Miltenyi Biotec

CliniMACS® CD34 Reagent System

Ex vivo T cell depletion for GVHD prophylaxis

For US healthcare professionals only

Allogeneic stem cell transplantation in AML

Nearly 13,000 people in the United States are diagnosed annually with acute myeloid leukemia (AML). Regrettably, survival rates for patients following an AML diagnosis are strikingly low. Two-thirds of young adults and 90% of older adults will die from their disease and its sequelae.[1]

Allogeneic hematopoietic stem cell transplantation (SCT) is regarded as the single most effective treatment for preventing relapse in AML patients in complete remission (CR) after induction therapy. Moreover, allogeneic SCT has a significant survival benefit for intermediate as well as poor-risk AML patients in CR1.[2]

Complications of allogeneic SCT
Graft-versus-host disease (GVHD) is a major complication associated with allogeneic transplants in which donor T cells in the graft identify the patient's cells as "non-self" and attack tissues and organs. The course of the disease can range from mild to severe and can lead to increased morbidity and mortality following transplantation.

Acute GVHD
The risk of acquiring acute GVHD following allogeneic transplantation from HLA-matched sibling donors is 20–60%, despite the use of immunosuppressive agents such as cyclosporin A, tacrolimus, methotrexate, antithymocyte globulin, and corticosteroids alone or in combination.[3,4] Moderate to severe (Grade II–IV) acute GVHD is associated with an increased risk of transplant-related mortality. Mortality rates among patients who develop severe GVHD can be as high as 75% if there is no response to immunosuppressive therapy.[5]

The CliniMACS CD34 Reagent System is available as prescription and *in vitro* use only. For complete prescribing information consult Instructions for Use. **Humanitarian Device:** Authorized by U.S. Federal law for use in the treatment of patients with acute myeloid leukemia (AML) in first complete remission. The effectiveness of the device for this use has not been demonstrated. **Indications for use:** The CliniMACS CD34 Reagent System is indicated for processing hematopoietic progenitor cells collected by apheresis (HPC, Apheresis) from an allogeneic, HLA-identical, sibling donor to obtain a $CD34^+$ cell-enriched population for hematopoietic reconstitution following a myeloablative preparative regimen without the need for additional graft versus host disease (GVHD) prophylaxis in patients with acute myeloid leukemia (AML) in first morphologic complete remission. **Contraindications:** Do not use $CD34^+$ cells prepared with CliniMACS CD34 Reagent System in patients with known hypersensitivity to murine (mouse) proteins or iron-dextran. **Warnings:** Do not infuse the CliniMACS CD34 Reagent or the CliniMACS PBS/EDTA Buffer into patients directly. Hypersensitivity reactions, including anaphylaxis, have been observed during infusion of $CD34^+$ cells from the CliniMACS CD34 Reagent System. Monitor the patient for hypersensitivity reactions, including anaphylaxis, during infusion of $CD34^+$ cells from the CliniMACS CD34 Reagent System. Failure to infuse an adequate number of functioning $CD34^+$ cells can result in engraftment failure. Collect sufficient HPC, Apheresis to yield at least 2.4×10^6 $CD34^+$ cells per kg of patient body weight after system processing. The clinical trial using the CliniMACS CD34 Reagent System to process HPC, Apheresis did not test allografts with less than 2.4×10^6 $CD34^+$ cells per kg of recipient body weight. Monitor patients for laboratory evidence of hematopoietic recovery after transplantation. Acute and chronic graft versus host disease (GVHD) can occur in patients who receive HPC, Apheresis processed using the CliniMACS CD34 Reagent System. Use pharmacologic prophylaxis if more than 1×10^5 $CD3^+$ cells per kilogram of recipient body weight are infused. Removal of T cells from the HPC, Apheresis can delay immune reconstitution after transplantation. Patients who receive the $CD34^+$ cell-enriched population prepared using the CliniMACS CD34 Reagent System are at risk for serious opportunistic viral infections, including post-transplant lymphoproliferative disorder caused by Epstein-Barr virus (EBV) and cytomegalovirus (CMV). Monitor for EBV and CMV in the peripheral blood of patients after transplantation and initiate appropriate treatment promptly. **Precautions:** Safety and probable benefit in children under the age of 17 years have not been established. Drugs may be incompatible with the CliniMACS PBS/EDTA Buffer. Do not add drugs to the buffer other than Human Serum Albumin as specified in the CliniMACS User Manual for the CD34 Reagent System. Do not use cryopreserved and thawed HPC, Apheresis because cryopreservation promotes cell clumping, which may lead to device performance issues. Process HPC, Apheresis as soon as available, but not longer than 24 hours after collection. Use only HPC, Apheresis from an allogeneic, HLA-identical sibling donor with the CliniMACS CD34 Reagent System. Collect HPC, Apheresis according to standard hospital or institutional leukapheresis procedures in standard leukapheresis collection bags. Do not include additional anticoagulants or blood additives, such as heparin, other than those normally used during leukapheresis. Keep the HPC, Apheresis at controlled room temperature (+19 °C to +25 °C (67° to 77° F)) if it has to be stored, e.g., overnight, before processing. Do not allow the concentration of leukocytes to exceed 0.2×10^9 cells per mL. Only trained operators should use the CliniMACS CD34 Reagent System to prepare $CD34^+$ cells for infusion. Operator training is provided by Miltenyi Biotec authorized personnel.

Chronic GVHD
Chronic GVHD is a serious long-term complication of allogeneic SCT which occurs in 30–70% of patients who survive 100 or more days post transplantation.[6,7] Nearly half of those affected have three or more organs impacted. Treatment typically requires immunosuppressive medications for a median of two to three years. In a subset of patients the treatment is prolonged, with 15% still receiving immunosuppressive therapies for seven or more years after the initial diagnosis of chronic GVHD.

Despite numerous pharmacologic approaches, treatment outcomes of chronic GVHD remain unsatisfactory. Corticosteroids are a therapy mainstay, but their effectiveness can vary and long-term use can lead to complications.[8] Moreover, chronic GVHD following peripheral blood stem cell transplantation may be more difficult to treat and requires a greater duration of corticosteroid use in comparison to bone marrow transplantation. This results in a higher risk of late complications.[9,10]

Use of T cell-depleted grafts to reduce GVHD
The goal of allogeneic stem cell transplantation in AML is to achieve disease-free survival in the absence of acute and chronic GVHD. Removal of T cells from the donor graft before transplantation has shown great promise in preventing the onset of GVHD.[11–13] Recent publications have suggested this approach results in a low risk of GVHD and relapse in patients with AML, particularly in those transplanted in remission.[11,14,15,16]

The CliniMACS® CD34 Reagent System is an FDA-approved medical device indicated for the enrichment of $CD34^+$ hematopoietic stem cells from hematopoietic progenitor cells collected by apheresis from an HLA-identical sibling donor. As the system enriches the $CD34^+$ stem cells, it passively depletes other cells such as T cells that can cause GVHD.

Clinical study using T cell-depleted grafts

Study details
The CliniMACS® CD34 Reagent System was evaluated as sole GVHD prophylaxis in a phase II clinical trial (BMT CTN 0303) of adult AML patients in first complete remission receiving a transplant from an HLA-identical sibling donor.

The multicenter clinical study was conducted in collaboration with the Blood and Marrow Transplant Clinical Trials Network (BMT CTN). The trial was designed to provide extensive T cell depletion using the CliniMACS CD34 Reagent System, to eliminate the requirement for post-transplantation pharmacological GVHD prophylaxis. The goal was to demonstrate that the side effects of T cell–depleted SCT would be reduced if combined with a conditioning regimen that was highly immunosuppressive and anti-leukemic.

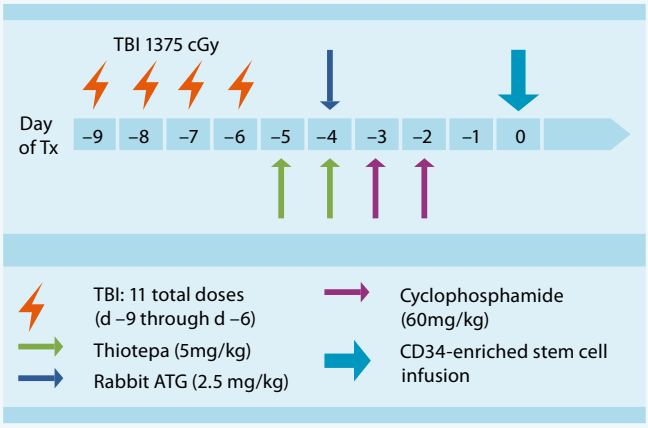


Figure 1: BMT CTN 0303 Conditioning Regimen

No pharmacological GVHD prophylaxis was given post transplantation.

The clinical trial included 37 subjects in first complete remission (CR) undergoing transplantation at eight different centers in the BMT CTN.

Patient characteristics		
Patient age	Median (range)	48 (21–60)
Gender	Male	14 (37.8%)
	Female	23 (62.2%)
Cytogenetic risk	Intermediate	68%
	Unfavorable	27%
	Unknown	5%

Table 1: Patient characteristics

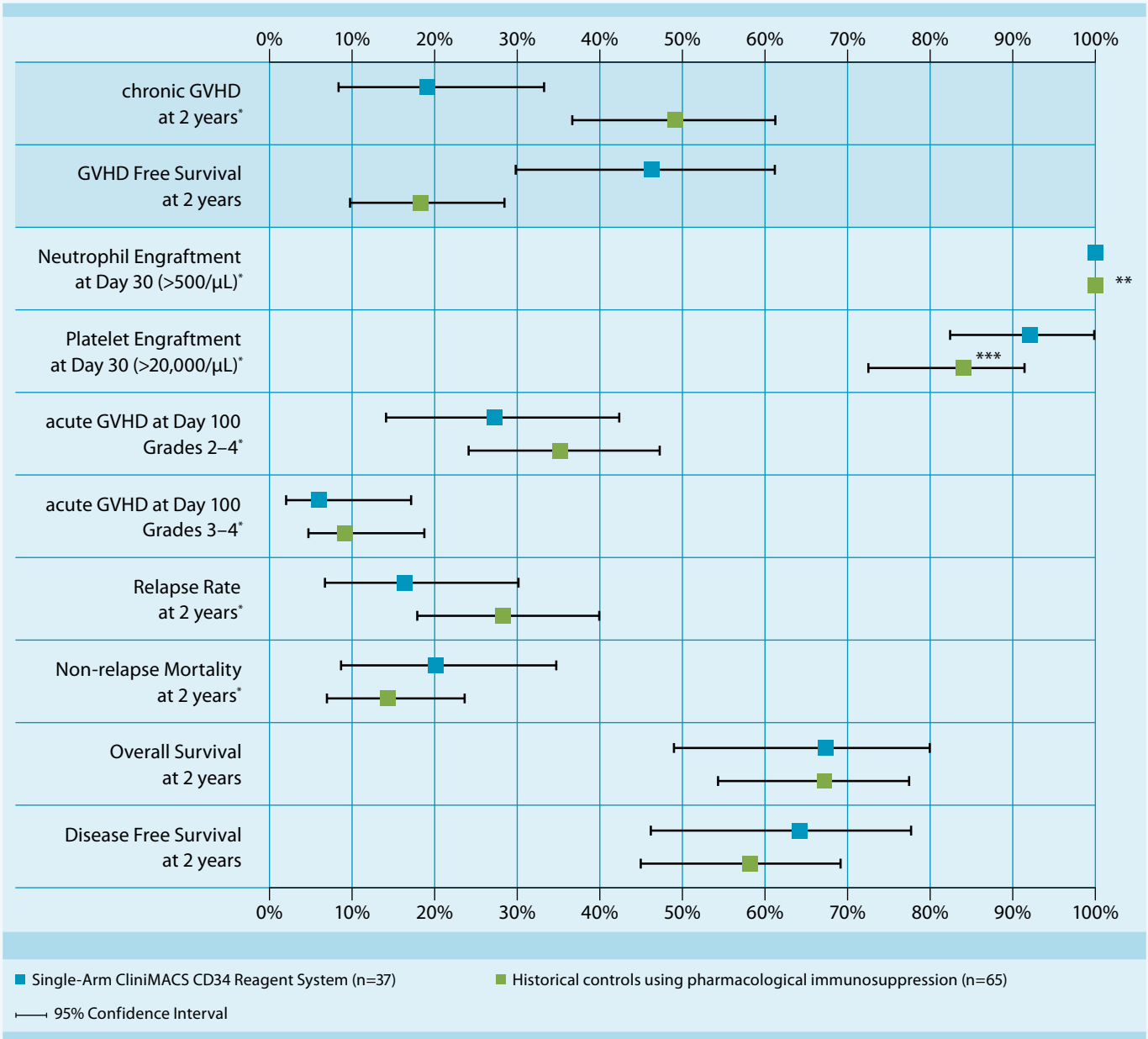
Summary of results
- The protocol was well tolerated.
- All patients engrafted neutrophils (>0.5×10^9 per liter) by day 21 after transplantation. The platelet count recovered to >20.000 per µl by day 30 post-transplantation for 92% of the patients.
- The incidence of both acute and chronic GVHD was low. The cumulative incidence of acute GVHD grades II–IV was 27% at 100 days follow-up, while grades III–IV were only 5%.
- Chronic GVHD was 19% at 2 years follow-up.
- The cumulative incidence of relapse at 2 years was low with 16%.
- Disease-free survival (DFS) was 82% at 6 months and 64% at 2 years.
- GVHD-free survival at 2 years was with 46% higher than historical controls (Figure 2).

Comparative analysis
A two-year comparative analysis was carried out by comparing the 37 patients from the BMT CTN 0303 study to a similar cohort of 65 patients receiving unselected grafts and enrolled in the BMT CTN 0101 study.[19] The inclusion criteria for these AML patients were matched based on age, disease status, and allograft characteristics. This patient cohort had been given standard pharmacologic immune suppression post transplantation, whereas the T cell-depleted cohort did not receive pharmacologic immune suppression.

The CliniMACS® CD34 Reagent System reduces the risk of chronic GVHD in patients with AML without negatively impacting post-transplantation outcome.

Major findings were:
- Substantially lower rate of chronic GVHD in the T cell depletion study
- Substantially higher rate in GVHD-free survival in the T cell depletion study
- No difference in relapse, treatment-related mortality, overall survival, and disease-free survival between T cell depletion and the comparison group

Single-Arm CliniMACS® CD34 Reagent System Study versus historical controls



* Cumulative Incidence
** Neutrophil data missing for two patients
*** Platelet data missing for one patient

Figure 2: Comparison of the Single-Arm CliniMACS® CD34 Reagent System Study to historical controls using pharmacological immunosuppression

Conclusion and perspectives

T cell depletion as a therapeutic approach is shown to result in a low risk of both acute and chronic GVHD and relapse for patients with AML in first complete remission. The BMT CTN 0303 study results demonstrate that T cell-depleted SCT following intensive myeloablative chemo-radiotherapy can be safely performed in a multicenter setting using the CliniMACS® CD34 Reagent System to deplete T cells as the sole means of GVHD prophylaxis. Particularly striking is the very low incidence of chronic GVHD at 2 years (19%), and the high rate of GVHD free survival at 2 years (46%). These data suggest the benefit of the therapy is achieved, without negatively affecting engraftment, relapse, overall survival, or disease-free survival.

Allogeneic SCT is a very complex therapy that is regarded as a costly procedure. An analysis of costs associated with SCT involving high dose chemotherapeutic regimens was recently published.[20] This single center analysis identified pre- and/or post-transplantation factors that led to higher costs within the first year of treatment. Costs incurred within the first 100 days comprised a substantial portion of the total amount. Of the major factors observed, post transplantation complications such as acute GVHD grade II to IV resulted in a cost increase of 30%. Likewise, costs associated later in therapy were often attributed to the incidence and severity of extensive chronic GVHD. Thus, an efficient GVHD prophylaxis that significantly reduces the incidence of GVHD without increasing other post-transplant complications may also reduce the costs of allogeneic transplantation.

T cell depletion without the need for immunosuppressive post-transplantation prophylaxis could contribute to better patient outcome. Furthermore, by avoiding the use of immunosuppressive drugs, the affiliated drug side effects are prevented.

"Reduction of GvHD rates without an increase in relapse rates and no requirement for post-transplant immunosuppression are distinct advantages of this method of TCD." [18]

"The low incidences of relapse and of acute and chronic GvHD in the absence of post- transplant prophylaxis are especially encouraging." [17]

References

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How does the CliniMACS® CD34 Reagent System work?

The CliniMACS® CD34 Reagent System employs a reagent consisting of an antibody that specifically binds to blood cells that express the CD34 surface marker (hematopoietic stem cells or blood stem cells). The CD34 antibody is conjugated to an iron-containing particle that is only nanometers in size and safe for infusion.

The enrichment of CD34⁺ cells is accomplished by passing the antibody/magnetically-labeled cell suspension through a magnetic separation column, which is provided as part of a single-use disposable tubing set. Magnetically labeled CD34⁺ target cells are retained within the separation column, while the unlabeled cells flow through. Recovery of CD34⁺ cells is achieved by removing the magnetic field and eluting the targeted CD34⁺ stem cells into a collection bag.

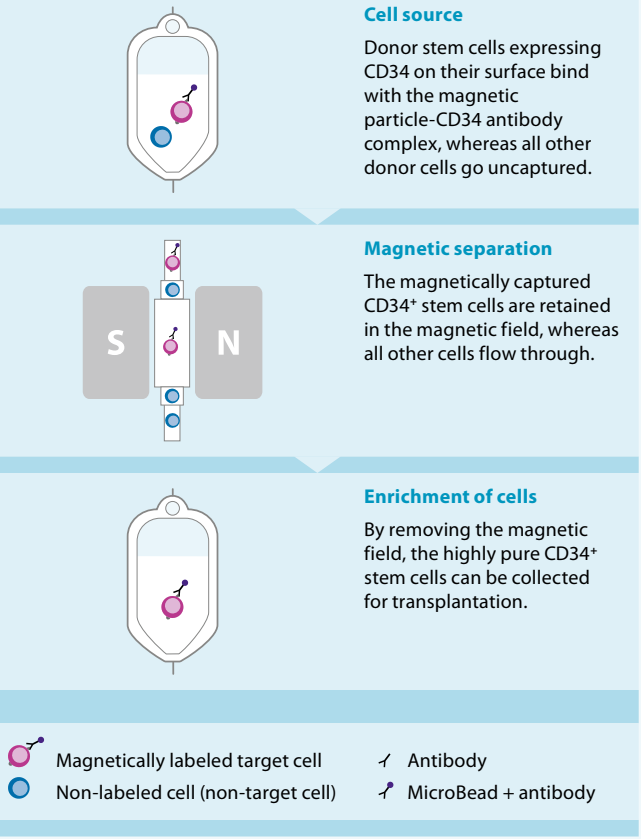

Figure 3: The principle of magnetic cell separation

Figure 4: The CliniMACS CD34 Reagent System is a medical device used for *in vitro* enrichment of CD34⁺ target cells from heterogeneous hematologic cell populations as clinically indicated.

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