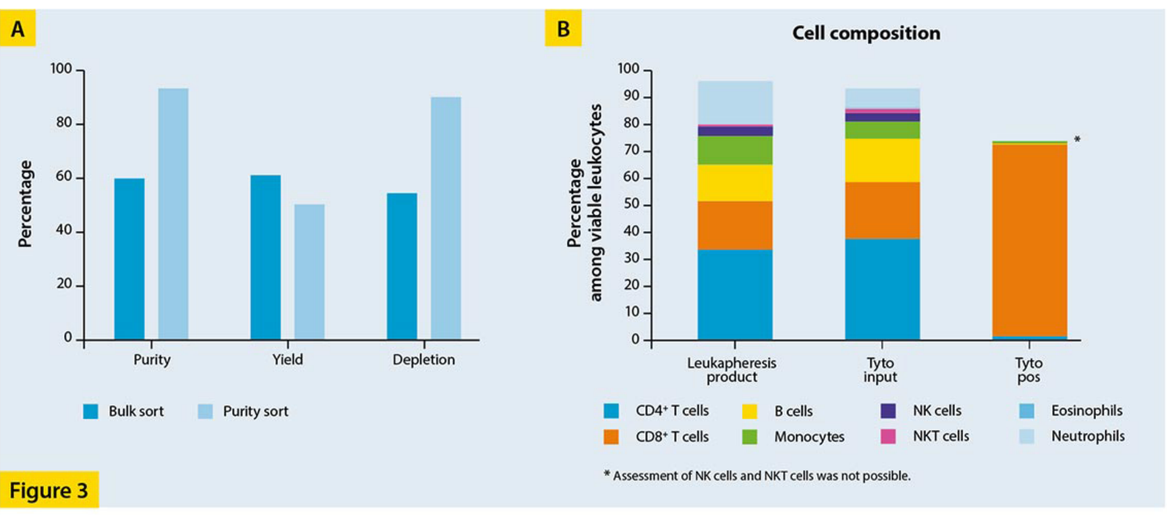


Figure 3

The bulk sort resulted in purities and yields of 60%. Non-target cells were depleted by 55% (A). Using the purity sort, a purity of 93% was obtained, with a corresponding yield of 53% and 90% depletion. Samples were analyzed on the MACSQuant Analyzer 10 as IPC. In addition, an antibody cocktail was used to analyze the cell composition of the original leukapheresis product, the leukapheresis product after stimulation with PepTivator Peptide Pools (Tyto input), and the sorted cells (Tyto pos) (B). For this purpose, the 7-Color Immunophenotyping Kit, human was adapted to include a viability marker, and CD20 was used instead of CD19. Due to the presence of MACS GMP CD8-APC and MACS GMP CD137-PE on the sorted cells, not all cell types, in particular NK and NKT cells, could be assessed. In the sorted fraction, percentages of potentially contaminating cells were as follows: 1.32% for CD4$^+$ T cells (96% reduction in comparison to Tyto input), 0.31% for B cells (98% reduction), 0.81% for monocytes (87% reduction) and 0.18% for eosinophils and neutrophils (50% and 97% reduction, respectively).

2 T cell expansion and phenotype

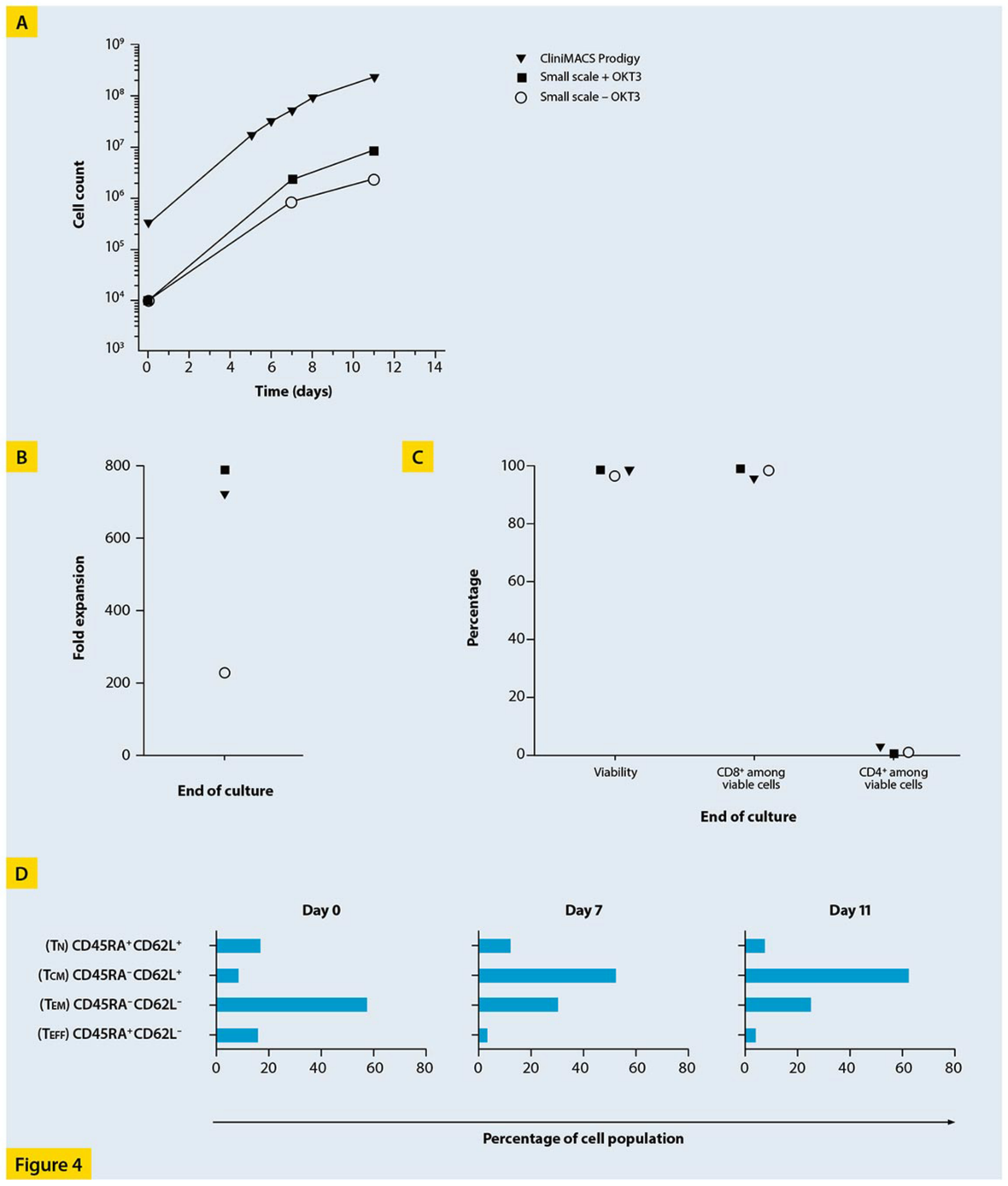


Figure 4

Cell numbers increased from 3×10^5 CD8$^+$CD137$^+$ cells to 2×10^8 cells within 11 days of T cell culture on the CliniMACS Prodigy®, reflecting a 720-fold expansion (A and B). In addition, small-scale expansion was done in parallel using 10,000 sorted cells with or without TCR stimulation (MACS GMP CD3 pure, OKT3), resulting in a 790-fold expansion with OKT3 and a 230-fold expansion without OKT3. Viability rates were high after 11 days (CliniMACS Prodigy and small-scale expansion with OKT3: 98.9%; small-scale expansion without OKT3: 97.5%) with a consistently high frequency of CD8$^+$ T cells (C).
During expansion, the frequency of naive (TN), effector memory (TEM), and effector (TEFF) T cells was reduced (from 17.5% to 7.1% for TN cells, from 57% to 27% for TEM cells and from 17% to 3.2% for TEFF cells, D). In contrast, the population frequency of central memory (TCM) T cells increased from 8.9% to 62.7%. Expression of PD-1 and Tim-3 did not increase significantly during expansion (data not shown).

3 *In vitro* functionality of expanded antigen-specific T cells

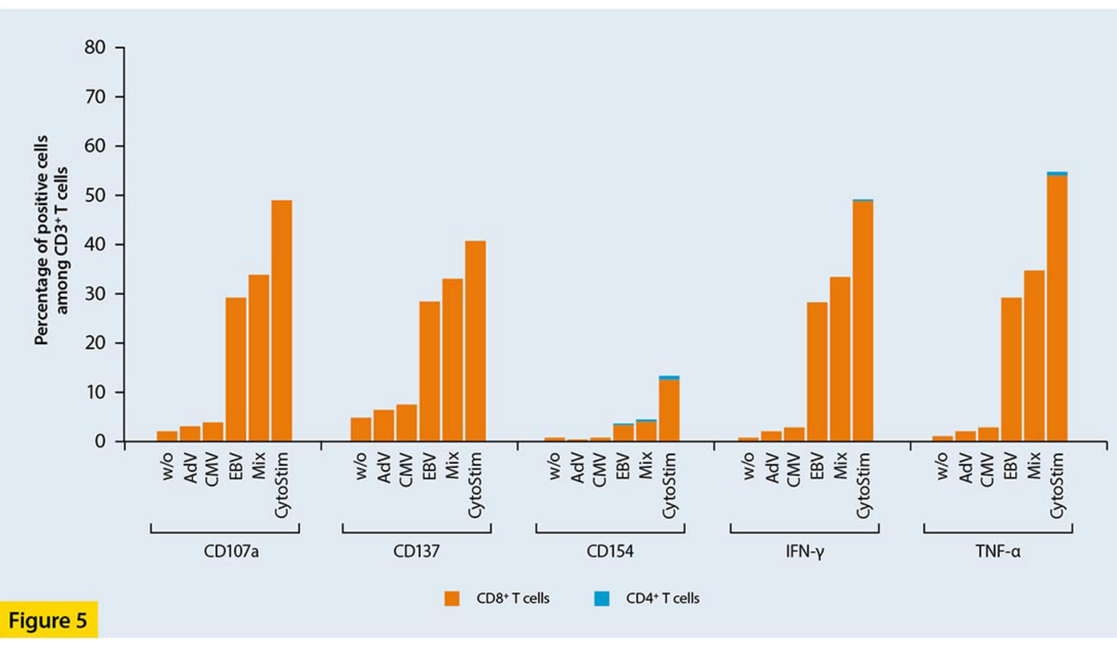


Figure 5

After 11 days of expansion, the cells displayed robust activation upon restimulation with PepTivator Peptide Pools, as the activation markers CD154 and CD137 were up-regulated, particularly after restimulation with the EBV peptides. The same trend was observed for the degranulation marker CD107a and the cytokines IFN-γ and TNF-α. This points not only to an effective activation profile but also to a potential cytotoxic capacity of the expanded cells.
Briefly, the cells were restimulated for six hours with all three MACS GMP PepTivator Peptide Pools individually, all three peptide pools mixed together, or a positive control (CytoStim™ Reagent). Expression of CD107a, CD137, CD154, IFN-γ, and TNF-α was assessed by flow cytometry.

Conclusion

Our results demonstrate a reliable process for the isolation of antigen-specific T cells with high purity from heterogeneous human blood products within a GMP-compliant manufacturing environment.

- GMP-compatible cell sorting and expansion using GMP-compliant reagents.
- Easy and effective isolation of antigen-specific T cells (3 h for 5×10^8 cells, 5 h for 1×10^9 total processed cells).
- Robust expansion of highly purified antigen-specific T cells.
- Expanded antigen-specific T-cells show a potent response to antigen restimulation.
- GMP-compliant MACS GMP Tyto Cartridge is now available.

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