NCI Protocol #: *N/A*

DF/HCC Protocol #: 18-270

TITLE: A Phase 2 Study of Ex Vivo TCR αβ T Cell Depletion for Graft-Versus-Host Disease (GVHD) Prophylaxis in Mismatched Donor Peripheral Blood Stem Cell Transplantation for Hematologic Malignancies

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Responsible Data Manager:

Agent(s): Mobilized peripheral blood stem cells from 8/10 or 9/10 matched related or unrelated donors depleted of TCRαβ T cells using the Miltenyi CliniMACS system

IDE #: 18107
IDE Sponsor: Vincent T. Ho, MD
Device Name: CliniMACS® TCRα/β Reagent Kit

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SCHEMA

This is a single-arm, two-stage phase II study of ex vivo αβ+ TCR T cell depleted (TCD) peripheral blood stem cell transplantation (PBSCT) as a GVHD prophylaxis for patients with hematologic malignancies undergoing allogeneic PBSCT. The primary objective is to evaluate the safety and efficacy of ex vivo αβ+ TCR TCD for patients receiving myeloablative PBSCT from related or unrelated mismatched (8/10 or 9/10) donor. The premise is that ex vivo αβ+ TCR TCD will reduce the incidence of grade 3-4 acute GVHD and/or death. The target accrual goal is 25 evaluable patients over 3.5-4 year period. All surviving patients will be followed for at least 100 days. Patients who meet the eligibility criteria but deemed unevaluable per Section 3.2 will be excluded from the study.

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1. OBJECTIVES

1.1 Study Design

This is a single-arm, two-stage phase II study of ex vivo αβ+ TCR T cell depleted (TCD) peripheral blood stem cell transplantation (PBSCT) as a GVHD prophylaxis for patients with hematologic malignancies undergoing allogeneic PBSCT. The primary objective is to evaluate the safety and efficacy of ex vivo αβ+ TCR TCD for patients receiving myeloablative PBSCT from related or unrelated mismatched (8/10 or 9/10) donor. The premise is that ex vivo αβ+ TCR TCD will reduce the incidence of grade 3-4 acute GVHD and/or death. The target accrual goal is 25 evaluable patients over 3.5-4 year period. All surviving patients will be followed for at least 100 days. Patients who meet the eligibility criteria but deemed unevaluable per Section 3.2 will be excluded from the study.

1.2 Primary Objective:

To assess the day 100 severe acute GVHD-free survival rate. Events considered for the primary endpoint are grade 3-4 acute GVHD or death from any cause.

1.3 Secondary Objectives:

- Grades II-IV and III-IV acute GVHD
- Chronic GVHD and moderate/severe chronic GVHD
- Chronic GVHD-free survival
- Immune suppression-free survival
- Hematologic recovery (neutrophil and platelet engraftment)
- Immune reconstitution
- Disease relapse
- Transplant-related mortality
- Organ Toxicity (e.g. SOS/VOD and IPS)
- Rates of infections (CMV and EBV reactivation)
- Disease-free and overall survival
- Graft-versus-host disease and relapse free survival (GRFS). GRFS will be defined as alive without having experienced grade 3-4 acute GVHD, moderate/severe chronic GvHD, or relapse of underlying malignancy.

2. BACKGROUND

Unrelated donor hematopoietic stem cell transplantation (HSCT), especially in the HLA mismatched setting, is a risky procedure with high mortality compared to transplantation from HLA identical sibling donors. The excess morbidity and mortality after URD HSCT are mainly attributable to increased incidence of infections and severe GVHD. Unfortunately, only about 30% of patients in North America will have an HLA-identical sibling, and most patients in need of an allogeneic HSCT must rely upon unrelated donors. With the development of an international network of registries worldwide, increasing numbers of unrelated HSCT are performed each year. For patients who do

not have a fully matched unrelated donor, transplantation options are restricted to mismatched unrelated donor (8-9/10 or 7/8 match), haplo-identical related donor, or mismatched umbilical cord blood transplantation. As of today, there have not been any published randomized trials to demonstrate superiority of any of these 3 options over each other, especially after myeloablative conditioning transplantation.

At the DFCI, we have continued to use 7/8 or 9/10 mismatched unrelated donor (or in some cases 1-2 antigen mismatched related donors) for transplantation in cases where a 10/10 match donor is not available. However, this approach is associated with a higher risk of acute and chronic GVHD, higher transplant related mortality and lower overall survival compared to full matched URD HSCT. [1] To overcome these hurdles, better techniques for GVHD prevention without exacerbating the risk of graft rejection and infections are needed.

2.1 Study Disease: GVHD Prophylaxis

Since T lymphocytes are crucial mediators of the graft-versus-host reaction, they are the primary targets of most strategies aimed at reducing GVHD. The current accepted standard regimen for GVHD prophylaxis involves the administration of methotrexate (MTX) and cyclosporine (CyA) or tacrolimus after transplantation. These pharmacologic agents prevent GVHD by suppressing donor T cell function, but are in themselves associated with infectious risks and other undesirable side effects. Over the past two decades, investigators have striven to alleviate our dependence from these pharmacologic agents by depleting donor T cells from the graft as GVHD prophylaxis. Although many types of T cell depletion have been shown to reduce GVHD, none have been established to date that improve overall survival relative to conventional MTX/CyA or MTX/tacrolimus. The few randomized trials comparing ex vivo TCD against standard pharmacologic prophylaxis have used broad specificity T cell depletion methods (counter flow centrifugal elutriation, T10B9 antibody) and have not demonstrated any improvement in long term overall survival after matched unrelated donor marrow transplantation, despite lower GVHD, primarily due to higher rates of infection and reciprocal increases in disease relapse, especially in patients with chronic phase CML.

2.1.1 Pharmacologic agents as GVHD prophylaxis

Methotrexate (MTX) was the first efficacious drug used for prevention of GVHD. However, MTX at higher doses delayed hematopoietic engraftment and worsen mucositis. With the advent of the calcineurin inhibitors, investigators subsequently found that in randomized trials, the combination of a short course of MTX (15 mg/m2 IV day +1, 10 mg/m2 IV days +3,6,11) with cyclosporine A (CyA) to be better tolerated and superior to using either agent alone.[2, 3] In the 1990s, tacrolimus (FK506) plus MTX also emerged as an effective regimen for GVHD prophylaxis. In a randomized trial involving unrelated donor BMT, patients receiving Tac/MTX had a significantly lower incidence of acute GVHD than those receiving CyA/MTX. [4] Based on these randomized studies, CyA/MTX or Tac/MTX have become the standard regimens for GVHD prophylaxis after allogeneic marrow or stem cell transplantation. However, acute

and chronic GVHD rates are still substantial and remained significant contributors to morbidity and mortality.

2.1.2 **Donor T cell depletion (TCD) as GVHD prophylaxis**

T cell depletion (TCD) of the donor graft represents an alternative, yet effective means of achieving GVHD prophylaxis. This approach relies primarily upon the ex vivo removal of putative effector cells of GVHD from the donor graft prior to transplantation. Many different methods of TCD have been used in human marrow transplantation. These include monoclonal antibodies with [5-8] or without complement,[9] immunotoxins,[10, 11] lectin agglutination, [12]and counter flow elutriation.[13] Most of these trials demonstrated a decrease in the incidence of acute and chronic GVHD. Unfortunately, the reduction in GVHD after TCD BMT has not resulted in improved overall survival due to unexpected increases in incidence of graft failure, post-transplant lymphoproliferative disorders (EBV-LPD) due to delayed immune reconstitution, and disease relapse from loss of GVL activity.

Because of the increased genetic disparity and increased risk for GVHD, unrelated donor (URD) or mismatched BMT has been grounds for many studies of TCD over past two decades. Cumulative data from the NMDP and IBMTR have indeed shown that TCD is associated with a lower incidence of GVHD after unrelated/mismatched donor BMT. [14, 15] However, it should be noted that many types of T cell depletion were included in these registry analyses, and that not all TCD methods are equivalent in terms of GVHD control and disease relapse. For example, Champlin et al. have reported on the IBMTR experience from 870 patients who underwent T-depleted unrelated or mismatched donor BMT for leukemia. They discovered that the leukemia free survival for patients whose grafts were T-depleted with "narrow specificity" antibodies (e.g. anti-CD5, CD6, CD8 etc.) were significantly higher than those whose grafts had been T depleted with "broad specificity" antibodies (e.g. anti-CD2, ATG, CAMPATH-1, elutriation, or lectin/SRBC agglutination). [14] These results suggest that in unrelated or mismatched BMT, TCD using "narrow specificity" antibodies is not associated with loss of GVL effect, but retains the advantage of decreased GVHD compared to conventional BMT.

2.2 IDE Agent

2.2.1 **T-Cell Receptor (TCR) αβ T-Cell Depletion**

Among the narrow specificity TCD methods, selective depletion of alpha/beta T cells has gained recent interest compared to CD34 selection methods since the selective depletion of alpha/beta T cells preserves NK cells and gamma/delta T cells, which may be important for preserving innate immunity and the GVL effect. The majority of T cells express alpha/beta T cell receptors (TCR), whereas the gamma/delta TCR is expressed by only 2-10% of all T cells in human peripheral blood. However, the gamma/delta T cells display a range of innate effector functions, including rapid secretion of chemokines and

cytokines, as well as target cell lysis. Recent studies suggest that gamma/delta T lymphocytes are potential beneficial effector cells after allogeneic hematopoietic stem cell transplantation.[16] These findings have spawned interest in the selective depletion of alpha/beta T lymphocytes in the graft for allogeneic transplantation.

Locatelli and colleagues have performed a clinical trial of T-cell depleted haplo-HSCT using the negative selection of TCRαβ T cells (NCT01810120).[17] They have transplanted more than 80 children with malignant and non-malignant disorders with negative depletion of TCRαβ T cells and CD19 B cell using the Miltenyi CliniMACS device. The data collected to date clearly demonstrates that this method of graft manipulation is reliable and reproducible, leading to around a 4.1-log and 3.1-log depletion of αβ TCR T cells and B cells, respectively. The residual T-cell content was below the safe threshold (i.e. $1x10^5$/Kg recipient body weight) for performing HLA-haploidentical HSCT without any post-transplant immune suppression in all donor depletions performed. The outcome of children with acute leukemia given TCRαβ T-cell and B-cell depleted haplo-HSCT demonstrated a low transplant-related mortality (less than 10%) and an acceptable risk of disease recurrence (on the order of 20%). No patient has developed acute GVHD with either gastrointestinal or liver involvement, with skin-only Grade I-II GVHD being recorded in around 30% of patients. No patient developed chronic GVHD.[17]

2.3 Rationale

Unrelated donor hematopoietic stem cell transplantation (HSCT), especially in the HLA mismatched setting, is a risky procedure with high mortality compared to transplantation from HLA identical sibling donors. The excess morbidity and mortality after URD HSCT are mainly attributable to increased incidence of infections and severe GVHD. Unfortunately, only about 30% of patients in North America will have an HLA-identical sibling, and most patients in need of an allogeneic HSCT must rely upon unrelated donors. With the development of an international network of registries worldwide, increasing numbers of unrelated HSCT are performed each year. For patients who do not have a fully matched unrelated donor, transplantation options are restricted to mismatched unrelated donor (8-9/10 or 7/8 match), haplo-identical related donor, or mismatched umbilical cord blood transplantation. As of today, there have not been any published randomized trials to demonstrate superiority of any of these 3 options over each other, especially after myeloablative conditioning transplantation.

At the DFCI, we have continued to use 7/8 or 9/10 mismatched unrelated donor (or in some cases 1-2 antigen mismatched related donors) for transplantation in cases where a 10/10 match donor is not available. However, this approach is associated with a higher risk of acute and chronic GVHD, higher transplant related mortality and lower overall survival compared to full matched URD HSCT. [1] To overcome these hurdles, better techniques for GVHD prevention without exacerbating the risk of graft rejection and infections are needed.

3. PARTICIPANT SELECTION

This study will enroll adults (age 18 or higher) with malignant hematological disorders who are deemed to be clinically eligible for a myeloablative allogeneic transplantation and who do NOT have 10/10 matched donor (related or unrelated) or whose disease status does not allow the extensive wait for a 10/10 matched unrelated donor to be identified.

3.1 Eligibility Criteria

Participants must meet the following criteria on screening examination to be eligible to participate in the study:

3.1.1 Diagnoses and stage at time of transplant admission:

- Acute leukemia (AML or ALL or MPAL) in first or subsequent remission
- Myelodysplastic syndromes (MDS) with <10% marrow blasts
- Myeloproliferative neoplasm (MPN) with <10% marrow blasts
- CMML with less than 10% marrow blast
- CML accelerated phase or second or subsequent chronic phase
- Non-Hodgkin's lymphoma in PR or CR2 or beyond
- Hodgkin lymphoma in PR or CR2 or beyond

3.1.2 Age 18-65 years

3.1.3 Patient has a related or unrelated donor who is 8 or 9 out of 10 match at HLA A, B, C, DRB1 and DQB1, based on allele level typing.

3.1.4 Patient ECOG performance status 0-2 (Karnofsky ≥60%, see Appendix A)

3.1.5 Patient deemed to be appropriate candidate for myeloablative conditioning transplantation.

3.1.6 Ability to understand and the willingness to sign a written informed consent document.

3.2 Exclusion Criteria

Participants who exhibit any of the following conditions at screening will not be eligible for admission into the study.

3.2.1 Patient with active HIV infection

3.2.2 Chronic active hepatitis B infection (HepB surface Ag+ or detectable Hep B viral load)

3.2.3 Prior allogeneic hematopoietic stem cell transplantation

3.2.4 Impaired cardiac function- ejection fraction < 40%

3.2.5 Impaired pulmonary function- pretransplant FEV1, DLCO < 50%

3.2.6 Impaired renal function, based on
Serum creatinine > 2.0 mg/dl

3.2.7 Impaired liver function unrelated to primary disease, based on
ALT or AST > 3x ULN, or
Total Bilirubin > 2.0mg/dl (with exception for known or suspected Gilbert's disease)

3.2.8 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

3.2.9 Women who are pregnant or breast feeding. Women of child bearing potential must have a negative serum pregnancy test at study entry.

3.2.10 Participants who are receiving any other investigational agents are eligible but such agent must be discontinued before admission for HSCT, and if resumption of investigation agent is planned after HSCT, this must be approved by the study PI.

3.2.11 Participants with known active CNS disease. CNS disease that has been treated is eligible.

3.3 Inclusion of Women and Minorities

Both men and women of all races and ethnic groups are eligible for this trial.

4. REGISTRATION PROCEDURES

4.1 General Guidelines for DF/HCC Institutions

Institutions will register eligible participants in the Clinical Trials Management System (CTMS) OnCore. Registrations must occur prior to the initiation of protocol therapy. Any participant not registered to the protocol before protocol therapy begins will be considered ineligible and registration will be denied.

An investigator will confirm eligibility criteria and a member of the study team will complete the protocol-specific eligibility checklist.

Following registration, participants may begin protocol therapy. Issues that would cause treatment delays should be discussed with the Overall Principal Investigator (PI). If a participant does not receive protocol therapy following registration, the participant's registration on the study must be canceled. Registration cancellations must be made in OnCore as soon as possible.

4.2 Registration Process for DF/HCC Institutions

DF/HCC Standard Operating Procedure for Human Subject Research Titled *Subject Protocol Registration* (SOP #: REGIST-101) must be followed.

4.3 General Guidelines for Other Investigative Sites

N/A

4.4 Registration Process for Other Investigative Sites

N/A

5. TREATMENT PLAN

5.1 Preparative Regimens- Myeloablative conditioning regimen will consist of one of 2 regimens, at the choice of the treating physician: **Bu/Flu/Mel or TBI/Flu/Thiotepa.**

- It is recommended that patients with myeloid malignancies receive Flu/Bu/Mel, and those with lymphoid malignancies receive Flu/TBI/Thiotepa.

- For patients who weigh less than 125% of their ideal body weight (IBW), dosing should be based on actual body weight. These dosing rules apply to all chemotherapy drugs used in this trial.

- For Patients ≥ 18.0 Years of Age
  Males IBW = 50 kg + 2.3 kg/inch over 5 feet (60 in)
  Females IBW = 45.5 kg + 2.3 kg/inch over 5 feet (60 in)
  For patients less than 5 feet, subtract 2.3 kg/inch

- For patients who weigh greater than or equal to 125% of their IBW, dosing should be based on the adjusted ideal body weight (AIBW).

- Adjusted Ideal Body Weight (AIBW) Formula:
  AIBW = IBW + [(0.25) x (actual body weight – IBW)]

5.1.1 Busulfan, Melphalan, Fludarabine Regimen:

- Days -9 to -7: Busulfan 0.8 mg/kg/dose Q6h IV x 12 doses (total 9.6mg/kg)
- Days -6 to -5: Melphalan 70 mg/m2/day x 2 (total 140mg/m2)
- Days -6 to -2: Fludarabine 25mg/m2/day x 5 (total 125mg/m2)

Busulfan will be infused over 2 hours. Phamacokinetics are not required.
Melphalan will be infused intravenously over approximately 30 minutes.
Fludarabine will be infused intravenously over approximately 30 minutes

5.1.2 TBI, Fludarabine, Thiotepa (Flu/TBI/Thio) regimen:

- Days -10 to -7: fractionated TBI total 1375 cGy
- Days -6 to -5: Thiotepa 5mg/kg/day x 2 days
- Days -6 to -2: Fludarabine 25mg/m2/day x 5 days (total 125mg/m2)

Fractionated TBI will be administered at a dose rate of < 20 cGy/minute. Doses of 125 cGy/fraction are administered at a minimum interval of 4 hours between fractions, three times/day for a total of 11 doses (1375 cGy) over 4 days.

Thiotepa will be administered at a dose of 5mg/kg/day IV over 4 hours for two consecutive days (Day -6 and -5).

Fludarabine will be administered at a dose of 25mg/m2/day IV over 30minutes for 5 days (Day -6 to -2)

5.2 Procurement of allogeneic PBSC

PBSC will be mobilized from the donor in accordance with standard protocol at the DFCI (related donors) or at the unrelated donor's collection center. Ideally, 2.5 blood volumes will be processed and a target of 6.0 x106 CD34+ cells/kg recipient

weight will be collected in one or two sessions. The product will be delivered immediately by courier to the Dana-Farber Cancer Institute for processing. The PBSC may not be cryopreserved before processing.

5.3 T cell depletion of allogeneic PBSC using Miltenyi Clinimacs

The manufacturing process of the TCRαβ T-cell depleted cell grafts will be performed in the CMCF-GMP laboratory at the DFCI. The manufacturing process and quality control will be performed according to validated procedures and documented in accordance with full GMP requirements.

The stem cell apheresis product will be depleted of TCRαβ T cells by negative selection using the automated CliniMACS® Plus device, the current version of the CliniMACS user manual and according to institutional Standard Operating Procedures (SOPs) in place and validated at the manufacturing site. A detailed protocol for the preparation and use of this system is provided in the CliniMACS® TCRαβ Reagent Kit Investigator's Brochure (Appendix B). Samples of the donor leukocyte preparation will be removed prior to processing and after completion. These samples will be used to determine cell viability and enumerate the total number of alpha/beta T cell, gamma/delta T cells, B cells, NK cells and CD34+ cells in the product before and after processing.

5.4 CD34+ and alpha/beta T cell target doses for graft composition

CD34+ stem cell counts will be obtained before and after processing with the Miltenyi ClinicMACs device.

If the initial CD34 count on the PBSC prior to TCRαβ depletion is $< 4.0 \times 10^6$ CD34+ cells/kg, then no T cell depletion will be performed and the patient will be started on standard pharmacologic GVHD prophylaxis. These patients are not evaluable for the primary endpoint and will be replaced on the study.

After processing, the volume of processed product infused will be adjusted to target a TCRαβ T-cells dose $\leq 1.0 \times 10^5$ cells/kg, while maintaining a minimal viable CD34+ cell dose $\geq 2.0 \times 10^6$ cells/kg recipient weight
The CD34+ cell dose may exceed 5.0×10^6 CD34+ cells/kg after selection so long as the final stem cell product contains $\leq 1.0 \times 10^5$ αβ + T cells/kg. If the product after processing contains $> 1.0 \times 10^5$ TCR αβ T cells/kg, then the volume of the product released for infusion should be adjusted to the cap T cell dose, as long as the CD34 dose remains $\geq 2.0 \times 10^6$ CD34+ cells/kg.

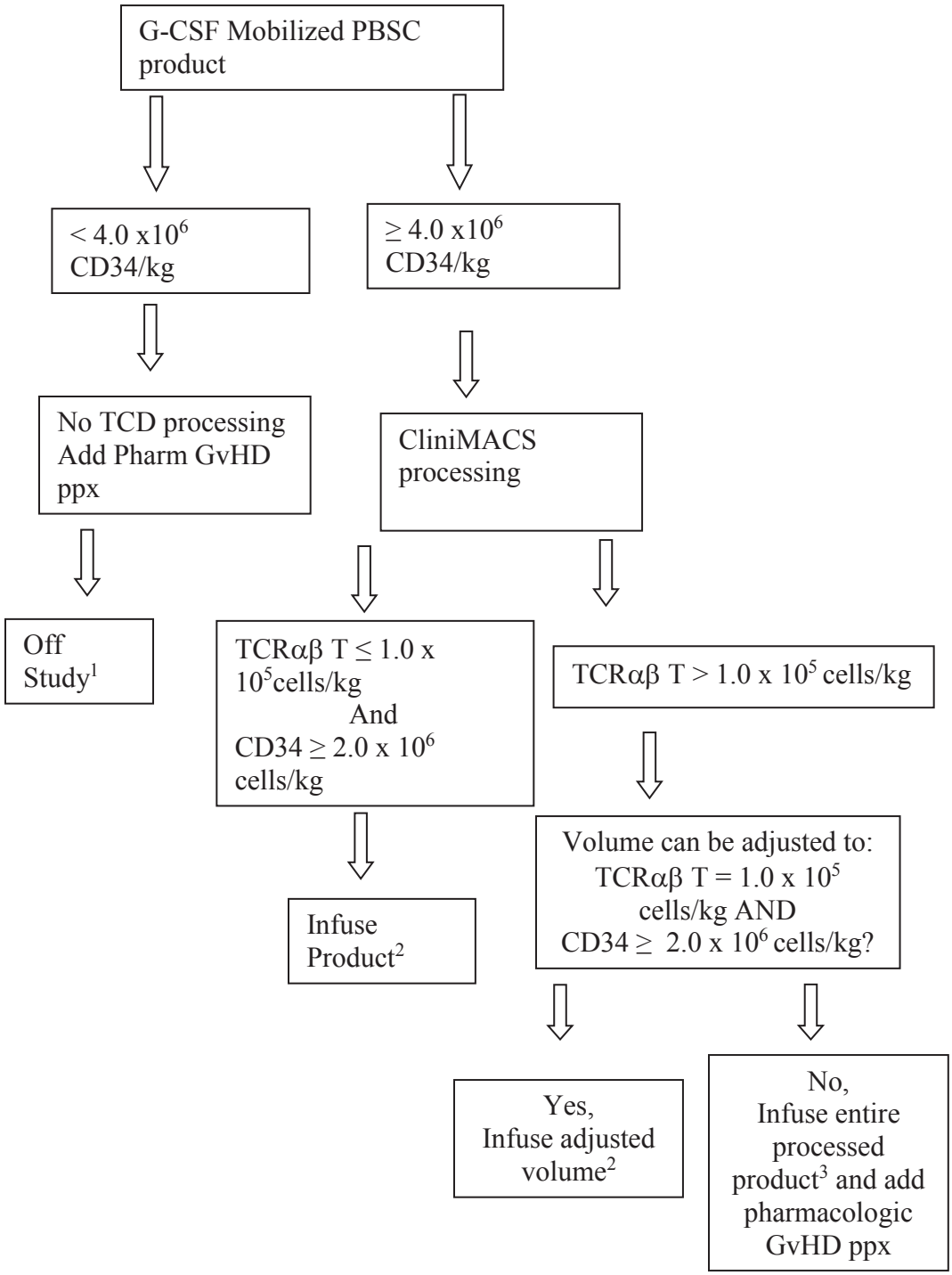
If CD34 dose of 2.0×10^6 CD34+ cells/kg cannot be attained when the T cell dose is 1.0×10^5 αβ + T cells/kg, then the patient should receive the whole processed product (containing $> 1.0 \times 10^5$ αβ + T cells/kg and $> 2.0 \times 10^6$ CD34+ cells/kg), and started on standard pharmacologic GVHD prophylaxis. These patients will not be evaluable

for the primary endpoint, and will be replaced. They will be included for the analysis of feasibility and intention to treat analysis since they received a processed product.

It is also possible that doses of CD34+ cells far exceeding 5 x 10^6/kg can be given without exceeding the maximum T-cell dose of 1.0 x 10^5 αβ + T cells/kg. High doses of CD34+ cells in extensively T-cell depleted transplants are postulated to hasten immune reconstitution, without altering the low risk of GVHD. Consequently, there is no upper limit for the dose of CD34+ cells/kg so long as the dose of αβ + T cells does not exceed 1.0 x 10^5/kg.

The mobilized T cell depleted PBSC product will be administered on Day 0, according to institutional guidelines. Stem cells are administered through an indwelling central venous catheter. If infusion occurs over two days, Day 0 is the day the last infusion is completed. For the purpose of cell infusion, the actual recipient body weight will be used to calculate cell dose.

The flow chart for the processing and infusion of the stem cell product is outlined in the flow chart below:



1. These patients will not be evaluable for the primary endpoint and will be replaced.
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3. These patients will not be evaluable for the primary endpoint, and will be replaced. They will be included for feasibility and intention to treat analysis since they received a processed product.

A target dose ≥ 5.0 x 10^6 CD34+ cells/kg after selection containing ≤ 1.0 x 10^5 alpha/beta T cells/kg is desired. The CD34+ cell dose can exceed 5.0 x 10^6 CD34+ cells/kg after selection so long as the final stem cell product contains ≤ 1.0 x 10^5 αβ + T cells/kg. If the product after processing contains > 1.0 x 10^5 TCR αβ T cells/kg, then the volume of the product released for infusion should be adjusted to the cap T cell dose, as long as the CD34 dose remains ≥ 2.0 x 10^6 CD34+ cells/kg.

If CD34 dose of 2.0 x 10^6 CD34+ cells/kg cannot be attained when the T cell dose is 1.0 x 10^5 αβ + T cells/kg, then the patient should receive the whole processed product (containing > 1.0 x 10^5 αβ + T cells/kg and > 2.0 x 10^6 CD34+ cells/kg), and started on standard pharmacologic GVHD prophylaxis. These patients will not be evaluable for the primary endpoint, and will be replaced. They will be included for the analysis of feasibility and intention to treat analysis since they received a processed product.

It is also possible that doses of CD34+ cells far exceeding 5 x 10^6/kg can be given without exceeding the maximum T-cell dose of 1.0 x 10^5 αβ + T cells/kg. High doses of CD34+ cells in extensively T-cell depleted transplants are postulated to hasten immune reconstitution, without altering the low risk of GVHD. Consequently, there is no upper limit for the dose of CD34+ cells/kg so long as the dose of αβ + T cells does not exceed 1.0 x 10^5/kg.

5.5 Pharmacologic GVHD prophylaxis

If the infused target of ≤ 1.0 x 10^5 TCR αβ T cells/kg is achieved in the infused product, then patients will not receive any additional pharmacologic GVHD prophylaxis after transplantation.

If the final infused product contains > 1.0 x 10^5 αβ +T cells/kg, or if no TCD is performed due to low starting CD34 dose in the donor product, then patients should receive tacrolimus and methotrexate as GVHD prophylaxis, in accordance with standard institutional practice. These patients will be unevaluable for the primary endpoint.

5.6 Growth Factor Support

Patients may receive growth factor support starting on day +5 using TBO-filgrastim (or filgrastim) at 5 mcg/kg/day IV/SC until ANC exceeds 1.0 x 10^6 neutrophils/ml for 2 consecutive days.

5.7 Supportive care

All patients will be maintained on HEPA-filtered units to decrease the risk of exposure to pathogenic organisms. All patients will receive prophylactic oral or IV antibiotics until they are placed on parenteral antibiotics. Total parenteral nutrition will be employed when deemed clinically appropriate. Blood product support will include red cell, platelet and granulocyte transfusions if necessary. All blood products will be irradiated.

5.8 Seizure Prophylaxis

Keppra (Levetiracetam) will be administered for the prevention of busulfan-associated seizures to all participants receiving busulfan, starting prior to starting busulfan, and stopping 1 days after the last dose of busulfan. Dosing of Levetiracetam will be administered as per the BMT guidelines. In case of allergic reactions/intolerance to levetiracetam, alternative anti-seizure medications will be used as clinically indicated.

5.9 Prophylaxis Against Infections

Patients will receive infection prophylaxis in accordance with institutional guidelines. Infection prophylaxis should include, but is not limited to, agents or strategies (e.g., PCR screening and preemptive therapy) to reduce the risk of bacterial, herpes simplex, CMV, EBV, Pneumocystis jiroveci, toxoplasmosis, and fungal infections:

5.9.1 Antifungal therapy: Prophylaxis with fluconazole or other antifungal agents can be given as per institutional guidelines, with appropriate dose adjustment in tacrolimus dosing if an azole is started on a patient who is receiving tacrolimus.

5.9.2 Cytomegalovirus (CMV): CMV viral load monitoring by PCR will be done weekly starting around day +20 to Day 90-100 and then at each clinical assessment until Day 180 post-transplant. Pre-emptive treatment is the preferred strategy for patients with CMV reactivation. Use of letemavir as CMV prophylaxis is allowed.

5.9.3 Epstein-Barr Virus (EBV): EBV monitoring through quantitative PCR should be done weekly starting around day +20 through Day 90- 100 and then at each clinical assessment until Day 180 post-transplant. EBV reactivation with rising levels above 1000 copies or evidence of PTLD should be treated with Rituximab, as per institutional standards

5.9.4 Pneumocystis jiroveci: Prophylaxis with agents against Pneumocystis jiroveci should be given as per institutional guidelines, until at least 1 year after HSCT.

5.9.5 Herpes virus (HSV or VZV): Patients should receive acyclovir or valacyclovir for at least 2 years after transplant as prophylaxis against HSV and VZV per institutional Guidelines.

5.9.6 Toxoplasmosis: In patients at risk (seropositive patient or donor) for toxoplasmosis. Prophylaxis against toxoplasmosis with Bactrim (trimethroprim/sulfamethoxzalole) should be given starting after transplant admission. **If patient cannot take Bactrim, toxoplasmosis monitoring by PCR every 1-2 weeks is recommended during the first 100 days after transplant**.

5.10 Intravenous Immune Globulin (IVIG)

IVIG administration for post HSCT hypogammaglobulinemia is recommended and should follow institutional standard practice.

5.11 Hepatic Veno-occlusive Disease (VOD) Prophylaxis

Prophylaxis against SOS/VOD with ursodiol is recommended per institutional practice, and should be started 1-2 weeks before start of conditioning. Ursodiol may be discontinued about 2-3 months post HSCT, at the discretion of the treating physician.

5.12 Immune Monitoring

Peripheral blood samples will be obtained to perform immunological tests for assessment of TCR repertoire and immune reconstitution. Both phenotypic and functional studies of peripheral blood lymphocytes will be performed. Blood samples will be banked periodically at around day +30, +60, day+100, and at about 6, 12, 18 and 24 months after transplantation.[18]

5.13 Management of GVHD

Acute and chronic GVHD will be managed per standard practice at the discretion of the treating transplant physician. It is recommended that for patients who develop overall grade 2-4 acute GVHD, that tacrolimus and corticosteroids be started together as initial therapy since patients on this trial do not routinely receive tacrolimus as GVHD prophylaxis.

5.14 Post-transplant maintenance therapy

For patients who have CML, Ph+ ALL, or FLT-3 mutation positive AML, maintenance therapy with TKIs or FLT-3 inhibitor agents for the prevention of relapse is permitted. It is recommended that maintenance therapy be started after day 42, after stable engraftment of WBC and Platelets, and only after a restaging marrow biopsy has been performed to rule out early relapse or minimal residual disease.

5.15 Criteria for Taking a Participant Off Protocol Therapy

Patients will be removed from this study prior to infusion of stem cells if for any reason, fresh or sufficient numbers of mobilized peripheral blood stem cells could not be obtained from the donor to allow for the T cell depletion processing. If this occurs, the patient will receive a standard transplant with unmanipulated bone marrow or PBSC, using tacrolimus and standard dose methotrexate (recommended), or other standard pharmacologic GVHD prophylaxis regimen at the discretion of the transplant physician.

Patients are free to discontinue participation or withdraw consent from the study at any time, for any reason, and without prejudice to further treatment.

Patients who discontinue/withdraw from the study will receive treatment as deemed appropriate by their treating physician

An ODQ Treatment Ended/Off Study Form will be filled out when a participant is removed from protocol therapy. This form can be found on the DF/HCC website at http://www.dfhcc.harvard.edu/research/clinical-research-support/document-library-forms-sops-etc/.

In the event of unusual or life-threatening complications, treating investigators must immediately notify the Overall PI, Vincent Ho, MD at 617-632-5938

5.16 Duration of Follow up

Participants will be followed for overall survival at Day 100 and after 1 year and 2 years after removal from protocol therapy or until death, whichever occurs first. Participants removed from protocol therapy for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event.

5.17 Criteria for Taking a Participant Off Study

Participation in the study also may be discontinued at any time at the discretion of the investigator. The following may be justifiable reasons for the Investigator to remove a subject from the study:

- The subject was erroneously included in the study
- The subject developed an exclusion criterion after having been considered eligible for the trial but before the start of the preparative regimen
- Disease progression

- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Participant demonstrates an inability or unwillingness to comply with the oral medication regimen and/or documentation requirements
- Participant decides to withdraw from the protocol therapy
- General or specific changes in the participant's condition render the participant unacceptable for further treatment in the judgment of the treating investigator

Participants will be removed from the protocol therapy when any of these criteria apply. The reason for removal from protocol therapy, and the date the participant was removed, must be documented in the case report form (CRF). In addition, the study team will ensure Off Treatment/Off Study information is updated in OnCore in accordance with DF/HCC policy REGIST-101. Alternative care options will be discussed with the participant.

6. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

6.1 Expected Toxicity of ablative chemotherapy

High dose chemotherapy will cause severe marrow aplasia with subsequent immunoablation and myeloablation, leading to pancytopenia and increased risk for bleeding and infections. Additional expected toxicities associated with chemotherapies used in the conditioning regimens of this study include:

- General toxicity:
  Alopecia, anorexia, dysgeusia
- GI toxicity:
  Nausea, vomiting, mucositis and esophagitis, abdominal pain, typhlitis, diarrhea
- Pulmonary toxicity:
  Pulmonary fibrosis, Interstitial pneumonitis
- Hepatic toxicity:
  Hepatic transaminase elevations, Hepatic veno-occlusive disease

- Neurologic toxicity:
  Seizure, peripheral neuropathy

- Dermatologic toxicity:
  Skin rash/irritation, darkening of skin

6.2 Expected toxicity of TBI

TBI will cause permanent ablation of normal bone marrow and profound immune suppression. Additional acute toxicities may include nausea, vomiting, diarrhea, parotitis, and skin erythema. Patients who receive TBI are likely to have permanent sterility. Delayed toxicity which occurs in some patients includes interstitial pneumonitis, hepatic veno-occlusive disease, radiation nephritis, and cataracts.

6.3 Expected toxicity of TCR αβ T depleted allogeneic PBSC transplantation

T cell depletion may increase the risk of graft failure or graft rejection. However, this risk may be mitigated with the increased intensity of the conditioning regimens used, and the assurance that sufficient CD34+ cells are infused with the graft. This study is designed such that it will be halted if the graft failure rate is significantly higher than expected.

Acute and chronic GVHD are major complications of transplantation. The major target organs of acute GVHD are the skin, alimentary tract, and liver. Treatment usually consists of corticosteroids and other immune suppressive medications. Depletion of TCR αβ T cells from donor graft may reduce the incidence of GVHD but it does not eliminate that risk.

Engraftment with T cell depleted grafts may impair reconstitution of the donor immune system and render the transplant recipient more susceptible to infections, and the development of lymphoma/post-transplant lymphoproliferative disease (PTLD). T cell depletion and may also impair graft-versus-tumor activity and subject patients to higher risk for relapse of their malignancy.

6.4 Adverse Event Characteristics

CTCAE term (AE description) and grade: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

6.4.1 Definitions of AE and SAE

Adverse Event- An AE is any unfavorable and unintended sign, symptom, or disease temporally associated with the use of an investigational medicinal product (IMP) or other protocol-imposed intervention, regardless of attribution. This includes the following:

1. AEs not previously observed in the subject that emerge during the protocol-specified AE reporting period, including signs or symptoms that were not present prior to the AE reporting period.

2. Pre-existing medical conditions judged by the investigator to have worsened in severity or frequency or changed in character during the protocol-specified AE reporting period.

Serious Adverse Event- An SAE is any AE that is any of the following:

1. Fatal (i.e., the AE actually causes or leads to death)

2. Life threatening (i.e. The AE, in the view of the investigator, places the subject at immediate risk of death)

3. Requires or prolongs inpatient hospitalization

4. Results in persistent or significant disability/incapacity (i.e., the AE results in substantial disruption of the subject's ability to conduct normal life functions)

5. A congenital anomaly/birth defect in a neonate/infant born to a mother exposed to the investigational product(s)

6. Considered a significant medical event by the investigator (e.g., may jeopardize the subject or may require medical/surgical intervention to prevent one of the outcomes listed above)

All AEs that do not meet any of the criteria for serious should be regarded as **non-serious AEs**. The terms "severe" and "serious" are not synonymous. Severity refers to the intensity of an AE (as in mild, moderate, or severe pain); the event itself may be of relatively minor medical significance (such as severe headache). "Serious" is a regulatory definition and is based on subject or event outcome or action criteria usually associated with events that pose a threat to a subject's life or vital functions. Seriousness (not severity) serves as the guide for defining regulatory reporting obligations. Severity and seriousness should be independently assessed when recording AEs and SAEs.

6.5 Adverse Event Reporting Guidelines

Since patients undergoing myeloablative conditioning stem cell transplantation are expected to experience many, and often severe, side effects from the procedure, we will restrict the reporting of AEs to those events which are **serious (CTC grade 3 or higher) AND unexpected** with regards to myeloablative stem cell transplantation. AEs that are listed in **section 6.0**, and/or are expected after myeloablative conditioning stem cell transplantation, are to be reported only if the adverse event varies in nature, intensity or frequency from the expected toxicity information.

Grade 3 or higher AEs that are expected and do not meet the above criteria for reporting will be recorded in the transplant database as per standard post HSCT data collection. Safety assessments will consist of monitoring and recording protocol-defined AEs and SAEs;

AEs will be reported directly to the DFCI Office for Human Research Studies (OHRS) per the DFCI IRB reporting policy. Events and time line for reporting are defined below:

Attribution of AE is defined below:

Definite – The AE *is clearly related* to the study treatment.
Probable – The AE *is likely related* to the study treatment.
Possible – The AE *may be related* to the study treatment.
Unlikely – The AE *is doubtfully related* to the study treatment.
Unrelated – The AE *is clearly NOT related* to the study treatment

6.6 Adverse Event Reporting

6.6.1 In the event of an unanticipated problem or life-threatening complications treating investigators must immediately notify the Overall PI.

6.6.2 Investigators **must** report to the Overall PI any adverse event (AE) that occurs after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment on the local institutional SAE form.

6.7 Reporting to the Food and Drug Administration (FDA)

The Overall PI, as study sponsor, will be responsible for all communications with the FDA. The Overall PI will report to the FDA, regardless of the site of occurrence, any serious adverse event that meets the FDA's criteria for expedited reporting following the reporting requirements and timelines set by the FDA.

6.8 Reporting to Hospital Risk Management

Participating investigators will report to their local Risk Management office any participant safety reports, sentinel events or unanticipated problems that require reporting per institutional policy.

6.9 Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine study data submissions to the Overall PI on the toxicity case report forms. **AEs reported through expedited processes (e.g., reported to the IRB, FDA, etc.) must also be reported in routine study data submissions.**

6.10 Expedited Adverse Event Reporting

Investigators must report to the Overall PI any serious adverse event (SAE) that occurs after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment on the local institutional SAE form.

7. PHARMACEUTICAL AND/OR IMAGING AGENT INFORMATION

7.1 T-Cell Receptor (TCR) αβ T-Cell Depletion

7.1.1 Description

TCR alpha/beta reagent system: Description of the TCR a/b reagent system based on the user manual is appended below.

CliniMACS® TCRα/β-Biotin

I. General information, warnings and precautions

General information

The CliniMACS® Plus TCRα/β-Biotin System including the CliniMACS ^{plus} Instrument, the CliniMACS TCRα/β-Biotin, the CliniMACS Anti-Biotin Reagent, the CliniMACS Depletion Tubing Set, and CliniMACS PBS/EDTA Buffer, is intended for the *in vitro* depletion of human TCRα/β positive cells from heterogeneous hematologic cell populations.

The TCRα/β is the T cell receptor heterodimer composed of two transmembrane glycoprotein chains, α and β. Both chains are members of the Ig superfamily and consist of a constant and a polymorphic variable region. The variable region of the TCRα/β receptor is involved in recognition of antigenic peptides presented by the MHC complex of antigen presenting cells. The TCRα/β is expressed on the majority of peripheral blood T cells.

The CliniMACS Plus TCRα/β-Biotin System uses murine monoclonal antibodies specific for the TCRα/β antigen conjugated to biotin in combination with the CliniMACS Anti-Biotin Reagent. TCRα/β positive cells are separated in an automated separation system provided by the CliniMACS ^{plus} Instrument and the CliniMACS Depletion Tubing Set and CliniMACS PBS/EDTA Buffer.

The TCRα/β positive cells are labeled for separation by incubation with the CliniMACS TCRα/β-Biotin. After unbound conjugate is removed from the suspension, the cells are magnetically labeled with the CliniMACS Anti-Biotin Reagent. After excess reagent is removed from the suspension, the cells are ready for the automated separation process.

The CliniMACS Plus TCRα/β-Biotin System passes the antibody-labeled cell suspension through the separation column in which strong magnetic gradients are generated. The separation column retains the magnetically labeled TCRα/β positive cells (non-target cells), while the unlabeled cells (target cells) flow through the column. Several automated washing steps are performed, collecting the TCRα/β negative target cells in the Cell Collection Bag.

7.1.2 Storage and Stability

The Cell Manipulation Core Facility (CMCF) of the Dana-Farber Cancer Institute is an FDA approved facility with laboratories equipped with state of the art equipment for generating cells and cellular products for clinical use. Dr. Jerome Ritz is director of the facility and Dr. Hélène Nègre is the Novel Cell Therapy Director.

The CMCF is accredited by The Joint Commission (JC) and FACT and is CLIA certified (ID #22D1010753). The CMCF laboratory was last surveyed by JC in January 2017. The laboratory was inspected by FACT in February 2016 and received a three-year accreditation as the cell processing laboratory for both the adult and pediatric transplant programs at DFCI. The laboratory is also FDA registered to package, process, store, label, and distribute 351 and 361 cell therapy products such as; peripheral blood stem cells, Cord Blood, somatic cellular products. The FDA registration number the CMCF is FEI 3003934255.

7.1.3 Handling

Qualified personnel, familiar with procedures that minimize undue exposure to themselves and the environment, should undertake the preparation, handling, and safe disposal of the chemotherapeutic agent in a self-contained and protective environment.

7.1.4 Availability

Materials are all commercially available and will be bought with internal grant funding from Miltenyi.

7.1.5 Preparation

Cell Manufacture: The blood stem cell product will be mobilized with G-CSF from the donor in accordance with standard protocol at the DFCI (related donors) or at the unrelated donor's collection center. Ideally, 2.5 blood volumes will be processed and a target of 6.0 x106 CD34+ cells/kg recipient weight will be collected in one or two sessions. The product will be delivered immediately by courier to the Dana-Farber Cancer Institute for processing. The PBSC product will not be cryopreserved before processing.

The manufacturing process of the TCRαβ T-cell depleted cell grafts will be performed in the CMCF-GMP laboratory at the DFCI. The manufacturing process and quality control will be performed according to validated procedures and documented in accordance with full GMP requirements.

The stem cell apheresis product will be depleted of TCRαβ T cells by negative selection using the automated CliniMACS® Plus device, the current version of the CliniMACS user manual and according to institutional Standard Operating

Procedures (SOPs) in place and validated at our manufacturing site. A detailed protocol for the preparation and use of this system is provided in the CliniMACS® TCRαβ Reagent Kit Investigator's Brochure. Samples of the donor leukocyte preparation will be removed prior to processing and after completion. These samples will be used to determine cell viability and enumerate the total number of alpha/beta T cell, gamma/delta T cells, B cells, NK cells and CD34+ cells in the product before and after processing. After processing, the TCRαβ T cell depleted product will be delivered immediately in a cooler at room temperature to the bedside for intravenous infusion to the designated transplant recipient.

7.1.6 Administration

Please reference Section 5, Treatment Plan.

7.1.7 Ordering

Product Tracking: When a patient's product is received in the Cell Manipulation Core Facility (CMCF), the product is assigned a unique number, if it does not have one from the collection facility. The product is inspected and signed in noting the products condition, study subject's name and identifiers (medical records). This information is recorded into the "CMCF Product Receipt Log" directly from the unit's label and the accompanying paper work. This information is compared with the identifiers on the order to process the cells for a patient. The products unique identifier, subject's name, MR #, and other required information are maintained on all intermediated labels during manufacturing and on the final product label along with the products name and "Caution: New Drug—Limited by Federal (or United States) law to investigational use." Additional information is recorded as part of the batch record during each manufacturing step.

7.1.8 Accountability

Experience with the Miltenyi ClinicMACs device: The CMCF has extensive experience with the Miltenyi CliniMacs device. We were a high accrual center for the national CTN phase II study that tested CD34 selection using CliniMACs in related donor transplantation, and we have conducted our own clinical trial of CD25 T reg selection using CliniMACs for infusion as a treatment for cGVHD. We also have a standard treatment plan for performing CD34 selection of allogeneic PBSC products for use in stem cell rescues or stem cell boosts for patients who experience graft failure after HSCT.

8. STUDY ENDPOINTS

8.1 Engraftment

Engraftment of neutrophils will be defined as the first of 3 consecutive days in which the absolute neutrophil count exceeds 500 x 10^6 neutrophils/ml. Patients will be considered to have graft failure if neutrophil engraftment is not achieved within 30 days of PBSC infusion. Engraftment of platelets will be defined as the first day of a seven-day span in which they maintain a platelet count over 20,000 x 10^6 platelets/ml without transfusion.

Primary graft failure is defined as the failure to achieve an ANC >500 cells/μL by Day +28. Secondary graft failure is defined as initial neutrophil engraftment followed by subsequent decline in neutrophil counts <500 cells/μL, unresponsive to growth factor therapy.

8.2 GVHD

Acute GVHD will be graded according to the modified Glucksberg criteria.[19] Beyond day +100, development of chronic GVHD will be assessed as per the NIH 2014 consensus criteria for cGVHD.[20]

8.3 Disease Relapse

Relapse of disease post-HSCT will be assessed by standard criteria, including physical exam, laboratory tests, radiographs, CT scans or MRI, and tissue (bone marrow, lymph node, etc.) biopsy deemed as appropriate for the patient's diagnosis. Disease assessment is required at approximately day +100, and at12 and 24 months after transplant, and whenever clinically appropriate at the discretion of the treating physician.

8.4 Survival

8.4.1 Overall survival (OS) is defined as time from transplantation to death or last follow-up and will be assessed at Day 100 and after 1 year and 2 years.

8.4.2 Disease-free survival (DFS) is defined as time from transplantation to relapse/ recurrence or death from any cause, whichever occurs first. Patients who are alive and disease free at the time of data analysis will be censored.

8.4.3 GVHD-Relapse-free-Survival (GRFS) is defined as time from transplantation to grade 3-4 acute GVHD, moderate-severe chronic GVHD, relapse or death of any cause, whichever occurs first. Patients who are alive and free of any of these events will be censored.

8.4.4 Chronic GVHD-free Survival (CRFS) is defined as time from transplantation to moderate-severe chronic GVHD or death of any cause, whichever occurs first. Patients who are alive and free of any of these events will be censored.

8.4.5 Immune suppression-free survival (IFS) is defined as time from transplantation to the first date of requiring immunosuppressant or death of any cause, whichever occurs first. Patients who are alive and free of any of these events will be censored.

8.5 Studies of immune reconstitution

Blood specimens will be obtained from patients weekly after day 0 during the transplant admission, then monthly up to about day 100, and then at about 6, 12, 18, and 24 months after transplantation. Immune reconstitution will be monitored using flow cytometry to examine recovery of CD4Treg, CD4Tcon, CD8 T cells, NK cells and B cells in peripheral blood samples. Recovery of T cells expressing α/β and γ/δ T cell receptors will also be monitored.

9. DATA REQUIREMENTS

9.1 Post-Transplant Evaluations

The following observations should be performed according to Table I:

- History and physical exam weekly through Day 100 post-transplant, then at around monthly intervals until 1 year, at 1.5 year and 2 years from transplant.
- GVHD assessments
  - GVHD should be assessed weekly from approximately Day 7 until Day 90-100 post-transplant, then at all routine clinical follow visits after 100 days as per standard care practice.
  - Acute and chronic GVHD should be monitored in accordance with institutional guidelines and whenever possible, recorded in the GVHD flowsheet in the Epic EMR system. Acute GVHD will be scored according to the standard Keystone criteria [19]and cGVHD will be assessed according to the 2014 NIH consensus criteria for cGVHD grading [20].
- CMV Monitoring: CMV monitoring through PCR viral load testing will be done weekly until about Day 90.

- EBV Monitoring: EBV monitoring through quantitative PCR viral load testing will be done weekly until about Day 90.

- Disease evaluation: Restaging studies with radiology imaging or bone marrow aspirate/biopsy as appropriate for the underlying disease should be performed at about day 180, 1 year, and 2 years post-transplant.

Table I. Required Data

	Pre-Study[c]	Weekly to day +90[d]	Day +30[d]	Day +60[d]	Day +100[e]	6 months[e]	12 months[e]	18 months[f]	24 months[f]
Informed consent	X								
Standard pre-HSCT FACT testing	X								
Medical history	X								
HCT-CI	X								
B-HCG[a]	X								
Donor and Recipient HLA and ABO typing	X								
HIV/HepB testing	X								
Physical exam	X	X	X	X	X	X	X	X	X
Performance status (ECOG)	X		X	X	X	X	X	X	X
ECHO	X								
PFT	X								
CBC w/diff	X	X	X	X	X	X	X	X	X
Serum Chemistry[b]	X	X	X	X	X	X	X	X	X
Primary Disease Staging[g]	X	Staging studies (marrow biopsy and/or radiology) appropriate for the patient's cancer type should be performed at about day 180, 12 months, and 24 months after HSCT.							
GVHD Assessment		X			X	X	X	X	X
Toxicity Assessment		X			X	X	X	X	X
Chimerism studies: PB, Gran, CD3 Chim			X	X	X	X	X	X	X
Viral Monitoring: CMV and EBV Quant PCR		X			X				
Immune reconstitution blood	X	Weekly during inpatient admission	X	X	X	X	X	X	X

a: Serum pregnancy test (women of childbearing potential only).

b: BMP panel including LFTs (AST, ALT, Alk Phos, BILT).
c. Baseline tests to be performed within 42 days of transplant day 0.
d: +/- 3 days.
e. +/- 7 days
f: +/- 2 weeks
g: For patients who have CML, Ph+ ALL, or FLT-3 mutation positive AML, maintenance therapy with TKIs or FLT-3 inhibitor agents for the prevention of relapse is permitted. It is recommended that maintenance therapy be started after day 42, after stable engraftment of WBC and Platelets, and only after a restaging marrow biopsy has been performed to rule out early relapse or minimal residual disease.

10. DATA REPORTING / REGULATORY REQUIREMENTS

10.1 Data Reporting

10.1.1 Method

The Office of Data Quality (ODQ) will collect, manage, and perform quality checks on the data for this study.

10.1.2 Responsibility for Data Submission

Investigative sites within DF/HCC or DF/PCC are responsible for submitting data and/or data forms to the Office of Data Quality (ODQ) in accordance with DF/HCC policies.

10.1.3 Data Safety Monitoring

The DF/HCC Data and Safety Monitoring Committee (DSMC) will review and monitor toxicity and accrual data from this study. The committee is composed of medical oncologists, research nurses, pharmacists and biostatisticians with direct experience in cancer clinical research. Information that raises any questions about participant safety will be addressed with the Overall PI and study team.

The DSMC will review each protocol up to four times a year with the frequency determined by the outcome of previous reviews. Information to be provided to the committee may include: up-to-date participant accrual; current dose level information; DLT information; all grade 2 or higher unexpected adverse events that have been reported; summary of all deaths occurring within 30 days of intervention for Phase I or II protocols; for gene therapy protocols, summary of all deaths while being treated and during active follow-up; any response information; audit results, and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

11. STATISTICAL CONSIDERATIONS

11.1 Study Design/Endpoints

This is a single-arm, two-stage phase II study of ex vivo αβ+ TCR T cell depleted (TCD) peripheral blood stem cell transplantation (PBSCT) as a GVHD prophylaxis for patients with hematologic malignancies undergoing allogeneic PBSCT. The primary objective is to evaluate the safety and efficacy of ex vivo αβ+ TCR TCD for patients receiving myeloablative PBSCT from related or unrelated mismatched (8/10 or 9/10) donor. The premise is that ex vivo αβ+ TCR TCD will reduce the incidence of grade 3-4 acute GVHD and/or death. The target accrual goal is 25 evaluable patients over 3.5- 4 year period. All surviving patients will be followed for at least 100 days. Patients who meet the eligibility criteria but deemed unevaluable per Section 5.4 will be excluded from the study.

11.2 Endpoints

11.2.1 **Primary Endpoint**: The primary endpoint of this study is day 100 severe acute GVHD-free survival rate. Events considered for the primary endpoint are grade 3-4 acute GVHD or death from any cause.

11.2.2 **Secondary Endpoints**

- Grades II-IV acute GVHD
- Chronic GVHD
- GRFS
- Immunosuppression-free survival
- Hematologic recovery (neutrophil and platelet engraftment)
- Immune reconstitution
- Disease relapse
- Transplant-related mortality
- Organ Toxicity (e.g. SOS/VOD and IPS)
- Rates of infections (CMV and EBV reactivation)
- Relapse-free and overall survival

11.3 Accrual

Based on the accrual of the DFCI transplant group between 2010 and 2015 who meet the eligibility criteria, the projected accrual rate will be 7-8 patients per year. Conservatively extrapolating this projection, it is anticipated that the accrual will complete in 3.5-4 years to enroll the targeted sample size of 25 patients.

11.4 Randomization

N/A

11.5 Sample Size and Power Calculation

A retrospective analysis of patients who underwent myeloablative PBSCT between 2010 and 2015 at DFCI who meet the eligibility criteria shows that the estimated day 100 severe acute GVHD-free survival rate is 67%. Basing this information as the null hypothesis, we hypothesize that with the ex vivo αβ+ TCR TCD, the day 100 severe acute GVHD-free survival rate will be increased by 20% from 67% to 87%. With the sample size of 25, there will be 82% power to detect a 20% difference in severe acute GVHD-free survival rate.

The study is a two-stage design with early stopping rules if the proposed therapy shows no efficacy after the first stage or excessive rate of graft failure. In the first stage, 10 patients will be accrued. If 7 or fewer patients are alive and severe acute GVHD-free by day 100 of transplantation, the study will be terminated early for lack of efficacy. If, however, 8 or more patients are alive without severe acute GVHD by day 100, then additional 15 patients will be accrued. After the second stage, if the total number of patients who are alive without sever acute GVHD is 20 or more, then proposed prophylaxis will be considered efficacious for preventing acute GVHD. Conversely, if 19 or fewer patients are alive and severe acute GVHD-free, then this regimen will be considered inefficacious. If 20 patients are alive and severe acute GVHD free after the second stage, the 95% confidence interval for this rate is (61%, 94%). This confidence interval is calculated based on the Atkinson and Brown method (Biometrics, 1985).

With this design, the probability of concluding the proposed prophylaxis efficacious is 0.82 if the true but unknown day 100 severe acute GVHD-free survival rate is 87% and 0.09 if the true rate is 67%. The probability of early stopping is 0.69 if the rate is 67% and 0.13 if the rate is 87%. Table 1 below presents the operating characteristics of this design.

Table 1. Operating Characteristics

	True but Unknown severe acute GVHF-free survival Rate				
	67%	72%	77%	82%	87%
Probability of stopping early (<=7 in 10)	0.69	0.56	0.41	0.26	0.13
Overall Probability of accepting the treatment	0.09	0.21	0.39	0.61	0.82

11.6 Monitoring Events

The occurrence of graft failure will be monitored closely and continuously throughout the study period. In particular, if 3 or more of the first 10 patients enrolled experience

graft failure, defined as lack of neutrophil engraftment by day+30, then the study will be halted for further review. With this design, the probability of early termination is 0.07 if the true but unknown unknown graft failure rate at day +30 is 10% but 0.62 if the true rate is 30%. This decision rule is calculated using an exact binomial distribution**.** Table 2 shows probabilities of stopping early under various scenarios.

Table 2.

	True but Unknown Graft Failure Rate				
	10%	15%	20%	25%	30%
Prob (≥3 graft failure in the first 10 patients)	0.07	0.18	0.32	0.47	0.62

11.7 Analysis of Primary Endpoints

When data are ready for analysis, the primary endpoint will be reported descriptively, reporting the proportion and confidence interval. Time to event endpoints such as chronic GVHD-free survival, GRFS, immunosuppression-free survival (IFS), disease-free survival (DFS) and overall survival will be estimated using the Kaplan-Meier method. Cumulative incidence of acute/chronic GVHD, relapse, and transplant-related mortality (TRM) will be estimated in the competing risks framework considering relapse or death without developing GVHD, TRM, and relapse as a competing event, respectively. Cumulative incidence of engraftment and CMV/EBV reactivation will also be estimated in the competing risks framework considering death without engraftment and CMV/EBV as a competing event, respectively. Other endpoints such as organ toxicity, immune reconstitution and quality of life will be analyzed descriptively.

11.8 Reporting and Exclusions

Precisely define the analysis population for the various endpoints and subset analyses. Participants who never start protocol therapy are usually excluded ("inevaluable") from most analyses in Phase I and Phase II studies.

11.7.1 Evaluation of Toxicity

Define "evaluable." For example: All participants will be evaluable for toxicity from the time of their first treatment.

11.7.2 Evaluation of the Primary Efficacy Endpoint

Analyses should be intent-to-treat unless justification can be provided for not doing so. Specifically, all eligible participants included in the study must be assessed for response/outcome to therapy, even if there are major protocol therapy deviations.

Subanalyses may then be performed on the basis of a subset of participants, excluding those for whom major protocol deviations have been identified (e.g., early death due to other reasons, early discontinuation of treatment, major protocol violations, etc.). However, these subanalyses may not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding participants from the analysis should be clearly reported. If applicable to the endpoint, the 95% confidence intervals should also be provided.

12. PUBLICATION PLAN

The results should be made public within 24 months of reaching the end of the study. The end of the study is the time point at which the last data items are to be reported, or after the outcome data are sufficiently mature for analysis, as defined in the section on Sample Size, Accrual Rate and Study Duration. If a report is planned to be published in a peer-reviewed journal, then that initial release may be an abstract that meets the requirements of the International Committee of Medical Journal Editors. A full report of the outcomes should be made public no later than three (3) years after the end of the study.

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14. APPENDIX

- **A.** Performance Status Criteria
- **B.** CliniMACS® TCRα/β Reagent Kit Investigator's Brochure

APPENDIX A PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (*e.g.*, light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

APPENDIX B CLINIMACS® TCRA/B REAGENT KIT INVESTIGATOR'S BROCHURE

INVESTIGATOR'S BROCHURE

CliniMACS® TCRα/β Reagent Kit

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Date: August 7, 2013
Version: 1.0

CliniMACS

TCRα/β Reagent Kit
Investigator's Brochure

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1. SUMMARY

1.1. *Investigational Product*

The CliniMACS® TCRα/β Reagent Kit consists of 1 vial of CliniMACS® TCRα/β-Biotin and 2 vials of CliniMACS Anti-Biotin Reagent which are used with the CliniMACS System. The CliniMACS® TCRα/β Reagent Kit is used to deplete human TCRα/β$^+$ T cells *in vitro* from heterogeneous hematological cell populations for stem cell transplantation and lymphocyte infusions in cases where this is clinically indicated.

Each CliniMACS® TCRα/β Reagent Kit is sufficient to label up to 24 x 10^9 TCRα/β$^+$ T cells out of 60 x 10^9 total white blood cells using an indirect labeling strategy. The CliniMACS® TCRα/β Reagent Kit is used with the CliniMACS® System in conjunction with the following CliniMACS® components.

- CliniMACS TCRα/β Reagent Kit
  - CliniMACS® TCRα/β-Biotin – a sterile solution containing biotinylated monoclonal antibodies specific for human TCRα/β$^+$ T cells
  - CliniMACS® Anti-Biotin Reagent – a sterile solution containing monoclonal antibodies specific for the biotin antigen, covalently linked to dextran beads having an iron oxide/hydroxide core.
- CliniMACS® ^{plus} Instrument – a software controlled instrument that processes the blood sample (cell product)
- CliniMACS® Depletion Tubing Set (DTS) – a single-use, sterile, disposable tubing set with proprietary cell selection columns
- CliniMACS® PBS/EDTA Buffer – a sterile, isotonic phosphate-buffered, 1 mM EDTA, saline solution, used as external wash and transport fluid for the *in vitro* preparation of blood cells

Following the depletion of TCRα/β$^+$ T cells using the ClinicMACS TCRα/β Reagent Kit, the use of the CliniMACS® CD19 Reagent is referenced for B cell depletion (refer to section 2.2, "Intended Use" and Section 6, "Summary and Guidance for the Investigator") when clinically indicated. Please note that *in vivo* methods for B cell depletion (i.e. Rituximab) may also be considered by the investigator.

1.2. *Physical, Chemical and Toxicological Information*

1.2.1. CliniMACS® TCRα/β-Biotin

TCRα/β-Biotin Description

The CliniMACS® TCRα/β-Biotin consists of biotinylated TCRα/β monoclonal antibody (mAb). The CliniMACS® TCRα/β-Biotin mAb is a mouse IgG_{2b} monoclonal, which is produced by the hybridoma cell line clone BMA031.H7.

CliniMACS® TCRα/β-Biotin is a sterile solution intended for the indirect magnetic labeling of human TCRα/β$^+$ T cells, in combination with the CliniMACS® Anti-Biotin Reagent. It is

supplied in single use vials which contain nominally 7.5 mL per vial. The vials used are 10 mL glass injection vials sealed with a chlorobutyl rubber stopper and flip-off aluminum seal.

Safety Testing of the TCRα/β Monoclonal Antibody

Cell banking, cell culture, as well as subsequent purification of the antibody, follow the applicable current international guidelines as described in Section 4. The testing of the TCRα/β Master Cell Bank, the TCRα/β Manufacturer's Working Cell Bank, the End of Production Cells, the TCRα/β mAb pooled cell culture harvest (unprocessed bulk), and the purified bulk TCRα/β monoclonal antibody has been completed and the purified monoclonal antibody has been released for manufacturing of the TCRα/β-Biotin. The safety testing requirements are listed in Table 1. Additionally, the viral inactivation/removal steps used in the purification of the TCRα/β monoclonal antibody have been validated.

Safety Testing of the TCRα/β-Biotin

Detailed toxicity studies have been undertaken to assess the safety of the CliniMACS® TCRα/β-Biotin when delivered in dosages significantly greater than the projected maximum dosage anticipated in clinical use. The testing was performed in accordance with 21 CFR§58, Good Laboratory Practices for Nonclinical Laboratory Studies. The results are detailed in Section 4.3 and summarized in the following table.

Table 1: Summary of Toxicology Testing Results of the CliniMACS® TCRα/β-Biotin

Test	Results
Cytotoxicity	Slight cytotoxicity (undiluted sample)
Hemocompatibility	Non-hemolytic
Irritation	Irritant (undiluted sample)
Systemic toxicity	No systemic toxicity
Sensitization	No sensitization

1.2.2. CliniMACS® Anti-Biotin Reagent

CliniMACS® Anti-Biotin Reagent Description

The CliniMACS® Anti-Biotin Reagent is composed of the Anti-Biotin monoclonal antibody chemically coupled to iron-dextran beads. The CliniMACS® Anti-Biotin Reagent has no therapeutic endpoint and is not intended for direct infusion into patients. The CliniMACS® Anti-Biotin Reagent is a flexible labeling system and is used as a secondary labeling antibody in conjunction with CliniMACS® TCRα/β-Biotin in the CliniMACS® TCRα/β Reagent Kit. After the indirect labeling, target cells are isolated using the CliniMACS® ^{plus} Instrument.

The CliniMACS® Anti-Biotin Reagent is a dark brown to black, non-viscous, colloidal solution containing the antibody conjugate in buffer. The conjugate consists of a mouse IgG_1 monoclonal antibody (specific for the biotin antigen) covalently linked to dextran beads having an iron oxide/hydroxide core. The CliniMACS® Anti-Biotin Reagent is supplied sterile in 10R glass

vials. Each vial contains 7.5 mL of the CliniMACS® Anti-Biotin Reagent. It is intended for single and *in vitro* use only.

Safety Testing of the Anti-Biotin Monoclonal Antibody

Cell banking, cell culture, as well as subsequent purification of the antibody, follow the applicable current international guidelines as described in Section 4. The testing of the Anti-Biotin Master Cell Bank, the Anti-Biotin Master Working Cell Bank, the End of Production Cells, the Anti-Biotin mAb pooled cell culture harvest (unprocessed bulk), and the purified Anti-Biotin monoclonal antibody has been completed and the purified monoclonal antibody has been released for manufacturing of the Anti-Biotin Reagent. The safety testing requirements are listed in Table 2. Additionally, the viral inactivation/removal steps used in the purification of the Anti-Biotin monoclonal antibody have been validated.

Safety Testing of the Anti-Biotin Reagent

Detailed toxicity studies have been undertaken to assess the safety of the Reagent when delivered in dosages significantly greater than the projected maximum dosage anticipated in clinical use. The testing was performed in accordance with 21 CFR§58, Good Laboratory Practices for Nonclinical Laboratory Studies. The results are detailed in Section 4.3 and summarized in the following table.

Table 2: Summary of Toxicology Testing Results of the CliniMACS® Anti-Biotin Reagent

Test	Results
Cytotoxicity	No cytotoxicity
Hemocompatibility	Non-hemolytic
Irritation or Intracutaneous reactivity	Slight (undiluted sample)
Systemic toxicity	No systemic toxicity
Sensitization	No sensitization

1.3. *Safety Testing of CliniMACS® System Components (Instrument, Reagent, Tubing Sets and PBS/EDTA Buffer)*

The CliniMACS® plus Instrument has been tested for electrical safety and the potential for fire, shock, explosion, or mechanical damage. Potential safety issues have been reduced by using a design to meet European standards and EN 60601-1 as well as EN 60601-1-2: 2007 6.1 + 6.2 (*EMC Requirement for Medical Devices*). The CliniMACS® plus Instrument is UL and CSA listed and approved.

Biocompatibility Testing of the CliniMACS Tubing Sets and CliniMACS PBS/EDTA Buffer was performed according to applicable guidelines. Final results are summarized in Tables 6-10 and were fulfilled for the use of CliniMACS® TCRα/β Reagent Kit with the CliniMACS® System.

1.4. *Overall Safety of the CliniMACS® TCRα/β Reagent Kit*

The results summarized in this Investigator's Brochure support that the CliniMACS® TCRα/β Reagent Kit is sufficiently safe for clinical use with human subjects. The potential applications of the CliniMACS® TCRα/β Reagent Kit are broad and individual risk analysis on the therapeutically used target cells isolated in conjunction with the CliniMACS® TCRα/β Reagent Kit should be considered by each user.

2. INTRODUCTION

2.1. *MACS® Technology*

The CliniMACS System is based on magnetic cell sorting (MACS®). MACS is a registered trademark of Miltenyi Biotec GmbH (Bergisch Gladbach, Germany) and is a powerful tool for the isolation or depletion of many cell types.[1] By using the specificity of antibody interaction with cell surface antigens, heterogeneous cell mixtures, e.g. leukapheresis products, can be separated in a magnetic field using an immunomagnetic label specific for the cell type of interest. The CliniMACS TCRα/β Reagent Kit is an indirect labeling system intended for the clinical scale separation of cells intended directly for therapeutic applications, or for the manufacture of cellular therapeutic products.

The CliniMACS System is comprised of a computer controlled instrument incorporating a strong permanent magnet, a closed-system sterile tubing set containing columns with a coated ferromagnetic matrix, and a paramagnetic, cell specific, labeling reagent(s). The instrument separates the cells in a fully automated process yielding either a highly enriched population of targeted cells or a selectively depleted cell population.

The cells to be isolated are specifically labeled with super-paramagnetic particles. The super-paramagnetic particles are small in size (about 50nm in diameter) and are composed of iron oxide/hydroxide and dextran conjugated to monoclonal antibodies. The super-paramagnetic particles form a stable colloidal solution and do not precipitate or aggregate in suspension or in magnetic fields. These antigen specific magnetic particles can be used to enrich or selectively deplete target cell types.

The cell population is labeled by incubating with a cell specific reagent. Note that since the CliniMACS® TCRα/β Reagent Kit applies an indirect labeling strategy, cells are first labeled with a biotinylated primary antibody (CliniMACS® TCRα/β-Biotin) and subsequently labeled with the secondary anti-biotin antibody covalently linked to dextran beads having an iron oxide/hydroxide core. (CliniMACS® Anti-Biotin Reagent). After magnetic labeling, the cells are pumped through a high-gradient magnetic column that is described below. The magnetically labeled cells are retained in the magnetized column while the unlabeled cells flow through.

The CliniMACS System incorporates a strong permanent magnet and a column with a ferromagnetic matrix to separate the cells labeled with the magnetic particles. Small ferromagnetic structures, as in the column matrix, when placed in a magnetic field, disturb the homogenous field and thereby produce high magnetic gradients. The high-gradient system allows the concentration of strong magnetic forces. In their immediate proximity, the ferromagnetic structures generate magnetic forces 10,000-fold greater than in the absence of those structures, thereby enabling the retention of magnetically labeled cells. After removing the column from the magnet, the rapid demagnetization of the column matrix allows the release of retained cells.

2.2. *Intended Use of the CliniMACS® TCRα/β Reagent Kit*

The intended use of the CliniMACS® TCRα/β Reagent Kit is to deplete human TCRα/β$^+$ T cells from human heterogeneous hematological cell populations for stem cell transplantation and lymphocyte infusions in cases where this is clinically indicated.

Allogeneic stem cell transplantation

During the last 30 years, allogeneic stem cell transplantation has been developed and improved for the curative treatment of patients with life-threatening hematological and non-hematological malignancies as well as non-malignant diseases. The stem cell transplant is intended to reconstitute a healthy and self-sustaining hematopoietic system after eradication of the underlying disease by chemo- and radiotherapy (conditioning). The success of allogeneic stem cell transplantation is limited by the potential development of life-threatening complications associated with the procedure. Infection, relapse, and graft-versus-host disease (GVHD) are still major causes of mortality and morbidity after allogeneic transplantation. Therefore, the speed of reconstitution of T, B, NK and other immune cells is of importance for the reduction of the risk for bacterial, viral and fungal infections as well as for mediating anti-tumor effects, especially in HLA mismatched and haploidentical transplantation.[2,3,4] Strategies to reduce the risk of GVHD development have been pursued. These strategies include graft engineering with the aim to deplete donor derived T cells which play a central role in GVHD development.

Pre-clinical and clinical research have been performed to evaluate the role that allo-reactive donor T cells play in the development of GVHD. In animal models for acute GVHD (aGVHD) it has been demonstrated that tissue damage caused by the underlying disease and the conditioning regimen leads to the release of pro-inflammatory cytokines that promote the activation and maturation of antigen-presenting-cells (APCs). The donor T cells which recognize the activated APCs proliferate, differentiate, and migrate to the GVHD target tissues. Direct cytotoxic activity and recruitment of other effector leukocytes lead to tissue destruction. The tissue damage is associated with increased inflammatory signals which contribute to the cytokine storm perpetuating the disease process.[5] The activation of donor T cells in response to APCs via the alpha/beta T cell receptor is one central step in GVHD development. HLA disparities, differences in minor histocompatibility antigens and genetic polymorphism of cytokine genes and proteins connected with innate immunity have been associated with aGVHD in humans. Pre-clinical animal models for chronic GVHD (cGVHD) are scarce.

GVHD remains a major cause of mortality and morbidity after transplantation with allogeneic peripheral blood or bone marrow for both matched and mismatched transplants.[6, 7]

For many patients, no HLA-matched sibling or HLA-matched unrelated donor is available but almost every patient has a suitable haploidentical donor within the family. In the haploidentical transplantation setting the high HLA class I and II disparity between donor and recipient causes strong graft-versus-host and host-versus-graft alloresponses. The combination of a megadose of CD34$^+$ stem cells which is extensively depleted of T cells in combination with myeloablative conditioning ensures high engraftment rates with the potential of not inducing GVHD.[8]

Historically, the passive removal of T cells by $CD34^+$ stem cell selection has been well documented in haploidentical transplantation. In one study, Aversa *et al*. treated forty-three patients with high-risk leukemia using a haploidentical donor and a conditioning regimen that included total body irradiation (TBI), thiotepa, fludarabine, and antithymocyte globulin (ATG).[9] The peripheral blood stem cell product was T cell depleted by e-rosetting followed by $CD34^+$ selection using the CellPro Ceprate® SC system. The resulting product was highly T cell depleted (4-log depletion) and yielded a high $CD34^+$ cell dose (median 10.0 x 10^6 $CD34^+$ cells/kg patient body weight). Importantly, no additional GVHD prophylaxis was administered. Engraftment was achieved in 95% of the patients and none of the evaluable patients developed acute or chronic GVHD, however, delayed immune reconstitution was the main cause of death and contributed to a 40% rate of transplant-related mortality (TRM). In 2005, a phase II trial of 104 haploidentical transplants was published by Aversa and colleagues. *In vitro* passive T cell depletion was performed by CD34 selection using the CliniMACS CD34 Reagent System or the Baxter Isolex® device. Of the 101 evaluable patients, 100 engrafted and the event free survival (EFS) for AML and ALL patients in remission was promising with 48% and 46%, respectively.[10]

Other studies utilizing haploidentical donors in both children and adult stem cell transplantations with mainly TBI-based myeloblative conditioning regimen followed by the infusion of $CD34^+$ selected stem cell products without additional GVHD prophylaxis also resulted in delayed immune reconstitution. This delayed immune reconstitution was associated with a high incidence of severe and lethal infections.[10, 11, 12, 13] Important findings noted from the above referenced studies using highly enriched $CD34^+$ stem cells was that, with positive selection, many potentially beneficial cells such as NK cells, CD34 negative stem cells and other cells that might be "graft facilitating" such as monocytes and antigen-presenting cells, are discarded. Putatively, these cells have a positive effect on engraftment, on immune reconstitution, provide a graft-versus-tumor effect and react against infections. The direct depletion of T cells with the CliniMACS CD3 Separation System enables the engineering of a stem cell graft for allogeneic stem cell transplantation containing all cell populations of an un-manipulated graft sparing CD3 positive T cells.

The combined CliniMACS CD3/CD19 Separation System depletes T and B cells. The clinical rationale for combining B cell depletion with T cell depletion is to reduce the risk of Epstein-Barr Virus (EBV) associated Post-transplant lymphoproliferative disorder (PTLD). In one of the first clinical trials using T cell depleted grafts, an increase in potentially life threatening PTLD was documented.[14] In a clinical trial the combination of B and T cell depletion led to a significant reduction of PTLD in comparison to a retrospective control group. In the study, 19 patients were transplanted with bone marrow from mismatched related and unrelated donors which was *ex vivo* depleted of B and T cells. None of the patients experienced EBV associated PTLD of donor origin. By comparison, in the control group that received T cell depleted bone marrow, 7 of 19 patients experienced PTLD from donor origin. Of these 7 patients, this was the cause of death in 4 cases.[15, 16] In a large-scale preclinical study using mobilized PBSC from five healthy donors, Barfield *et al* demonstrated that simultaneous negative depletion of T and B lymphocytes using the CliniMACS CD3/CD19 Separation System can be achieved, leaving graft facilitating cells and immune effector cells like NK cells in the graft, allowing for the potential benefit of $CD34^-$ stem cells, NK cells and other cell populations, which may contribute to anti-leukemic effects and may support immune reconstitution.[17]

The first clinical trial using CD3 depleted grafts and Rituximab as *in vivo* B-cell depletion to prevent PTLD was performed in 20 children with hematological malignancies.[18] The conditioning regimen was myeloablative and TBI based. Of 19 evaluable patients all engrafted, the TRM rate was 30%, 20% of the patients died due to disease recurrence and 50% of the patients were alive at 3 years follow up.

The high need for the development of a haploidentical transplant protocol with reduced toxicity for children, elderly patients, heavily pretreated and comorbid patients remained. This also encompassed the need of reaching stable engraftment, reducing transplant related mortality (TRM), especially TRM due to infections, and providing improved anti-leukemic effects possibly, by retaining NK cells in the graft. To address these problems, recently an alternative method for haploidentical stem cell transplantation using reduced intensity conditioning (RIC) in combination with OKT-3 (an immunosuppressive drug) was developed.[19, 20]

Engraftment rates in adult and pediatric patients of more than 90% were described for the CD3/CD19 depletion approach combined with RIC in haploidentical transplantation. The reconstitution of T and B cells started early, within the first 2 months post-transplantation.[21, 21, 22]

NK cells recovered early and fast. Low TRM rates between 2.6% and 25% were reported for children.[23, 24] Data published for adult patients describe a cohort of 61 heavily pretreated patients with advanced disease. Transplant related mortality (TRM) was 23% at day 100 post-transplantation and 42% after 2 years. Primary engraftment was fast. In comparison to data from patients transplanted with $CD34^+$-selected haploidentical grafts, T cell reconstitution appears to have been faster. The number of $CD34^+$ cells transplanted ranged from 3.2 to 22 x 10^6/kg BW of the recipient. With this approach, adult patients also suffering from non-malignant and malignant diseases with a higher risk of relapse and other comorbidities could be treated. Incidences and degree of GVHD after haploidentical transplantation with CD3/CD19 depleted grafts was higher compared to the low incidences of <10% in patients receiving CD34 depleted grafts.[25] This may be related to the less effective T cell depletion as compared to the $CD34^+$-enrichment approach. It should be noted that, because of short follow-up periods of closed clinical trials or due to ongoing enrollment in open clinical trials, the number of publications describing clinical data on immune reconstitution, occurrence of GVHD and infectious complications and outcome of transplantation using CD3/CD19 depleted grafts is limited.

It has been shown, however, that haploidentical transplantation using T cell and B cell depleted grafts in combination with a reduced intensity conditioning regimen is safe and feasible in adult and pediatric patients. The published data indicate, that the combination of a CD3/CD19 depleted graft and the conditioning regimen has a positive impact on time to engraftment, immune reconstitution and TRM in children and adults.[20, 22, 24, 25] In the adult setting the low toxicity of this approach allows its use even in the elderly and pretreated patients, and immune reconstitution seems to be improved in comparison to data from $CD34^+$ selected grafts. Since active depletion of $CD3^+$ T cells from the graft by using the CD3/CD19 method is less stringent as compared to the $CD34^+$ enrichment, post-transplantation application of a moderate pharmacologic GVHD prophylaxis (MMF) has been used in patients when the T cell content of the graft was above specific thresholds defined by the investigators.[20]

To further improve outcomes of haploidentical transplantation, a new method for the depletion of unwanted T cells triggering GVHD was developed, namely the selective depletion of TCRα/β$^+$ T cells, which constitute a subpopulation of CD3$^+$ cells. Depletion of TCRα/β$^+$ T cells in this setting is very efficient and selective with a 4.5 – 5 log T cell reduction.[26] The efficiency of depletion is comparable to that achieved with CD34$^+$ cell enrichment. This may allow further reduction of pharmacological immunosuppression post-transplantation including its numerous negative short- and long-term side effects. This method of combined TCRα/β$^+$ and CD19$^+$ cell depletion leads to stem cell grafts, which strongly resemble unmanipulated preparations. For example, CD3$^+$TCRγ/δ$^+$ T cells are preserved which are removed with the previously described approach using CD3$^+$/CD19$^+$ depletion.[27]

In-vitro, when TCRγ/δ$^+$ T cells were cultivated in co-culture with primary leukemia blasts they proliferated and exhibited cytotoxicity against the blasts.[28] In addition, TCRγ/δ$^+$ T cells and NK cells have been shown to synergistically enhance their anti-leukemic and anti-viral effector cell function *in vitro*.[29] Furthermore, it has been reported that TCRγ/δ$^+$ T cells seem to exert anti-leukemic effects in partially mismatched PBMC *in vivo*. In a retrospective analysis, patients with a high number of TCRγ/δ$^+$ T cells post-transplantation had an improved 5-year survival compared to those with normal or low counts (70.8% vs. 19.6%). Therefore, a high content of TCRγ/δ$^+$ T cells in the graft might have a beneficial effect on survival.[30] Since TCRγ/δ$^+$ T cells co-express CD3, but do not present the α/β T cell receptor (TCRα/β), they do not provoke GVHD driven by alloantigen T cell receptor activation. Additionally, TCRγ/δ$^+$ T cells may support engraftment, may provide protection against infections and bridge the time to the immune reconstitution of TCRα/β$^+$ T cells by providing protection against infections and they may exert GVT effects.[31, 32, 33] It has been shown, that similar effects are exerted by NK cells retained in the graft.[34]

In addition, the use of a reduced intensity conditioning based on fludarabine, thiotepa and melphalan could lead to reduction of TRM. Such an effect has already been observed in transplantations performed with the same conditioning regimen and the infusion of CD3$^+$/CD19$^+$ cell depleted peripheral blood stem cells.

Data from the first clinical applications of TCRα/β$^+$/CD19$^+$ depleted grafts in 23 heavily pre-treated and high-risk pediatric patients have been published. The results confirm the initial assumptions shown in the rapid and sustained hematopoietic engraftment, rapid immune reconstitution and low grades of GVHD (only 2 patients with grade 1 aGVHD).[35, 36]

During the EBMT 2012, in an oral presentation, Professor Lang reported the outcome of 24 heavily pre-treated and high-risk pediatric patients after transplantation with TCRα/β$^+$/CD19$^+$ depleted grafts. Rapid and sustained hematopoietic engraftment and rapid immune reconstitution were observed in these patients as expected by previous reports. One of the 24 patients developed GVHD grade IV (4%), three patients developed GVHD grade III (12%), three patients developed GVHD grade II (12%), 12 patients developed GVHD grade I (50%) and in 5 of the 24 patients, no GVHD was observed (21%). In evaluating these results it is important to note that 22 patients of these 24 patients were treated without pharmacological immune suppression post-transplantation.

First results of haploidentical transplantation with myeloablative conditioning and TCRα/β$^+$/CD19$^+$ depleted graft were reported during ASH Annual Meeting 2012 (ClinicalTrials.gov Identifier: NCT01810120); 23 patients with hematological malignancies and nonmalignant disorders were transplanted, all patients engrafted with a median time to reach 500 neutrophils/µl and 50.000 platelets/µl of 12 days (range 10-16) and 13 days (range 12-18), respectively. Two patients developed grade I/II acute GVHD. With a median follow up of 6 months (range 2-8) no TRM or EBV related PTLD occurred.[37]

Since introduction of the CliniMACS® TCRα/β Reagent Kit clinical trials using TCRα/β$^+$/ CD19$^+$ depleted grafts in allogeneic transplant settings have been initiated in Europe. It should be noted, that because of short follow-up periods of closed clinical trials or due to ongoing enrollment in open clinical trials the number of publications describing clinical data is limited.

Additionally, following the depletion of TCRα/β$^+$ T cells using the ClinicMACS TCRα/β Reagent Kit, the use of the CliniMACS® CD19 Reagent is referenced for B cell depletion when clinically indicated, however, please note that *in vivo* methods for B cell depletion (i.e. Rituximab) may be considered by the investigator.

Lymphocyte infusion

The CliniMACS® TCRα/β Reagent Kit may be used to generate lymphocyte infusions depleted of TCRα/β$^+$ T cells.

Donor lymphocyte infusion after allogeneic transplantation

Unmodified donor lymphocyte infusions (DLI) have been used in T cell depleted and repleted allogeneic transplantation in case of decreasing donor chimerism, signs of minimal residual disease and therapy refractory infections.[38, 39, 40, 41] GVHD remains the most common and serious adverse event of unmodified DLI after allogeneic transplantation.[42] Different strategies have been used with the aim to dissociate the beneficial effects of DLI´s such as graft versus malignancy activity, from the unwanted, harmful effects, such as GVHD. These approaches encompass the use of escalating doses of DLI´s, CD8$^+$ T cell depleted DLI´s, CD3$^+$ T cell depleted DLI´s and NK cell DLI´s.[40, 43, 44]

The depletion of TCRα/β$^+$ T cells with the CliniMACS® TCRα/β Reagent Kit is very efficient and NK cells and TCRγ/δ$^+$ T cells seem to exert anti-leukemic effects without inducing GVHD. Clinical investigators are interested in performing clinical trials using TCRα/β$^+$ T cell depleted DLI´s for the prevention or treatment of minimal residual disease or decreasing donor chimerism after allogeneic transplantation.

At the time of publication, literature regarding the clinical use of TCRα/β$^+$ T cell depleted DLI´s administered after allogeneic stem cell transplantation was not available.

Donor lymphocyte infusion in the non-transplant setting

Lymphocyte infusions with separated or enriched donor or patient derived NK cells in the non-transplant setting are under clinical investigation in patients with hematologic diseases and solid tumors. The aim of these trials is to investigate the anti-leukemic and anti-tumor effects displayed by NK cells, which are part of the innate immunity. NK cell products can be generated by direct enrichment of NK cells via the CliniMACS CD56 Separation System, by T

cell depletion with the CliniMACS CD3 Separation System followed by CD56 enrichment, by expansion protocols, or by passive enrichment of NK cells by depleting potentially harmful $CD3^+$ T cells using the CliniMACS CD3 Separation System.[45, 46]

Results of clinical trials which have been published to date indicate that the application of NK cell products in the non-transplant setting is feasible and safe and no GVHD induction was observed.[47, 48, 49, 50]

Preclinical studies and retrospective analysis of clinical trials have shown that TCRγ/δ T cells display anti-leukemic and anti-tumor effects as well. The depletion of $TCR\alpha/\beta^+$ T cells with the CliniMACS® TCRα/β Reagent Kit is very efficient, resulting in a cellular product depleted of potentially harmful $TCR\alpha/\beta^+$ T cells, without affecting NK cells, NK-T cells and $TCR\gamma/\delta^+$ T cells.[26]

Clinical investigators are interested in performing clinical trials using $TCR\alpha/\beta^+$ T cell depleted DLI's for the treatment of hematologic diseases and solid tumors. At the time of publication, literature regarding the clinical use of $TCR\alpha/\beta^+$ T cell depleted DLI's for the treatment of malignant diseases was not available.

2.3. *Investigational Product*

The CliniMACS® TCRα/β Reagent Kit is used to deplete human $TCR\alpha/\beta^+$ T cells *in vitro* from heterogeneous hematological cell populations for stem cell transplantation and lymphocyte infusions in cases where this is clinically indicated.

Each CliniMACS® TCRα/β Reagent Kit is sufficient to label up to 24×10^9 $TCR\alpha/\beta^+$ T cells out of 60×10^9 total white blood cells using an indirect labeling strategy. The CliniMACS® TCRα/β Reagent Kit is used with the CliniMACS® System in conjunction with the following CliniMACS® components.

- CliniMACS TCRα/β Reagent Kit
  - CliniMACS® TCRα/β-Biotin – a sterile solution containing biotinylated monoclonal antibodies specific for human $TCR\alpha/\beta^+$ T cells
  - CliniMACS® Anti-Biotin Reagent – a sterile solution containing monoclonal antibodies specific for the biotin antigen, covalently linked to dextran beads having an iron oxide/hydroxide core.
- CliniMACS® ^{plus} Instrument – a software controlled instrument that processes the blood sample (cell product)
- CliniMACS® Depletion Tubing Set (DTS) – a single-use, sterile, disposable tubing set with proprietary cell selection columns
- CliniMACS® PBS/EDTA Buffer – a sterile, isotonic phosphate-buffered, 1 mM EDTA, saline solution, used as external wash and transport fluid for the *in vitro* preparation of blood cells

A brief description of each component is provided below. Additional items required for use with the CliniMACS® TCRα/β Reagent Kit include: transfer bags, luer connectors, a Pall filter, IgG and human serum albumin (HSA). These items are not part of the CliniMACS TCRα/β Reagent Kit and must be provided by the clinical site.

2.3.1. CliniMACS® TCRα/β-Biotin

The CliniMACS® TCRα/β-Biotin consists of biotinylated TCRα/β mAb which is a mouse IgG_{2b} monoclonal, produced by the hybridoma cell line clone BMA031.H7.

CliniMACS® TCRα/β-Biotin is a sterile solution intended for the indirect magnetic labeling of human TCRα/β^+ T cells, in combination with the CliniMACS® Anti-Biotin Reagent. It is supplied in single use vials which contain nominally 7.5 mL per vial. The vials used are 10 mL glass injection vials sealed with a chlorobutyl rubber stopper and flip-off aluminum seal.

2.3.2. CliniMACS® Anti-Biotin Reagent

The CliniMACS® Anti-Biotin Reagent is composed of the Anti-Biotin monoclonal antibody chemically coupled to iron-dextran beads and is used as a secondary labeling antibody in conjunction with CliniMACS® TCRα/β-Biotin in the CliniMACS® TCRα/β Reagent Kit. After the indirect labeling, target cells are isolated using the CliniMACS® plus Instrument.

The CliniMACS® Anti-Biotin Reagent is a dark brown to black, non-viscous, colloidal solution containing the antibody conjugate in buffer. The conjugate consists of a mouse IgG_1 monoclonal antibody (specific for the biotin antigen) covalently linked to dextran beads having an iron oxide/hydroxide core. The CliniMACS® Anti-Biotin Reagent is supplied sterile in 10R glass vials. Each vial contains 7.5 mL of the CliniMACS® Anti-Biotin Reagent. It is intended for single and *in vitro* use only.

2.3.3. CliniMACS® plus Instrument

The CliniMACS® plus Instrument is an electro-mechanical instrument that controls the processing of the donor leukapheresis sample after it has been labeled with a CliniMACS® Reagent(s). Resident software on a PC controller board in the instrument console controls the operation of the electro-mechanical components of the instrument during a cell processing run.

2.3.4. CliniMACS® Depletion Tubing Set (DTS)

The CliniMACS Tubing Sets are a combination of PVC and silicone tubing lengths with plastic connectors and appendices as necessary to meet the design requirements. Two cell selection columns (CliniMACS Columns) are placed at designated locations to pre-filter the cell sample and isolate the labeled cells respectively.

2.3.5. CliniMACS® PBS/EDTA Buffer

The CliniMACS PBS/EDTA Buffer is an isotonic, clear, and colorless solution supplied sterile in one liter bags. The bags are made of medical grade materials designed for sterile solutions. The CliniMACS PBS/EDTA Buffer is formulated in a controlled environment and is terminally sterilized by autoclaving. All buffer components are USP and European Pharmacopoeia grade and tested according to these following specifications.

Table 3: Formulations of the CliniMACS® PBS/EDTA Buffer

Ingredient	Compendial	Amount
NaCl	Ph. Eur. / USP	8.0 g/L
KCl	Ph. Eur. / USP	0.19g/L
Na_2HPO_4 anhy.	Ph. Eur. / USP	1.15g/L
KH_2PO_4	Ph. Eur. / USP	0.19g/L
Na_2EDTA	Ph. Eur. / USP	0.37g/L
Water for Injection	Ph. Eur. / USP	*ad* 1L

The CliniMACS PBS/EDTA Buffer is used as external wash and transport fluid for the *in vitro* preparation of human heterogeneous cell populations intended to be separated using the CliniMACS® TCRα/β Reagent Kit in combination with the CliniMACS® System.

2.3.6. Principles of Operation

The CliniMACS® TCRα/β Reagent Kit is used to deplete human TCRα/β^+ T cells from heterogeneous cell populations. The detailed procedures are provided in the CliniMACS User Manual (US-Edition) that accompanies the CliniMACS® plus Instrument. Provided below is a brief summary of the procedure. The depletion process involves two phases: magnetic cell labeling of the TCRα/β^+ T cells prior to depletion (phase 1) and the automated cell depletion process (phase 2).

Phase I – Magnetic Labeling

The first phase is the two step indirect cell labeling procedure. The first step of the labeling procedure involves combining the CliniMACS® TCRα/β-Biotin with the cellular harvest from the donor or patient. The blood product is incubated for thirty minutes at room temperature with the CliniMACS® TCRα/β-Biotin during which time the antibodies selectively bind to cells expressing the TCRα/β antigen. The mixture is then washed twice in the CliniMACS® PBS/EDTA buffer that is supplemented with HSA by the user to remove excess conjugate. The volume is adjusted and the next step involves labeling the blood product with the CliniMACS® Anti-Biotin Reagent (the secondary antibody, anti-biotin, is covalently linked to dextran beads having an iron oxide/hydroxide core). The blood product is again incubated for thirty minutes at room temperature with the reagent during which time the antibodies selectively bind to the biotin labeled cells. The mixture is then washed once in the CliniMACS® PBS/EDTA buffer (supplemented with HSA). Following centrifugation, the resulting cell pellet is resuspended in the CliniMACS® PBS/EDTA buffer (supplemented with HSA) and the labeled product is ready for cell depletion using the CliniMACS® Depletion Tubing Set.

Phase II – CliniMACS® Run

Using CliniMACS® Depletion Tubing Set

In combination with software sequence 3.1 on CliniMACS® plus Instrument, the bag containing the labeled cell product is attached to the sterile CliniMACS® Depletion Tubing Set (REF 261-01). Following a series of automated priming steps, the cell product is passed through a blood filter to remove any cell aggregates that may be present. Using the CliniMACS Depletion Tubing Set in combination with a depletion program, the cells are applied onto the Selection Column twice. First, cells are applied in a bulk load and then applied a second time using a sensitive load.

The pump draws the cells into the tubing set where the magnetically labeled cells are retained on the Selection Column placed in the magnetic field, while unlabeled cells pass through and are collected in the Reapplication Bag. If the number of magnetically labeled cells (calculated by the internal computer) exceeds the binding capacity of the Selection Column, the separation program automatically loads and separates the cell sample in smaller portions. Sample loading is stopped when the capacity of the Selection Column is reached and the separation program then proceeds to the next step of the separation sequence.

Between the staged loading and sensitive loading stage, the Selection Column is washed to remove all labeled cells. The labeled cells are eluted and held in the Non-Target Cell Bag. For further depletion, the cells held in the Reapplication Bag are loaded onto the Selection Column a second time and the unlabeled cells flow through the magnetic field and are collected in the Cell Collection Bag. The magnetic field is removed and labeled cells are eluted into the Non-Target Cell Bag.

The target fraction, depleted of TCRα/β^+ T cells, can be used immediately after analysis or cryopreserved for later infusion into the patient. All parts of the CliniMACS® TCRα/β Reagent Kit and CliniMACS® System are non-invasive in all aspects involving processing of the cellular harvest and are not connected to the patient at any time.

3. OVERVIEW OF THE CLINIMACS® TCRα/β REAGENT KIT

Miltenyi Biotec GmbH - Clinical Products has developed a cell separation technology utilizing paramagnetic iron dextran beads that are coupled with monoclonal antibody. The CliniMACS® TCRα/β Reagent Kit provides the user the ability to use the MACS technology for clinical applications for the separation of specific cell populations and has potential clinical utility in various applications including the *in vitro* depletion of TCRα/β$^+$ T cells from heterogeneous hematological cell populations for stem cell transplantation and lymphocyte infusions in cases where this is clinically indicated.

Each CliniMACS® TCRα/β Reagent Kit is sufficient to label up to 24 x 10^9 TCRα/β$^+$ T cells out of 60 x 10^9 total white blood cells using an indirect labeling strategy. The CliniMACS® TCRα/β Reagent Kit is used with the CliniMACS® System in conjunction with the following CliniMACS® components.

- CliniMACS TCRα/β Reagent Kit
  - CliniMACS® TCRα/β-Biotin – a sterile solution containing biotinylated monoclonal antibodies specific for human TCRα/β$^+$ T cells
  - CliniMACS® Anti-Biotin Reagent – a sterile solution containing monoclonal antibodies specific for the biotin antigen, covalently linked to dextran beads having an iron oxide/hydroxide core.
- CliniMACS® ^{plus} Instrument – a software controlled instrument that processes the blood sample (cell product)
- CliniMACS® Depletion Tubing Set (DTS) – a single-use, sterile, disposable tubing set with proprietary cell selection columns
- CliniMACS® PBS/EDTA Buffer – a sterile, isotonic phosphate-buffered, 1 mM EDTA, saline solution, used as external wash and transport fluid for the *in vitro* preparation of blood cells

Miltenyi Biotec GmbH – Branch Teterow of Teterow, Germany (a division of Miltenyi Biotec GmbH) and Miltenyi Biotec GmbH of Bergisch Gladbach, Germany are the sites of manufacture and Miltenyi Biotec GmbH - Clinical Products of Bergisch Gladbach, Germany (a wholly owned subsidiary of Miltenyi Biotec GmbH) has primary responsibility for production and quality control of all components.

3.1. *CliniMACS® TCRα/β-Biotin*

The CliniMACS® TCRα/β-Biotin consists of biotinylated TCRα/β mAb which is a mouse IgG_{2b} monoclonal, produced by the hybridoma cell line clone BMA031.H7. The concentration of the final filled conjugate can range from 140 to 180 micrograms (µg) of antibody protein per mL.

The CliniMACS® TCRα/β-Biotin is buffered in a phosphate-buffered saline (PBS) containing ethylene diamine tetraacetic acid (EDTA) and Poloxamer 188. The nominal concentrations of its components are 0.0095 M Phosphate, 0.004 M Potassium, 0.163 M Sodium, 0.139 M Chloride, 0.005 M EDTA and 0.03% (w/v) Poloxamer 188. Poloaxmer 188 is added to the CliniMACS® TCRα/β-Biotin to stabilize it during shipping, handling and storage. The pH is 7.3-7.7.

The CliniMACS® TCRα/β-Biotin is supplied sterile and endotoxin free in glass vials containing 7.5 mL conjugate. The sterile CliniMACS® TCRα/β-Biotin is tested and released according to release specifications.

3.1.1. BMA031.H7 Monoclonal Antibody

Miltenyi Biotec GmbH - Clinical Products (MBCP) has developed an in-house clone (BMA031.H7) adapted to protein-free media and has established cGMP production of the antibody (including cell-culture, purification and testing) for the production of the CliniMACS® TCRα/β-Biotin. The starting BMA031 hybridoma cell line was purchased from Behringwerke AG. This murine hybridoma cell line had been manufactured by fusing P3x63Ag8.653 mouse myeloma cells with splenocytes from Balb/c mice and immunized with human peripheral blood lymphocytes and produces an IgG_{2b} monoclonal antibody directed against human $TCR\alpha/\beta^+$ T cells in peripheral blood, bone marrow and peripheral lymphoid tissues.

3.2. *CliniMACS® Anti-Biotin Reagent*

The CliniMACS® Anti-Biotin Reagent is composed of the Anti-Biotin monoclonal antibody chemically coupled to iron-dextran beads and is used as a secondary labeling antibody in conjunction with CliniMACS® TCRα/β-Biotin in the CliniMACS® TCRα/β Reagent Kit. After the indirect labeling, target cells are isolated using the CliniMACS® plus Instrument.

The concentration of the final filled conjugate is in the range of 20 to 52 micrograms (µg) of antibody protein per vial, 800 µg/mL of dextran and 800 µg/mL of iron (as Fe^{II}/Fe^{III} hydroxide-oxide polymer). The colloid is buffered in a phosphate-buffered saline (PBS) containing ethylenediaminetetraacetic acid (EDTA) and Poloxamer 188. The nominal concentrations of its components are 0.0095 M phosphate, 0.004 M potassium, 0.163 M sodium, 0.139 M chloride, 0.005 M EDTA; 0.03% Poloxamer 188 (w/v). Poloxamer 188 is added to the CliniMACS Anti-Biotin Reagent to stabilize it during shipping, handling and storage. The pH is 7.1– 7.9.

The CliniMACS Anti-Biotin Reagent is supplied sterile in 10R glass vials. Each vial contains 7.5 mL of the CliniMACS Anti-Biotin Reagent. The sterile CliniMACS® Anti-Biotin Reagent is tested and released according to release specifications.

3.2.1. Bio3-18E7 Monoclonal Antibody

Miltenyi Biotec GmbH - Clinical Products (MBCP) has developed an in-house clone (Bio3-18E7) adapted to protein-free media and has established cGMP production of the antibody (including cell-culture, purification and testing) for the production of the CliniMACS® Anti-Biotin Reagent. The starting Bio3-18E7 hybridoma cell line was derived by fusion of the Sp2/0 Ag 14 myeloma cell line with spleen cells from a BALB/c mouse that were immunized with a Keyhole Limpet Hemocyanin (KLH)-Biotin Aluminum hydroxide (Alum) precipitate. The cell line was developed by Miltenyi Biotec GmbH. It produces an IgG_2 monoclonal antibody directed against positive human T lymphocytes.

3.3. *CliniMACS® Depletion Tubing Set*

The CliniMACS® Depletion Tubing Set (DTS) consists of a tubing element combined with a separation column (28mm diameter). The separation column consists of a Grilamid® TR90 (polyamide derivate) column housing with polypropylene frits at each end. The interior of the column housing is filled with a matrix of submillimeter sieved iron shot coated with epoxy resin lacquer. The column is located at an appropriate place in the tubing set to facilitate the cell separation process and the column performs the actual cell separation in a magnetic field.

The CliniMACS® DTS consists of a series of tubes, connectors, spikes, Luer locks, and collection bags. The principal constituents are polyvinyl chloride (PVC) for tubes, bags and some connectors, and silicone for the pump tube. Some connectors are made of Acrylonitrile-butadiene-styrene (ABS). The silicone pump tubing is softened with petroleum ether for assembly. PVC tubes are solvent bound with cyclohexanone.

The CliniMACS® DTS has been qualified for use according to applicable guidelines as indicated in Table 4. For TCRα/β^+ T cell depletion applications performed in combination with software sequence 3.1, the CliniMACS Depletion Tubing Set must be used.

The CliniMACS® Depletion Tubing Set is packed in a thermoform tray and heat-sealed with a Tyvek® lid. The DTS is sterilized by ethylene oxide gas and supplied as a single-use component for the depletion of TCRα/β^+ T cells in combination with the CliniMACS® TCRα/β Reagent Kit and the CliniMACS® ^{plus} Instrument.

3.4. *CliniMACS® PBS/EDTA Buffer*

The CliniMACS® PBS/EDTA Buffer is an isotonic, clear, and colorless solution supplied sterile in one liter bags. The bags are made of medical grade materials designed for sterile solutions. The CliniMACS® PBS/EDTA Buffer is formulated in a controlled environment under a quality system and is terminally sterilized by autoclaving. All substances are USP and/or European Pharmacopoeia grade and tested according to these specifications.

The CliniMACS® PBS/EDTA Buffer is packaged in bags made of a laminated polypropylene (PP) material (Nexcel® formerly called Cryovac®) specifically designed and developed for sterile solutions. The buffer is double-bagged to minimize damage to the bags. The CliniMACS® PBS/EDTA Buffer is designed and manufactured in such a way to alleviate any unwanted by-products. Such unwanted by-products could be introduced by the raw materials used in manufacturing, by the container or through the autoclaving (sterilization) process. Compendial grade materials are used for formulation and manufacturing in a controlled environment suitable for the manufacture of sterile solutions. The PP bags comply with European Pharmacopoeia requirements for blood bags. Nexcel® material is tested according to USP, EP, ISO 10993, and JP. (The FDA Master File Number is MF-9075 Type III).

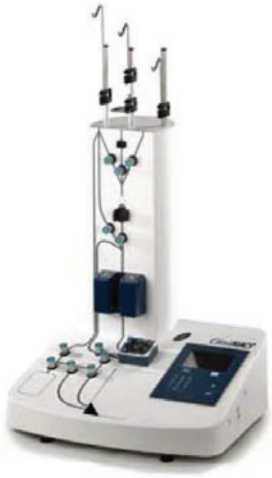
Although the heterogeneous white blood cells are collected in a leukapheresis process that inhibits clotting during collection, the PBS also contains EDTA to preclude clotting during subsequent-cell processing.

The CliniMACS® PBS/EDTA Buffer is a component of the CliniMACS® TCRα/β Reagent Kit that facilitates, but does not in itself take an active part in the separation of the TCRα/β$^+$ T cells from a population of heterogeneous mononuclear cells.

3.5. *CliniMACS® plus Instrument*

The CliniMACS® plus Instrument is an electro-mechanical instrument that controls the processing of the patient sample after it has been labeled with the CliniMACS® TCRα/β-Biotin and the CliniMACS Anti-Biotin Reagent. Resident software on a PC controller board in the instrument console controls the operation of the electro-mechanical components of the instrument during a cell processing run. The result of the processing run is to deplete the patient's labeled TCRα/β$^+$ T cells. A picture of the CliniMACS® plus Instrument is provided in Figure 1.

Figure 1: The CliniMACS® plus Instrument



Dosage and Handling: CliniMACS® TCRα/β Reagent Kit

3.6.1. Labeling Preparation

The CliniMACS® TCRα/β Reagent Kit, comprised of one vial of CliniMACS® TCRα/β-Biotin and two vials of CliniMACS® Anti-Biotin Reagent, is sufficient for depleting up to 24 x 10^9 TCRα/β$^+$ T cells out of a total cell number of 60 x 10^9 cells (WBC). The CliniMACS® Depletion Tubing Set must be used in combination with the CliniMACS® TCRα/β Reagent Kit .

Both the CliniMACS® TCRα/β-Biotin and the CliniMACS® Anti-Biotin Reagents are for single use only and should be stored at 2 - 8 °C and never frozen.

Prior to opening a vial of CliniMACS® TCRα/β-Biotin or CliniMACS® Anti-Biotin Reagent, inspect the cover for puncture or tears. A damaged cover could signal that the vial of reagent is no longer sterile and should not be used for cell depletion.

4. NON-CLINICAL AND TOXICOLOGY STUDIES

4.1. *Overview*

The CliniMACS® TCRα/β-Biotin consists of biotinylated TCRα/β mAb, which is a mouse IgG_{2b} mAb produced by the hybridoma cell line clone BMA031.H7. The BMA031.H7 antibody is a mouse IgG_{2b} monoclonal antibody directed towards an epitope of the human TCRα/β antigen.

The CliniMACS® Anti- Biotin Reagent consists of Bio3-18E7 mouse IgG_1 antibody bound to iron dextran beads and is used to deplete $TCR\alpha/\beta^+$ T cells, previously labeled with the CliniMACS® TCRα/β-Biotin Reagent, from heterogeneous hematologic cell populations. The iron dextran beads are highly purified colloidal dextran beads having an iron oxide/hydroxide core. The Bio3-18E7 antibody is a mouse IgG_1 monoclonal antibody directed toward biotin conjugated to TCRα/β mAb in the primary TCRα/β Biotin.

The CliniMACS® TCRα/β Reagent Kit components are manufactured in accordance with the applicable International Harmonized Standards. The CliniMACS® TCRα/β-Biotin, CliniMACS® Anti-Biotin Reagent, CliniMACS® DTS Tubing Set, CliniMACS® ^{plus} Instrument and the CliniMACS® PBS/EDTA Buffer are tested according to these standards at the appropriate time during the manufacturing process. The CliniMACS® TCRα/β Reagent Kit complies with the appropriate essential requirements for device manufacture as described in Annex II of the Council Directive 93/42/EEC, *Medical Devices Regulations* and the applicable sections of US FDA Code of Federal Regulations Title 21. Miltenyi Biotec GmbH is annually inspected by Miltenyi Biotec's Notified Body (TÜV-PS) according to Annex II.3 of the MDD 93/42/EEC, ISO 13485, and ISO 9001 for the development, production, testing, and release of medical devices.

4.2. *Safety Testing of the TCRα/β and Anti-Biotin Monoclonal Antibodies*

This section summarizes the nonclinical or preclinical safety studies performed on the TCR and Anti-Biotin cell banks and the TCRα/β and Anti-Biotin monoclonal antibodies (mAb). All testing was performed in accordance with the FDA's Good Laboratory Practice (GLP) for Nonclinical Laboratory Studies (21CFR §58) and/or United Kingdom's GLP Compliance Program. Veterinary control, surveillance, processing, preservation, testing, and handling of all tissues, cells, and substances of animal origin were conducted according to established guidelines during qualification of the TCRα/β and Anti-Biotin cell lines for production of the murine monoclonal antibodies (mAb).

4.2.1. TCRα/β and Anti-Biotin Cell Banks

The TCRα/β and Anti-Biotin Master Cell Banks (MCB), Manufacturer's Working Cell Banks (MWCB), and End of Production Cell Banks (EPC) were developed and tested in conformance with US FDA's Points to Consider (PTC) for the Manufacture and Testing of Monoclonal Antibody Products for Human Use, February 27, 1997.

Viral, bacterial, fungal, and mycoplasma contamination, as well as species-specific viruses, retroviruses and adventitious agents were tested for each cell bank as identified in the PTC guidance document and the specifications for this testing are listed in the following tables. In addition, the authenticity of the TCRα/β cell line and the Anti-Biotin cell line was determined after testing at reference laboratories. The results indicate that the TCRα/β and Anti-Biotin antibody manufacturing process satisfies safety criteria with regard to viruses, other transferable agents, and authenticity.

Table 4: TCRα/β & Anti-Biotin Cell Banks Safety Testing According to FDA's Points To Consider (PTC)

Testing	Master Cell Bank	Manufacturer's Working Cell Bank	EPC End of Production Cells
1. Authenticity	Pass	Pass	Pass
2. Sterility, USP	Sterile	Sterile	Sterile
3. Mycoplasma	Negative	Negative	Negative
4. Thin Section Electron Microscopy (qualitative)	For Cell Line Characterization	Not Required	For Cell Line Characterization
5. *In vitro* virus assay	Negative	Not Required	Negative
6. *In vivo* virus assay	Negative	Not Required	Negative
7. MAP test	Negative	Not Required	Not Required
8. Viability (4 days post thaw)	Not Required	Pass	Not Required

4.2.2. TCRα/β Monoclonal Antibody – Clone BMA031.H7 and Anti-Biotin Monoclonal Antibody – Clone 18E07.7.4.4

The TCRα/β mAb and the Anti-Biotin mAb are tested in conformance with US FDA's Points to Consider (PTC) for the Manufacture and Testing of Monoclonal Antibody Products for Human Use, February 27, 1997.

Viral, bacterial, fungal, and mycoplasma contamination, as well as species-specific viruses, retroviruses and adventitious agents were tested as summarized below. The results indicate that the TCRα/β and Anti-Biotin antibody manufacturing purification process satisfies safety criteria with regard to viruses and other transferable agents.

Table 5: Safety Testing of the TCRα/β Monoclonal Antibody & Anti-Biotin Monoclonal Antibody According to FDA's PTC

Testing	Unprocessed Bulk TCRα/β or Anti-Biotin mAb	Purified Bulk TCRα/β or Anti-Biotin mAb
1. IgG isotype	For information only	[1]Mouse IgG_{2b} only [2]Mouse IgG_1 only
2. Bioburden	NMT 2 CFU/mL	Not Required
3. Endotoxin	For information only	NMT 2 EU/mL
4. Mycoplasma -cultivable -non-cultivable	Negative	Not Required
5. In vitro virus assay	Negative	Not Required
6. In vivo virus assay	Negative	Not Required
7. Transmission Electron Microscopy	For Information purposes	Not Required
8. Mouse DNA Probe Assay	Not Required	NMT 10 pg/mg IgG
9. Sterility	Not Required	Sterile

[1] Purified Bulk TCRα/β mAb
[2] Purified Bulk Anti-Biotin mAb

4.2.3. Virus Removal / Inactivation During the Purification Process for TCRα/β mAb and the Anti-Biotin mAb

Miltenyi Biotec, Inc. submitted a Viral Clearance Master File (BB-MF 11159) dated July 7, 2003. In this submission, Miltenyi Biotec, Inc. presented a justification for use of a single generic virus validation on common viral inactivation/removal steps that are used in the purification of all mAbs. This approach eliminates the need to perform virus validation on each individual mAb used in the CliniMACS Reagent System. Quantitation of starting viral load is performed on each manufactured bulk unpurified mAb to define the viral load specification. Additionally, a line comparison between each of the generic steps and the corresponding TCRα/β mAb and the Anti-Biotin mAb steps is included to demonstrate that the virus removal/inactivation steps for TCRα/β mAb and the Anti-Biotin mAb are identical to those generic steps stated in the Viral Clearance Master File.

4.3. *Safety Testing of the CliniMACS® TCRα/β-Biotin and the CliniMACS® Anti-Biotin Reagent*

Detailed toxicity studies have been undertaken to assess the safety of the CliniMACS® TCRα/β-Biotin and the CliniMACS® Anti-Biotin Reagent when delivered in dosages greater than the projected maximum human dose anticipated for clinical use.

Testing of the CliniMACS® TCRα/β-Biotin and the CliniMACS® Anti-Biotin Reagent has been performed based on ISO 10993, Biological Evaluation of Medical Devices, and included cytotoxicity, sensitization, systemic toxicity, local irritation study in rabbits and hemolysis. All testing resulted in an acceptable safety profile for the reagent. Biocompatibility testing as summarized in this section was performed with CliniMACS® TCRα/β-Biotin and the CliniMACS® Anti-Biotin Reagent. The testing of the CliniMACS® TCRα/β-Biotin was performed at Nelson Laboratories (Salt Lake City, Utah). Testing of the CliniMACS® Anti-Biotin Reagent was performed at Nelson Laboratories (Salt Lake City, Utah) and mdt (medical device testing, Ochsenhausen, Germany). The results of these studies are summarized below in Table 6.

Table 6: Summary of Toxicology and Studies using the CliniMACS® TCRα/β-Biotin and the CliniMACS® Anti-Biotin Reagent

Toxicological Test	Species/Method	Duration of Study	CliniMACS® TCRα/β-Biotin Results	CliniMACS® Anti-Biotin Reagent Results
Cytotoxicity	Mouse embryo fibroblasts	*In Vitro*/24-26 hours	Slight cytotoxicity (undiluted sample)	No cytotoxicity
Sensitization	Guinea Pig	ID/24 days Topical/24 days	No sensitization	No sensitization
Intracutaneous Irritation	Rabbit	IC/72 hours	Irritant (undiluted sample)	Slight
Systemic toxicity	Mice	IV/Single Dose	No systemic toxicity	No systemic toxicity
Hemocompatibility	Human blood	*Ex vivo*/3 hrs	Non-hemolytic	Non-hemolytic

4.3.1. Cytotoxicity

The purpose of this study was to examine the cytotoxic potential of the CliniMACS® TCRα/β-Biotin (USP 31[2008] <87>, including "Agar Diffusion Test") and the CliniMACS Anti-Biotin Reagent (USP 24-NF 19 <87>, Biological Reactivity Tests, *in vitro* pp. 1831-1832) using the agar overlay test). This test evaluates the cytotoxic potential of the diffusible components of the test material on cell culture monolayers. Mouse embryo fibroblast cells were cultured by standard compendial methods. An agar overlay was poured over the cell monolayers to act as a cushion from mechanical injury. The test material (TCRα/β-Biotin or Anti-Biotin Reagent) or the control material (CliniMACS® PBS/EDTA Buffer) were placed on top of the agar layer and incubated at 37°C for 24-26 hours. Cytotoxicity, if present, is scored as a degree of cellular damage or cytopathic effects.

The CliniMACS TCRα/β-Biotin gave a cytotoxicity score of 1 (some malformed or degenerate cells under the sample) compared to positive and negative control substances. The results from this study indicate that CliniMACS TCRα/β-Biotin meets USP requirements as none of the cell culture exposed to the sample showed more than a mild reactivity (grade 2). The reagent is slightly cytotoxic at a concentration that exceeds the maximum possible level during incubation with the blood and/or marrow cells from donor/patient. The reagent used in the assay is undiluted which is not the case in a real application where it is diluted 1:13.67 during cell labeling.

The CliniMACS Anti-Biotin Reagent gave a cytotoxicity score of 0 compared to positive and negative control substances. The results from this study indicate that the CliniMACS Anti-Biotin Reagent is not considered to be cytotoxic at a concentration that reflects the maximum levels of exposure during incubation with the blood and/or marrow cells from donor/patient.

4.3.2. Sensitization

The purpose of the Sensitization: Maximization Test ISO (Magnusson Kligman Method ASTM F720-81 [2002]) was to determine to what extent the test material (CliniMACS® TCRα/β-Biotin and the CliniMACS Anti-Biotin Reagent) has the potential for acting as a contact sensitizer in guinea pigs. The animals were initially exposed to the test material and the control solution in an induction phase lasting 1 week (± 1 day). In this phase, the test and controls solutions were given undiluted or emulsified with Freund's Complete Adjuvant (FCA) by intradermal injection (ID) on day one and topical exposure for 48 hours (± 2 h) to the ID sights on day seven. The animals were challenged 14 days (±1 day) after topical exposure with another topical exposure of test and control samples for 24 hours (± 2 h) to untreated sites. The animals were scored after 24 ± 2 h and 48 ± 2 h for skin reactions (e.g. erythema). Additionally, the animals exposed to the CliniMACS® Anti-Biotin Reagent were scored after 72 hours for skin reactions.

The test group animals, exposed to the undiluted CliniMACS® TCRα/β-Biotin, did not receive scores higher than negative control animals (exposed to CliniMACS® PBS/EDTA Buffer). According to the criteria of this test, the sensitization potential of the CliniMACS® TCRα/β-Biotin for the animals used in this study was classified as Grade I (no different than negative controls). Based on the results, the CliniMACS TCRα/β-Biotin does not produce contact sensitization and is considered to be a non-sensitizer (Grade I) according to established guidelines.

The test group animals, exposed to the undiluted CliniMACS Anti-Biotin Reagent, did not receive scores higher than negative control animals (CliniMACS® PBS/EDTA Buffer). According to the criteria of this test, the sensitization potential of the CliniMACS Anti-Biotin Reagent for the animals used in this study was classified as Grade I (no different than negative controls). Based on the results, the CliniMACS Anti-Biotin Reagent does not produce contact sensitization and is considered to be a non-sensitizer (Grade I) according to established guidelines.

4.3.3. Systemic Toxicity

Systemic toxicity of the CliniMACS® TCRα/β-Biotin was analyzed by the USP/ISO "Systemic Injection Test" (USP 31 [2008] <88> including "Systemic Injection Test"). This test evaluated

systemic responses to extracts of the test material (CliniMACS® TCRα/β-Biotin) or negative control (CliniMACS® PBS/EDTA Buffer) following injection into mice. Ten Swiss Webster albino mice (five per sample and five per corresponding control) were injected. Each animal was injected with 50 mL of diluted CliniMACS® TCRα/β-Biotin per kg of body weight (dilution 1:23) or control (CliniMACS® PBS/EDTA Buffer). The animals were observed immediately after and at 4 ± 0.75, 24 ± 2, 48 ± 2, and 72 ± 2 hours following injection.

No toxic signs were observed during the 72-hour clinical observation period in any of the mice injected. The results indicate no systemic toxicity was observed in any of the mice at levels that reflect approximately twenty times the maximum estimated clinical dose per infusion.

Systemic toxicity of the CliniMACS® Anti-Biotin Reagent was analyzed by the USP/ISO "Systemic Injection Test" (USP 31 [2008] <88> including "Systemic Injection Test"). This test evaluated systemic responses to extracts of the test material (CliniMACS® Anti-Biotin Reagent) or negative control (CliniMACS® PBS/EDTA Buffer with 0.03% Poloxamer) following injection into mice. Ten Swiss Webster albino mice (five per sample and five per corresponding control) were injected. Each animal was injected with 50 mL of the CliniMACS® Anti-Biotin Reagent per kg of body weight (dilution 1:25) or with control (CliniMACS® PBS/EDTA buffer). The animals were observed for toxic signs immediately after and at 4, 24, 48, and 72 hours following injection.

No toxic signs were observed during the 72-hour clinical observation period in any of the mice injected. The results indicate no systemic toxicity was observed in any of the mice at levels that reflect approximately twenty times the maximum estimated clinical dose per infusion.

4.3.4. Local Irritation Study in Rabbits

The purpose of the Intracutaneous (Intradermal) Reactivity Test (ANSI/AAMI BE78:2002) was to evaluate the potential of the CliniMACS® TCRα/β-Biotin to produce irritation following intracutaneous injections into two New Zealand white rabbits. The day of the injections, the fur along the spinal column of the rabbits was removed on both sides. Five injections of the test material (CliniMACS® TCRα/β-Biotin) or the corresponding control (CliniMACS® PBS/EDTA Buffer) were given intracutaneously to the rabbits at five distinct sites along the spinal column. The injection sites were observed immediately after the injection for erythema and edema and scored at 24, 48 and 72 hours.

All test animals (exposed to the CliniMACS® TCRα/β-Biotin) received a primary irritation index of 1.0 (0.97) and the difference between the test article and the control mean score was below 1 as required (mean score of the negative control was 0). The requirements of the ISO Intracutaneous (Intradermal) Reactivity Test were met by the TCRα/β-Biotin. The assay was conducted with the undiluted reagent as a worst case scenario as this will not be the case in a real application.

The purpose of the Intracutaneous (Intradermal) Reactivity Test (ANSI/AAMI BE78:2002) was to evaluate the potential of the CliniMACS® Anti-Biotin Reagent to produce irritation following intracutaneous injections into three New Zealand white rabbits. The day of the injections, the fur along the spinal column of the rabbits was removed on both sides. Five injections of the test material (CliniMACS® Anti-Biotin Reagent) or the corresponding control (CliniMACS®

PBS/EDTA Buffer) were given intracutaneously to the rabbits at five distinct sites along the spinal column. The injection sites were observed immediately after the injection for erythema, eschar formation, edema and necrosis and scored at 24, 48 and 72 hours.

All test animals (exposed to the CliniMACS® Anti-Biotin Reagent) received scores of 0-2 for each indices. These scores reflect zero to slight signs of irritation for direct contact with the CliniMACS Anti-Biotin Reagent. According to ISO Intracutaneous (Intradermal) Reactivity Test standards, the primary irritation response to the CliniMACS® Anti-Biotin was slight at levels that exceed human exposure by a very high factor. The assay was conducted with the undiluted reagent as a worst case scenario as this will not be the case in a real application.

4.3.5. Hemocompatibility

Hemolysis testing of the CliniMACS® TCRα/β-Biotin was conducted to determine the hemolytic potential of the reagent. The ASTM Hemolysis assay (ASTM F 756-00 [July 2000]) was conducted using anti-coagulated human blood containing a defined amount of hemoglobin. The tests were performed with appropriate controls. The test sample (CliniMACS® TCRα/β-Biotin) and negative control (CliniMACS® PBS/EDTA Buffer) were incubated for 3 hours at 37°C. The samples were then centrifuged and cyanmethemoglobin was added to the supernatant aliquot and the absorbance was read at OD_{540}. The concentration of hemoglobin released was calculated with the Lambert-Beer Law and the molar extinction coefficient of hemoglobin. Additionally the hemolytic index was calculated.

Test hemolytic index of test samples and negative controls was calculated using the regression output of the hemoglobin standard curve. The CliniMACS® TCRα/β-Biotin had a hemolytic index of 0.57 (non-hemolytic index; 0-2). These results indicate that the CliniMACS® TCRα/β-Biotin was non-hemolytic at the labeling concentration onto cells (dilution factor 13.7).

Hemolysis testing of the CliniMACS® Anti-Biotin Reagent was conducted to determine the hemolytic potential of the reagent. The ASTM Hemolysis assay (ASTM F756-00 [Draft, June 1999]) was conducted using anticoagulated human blood containing a defined amount of hemoglobin. The tests were performed with appropriate controls. The test sample (CliniMACS® Anti-Biotin Reagent) and negative control (CliniMACS® PBS/EDTA Buffer) were incubated for 3 hours at 37°C. The samples were then centrifuged at 700-800 x g for 5 minutes and the amount of hemoglobin released was calculated based on OD_{540} measurements and using a standard hemoglobin curve. The lowest OD_{540} of the standard curve was 0.018 corresponding to a hemoglobin concentration of 0.03 mg/ml (hemoglobin concentrations used for the standard curve: from 0.03 mg/ml to 1.44 mg/ml). The hemolytic index of the test sample and negative control were calculated using the regression output of the hemoglobin standard curve.

Results indicated that the reagent was non-hemolytic to blood. Test sample and negative control showed $0D_{540}$ values of 0.011; lower than the lowest value of the standard curve. Therefore, the CliniMACS® Anti-Biotin Reagent was not hemolytic to blood cells at a level that represents maximum human exposure.

4.4. *Safety Testing of the CliniMACS® Tubing Set, Separation Column, and PBS/EDTA Buffer*

4.4.1. Safety Testing of the CliniMACS® Tubing Set and Separation Column

This section summarizes the nonclinical or preclinical safety studies performed on the CliniMACS Tubing Sets and CliniMACS Column. All testing was performed in accordance with the FDA's Good Laboratory Practice (GLP) for Nonclinical Laboratory Studies (21CFR §58) and/or United Kingdom's GLP Compliance Program. Veterinary control, surveillance, processing, preservation, testing, and handling of all tissues, cells, and substances of animal origin were conducted according to established guidelines during qualification of the CliniMACS Tubing Set and CliniMACS Column.

CliniMACS® Tubing Set

The CliniMACS Tubing Set and the CliniMACS Column were tested for biocompatibility in two independent series of tests. The selection and methodology of the tests performed were based on the guidance found in the Center for Devices and Radiological Health's General Program Memorandum #G95–1 on Use of International Standard ISO 10993, Biological Evaluation for Medical Devices: Part 1 – Evaluation and Testing. Following ISO 10993, the CliniMACS Tubing Set was defined as an external communicating device that makes contact with circulating blood for limited duration (< 24 hours).

The tests conducted in accordance with the standard were:

- Cytotoxicity
- Hemolysis
- Intracutaneous Irritation
- Systemic Toxicity
- Sensitization

In accordance with guidance from ISO/DIS 10993–12, an extract was prepared from the tubing used in the CliniMACS Tubing Set. The complete CliniMACS Tubing set, minus columns and bags, was cut into 4.0 cm sections and incubated in approximately 345 mL of sterile PBS at 37°C for 72 hours. The volume corresponds approximately to the internal volume of the CliniMACS Tubing Set. Biocompatibility testing was conducted within 24 hours of extraction. This testing was conducted on the Standard Scale Tubing Set. However, because the raw materials, construction and the function of the Standard and Large Scale Tubing Sets are virtually identical, the testing is applicable to both CliniMACS Tubing Sets. The CliniMACS Depletion Tubing Set is manufactured with raw material components and with the same manufacturing procedure and facilities as used for the Standard Tubing Set. All testing was performed in accordance with 21 CFR §58, Good Laboratory Practice for Nonclinical Laboratory Study. The results were non–toxic in all cases.

Table 7: Summary of Toxicology and Biocompatibility Studies Conducted With Eluate[a] Collected from the CliniMACS® Tubing Set			
Test	**Species/Method**	**Duration of Study**	**Results**
Cytotoxicity	MEM Elution Mouse embryo fibroblasts	*In Vitro*/ 48 hours *In Vitro*/ 24 hours	No Cytotoxicity No Cytotoxicity
Hemolysis	Human Blood Rabbit Blood	*Ex Vivo*/1 hour *Ex Vivo*/1 hour	Not Hemolytic Not Hemolytic
Intracutaneous Irritation	Rabbit	IC/72 hours	No Irritation
Systemic Toxicity	Mice	IV/ Single Dose	No Toxicity
Sensitization Study	Guinea Pig	ID/24 days Topical/24 days	No Sensitivity

IV: Intravenous:; ID: Intradermal; IC: Intracutaneous
a: The eluate was Phosphate Buffered Saline incubated in the CliniMACS System Tubing Set for 72 hours at 37°C.

Separation Column (Grilamid® TR90 Column Material)

Column eluate studies were undertaken to characterize the safety profile of the potential extractables from the CliniMACS Column used in the CliniMACS Tubing Set. The results, summarized in Table 8, indicated no toxic responses to the CliniMACS Column (with the Ultem® Column Housing).

Table 8: Summary of Toxicology and Biocompatibility Studies Conducted With Eluate[a] Collected from the CliniMACS® Column (with the Ultem® Column Housing)			
Test	**Species/Method**	**Duration of Study**	**Results**
Cytotoxicity	Mouse embryo fibroblasts	*In Vitro*/ 48 hours	No Cytotoxicity
Hemolysis	Human Blood	*Ex Vivo*/1 hour	Not Hemolytic
Intravenous Irritation	Rabbit	IV/ Single Dose (8 Days)	No Irritation
Systemic Toxicity	Mice	IV/ Single Dose (72 Hours)	No Toxicity
Sensitization Study	Guinea Pig	ID/24 days and Topical/24 days	No Sensitivity

IV: Intravenous; ID: Intradermal; IC: Intracutaneous
a: The eluate was Dextrose Lactated Ringers Solution incubated in the CliniMACS System Tubing Set for 72 hours at 37°C.

After the original biocompatibility test was completed on the CliniMACS Column, the plastic material used to form the column housing was changed from Ultem® to Cristamid® to Grilamid® TR90 (the current CliniMACS Column component). Grilamid® TR90 was subjected to a series of tests conducted by mdt (medical device testing) in Germany according to USP 24, Class VI Guidelines. The following tests were performed: systemic toxicity, intracutaneous irritation, and intramuscular implantation. The results, summarized in Table 9, indicated no toxic responses to the Grilamid® TR90 that is used in the CliniMACS Column.

Table 9: Summary of Toxicology and Biocompatibility Studies Conducted With Eluate[a] Collected from Grilamid® Resin			
Test	**Species/ Method**	**Duration of Study**	**Results**
Systemic Toxicity	Mice	IV/ Single Dose	No Toxicity
Intracutaneous Irritation	Rabbit	IC/72 hours	No Irritation
Intramuscular Implantation	Rabbit	Implant 4 strips/≥ 120 hours	No Sensitivity

IV: Intravenous; IC: Intracutaneous
a: The eluate was 0.9% Saline incubated with Grimalid TR90 for 72 hours at 50°C

CliniMACS® Tubing Set and CliniMACS® Column

The testing described above was supplemented with an additional biocompatibility evaluation performed at Hatano Research Institute, Food and Drug Safety Center (Japan) according to the Japanese Guideline: Guidelines for Biological Tests Required for Application for Approval to Manufacture (Import) Medical Devices (1995/6/27 PAB Notification No. 99) and the results are summarized in the following table.

Table 10: Additional Biocompatibility Testing of the CliniMACS® Tubing Set (TS) Eluate According to Japanese Guidelines

Test	Test Article	Species/ Method	Extraction Medium	Results
Cytotoxicity	TS with column	MEM Elution Test (V79 cell)	Eagle's MEM/ 5% calf serum/ 1 mM Sodium pyruvate	No Cytotoxicity
	Grilamid® TR90 resin			No Cytotoxicity
Sensitization	TS with column	Guinea pig Skin Sensitization Test (Maximization Test)	Methanol and Hexane	Sensitizer at 1% or above
	TS without column		Methanol	Non-sensitizer
	CliniMACS® Column		Methanol	Sensitizer at 0.1% or above
	CliniMACS® Column		PBS/EDTA and Albumin	Non-sensitizer
	Grilamid® TR90 resin		Methanol and Chloroform	Non-sensitizer
Irritation	TS with column	Intracutaneous reactivity (Rabbit)	Normal saline and sesame oil	Non-irritant
Acute Systemic toxicity	TS with column	Acute Systemic Toxicity Test (ICR mouse)	Normal saline and sesame oil	No acute systemic toxicity
Hemocompatibility	TS with column	Hemolysis (Rabbit)	Normal saline	Non-hemolytic
(*in vitro* hemolysis)	Grilamid® TR90 resin		Normal saline	Non-hemolytic

4.4.2. Safety Testing of the CliniMACS® PBS/EDTA Buffer

This section summarizes the nonclinical or preclinical safety studies performed on the CliniMACS PBS/EDTA Buffer. All testing was performed in accordance with the FDA's Good Laboratory Practice (GLP) for Nonclinical Laboratory Studies (21CFR §58) and/or United Kingdom's GLP Compliance Program. Veterinary control, surveillance, processing, preservation, testing, and handling of all tissues, cells, and substances of animal origin were conducted according to established guidelines during qualification of the CliniMACS PBS/EDTA Buffer. The results summarized in Table 11 indicated no toxic responses to the CliniMACS PBS/EDTA Buffer.

Table 11: Summary of Toxicology and Biocompatibility Studies Conducted with the CliniMACS® PBS/EDTA Buffer			
Test	**Species/Method**	**Duration of Study**	**Results**
Cytotoxicity	Mouse embryo fibroblasts	*In Vitro*/ 24 hours	No Cytotoxicity
Hemolysis	Human Blood Rabbit Blood	*Ex Vivo*/1 hour	Not Hemolytic
Intracutaneous Irritation	Rabbit	IC/72 hours	No Irritation
Systemic Toxicity	Mice	IV/ Single Dose	No Toxicity
Sensitization Study	Guinea Pig	ID/24 days and Topical/24 days	No Sensitivity

IV: Intravenous; IC: Intracutaneous, ID: Intradermal

4.5. *Pre-Clinical Performance Testing*

The CliniMACS® TCRα/β Reagent Kit is used to deplete human TCRα/β$^+$ T cells *in vitro* from heterogeneous hematological cell populations for stem cell transplantation and lymphocyte infusions in cases where the depletion of this population of T cells is clinically indicated.

Each CliniMACS® TCRα/β Reagent Kit is sufficient to label up to 24 x 10^9 TCRα/β$^+$ T cells out of 60 x 10^9 total white blood cells.

4.5.1. Optimization of the TCRα/β depletion with the CliniMACS® TCRα/β-Biotin (clone BMA031.H7) using the CliniMACS System

Initial development was performed to optimize and verify sample preparation and flow cytometric analysis following indirect labeling using the CliniMACS® TCRα/β-Biotin in combination with the CliniMACS® Anti-Biotin Reagent. To optimize the depletion of TCRα/β$^+$ T cells using the CliniMACS® System, several experiments were performed using different labeling and/or separation conditions.

The results demonstrated that the CliniMACS® TCRα/β-Biotin in combination with the CliniMACS® Anti-Biotin Reagent, the CliniMACS® ^{plus} Instrument and the separation program 3.1 with the CliniMACS® Depletion Tubing Set showed high performance with regard to purity, recovery, -logP and FAB values using 10µg/mL on the cells.

The capacity of the CliniMACS® Anti-Biotin Reagent in combination with the CliniMACS® TCRα/β-Biotin was also evaluated using a small-scale system and results demonstrated that one vial of TCRα/β-Biotin Reagent and two vials CliniMACS® Anti-Biotin Reagent are required for labeling 36 x 10^{10} TCRα/β$^+$ T cells out of a total leukocyte number of up to 60 x 10^9 cells. The findings of this small-scale test were noted to require further verification.

For the staining and flow cytometric analysis of the TCRα/β depleted cells with the CliniMACS® TCRα/β$^+$ Biotin, PE-conjugated TCRα/β antibody clone BMA031 was recommended. Precise analysis of the Target Cell Fraction was shown to require the exclusion of monocytes, granulocytes, B cells and NK cells using an exclusion marker cocktail consisting of CD14/15/16/19-FITC. Additionally, the use of CD45-PerCP for the staining of all white blood cells was recommended for the exclusion of platelets and cellular debris.

4.5.2. Design Verification: Depletion with the CliniMACS TCRα/β-Biotin using the CliniMACS® System

The verification of the TCRα/β depletion procedure using the CliniMACS® TCRα/β-Biotin and CliniMACS® Anti-Biotin Reagent in combination with the CliniMACS® plus Instrument and the separation program 3.1 with the CliniMACS® Depletion Tubing Set was performed.

A total of six depletions were performed using three unmobilized leukaphersis products and three G-CSF mobilized leukapheresis products as the starting cellular product. A summary of the product characteristics including the total amount of WBCs and TCRα/β$^+$ T cell starting frequencies is provided in the following table.

Table 12: Summary of Starting Product Characteristics

Product Number	Mobilized (Yes/No)	Total WBCs (x 10^{10})	Starting Viability WBCs (%)	Starting Frequency TCRα/β$^+$ T cells (%)	Total TCRα/β+ T cells (x 10^9)
1	No	1.1	99.2	62.1	6.8
2	No	1.3	97.1	66.9	8.4
3	No	0.96	99.0	51.5	4.9
4	Yes	5.6	28.1	25.1	3.9
5	Yes	2.6	95.5	44.0	10.9
6	Yes	3.6	98.1	42.0	15.1

The products were labeled using one vial of CliniMACS® TCRα/β-Biotin followed by labeling with two vials of CliniMACS® Anti-Biotin Reagent. Depletion was performed using the CliniMACS® plus Instrument with the CliniMACS® Depletion Tubing Set. Separation performance results are shown in the following table.

Table 13: Depletion Efficiency of the CliniMACS® TCRα/β Reagent Kit

Product Number	Viability (%)	TCRα/β$^+$ T cells (%)	Total TCRα/β+ T cells (x 10^6)	-logP
1	98.4	62.1	6.8	5.58
2	96.7	66.9	8.4	4.47
3	98.4	51.5	4.9	4.33
4*	28.5	25.1	3.9	3.17
5	96.3	44.0	10.9	4.50
6	95.7	42.0	15.1	4.22

*Leukapheresis product had a very low viability

The recovery of CD34$^+$ cells, NK cells and TCRγ/δ$^+$ T cells following depletion of the TCRα/β$^+$ T cells are shown in the following table.

Table 14: Recovery of CD34$^+$ cells, NK cells and TCRγ/δ$^+$ T cells			
Product Number	**CD34$^+$ Cells (%)**	**NK Cells (%)**	**TCRγ/δ$^+$ T cells (%)**
1	Not done	71	55
2	31	74	54
3	48	14	100
4	Not done due to very low starting viability		
5	67	87	100
6	94	76	55

Excluding the yield of CD34$^+$ cells (Product #1) and the NK cell yield (Product #3), the recoveries of target cells were acceptable

In summary, the results of the six processed TCRα/β depletions using unmobilized (3) and mobilized leukapheresis products (3) demonstrated that the cell sample preparation and separation protocol for the depletion of TCRα/β$^+$ T cells using the CliniMACS® TCRα/β-Biotin with CliniMACS® Anti-Biotin Reagent in combination with the CliniMACS® plus Instrument and the separation program DEPLETION 3.1 with the CliniMACS® Depletion Tubing were suitable for their intended use.

Additionally, the staining and flow cytometric analysis procedures, both before and after depletion of TCRα/β$^+$ T cells, were reviewed and recommendations were made regarding the revision of procedures

4.5.3. Performance Validation: TCRα/β Depletion and TCRα/β/CD19 combined Depletion using the CliniMACS® System

Three validation runs were performed with the CliniMACS® TCRα/β-Biotin in combination with the CliniMACS® Anti-Biotin Reagent to deplete TCRα/β$^+$ T cells. In addition, three validation runs were performed using a combined depletion labeling strategy with CliniMACS TCRα/β-Biotin/Anti-Biotin Reagent and the CliniMACS CD19 Reagent for the simultaneous depletion of TCRα/β$^+$ T cells and CD19$^+$ B cells. All validations were performed with the CliniMACS® plus Instrument and the separation program DEPLETION 3.1 with the CliniMACS® Depletion Tubing Set.

The validations were performed using leukapheresis products with the composition of each product meeting set specifications for the starting material (unmobilized LP, cell count not less than 5 x 10^9 WBCs and viability > 90%).

The results of the depletion efficiency and viability in target cell fraction of the three processed TCRα/β depletions and three combined TCRα/β/CD19 depletions, respectively, are summarized in the following table.

Table 15: Separation Performance Results Regarding Viability and Depletion Efficiency						
Date	**Viability in TCF [%]**	**Loss of viability in TCF [%]**	**TCRα/β$^+$ T cells [%]**	**TCRα/β$^+$ T cells [x 10^6]**	**TCRα/β$^+$ T cells depletion -logP**	**CD19$^+$ B cells depletion -logP**
09.12.2009	80.6	18.1	0.0050	0.18	4.3	N/A
10.12.2009	92.7	1.6	0.0053	0.05	4.7	N/A
16.12.2009	98.9	0.6	0.0018	0.11	4.7	N/A
22.12.2009	97.5	2.0	0.0020	0.05	4.8	3.5
05.01.2010	94.5	3.7	0.0016	0.04	5.1	3.4
06.01.2010	97.7	1.6	0.0018	0.06	4.9	3.6
Mean	**93.7**	**4.6**	**0.0029**	**0.08**	**4.8**	**3.5**

The following table summarize the recovery of non-TCRα/β$^+$ and non-TCRα/β$^+$/CD19$^+$ cells, respectively and the recovery of TCRα/β$^+$ T cells in the target cell fraction in terms of the starting material. The performance validation results are all within the acceptance criteria and therefore, the system is suitable for its intended use.

Table 16: Recovery Summaries for the Relevant Cell Populations			
Date	**Non - TCRα/β$^+$ [%]**	**Non - TCRα/β$^+$ / CD19$^+$ [%]**	**TCRγ/δ$^+$ cells**
09.12.2009	94.3	N/A	95
10.12.2009	94.1	N/A	100
16.12.2009	96.8	N/A	100
22.12.2009	N/A	95.9	95
05.01.2010	N/A	93.8	98
06.01.2010	N/A	90.5	90
Mean	**95.1**	**93.4**	**96.3**

5. PREVIOUS HUMAN EXPERIENCE

A phase I/II safety and feasibility trial is being pursued by Miltenyi Biotec GmbH using the CliniMACS® TCRα/β Reagent Kit and the CliniMACS® CD19 Reagent (EudraCT No.: 2011-005562-38). The trial is designed to test the safety and feasibility of TCRα/β and CD19 depleted stem cell grafts from haploidentical donors for hematopoietic progenitor cell transplantation in children and adults. Additional physician sponsored studies are ongoing in Europe.

Please note that while the above sponsored trial references use of the CliniMACS® CD19 Reagent for B cell depletion, *in vivo* methods (i.e. Rituximab) may also be considered by the physician.

5.1. *Marketing History*

The CliniMACS® TCRα/β Reagent Kit has not been approved for marketing in the United States. However, CliniMACS® TCRα/β Reagent Kit has received CE-marking in Europe.

6. SUMMARY AND GUIDANCE FOR THE INVESTIGATOR

6.1. *Overview*

The CliniMACS® TCRα/β Reagent Kit is a system of integrated components designed for the *in vitro* depletion of TCRα/β$^+$ T cells from heterogeneous hematopoietic cell populations (e.g. peripheral blood) for subsequent infusion into patients. This section describes potential risks associated with the CliniMACS TCRα/β-Biotin and CliniMACS Anti-Biotin Reagent to provide the investigator with an understanding of the specific tests, observations and precautions that may be needed for a clinical trial. Although the risks associated with the product are considered to be minimal, the investigator is encouraged to be aware of the potential risks associated with processing the cellular product using the CliniMACS® TCRα/β Reagent Kit.

6.2. *Risk Analysis*

6.2.1. Risks to the Operator

Theoretical risks to the operator of the CliniMACS® TCRα/β Reagent Kit in combination with the CliniMACS® System include exposure to blood products because of leaking from the disposable tubing set or injury due to mechanical failure of the hardware of the device. Such risks have been mitigated to the greatest extent possible, by the design of the system components.

The priming step allows the operator to check for leaks in the disposable tubing set before the patient sample is attached. An automated integrity test of the installed tubing set may be performed prior to the processing run to further check for leaks in the tubing set. Operators should follow standard laboratory safety procedures during the handling of blood products. Proper precautions should be followed when handling the cells, waste products, and any contaminated fittings, tubing, connectors, or containers according to standard institutional practices.

All disposable tubing and columns are supplied in sterile packaging. In the event of the packaging being compromised, the tubing should be replaced. The compromised tubing set should be returned to Miltenyi Biotec Inc.

The CliniMACS® plus Instrument is equipped with an extremely powerful permanent magnet. Users must keep any magnetic information carriers (such as credit cards, magnetic tapes and floppy disks), any electronic equipment (such as hearing aids, pacemakers, cerebral/brain shunts, measuring and control instruments, computers and watches) and magnetizable tools and objects at a distance of at least 30 cm from the device. These items may be affected or damaged by the magnetic field.

The CliniMACS® plus Instrument has been tested for electrical safety and the potential for fire, shock, explosion, or mechanical damage has been reduced by using a design to meet European standards EN 60601-1as well as EN 60601-1-2: 2007 6.1 + 6.2 (EMC Requirement for Medical Devices). The CliniMACS plus Instrument is UL and CSA listed and approved.

6.2.2. Risks to the Patient

Theoretical risks to the patient when using the CliniMACS® TCRα/β Reagent Kit and the CliniMACS® CD19 Reagent are postulated from possible events resulting from system failure, user error or patient reaction to the depleted product. These risks are summarized below followed by the risk reduction measures that have been implemented. Furthermore, recipients of allogeneic transplants are subject to risks from the transplant related procedures and medication utilized. These risks are independent of the TCRα/β$^+$ T cell depletion using the CliniMACS® TCRα/β Reagent Kit.

Allogeneic Transplantation with TCRα/β and CD19 depleted stem cell grafts

Graft Failure

When protocols for allogeneic transplantation including T cell depletion were developed, a higher incidence of graft failure in comparison to protocols with T cell replete grafts was observed.[51] One reason for graft failure seems to be rejection of the graft by host lymphoid cells. Supporting this is the identification of host cytotoxic T cells exhibiting donor-specific cytotoxicity at the time of rejection.[52] Strategies to intensify and diversify conditioning regimens have been applied to patients receiving TCD grafts, especially in the haploidentical setting. Among these include the use of ATG or CAMPATH 1-H for *in vivo* depletion of host T cells prior to allogeneic stem cell infusion. Aversa *et al* were able to achieve reliable engraftment in haploidentical transplants using high number of highly purified CD34$^+$ cells obtained from mobilized peripheral blood stem cells in combination with myeloablative conditioning and *in vivo* T cell depletion.[9] Thus, improving the conditioning regimen of the patient and increasing the stem cell dose can minimize graft failure of TCD stem cells.

The direct T cell depletion approach using the CliniMACS CD3/CD19 Separation System in combination with a conditioning regimen of reduced intensity was developed for patients with comorbidities and heavily pretreated patients. Using this approach immune competent cells such as NK cells and graft facilitating cells remain in the graft. Primary engraftment rates after haploidentical transplantation with CD3/CD19 depleted grafts ranging from 83% to 100% are comparable to engraftment rates in haploidentical transplantation performed with CD34 selection and myeloablative conditioning[22, 24, 25]

The TCRα/β and CD19 depletion approach for haploidentical transplantation depletes the TCRα/β$^+$ subpopulation of CD3$^+$ T cells, which is involved in GVHD development. Additionally, B cells are eliminated, by means of CD19 depletion (i.e., CliniMACS CD19 Reagent System or Rituximab) which may be involved in PTLD development. The combination of direct TCRα/β$^+$ and B cell depletion leaves immune competent cells such as TCRγ/δ$^+$ T cells, NK cells and graft facilitating cells in the graft. With this method a 4-5 log depletion of TCRα/β$^+$ T cells can be achieved, which is in the range of T cell depletion resulting from CD34 selection.[36] In first clinical trials TCRα/β$^+$ T cell and B cell depleted grafts from haploidentical donors were applied to pediatric patients after a conditioning regimen of reduced intensity (RIC) and myeloablative intensity. First results from these trials were presented at the ASH Annual Meeting and the Annual Meeting of the European Group for Blood and Marrow Transplantation.

Fifteen poor-prognosis patients with advanced refractory leukemias were treated in the RIC setting (Tübingen protocol), and 17 patients with refractory/relapsed ALL, AML and NHL were treated according to the Rome protocol with myeloablative conditioning. All patients in Tübingen engrafted with a median time to PMN recovery of 10 days (8-12) and a median time to platelet recovery of 11 days (6-28). In the Rome cohort, 16 of 17 patients engrafted with median times to PMN and platelet recovery of 12 days (10-18) and 13 days (8-15), and in total 31 of 32 patients showed primary engraftment.[53]

Data from 16 patients with non-malignant disorders transplanted with TCRα/β/CD19 depleted grafts were presented at the Annual Meeting of the European Group for Blood and Marrow Transplantation 2013.[54] Thirteen of 16 patients had primary engraftment with median time to neutrophil and platelet recovery of 13 days (8-19) and 11 days (7-40), respectively. The 3 patients with primary graft failure were successfully retransplanted with TCRα/β/CD19 depleted grafts.

These early data are comparable to engraftment rates in haploidentical transplantation performed with CD34 selection and myeloablative conditioning.[10] and to engraftment rates achieved with CD3/CD19 depletion with reduced intensity conditioning.[22,24,]

Engraftment of a TCRα/β^+ T cell and B cell depleted product may also be delayed or fail completely because of contamination or accidental cell loss during cell processing. The infusion of inadequate low numbers of depleted hematopoietic stem cells may also be a risk factor for graft failure. The risks of such failures are minimized as the CliniMACS® TCRα/β Reagent Kit in combination with the CliniMACS® System retains cells in a sterile, closed environment. In addition, the storage of another leukapheresis product as a backup may be recommended to mitigate the risks associated with the loss of the depleted product.

As a final safeguard, the TCRαβ/CD19 depleted product undergoes release testing prior to infusion into the patient. All release testing performed on the final TCRα/β/CD19 depleted product is performed by the clinical site and released for infusion according to their established release criteria.

GVHD

The reported incidence of GVHD in haploidentical transplantation with CD34 selected grafts is below 10% reflecting the high degree of T cell depletion achieved with this procedure.[10] The incidence and degree of GVHD observed in clinical trials with CD3/CD19 depleted grafts is higher which may be related to the higher median T cell dose transplanted within the framework of these protocols. Additional GVHD prophylaxis with Mycophenolate Mofetil is foreseen in these trials when the applied T cell dose is higher than 5.0×10^4 $CD3^+$ cells/kg bodyweight in adults and higher than 2.5×10^4 $CD3^+$ cells/kg bodyweight in children.[10] Maximum thresholds of residual T cells in the graft are further defined in the respective clinical protocols.

The Tübingen and Rome groups reported efficient TCRα/β$^+$ T cell depletion of 4.1-5 log by the TCRα/β/CD19 approach, which is comparable to the values achieved by CD34 positive selection.[26,,55] Data on GVHD development were reported for a cohort of 15 patients which were transplanted with TCRα/β/ CD19 depleted grafts from haploidentical donors after reduced intensity conditioning. Three patients experienced grade II aGVHD and 1 patient grade III aGVHD, three patients experienced chronic GVHD including one case of extensive chronic GVHD. From the 17 patients transplanted after myeloablative conditioning 1 patient experienced grade I aGVHD and no chronic GVHD was reported.[53] A cohort of 16 patients with nonmalignant diseases was transplanted with TCRα/β/CD19 depleted grafts after myeloablative conditioning. No pharmacological GVHD prophylaxis was applied. Four patients experienced grades I/II aGVHD of the skin and one patient experienced limited skin chronic GVHD, no visceral acute GVHD was monitored.[54] The reported incidence of acute and chronic GVHD in these 48 patients transplanted from haploidentical donors after reduced intensity and myeloablative conditioning is low and in most cases moderate (8 patients with grades I/II aGVHD, 1 patient with grade III aGVHD, 3 patients with limited chronic GVHD and 1 patient with extensive chronic GVHD), and seems to be lower or comparable to GVHD incidences reported from haploidentical transplantations with CD3/CD19 depleted grafts.

Since the incidence of GVHD depends on the residual numbers of T cells in the graft, release testing of the TCRα/β/CD19 depleted product is an import and final safeguard. All release testing performed on the final TCRα/β/CD19 depleted product is performed by the clinical site and released for infusion according to their established release criteria. It may be recommended to define maximum thresholds of residual T cells in the graft in the respective clinical protocols.

Infections

In haploidentical transplantation protocols using CD34 selected stem cells delayed immune reconstitution associated with a high incidence of severe and lethal infectious complications is monitored.[10] One aim for the development of CD3/CD19 depletion and TCRα/β/CD19 depletion in haploidentical transplantation was to reduce TRM by providing a graft which contains immune effector cells such NK cells and other cell populations which might accelerate immune recovery.[24]

In clinical trials using CD3/CD19 depleted grafts in haploidentical transplantation after reduced intensity conditioning in children and adults fast immune recovery and promising data on low incidences of lethal infections have been reported.[36] In a study with 38 children no lethal viral infections occurred and one patient experienced transplant related death[24] and a further study including 59 pediatric patients reported the cumulative incidence of TRM of 10.7%.[22]

In the above described three cohorts of patients transplanted with TCRα/β/CD19 depleted grafts after haploidentical transplantation 1 patient out of 15 in the Tübingen cohort died from multi-organ failure, in the Rome cohort one patient out of 17 died from lung aspergillosis and in the group of 16 patients with nonmalignant diseases two patient died because of respiratory failure secondary to viral infections.

One may conclude from the published results of patients transplanted with TCRα/β/CD19 depleted products in comparison to results from transplants with CD3/CD19 depleted grafts that the incidence of lethal infectious complications is comparable using the TCRα/β and B cell depletion strategy. As a precaution, infection surveillance and adequate preventive and preemptive therapeutic strategies should be considered in patients receiving a T cell and B cell depleted allograft.

Additional Risks

A further complication of allogeneic stem cell transplantation is veno-occlusive disease (VOD) of the liver. VOD is a manifestation of damage to the liver which can be caused by the conditioning regimen used in haploidentical stem cell transplantation. Usually it develops within two weeks after transplantation and is characterized by at least two of the following:

- Hyperbilirubinemia (total bilirubine >2 mg/dL)
- Hepatomegaly or right upper quadrant pain
- Sudden weight gain (>5% above baseline)

Recipients developing VOD must be monitored closely and receive appropriate supportive care and careful fluid management.

In haploidentical stem cell transplantation, end organ damage to all or any of the major organs, including the brain, may occur as a result of cumulative toxicity from the anti-neoplastic therapy, reactions to other drugs, and as a result of destructive processes (e.g. infection, GVHD, etc.). It may have a fatal outcome. Toxicities may occur in individual patients due to multiple events and cumulative effects that may involve any and all organs, including the brain. Brain damage can result in severe loss of cognitive or neurologic function. Preliminary data from published studies do not suggest that the risk of end organ damage is appreciably affected by TCRα/β/CD19 depleted grafts or the preparative regimens used in these studies.

After haploidentical stem cell transplantation patients have a considerable risk of non-relapse mortality within the first year after transplantation. This results from severe regimen related toxicity, and risks of hemorrhage, opportunistic infections, or other complications.

Iron/Dextran

Use of the CliniMACS® TCRα/β Reagent Kit enables the infusion of cells depleted of TCRα/β$^+$ T cells that may have residual amounts of unbound CliniMACS Anti-Biotin Reagent (murine monoclonal antibody conjugated to an iron-dextran moiety). A vial of CliniMACS Anti-Biotin Reagent contains 7.5 mL of CliniMACS Anti-Biotin Reagent at a concentration range between 20 to 52 micrograms (µg) of antibody protein per vial, 800 µg/mL of dextran and 800 µg/mL of iron Patient reactions to therapeutic levels of iron dextran products and murine monoclonal antibodies have been reported in the literature and are discussed below. The amounts of murine monoclonal antibody and iron-dextran in the CliniMACS Anti-Biotin Reagent to which the patient could theoretically be exposed is significantly less than products intended for therapeutic uses.

Iron dextran is commercially available as a sterile solution of an iron dextran complex for the treatment of severe iron-deficient syndromes. It contains 5% iron and 20% dextran and its safety profile has been well characterized. The iron dextran solution contains 50mg/mL of elemental iron, most of which is present in the ferric state. In the blood, iron dextran is a highly stable complex from which iron is slowly released to the endogenous carrier protein, transferrin.[56] Iron dextran is cleared from the circulation by the reticuloendothelial system and appears inside macrophages of the liver and spleen within 1 hour after injection. In healthy subjects, the mean clearance half-life is approximately 6 hours with 40% of the injected dose bound to transferrin 11 hours later.[57] The total dose of iron-dextran for treatment of severe iron deficiency for the average 70-kg person is calculated to be approximately 2 g over several days (single dose of 50 mg).

Hypersensitivity reactions resulting from iron dextran therapy have been reported[58]. The most dangerous complication is an anaphylactoid reaction similar to contrast media reactions. The anaphylactoid reaction is usually manifested during the first few minutes of the infusion (often during test dose administration). The mechanisms of these reactions have not been established, although immunologic and pathologic evidence suggests that the IgG-mediated type III immune complex mechanism is most likely the cause, at least for most severe systemic reactions. An IgE-induced type I reaction has also been postulated. The most common hypersensitivity reaction occurring in more than 30% of patients given therapeutic levels of iron dextran is the development of arthralgia and fever within 24 to 48 hours after initiation of the intravenous therapy. In iron dextran therapy, it is theoretically possible, that a patient could have a life-threatening anaphylactic reaction; but again, these reactions occur primarily during the test dose administration of the contrast media with an overall incidence between 0.1% and 0.6%. Individuals with a history of allergies, asthma, or active inflammatory disease appear to be highly susceptible to the adverse effects of iron dextran.[59] Other high-risk patients are those with active rheumatoid arthritis or active systemic lupus erythematosus.[56]

Iron dextran exposure from the CliniMACS® Anti-Biotin Reagent is unlikely because the intended use of the reagent in combination with the CliniMACS® TCRα/β-Biotin is to deplete the TCRα/β^+ T cells. The CliniMACS TCRα/β Reagent Kit in combination with the CliniMACS Separation System are used for the *in vitro* depletion of TCRα/β^+ T cells from heterogeneous cell populations. This is achieved by the retention of the TCRα/β^+ T cells as well as the excess CliniMACS Anti-Biotin Reagent in the magnetic field. Cells that are not retained in the magnetic field are collected and infused; therefore, the exposure of a patient to iron-dextran is minimal.

BMA031.H7 and Bio3-18E7

The CliniMACS® TCRα/β-Biotin and the CliniMACS Anti-Biotin Reagent each contain a murine monoclonal antibody, BMA031.H7 and Bio3-18E7, respectively. Murine monoclonal antibodies have been used clinically in the prevention of organ transplant rejection, cancer therapy and in clinical trials for several indications (e.g. sepsis, auto-immune disease, and inflammatory diseases). One of the problems limiting the therapeutic use of murine monoclonal antibodies (mAbs) has been the generation of human anti-mouse antibodies (HAMA) and anaphylactic reactions[60, 61, 62]. Systemic reactions appear to be related to the dose and rapidity of administration. Therapeutic levels of mAb are reported to be in the range of 2.5 to 5 mg/kg. The

most commonly reported adverse effects have been myalgia, arthralgia, increased peripheral edema and flu-like symptoms, the latter involving fever (70% to 73%), chills (46% to 53%), chest pain and tightness (14%), wheezing (11% to 14%) and vomiting (13% to 32%).[63, 64, 65]

One vial of CliniMACS TCRα/β-Biotin contains 7.5 mL of antibody reagent at a concentration of approximately 140-180 µg/mL of protein. One vial of CliniMACS Anti-Biotin contains 7.5 mL of antibody reagent at a concentration of approximately 2.0-4.5 µg/OD_{450} of protein with a filling concentration of 10.0-11.5 OD_{450}. For a single TCRα/β^+ T cell depletion run, one vial of CliniMACS TCRα/β-Biotin and two vials of CliniMACS Anti-Biotin Reagent are incubated with an apheresis product prior to processing on the CliniMACS Separation System. If all the TCRα/β-Biotin mAb disassociated from the anti-Biotin, a maximum of 1350 µg of monoclonal antibody could be infused into the subject. Two vials of the Anti-Biotin mAB are used therefore, if all of the Anti-Biotin mAB is disassociated from the beads, a maximum of 776.25 µg of monoclonal antibody could be infused into the subject. Because only the depleted fraction of the separation procedure is infused, it is unlikely that reagent would be infused along with the cells since the excess reagent and the labeled cells and remain bound to the separation column. To mitigate the risk of infusing any unbound reagent, there is a washing step following the labeling procedure to remove unbound reagent.

In addition to the above, biohazards could be introduced to the patient through a contaminated blood product. To prevent this, use of current Good Tissue Practices (21 CFR 1271) for cell processing and handling of blood products should be followed. Additionally, the proposed rule published in Federal Register Volume 64: No. 198, 52696-52723 (September 30, 1999 "Suitability Determination for Donors of Human Cellular and Tissue-Based Products") should be followed.

Finally, it should be noted that, as with any clinical product, adverse experiences not seen in pre-clinical testing may occur during clinical testing, and it is the responsibility of the sponsor and investigator to report such events promptly.

Risks to the Patient in a Non-Transplant Setting: TCRα/β – and TCRα/β/CD19 depleted lymphocyte infusions

Theoretical risks to the patient when using the CliniMACS® TCRα/β or CliniMACS® TCRα/β /CD19 reagents for the generation of TCRα/β^+ T- and T/B-cell depleted lymphocyte infusions in a non-transplant setting are postulated from possible events resulting from system failure, user error or patient reaction to the TCRα/β/CD19 depleted cell product. These risks are similar to those summarized above and one must consider exposure to iron dextran as well as the monoclonal antibodies present in the CliniMACS® TCRα/β Reagent Kit (BMA031.H7 and Bio3-18E7) and the CliniMACS® CD19 Reagent (SJ25-C1) .

GVHD is usually associated with the administration of allogeneic T cells during allogeneic stem cell transplantation. TCRα/β depleted cell products, which may be administered in the non-transplant setting are depleted of TCRα/β^+ T cells, which are involved in the development of GVHD. However, residual T cells are infused with the cellular product. A further complication in allogeneic transplantation with T cell depleted but non B cell depleted grafts is the development of EBV associated PTLD. Therefore, the combined direct B- and T cell depletion methods, the CliniMACS® CD3/CD19 Separation System and TCRα/β Reagent Kit /CD19

Reagent, have been developed. As previously described, please note that *in vivo* methods for B cell depletion (i.e. Rituximab) may be considered by the investigator.

The application of B cell containing, but T cell depleted lymphocyte infusions in the non-transplant setting may bear the risks of EBV associated PTLD development and Passenger Lymphocyte Syndrome (PLS), an autoimmune hemolytic anemia resulting of antibody production by donor B lymphocytes in response to the recipient red blood cell antigens.

Since no clinical data on the application of TCRα/β- or TCRα/β/CD19 depleted lymphocyte infusions in the non-transplant setting are published so far, the results of clinical trials performed with CD3 depleted lymphocyte infusions are summarized below. The summary includes data of GVHD incidence, PTLD and PLS.

Clinical trials have been performed using the CliniMACS CD3 Reagent System to deplete $CD3^+$ T cells from donor derived unmobilized leukapheresis products to generate T cell depleted lymphocyte infusions enriched for NK cells for the treatment of patients with malignant diseases.

In a clinical safety trial escalating doses of NK DLIs from haploidentical donors were administered to patients with melanoma, renal cell carcinoma and Hodgkin disease.[66] For the generation of NK cell products leukapheresis collections from haploidentical donors with up to 2.0×10^{10} PBMC were T cell depleted by using the CliniMACS CD3 Separation System. The final T cell dose in the cellular products was $1.75 +/- 0.3 \times 10^5$ $CD3^+$ cells/kg bodyweight. Before infusion of NK DLIs the patients were pretreated with a low intensity cyclophosphamide/methylprednisolone regimen with the intent to prevent immediate rejection of the donor cells. The lowest dose level which was applied to three patients was 1.0×10^5 cells/kg, three patients received 1.0×10^6 cells/kg, six patients received 1.0×10^7 cells/kg and the last dose level of 2.0×10^7 cells/kg was applied to nine patients. Four patients received a second dose of NK DLI. No adverse reaction during or after cell infusion was monitored and no GVHD was observed. To support the activity of the haploidentical NK cells in the patients, low dose IL-2 injections were applied daily for 14 days. Side effects after IL-2 injections were tolerable and were common as observed with the described regimen without NK cell infusions. Since no dose-limiting-toxicity was seen in the dose escalation trial, the dose level of 2.0×10^7 cells/kg was used for subsequent patient cohorts.[55] In seven patients with renal cell carcinoma a preparative regimen consisting of Fludarabine was used and in nineteen patients with poor-prognosis AML a regimen of higher intensity comprised of Cyclophosphamide and Fludarabine was applied. No unexpected toxicities appeared in the patient cohort receiving the Fludarabine regime, and no hospitalization was necessary. The Fludarabine regimen induced lymphopenia but no neutropenia. In contrast, AML patients treated with the combined Cyclophosphamide/Fludarabine regimen developed pancytopenia and experienced more toxicities associated with IL-2 administration, therefore the IL-2 dose was reduced. The AML patients were hospitalized throughout the treatment (as AML patients receiving induction therapy). One AML patient developed pleural effusions and hypoxemia and after stopping IL-2 administration the acute event grade 3 resolved. A grade 2 shortness of breath and hypoxemia was developed by another AML patient and symptoms resolved after completion of IL-2 therapy. None of the patients in either treatment group developed GVHD. The applied T cell dose within the NK cell DLIs was $2.1 +/-0.3 \times 10^5$ cells/kg recipient bodyweight.[55] The authors stated that in haploidentical transplantation containing the above referenced T cell doses in the graft, GVHD

development would be expected, but that the T cells in the NK DLI approach did not contribute significantly to engraftment (in contrast to NK cells), which could explain the lack of GVHD.

One patient in the AML cohort received a NK DLI from his sister, after being transplanted with double cord and experiencing a subsequent bone marrow relapse. NK cell engraftment from the haploidentical donor was observed. The patient died due to EBV reactivation 126 days after NK cell infusion without evidence of leukemic relapse. In the bone marrow of this patient, 8% haploidentical donor cells were found and in the periphery, a large number of $CD19^+$ B cells of haploidentical origin but not cord blood or recipient origin were identified. The authors concluded that the CD3 depleted cellular product enriched for NK cells but containing B cells, may require further purification to decrease the possibility of clinical complications due to EBV reactivation.

A pilot trial using haploidentical NK DLI in patients with refractory B cell lymphoma was published in 2010.[67] The NK DLI were passively enriched for NK cells by CD3 depletion using the CliniMACS® CD3 Separation System. Six patients with advanced Non Hodgkin Lymphoma (NHL) resistant to rituximab-containing salvage chemotherapy regimens were included in the study. Three of them were also resistant to Fludarabine and all were not ineligible for autologous or allogeneic transplantation. The preparative regimen consisted of rituximab, cyclophosphamide and fludarabine. In previous publications it has been shown, that high doses of IL-2 are synergistic with rituximab against rituximab resistant cell lines supporting the idea that IL-2 provides a stimulus for effective NK-mediated antibody-dependent cellular cytotoxicity (ADCC).[68] The high dose chemotherapy and NK DLI infusions did not induce unexpected toxicities and no prolonged marrow aplasia or GVHD occurred. Grade 3 neutropenia and thrombocytopenia was documented in all treated patients. One patient experienced delayed hematologic recovery and a human herpes virus-6 infection which resolved after therapy with foscarnet. The reported non hematologic toxicities grades 1-3 (NCI CTCAE) were rigors and fever with the NK cell infusion, skin redness and swelling at the IL-2 injection site, fatigue, sepsis, hypertension, bigeminy and tumor induced airway obstruction in one patient. In four patients, objective clinical responses were reported, two patients achieved CR, and two patients achieved PR. Three of the responding patients were treated with allogeneic transplantation, two of which were alive and in CR 5 and 6 months after transplantation. The authors discussed that NK DLI infusions after immune suppressive chemo-immunotherapy in NHL patients followed by IL-2 injections is safe and feasible, however sustained NK cell expansion in the periphery was not monitored making it difficult to associate the clinical responses directly with the NK cell therapy.

The antitumor activity of allogeneic NK cell DLI was further evaluated in a Phase II in 20 patients with recurrent ovarian and breast cancer was performed. The NK cell products were generated from leukapheresis products collected from haploidentical donors by CD3 depletion using the CliniMACS CD3 Separation System. The cellular products contained a median 33% of NK cells and median of 0.11% $CD3^+$ T cells. The median infused T cell dose 5.59 x 10^4 T cells/kg (range 2.35 x 10^4 T cells/kg – 5.69 x 10^5 T cells/kg). Before NK cell infusion the patients received a lymphodepleting preparative regimen consisting of cyclophosphamide and fludarabine and seven patients received additional 200 cGy TBI. To promote expansion of NK cells the patients received IL-2 injections three times per week. The expected toxicities induced

by the preparative regimen and IL-2 injections were low-grade in most patients and 11 patients experienced grade 3 side effects. Ten unanticipated severe adverse events (SAEs) occurred, eight of them following the addition of TBI. One grade 5 toxicity was ascribed to tumor lysis syndrome (TLS) in a patient with high ovarian cancer burden. The authors assumed that the patient died as a result of metabolic derangement consistent with TLS as a result of NK cell therapy. Two other patients experienced passenger lymphocyte syndrome (PLS), an autoimmune hemolytic anemia resulting of antibody production by donor B lymphocytes in response to the recipient red blood cell antigens. In one patient the hemolysis resolved after treatment with high dose methylprednisolone and rituximab, in the second patient the event resolved spontaneously within one day. Five patients experienced grade 4 toxicities (neutropenia beyond day 28), four of them received TBI. Four patients with ovarian cancer achieved partial remission after NK cell therapy, 12 patients had stable disease, and three patients experienced progressive disease with a median time to progression of 2 months. The authors stated that the combination of a chemotherapy based lymphodepleting regimen with subsequent NK DLI application was tolerated well overall but that two significant unexpected toxicities, PLS and TLS, are possible consequences of this approach. For prevention of TLS allopurinol was given to the patients and no further episodes have occurred. The adoptive-cell therapy-related PLS monitored in two patients and resulting in autoimmune hemolysis was not reported previously. The authors concluded that B cell derived antibody production in ABO-incompatible donors should be considered because of the potential toxicity. As a consequence, NK DLIs from ABO incompatible donors should be depleted of B cells.[56]

In the 69 reported cases where patients received CD3 depleted NK cell DLI no GVHD occurred. The depletion of TCRα/β^+ T cells is very efficient and selective with a 4.5 – 5 log T cell reduction, depleting more efficiently potentially harmful T cells in comparison to CD3 depletion with a median log depletion of 4. [34]Therefore, the risk of GVHD development in the non-transplant setting using TCRα/β^+ T depleted lymphocyte infusions can be considered low. In the event patients receiving TCRα/β^+ depleted lymphocyte infusions are treated with immune suppressive regimens to promote persistence of γ/δ^+ T cells and NK cells, infection surveillance and adequate preventive and preemptive therapeutic strategies should be considered.

To reduce the risk of EBV associated PTLD development of donor origin and the risk of PLS, combined B and T cell depletion of lymphocyte preparations should be taken into consideration by the investigators. Alternatively, *in vivo* pharmacologic B cell depletion strategies may be considered.

Additionally, patients should be monitored closely because of known side effects caused by the immune suppressive chemotherapy and the IL2 therapy and adequate therapeutic strategies should be taken into consideration by the responsible investigators.

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