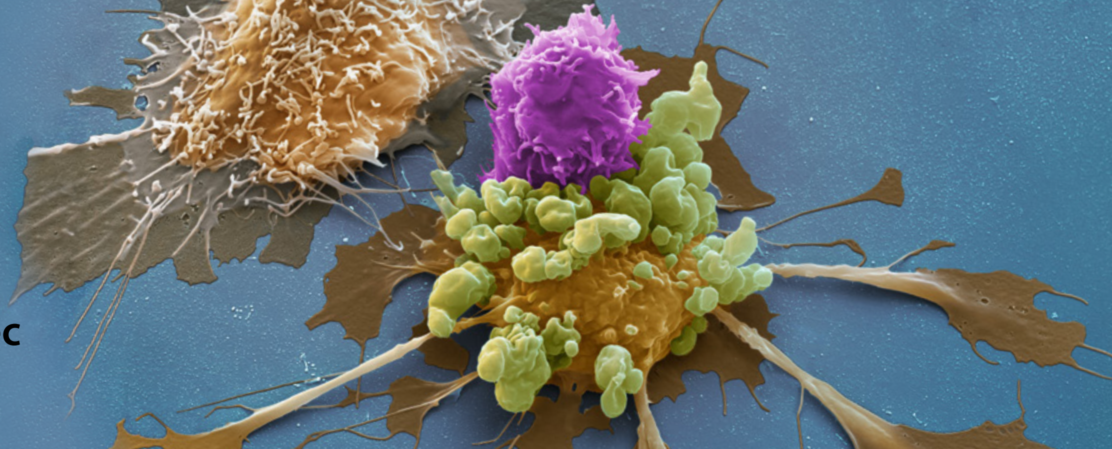
MACS
Miltenyi Biotec



Copyright © Eye of Science

Generation of functional CAR T cells from pan T cells isolated by MACS® technology

Background

As effectors of the immune system, T cells play a pivotal role in the fight against cancer. Recently, T cells have been redirected against cancer by genetic engineering. Equipped with a transgenic chimeric antigen receptor (CAR) recognizing a specific tumor antigen, these CAR T cells are a promising new approach for an efficient cancer therapy. However, research is still ongoing to define the optimal parameters for the generation, cultivation and analysis of CAR T cells in order to translate the most efficient approaches into immune therapy. Automation and easy translation from basic research into clinics are important challenges to address.

Here we show how human pan T cells isolated with the Pan T Cell Isolation Kit and cultured in serum-free TexMACS™ Medium, are efficiently used to generate functional CD19 CAR T cells. Using the autoMACS® Pro Separator and MACS® GMP reagents, we demonstrate easy automation and translation.

Materials and methods

Materials

- Pan T Cell Isolation Kit, human
- autoMACS Pro Separator
- TexMACS Medium
- Human IL-7, premium grade
- Human IL-15, premium grade
- T Cell TransAct, human
- CAR vector, e.g., lentiviral transduction
- GFP⁺/CD19⁺ JeKo-1 mantle cell lymphoma target cell line
- REAfinity Recombinant Antibodies
- MACSQuant Analyzer 10

Methods

1. Pan T cells from peripheral blood mononuclear cells (PBMC) were isolated using the Pan T cell Isolation Kit, human (day 1).
2. Pan T cells were cultivated in TexMACS™ Medium supplemented with IL-7 and IL-15. Cells were activated with T Cell TransAct™ (day 1).
3. Mock transduction completed with CD19 CAR construct, using a lentiviral vector (day 2).
4. Culture wash and removal of T Cell TransAct.
5. Expansion of T cells in TexMACS Medium supplemented with IL-7 and IL-15. Cells are split every 2–3 days (days 4–14).
6. Culture wash and removal of cytokines. Co-culture with target cells for 48 h (day 14).
7. Analysis of killing of target cells (day 16).

Results

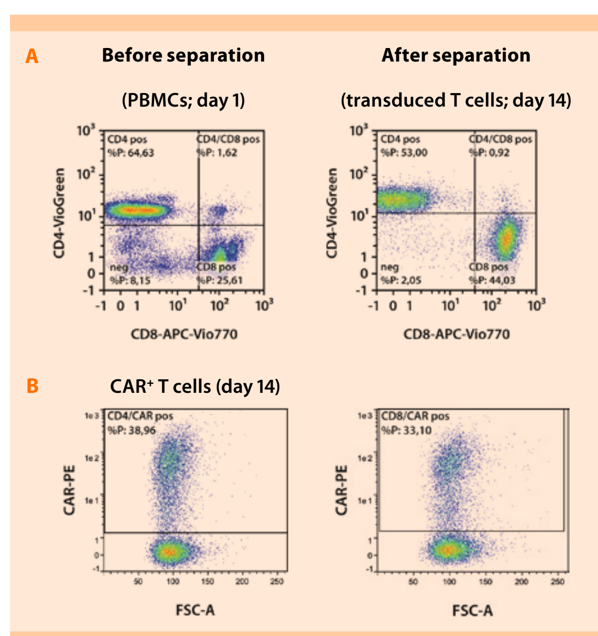


Figure 1: Phenotypical analysis of T cells before and after separation and transduction on days 1 and 14.

In order to generate functional CAR T cells, human pan T cells were isolated from PBMCs using the Pan T cell Isolation Kit, human on the autoMACS® Pro Separator, activated with TransAct™ T cell Reagent and either mock transduced or with a CD19 CAR using a lentiviral vector. After 13 days of expansion, transduced T cells were functionally tested in a killing assay.
Before and after separation purity of CD4⁺ and CD8⁺ T cells was assessed on the MACSQuant® flow cytometer using REAfinity™ Recombinant Antibodies fig.1. Separated CD4⁺ and CD8⁺ T cells exhibited a purity of over 97% on day 13 fig. 1A. After transduction and 12 days of culture, T cells were also analyzed for CAR expression on day 13. Over 38% of the CD4⁺ T cells expressed the tranduced CAR, while over 33% of the CD8 T cells were CAR-positive.

CD19 CAR transduced or mock transduced T cells were co-cultured for 48 h with the GFP⁺/CD19⁺ JeKo-1 mantle cell lymphoma target cell line at indicated CAR T: JeKo-1 cell ratios fig. 2. Analysis of green fluorescence protein (GFP) on the MACSQuant® flow cytometer indicated specific killing of the target cells by the CAR transduced T cells, at a 5:1 CAR T: target cell ratio, average killing of target cells amounted to over 98%, whereas at a 1:1 ratio over 85% of target cells were killed and at a 0.2:1 ratio killing was reduced to over 35%. In contrast, killing by the mock transduced T cells was significantly lower, indicating that the generated CD19 CAR T cells are functional at killing in an antigen-specific manner.

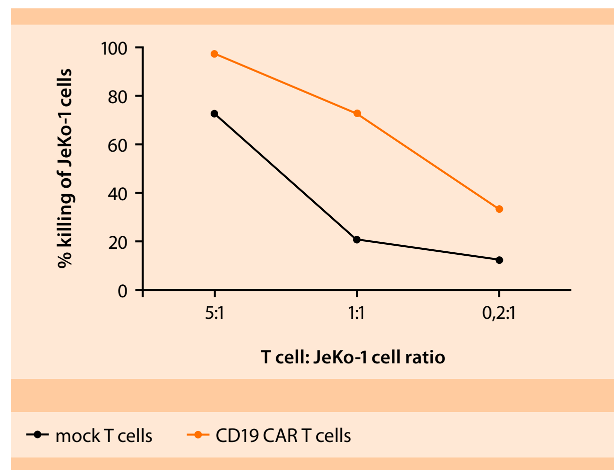


Figure 2: Killing of GFP⁺ JeKo.1 target cells by CD19 CAR and mock transduced T cells on day 16.

Conclusions

Using MACS® technology, untouched isolated pan T cells are suitable for the generation of functional and specific CD19 CAR T cells.

Miltenyi Biotec

Miltenyi Biotec provides products and services worldwide. Visit **www.miltenyibiotec.com/local** to find your nearest Miltenyi Biotec contact.

Unless otherwise specifically indicated, Miltenyi Biotec products and services are for research use only and not for therapeutic or diagnostic use. autoMACS, MACS, MACSQuant. REAfinity, TexMACS, TransAct and the MACS logo 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.

V.01