Miltenyi Biotec

Advancing stem cell transplantation with CliniMACS® Technology



Overcome the challenges of HSCT

Enrichment of CD34+ stem cells

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, relapse, and poor graft function (PGF) remain challenges of the treatment. We developed our CliniMACS Systems for GMP-compliant cell depletion and enrichment strategies to help clinicians overcome these challenges.

Passive T and B cell depletion as GVHD prophylaxis

The immunomagnetic *in vitro* enrichment of CD34+ cells, using the CliniMACS System, is a very potent and robust technology for T cell depletion. Studies have shown 10^1 to 10^4 depletion rates,[1] leading to an effective GVHD prevention.[2–8] In the US, the CliniMACS CD34+ Reagent System was registered as sole GVHD prophylaxis in allogeneic HSCT, from an HLA-identical sibling donor in adult patients with acute myeloid leukemia (AML) in first complete remission. This technique has been widely used for more than two decades and is continuously improving in terms of automation on the CliniMACS Prodigy®. However, the use of CD34+ cell enrichment as GVHD prevention strategy, may contribute to delayed immune reconstitution and higher non-relapse mortality (NRM).[2]



Figure 1: Connecting CliniMACS CD34 Reagents to the CliniMACS Prodigy.

Memory T cells for immune reconstitution

Adding memory T cells to the graft may be one option to solve this challenge. Memory T cells were shown to support the immune reconstitution and significantly improve the NRM rate, while maintaining excellent GVHD prevention.[3]

"Add back" of donor T cells

Additional strategies have been applied in combination with CD34-enriched HSCT as a backbone. The adoptive transfer of donor regulatory T cells allows for the infusion of donor conventional T cells with no or limited use of post-transplant pharmacologic immune suppression. This strategy helped to reduce leukemia relapse and chronic GVHD rates. Regulatory T cells can be infused 2–3 days before conventional T cells or at the time of transplant, together with CD34+ cells.[4,5] Adding CD3+ T cells to the CD34-enriched stem cell graft can lead to improved engraftment and immune reconstitution.[6]

Stem cell boost (SCB) – rescue therapy for PGF

PGF is a complication after allogeneic HSCT, occurring in 5–27% of the cases.[7] It's 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.[8–12]

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[20]

As published, the application of stem cell boost was applied independently of donor type and transplantation protocols, 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.[14] Three-year overall survival rates of 40–63% were reported. In addition, immune reconstitution of all major lymphocyte populations was shown to be improved in 80% of the cases within four weeks.[7–14]

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-enriched SCBs for PGF after allogeneic HSCT, from seven studies (N = 209), adapted from Shahzad *et al.* (2021).[15]

SCB – rescue therapy for long-term cytopenia after CAR T cell therapy

Long-term cytopenia is a severe side effect after CAR T cell therapy, and SCB with selected CD34$^+$ cells has been used as a treatment option in this setting. Different case studies show that the time to recovery of neutropenia and transfusion independence ranged between 14–33 days after CD34$^+$ stem cell boost.[16–19]

Autologous HSCT in autoimmune diseases

CD34$^+$ cell enrichment is also used to deplete autoreactive cells from stem cell grafts for severe refractory autoimmune diseases, like systemic lupus erythematosus and systemic sclerosis, with good results in progression-free survival (PFS) and improvement in skin sclerosis.[20–22]

LEARN MORE

Learn more about stem cell enrichment for improved HSCT.

▸ **miltenyibiotec.com/cd34enrichment**



Effectively prevent GVHD

Active T and B cell depletion

After active T and B cell depletion, the graft contains CD34$^+$ stem cells, CD34$^-$ stem cells, other progenitor cells, TCRγ/δ$^+$ T cells, natural killer (NK) cells, and additional 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 TCRα/β/CD19 Depletion System.[23, 24]

Active depletion – TCRα/β/CD19 cell depletion

The process results in the depletion of alloreactive TCRα/β$^+$ T cells. At this, various cell populations like stem cells and immune effector cells are retained in the cellular product. Immune effector cells, such as NK cells and the TCRγ/δ$^+$ T cells, are reported to induce graft-versus-leukemia (GVL) or graft-versus-tumor (GVT) effects while the potential risk of inducing GVHD may be reduced.[25]

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.[26]

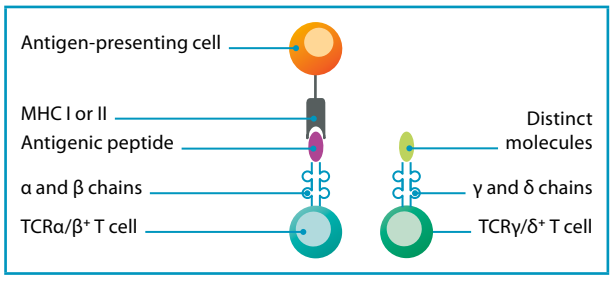


Figure 2: T cell receptors of TCRα/β$^+$ and TCRγ/δ$^+$ T cells.
One major difference lies in the T cell receptor which is composed of different chains, α and β or γ and δ. TCRγ/δ$^+$ T cells are not activated by MHC-presented antigens, which might contribute to the low alloreactivity of TCRγ/δ$^+$ T 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.[27] 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 28–34)

Patients with non-malignant diseases

Since the first publication by Bertaina *et al.*[28] in 2014, various publications have reported results from treatments of patients suffering from different forms of non-malignant diseases. These studies have shown 'fast' engraftment, with ten to 16 days for neutrophil recovery[33,36–40]. Despite the heterogeneity of the diseases being treated, the GVHD rate is remarkably low. Merli *et al.*[29] reported 14.4% of acute GVHD grade I–II in a cohort of 70 pediatric patients, with no cases of grade III–IV and only one case of mild chronic GVHD.

A group from Newcastle[30] reported 11% grade II–IV acute GVHD and 91% GVHD- and event-free survival (GEFS) in SCID patients. This led them to conclude that the outcomes of TCRαβ-Haplo SCT are comparable to those of MUD-based transplants.

Patients with malignant diseases

Merli *et al.* reported on a cohort of 213 pediatric patients with acute leukemias and a median follow-up of nearly five years. The 5-year cumulative incidence (CI) of non-relapse mortality (NRM) was low, at only 5.2%. With a relapse rate of 22.7%, this resulted in favorable survival outcomes. Projected 10-year overall (OS) and disease-free survival (DFS) were 75.4% and 71.6%, respectively. Importantly, GVHD rates were low, with a CI of grade II-IV acute and chronic GVHD of 14.7% and 8.1%.[31]

Similar results have been reported from a prospective US multicenter trial involving 51 pediatric patients with acute leukemias or MDS. Two-year DFS and OS were 79% and 82%, respectively, with transplant-related mortality and chronic GVHD rates below 10%. The authors noted that non-radiation, low-toxicity approaches resulted in better DFS and OS compared to traditional myeloablative approaches.[32]

A group from the Netherlands reported results from a cohort of 35 adult patients with various malignant hematological diseases receiving a TCRα/β and CD19-depleted graft from a matched donor.[33] The authors noted an incidence of aGVHD grade II-IV of 26% at day 100, and of moderate and severe cGVHD at 2 years of 17% and 0%, respectively. Remarkably, the low rate of GVHD allowed for administration of prophylactic Donor lymphocyte infusions, which was considered important in this group of patients with heterogeneous diseases and contributed to a 2-year OS of 54%.[33]

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Discover the automated depletion of T and B cells for precision graft manufacturing.

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Transfer immunity against opportunistic infections

Manufacturing of memory T cell products

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 have a reduced alloreactive potential in an allogeneic HSCT setting.[35–37] Cell products, depleted with the CliniMACS CD45RA System have been used to treat and prevent opportunistic infections and to enhance immune reconstitution in the setting of allogeneic HSCT.[3,37–39]

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.[35,37,40–43]

Log depletion	Reference
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.[35,36,44]

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.

- To enhance immune reconstitution, Marie Bleakley *et al.* were the first to describe a combination approach: enriching CD34$^+$ cells and subsequently depleting CD45RA$^+$ cells from the CD34-negative fraction of the same mobilized leukapheresis product.[35] This approach has been used in different transplantation settings, as matched related and haploidentical transplantations.[3,37,45,46]
- 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.[47–49]
- Other researchers developed strategies to improve immune reconstitution by combining TCRα/β-depleted grafts with CD45RA-depleted memory T cells. This approach was mainly used in haploidentical transplantation settings.[50,51]

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 repeated infusion of high doses of memory T cells, with a median infused dose of 8.96×10^6/kg CD3$^+$CD45RO$^+$ and 3.81×10^3/kg CD3$^+$CD45RA$^+$ in pediatric patients with malignant and non-malignant disorders, was published jointly by the La Paz University Hospital in Madrid, Spain, and the St. Jude Children's Hospital in Memphis, US.[52]
- The children's hospital in Moscow, Russia, reported on prophylactic infusions of low-dose 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.[53]
- 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.[54]
- 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 GVHD prophylaxis.[55]

The use of memory T cells to treat active drug-resistant infections was reported too. In several case reports, the treatment of active infections has been described for patients after HSCT and SOT as well as in an immunocompromised patient receiving an anti-lymphoma treatment. In these cases, the memory T cell products were obtained from original stem cell donors.[56,57]

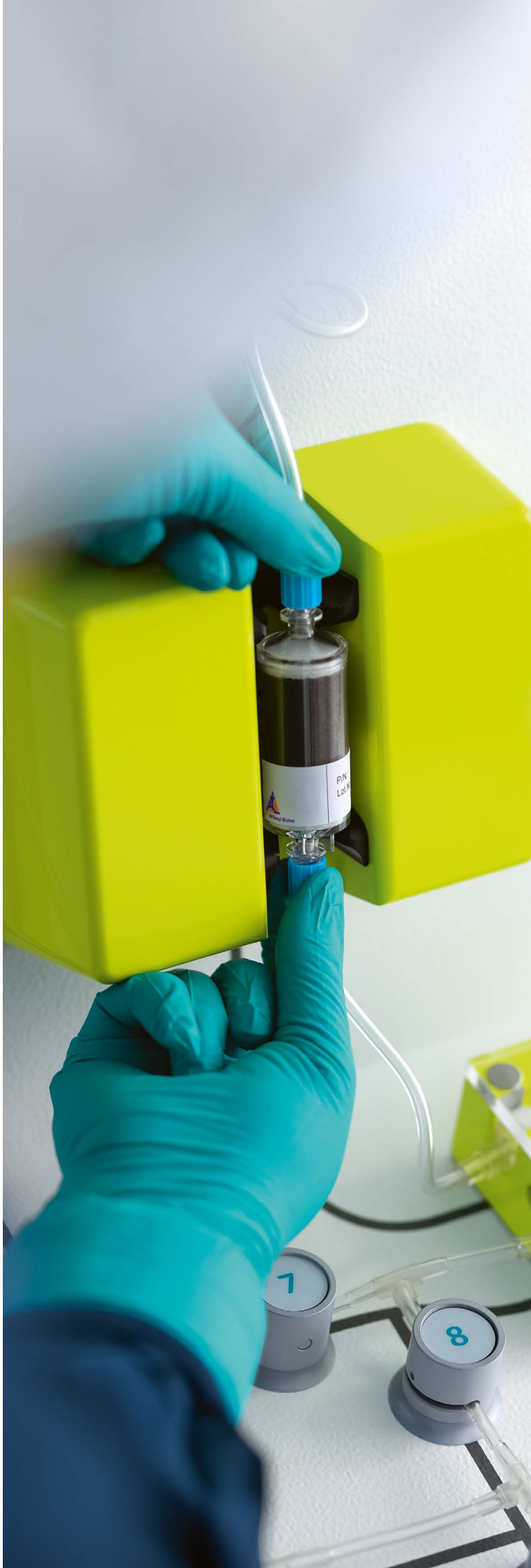
Memory T cells in COVID-19

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.[59] The clinical use of these cells has been reported within phase I and II clinical trials.[60,61]

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Efficiently fight infections after HSCT with CD45RA$^+$ cell depletion.

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Enable anti-viral immunity

Enrichment of antigen-specific T cells

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 PepTivator Peptide Pools 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.

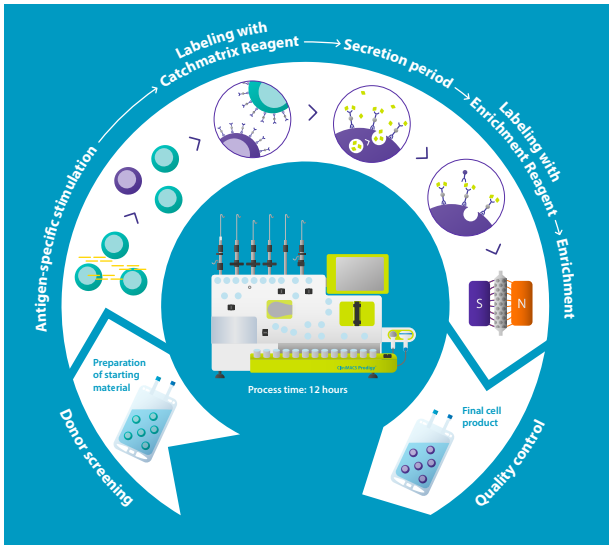


Figure 3: Overview of the automated workflow steps of the CliniMACS Prodigy CCS (IFN-gamma) System. Virus-specific T cells can be enriched based on their secretion of IFN-γ after restimulation with appropriate antigens.

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.[62–64] Today, an increasing number of reports show the possibility of using third-party donor-derived cell products providing different types of biobanks.[65,66] Currently a multinational clinical phase III trial is investigating efficacy and safety of adoptive T cell transfer in immunocompromised individuals.[67]

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.[68–70]

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

Several groups have developed manufacturing processes for cell products from convalescent donors, targeting SARS-CoV-2 infections. Wing Leung and his coworkers describe a process for flexible and rapid manufacturing of virus-specific T cells (VST) once a donor is available.[71] Cooper *et al.* describe 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.[72] Clinical use has been demonstrated in three stem cell transplant recipients diagnosed with COVID-19.[73]

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Get the details of virus-specific T cell isolation.

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

CliniMACS Plus System

The industry standard for automated cell enrichment and depletion

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



Flexible 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 of 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 set.

Key features of the CliniMACS Plus System

- Clinical-scale cell separation
- Automated separation procedures
- Compatible for use in a GMP setting
- Closed system, using CE-marked consumables
- 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

VISIT

Find out why the CliniMACS Plus is the benchmark for cell separation.

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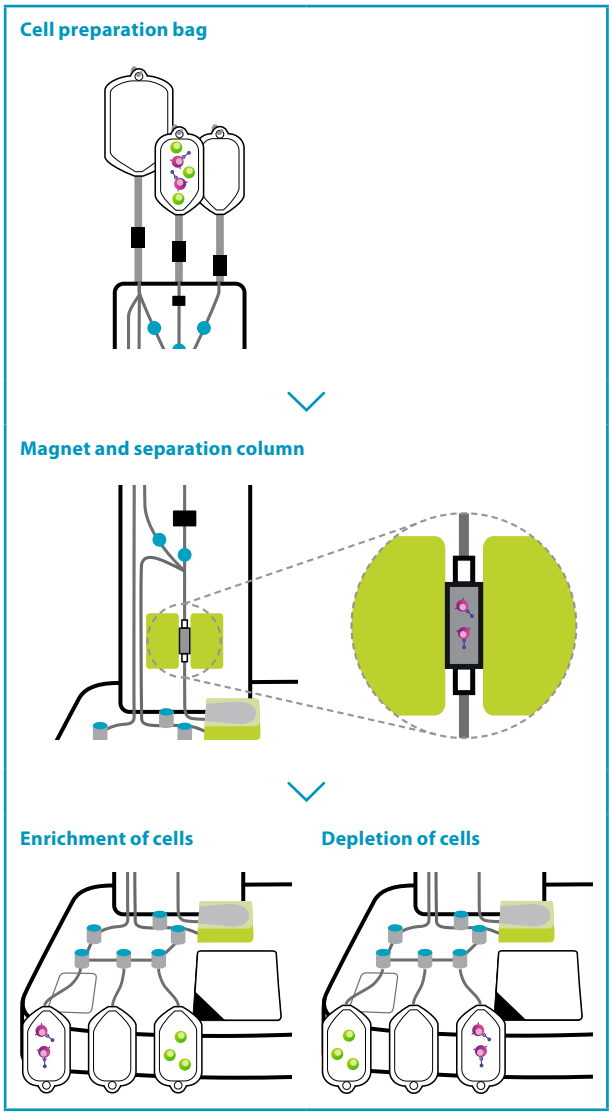
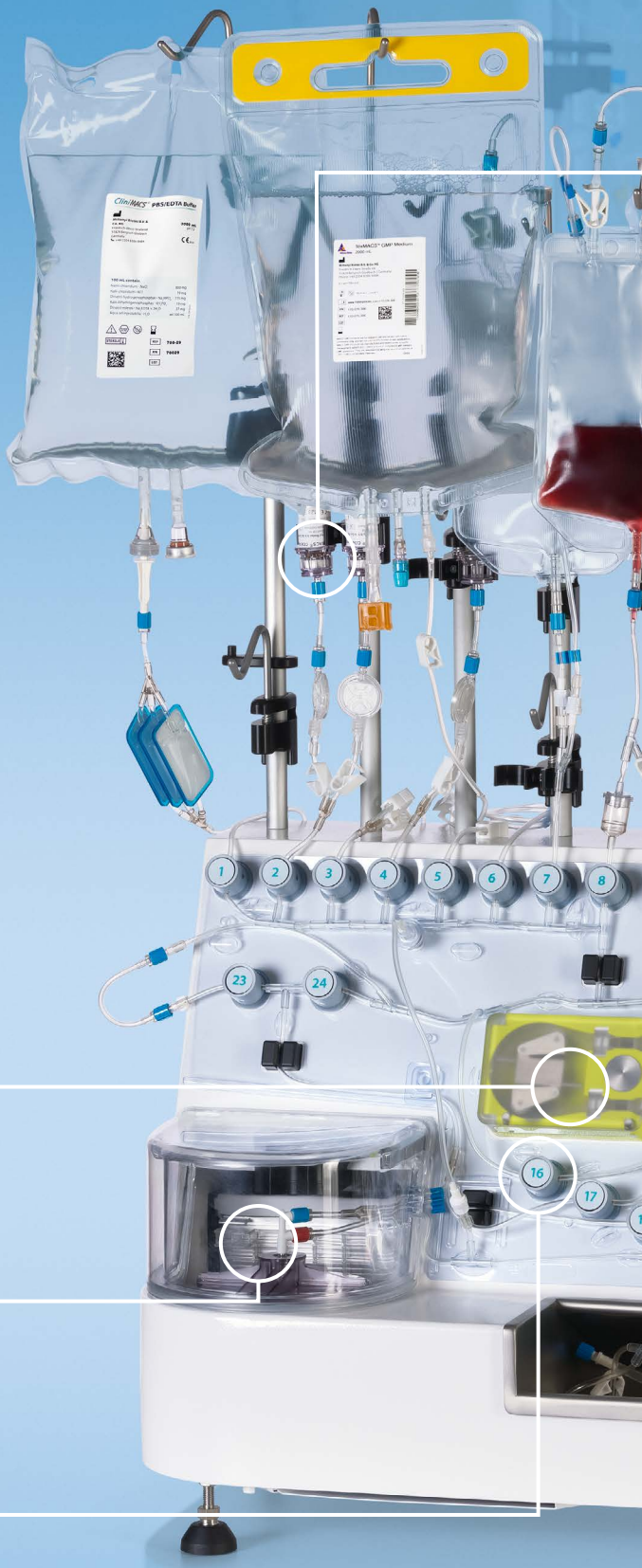


Figure 4: Workflow on the CliniMACS Plus System: The process begins with a bag containing magnetically labeled cells being connected to the tubing set. The cells are then applied to the separation column while the magnetic field is active, causing the labeled cells to be retained in the column while unlabeled cells flow through and are collected. Once the separation is complete, the magnetic field is deactivated to allow the labeled cells to be eluted from the column and collected in a separate bag.

CliniMACS Prodigy® Platform

The no. 1 platform for clinical cell manufacturing

The CliniMACS Prodigy integrates all cell processing steps, including cell preparation, washing, density gradient centrifugation, magnetic separation, activation, genetic modification, cultivation, and cell product formulation. The automated processes provide a high level of standardization and reproducibility, while hands-on time is significantly reduced. Since all steps occur within a single-use, closed, and sterile tubing set, the instrument also notably decreases cleanroom requirements.
The GMP and 21 CFR Part 11-supportive software, with user management and audit trail features, simplifies regulatory compliance and supports network functions.



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 multipurpose 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 barcode reader.

Sterile sealing

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

VISIT

Discover the no. 1 platform for automated cell manufacturing.

▸ **miltenyibiotec.com/Prodigy**

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