

# Chimeric Antigen Receptor T cells

## See how easy CAR T cell production is!

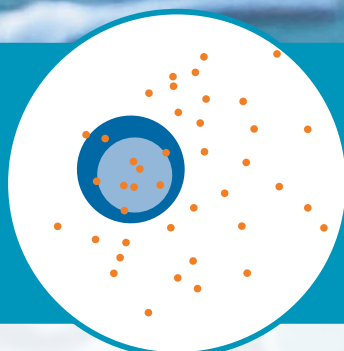
Treatment of cancer patients with T cells expressing a Chimeric Antigen Receptor (CAR) is one of the most promising adoptive cellular therapy approaches.<sup>1,2</sup>

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.<sup>3,4,5</sup> This simplifies logistics and potential contamination issues for the sensitive patient material.

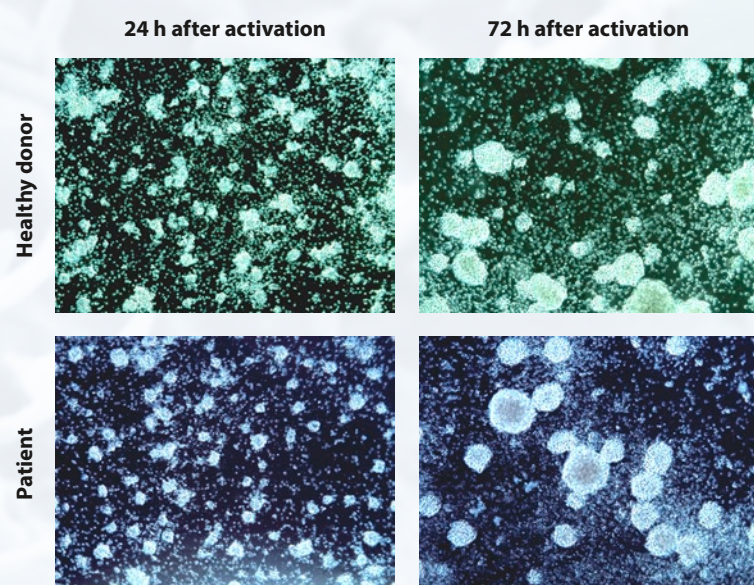
1. Anurathapan, U. et al. (2014) Cytotherapy 16: 713–733.
2. Maus, M.V. et al. (2014) Blood 123: 2625–2635.
3. Mock, U. et al. (2016) Cytotherapy 18: 1002–1011.
4. Lock, L. & Mochel-Tenbrink, N. et al. (2017) Hum. Gene Ther. 28: 914–925.
5. Priesner, C. et al. (2016) Hum. Gene Ther. 27: 860–869.

## 2 T cell activation



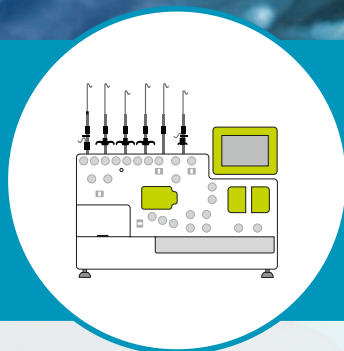
Activation of T cells is essential for successful viral transduction. MACS® GMP T Cell TransAct™ is a colloidal polymeric nano-matrix that ensures physiological and effective stimulation of T cells while maintaining high cell viability. Key benefits of MACS GMP T Cell TransAct are:

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



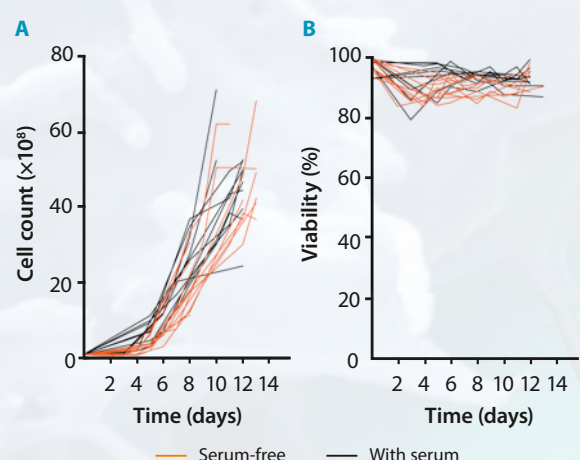
**Figure 2:** Cluster formation of activated CD4<sup>+</sup> and CD8<sup>+</sup> T cells from a healthy donor (upper row) and a melanoma patient (bottom row). T cells were activated with MACS GMP T Cell TransAct and cultured in TexMACS® GMP Medium supplemented with MACS GMP Recombinant Human IL-7 and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

## 4 T cell expansion

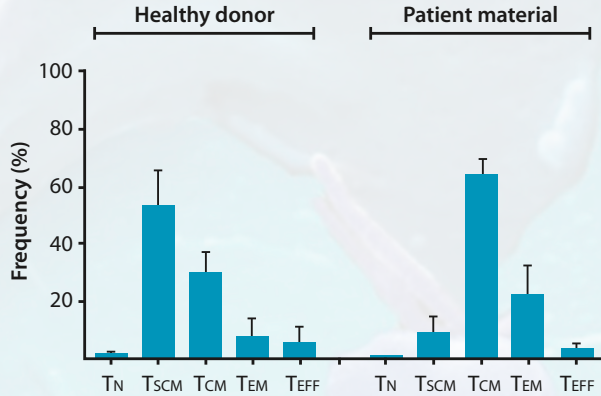


Clinical-scale expansion of transduced T cells is essential for a CAR T cell product. Optimal cultivation and expansion of transduced T cells rely on the strong synergy of MACS GMP T Cell TransAct, TexMACS® GMP Medium, and

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.



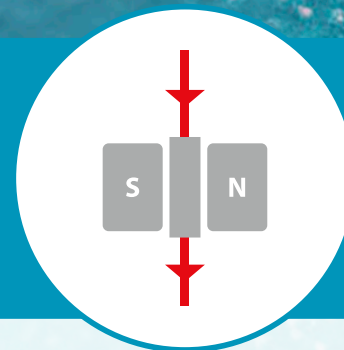
**Figure 5:** The CliniMACS Prodigy TCT Process expands CAR T cells and provides a favorable phenotype of the final product. Expanded T cells from healthy donor or patient material show large frequencies of early differentiated T cells, such as central memory T cells.

## 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



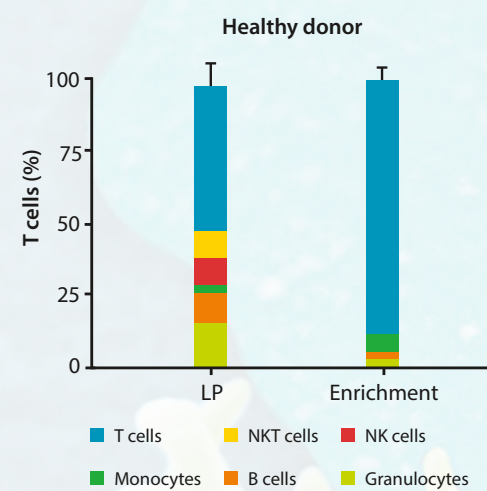
## 1 T cell selection



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<sup>+</sup> and CD8<sup>+</sup> 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.

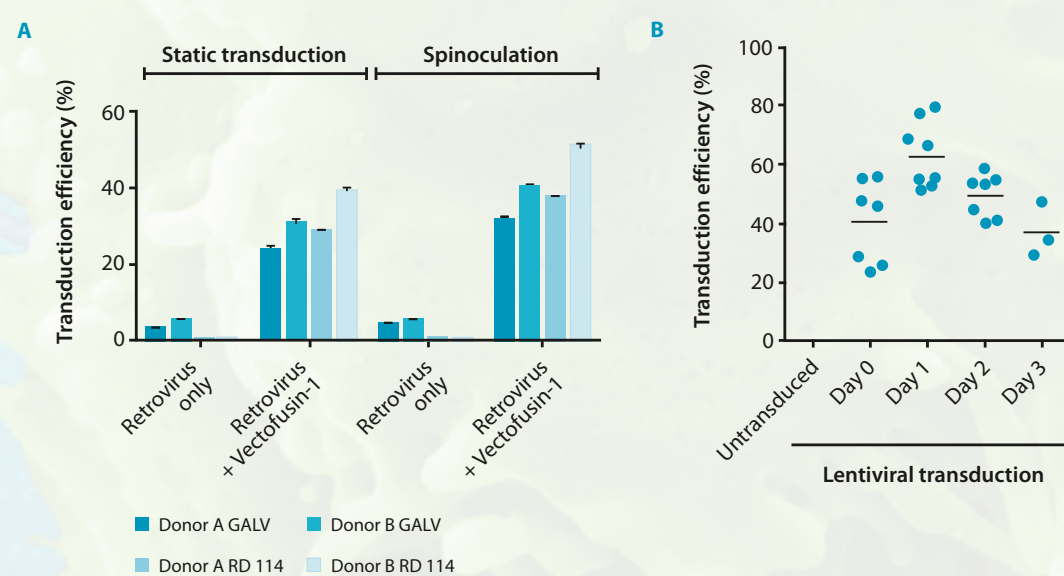
## 3 T cell transduction



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

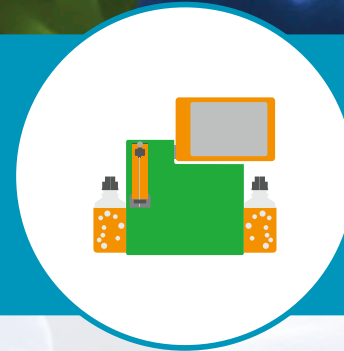
- Gamma-retroviral vectors (RV)
- Lentiviral vectors (LV)

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.

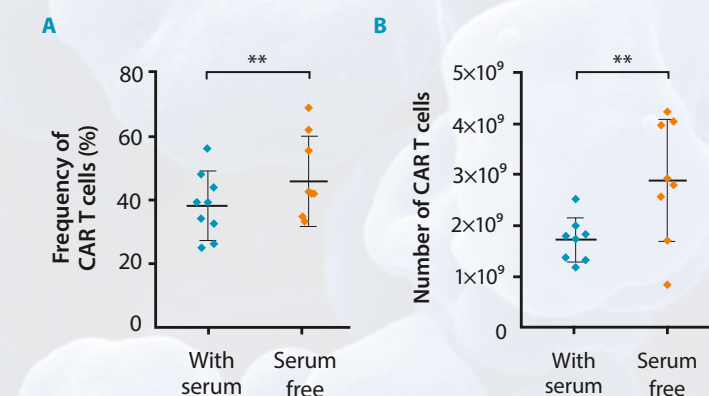


**Figure 3:** Transduction efficiency of enriched T cells activated with MACS GMP T Cell TransAct is greater than 40% with retroviral (A) or lentiviral (B) vector. Spinoculation with MACS GMP Vectofusin-1 assists with retroviral transduction efficiency. Lentiviral transduction efficiency is improved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

## 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.