Chimeric Antigen Receptor T cells

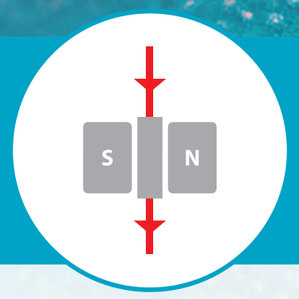
See how easy CAR T cell production is!

Treatment of cancer patients with T cells expressing a Chimeric Antigen Receptor (CAR) is one of the most promising adoptive cellular therapy approaches.[1,2]

Reproducible production of these genetically modified T cells in high-quality and clinical-grade is a prerequisite for a wide range of applications. With the CliniMACS Prodigy® all complex cell production steps can be processed automatically in a closed system.[3,4,5] This simplifies logistics and potential contamination issues for the sensitive patient material.

1. Anurathapan, U. *et al.* (2014) Cytotherapy 16: 713–733.
2. Maus, M.V. *et al.* (2014) Blood 123: 2625–2635.
3. Mock, U. *et al.* (2016) Cytotherapy 18: 1002–1011.
4. Lock, L. & Mockel-Tenbrinck, N. *et al.* (2017) Hum. Gene Ther. 28: 914-925.
5. Priesner, C. *et al.* (2016) Hum. Gene Ther. 27: 860–869.

1 T cell selection



A well-defined enriched population is key for producing CAR T cells and provides:

- Specific expansion
- Higher reproducibility

T cells are automatically labelled and selected with CliniMACS® CD4 and CliniMACS® CD8 Reagents.

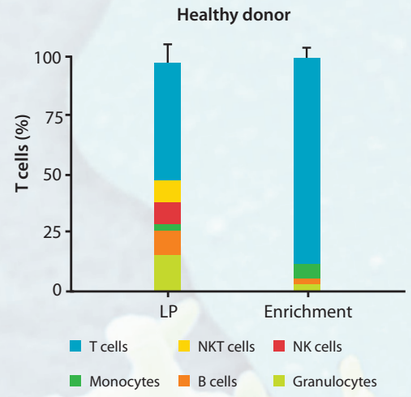
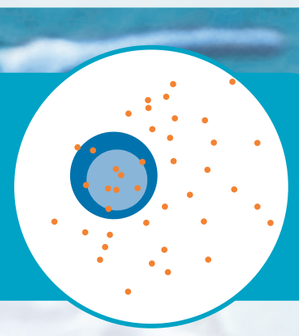


Figure 1: Automated enrichment of CD4+ and CD8+ T cells from leukapheresis (LP) samples is performed by the CliniMACS Prodigy and results in a single-cell suspension of over 90% purity of T cells – the optimal starting material for transducing T cells.

2 T cell activation



Activation of T cells is essential for successful viral transduction. MACS® GMP T Cell TransAct™ is a colloidal polymeric nano-matrix that ensures physio-logical and effective stimulation of T cells while maintaining high cell viability. Key benefits of MACS GMP T Cell TransAct are:

- Volumetric dosing
- Removal by simple washing
- Can be sterile filtered

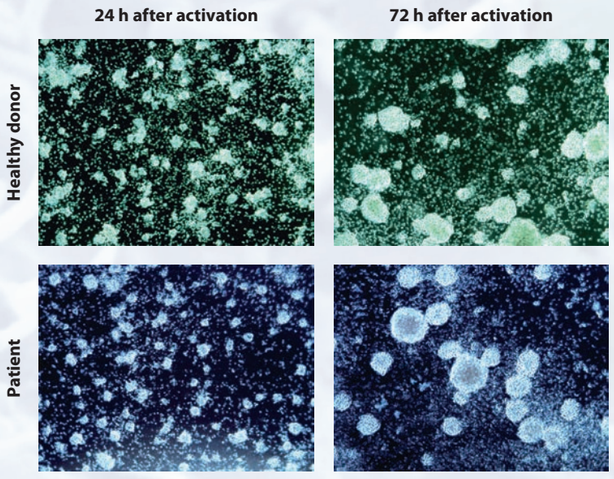


Figure 2: Cluster formation of activated CD4+ and CD8+ T cells from a healthy donor (upper row) and a melanoma patient (bottom row). T cells were activated with MACS GMP T Cell TransAct and cultured in TexMACS™ GMP Medium supplemented with MACS GMP Recombinant Human IL-7 and IL-15. Pictures were taken with the integrated microscope camera of the CliniMACS Prodigy 24 and 72 hours after activation.

3 T cell transduction

The long-term expression of CARs relies on a stable genomic insertion. Typically, two types of viral vectors are used:

- Gamma-retroviral vectors (RV)
- Lentiviral vectors (LV)

MACS GMP Vectofusin-1® can be used with spinoculation to enhance retroviral transduction. Both transduction processes are completely compatible with the CliniMACS Prodigy for efficient transduction of activated T cells.

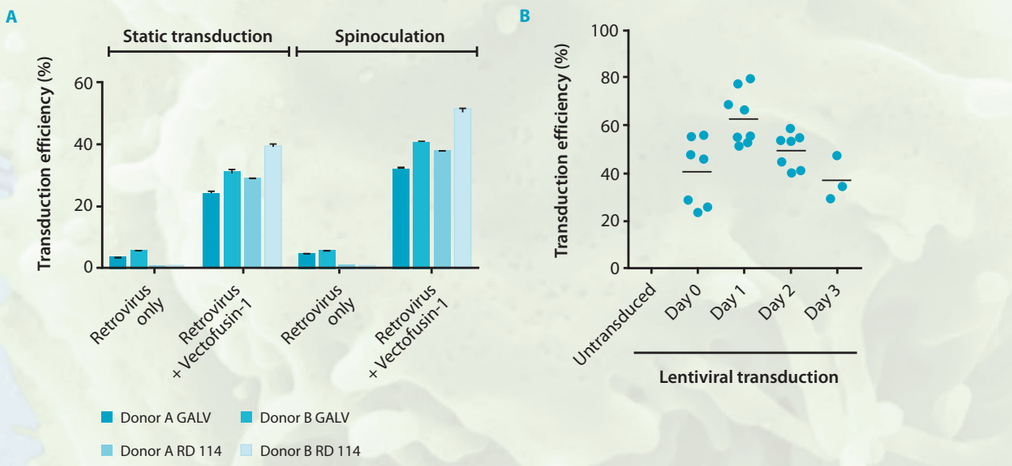
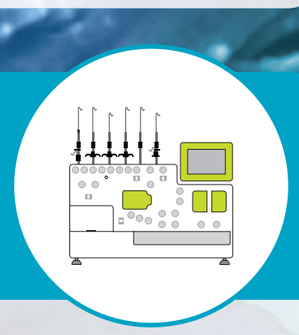


Figure 3: Transduction efficiency of enriched T cells activated with MACS GMP T Cell TransAct is greater than 40% with retroviral (A) or lentiviral (B) vector.Spinoculation with MACS GMP Vectofusin-1 assists with retroviral transduction efficiency. Lentiviral transduction efficiency is improved by transducing T cells the day after stimulation with MACS GMP T Cell TransAct.

4 T cell expansion



Clinical-scale expansion of transduced T cells is essential for a CAR T cell product. Optimal cultivation and expansion of transduced T cells rely on the strong synergy of MACS® GMP T Cell TransAct™, TexMACS™ GMP Medium, and MACS GMP Cytokines. CAR T cells can be expanded in TexMACS GMP Medium supplemented with IL-7 and IL-15 without the need for additional human AB serum or animal derived components.

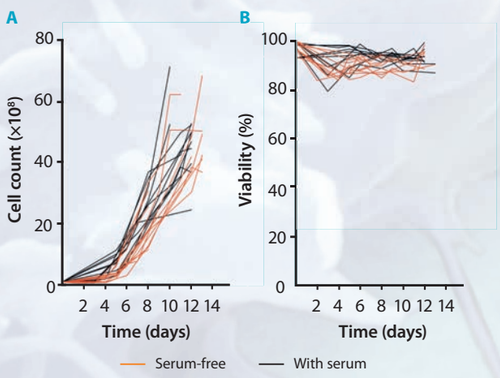


Figure 4: CAR T cells were expanded in serum-free TexMACS GMP Medium or TexMACS GMP Medium supplemented with 3% human AB serum. Cell count (A) and viability (B) were measured up to 13 days and cells were cultured in the presence of IL-7 and IL-15. Cell density and viability were similar between serum-free TexMACS Medium or supplemented with human AB serum. The T cell culture was monitored at different time points and multiple runs.

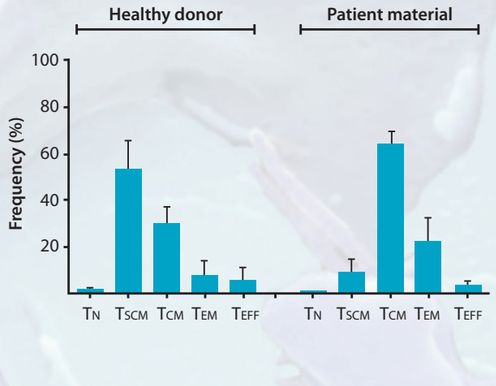


Figure 5: The CliniMACS Prodigy® TCT Process expands CAR T cells and provides a favorable phenotype of the final product. Expanded T cells from healthy donor or patient material show large frequencies of early differentiated T cells, such as central memory T cells.

5 Cell characterization

The quality of the CAR T cell product needs to be carefully monitored during and after expansion. Our broad range of tools for flow cytometry like the MACSQuant® Analyzer 10 and REAfinity™ Recombinant Antibodies allow for a detailed analysis of CAR T cells.

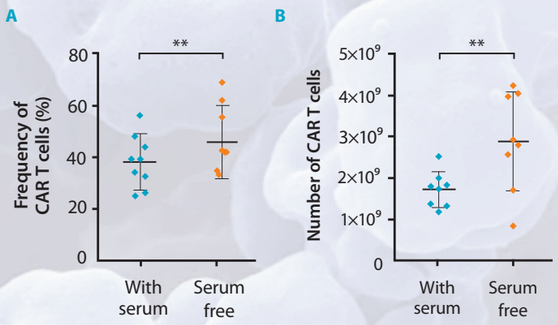


Figure 6: The TCT Process is optimized for expansion of CAR T cells in serum-free conditions. T cells were activated with MACS GMP T Cell TransAct and transduced with CAR lentiviral vector. TexMACS GMP Medium supplemented with IL-7 and IL-15 was used to cultivate T cells for 13 days. A significantly greater frequency (A) and number (B) of CAR T cells were detected when expanded following the TCT process in the absence of human AB serum or animal derived components.

CliniMACS Prodigy®

- Fully automated and closed cell production from sample to formulation
- Integrated enrichment or depletion by cell surface markers
- Instant up- and out-scaling capability with easy parameterization

▶ miltenyibiotec.com/tct

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