Miltenyi Biotec

CliniMACS® Technology in stem cell transplantation

Enrichment of CD34+ stem cells

Overcome 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 clinicians overcome 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. Studies have shown 10^4 to 10^5-fold depletion rates[1], leading to an effective GVHD prevention.[2–5] 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 full automation on the CliniMACS Prodigy.[6] However, the use of CD34+ cell enrichment as GVHD prevention strategy, may contribute to delayed immune reconstitution and higher non-relapse mortality (NRM).[7] Yet, the addition of memory T cells to the graft may solve this challenge. Memory T cells were shown to support the immune reconstitution and significantly improve the NRM rate, while maintaining excellent GVHD prevention.[8]

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

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.[9] 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.[10]

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, 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.[10–16] In addition, immune reconstitution of all major lymphocyte populations was shown to be improved in 80% of the cases within four weeks.[17]

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.[18,19]

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).[27]

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. [21–23]
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.[21–23]

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[24,25] 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.[4,5,26]

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.[28,29]

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.[30,31]

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.

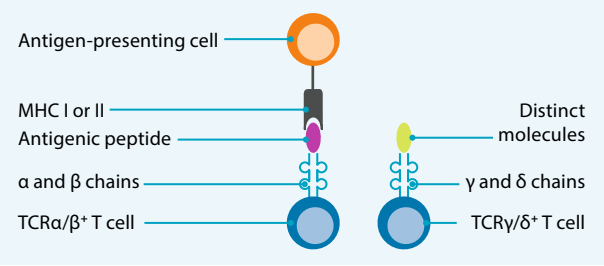


Figure 2: T cell receptors of TCRα/β⁺ and TCRγ/δ⁺ T cells. One major difference lies in the T cell receptor which is composed of different chains, α and β or γ and δ. TCRγ/δ⁺ T cells are not activated by MHC-presented antigens, which might contribute to the low alloreactivity of TCRγ/δ⁺ T cells. These 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.[32] 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 30–40)

	Rome[33]	Newcastle[34]	Utrecht[35]
CD34⁺ stem cells ×10⁶/kg	15.8	17.8	6.1
TCRαβ⁺ T cells ×10³/kg	40	3.3	20
TCRγ/δ⁺ T cells ×10⁶/kg	9.4	n/a	5.1
NK cells ×10⁶/kg	38.2	5.15	18.4
CD20⁺ B cells ×10⁴/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.[33,36–40] Also, the immune reconstitution data are rated as 'remarkably fast' by Balashov *et al.*[38] Bertaina *et al.*[33] 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.[37]

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]

Manufacturing 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.[41–43] 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.[43–45]

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.[41,43,46–48]

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.[41,42,49]

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.*[41] This approach has been used in different transplantation settings, as matched related and haploidentical transplantations (Seattle, Madrid, Paris).[8,43,50,51]
- 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.[52,53]
- 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.[54]

Donor Lymphocyte Infusion (DLI) – memory T cells posttransplant

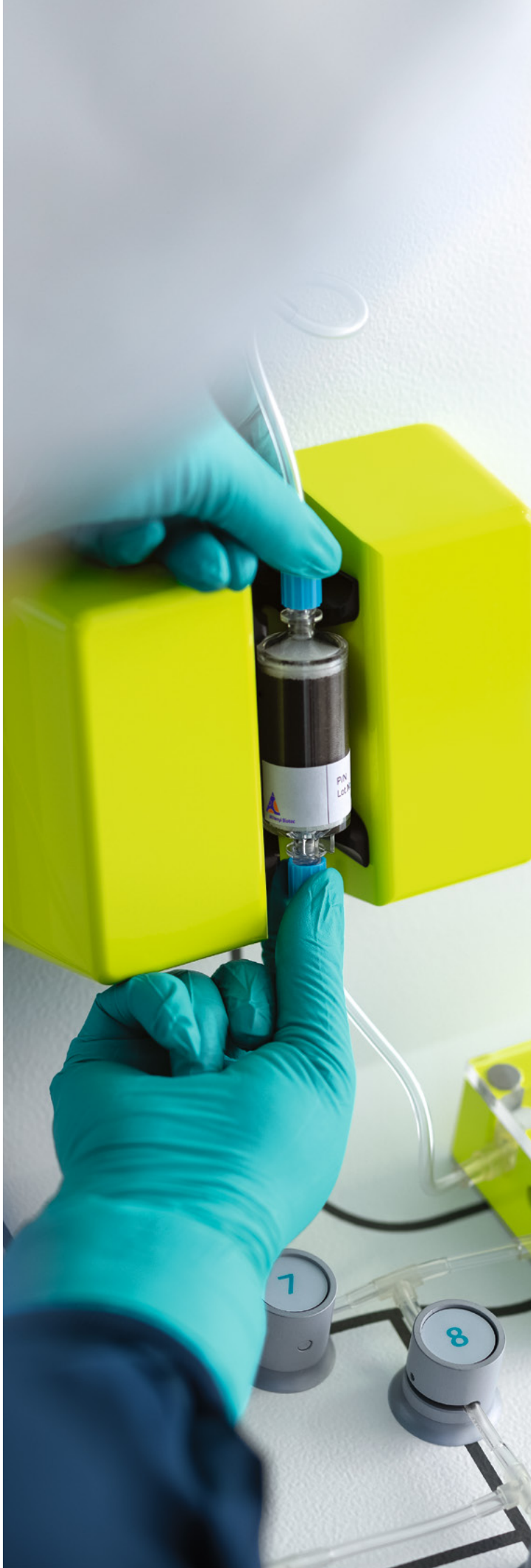
DLIs, depleted of CD45RA$^+$ cells, have been infused posttransplant as infection prophylaxis. This approach might be applied in combination with any kind of GVHD prophylaxis, such as *ex vivo* or *in vivo* T cell depletion strategies.

- 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.[44]
- 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.[55]
- 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.[56]

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.[45,57]

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.[58] The clinical use of these cells has been reported within a phase I clinical trial.[59]



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.

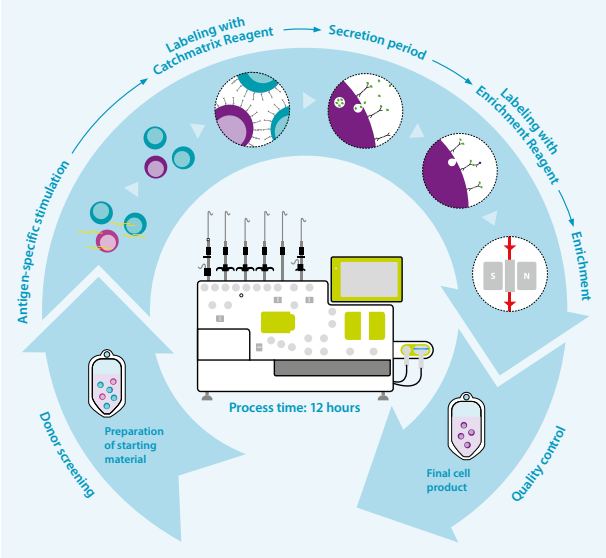


Figure 3: Overview of the automated workflow steps of the CliniMACS Prodigy CCS (IFN-gamma) System. Virus-specific T cells can be enriched based on their secretion of IFN-γ after restimulation with appropriate antigens.

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.[60–63] Today, an increasing number of reports show the possibility of using third-party donor-derived cell products that can provided different types of biobanks.[64,65] Currently a multi-national clinical phase III trial is investigating efficacy and safety of adoptive T cell transfer in immunocompromised individuals.[66]

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.[67,68]

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.[69] 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.[70] First phase I clinical trials have been started.[71,72]

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, using CE-marked medical devices
- 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

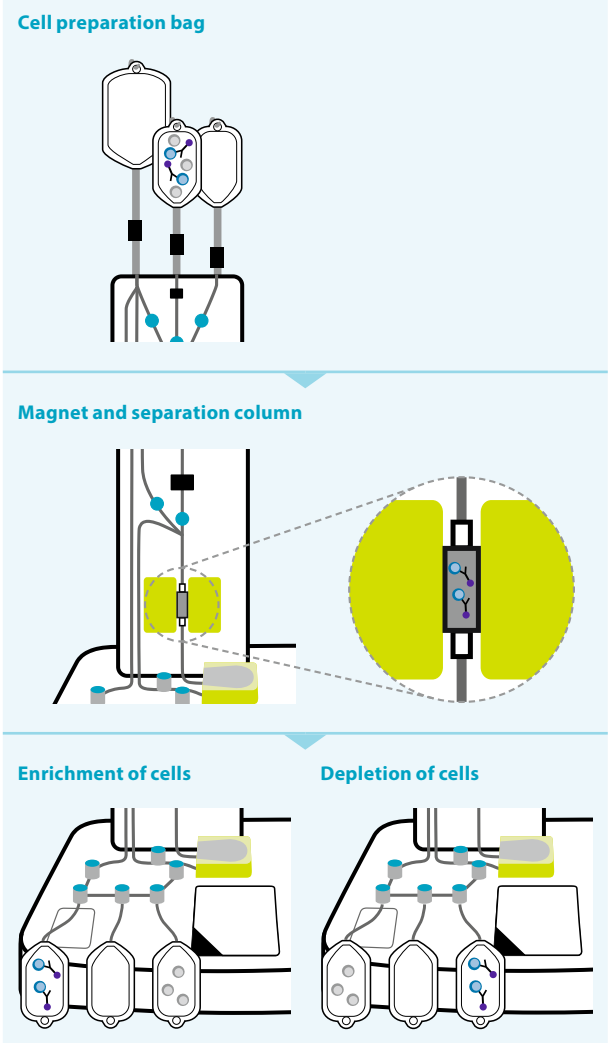


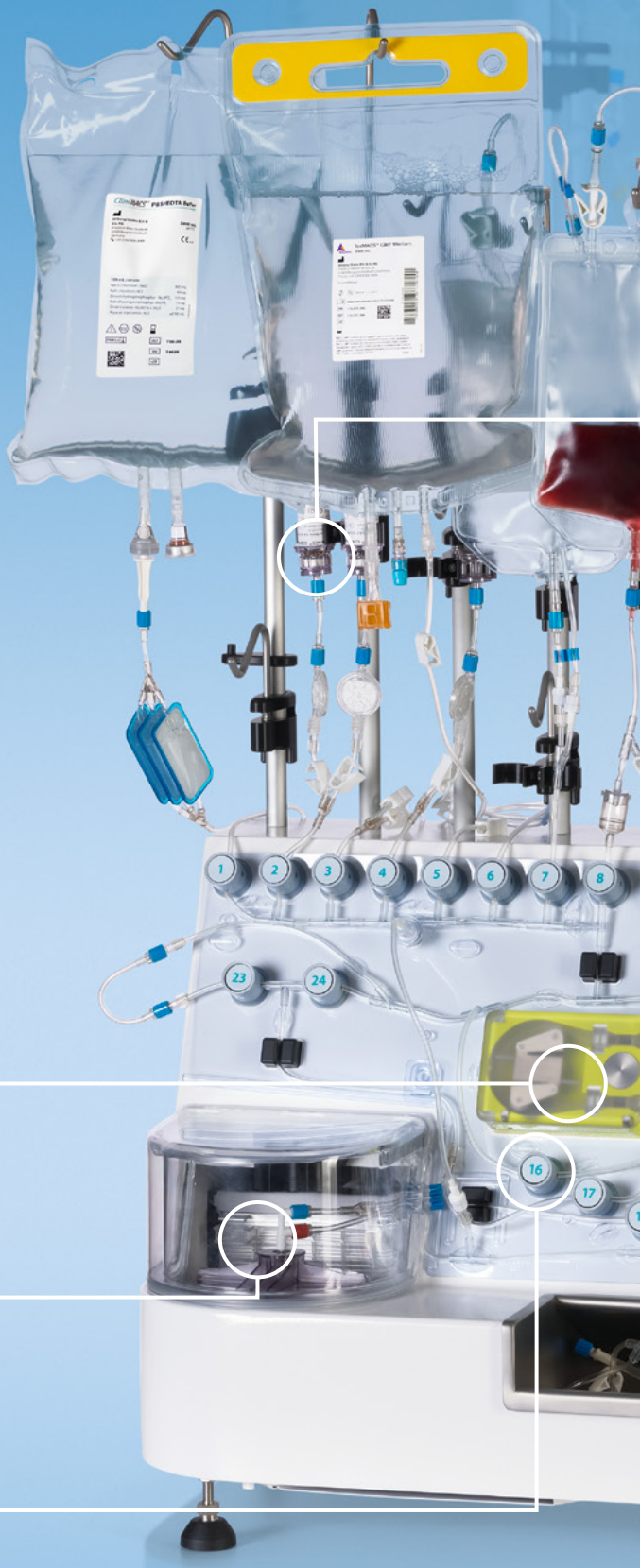
Figure 5: Workflow on the CliniMACS Plus System. Target cells are always collected in the left-most bag.

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 CE-certified medical devices and 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.

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