

CliniMACS[®]

Newsletter

Vol. 6 No. 1/2006

Customer report

Isolation of CD4⁺CD25⁺ regulatory T cells
under GMP conditions

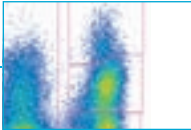

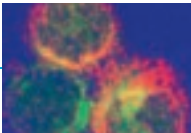
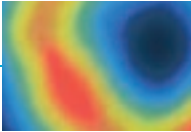



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Editorial



I am pleased to be able to provide you with this new issue of our CliniMACS® Newsletter. The last years have shown that cellular therapy is a treatment option, that has gained wider acceptance in the community. Novel ideas have been transferred from research via preclinical investigation to clinical protocols. New and more complex therapeutic approaches have been developed.

We at Miltenyi Biotec continuously develop the products needed and broaden our portfolio of clinical-grade reagents to allow these ideas to actually make their way into the clinic.

This is only possible because of our close relationship to you—thank you very much. One forum for discussing ideas and sharing of new results are the satellite symposia organized and supported by Miltenyi Biotec at the major conferences including EBMT, ISCT, ESC and ASH.

CliniMACS products for cellular approaches are now not only used in the hemato-oncology field but are being applied in areas such as tissue regeneration. More and more interest comes from the field of cardiology. Here several protocols have been developed for the treatment of acute myocardial infarction with CD133⁺ progenitor cells.

A symposium addressing the use of such progenitor cells for cardiac applications preceded the annual meeting of the European Society of Cardiology (ESC) in September 2005. Minutes of the Miltenyi Biotec satellite symposium at the ESC meeting are included in this issue of the Newsletter, as well as a summary of our symposium preceding the last ASH meeting. You will also find a preview on the upcoming symposia organized by Miltenyi Biotec at the EBMT and ISCT conferences.

Our customer report describes the isolation of CD4⁺CD25⁺ regulatory T cells with the CliniMACS Cell Selection System. Also included is an overview about all the CliniMACS T cell products.

I hope this new issue of our CliniMACS Newsletter is to your liking, and as always, if you have any suggestions, please do not hesitate to contact us.

Dr. Johannes Irsch
Clinical Marketing Manager
Miltenyi Biotec GmbH

Isolation of CD4⁺CD25⁺ regulatory T cells under GMP conditions

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Introduction

Natural CD4⁺CD25⁺ regulatory T cells (T_{reg}) represent a thymus-derived T cell lineage that efficiently suppresses the proliferation and effector function of other T cell populations. Their adoptive transfer has been shown to protect from autoimmunity and to promote tolerance after allogeneic organ and stem cell transplantation in animal models.¹ Human T_{reg} cells constitutively express high levels of intracellular CTLA-4 as well as FOXP3, a transcription factor that is essential for their thymic generation and peripheral function.² In peripheral blood from healthy individuals natural T_{reg} cells reside predominantly within the CD4⁺ T cell population with high CD25 expression levels,^{3,4} and since no exclusive surface marker is known, all isolation strategies developed thus far are based on this characteristic. Magnetic bead separation is an established method for the isolation of this population for experimental purposes.^{5,6} Here we examined whether T_{reg} cells could also be isolated under GMP conditions from leukapheresis products using the CliniMACS[®] Plus Instrument. At our institution, we examine the adoptive transfer of such cell products in recipients of allogeneic stem cell grafts. Since we and others have shown before that co-transfer of T_{reg} cells and effector T cells at a 1:1 ratio results in protection from graft-versus-host disease (GVHD) in mouse models of allogeneic bone marrow transplantation⁷⁻¹⁰, we aimed for a 50% enrichment of T_{reg} cells by use of this procedure.

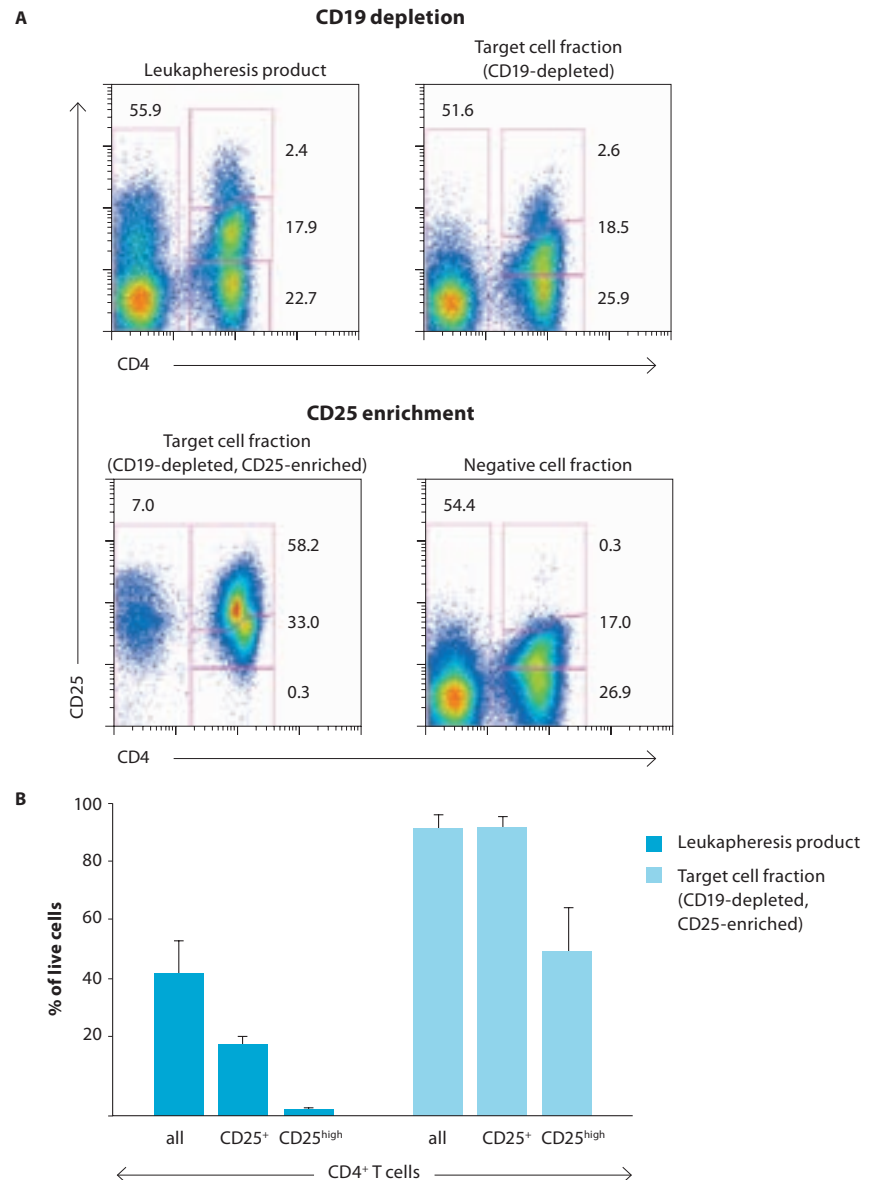


Figure 1: Preferential enrichment of CD4⁺CD25^{high} T cells by magnetic cell separation under GMP conditions using the CliniMACS[®] Plus Instrument.

A. Cells from a leukapheresis product of a healthy donor were depleted of CD19⁺ cells followed by enrichment for CD25⁺ cells. Aliquots were taken at each step of the isolation procedure and analyzed by FACS.

B. The percentage of CD4⁺ T cells (all) as well as CD4⁺CD25⁺ and CD4⁺CD25^{high} T cell subsets in the original leukapheresis products as compared to the target cell fractions after magnetic bead separation under GMP conditions are depicted. Means + SD from all six isolations are shown.

Methods

Magnetic cell separation

Leukapheresis products from six volunteers were adjusted to 95 mL in CliniMACS® PBS/EDTA Buffer (2% HSA) (Miltenyi Biotec), labeled with 7.5 mL CliniMACS CD19 Reagent for 30 min. at RT on an orbital shaker, washed, and resuspended in 100 mL buffer. B cells were depleted using an LS tubing set and the depletion program DEPLETION 2.1 of the CliniMACS Plus Instrument. The target cell fraction comprising B cell-depleted cells was suspended in 190 mL buffer, labeled with 7.5 mL CliniMACS CD25 Reagent, washed and resuspended in 100 mL buffer. Using the enrichment program ENRICHMENT 3.1, consisting of three automatic cycles of positive selection, CD25⁺ cells were isolated. Aliquots for FACS analysis and enumeration were retrieved during cell processing.

Antibodies and flow cytometry (FACS®)

FACS analysis was performed as published previously⁴ with some modifications: For analysis of CD25 expression after magnetic cell separation CD25-Biotin (4E3, Miltenyi Biotec) followed by phycoerythrin (PE)-labeled Anti-Biotin (Bio3-18E7.2, Miltenyi Biotec) was used to enhance fluorescence intensity. Anti-human FOXP3-PE antibody (clone PCH101) was from eBioscience. FACS sorts were performed on a FACS Aria (Becton Dickinson) and sort gates were set as described⁴.

Suppression assay

CD4⁺ responder T cells (T_{resp}) were isolated from the CD19/CD25-depleted cell fraction (Non-target cell fraction) by the use of CD4 MicroBeads (Miltenyi Biotec) and labeled with 2 μM CFSE. Cultures of 5 × 10⁴ T_{resp} cells were stimulated with 100 ng/ml anti-CD3 (OKT-3; Ortho Biotech) in the presence of 5 × 10⁴ autologous, irradiated (30 Gy) PBMC in 96-well round-bottom microtiter plates in RPMI-1640 culture medium with 10% FCS. Where indicated, graded numbers of CliniMACS-purified CD4⁺CD25⁺ T cells, FACS-purified CD4⁺CD25^{high} or CD4⁺CD25^{neg} T cells were added to obtain ratios of 1:1 and 1:4 T_{reg}:T_{resp} cells. After 4 days in culture the cells were harvested, stained with CD4-PE and CD25-APC (both

BD Life Sciences) and analyzed using a FACSCalibur (Becton Dickinson).

Results and Discussion

CD4⁺ cells in peripheral blood co-expressing CD25 mainly consist of activated B cells. These activated B cells could contaminate a CD25-enriched cell product to variable degrees depending on the blood composition of the donor. Since such B cells also endanger patients to develop EBV-associated lymphoproliferative disease after allogeneic stem cell transplantation (SCT), we performed a B cell depletion cycle followed by three repetitive and automated enrichment cycles for CD25⁺ cells for the isolation of T_{reg} cells. The leukapheresis products derived from six different volunteers contained 4.4 to 15.8 × 10⁹ nucleated cells and on average 1.9% CD4⁺CD25^{high} T cells (range 1.1–2.9%). After magnetic cell separation, between 63 and 262 × 10⁶ cells were recovered in the

target cell fraction (average 1.72% of the starting population, range 0.89–2.47 %), which contained 7.3% CD4⁺ cells (range 2.9–14.3%), less than 0.01% B cells, and 92% CD4⁺ T cells (range 85.5–96.0%) of which only 0.5% were CD25⁺ (range 0.2–1.1%). On average 91.5% of the cells were CD4⁺CD25⁺ (range 85.2–95.6%) and highly enriched for CD4⁺CD25^{high} T cells (49.5%, range 30.3–63.1%). Recovery of CD4⁺CD25^{high} T cells as calculated from their frequency in the initial leukapheresis product was 46.5% (range 25–66%). Representative FACS data generated from cells at different steps of the isolation procedure are depicted in fig. 1A and data regarding the CD4 T cell content of all six leukapheresis products and corresponding target cell fractions are summarized in fig. 1B. For further phenotypic analysis cells in the target cell fraction were stained for intracellular CTLA-4 and FOXP3. As already expected from their CD25 expression profile, magnetically isolated cells were highly

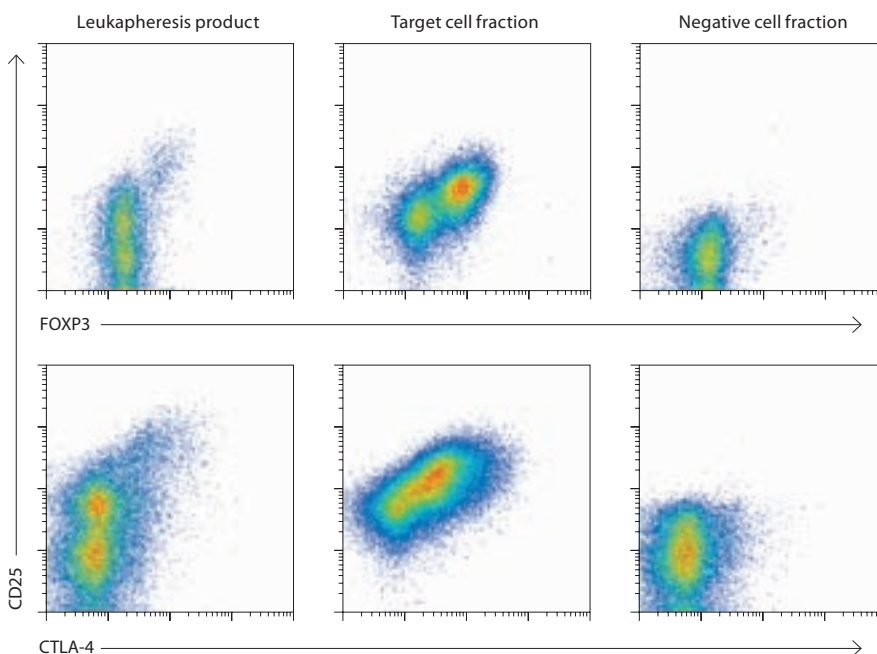


Figure 2: CD4⁺CD25⁺ T cells isolated under GMP conditions show a preferential expression of intracellular CTLA-4 and FOXP3.

Cells from a representative leukapheresis product, the corresponding target cell fraction and the CD19/CD25-depleted (negative) cell fraction were stained for CD4, CD25 and CTLA-4 (A) or FOXP3 (B), respectively. Dot plots show gated CD4⁺ T cells.

enriched for both CTLA-4 and FOXP3 expressing T_{reg} cells (fig. 2 A and B). To analyze the functional activity of the isolated cells, inhibition of proliferation of autologous $CD4^+CD25^-$ T cells was examined in CFSE-dilution assays where the magnetically enriched $CD4^+CD25^+$ T cells were compared to FACS-purified $CD4^+CD25^{high}$ T cells. $CD4^+CD25^-$ T_{resp} cells proliferated vigorously when stimulated with anti-CD3 antibody in the presence of autologous APCs. An equally efficient inhibition of this proliferation occurred when T_{reg} cells enriched with the CliniMACS® Plus Instrument or $CD4^+CD25^{high}$ T cells further purified by FACS were added to the responder cells, while the addition of unlabeled $CD4^+CD25^-$ T cells did not suppress T_{resp} cell proliferation (fig. 3).

In summary, these data show that depletion of B cells followed by enrichment for $CD25^+$ cells using magnetic bead separation under GMP conditions results in a >90% pure $CD4^+CD25^+$ T cell population. Preferential retention of $CD4^+CD25^{high}$ T_{reg} cells within such cell products increases their frequency to approximately 50% and results in a suppressive activity comparable to that of FACS-purified $CD4^+CD25^{high}$ T cells. The ability to isolate $CD4^+CD25^+$ T cells in clinically relevant numbers under GMP conditions now enables first clinical trials. Our current phase I study addresses feasibility and safety of the adoptive transfer of T_{reg} cells into allogeneic stem cell recipients. The final goal of such strategies is to reduce the incidence and severity of GVHD after SCT without abrogating beneficial donor T cell effects, such as immunity to infection, facilitation of engraftment or graft-versus-tumor activity.

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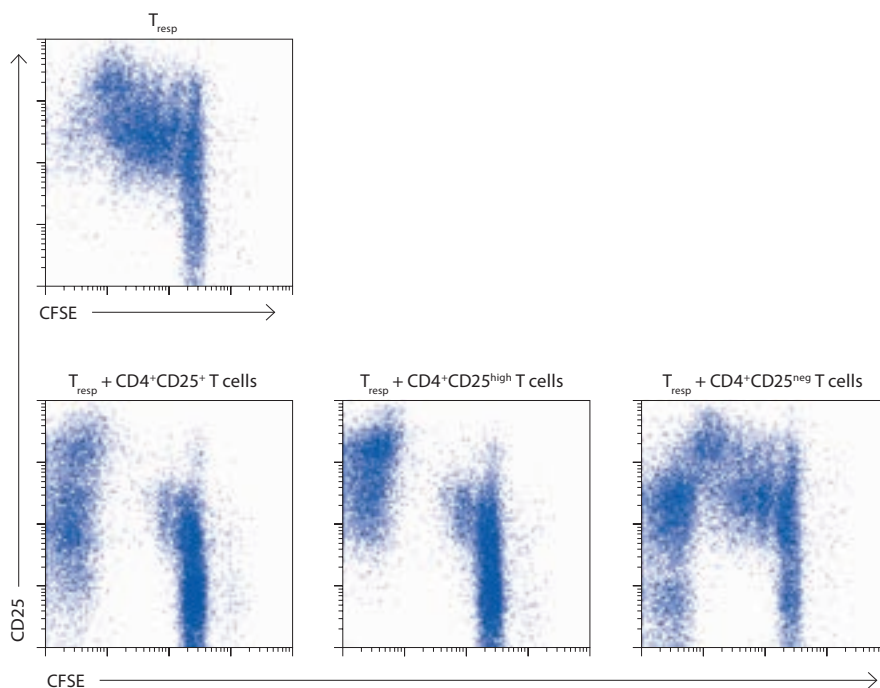


Figure 3: The suppressive activity of $CD4^+CD25^+$ T cells isolated by magnetic cell separation under GMP conditions is comparable to that of further FACS-purified $CD4^+CD25^{high}$ T cells.

Magnetically enriched $CD4^+CD25^+$ T cells (lower left panel) or further FACS-purified $CD4^+CD25^{high}$ (lower middle panel) or $CD4^+CD25^{neg}$ T cells (lower right panel) were co-cultured with CFSE-labeled T_{resp} cells and stimulated with anti-CD3 in the presence of autologous APC for 4 days. Proliferation of T_{resp} cells, as determined by CFSE-dilution, was profoundly inhibited in the presence of $CD4^+CD25^+$ and $CD4^+CD25^{high}$ T cells, but not in the presence of $CD4^+CD25^{neg}$ T cells.

New perception/aspects of T cells in cellular therapy

The continuing increase in activity in the field of immunotherapy has led to strengthened interest in methods that accurately assess T cell function and allow for engineering and the effective use of different T cell subsets, for instance in the transplant setting.

T cells play critical roles in the regulation of immune responses. They act as effectors either protective against pathogens or the development of tumor, or destructive as in the case of graft versus host disease (GVHD) and some autoimmune diseases. The different T cell subsets have a wide spectrum of capabilities and therefore become ever more interesting as novel approaches in cellular therapy emerge. The translation of research into the clinic and the possible utilization of T cell subsets in the field of transplantation require highly sophisticated reagents and technologies to enable clinical-grade enrichment or depletion of T cell subsets and assessment of their function.

Allogeneic transplantation protocols are increasing; registry data show an average increase of 10% per year.¹ Graft engineering may favor graft versus leukemia (GVL) effects by employing alloimmune effector lymphocytes to eliminate the tumor cells. Donor lymphocyte infusions (DLI) may prevent or fight relapses after stem cell transplantation (SCT) and may induce sustained remissions in patients.² Unfortunately, GVHD still is a common complication following allogeneic transplantations.²

To date, prevention of clinically significant GVHD without compromising engraftment, or anti-tumor activity, and without increasing the risk for infections is one of the most important goals of allogeneic transplantation.

The development of moderate to severe GVHD can have a negative impact on the survival of patients after hematopoietic stem cell transplantation (HSCT). Donor T cells are implicated in the pathogenesis of GVHD.

The most effective means of preventing GVHD may be the removal of T lymphocytes from the donor graft. However, in some studies these reductions in GVHD have been counterbalanced by unexpectedly high rates of graft failure, immune deficiency, and disease recurrence.³

Thus, the goal of current clinical research is the promotion of the desired GVL effect and the inhibition of GVHD. The key to this may be the separation of different cellular components of the graft or the specific tailoring of the DLI. The benefits of a GVL effect may be sustained without inducing GVHD via transfer of DLI either depleted of or enriched for specific T cell subsets or selected for antigen-specific T cells that recognize leukemia-specific antigens or minor histocompatibility antigens (mHAgs).

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The Miltenyi Biotec CliniMACS® Cell Selection System enables enrichment and depletion of different T cell subsets and thus provides the basis for a variety of different applications for investigational use.

CliniMACS® CD34 Reagent (CE)

The CliniMACS® CD34 selection technology is a very effective method for achieving high purity stem cell grafts with very low T cell numbers (up to 5 log depletion of the initial T cell number).¹ The T cell depletion of the graft may provide control over the T cell dose reinfused into the patient, reducing the potential risk of GVHD.²

In allogeneic settings, CD34 selection of stem cell grafts is currently being evaluated in:

- HLA-identical sibling transplantation.³
- Matched unrelated donor (MUD) transplantation.⁴
- Partially mismatched related and haplo-identical donor transplantation.⁵

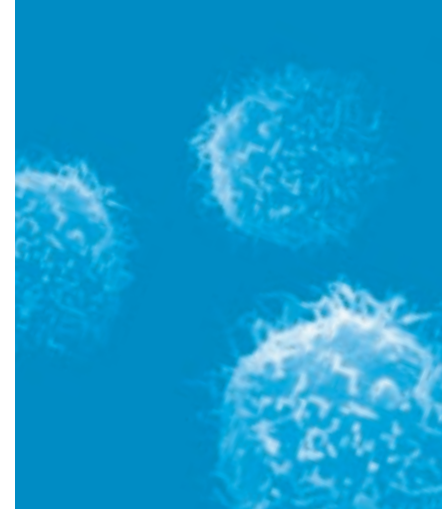
A further application is the use of CD34 selections to provide T cell-depleted stem cell boosts in order to treat delayed or insufficient engraftment after allogeneic stem cell transplantation without increasing the risk for GVHD.^{6,7}

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CliniMACS® CD3 Reagent (CE)

The CliniMACS® CD3 Reagent is suitable for bulk depletion of T cells from either unmobilized or mobilized leukapheresis products. In combination with the CliniMACS CD19 Reagent it can be used for the simultaneous depletion of T and B cells. Also, the CD3 Reagent is an important tool for the enrichment of NK cells. Use of the CD3 Reagent to deplete CD3⁺ T cells prior to CD56 selection results in a highly enriched CD3⁻CD56⁺ natural killer (NK) cell fraction. G-CSF-mobilized peripheral blood stem cells (PBSCs) are increasingly used as a stem cell source. Compared with transplantation of bone marrow, transplantation of allogeneic mobilized PBSC is associated with earlier hematopoietic recovery and shorter hospitalization.¹ However, due to the higher numbers of T cells in PBSC grafts, the risk of



acute and chronic GVHD increases, especially in HLA mismatched transplantation settings.^{2,3} One very effective means for prevention of acute and chronic GVHD is *in vitro* depletion of T lymphocytes from the graft.⁴

Several reports describe anti-leukemic effects and graft-facilitating effects of alloreactive NK cells derived from KIR-ligand-mismatched haploidentical donors.⁵ Clinical investigators are currently analyzing whether a simultaneous transplantation of NK cells with large numbers of stem cells and low numbers of T cells exerts a potent anti-leukemic effect, potentially reducing relapse rates without inducing GVHD.⁶⁻⁸ The engraftment-facilitating effect of alloreactive NK cells might also allow a reduction of conditioning intensity without compromising engraftment. This could pave the way for reduced intensity conditioning, thus decreasing transplant-related toxicity of haploidentical stem cell transplantation in malignant and non-malignant diseases.⁹ The combined use of the CD3 and CD19 Reagents to deplete T and B cells preserves NK cells and other effector cells in the graft.⁹ Feasibility of CD3/CD19-depleted grafts in the haploidentical and unrelated donor setting was shown in a number of clinical applications.¹⁰⁻¹² By combining CD34-selected with T cell-depleted and B cell-depleted products it is possible to tailor the graft even more specifically. The combination of high doses of selected stem cells with a cellular product depleted of T and B cells may result in a graft specifically suited to serve the requirements for allogeneic transplantations.

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CliniMACS® CD4 Reagent

CliniMACS® CD4 Reagent may provide a novel option in clinical research for investigating means to promote GVL effects and to inhibit GVHD. In this context it has been reported that CD4⁺ T cells may be capable of mounting anti-tumor responses, concomitantly reducing the risk of GVHD¹ and quickly and efficiently reconstituting the recipient's immune system². Another report showed that *in vitro* generated Th2 cells have been successfully used to support stem cell grafts³; the addition of IL-4 to selected CD4⁺ T cells allows for skewing towards a Th2 type. The combined depletion of CD4⁺ and CD8⁺ T cells is another option for the use of CliniMACS CD4 Reagent; this approach results in a target cell fraction enriched for major components of the innate immune system, the NK cells and TCRγδ⁺ T cells. Besides NK cells, TCRγδ⁺ T cells are thought to have potent anti-tumor effects without causing GVHD.⁴ In the haploidentical allogeneic setting the enrichment of TCRγδ⁺ T cells and/or NK cells may be useful in graft modulation, as well as for tailoring DLI. In addition, the use of CliniMACS CD4 Reagent may be the basis in various gene-therapeutic approaches. In the HIV setting a highly enriched CD4 population may be transduced with vectors delivering resistance genes aiming at protection of CD4⁺ T cells against HIV infection.⁵⁻⁷ A pure and viable CD4⁺ T cell population may be the critical step in transduction protocols. Enrichment of CD4⁺ T cells may also be useful in the generation of antigen-specific CD4 T cell lines.

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CliniMACS® CD8 Reagent (CE coming soon)

The CliniMACS® CD8 Reagent allows the positive or negative selection of CD8⁺ T cells.

The presence of high numbers of CD8⁺ T cells in peripheral blood early post-BMT has been associated with the subsequent development of GVHD.¹

In the allogeneic transplantation setting CD8⁺ T cells can be removed from a graft as a mode of graft engineering or DLI to prevent development of GVHD following transplantation.² It was shown in a clinical study that the depletion of CD8⁺ T cells for DLI may diminish GVHD while maintaining the desirable GVL effect.¹ This may be due to the retained major players of the innate immune system, the NK cells and the TCRγδ⁺ T cells. First results from a phase I study, investigating the early preemptive transfer of CD8-depleted DLI after T cell-depleted RIC allogeneic transplantation to improve immune reconstitution and reduce incidence of GVHD were presented at ASH 2005. The results suggest that the preemptive use of clinical-grade CD8-depleted DLIs accelerates immune reconstitution after Alemtuzumab-based reduced intensity conditioning, thereby increasing virus-specific immunity and maintenance of donor chimerism with a low risk of GVHD.³ Another study⁴ investigated the preemptive transfer of CD8-depleted DLI using the CliniMACS CD8 Reagent after haploidentical SCT (n=9) using a reduced intensity conditioning in a dose-escalating regimen. Results suggest that the procedure is feasible with a high rate of engraftment and a low acute GVHD incidence as well as a fast immune recovery.

In a pilot study Baron and colleagues⁵ investigated the feasibility of CD34-selected peripheral blood stem cell transplantation followed by preemptive CD8-depleted DLI with reduced intensity conditioning. The results indicated that the procedure is feasible and preserves engraftment and apparently also the graft versus leukemia effect. CD8 depletion might also be a useful step in enrichment strategies for regulatory T cells, where the depletion of unwanted cell types (CD8 cells expressing CD25) precedes CD25 enrichment.

Enrichment of CD8⁺ T cells may also be an option for the generation of T cell lines.⁶

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CliniMACS® CD25 Reagent

CD25 was first described as a T cell proliferation marker. It is now regarded as a marker that defines activated/reactive T cells. CliniMACS CD25 Reagent was developed for the positive selection of activated T cells and for the depletion of alloreactive T cells. In this respect CD25 T cell depletion may be an interesting option in the allogeneic transplantation setting where alloreactive CD25⁺ T cells are removed from a graft or DLI with the goal of preventing GVHD^{1,2} without diminishing the graft versus tumor (GVT) effect³. Removing these cells results in a passive enrichment of cells possibly mediating the GVT effect.

A subpopulation of naïve CD4⁺ T cells co-expressing CD25 has been shown to have potent suppressor activity⁴; these naturally occurring CD25^{hi}CD4⁺ regulatory T cells are thought to prevent GVHD by suppression of activated CD4⁺ and CD8⁺ effector T cells.

Data generated from studies in mice⁴ encouraged clinicians to further investigate the potential role of CD25^{hi}CD4⁺ regulatory T cells for the improvement of stem cell transplantations. The aim is to modulate GVHD alloresponses through the addition of CD25^{hi}CD4⁺ regulatory T cells, either to the graft or as DLI.⁵

CD25 Reagent can also be used for the enrichment of these CD25^{hi}CD4⁺ regulatory T cells, either in a one-step protocol or after a pre-separation step with, for example, CliniMACS CD8 Reagent or CliniMACS CD19 Reagent, in order to first deplete other unwanted CD25-positive cell populations.

On the other hand, the depletion of CD25^{hi}CD4⁺ regulatory T cells using CD25 Reagent is being investigated as a means to enhance cytotoxic effects of tumor-specific T cells. Powell *et al.*⁶ described the efficient depletion of CD25^{hi}CD4⁺ regulatory T cells from patient leukapheresis samples. Using the CliniMACS CD25 Reagent they were able to deplete the CD25^{hi}CD4⁺ regulatory T cells and gained a cellular product ready for their immunotherapeutic approaches. In

theory, depletion of the CD25^{hi}CD4⁺ regulatory T cells should decrease the suppressive effects observed *in vivo* in some malignancies and should allow for antitumor responses.

Additionally, several protocols examine the versatile potential of CD25 Reagent for improving autoimmune disorders, as naturally arising CD25^{hi}CD4⁺ regulatory T cells are known to be important for induction and maintenance of self-tolerance.⁷ Thus they play a pivotal role in preventing organ-specific autoimmune diseases and in inducing tolerance to allogeneic organ transplants.⁷

Also see the customer report in this issue on pages 4–6 by P. Hoffmann *et al.* from Regensburg, Germany.

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CliniMACS® Cytokine Capture System (IFN-gamma) (CE coming soon)

The CliniMACS® Cytokine Capture System (IFN-gamma) (CCS) enables the simultaneous enrichment of viable antigen-specific CD4⁺ and CD8⁺ T cells for immune therapy^{1–4}. Since the application of CD4⁺ T cells together with CD8⁺ T cells is crucial for a sustained antigen-specific immune reaction⁵, the system may enable better immune responses than the infusion of CD8⁺ cells alone. Subsequent to enrichment, *in vitro* expansion of the antigen-specific T cells can be performed, if indicated.

Viral infections continue to be a source of morbidity and mortality after allogeneic transplantation of solid organs and hematopoietic cells. These viral infections are presumed to be in part due to profound immunosuppression. Antigen-specific T cells have been described as very useful for the treatment and/or prevention of infections after allogeneic HSCT^{6,7} and also after solid

organ transplantation^{8,9}. Thus the enrichment of T cells specific for herpes viruses, especially for CMV, EBV, or Adenovirus, is an important goal.

The adoptive transfer of virus-specific T cells offers the potential for accelerating reconstitution of antigen-specific immunity and limiting the morbidity and mortality from viral infections following allogeneic stem cell transplantation and following solid organ transplantation. Selection and adoptive transfer of antigen-specific T cells according to the production of IFN-gamma allows very specific enrichment of these cells with low risk of unwanted allogeneic effects.

Recently, the first study has been published that describes adoptive T cell therapy not only for viral but also for invasive fungal infection. Perruccio and colleagues¹⁰ for the first time documented antifungal efficacy of T cell therapy after the adoptive immunotherapy with donor-derived T cell lines following haploidentical stem cell transplantation.

The Cytokine Capture System (IFN-gamma) may also be of value in the field of oncology. In contrast to the virology setting the main challenge in the oncological setting is the identification of the most immunogenic antigens. Many studies are under way addressing the question of the most suitable antigens for specific malignant diseases.

The possibility to simultaneously enrich for both CD4⁺ and CD8⁺ antigen-specific T cells is a milestone in T cell therapy, simplifying the often tedious and time-consuming generation of specific T cell lines.

The CliniMACS Cytokine Capture System (IFN-gamma) is not available in the United States.

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Satellite Symposium ASH 2005

Cellular Therapy: Present and Future

The 47th Annual Meeting of the American Society of Hematology, which took place from December 10 to 13, 2005, in Atlanta, GA, hosted the Satellite Symposium "Cellular Therapy: Present and Future" organized by Miltenyi Biotec.

Miltenyi Biotec was honored that Jeffrey S. Miller, Professor of Medicine, University of Minnesota, agreed to chair the session.

More than 450 participants attended the presentations on new trends in stem cell transplantation, cellular therapy and graft engineering. The speakers discussed current strategies in cellular therapy and the clinical potential of T cell subsets, NK and NKT cells and stem cell populations to treat such diverse clinical challenges as cancer relapse, viral infections, graft rejection, and graft versus host disease. Dr. Miller welcomed the opportunity to come together to discuss different aspects of novel cell-based therapies.

Miltenyi Biotec has prepared a booklet with the summaries of the presentations. It may be requested using the fax-back form on the inside of the back cover.

Robert Lowsky, Robert Negrin, Maria Milan, Karl Blume, and Samuel Strober, Stanford University School of Medicine, Stanford, CA, USA

TLI conditioning for graft versus host disease protection and induction of tolerance following combined blood stem cell and kidney transplantation for all patients.

The immunosuppressive effects of total lymphoid irradiation (TLI) for inducing transplantation tolerance following bone marrow and solid organ allografts have been studied for years.

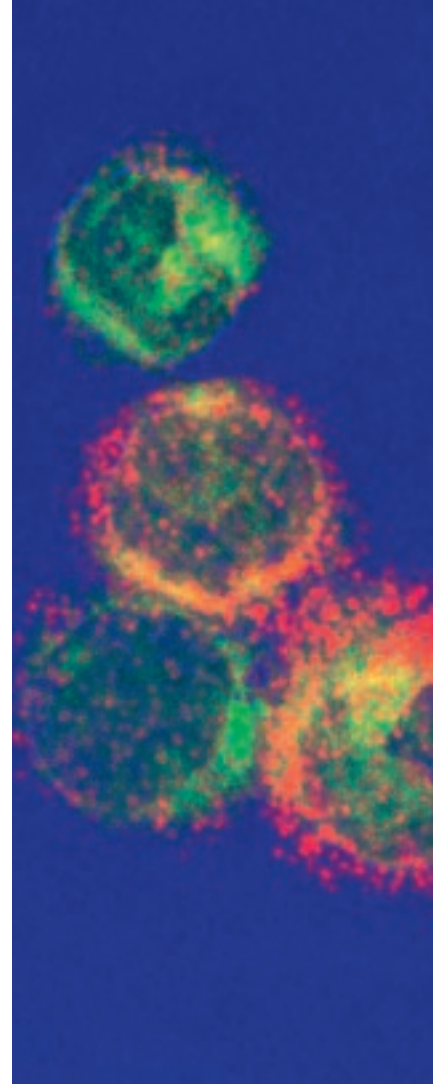
Using a non-myeloablative regimen consisting of TLI (800 cGy in 10 fractions)

and anti-thymocyte globulin (ATG) (1.5 mg/kg/d on days -11 to -7) and grafts from HLA-matched related, unrelated and haploidentical donors, the conditions for a successful hematopoietic cell engraftment could be established by Dr. Lowsky and colleagues. Dr. Lowsky presented data showing that the low incidence of aGVHD is associated with a significant alteration in residual host T cell subsets favoring NKT cells (mean 0.01% NKT before TLI to 0.5% after TLI). In summary, the regimen of TLI and ATG is non-myeloablative, potentially results in a marked reduction of GVHD incidence and could favor anti-tumor activity. Finally, Dr. Lowsky briefly touched on clinical data achieved through the combination of hematopoietic stem cell transplantation (HSCT) and kidney transplantation.

Ann Woolfrey, Shelly Heimfeld (presenting author), Clinical Research Division, Fred Hutchinson Cancer Research Center (FHCRC), Seattle, WA, USA

Partial T cell depletion of G-CSF mobilized peripheral blood mononuclear cells (G-PBMC) for patients with advanced MDS and other myeloid malignancies transplanted from HLA-identical siblings

Randomized clinical trials have shown superior outcome in patients who were transplanted with G-CSF mobilized peripheral blood mononuclear cells (G-PBMC) compared to patients transplanted with bone marrow from HLA-identical related donors. Even though the number of T Lymphocytes in G-PBMC is more than 10-fold higher, the incidence of acute GVHD does not increase in these protocols. One hypothesis is that G-PBMC may contain proportionally higher numbers of immunosuppressive cells.



Dr. Heimfeld presented data of four patients enrolled in a protocol at the FHCRC. The protocol includes T lymphocyte reduction of G-PBMC by depletion of CD3⁺ cells using the CliniMACS® System. In order to achieve a 10-fold overall reduction of T lymphocytes, only 90% of the G-PBMC is processed; after testing, the CD3-depleted fraction is recombined with the non-manipulated G-PBMC. The idea is to reduce the T cell number to bone marrow level. With the CliniMACS Plus Instrument an average T cell depletion of 3.7 log has been achieved (range 3.2–4.0), whereas the majority of the progenitor cells and other immune effector populations (>70%) could be retained in the graft.

The preliminary results showed good engraftment and no evidence of graft rejection; unfortunately the time after transplantation was too short to comment on relapse and day 100 non-relapse mortality.

Robert Soiffer, Department of Medical Oncology, Dana Farber Cancer Institute, Boston, MA, USA

CD8⁺ T cell depletion as GVHD prophylaxis

The most effective means for preventing GVHD is the removal of T lymphocytes from the donor graft. The decreased incidence and severity of GVHD, however, is counterbalanced by an increase in graft rejection, relapse and a prolonged time to immune reconstitution. Positive results from several studies led Robert Soiffer's group to explore CD8 depletion as a mode of graft engineering to prevent GVHD following transplantation of mobilized peripheral blood. Using the Eligix™ Cell Selection System to deplete CD8⁺ cells from the donor graft, engraftment and relapse rates were unaltered. Although results for event-free survival were encouraging, grades 2–4 acute GVHD and chronic GVHD were not diminished to the anticipated degree. Analyses revealed that the median number of CD8⁺ T cells infused was still high with 4×10^5 cells/kg.

In order to improve outcome, Dr. Soiffer recently started accruing patients for a study protocol using the CliniMACS® System for CD8⁺ T cell depletion of allografts aiming at $<1.0 \times 10^5$ CD8⁺ T cells/kg as GVHD prophylaxis in recipients of unrelated PBSCs after non-myeloablative conditioning.

Jeffrey S. Miller, University of Minnesota Cancer Center, Blood and Marrow Transplant Program, Minneapolis, MN, USA

NK cells, their receptors and implications for cancer therapy and hematopoietic cell transplantation

Natural killer (NK) cells produce cytokines and express killer immunoglobulin-like receptors (KIRs) that regulate their cytotoxicity. It is hypothesized that T cells in the graft affect NK cell reconstitution and function *in vivo*. Using a non-transplant strategy aimed at treating advanced myeloid leukemia patients with haploidentical NK cells, Dr. Miller's group found that a lymphocyte-depleting regimen is required to induce *in vivo* expansion of NK cells. In the study presented, NK cells were enriched passively from donor apheresis products

using CD3 depletion alone or a combined strategy consisting of CD3 depletion followed by CD56 selection. All patients received IL-2 after the NK cell infusions. Remission in these patients who were transplanted with the CD3-depleted product correlated positively with *in vivo* expansion of functional NK cells. With the CD3/CD56 strategy no patient achieved remission. Dr. Miller hypothesized that accessory cells in the CD3-depleted product might be mandatory to support long-term proliferation of NK cells. In his presentation Dr. Miller also briefly touched on an open trial using allogeneic NK cells plus NK precursors from umbilical cord blood passively enriched by CD3 depletion.

Bruce R. Blazar, Department of Pediatrics, Pediatric Blood and Marrow Transplantation Program, University of Minnesota, MN, USA

Application of CD4⁺CD25⁺ regulatory T cells in allogeneic bone marrow transplantation

A major limitation for the clinical application of human CD4⁺CD25^{hi} regulatory T cells (Treg) has been their low frequency in blood. Recent approaches have solved this problem. Dr. Blazar's group isolated Treg from adult peripheral blood mononuclear cells using the CliniMACS® System. Without additional sorting procedures the enriched cells could be expanded more than 100-fold in 2–3 weeks with a protocol using CD3/CD28 stimulation + feeder cells + IL-2. In mixed lymphocyte reaction (MLR) cultures the expanded Treg showed higher suppressive capacity than fresh cells and retained a high level expression of characteristic lymph node

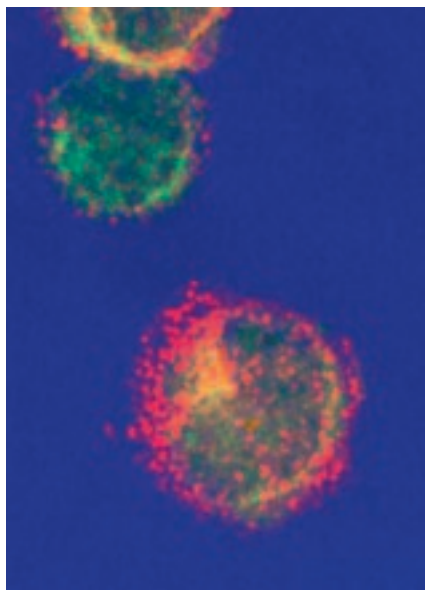
homing receptors like L-selectin. Also, umbilical cord blood (UCB) was discussed as a source of Treg. Dr. Blazar's data showed that the UCB Treg lead to a uniformly high level of suppression. Hopes are that these cells perform superiorly in clinical trials aimed at inhibiting GVHD and graft rejection. Dr. Blazar also talked about the receptor signaling properties of human Treg.

S. Mackinnon, K. Thomson, S. Verfuert, R. Chakraverty, K. Peggs, A. Fielding, M. Lowdell, Royal Free and University College London Medical School, London, United Kingdom

Rapid selection of CMV-specific T cells: preliminary results of adoptive cellular therapy for CMV infection following allogeneic stem cell transplantation

Adoptive transfer of virus-specific T cells offers the potential for accelerating reconstitution of antigen-specific immunity following allogeneic stem cell transplantation. Dr. Mackinnon reported on a study his group has recently embarked on. In their protocol CMV-specific T cells were selected on the basis of IFN-gamma secretion from donor leukapheresis using the CliniMACS® Cytokine Capture System (IFN-gamma)*. The preliminary data presented—5 patients with a maximum of 3 months follow-up—demonstrate that T cell immunity to CMV might be reconstituted by transfer of CMV-specific T cells even in the very low dose of 1×10^4 cells/kg. After selection of the CMV-specific cells, aliquots of 1×10^4 and 3×10^4 cells/kg recipient weight were frozen and either infused prophylactically between d40 and d50 or preemptively, in case the patient developed a CMV viremia following transplant. Ganciclovir was applied if the viral titer increased for two consecutive days after transfer of the cells. Up to now, no toxicities related to the cellular product and no GVHD were observed. Dr. Mackinnon reported on a rise in T cell number after adoptive transfer probably due to an *in vivo* expansion of the transferred cells with restoration of anti-viral immunity following adoptive transfer.

*The CliniMACS Cytokine Capture System (IFN-gamma) is not available in the United States.



Satellite Symposium European Society of Cardiology, 2005

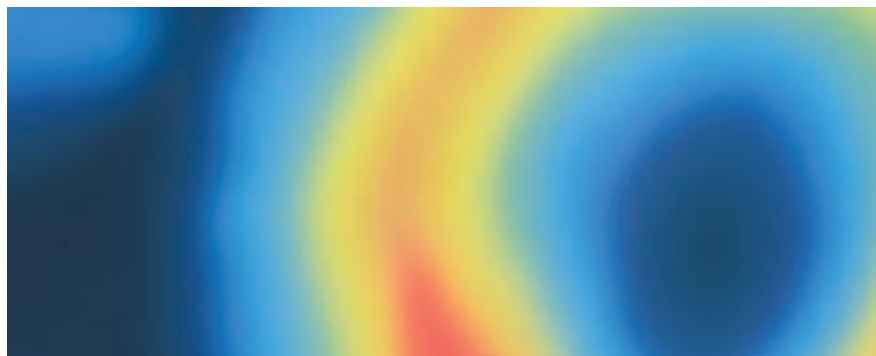
“Stem cell therapy in cardiac regeneration”

The annual meeting of the European Society of Cardiology (ESC), which took place from September 3 to 7, 2005, in Stockholm, Sweden, also hosted the Miltenyi Biotec symposium “Stem cell therapy in cardiac regeneration”.

The symposium was chaired by Jozef Bartunek, Associate Director at the OLV Hospital Cardiovascular Center in Aalst, Belgium, and Warren Sherman, Director of Cardiac Cell-Based Endovascular Therapies at the Columbia University College of Physicians and Surgeons, New York, USA.

The session conveyed an overview on current clinical experience using CD133⁺ stem cells, and gave rise to an expert discussion on future perspectives of myocardial regeneration as cardiac surgeons as well as interventional cardiologists presented their clinical results.

Miltenyi Biotec has prepared a booklet with summaries of the presentations. It may be requested using the fax-back form on the inside of the back cover.



Christof Stamm
Assistant Professor of Cardiac Surgery at the University of Rostock, Germany
Stem cell therapy for ischemic heart disease: Beginning or the end of the road?

Dr. Stamm gave an overview on the work he and his colleagues in Rostock pioneered using CD133-selected stem cells for patients with subchronic heart failure after myocardial infarction. Their approach is the intramyocardial application of bone marrow-derived CD133⁺ stem cells in patients who undergo coronary bypass surgery. The results Dr. Stamm presented of their clinical phase I study demonstrate the feasibility of the approach. The preliminary results of an ongoing controlled phase II study enrolling 100 patients were also discussed. Dr. Stamm concluded that the treatment might provide evidence of a pronounced effect on blood supply to the ischemic myocardial tissue as measured by SPECT associated with an improvement of global contractile function. The effect on global left ventricular function was more pronounced in patients with

preoperative ejection fraction < 30%. Dr. Stamm emphasized that the potential of stem cells in heart diseases needs to be explored in close relationship to basic science.

Hans-Michael Klein
Professor of the Departments of Thoracic and Cardiovascular Surgery at the University of Düsseldorf, Germany
Stem cell therapy for severe ischemic cardiomyopathy: Safety and enhanced left ventricular function.

Dr. Klein focused on the treatment of patients with severe ischemic heart failure. His group performed a prospective, non-randomized, open-label study in nine heart-transplant candidates, not amenable to coronary artery bypass grafting (CABG). The application of CD133⁺ stem cells into predefined regions within hibernating myocardium was performed during lateral thoracotomy. Three months after the patients received stem cells an improvement of cardiac function was evident, reflected by a mean improvement of left ventricular ejection fraction (LVEF) from

18.3% preoperative to 26% postoperative. In addition, the mean left ventricular end-diastolic diameter decreased from 79.2 mm preoperative to 57.4 mm postoperative. Dr. Klein concluded that the sole treatment of patients with purified stem cells might hold promise for the management of patients with ischemic heart failure who are ineligible for conventional revascularization.

Alessandro Colombo
Senior Cardiologist at the
Catheterization Laboratory, “Luigi
Sacco” Hospital, Milan, Italy
Intracoronary transfer of autologous
CD133⁺ stem cells in recent myocardial
infarction with extensive damage:
A pilot controlled phase I/II trial.

Dr. Colombo summarized the potential of stem cells for cellular therapy in patients with acute myocardial infarction (AMI) without efficient reperfusion after primary percutaneous intervention (PCI). In close collaboration with other hospitals in Milan, Dr. Colombo and his colleagues conducted a randomized controlled phase I study based on intracoronary administration of CD133⁺-enriched stem cells from mobilized peripheral blood or bone marrow. Patients with recent large AMI treated by optimal vessel recanalization through coronary stenting within 12 hours, yet unsatisfactory postprocedural reperfusion, were enrolled. The patients were randomly assigned to the following groups: A) bone marrow-derived CD133⁺ cells, B) peripheral blood-derived CD133⁺ cells and C) optimal medical therapy. Results of nine patients (three in each group) treated up to now were presented. The processing and administration of the stem

cells were shown to be safe and feasible in this study. Results on regional and global left ventricular function, and perfusion studies showed promising results. Dr. Colombo concluded that there is a necessity to conduct more treatments for a better assessment of these preliminary results.

Marc Vanderheyden
Associate Director and Cardiologist at
the OLV Hospital Cardiovascular Center,
Aalst, Belgium

Enriched CD133⁺ cells for cardiac
recovery in reperfused myocardial
infarction: Clinical rationale, potential
and safety.

Dr. Vanderheyden gave a summary on recent results in stem cell therapy in interventional cardiology and on the motives to perform clinical studies using purified CD133⁺ stem cells. He presented clinical results of a phase I/II study which addressed the feasibility, safety, and functional effects of intracoronary stem cell administration. Among 35 AMI patients with PCI, 19 patients underwent intracoronary administration of CD133⁺ stem cells, whereas 16 did not receive stem cells. Some patients treated with stem cells showed in-stent reocclusion and distal lumen loss, whereas the control group showed slightly less in-stent reocclusion and no distal lumen loss. Despite this observation, the patients in the treatment group showed improved left ventricular function paralleled by increased myocardial perfusion and viability. Dr. Vanderheyden stated that the observed results need to be followed more closely in studies enrolling larger patient numbers in the future.

Frequently asked questions

Q.: I performed a T and B cell depletion from a mobilized apheresis product with 20×10^9 labeled cells. I used separation program DEPLETION 2.1 and the CliniMACS® Tubing Set LS for this approach. The depletion process took about five hours. How can I speed up this procedure?

A.: The use of the new CliniMACS Depletion Tubing Set and selection program DEPLETION 3.1 significantly shortens depletion procedures, particularly of products with high T and/or B cell counts.*

A depletion procedure with 20×10^9 labeled cells will take you 1.25 hours compared to five hours when using the CliniMACS Tubing Set LS.

*In the U.S.A., the CliniMACS Depletion Tubing Set may only be used for depletion of CD3-positive cells.

Q.: What is the maximal capacity of the new CliniMACS® Depletion Tubing Set?

A.: Using this type of tubing set up to 40×10^9 labeled cells can be depleted from up to 120×10^9 white blood cells.

Q.: I have recognized that with the CliniMACS® Tubing Set and the CliniMACS Tubing Set LS the bubble trap and the spike on top have changed. Why has this been done?

A.: Based on customer requests for a more convenient connection of the pre-system filter to the tubing set, a component change has been implemented. Both the new bubble trap and the spike meet all specifications according to relevant manufacturing requirements. The new spike allows for a very easy and convenient insertion into the Pre-System Filter, as it requires less force for connection.

Q.: How can I reliably test the CliniMACS® Tubing Set for leakage before patient material enters the system?

A.: For software 2.31 and higher a so-called “Integrity Test” has been integrated. This short and fully automated leakage check can be performed directly after the priming sequence of the instrument. In the very unlikely event of leakage a new tubing set can be installed.

Q.: Which sample parameters do I have to feed into the CliniMACS® Plus Instrument when doing a depletion procedure?

A.: As volume and corresponding cell concentration of the product in the Cell Preparation Bag (product to be loaded onto the CliniMACS Plus Instrument, ORIGINAL sample) are different compared with the original leukapheresis product, the WBC concentration and the sample loading volume have to be determined in the ORIGINAL sample. In order to save valuable time, however, the percentage of labeled cells can be inferred from the starting leukapheresis product (LEUKAPHERESIS PRODUCT sample) by assuming that the composition of the product does not change during the procedure.

Q.: Using the depletion tubing set (DTS) I realized that the Pre-system Filter has to be installed upside down, while it is installed in the opposite direction when using, for example, the large-scale tubing set (Tubing Set LS). Does this influence the filtration process?

A.: The Pre-System Filter is used for retaining cell aggregates. The inverted installation of the Pre-System Filter does not affect the filtration performance and even serves as a bubble trap.

Q.: The CD34 selection procedure was aborted by an unexpected power failure. How can I rescue my labeled target cells from the tubing set, thereby assuring they stay sterile?

A.: You have to reset and restart the CliniMACS® Plus Instrument. Select the “Emergency Program” and ensure that the Cell Collection Bag attached is capable to hold another 100 mL. The “Emergency Program” guarantees that all cells retained on the column will be eluted into the Cell Collection Bag. Combine the contents of the Cell Collection Bag with the remainder of the Cell Preparation Bag and start a new selection using a new tubing set. Please note that the use of the “Emergency Program” is expedient for enrichment procedures only, not for depletions.

The Clinical Technical Support Team brings their experience in immunology, molecular biology and engineering to your research and clinical applications. As researchers themselves the team understands your need for high-quality technical support, customer service, and cutting edge product design.

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ASTIS Trial

Autologous Stem Cell Transplantation International Scleroderma Trial

The ASTIS Trial

This multicenter prospective randomized controlled phase III study will compare efficacy and safety of high-dose immunoablation and autologous hematopoietic stem cell transplantation (HSCT)(considered the investigational treatment), versus monthly intravenous pulse-therapy cyclophosphamide (CYC) (considered the standard treatment) in patients with diffuse systemic sclerosis and heart, lung or kidney involvement. These patients are at risk for severe organ dysfunction and as a consequence premature mortality. The goal of the treatment is to prolong survival by arresting or retarding the disease process. It is postulated that the investigational treatment has superior efficacy based on observations of long-term remissions in a number of patients, although this has to be balanced against potentially higher toxicity.

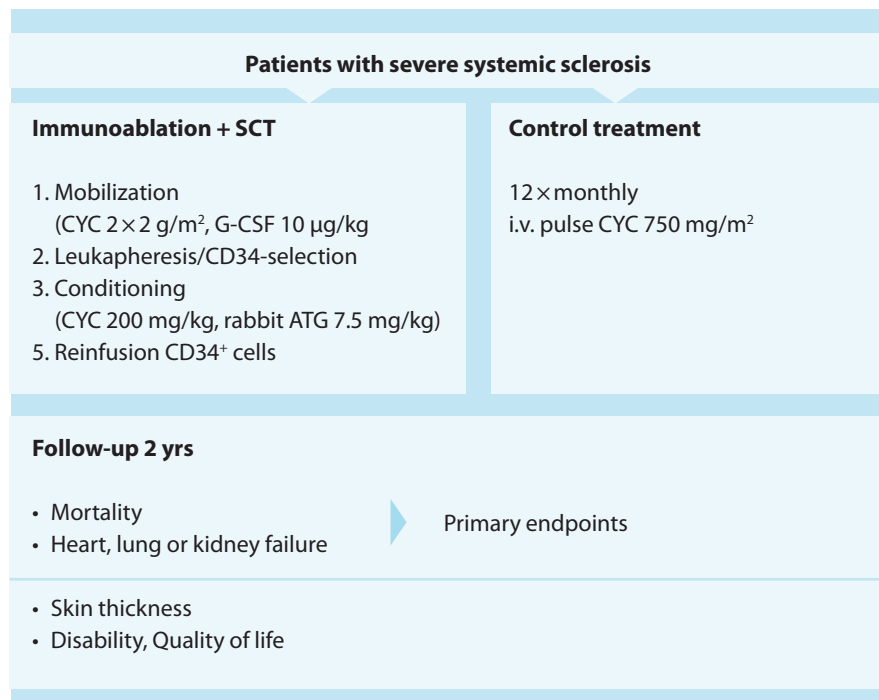
Miltenyi Biotec GmbH recently signed an agreement with the ASTIS study administration office. Miltenyi Biotec will give a limited commercial support for the CD34 selection.

For further information please contact the study chair of the EBMT/EULAR Scleroderma Study Group,

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Study flow chart



With permission from Dr. J.M. van Laar.

Miltenyi Biotec Satellite Symposium EBMT 2006, Hamburg, Germany

Innovative strategies for cellular therapy

Sunday, March 19, 2006
11:00–13:00 h
Congress Center Hamburg, Hall 4

Chair:

J. H. Frederik Falkenburg, MD, PhD
Professor of Hematology, Leiden
University Medical Center, Department
of Hematology, Leiden, Netherlands

Topics

Graft engineering to improve haploidentical transplantation

Rupert Handgretinger, MD, Pediatrics,
University Tübingen, Germany

CD8-depleted donor lymphocyte infusion to reverse mixed chimerism after allogeneic stem cell transplantation

Mark W. Lowdell, MD, PhD MRCPATH,
Department of Hematology, Royal
Free Hospital and School of Medicine,
London, United Kingdom

Isolation of human CD4⁺CD25⁺ regulatory T cells for clinical trials

Matthias Edinger, MD,
Department of Hematology and
Oncology, University Hospital
Regensburg, Regensburg, Germany

Enrichment of antigen-specific T cells for adoptive immunotherapy after allogeneic stem cell transplantation

J. H. Frederik Falkenburg, MD, PhD,
Department of Hematology, Leiden
University Medical Center, Leiden, The
Netherlands

Intramyocardial stem cell transplantation in patients with chronic ischemic heart disease

Christof Stamm, MD, German Heart
Institute, Berlin, Germany

Miltenyi Biotec Satellite Symposium ISCT 2006, Berlin, Germany

Novel routes in cellular therapy research

Thursday, May 4, 2006
16:00 – 18:00 h
Hotel Maritim proArte Berlin

Chair:
Nicolaus Kröger, MD, Hamburg,
Germany

Haploidentical allogeneic stem cell transplantation with CD3/CD19 depleted grafts

Wolfgang A. Bethge, MD, University of Tübingen, Tübingen, Germany

Serum-free generation of mature dendritic cells for clinical application in malignant glioma patients

Rüdiger V. Sorg, PhD, Heinrich Heine University, Düsseldorf, Germany

Adoptive transfer of CD4-enriched donor lymphocyte infusions after allogeneic stem cell transplantation

Nicolaus Kröger, MD, University Hospital Hamburg-Eppendorf, Hamburg, Germany

Preemptive CD8-depleted donor lymphocyte infusions after haploidentical stem cell transplantation using a reduced-intensity conditioning regimen

Paolo Corradini, MD, Istituto Tumori, Milan, Italy

Initial experience with CD133⁺ hematopoietic progenitor cell administration prior to extensive liver resection

Wolfram T. Knoefel, MD, Heinrich Heine University, Düsseldorf, Germany



Upcoming meetings – meet us at the booth!

Date	Congress	Webpage
April 30–May 2	5th Bi-annual Symposium on Childhood Leukemia, Noordwijkerhout, Netherlands	www.congresscare.com/congres_template.php?nieuwsID=1581
May 4–5	4th CIMT Symposium, Mainz, Germany	http://www.kimt.de/
May 4–7	ISCT, 12th Annual Meeting, Berlin, Germany	www.celltherapy.org/
May 18–20	8th International Congress of the Cell Transplant Society, Milan, Italy	http://www.transplantation-soc.org/meetings.php
June 21–24	Annual European Congress of Rheumatology, Amsterdam, Netherlands	www.eular.org/eular2006/
July 24–27	43rd Meeting of the Society for Cryobiology in association with the Society for Low Temperature Biology, Hamburg, Germany	www.sltb.info/PDF/Cryo2006_Flyer.pdf www.cryo2006.org
August 17–20	16th World Congress Cardio-Thoracic Surgeons, Ottawa, Canada	www.wscts2006.com
September 2–6	World Congress of Cardiology, Barcelona, Spain	www.escardio.org/congresses/World_Congress_Cardiology_2006/
October 5–7	20th Annual Meeting of the EMDS, European Macrophage & Dendritic Cell Society, Freiburg, Germany	www.immunologie.de/frames/Tagungen-Dateien/EMDS_2006_Flyer.pdf
October 26–28	National Hematology Congress, Granada, Spain	www.aehh.org
November 4–8	DGHO, Leipzig, Germany	www.haematologie-onkologie-2006.de/
December 9–12	ASH, 48th Annual Meeting, Orlando, Florida, USA	www.hematology.org/meeting

Fax-back form

CliniMACS® Newsletter Vol. 6 No.1/2006

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Marketing Department, Brigitte Borchert
Fax no. + 49 2204 85197

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ISCT 2005 130-092-264	Abstract booklet of Miltenyi Biotec symposium "Cellular Therapy: New insights and strategies"	<input type="checkbox"/>
ASH 2005 130-092-542	Abstract booklet of Miltenyi Biotec symposium "Cellular Therapy: Present and Future"	<input type="checkbox"/>
ESC 2005 130-092-432	Abstract booklet of Miltenyi Biotec symposium "Stem cell therapy in cardiac regeneration"	<input type="checkbox"/>
CliniMACS® Products 2005/2006 130-090-666		<input type="checkbox"/>
CliniMACS® Flyer 130-091-674		<input type="checkbox"/>
Cell Culture Bags Flyer 130-092-232		<input type="checkbox"/>
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Please check the box if you wish to receive additional information. Your local Miltenyi Biotec representative is available to visit your site and discuss our products in more detail.		<input type="checkbox"/>

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