Miltenyi Biotec

CliniMACS® Technology in stem cell transplantation research

Enrichment of CD34⁺ stem cells

Overcoming the challenges of HSCT

The scope of hematopoietic stem cell transplantation (HSCT) keeps expanding from malignant to non-malignant diseases. However, severe side effects like graft-versus-host disease (GVHD), infections, and poor graft function remain challenges of the treatment. We developed our CliniMACS Systems for GMP-compliant cell depletion and enrichment strategies to help scientists and clinicians with researching these challenges.

Passive T and B cell depletion

The immunomagnetic *in vitro* enrichment of CD34⁺ cells, using the CliniMACS System, is a very potent and robust technology for T cell depletion. The purity of CD34⁺ enriched stem cell product is often higher than 95% and the passive T cell depletion results in a log10 depletion of about 4.5.[1]

Stem cell boost (SCB) – rescue therapy for poor graft function (PGF)

PGF is a complication after allogeneic HSCT, occurring in 5–27% of the cases.[7] It is associated with increased morbidity and mortality due to severe infections, hemorrhagic complications, and organ failure caused by iron-overload. A boost of selected CD34⁺ stem cells from the initial donor, fresh or cryopreserved, without any further conditioning and GVHD prophylaxis, has been described as an option to overcome persistent PGF.[8]

Figure 1: Process set up is easy with the CliniMACS Prodigy touchscreen.

Poor graft function

Measures to diagnose PGF: two or three cytopenias >2 weeks, after day +28 in the presence of donor chimerism >5%

EBMT Handbook 2019[18]

As published, application of stem cell boost was applied independent of donor type and transplantation approach, also after failed previous therapy with growth factors. Clinical results show a 72–100% rapid and sustained lineage recovery with low rates of GVHD, even in high risk and mixed chimerism patients. Three years overall survival rates of 40–63% were reported.[8–14] In addition, immune reconstitution of all major lymphocyte populations was shown to be improved in 80% of the cases within four weeks.[15]

Other approaches like SCB as prevention of PGF after post-transplantation cyclophosphamide (PTCy) or treatment of mixed chimerism of secondary immunodeficiency (SID) patients after reduced intensity conditioning (RIC) HSCT are currently being investigated.[16,17]

Overall response	Complete response	aGVHD	cGVDH	Survival rate, median follow up 42 months
80%	72%	17%	18%	54%

Table 1: Efficacy and safety of CD34⁺ selected SCB for PGF after allo-HSCT, from seven studies (N = 209) adapted from Shahzad, M. *et al.* (2021).[25]

The haplo-cord approach

CD34+ cell enrichment has also been applied in combined haplo-cord transplantations, in which a CD34+ cell-enriched haploidentical transplant bridges the gap until the primary engraftment of the cord blood transplant takes place. [19-21]
As described, the idea of a combined haplo-cord transplantation is based on the observation that engraftment after haploidentical transplantation occurs faster than after cord blood transplantation. To provide the benefit of faster engraftment, the patient receives a bridging haploidentical transplant together with the cord blood at the same time. Clinical data show that with this approach even cord blood units containing very low stem cell numbers can be transplanted successfully and lead to sustained engraftment.[19-21]

CD34+ cell enrichment in autologous HSCT

CD34+ cell enrichment was originally developed for passive tumor cell depletion from autologous stem cell grafts. Currently, the technique is used for some tumors of early childhood[22,23] or non-Hodgkin lymphoma and other diseases in adults.[2,3]
Furthermore, CD34+ cell enrichment has been used to deplete autoreactive cells from stem cell grafts for severe refractory autoimmune diseases, like systemic lupus erythematosus and systemic sclerosis.[4,5,24]

Active T and B cell depletion

Prevention of GVHD

After active T and B cell depletion, the graft contains CD34⁺ stem cells, CD34⁻ stem cells, other progenitor cells, natural killer (NK) cells, and other members of the innate immune system that might have engraftment facilitating effects. Several strategies for depletion of distinct T cell subsets have been developed, such as the CliniMACS TCR α/β/CD19 Depletion System.[26,27]

Active depletion – TCRα/β/CD19 depletion

The process results in the depletion of alloreactive TCRα/β⁺ T cells. At this, various cell populations like stem cells and immune effectors cells are retained in the cellular product. Immune effector cells, such as NK cells and the TCRγ/δ⁺ T cells, are reported to induce GVL/T effects while the potential risk of inducing GVHD may be reduced.[28,29]

TCRγ/δ⁺ T cells – a fascinating cell type

Their unique set of functions include the ability to directly lyse infected or stressed cells, the production of cytokines and chemokines, and antigen presentation comparable to dendritic cells. Furthermore, they are thought to be highly effective against tumor cells and common infections.

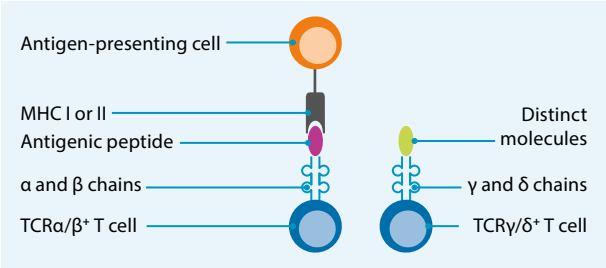


Figure 2: T cell receptors of TCRα/β⁺ and TCRγ/δ⁺ T cells. One major difference lies in the T cell receptor which is composed of different chains, α and β or γ and δ. TCRγ/δ⁺ T cells are not activated by MHC-presented antigens, which might contribute to the low alloreactivity of TCRγ/δ⁺ T cells. These cells become activated by structures of bacterial walls or heat shock proteins, for example.

Results from clinical applications

First clinical results using TCRα/β or TCRα/β/CD19 depleted stem cell grafts were published in 2011 by Lang *et al.*, from the Children's Hospital University Tübingen, Germany.[30] Since then, the number of publications has increased tremendously.
The technique has been applied in:

- malignant and non-malignant diseases
- myeloablative and reduced intensity conditioning regimens
- pediatric and adult patients
- settings with reduced or no post-transplant immune suppression
- haploidentical and matched unrelated HSCT

(see references 28–38)

	Rome[31]	Newcastle[32]	Utrecht[33]
CD34⁺ stem cells $\times 10^6$/kg	15.8	17.8	6.1
TCRαβ⁺ T cells $\times 10^3$/kg	40	3.3	20
TCRγ/δ⁺ T cells $\times 10^6$/kg	9.4	n/a	5.1
NK cells $\times 10^6$/kg	38.2	5.15	18.4
CD20⁺ B cells $\times 10^4$/kg	4	4	41.3
No. of patients	23	25	35

Table 2: Graft composition of haploidentical HSCT from three different sites.

The observed rates for primary engraftment are described as 'fast' in various publications, with ten to 16 days for neutrophil recovery.[31,34–38] Also, the immune reconstitution data are rated as 'remarkably fast' by Balashov *et al.*[36]Bertaina *et al.*[31] published the clinical results from 23 children with non-malignant disorders, receiving haploidentical HSCT and reported a two-year probability of disease-free survival of 91.1%. In a study with an adult patient population suffering from high-risk leukemias, it was shown that infusion of grafts depleted of TCRα/β⁺/CD19⁺ cells was safe and effective, resulting in rapid donor hematopoietic engraftment and early expansion of donor-derived T lymphocytes.[35]

High rates of disease-free survival, overall survival, and GVHD-free-relapse-free survival rates were observed in the company initiated multicenter prospective trial. Included were heavily pretreated adult and pediatric patients with malignant and non-malignant diseases using reduced-intensity conditioning regimen. The results showed a fast reconstitution of TCRγδ^{+} T cells and NK cells in the early posttransplant period, which translated into a favorable incidence of infectious complications. The low rate of 10% acute graft-versus-host disease (aGVHD) and no cases of grade III–IV aGVHD impressively highlights the strength of *ex vivo* TCRα/β^{+}/CD19^{+} depletion.[40]

Isolation of memory T cell products

A versatile concept for transfering immunity

Memory T cells provide immunity against many viral infections that the donors have experienced during their lifetime. In contrast to naive T cells, memory T cells are less alloreactive in an allogeneic HSCT setting.[39–41]CliniMACS CD45RA-depleted cell products have been used to treat and prevent opportunistic infections and to enhance immune reconstitution in the setting of allogeneic HSCT.[41–43]

CD45RA is an isoform of the CD45 family. It is present on naive T cells, on other leukocytes, and on some parts of hematopoietic stem cells in mobilized leukapheresis products. CD45RA is a proven marker to deplete alloreactivity in one step while central and effector memory cells are preserved.[39,41,44–46]

Log depletion	Leukapheresis product
4.7 (naive T cells)	(Bleakley, 2014)
4.4 (3.4–4.7; CD45RA$^+$ cells)	(Teschner, 2013)
3.6 (2.3–3.95; CD45RA$^+$ cells)	(Triplett, 2015)

Table 3: Log depletions obtained by CliniMACS Plus CD45RA Depletion of mobilized leukapheresis products.[39,40,47]

Graft engineering approach on an *ex vivo* T cell depletion platform

The intention of an *ex vivo* T cell depletion strategy is to provide grafts consisting of stem cells, combined with memory T cells, depleted of alloreactive naive T cell. The CliniMACS Platform allows for various combinations.

- The combination of CD34$^+$ cell enrichment and subsequent CD45RA$^+$ cell depletion from the CD34-negative cell fraction of the same mobilized leukapheresis products, has first been described by Marie Bleakley *et al.*[39] This approach has been used in different transplantation settings, as matched related and haploidentical transplantations (Seattle, Madrid, Paris).[6,41,48,49]
- Researcher from St. Judes Children's Hospital combined a CD34 enriched graft with CD45RA$^+$ depleted memory T cells that where obtained from an additional leukapheresis product. In this treatment protocol, CD56$^+$ enriched NK cells were added as well.[50,51]
- Other researchers developed strategies in which TCRα/β-depleted grafts were combined with CD45RA-depleted memory T cells. This approach was mainly used in haploidentical transplantation settings.[52]

Donor Lymphocyte Infusion (DLI) – memory T cells posttransplant

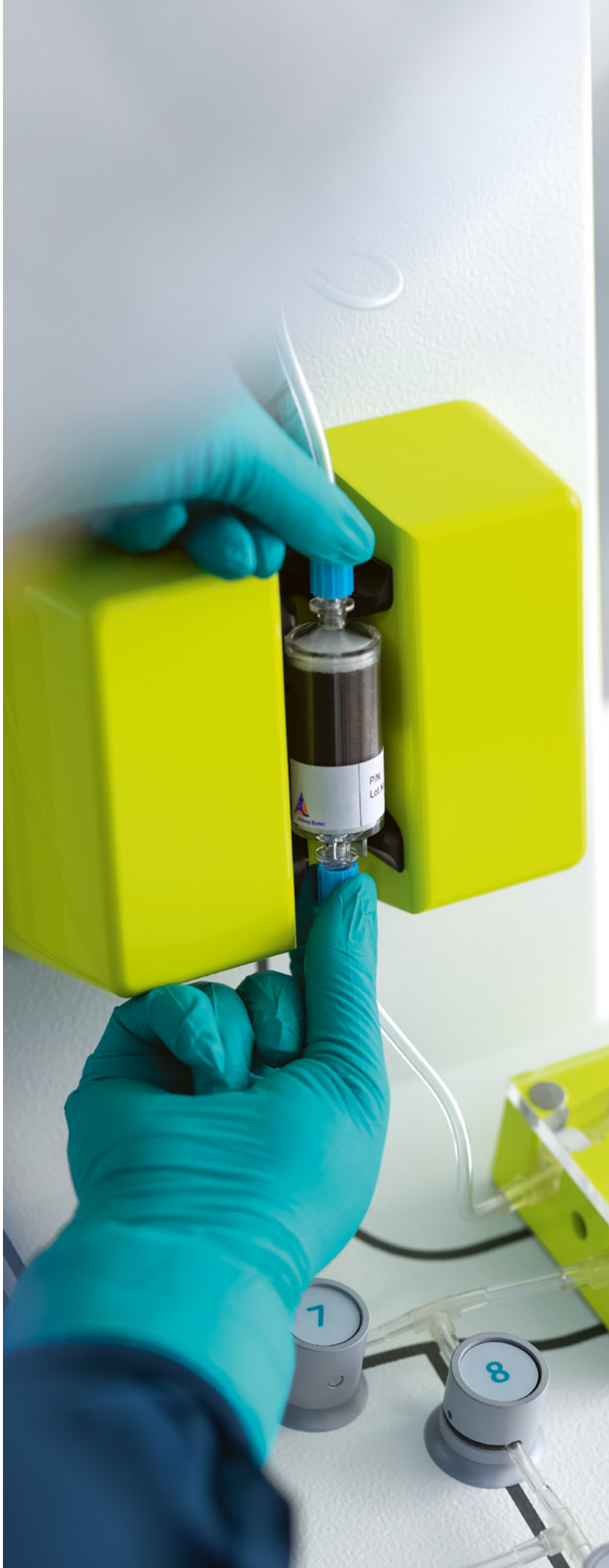
DLIs, depleted of CD45RA$^+$ cells, have been infused posttransplant as infection prophylaxis. This approach might be applied in combination with any kind of GVHD prophylaxis, such as *ex vivo* or *in vivo* T cell depletion strategies.

- The children's hospital in Moscow, Russia, reported on prophylactic infusions of memory T cell DLI, depleted of CD45RA$^+$ cells, after receiving a TCRα/β-depleted haploidentical transplant. The patients received up to three infusions in monthly intervals.[42]
- Prophylactic infusions of memory T cells for patients receiving T cell-depleted grafts from identical sibling donors have also been reported for the Duke University Medical Center, US.[53]
- In a study from Milan, Italy, patients received prophylactic memory T cell infusions after treatment with a haploidentical transplant that was based on a post-cyclophosphamide based GVHD prophylaxis.[54]

The use of memory T cells to treat active drug-resistant infections was reported too. The treatment of active infections after haploidentical HSCT in steroid-refractory cases has been demonstrated in several case reports. In these cases, the memory T cell products were obtained from original stem cell donors.[43,55]

Memory T cells in COVID-19

It was demonstrated that donor cells in a non-transplant setting can be helpful to overcome infections. Antonio Perez and his collaborators developed a method for delivering cell therapy products to COVID-19 patients. Using CliniMACS CD45RA Depletion, cell products were generated that are stored within a COVID-19 biobank from convalescent donors.[56] The clinical use of these cells has been reported within a phase I clinical trial.[57]



Enrichment of antigen-specific T cells

Enable anti-viral immunity

Antigen-specific T cells can be enriched using the CliniMACS Cytokine Capture System(CCS) (IFN-gamma) Technology. The CliniMACS Prodigy CCS (IFN-gamma) System allows for a fully automated specific labeling and enrichment procedure for antigen-specific IFN-γ secreting $CD4^+$ and $CD8^+$ T cells. The specificity of enriched T cells can be determined by choosing the respective MACS® GMP PepTivator® Peptide Pools. A growing repertoire of PepTivators for various viral antigens like AdV, BKV, EBV, and HCMV is available. Additionally, by using MACS GMP PepTivator NY-ESO-1 or WT1, cancer antigen-specific T cells may also be isolated.

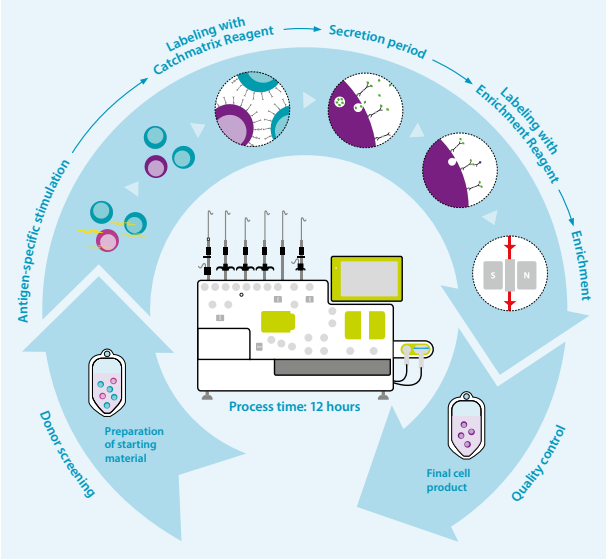


Figure 3: Overview of the automated workflow steps of the CliniMACS Prodigy CCS (IFN-gamma) System. Virus-specific T cells can be enriched based on their secretion of IFN-γ after restimulation with appropriate antigens.

Treatment of drug-resistant infections after allogeneic HSCT

Published clinical data are available for patients suffering from CMV, AdV, BKV, and EBV infections after receiving allogeneic HSCT. In these cases the infections could not be resolved by standard antiviral treatment. Initially, cells were obtained from the original stem cell donor.[58–61] Today, an increasing number of reports show the possibility of using third-party donor-derived cell products that can provided different types of biobanks.[62,63] Currently a multi-national clinical phase III trial is investigating efficacy and safety of adoptive T cell transfer in immunocompromised individuals.[64]

Treatment of patients after solid organ transplantation

Based on the experiences with HSCT patients, new strategies have been developed, using third party donor-derived cell products for patients post solid organ transplantation. Case reports are available for EBV-related lymphoma that has been treated with CCS derived antigen-specific T cells.[65,66]

SARS-CoV-2 specific T cells against COVID-19 disease

Several groups have developed manufacturing processes for cell products from convalescent donors, targeting SARS-CoV-2 infections. Wing Leung and his co-workers describe a process for flexible and rapid manufacturing of virus-specific T cells (VST) once a donor is available.[67] Cooper *et al.* describes a process that starts with the enrichment of VSTs using the CliniMACS Cytokine Capture System. A culturing step is added to increase the harvest of VST. The final products can be provided within a biobank.[68] First phase I clinical trials have been started.[69,70]

PepTivator Peptide Pools are optimal for effective stimulation of antigen-specific T cells.

CliniMACS® Plus System

Automated cell separation for clinical-scale cell enrichment or depletion

The CliniMACS Plus System automates cell separation for clinical-scale enrichment of target cells or depletion of unwanted cells from blood products. Cell separation occurs in a closed and sterile system. A single-use tubing set with its integrated separation column enables the instrument to separate target cells from unwanted cells and collect the fractions in different bags.

Sterile connections

Cellular starting material and buffers are connected for cell processing.

Ease of use

The software guides the user through interactions.

Flow of liquids

A peristaltic pump takes care for the precise flow of liquids.

Automated cell separation

The magnet unit houses the separation column for immunomagnetic cell separation

Controlled pathways

Pinch valves automatically close and open for a controlled fluid pathway within the tubing sets.

Key features of the CliniMACS Plus System

- Clinical-scale cell separation
- Automated separation procedure
- Compatible for use in a GMP setting
- Closed system
- Enrichment or depletion of dedicated cell populations
- Reproducible high purities, excellent yields and profound depletion efficiencies
- Established platform for *ex vivo* T cell depletion since 1997

Modular platform

- Variable cell sources
- Wide range of applications
- Versatile separation strategies

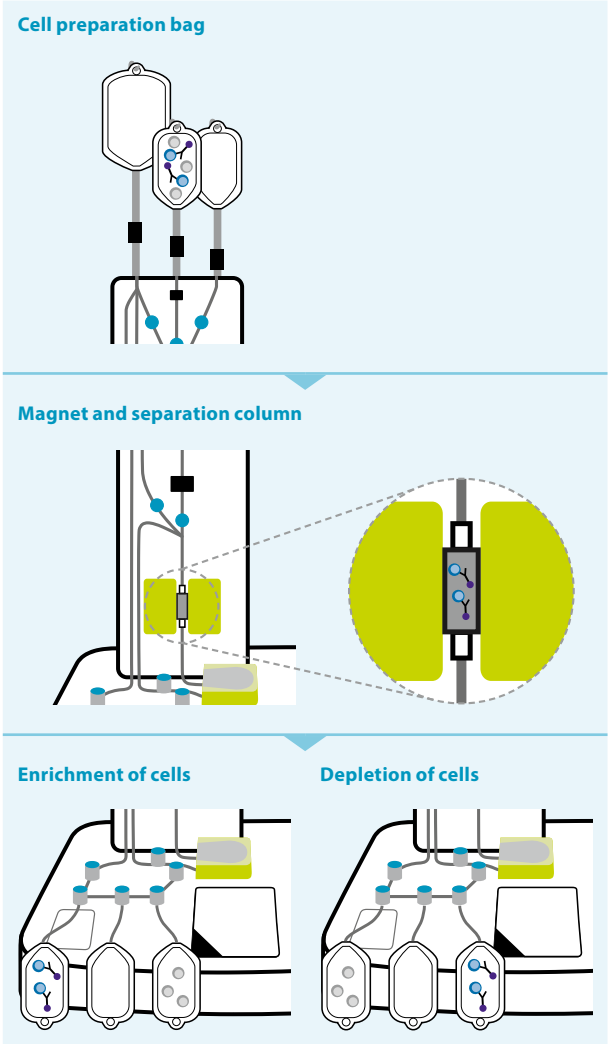


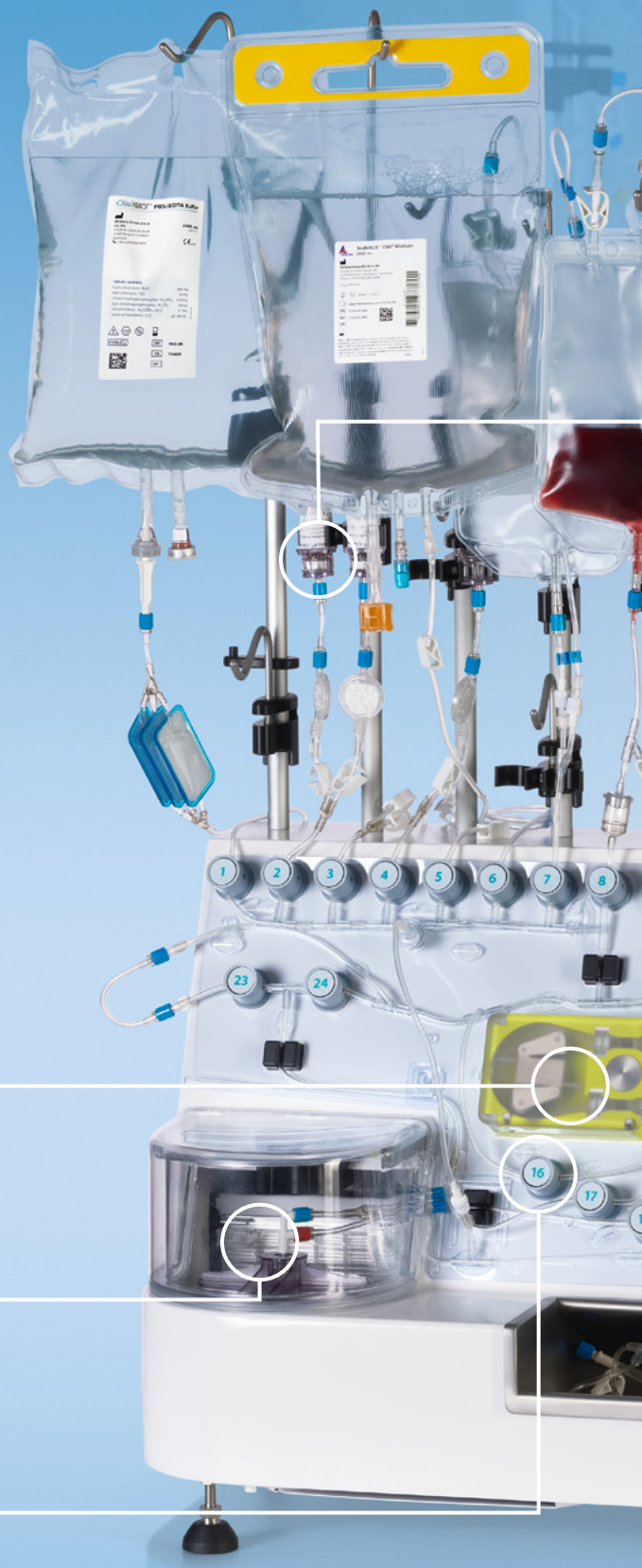
Figure 5: Workflow on the CliniMACS Plus System. Target cells are always collected in the left-most bag.

CliniMACS Prodigy® Platform

Master the complexity of cell processing

The CliniMACS Prodigy integrates all cell processing steps, including sample preparation, cell washing, density gradient centrifugation, magnetic cell separation, cell activation, genetic modification, cell culture, and cell product formulation. The fully automated, sensor-controlled processes provide a high level of standardization and reproducibility. Hands-on time is reduced substantially.

As all steps are performed in single-use, closed and sterile tubing sets, the instrument also reduces cleanroom requirements significantly. In combination with the wide variety of MACS GMP Products manufactured by Miltenyi Biotec, the CliniMACS Prodigy facilitates the implementation of GMP-compliant cell processing.



Network connectivity

The instrument is equipped to be connected to your local area network for process surveillance and data export.

Flow of liquids

The peristaltic pump directs accurate volumes of liquids through the tubing set.

Centrifugation and cell processing

The CentriCult™ Unit is a multi-purpose entity for cell processing and cell cultivation.

Controlled pathways

Pinch valves automatically close and open for a controlled fluid pathway within the tubing set.

Safe and sterile connections

Reagents, buffers, media and other materials can be easily connected via sterile spike ports or sterile welding.

Easy to operate

The user-friendly software guides the operator through interactions. Predefined processes and the flexible programming suite allow for convenient and tailored cell manufacturing.

Automated cell selection

The magnet unit houses the separation column for immunomagnetic cell separation.

Effortless documentation

CliniMACS Materials are scanned for identification and documentation with the bar code reader.

Sterile sealing

The MACS Tube Sealer is used for sealing and sterile removal of tubing components such as QC pouches.

Selected references

1. Hümmer, C. *et al.* (2016) Automation of cellular therapy product manufacturing: results of a split validation comparing CD34 selection of peripheral blood stem cell apheresis product with a semi-manual vs. an automatic procedure. J. Transl. Med. 14: 76. **https://doi.org/10.1186/s12967-016-0826-8**
2. Yahng, S. A. *et al.* (2014) Influence of *ex vivo* purging with CliniMACS CD34(+) selection on outcome after autologous stem cell transplantation in non-Hodgkin lymphoma. Br. J. Haematol. 164: 555–564. **https://pubmed.ncbi.nlm.nih.gov/24266323/**
3. Berger, M. D. *et al.* (2015) CD34+ selected versus unselected autologous stem cell transplantation in patients with advanced-stage mantle cell and diffuse large B-cell lymphoma. Leuk. Res. 39: 561–567. **https://doi.org/10.1016/j.leukres.2015.03.004**
4. Alchi, B. *et al.* (2013) Autologous haematopoietic stem cell transplantation for systemic lupus erythematosus: data from the European Group for Blood and Marrow Transplantation registry. Lupus 22: 245–253. **https://doi.org/10.1177/0961203312470729**
5. Henes, J. C. *et al.* (2012) Optimization of autologous stem cell transplantation for systemic sclerosis — a single-center longterm experience in 26 Patients with severe organ manifestations. J. Rheumatol. 39: 269–275. **https://doi.org/10.3899/jrheum.110868**
6. Bleakley, M. *et al.* (2022) Naive t-cell depletion to prevent chronic graft-versus-host disease. J. Clin. Oncol. JCO2101755. **https://doi.org/10.1200/JCO.21.01755.**
7. Lee, K. H. *et al.* (2004) Failure of trilineage blood cell reconstitution after initial neutrophil engraftment in patients undergoing allogeneic hematopoietic cell transplantation - Frequency and outcomes. Bone Marrow Transplant. 33(7): 729–734. **https://doi.org/10.1038/sj.bmt.1704428**
8. Ghobadi, A. *et al.* (2017) Fresh or cryopreserved CD34+-selected mobilized peripheral blood stem and progenitor cells for the treatment of poor graft function after allogeneic hematopoietic cell transplantation. Biol. Blood Marrow Transplant. 23(7): 1072–1077. **https://doi.org/10.1016/j.bbmt.2017.03.019**
9. Stasia, A. *et al.* (2014) CD34 selected cells for the treatment of poor graft function after allogeneic stem cell transplantation. Biol. Blood Marrow Transplant. 20: 1440–1443. **https://doi.org/10.1016/j.bbmt.2014.05.016**
10. Klyuchnikov, E. *et al.* (2014) CD34+-selected stem cell boost without further conditioning for poor graft function after allogeneic stem cell transplantation in patients with hematological malignancies. Biol. Blood Marrow Transplant. 20: 382–386. **https://doi.org/10.1016/j.bbmt.2013.11.034**
11. Haen, S.P. *et al.* (2015) Poor graft function can be durably and safely improved by CD34+-selected stem cell boosts after allogeneic unrelated matched or mismatched hematopoietic cell transplantation. J. Cancer Res. Clin. Oncol. 141: 2241–2251. **https://doi.org/10.1007/s00432-015-2027-x**
12. Askaa, B. *et al.* (2014) Treatment of poor graft function after allogeneic hematopoietic cell transplantation with a booster of CD34-selected cells infused without conditioning. Bone Marrow Transplant. 49: 720–721. **https://doi.org/10.1038/bmt.2014.5**
13. Chandra, S. *et al.* (2018) Post-transplant CD34+ selected stem cell "boost" for mixed chimerism after reduced-intensity conditioning hematopoietic stem cell transplantation in children and young adults with primary immune deficiencies. Biol. Blood Marrow Transplant. 24: 1527–1529. **https://doi.org/10.1016/j.bbmt.2018.03.013**
14. Mohty, R. *et al.* (2019) CD34+-selected stem cell "boost" for poor graft function after allogeneic hematopoietic stem cell transplantation. Curr. Res. Transl. Med. 67: 112–114. **https://doi.org/10.1016/j.retram.2018.12.003**
15. Cuadrado, M. *et al.* (2020) Predictors of recovery following allogeneic CD34+-selected cell infusion without conditioning to correct poor graft function. Haematologica 105: 2639–2646. **https://doi.org/10.3324/haematol.2019.226340**
16. Mainardi, C. *et al.* (2018) CD34+ selected stem cell boosts can improve poor graft function after paediatric allogeneic stem cell transplantation. Br. J. Haematol. 180: 90–99. **https://doi.org/10.1111/bjh.15012**
17. Abboud, R. *et al.* (2021) Can planned CD34+ stem cell boost prevent poor graft function after peripheral blood haploidentical hematopoietic transplantation? Leuk. Lymphoma 62: 749–751. **https://doi.org/10.1080/10428194.2020.1839657**
18. Carreras, E. *et al.* (2019) EBMT Handbook 2019. Hematopoietic stem cell transplantation and cellular therapies. 311. **https://doi.org/10.1007/978-3-030-02278-5**
19. Liu, H. & van Besien, K. (2015) Alternative donor transplantation – "mixing and matching": the role of combined cord blood and haplo-identical donor transplantation (haplo-cord SCT) as a treatment strategy for patients lacking standard donors? Curr. Hematol. Malig. Rep. 10: 1–7. **https://doi.org/10.1007/s11899-014-0245-y**
20. Kwon, M. *et al.* (2014) Haplo-cord transplantation using CD34+ cells from a third-party donor to speed engraftment in high-risk patients with hematologic disorders. Biol. Blood Marrow Transplant. 20: 2015–2022. **https://doi.org/10.1016/j.bbmt.2014.08.024**
21. Van Besien, K. *et al.* (2019) Haploidentical vs haplo-cord transplant in adults under 60 years receiving fludarabine and melphalan conditioning. Blood Adv. 3: 1858–1867. **https://doi.org/10.1182/bloodadvances.2019000200**
22. Ballova, V. *et al.* (2008) Autologous stem cell transplantation with selected CD34+ cells and unmanipulated peripheral blood stem cells in patients with relapsed and refractory Hodgkin's lymphoma: a single centre experience. Neoplasma 55: 428–436. **https://pubmed.ncbi.nlm.nih.gov/18665754/**
23. Marabelle, A. *et al.* (2011) CD34+ immunoselection of autologous grafts for the treatment of high-risk neuroblastoma. Pediatr. Blood Cancer 56: 134–142. **https://doi.org/10.1002/pbc.22840**
24. van Laar, J. M. *et al.* (2014) Autologous hematopoietic stem cell transplantation vs intravenous pulse cyclophosphamide in diffuse cutaneous systemic sclerosis. A randomized clinical trial. JAMA 311: 2490–2498. **https://doi.org/10.1001/jama.2014.6368**
25. Shahzad, M. *et al.* (2021) Outcomes with CD34-selected stem cell boost for poor graft function after allogeneic hematopoietic stem cell transplantation: a systematic review and meta-analysis. Transplant. Cell. Ther. 27: 877.e1-877.e8. **https://doi.org/10.1016/j.jtct.2021.07.012**
26. Chaleff, S. *et al.* (2007) A large-scale method for the selective depletion of αβ T lymphocytes from PBSC for allogeneic transplantation. Cytotherapy 9:746–754. **https://doi.org/10.1080/14653240701644000**
27. Schumm, M. *et al.* (2013) Depletion of T-cell receptor alpha/beta and CD19 positive cells from apheresis products with the CliniMACS device. Cytotherapy 15: 1253–1258. **https://doi.org/10.1016/j.jcyt.2013.05.014**
28. Locatelli, F. *et al.* (2013) At the bedside: innate immunity as an immunotherapy tool for hematological malignancies. J. Leukoc. Biol. 94: 1141–1157. **https://doi.org/10.1189/jlb.0613343**
29. Vantourout, P. *et al.* (2013) Six-of-the-best: unique contributions of γδ T cells to immunology. Nat. Rev. Immunol. 13: 88–100. **https://doi.org/10.1038/nri3384**
30. Lang, P. *et al.* (2011) First clinical results with αβ+ T-cell depleted haploidentical stem cells in children. Bone Marrow Transplant. 46: P558–S135. **https://doi.org/10.1038/bmt.2011.47**
31. Bertaina, A. *et al.* (2014) HLA-haploidentical stem cell transplantation after removal of αβ+ T and B cells in children with nonmalignant disorders. Blood 124: 822–826. **https://doi.org/10.1182/blood-2014-03-563817**
32. Shah, R. *et al.* (2017) T-cell receptor αβ+ and CD19+ cell–depleted haploidentical and mismatched hematopoietic stem cell transplantation in primary immune deficiency. J. Allergy Clin. Immunol. 141: 1417–1426.e1. **https://doi.org/10.1016/j.jaci.2017.07.008**
33. De Witte, M. A. *et al.* (2021) αβ T-cell graft depletion for allogeneic HSCT in adults with hematological malignancies. Blood Adv. 5: 240–249. **https://doi.org/10.1182/bloodadvances.2020002444**
34. Lang, P. *et al.* (2015) Improved immune recovery after transplantation of TCRαβ/CD19-depleted allografts from haploidentical donors in pediatric patients. Bone Marrow Transplant. 50: 6–10. **https://doi.org/10.1038/bmt.2015.87**
35. Prezioso, L. *et al.* (2019) Haploidentical hematopoietic stem cell transplantation in adults using the αβTCR/CD19-based depletion of G-CSF-mobilized peripheral blood progenitor cells. Bone Marrow Transplant. 54: 698–702. **https://doi.org/10.1038/s41409-019-0608-z**
36. Balashov, D. *et al.* (2015) Single-center experience of unrelated and haploidentical stem cell transplantation with TCRαβ and CD19 depletion in children with primary immunodeficiency syndromes. Biol. Blood Marrow Transplant. 21: 1955–1962. **https://doi.org/10.1016/j.bbmt.2015.07.008**

37. Maschan, M. *et al.* (2016) TCR-alpha/beta and CD19 depletion and treosulfan-based conditioning regimen in unrelated and haploidentical transplantation in children with acute myeloid leukemia. Bone Marrow Transplant. 51: 668–674. **https://doi.org/10.1038/bmt.2015.343**
38. Bethge, W. A. *et al.* (2021) Results of a multicenter phase I/II trial of TCRαβ and CD19-depleted haploidentical hematopoietic stem cell transplantation for adult and pediatric patients. Bone Marrow Transplant. Online ahead of print **https://doi.org/10.1038/s41409-021-01551-z**
39. Bleakley, M. *et al.* (2014) Engineering human peripheral blood stem cell grafts that are depleted of naïve T cells and retain functional pathogen-specific memory T cells. Biol. Blood Marrow Transplant. 20: 705–716. **https://doi.org/10.1016/j.bbmt.2014.01.032**
40. Teschner, D. *et al.* (2013) Depletion of naive T cells using clinical grade magnetic CD45RA beads: a new approach for GVHD prophylaxis. Bone Marrow Transplant. 49: 138–144. **https://doi.org/10.1038/bmt.2013.114**
41. Bleakley, M. *et al.* (2015) Outcomes of acute leukemia patients transplanted with naive T cell–depleted stem cell grafts. J. Clin. Invest. 125: 2677–2689. **https://doi.org/10.1172/JCI81229**
42. Dunaikina, M. *et al.* (2021) Safety and efficacy of the low-dose memory (CD45RA-depleted) donor lymphocyte infusion in recipients of αβ T cell-depleted haploidentical grafts: results of a prospective randomized trial in high-risk childhood leukemia. Bone Marrow Transplant. 56: 1614–1624. **https://doi.org/10.1038/s41409-021-01232-x**
43. Brodszki, N. *et al.* (2016) Novel treatment of severe combined immunodeficiency utilizing *ex vivo* T-cell depleted haploidentical hematopoietic stem cell transplantation and CD45RA$^+$ depleted donor lymphocyte infusions. Orphanet J. Rare Dis. 11: 5. **https://doi.org/10.1186/s13023-016-0385-3**
44. Fritsch, G. *et al.* (1993) Rapid discrimination of early CD34$^+$ myeloid progenitors using CD45-RA analysis. Blood 81: 2301–2309. **https://doi.org/10.1182/blood.V81.9.2301.2301**
45. Distler, E. *et al.* (2011) Alloreactive and leukemia-reactive T cells are preferentially derived from naive precursors in healthy donors: implications for immunotherapy with memory T cells. Haematologica 96: 1024–1032. **https://doi.org/10.3324%2Fhaematol.2010.037481**
46. Bremm, M. *et al.* (2021) Depletion of CD45RA$^+$ T cells: Advantages and disadvantages of different purification methods. Journal of Immunological Methods 492: 112960. **https://doi.org/10.1016/j.jim.2021.112960**
47. Triplett, B. M. *et al.* (2015) Rapid memory T-cell reconstitution recapitulating CD45RA-depleted haploidentical transplant graft content in patients with hematologic malignancies. Bone Marrow Transplant 50: 968–977. **https://doi.org/10.1038/bmt.2014.324**
48. Touzot, F. *et al.* (2015) CD45RA depletion in HLA-mismatched allogeneic hematopoietic stem cell transplantation for primary combined immunodeficiency: a preliminary study. J. Allergy Clin. Immunol. 135: 1303–1309. **https://doi.org/10.1016/j.jaci.2014.08.019**
49. Sisinni, L. *et al.* (2018) Unexpected high incidence of human herpesvirus-6 encephalitis after naive T cell–depleted graft of haploidentical stem cell transplantation in pediatric patients. Biol. Blood Marrow Transplant. 24: 2316–2323. **https://doi.org/10.1016/j.bbmt.2018.07.016**
50. Triplett, B. M. *et al.* (2018) Selective T-cell depletion targeting CD45RA reduces viremia and enhances early T-cell recovery compared with CD3-targeted T-cell depletion. Transpl. Infect. Dis. 2018: e12823. **https://doi.org/10.1111/tid.12823**
51. Mamcarz, E. *et al.* (2020) Improved survival rate in T-cell depleted haploidentical hematopoietic cell transplantation over the last 15 years at a single institution. Bone Marrow Transplant 55: 929–938. **https://doi.org/10.1038/s41409-019-0750-7**
52. Wu, I. Q. *et al.* (2021) HLA-Haploidentical hematopoietic cell transplantation of *ex vivo* Tcrαβ-depleted grafts with CD45RA-depleted memory T cell add-back in adults and children with hematological malignancies: 4-year follow-up of multicenter study in Singapore. Blood 138 (suppl. 1): 555. **https://doi.org/10.1182/blood-2021-152372**
53. Maung, K. K. *et al.* (2021) Phase I dose escalation study of naive T-cell depleted donor lymphocyte infusion following allogeneic stem cell transplantation. Bone Marrow Transplant 56: 137–143. **https://doi.org/10.1038/s41409-020-0991-5**
54. Castagna, L. *et al.* (2021) Feasibility and efficacy of CD45RA$^+$ depleted donor lymphocytes infusion after haploidentical transplantation with post-transplantation cyclophosphamide in patients with hematological malignancies. Transplantation and Cellular Therapy 27: 478.e1–478.e5. **https://doi.org/10.1016/j.jtct.2021.03.010**
55. Park, H. J. *et al.* (2020) Successful treatment of refractory CMV colitis after haploidentical HSCT with post-transplant cyclophosphamide using CD45RA$^+$ depleted donor lymphocyte infusion. Bone Marrow Transplant 55: 1674–1676. **https://doi.org/10.1038/s41409-019-0685-z**
56. Ferreras, C. *et al.* (2021) SARS-CoV-2-specific memory T lymphocytes from COVID-19 convalescent donors: identification, biobanking, and large-scale production for adoptive cell therapy. Front. Cell Dev. Biol. 9: 620730. **https://doi.org/10.3389/fcell.2021.620730**
57. Perez-Martínez, A. *et al.* (2021) Phase I dose-escalation single centre clinical trial to evaluate the safety of infusion of memory T cells as adoptive therapy in COVID-19 (RELEASE). EClinicalMedicine 39: 101086. **https://doi.org/10.1016/j.eclinm.2021.101086**
58. Feuchtinger, T. *et al.* (2010) Adoptive transfer of pp65-specific T cells for the treatment of chemorefractory cytomegalovirus disease or reactivation after haploidentical and matched unrelated stem cell transplantation. Blood 116: 4360–4367. **https://doi.org/10.1182/blood-2010-01-262089**
59. Meiji, P. *et al.* (2012) Effective treatment of refractory CMV reactivation after allogeneic stem cell transplantation with in vitro-generated CMV pp65-specific CD8$^+$ T-cell Lines. J. Immunther. 35: 621–628. **https://doi.org/10.1097/CJI.0b013e31826e35f6**
60. Icheva, V. *et al.* (2012) Adoptive transfer of Epstein-Barr virus (EBV) nuclear antigen 1-specific T cells as treatment for EBV reactivation and lymphoproliferative disorders after allogeneic stem-cell transplantation. J. Clin. Oncol. 31: 39–48. **https://doi.org/10.1200/JCO.2011.39.8495**
61. Pello, O. M. *et al.* (2017) BKV-specific T cells in the treatment of severe refractory haemorrhagic cystitis after HLA-haploidentical haematopoietic cell transplantation. Eur. J. Heam. 98: 632–634 **https://doi.org/10.1111/ejh.12848**
62. Qian, C. *et al.* (2017) Curative or pre-emptive adenovirus-specific T cell transfer from matched unrelated or third party haploidentical donors after HSCT, including UCB transplantations: a successful phase I/II multicenter clinical trial. Journal of Hematology & Oncology 10: 102. **https://doi.org/10.1186/s13045-017-0469-0**
63. Kállay, K. *et al.* (2018) Early experience with CliniMACS Prodigy CCS (IFN-gamma) system in selection of virus-specific T cells From third-party donors for pediatric patients with severe viral infections after hematopoietic stem cell transplantation. J. Immunother. 41: 158–163. **https://doi.org/10.1097/CJI.0000000000000197**
64. Kaeuferle, T. *et al.* (2019) Strategies of adoptive T -cell transfer to treat refractory viral infections post allogeneic stem cell transplantation. Journal of Hematology & Oncology 12: 13. **https://doi.org/10.1186/s13045-019-0701-1**
65. Hansen, B. T. *et al.* (2021) Adoptive cell transfer of allogeneic epstein-barr virus-specific T lymphocytes for treatment of refractory EBV-associated posttransplant smooth muscle tumors: a case report. Front. Immunol. 12: 727814. **https://doi.org/10.3389/fimmu.2021.727814**
66. Schultze-Florey, R. E. *et al.* (2018) Dissecting Epstein-Barr virus-specific T-cell responses after allogeneic EBV-specific T-cell transfer for central nervous system posttransplant lymphoproliferative disease. Front. Immunol. 9: 1475. **https://doi.org/10.3389/fimmu.2018.01475**
67. Leung, W. *et al.* (2020) Rapid production of clinical-grade SARS-CoV-2-specific T cells. Adv. Cell Gene. Ther. 2020; 00: e101 **https://doi.org/10.1002/acg2.101**
68. Cooper, R. S. *et al.* (2021) Rapid GMP-compliant expansion of SARS-CoV-2-specific T cells from convalescent donors for use as an allogeneic cell therapy for COVID-19. Front. Immunol. 11: 598402 **https://doi.org/10.3389/fimmu.2020.598402**
69. clinicaltrials.gov: **NCT04324463**
70. clinicaltrials.gov: **NCT04742595**

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