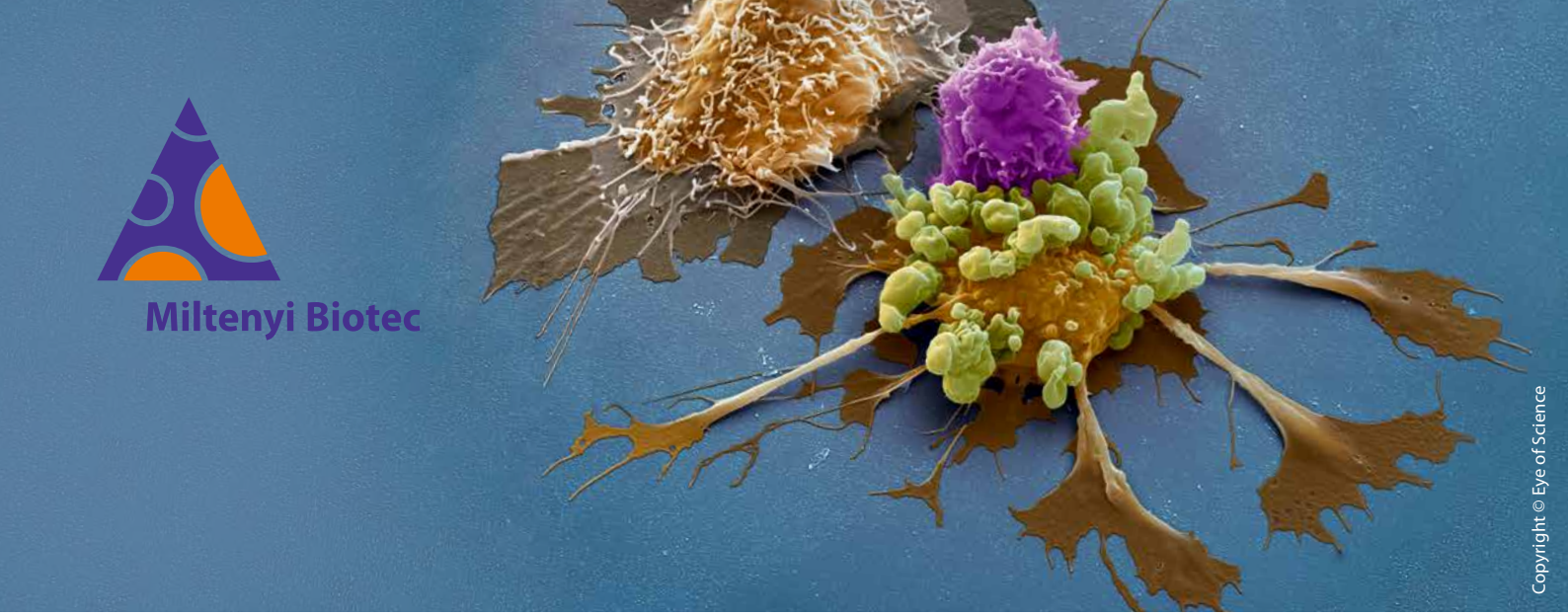

Generation of functional CAR T cells from CD4+ and CD8+ T cells

Background

T cells are central effectors of the immune system and play a pivotal role in the fight against cancer. Recently, T cells have been redirected against tumor cells by genetic engineering. Equipped with a transgenic chimeric antigen receptor (CAR) that recognizes a specific tumor antigen, CAR T cells are a promising new tool for a precise and efficient cancer therapy. However, optimal parameters for the generation, cultivation, and analysis of CAR T cells are still to be defined in order to translate the most efficient approaches into immunotherapy. Automation and easy translation from basic research into a clinical setting are particularly important challenges to address.

Here we demonstrate that human CD4+ and CD8+ T cells isolated with MACS® Technology are efficiently used to generate functional CAR T cells. MACS Technology enables fully automated cell separation with the autoMACS® Pro Separator, as well as straightforward translation into clinical settings thanks to MACS GMP Products and the CliniMACS Prodigy®.

Method

T cells were isolated from peripheral blood mononuclear cells (PBMCs) with CD4+ and CD8+ MicroBeads, using the autoMACS Pro Separator. The purity of isolated T cells was assessed by flow analysis using REAfinity™ Recombinant Antibodies and the MACSQuant® Analyzer. Enriched T cells were resuspended in TexMACS™ Medium supplemented with MACS Cytokines (IL-7, IL-15) and the T cell activation reagent T Cell TransAct™ (day 1). After a 24 hour incubation period (day 2), T cells were transduced with the CD19 CAR construct using a lentiviral vector and incubated for 48 hours (day 4). On day 4, the cell culture supernatant, containing T Cell TransAct and the lentiviral vector, was removed. CAR T cells were expanded in TexMACS Medium supplemented with IL-7 and IL-15 for an additional 8 days with splitting every 2 to 3 days.

On day 12, transduction efficiency was assessed by flow cytometry. Additionally, functionality and antigen specific target cell killing was assessed via a killing assay. Transduced T cells were co-cultured with either the GFP+CD19+ JeKo-1 mantle cell lymphoma target cell line or a GFP+CD19 knock out (k.o.) JeKo-1 variant control at indicated ratios. Using the MACSQuant Analyzer, the killing rate was determined by analyzing the green fluorescent protein (GFP) fluorescence intensity of the target cells or control cells respectively.

Results

Isolated CD4+ and CD8+ T cells exhibited a purity of >98% (fig. 1B). After transduction and expansion, cells were analyzed for CD4+, CD8+, and expression of CD19 CAR. More than 99% of all viable leucocytes were CD4+ or CD8+ (based on CD45 expression, 7-AAD fluorescence, and scatter signal) and more than 56% of all CD3+ cells expressed the CD19 CAR construct (fig. 2B).

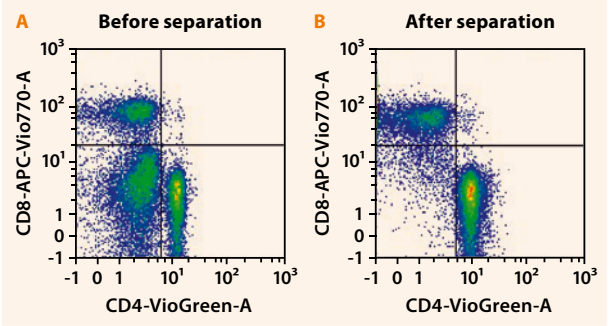

Figure 1: Flow cytometry analysis of T cells. CD4+ and CD8+ T cells before (A) and after (B) separation on day 1. Cells were labeled with CD8-APC-Vio® 770 and CD4-VioGreen™ as indicated.

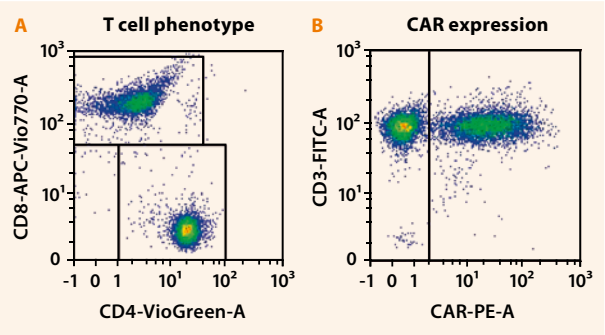

Figure 2: CD4⁺, CD8⁺ and CAR expression of transduced T cells after expansion on day 12.

CD19 CAR T cells were functionally tested in a killing assay. Flow cytometry analysis showed approximately 80% killing of target cells (GFP⁺CD19⁺ JeKo-1) at a 5-fold excess of CD19-directed CAR T cells (fig. 3). When changing the ratio by decreasing the number of CAR T cells, a maximum of 18% of the target cells were killed. In contrast, the GFP⁺CD19⁺ JeKo-1 control cells were not killed by the CD19 CAR T cells, highlighting the specificity of the CAR T cells.

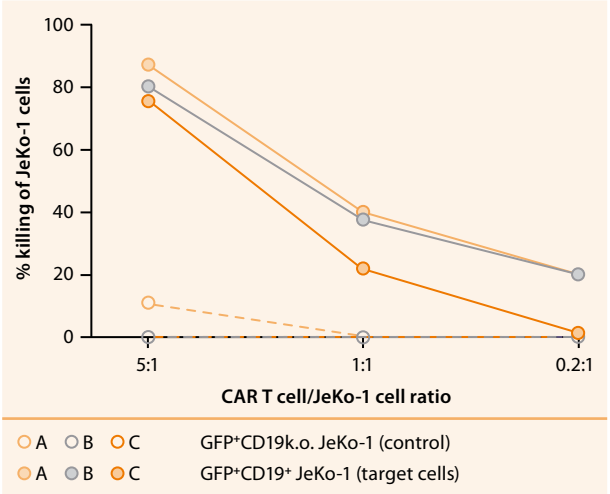

Figure 3: Killing of GFP⁺CD19⁺ JeKo-1 target cells by CD19 CAR T cells in a ratio-dependent manner. CAR T cells were derived from isolated CD4⁺ and CD8⁺ T cells from three independent donors (A, B, and C).

Conclusion

The autoMACS Pro Separator provides an automated solution to isolate pure CD4⁺ and CD8⁺ T cells for manufacturing functional CAR T cells. It enables reliable, fully automated separation of T cells from PBMCs with minimal hands-on time. Minimal labelling ensures preservation of T cell functionality, enabling downstream compatibility for CAR T cell generation.

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