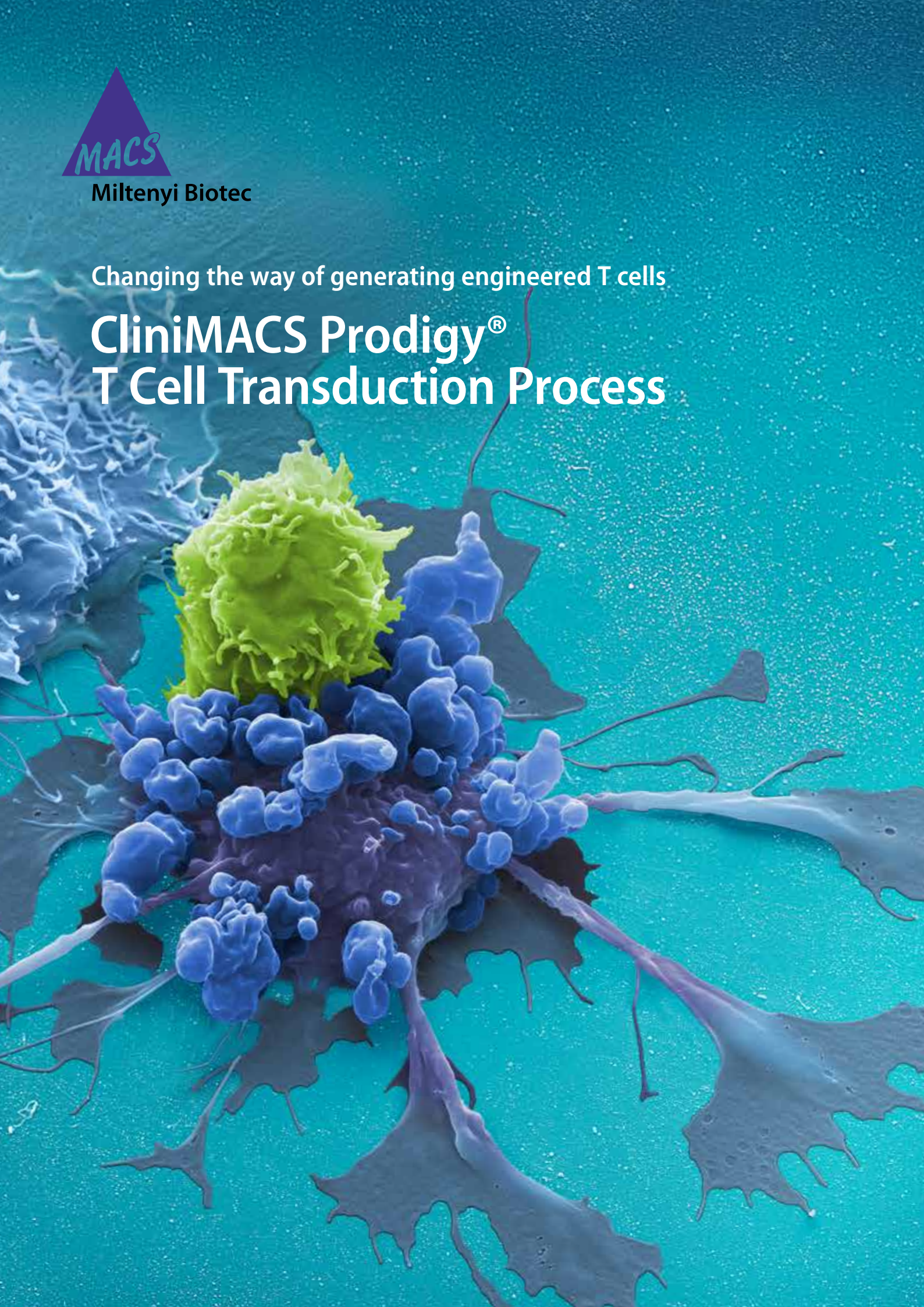
Miltenyi Biotec

Changing the way of generating engineered T cells

CliniMACS Prodigy®
T Cell Transduction Process



CliniMACS Prodigy® T Cell Transduction Process

It is happening – a new era of cell therapy

Chimeric antigen receptor (CAR) T cell therapy is undoubtedly leading a revolution in cancer therapy[1, 2]. The recent successes of CAR T cell therapy in fighting hematologic malignancies have led to a tremendous increase in interest in the immunotherapeutic field[3]. The great potential of genetically modified T cells now even expands into the area of solid tumors and infectious diseases[4].

Currently, the manufacturing process of engineered T cells consists of various complex procedures, is labor intensive, and represents one of the biggest challenges in this area.

The CliniMACS Prodigy® T Cell Transduction (TCT) Process provides a unique all-in-one solution to this challenge. The CliniMACS Prodigy Platform enables the generation of gene-modified T cells in a standardized and fully automated way. It is a breakthrough in cell manufacturing, a leap to the next generation of engineered cell manufacturing[5, 6].

The CliniMACS Prodigy TCT Process, a complete manufacturing solution – ready for the future.

References

1. Anurathapan, U. *et al.* (2014) Cytotherapy 16: 713–733.
2. Maus, M.V. *et al.* (2014) Blood 123: 2625–2635.
3. Barrett, D.M. *et al.* (2015) J. Immunol. 195: 755–761.
4. Whilding, L.M. and Maher, J. (2015) Mol. Oncol. 9: 1994–2018.
5. Mock, U. *et al.* (2016) Cytotherapy 18: 1002–1011.
6. Priesner, C. *et al.* (2016) Hum. Gene Ther. 27: 860–869.

Benefits of the TCT Process

Automation
- Simplifying a complex procedure.

Robustness
- Standardized procedure.

GMP compliance
- High-quality raw materials available.

Closed system
- Reliable product safety.
- Reduced hands-on time.

Transferable and scalable
- Supports centralized and de-centralized manufacturing models.

VIDEO

See the CliniMACS Prodigy® TCT Process in action!

Generate gene-modified T cells in a simple and automated fashion. Easy to use, this unique process will surely change the way you work.

▸ miltenyibiotec.com/tct

Automated workflow at a glance

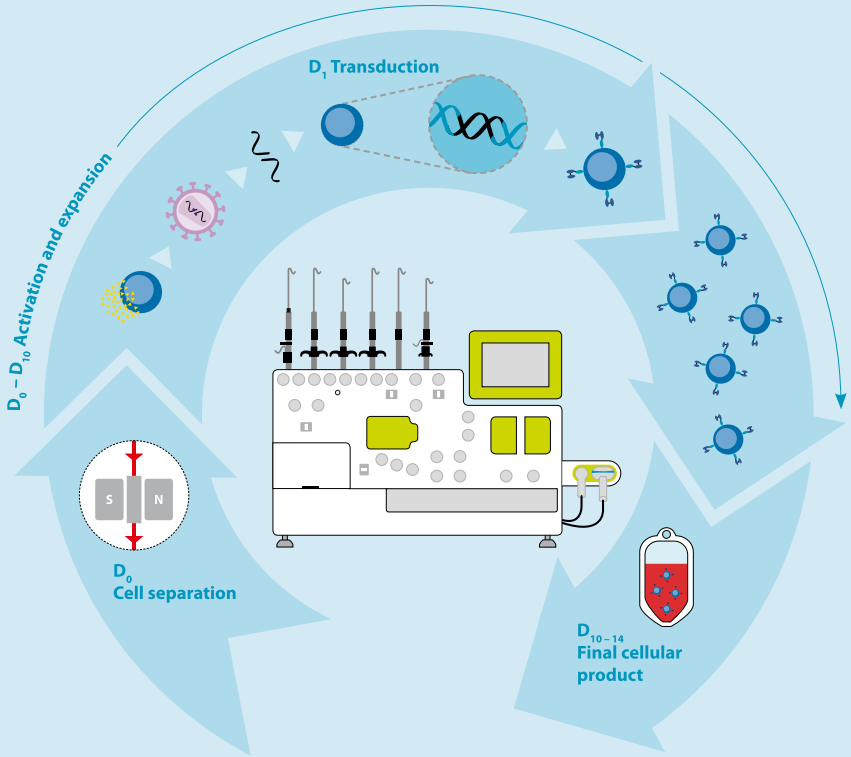
Ready-to-use process to manufacture engineered T cells

The CliniMACS Prodigy® TCT Process enables the automated generation of engineered T cells. T cells are selected, transduced, expanded and the end product is reconstituted in its final formulation. Various starting materials can be used, such as leukapheresis or whole blood (WB). Final yields of up to 3×10^9 T cells are generated.

Specifications of the CliniMACS Prodigy® TCT Process

Starting material and volume		Final product	
Total cells	up to 20×10^9 total leukocytes	Total cells	on average 3×10^9
Volume	50–280 mL	Elution volume	100 mL
Material	WB, PBMCs, apheresis	Process time	10–14 days
Max. cell number for labeling:	3×10^9	Hands-on time	about 4 h (including material preparation)

Day	Step	Hands-on time at the instrument
Day 0	Cell enrichment	1 h
Day 0	Cell activation	20 min
Day 1 or 2	Cell transduction	10 min
Day 2–10	Cell expansion	10 min
Day 10–14	Final formulation	15 min



Cell enrichment

Select the best

To obtain robust, standardized, and reproducible results in the manufacture of engineered T cells, the key is to start with a well-defined enriched T cell population:

- **Efficiency:** Improves transduction performance
- **Cost-saving:** Avoids vector consumption by unwanted cells
- **Reproducibility:** Reduces variability of final cellular products between manufacturing runs

T cell subsets can be enriched by a clinical-scale magnetic cell separation system. Process-compatible reagents are:

- CliniMACS® CD4 Reagent
- CliniMACS CD8 Reagent
- CliniMACS CD62L Reagent

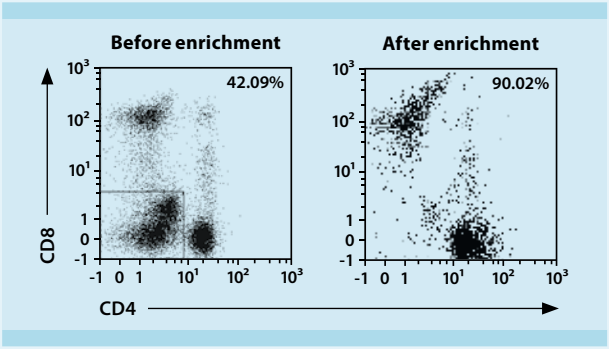


Figure 1: Flow cytometric analysis shows purity of CD4+ and CD8+ T cells enriched in an automated fashion by the CliniMACS Prodigy® TCT Process.

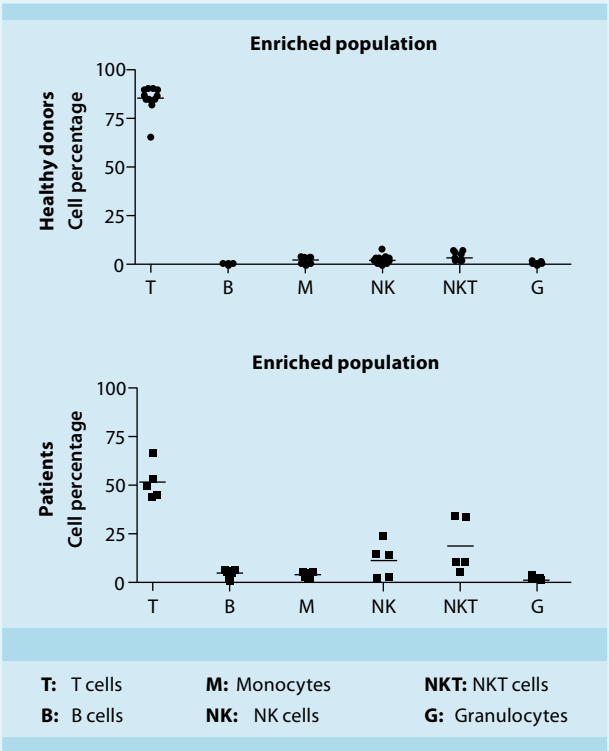


Figure 2: Composition of the cell populations after an automated co-enrichment with CliniMACS CD4 and CD8 Reagent on the CliniMACS Prodigy. Results from samples obtained from healthy donors (top) or patients (bottom) are shown. Each symbol represents one run on the CliniMACS Prodigy.

Cell activation

Effective activation and expansion

MACS® GMP T Cell TransAct™, with its unique features, enables biologically appropriate activation for your T cell manufacturing.

- **Unique format:** A colloidal polymeric nanomatrix conjugated to humanized, recombinant CD3 and CD28 agonists.
- **Ease of use:** Effective stimulation for a large range of cell densities.
- **Convenience:** Excess reagent is simply washed off.

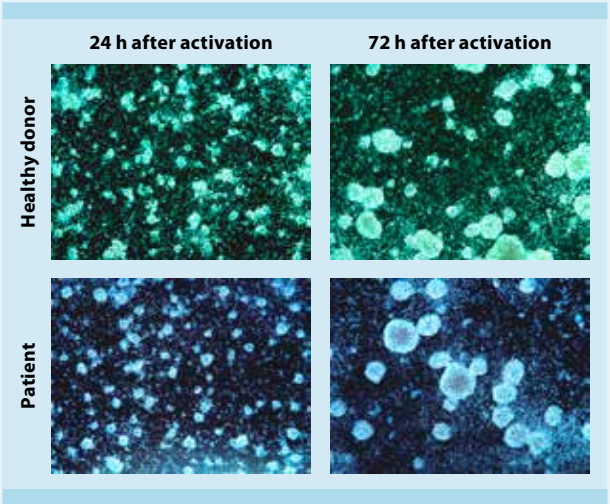


Figure 3: CD4⁺ and CD8⁺ T cells from a healthy donor (upper row) or a melanoma patient (lower row) were enriched and cultured in TexMACS™ GMP Medium supplemented with IL-7 and IL-15 and activated using the TransAct™ T Cell Reagent. Pictures were taken with the integrated microscope camera of the CliniMACS Prodigy® 24 and 72 hours after activation.

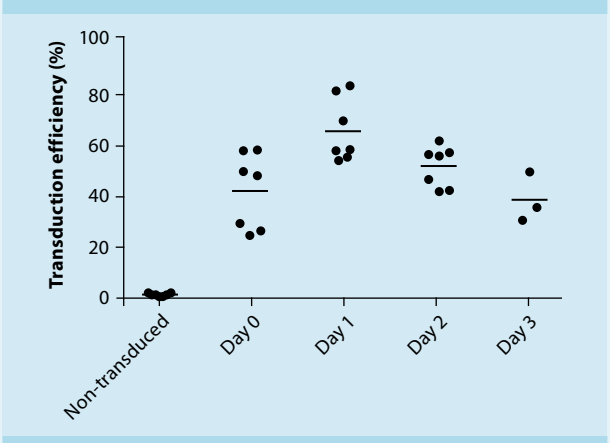


Figure 4: The optimal transduction efficiency is obtained on day 1 after the addition of T Cell TransAct. Lentiviral vector encoding GFP was added to CD4⁺ and CD8⁺ T cells, co-enriched with CliniMACS® CD4 and CD8 Reagents, at different time points. Cells were cultured in TexMACS Medium supplemented with IL-7 and IL-15 and in the presence of T Cell TransAct. The transduction efficiency was determined on day 14 by flow cytometric analysis.

Cell transduction

Seamless integration

To achieve long-term gene expression through stable genomic insertion two types of gene transduction tools are commonly used: gamma-retroviral vectors (RV) or lentiviral vectors (LV). The CliniMACS Prodigy® TCT Process supports transduction with both vectors.

For easy integration of RV-based transduction into the automated workflow of the CliniMACS Prodigy TCT Process, Vectofusin-1® enhances your RV transduction efficiency. Vectofusin-1 is compatible with both static and spinoculation protocols.

For seamless integration into the automated CliniMACS Prodigy TCT Process we deliver tailored LV solutions. Customers profit from scalable LV manufacturing that meets US and EU GMP requirements and from a strong expertise in advancing LV design and technologies.

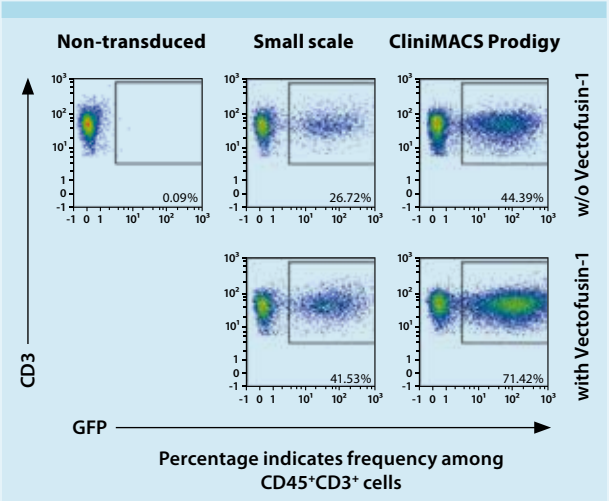


Figure 5: Transduction rates of enriched CD4+ and CD8+ T cells transduced on day 2 with gamma-retroviral GFP vector (GALV) could be increased with or without Vectofusin-1. Data shown for transduction performed in a small scale or on the CliniMACS Prodigy. This effect is further increased in an automated TCT Process on the CliniMACS Prodigy.

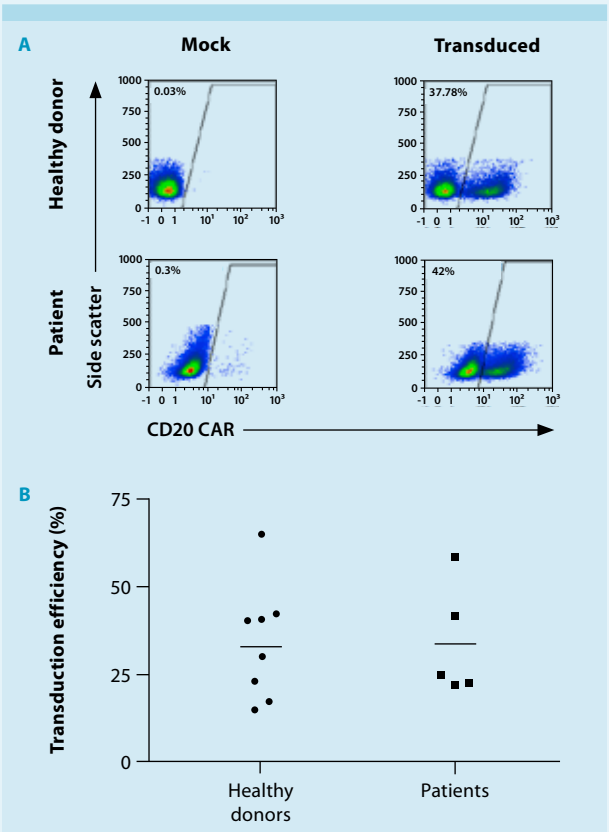


Figure 6: Enriched CD4+ and CD8+ T cells stimulated with MACS® GMP T Cell TransAct™ were transduced on day 1 with a LV encoding CD20 CAR. (A) Transduced T cells from healthy donor or lymphoma patients were analyzed by flow cytometry, (B) comparable transduction efficiencies of CD20 CAR–modified T cells were observed (healthy donors: n = 9; patients: n = 5).

Cell expansion

Culture is key

Optimal cultivation and expansion of transduced T cells rely on the strong synergy of MACS® GMP T Cell TransAct™ (page 6), TexMACS™ GMP Medium, and MACS GMP Cytokines.

TexMACS™ GMP Medium

- Specifically developed for T cell cultivation
- Serum- and xeno-component free
- Pharmaceutical-grade human serum albumin
- QC functionality test on every batch

MACS® GMP Cytokines

- Lot-to-lot consistency and lot-specific certificates of analysis
- Designed according to the recommendations of <USP 1043> on ancillary materials
- Manufactured and tested under a certified ISO 13485 quality system

Cell expansion takes place in the CentriCult Unit (CCU), an integrated cell cultivation chamber, which is part of the CliniMACS Prodigy® Tubing Set in order to maintain a closed system. The CCU allows culture volumes of 50 mL to 250 mL with an expansion capacity of up to 2×10^7 cells/mL. The culture duration is flexible and programmable, along with medium supply and exchange. The CCU also enables different agitation settings to maintain optimal culture conditions.

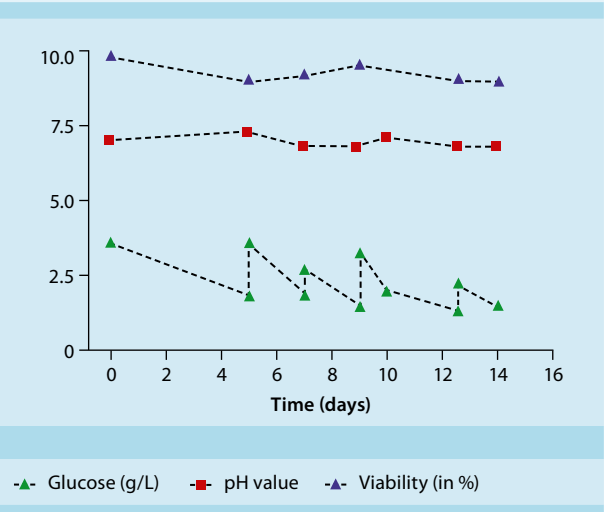


Figure 7: Enriched CD62L⁺ cells were cultured in the CliniMACS Prodigy during the TCT Process. Cell viability, pH-value, and glucose concentration throughout the whole process are shown.

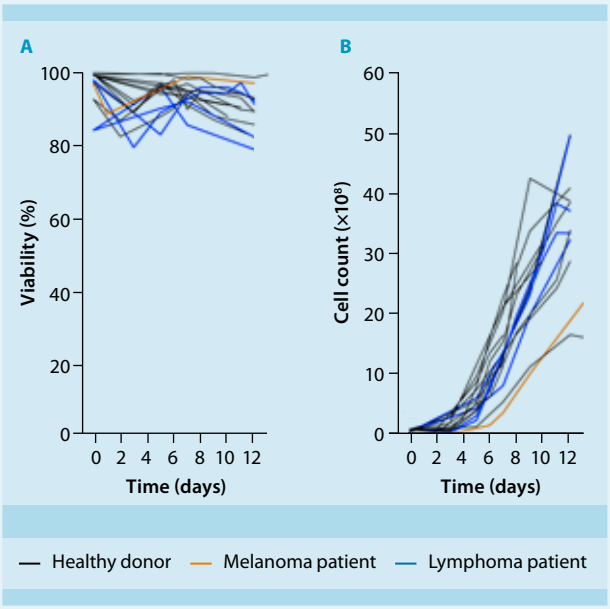


Figure 8: Enriched CD4⁺ and CD8⁺ T cells were automatically expanded in the CliniMACS Prodigy after polyclonal stimulation, and transduction. Material from either healthy donors (n = 12) or patients (n = 6) was used. The T cell culture was monitored at different time points to determine the viability (A). The absolute cell count of viable T cells was calculated (B).

At the end of the CliniMACS Prodigy TCT Process, the final product is harvested in 100 mL buffer of your choice. Gene-engineered T cells are then ready for cryopreservation or direct transfusion.

- Up to 3×10^9 T cells
- Robust, reliable, and reproducible results
- High cell viability
- Automated harvest

In-process and quality control

Reliable reproducibility

In-process control (IPC) and quality control (QC) are required for reliable cell manufacturing. As an ideal partner for IPC/QC, Miltenyi Biotec with its MACS® Flow Cytometry portfolio provides complete solutions with unmatched convenience. Reach out to your local Miltenyi Biotec flow cytometry specialists to obtain detailed information on flow antibody panels relevant for CAR T cell manufacturing. When using the MACSQuant® Instrument, Express Modes allow you to fully automate and standardize your flow cytometry process. The MACSQuant Instrument and the MACS Flow Cytometry portfolio are for research use only.

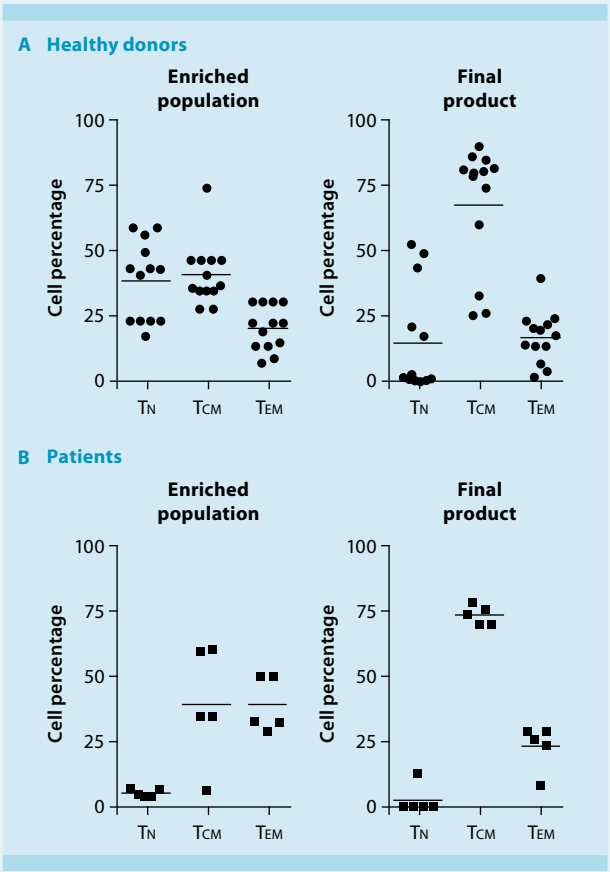


Figure 9: Phenotypes of enriched CD4⁺ and CD8⁺ cells immediately after enrichment (left) or after stimulation, transduction, and expansion (final product, right). The frequency of naive (T_N: D62L⁺CD45RO⁻), central memory (T_{CM}: CD62L⁺CD45RO⁺), and effector memory (T_{EM}: CD62L⁻ CD45RO⁺) T cells in the enriched population vs. the final product, manufactured in the CliniMACS Prodigy®, was analyzed by flow cytometry. Healthy donors, n = 13 (A). Patients, n = 5 (B).

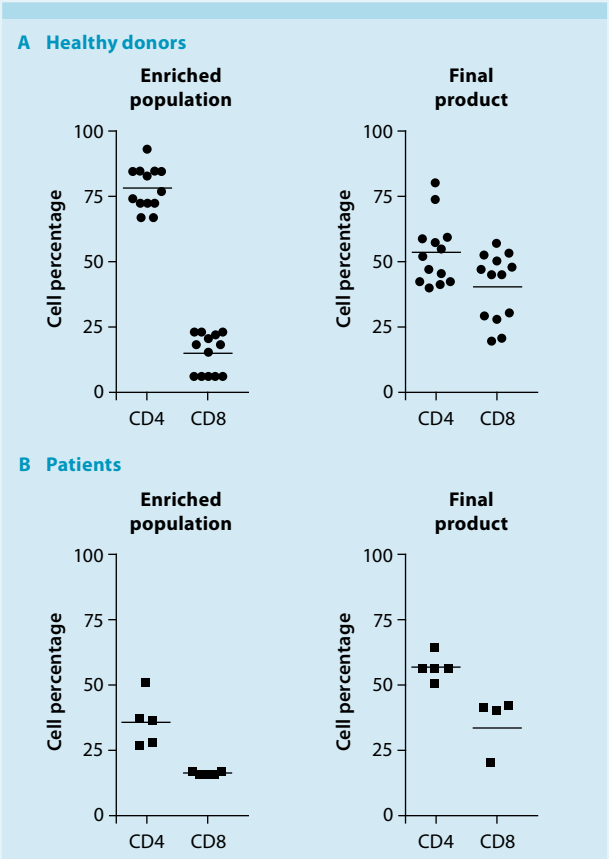


Figure 10: Percentages of CD4⁺ and CD8⁺ T cells in the enriched population vs. the final product manufactured in the CliniMACS Prodigy were determined. Healthy donors, n = 13 (A). Patients, n= 5 (B).

Figure 11: The MACSQuant Analyzer 10 (RUO) is a powerful benchtop flow cytometer for highly sensitive, multiparameter cell analysis.

Functionality of manufactured cells

Effectiveness that matters

Gene engineered T cells are assessed via cytokine secretion and their tumor cell killing.

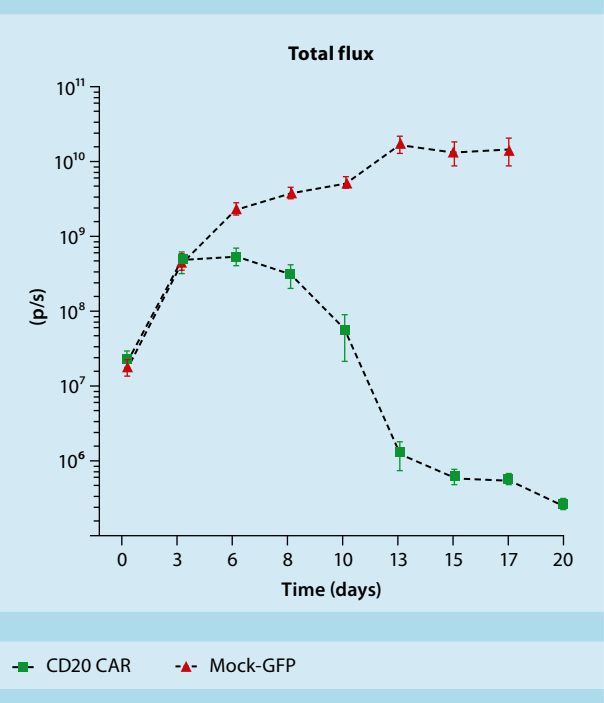


Figure 12: Longitudinal monitoring of tumor burden, mock GFP vs. CD20 CAR therapeutic effect from day 3 post therapy onwards. Mock-GFP fails to have any therapeutic effect; all animals had to be sacrificed by day 17.

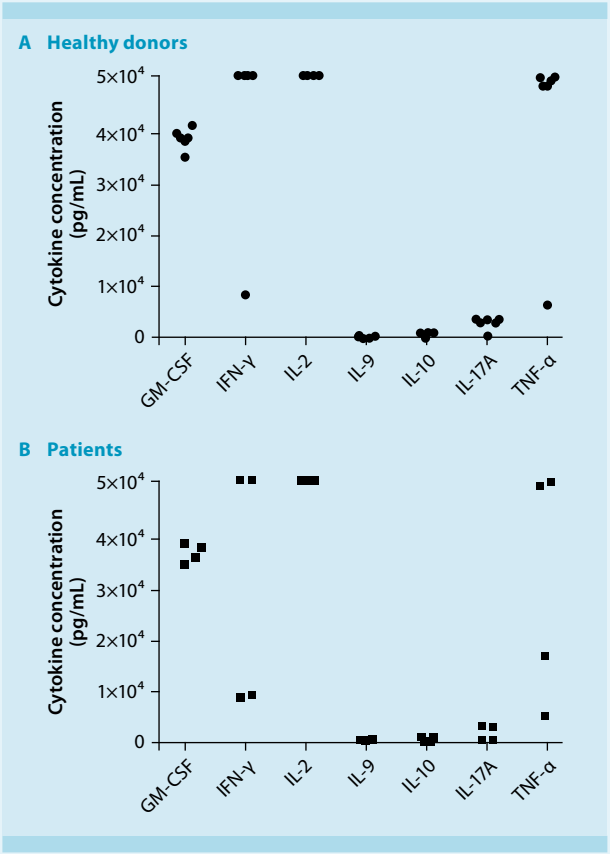


Figure 13: Automatically manufactured CD20 CAR T cells are fully functional in terms of cytokine secretion. CD20 CAR T cells, derived from healthy donor (A, n = 5) or patient (B, n = 4) samples, were cocultured with JeKo-1 as the target cell line (effector:target = 1:1), and cytokine secretion was analyzed using the MACSPlex Cytokine 12 Kit, human.

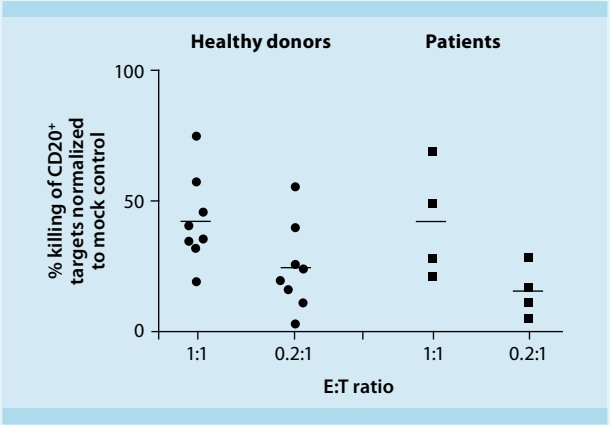


Figure 14: Cytotoxicity assay demonstrates an efficient and specific tumor cell killing by automatically manufactured CAR T cells. CD20 CAR T cells, which were derived from either healthy (n = 8) or patient material (n = 4), were co-culutured with JeKo-1 as the target cell line at the indicated effector: target cell (E:T) ratios for 24 hours. Percentage of tumor cell killing was determined by flow cytometry.

Consumables and software

Essentials for success

CliniMACS Prodigy® TS 520

- One single-use tubing set for the entire workflow
- Sterile filters at access ports
- A truly closed system
- Integrated sampling pouches allowing IPC/QC anytime

CliniMACS Prodigy® TCT Process

- Tailored for the manufacture of engineered T cells
- Enrichment of different T cell subsets possible
- Compatible with both RV and LV transduction applications
- GMP-compliant cytokines and cell culture media are available to support the manufacturing process

Figure 15: Tubing Set installed on the CliniMACS Prodigy Platform.

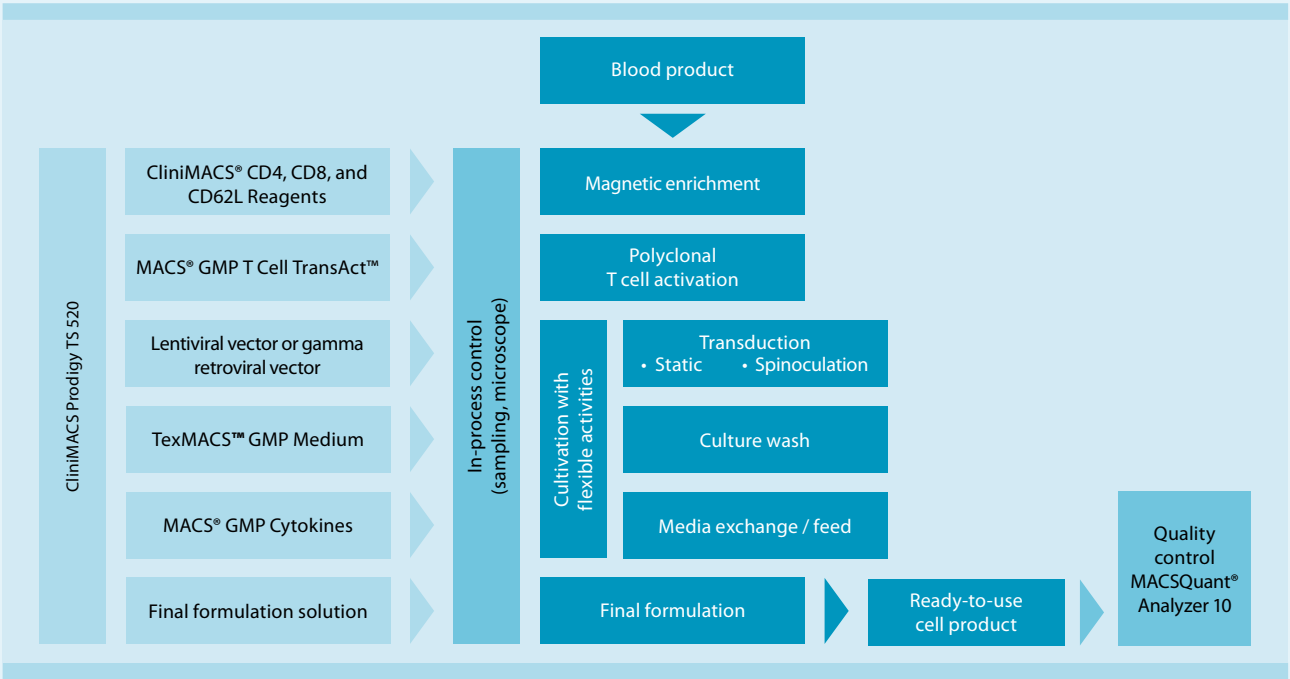


Figure 16: The complete process (indicated in dark blue) to generate genetically engineered T cells in a closed system, using the Tubing Set TS 520 on the CliniMACS Prodigy Platform. Spinoculation is an optional activity for the TCT Process.

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