

CliniMACS[®]

Newsletter

Vol. 6 No. 2/2006

Customer report

Cell boosting with selected CD34⁺ stem cells for poor graft function after allogeneic stem cell transplantation

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Editorial

Dear Colleagues,

for us at Miltenyi Biotec it has always been a great pleasure working with you, sharing the exciting progress in biomedical science, and developing novel therapies for the treatment of – so far – incurable diseases.

Our goal is to supply you with the right tools that can decipher the cellular and molecular background of most diseases. We hope that our tools for cellular therapies will contribute to your attempts at finding better treatment schemes for the future.

With your continuing support Miltenyi Biotec has grown to nearly 1000 people worldwide. As such, we are now able to offer you even more products and services supporting your day-to-day scientific and clinical work.

The Miltenyi Biotec team covers many disciplines from physics and chemistry to molecular biology, immunology, and cell biology, from instrument and plastic engineering to software development, from basic research to GMP production, regulatory and clinical trial support.

We have recently introduced our new and unique expression profiling service for RNA analysis on a single cell level, which can also be used for cells from tissue sections. We are working on providing you with GMP cell culture media and systems for cell expansion,

including recombinant cytokines, antigens, and peptides in the near future. Our interdisciplinary teams are developing new research and clinical cell separation platforms, as well as new systems for cell culture and cell manipulation.

Our clinical development team is looking forward to working with you on more exciting clinical trials using CliniMACS® Technology for various protocols and diseases.

Immunologically based therapies and regenerative medicine will continue to be the focus for our clinical product developments in the future.

I would like to ask you to continue and extend your dialogue with us. Share your vision in cellular therapies with us and let us know your needs for the future! We will try our best to give you the appropriate tools when you need them.

I look forward to continuing our successful cooperation,

Stefan Miltenyi

Cell boosting with selected CD34⁺ stem cells for poor graft function after allogeneic stem cell transplantation

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Introduction

Poor graft function after allogeneic stem cell transplantation remains a major complication because it is associated with considerable morbidity and mortality. To treat poor graft function, secondary hematopoietic stem cell transplantation has been evaluated resulting in a survival of 53% at three years [1]. Another option is treatment with a boost of donor stem cells without prior conditioning. However, due to the high amount of T cells in unmanipulated stem cell products, the probability of acute and chronic graft versus host disease (GVHD) is considerable [2, 3]. Several case reports showed a high efficacy of giving immunoselected CD34⁺ cells for poor graft function after allogeneic stem cell transplantation [4, 5].

Patients and Methods

We investigated the selected CD34⁺ stem cell boost in eight consecutive patients, who experienced poor graft function after allogeneic stem cell transplantation at the Department of Bone Marrow Transplantation, University Hospital Hamburg, Germany from June 2001 to May 2004. A detailed analysis was published recently [6]. The diagnoses of patients were myelofibrosis (n=3), acute lymphoblastic leukemia (n=1), and secondary acute myeloid leukemia (n=1), multiple myeloma (n=1) and non-Hodgkin's lymphoma (n=1). After a median of 128 days (range, 70–203), there was a poor graft function defined as persistent thrombocytopenia (median, $18 \times 10^9/L$ [range, 13–26]) and/or leucopenia (median, $2.1 \times 10^9/L$ [range, 0.9–6.4]). The indication for selected CD34⁺ stem cell boost was thrombocytopenia ($<30 \times 10^9/L$) in all patients which was associated with a leukocyte count of $<2 \times 10^9/L$ in five patients. Despite low graft function, all patients had complete donor chimerism defined by real-time PCR [7]. In all patients, low graft function was further determined by hypocellular bone marrow; cytopenia due to GVHD, viral infections or drugs could be excluded. The CD34⁺ cell boost was obtained from PBSCs in five patients and from bone marrow in three patients. The donor-PBSCs were mobilized by administration of 10 µg/kg G-CSF daily for five days. Prior to CD34⁺ cell selection, bone marrow was depleted from erythrocytes using the COBE Cell

Processor 2991 (Baxter, Munich, Germany). CD34⁺ stem cells were selected using CliniMACS CD34 Reagent and the automated CliniMACS[®] Plus Instrument (Miltenyi Biotec, Bergisch Gladbach, Germany) under good manufacturing practice (GMP), which has been previously described and reported [8].

Results and Discussion

The patients received a median of $1.71 \times 10^6/kg$ (range, $0.49-5.43 \times 10^6$) of positively selected CD34⁺ stem cells. The median purity of the positively selected CD34⁺ cells after separation was 98.45% (range, 98.47–99.62%), and the viability of the cells was consistently $>95\%$. The median recovery of CD34⁺ stem cells was 44.8% (range, 37.3–84.9%). The CD34⁺ selection of PBSC products lead to a higher purity (median 99.2%) compared to bone marrow products (median 91.7%). The degree of T cell depletion resulted in a median of 2.4×10^3 CD3⁺ cells/kg (range, $0.5-10.1 \times 10^3$ CD3⁺ cells/kg).

Figure 1 shows a consistent increase of median leukocyte and platelet count after one, two, and three months following a CD34⁺ stem cell boost. All patients had complete donor chimerism at time of CD34⁺ cell boosting. None of the patients developed acute GVHD after a median follow-up of 144 days (range, 35–585). None of the evaluable patients developed chronic GVHD. Five out of eight patients are alive

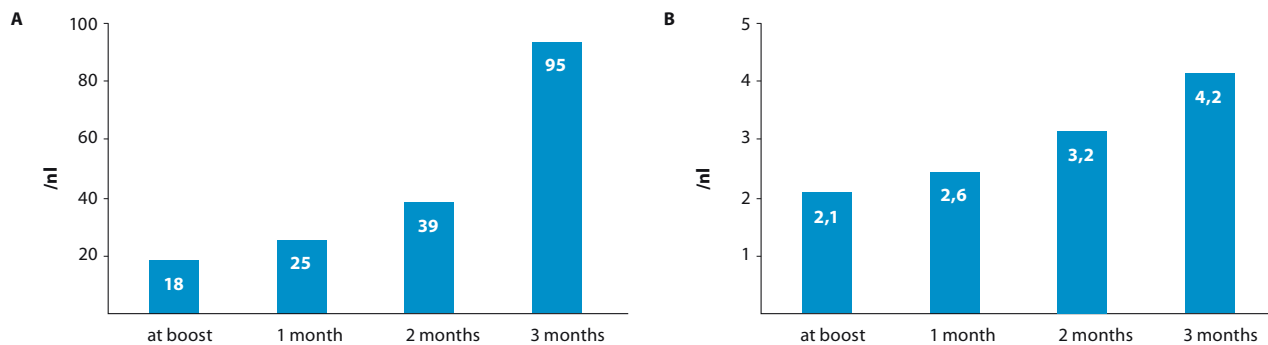


Figure 1: Consistent increase of median leukocyte and platelet count after one, two, and three months following a CD34⁺ stem cell boost. A: Median platelet count after selected CD34⁺ cell boost. B: Median leukocyte count after selected CD34⁺ cell boost.

and disease-free after a median follow-up of six months. Two patients died of mycosis, which was already present at time of boosting. Another patient died of relapse.

This small study shows that the administration of selected CD34⁺ stem cells is effective in improving poor graft function after allogeneic stem cell transplantation in case of complete donor chimerism. The high T cell depletion resulted in no acute or chronic graft versus host disease. More recently, the boosting of selected CD34⁺ peripheral stem cells to treat poor graft function has been confirmed by a retrospective study which reported a high chance of trilineage recovery and a low risk of acute GVHD [9]. Furthermore, in comparison to patients who did not receive a stem cell boost or to those who received an unmanipulated stem cell boost, the treatment-related mortality was reduced (20% vs. 55% and 64%, respectively).

Since poor graft function is associated with a high risk of morbidity and mortality due to infection or bleeding complications, CD34⁺ cell boosts should be considered in patients with poor graft function.

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Graft engineering and T cell-depleted stem cell boosts in allogeneic stem cell transplantation

Despite many advances in allogeneic stem cell transplantation (SCT), graft versus host disease (GVHD) and poor graft function (PGF) still remain clinical problems associated with significant morbidity and mortality. Graft engineering and stem cell boosts employing T cell depletion may be promising approaches to address these problems.

Graft engineering

In allogeneic SCT it has been shown that *in vitro* T cell depletion of the graft may reduce the incidence and severity of both acute and chronic GVHD[1]. Combined with B cell depletion, this procedure may reduce the incidence of EBV-related Lympho-Proliferative Disease (EBV-LPD) [2]. Positive selection of stem cells (CD34 selection) and depletion of T cells (CD3 depletion) are both methods to remove T cells from the graft.

CD34 selection

Since its introduction, CliniMACS® CD34 selection has been widely used to provide high-purity stem cell grafts with a high yield of CD34⁺ progenitor cells (see table 1). CliniMACS CD34 selection provides passive T cell depletion efficiencies of up to 5 logs (table 1). Not only T cells, but also B cells are passively depleted from the graft. A median of 3.2 log B cell depletion can be achieved (table 1).

In allogeneic SCT, CD34 selection allows the transplantation of T cells doses between 0.9 and 1.3×10⁴ CD3/kg bodyweight and stem cell doses of up to 19.5×10⁶ CD34/kg bodyweight (median values from three centers, see table 2) and is often used in haploidentical transplantation [6, 7].

	n = 73	n = 136	n = 30	n = 335
Purity CD34 (%)	92	90	96	93
Recovery CD34 (%)	71	81	64	81
CD3 log depl	5.1	4.6	5.0	4.8
CD19 log depl	3.7	3.2	3.2	3.3
Reference	3	4	5	6

Table 1: Results from CliniMACS CD34 selection procedures (median results); starting material = G-CSF-mobilized PBSCs grafts

/kg bodyweight		Transplant setting	n	Ref
CD34×10 ⁶	CD3×10 ⁴			
13.1 (5.1–29.7)	0.9 (0.04–3)	Haploidentical donors, adult	335	6
19.5 (5–50)	1 (0.18–3.2)	Haploidentical donors, pediatric	500	7
8 (1.3–18)	1.3 (0.17–65.8)	Haploidentical, mismatched related, and MUD, adult	102	8

Table 2: Graft composition after CD34 selection in the allogeneic setting (median results); starting material = G-CSF-mobilized PBSCs grafts

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CD3/CD19 depletion

Another option for T cell depletion *in vitro* is the direct immunomagnetic depletion of T cells, which can be combined with B cell depletion. Compared with positive stem cell selection, this method removes T and B cells only, preserving NK cells and other effector cells in the graft (table 3). T cell depletion is in the range of 3 to 4.1 logs, and B cells are depleted with an efficiency of 2.2 to 3.7 logs (median values, four centers, table 3).

CD3/CD19 depletion resulted in grafts with median T cell doses of 3 to 12.2×10^4 CD3/kg (range 0.006–45) and with median CD34 doses of 7.5 to 18×10^6 /kg (range 2.2–42.2.5, median values of four centers, see table 4). Safety and feasibility of CD3/CD19-depleted grafts in the haploidentical and unrelated transplantation setting has been already shown in a number of clinical trials [5–8]. The CliniMACS® Depletion Tubing Set (DTS) was developed for these large-scale depletion procedures, being able to process high cell numbers. A depletion of 40×10^9 T and B cells from 80×10^9 total cells is possible in 2 hours. CliniMACS Software version 2.40/2.41 is mandatory for this procedure.

In summary, the most efficient T cell depletion is achieved by positive selection of CD34⁺ cells with depletion efficiencies of up to 5 logs. However, if protocols favor maintaining in the graft NK cells and other effector cells, such as monocytes and dendritic cells in addition to the hematopoietic stem cells, CD3 depletion (in conjunction with CD19 depletion) may be the preferred approach (see table 4). These grafts contain similar numbers of stem cells with roughly three to ten times more T cells and remarkable numbers of NK cells

and monocytes/granulocytes are retained. Moreover, it is possible to “tailor” the graft by combining both methodologies. Part of the apheresis product(s) are submitted to CD34 selection while the other part is depleted of CD3⁺ and CD19⁺ cells. The selected CD34 cells and the CD3/CD19-depleted products may then be combined and adjusted according to the clinical needs.

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	n = 5	n = 7	n = 14	n = 115
Cell Source	PBSC	PBSC	BM	PBSC
Recovery CD34 (%)	69	53	67	61
Recovery NK cells (%)	36	67	75	64
Remaining T cells (%)	0.02	nr	0.11	0.0076
Remaining B cells (%)	0.1	nr	1.6	0.035
CD3 log depl	3.4	3.7	3.0	4.1
CD19 log depl	2.2	3.7	nr	3.7
Reference	1	2	3	4

Table 3: Results from CliniMACS® CD3/CD19 depletion procedures (median results, obtained with Tubing Set LS and program DEPLETION 2.1); nr = not reported

/kg bodyweight				Setting	n	Ref
CD34×10 ⁶	CD3×10 ⁴	NK×10 ⁶	Monocytes/ Granulocytes ×10 ⁶			
12.3	5	70	nr	Haploidentical pediatric	4	2
13.6 (2.2–42.5)	12.2 (0.6–45)	nr	nr	Haploidentical pediatric	25	5
18 (10–41)	4 (0.7–16)	106 (10–290)	600 (60–1300)	Haploidentical pediatric	20	6
10.6 (4.6–20)	nr	17.2	446.5	Haploidentical pediatric	10	7
7.5 (4.9–17)	3 (0.006–44)	53 (2–86)	nr	Haploidentical adult	12	8

Table 4: Graft composition after CD3/CD19 depletion in the allogeneic setting (median results and range); starting material = G-CSF–mobilized PBSCs grafts , nr = not reported

T cell–depleted stem cell boosts

Poor graft function (PGF) is a complication of stem cell transplantation that has been reported in about 5–27% of patients [1]. All three blood lineages may be affected leading to thrombocytopenia, neutropenia, and anemia requiring continuous blood transfusions. Prolonged and profound cytopenia is associated with considerable morbidity and mortality related to infections and hemorrhagic complications [1]. An infusion of stem cells may overcome cytopenia and does not require reconditioning [2, 3]. CD34 and CD133 selection has been used to deplete T cells from the stem cell boost in order to reduce the risk of acute and chronic GVHD [1, 4–7]. Table 5 summarizes the published experience so far.

Larocca et al. have reported the outcome of 54 patients with poor graft function. Twenty patients received selected CD34 stem cell boosts, 14 patients were treated with unmanipulated infusions and 20 received no additional stem cell infusion. The use of selected CD34+ cells, as compared to non-separated cells, was associated with a greater chance of trilineage recovery (75% vs 36%, $p = 0.02$). In multivariate analysis, infusion of selected CD34+ cells appeared as the only significant predictor for low Non-Relapse Mortality. This translates into a better 5-year survival (65%) compared with the group receiving unmanipulated stem cells (29%) or the group receiving no stem cell infusion (45%) ($p = 0.01$). In five published reports describing the application

of selected CD34+ stem cell boosts no acute GVHD was reported [1, 4–7]. Only Larocca et al. reported occurrence of chronic GVHD in three of 20 patients [1].

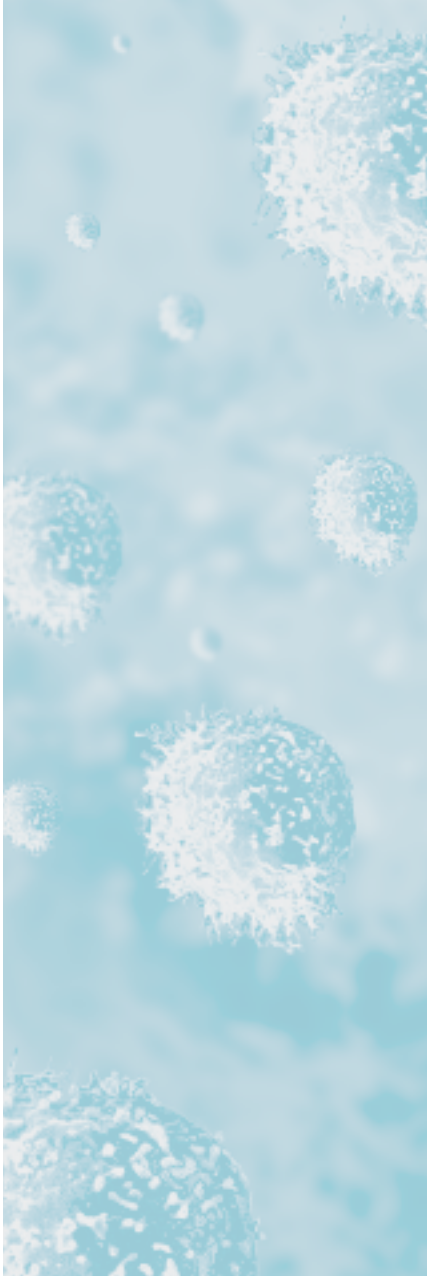
In summary, the application of selected CD34+ stem cell boosts helped to promote trilineage engraftment without inducing acute GVHD when used in a situation of poor graft function [1–7].

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Number of patients	Median CD34 dose ($\times 10^6$ /kg)	Median T cell dose ($\times 10^3$ /kg)	Stem cell source (PBSC/ BM)	Donors	Median Follow-up (days)	aGVHD (%)	Extensive cGVHD (%)	Ref
20	2.57	<10	19 PBSC 1 BM	7 HLAidsib 5 mm related 8 MUD	696 (98–2345)	0	15	1
8	14.54	nr	5 PBSC 3 BM	3 mm related 5 MUD	8270 (1460–21535)	0	0	4
8	1.7	2.5	1 PBSC	6 HLAidsib 2 mm related	144 (35–585)	0	0	5
14	3 (1–28)	1.25 (0.1–11)	13 PBSC 1 BM	10 MUD 4 Haplo	nr	0	0	6
8	3.9 (3.3–7.1)	6.5 (0–1.5)	8 PBSC	5 HLAidsib 3 Haplo	nr	0	0	7

Table 5: Selected CD34+ stem cell boosts for the treatment of poor graft function (median results and range); nr = not reported, HLAidsib = HLA identical sibling donors, mm related = mismatched related donors, MUD = matched unrelated donors, Haplo = haploidentical donors



CliniMACS® System: Enhanced portfolio of CE-marked products for clinical-scale cell separation

Target cells are selectable via surface markers using the CliniMACS Plus Instrument.

Marker/System	Target cells	Status
CD34	Progenitor cells	CE
CD133		CE
CD14	Monocytes > Dendritic cells	CE
CD19	B cells	CE
CD56	Natural killer cells	CE
Anti-Biotin (Flexible Labeling System)	Any cell type	CE
CD3	T cells	CE
CD8	T cell subsets	CE
CD4		For research use only
CD25		For research use only
CCS (Cytokine Capture System, IFN-gamma)	Antigen-specific T cells	CE, launch in preparation
CD1c (BDCA-1)	Direct Blood DC progenitors	For research use only
BDCA-4		For research use only

CliniMACS® Reagents, Tubing Sets, Instruments and PBS/EDTA Buffer are manufactured and controlled under an ISO 13485 certified quality system. In Europe, the CliniMACS® System and Reagents are available as CE-marked medical devices. In the USA, the CliniMACS® System components including the CliniMACS® Reagents are available for use only under an approved Investigational New Drug (IND) application or Investigational Device Exemption (IDE). CliniMACS® MicroBeads are for research use only and not for use in humans.

Miltenyi Biotec cell culture bags CE, launch in preparation

The unique features of the CE-marked Cell Expansion Bags and Cell Differentiation Bags make them valuable tools for any investigator culturing cells.

Culturing cell suspensions, as well as culturing cells with adherent properties, is possible in Miltenyi Biotec Cell Culture Bags.

The bags are gas permeable, transparent for microscopy, and allow for a closed culture system resulting in reduced risk of contamination.

Both the Cell Expansion Bag and Cell Differentiation Bag come in single-tube format or with six needleless access connectors fitting standard laboratory requirement.

Cell Expansion Bags are compartmentalized with easy to open seals for expandable culture volumes to accommodate the increase in cell number during the culture period; therefore the culture vessel does not have to be changed. Cell Differentiation Bags are available for three different culture volumes. The bags are individually packed, are sterile and tested for endotoxins.

CliniMACS® Reagents, Tubing Sets, Instruments and PBS/EDTA Buffer are manufactured and controlled under an ISO 13485 certified quality system. In Europe, the CliniMACS® System and Reagents are available as CE-marked medical devices.

In the USA, the CliniMACS® System components including the CliniMACS® Reagents are available for use only under an approved Investigational New Drug (IND) application or Investigational Device Exemption (IDE). CliniMACS® MicroBeads are for research use only and not for use in humans.

Cell Expansion Bags

(tube and 6 ports) are compartmentalized for expandable culture volumes.

15mL / 27 cm ² ; 50mL / 59 cm ² ; 100mL / 116 cm ²

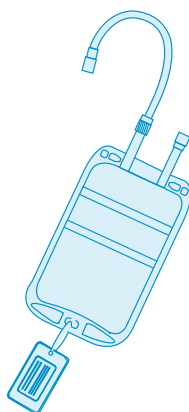


Figure 1: Cell Expansion Bag (tube) with two seals for expandable culture volumes.

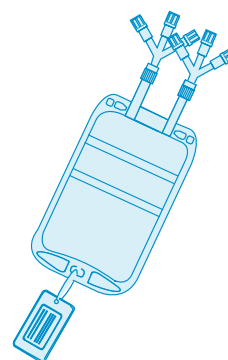


Figure 2: Cell Expansion Bag (6 ports) with six needleless access connectors. With two seals for expandable culture volumes.

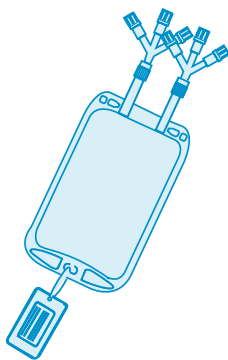
Cell Differentiation Bags

(tube and 6 ports) are available for three different culture volumes:

100mL / 113 cm ²	250mL / 156 cm ²	500mL / 230 cm ²
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Figure 3: Cell Differentiation Bag (tube).



Cell Differentiation Bag (6 ports) with six needleless access connectors.

Satellite Symposium, EBMT 2006

Innovative strategies for cellular therapy

This year's Satellite Symposium, organized by Miltenyi Biotec GmbH, preceding the 32nd annual meeting of the EBMT, took place on March 19, 2006, in Hamburg, Germany.

Miltenyi Biotec is grateful that J.H. Frederik Falkenburg, Professor of Hematology, Leiden University Medical Center, Leiden, The Netherlands, agreed to chair the very informative symposium, which focused on novel protocols for cellular therapy of hematological, viral, and cardiovascular diseases. Dr. Falkenburg stated that sophisticated manipulations of cell populations in the near future may lead to broad applications of old and new therapeutic strategies.

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.

Rupert Handgretinger, Children's University Hospital, Tübingen, Germany **Graft engineering strategies to improve haploidentical transplantation**

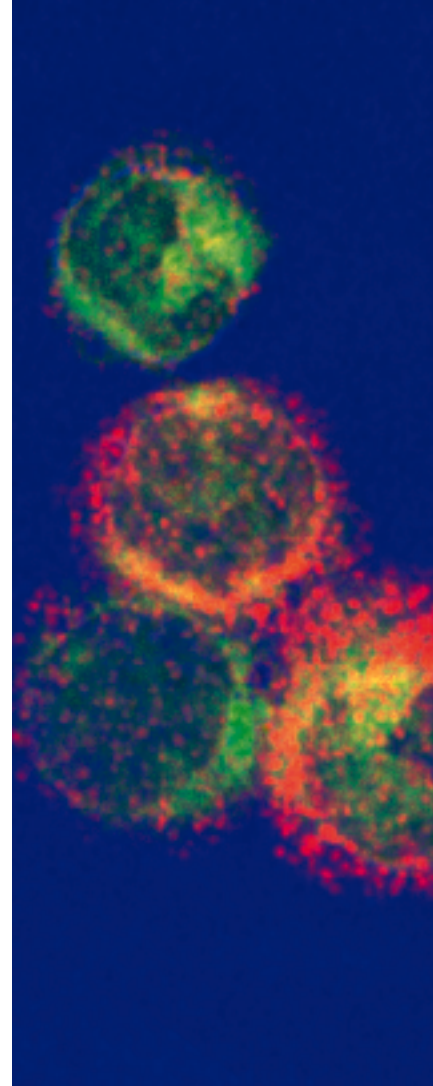
Dr. Handgretinger summarized the experiences from transplantations made with CD34⁺-selected grafts in the haploidentical setting. He pointed out that haploidentical transplantation is feasible without inducing graft versus host disease. A delayed immune reconstitution, however, still poses a high risk of infection. To improve immune reconstitution, an optimized graft should contain a large number of stem cells and NK cells. In addition, it should be effectively T cell-depleted. Based on these assumptions, Dr. Handgretinger has changed the procedure for the preparation from CD34 selection to CD3 depletion; this leaves CD34⁺ and CD34⁻ progenitors as well as NK cells in the graft. During the symposium Dr. Handgretinger reported on 25 patients transplanted with a

reduced intensity conditioning (RIC) with CD3-depleted haploidentical grafts at St. Jude in Memphis and on 20 children treated in Tübingen with a CD3/CD19-depleted graft. The patients showed a rapid reconstitution of T cells and NK cells; no lethal infection has occurred thus far. The conclusions drawn at this point were: haploidentical transplantation with a RIC can be a therapeutic option for the treatment of malignant and nonmalignant diseases for such cases where only family donors are available.

Mark W. Lowdell, Department of Haematology, Royal Free Hospital and School of Medicine, London, United Kingdom

CD8-depleted DLI for reversal of mixed chimerism after allogeneic SCT

Approximately 50% of reduced intensity conditioning (RIC) transplants result in mixed chimerism. Donor lymphocyte infusions (DLI) can help convert mixed to full donor chimeras, but at an increased risk of GVHD. Dr. Lowdell presented preliminary data of his study that aims at evaluating the safety and toxicity of CD8-depleted DLI after a RIC allogeneic SCT. The study looks at also the incidence of GVHD and the effect on immune reconstitution, as well as the anti-tumor activity of CD8-depleted DLI. To date, eight patients who had evidence of disease relapse or who were identified as mixed chimeras at six months after RIC allogeneic SCT, received the CD8-depleted DLI. For this purpose leukapheresis product from the original hematopoietic stem cell donor was depleted of CD8⁺ T cells by use of the CliniMACS[®] CD8 Reagent and the CliniMACS Plus Instrument. Dr. Lowdell concluded that CD8-depleted DLI are associated with a low incidence of GVHD and are capable of *in vivo* alloreactivity,



resulting in demonstrable GVL effect and in resolution of mixed chimerism.

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

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

In mouse models of allogeneic bone marrow transplantation, several groups demonstrated that adoptively transferred CD4⁺CD25⁺ regulatory T cells do not induce graft versus host disease (GVHD), but actively suppress GVHD initiated by co-transplanted conventional T cells. Recently CD4⁺CD25⁺ T cells with suppressive capacity have also been identified in human peripheral blood. These cells show similar phenotypic and functional characteristics. Thus, adoptive transfer for the prevention of GVHD in allogeneic

hematopoietic stem cell recipients seems promising. In humans, the true regulatory T cell population resides primarily within the CD25^{high} fraction. Dr. Edinger presented the preferential clinical-scale enrichment of CD25^{high}CD4⁺ T cells; in his protocol, the unwanted CD19⁺ cells are depleted followed by enrichment of CD25⁺ cells (all CliniMACS[®] Reagents). These results as well as preliminary data from a phase I clinical trial were discussed.

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

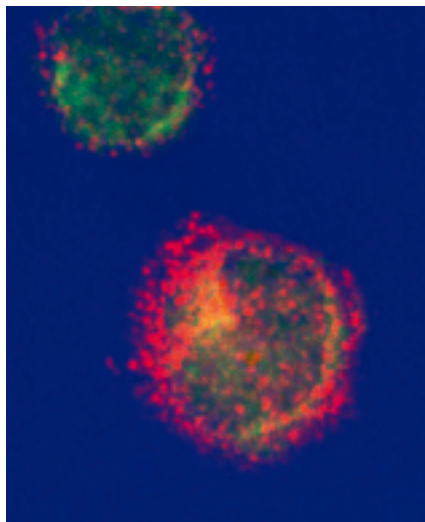
**Enrichment of antigen-specific T cells
for adoptive immunotherapy after
allogeneic stem cell transplantation**

Dr. Falkenburg introduced his concept of controlling infectious complications and malignant disorders by the administration of antigen-specific T cells. These T cells can be selected from T cell populations by their ability to secrete cytokines in response to antigenic restimulation using the CliniMACS[®] Cytokine Capture System (IFN-gamma). By using the CMV pp65 recombinant protein (Miltenyi Biotec) with this technology both CD8⁺ and CD4⁺ T cells from CMV positive donors could be selected with 60–80% purity. In a recently initiated clinical study, three patients were treated with 3–8×10⁶ CMV-specific T cells. In this setting antigenic restimulation was carried out using CMV peptides, resulting in CD8⁺ CMV-specific cells. The patients cleared the CMV infection, and no severe events occurred. In a similar approach, allogeneic leukemia-reactive T cell lines for adoptive transfer are generated. Adoptive transfer of these cells may allow treatment of residual or persistent disease shortly after allogeneic stem cell transplantation with limited risk of GVHD.

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

**Intramyocardial stem cell
transplantation in patients with chronic
ischemic heart disease**

Despite improvements in emergency treatment, myocardial infarction is often the first step of a downward spiral leading to congestive heart failure. Based on the promising phase I results, which have been presented earlier, a 100 patient phase II clinical trial was initiated. Dr. Stamm presented interim results of the first 20 patients treated in each arm; these results show that coronary artery bypass grafting (CABG) plus injections of CD133⁺ stem cells enriched from bone marrow result in better left ventricular (LV) contractility and perfusion compared to CABG alone. Furthermore, Dr. Stamm presented the case of a 14-year old patient who presented with dilated, non-ischemic cardiomyopathy. During the implantation of a left ventricular assist device (LVAD) CD133⁺ hematopoietic stem cells were implanted at 72 sites in the myocardium. After six months the boy could be discharged without needing the LVAD anymore and with stable normal cardiac dimensions and functions. This suggests that BM cell transplantation may also be life saving in terminal non-ischemic heart failure.



Satellite Symposium, ISCT 2006

Novel routes in cellular therapy

The Annual Meeting of the International Society of Cellular Therapy (ISCT), which took place from May 4 to 7, 2006, in Berlin, Germany, also hosted the Symposium “Novel routes in cellular therapy” organized by Miltenyi Biotec.

Miltenyi Biotec is honored that Nicolaus Kröger, Assistant Professor and Deputy Medical Director of the Bone Marrow Transplantation Center at the University Hospital in Hamburg, Germany, agreed to chair the session.

More than 200 participants attended the presentations covering various approaches in cellular therapy. The speakers discussed the clinical potential of T cell subsets, dendritic cell vaccines, graft manipulation, and stem cell populations in the context of diverse clinical challenges, such as cancer relapse, graft rejection, immune reconstitution, graft versus host disease, and tissue regeneration.

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.

Wolfgang A. Bethge, University of Tübingen, Tübingen, Germany
Haploidentical allogeneic stem cell transplantation with CD3/CD19-depleted grafts and reduced-intensity conditioning: fast engraftment and low toxicity

Haploidentical hematopoietic stem cell transplantation (HSCT) with megadoses of selected CD34⁺ stem cells has been proven to be feasible. Nevertheless, treatment-related complications are challenging. Dr. Bethge discussed an approach to improve engraftment, immune reconstitution, and anti-leukemic effects that may also allow for the reduction of the intensity of the

conditioning regimen. In Tübingen, 19 high-risk adult patients with refractory hematological malignancies or relapse were treated with CD3/CD19-depleted haploidentical grafts and reduced intensity conditioning (RIC). Conclusions from the first 19 patients were summarized as follows: i) Haploidentical HSCT in the RIC setting is feasible with low toxicity ii) Fast and sustained engraftment could be achieved even without megadoses of CD34⁺ cells iii) The GVHD rate was moderate and GVHD responded to steroids iv) Immune reconstitution was fast v) Relapse rates and outcome look promising. Only one patient died due to aGVHD grade IV; this patient received the highest T cell dose with 4×10^5 cells/kg bw. The study is ongoing and further patients and longer follow-up is needed to assess the benefit of this approach.

Nicolaus Kröger, Bone Marrow Transplantation, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Purification of CD4⁺ T cells for adoptive immunotherapy after allogeneic stem cell transplantation

Donor lymphocyte infusions (DLI) have been widely used to prevent or treat relapses and infections after allogeneic stem cell transplantation. However, unmanipulated DLI are associated with the risk of acute and chronic graft versus host disease (GVHD) in which donor CD8⁺ T cells may play a major role as some data suggest. In the set of data Dr. Kröger presented, the CliniMACS[®] CD4 Reagent was used to prepare CD4-enriched DLI for adoptive transfer (d63–d1127) into nine patients with hematological malignancies after allogeneic transplantation. In this study no infusion-related toxicity was observed after transfer of 1×10^6 CD4⁺ T cells/kg bw



and 1×10^3 CD8⁺ T cells/kg bw. Administration of CD4⁺ cell-enriched DLI was associated with a low incidence of chronic GVHD and no acute GVHD was observed, while graft versus tumor and graft versus hematopoiesis effects were retained. Further studies planned by Dr. Kröger will focus on early transfer of DLI after transplantation to improve immune reconstitution.

Paolo Corradini, Department of Hematology and Bone Marrow Transplantation, University of Milano, Istituto Nazionale per lo Studio e la Cura dei Tumori, Milano, Italy

Preemptive CD8-depleted donor lymphocyte infusions (DLIs) after haploidentical stem cell transplantation (SCT) using a reduced-intensity conditioning (RIC) regimen

Until now, the use of unselected DLI in the haplo-setting has been limited by the high risk of GVHD. Dr. Corradini presented data from his phase I–II pilot study for patients with advanced hematological malignancies, employing a reduced intensity conditioning and early preemptive infusions of CD8-depleted lymphocytes to boost immune reconstitution after haploidentical CD34-selected stem cell transplantation (all performed with CliniMACS[®] Reagents). The aim of this study was to improve immune reconstitution thereby reducing transplant-related mortality in this patient cohort. A total of 28 CD8-depleted DLI ($1\text{--}5 \times 10^4$ CD8-depleted cells/kg on days 45, 75, and 105) were administered to 14 patients without any toxicity. Dr. Corradini concludes that haploidentical SCT with a RIC regimen is feasible and leads to a high rate of engraftment. Moreover, the data show that preemptive transfer of CD8-depleted DLI is safe, does

not induce severe GVHD or graft rejection and improves immune reconstitution.

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

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

In hepatocarcinoma, the goals of liver resection are the complete removal of all tumor-bearing hepatic tissue, while preserving the supplying and draining structures of the remaining liver parenchyma as well as an adequate liver mass. Portal vein embolization (PVE) of the contralateral liver segment I and IV to VIII before right trisegmentectomy is increasingly performed as a safe and effective concept to provide a proliferation stimulus to the healthy tissue. Risks are that in advanced liver malignancy the adequate future remnant liver volume (FRLV) and an adequate left lateral hepatic mass may not be achieved in time. Dr. Knoefel reported on the first experience with the administration of autologous CD133⁺ bone marrow-derived stem cells (BMSC) into the left-lateral branches of three patients in addition to PVE and prior to extended right hepatectomy. A two-armed study (PVE with or without CD133⁺ BMSC) was conducted, and the results of the first 7 patients in each arm were presented in the symposium. The application of CD133⁺ BMSC was well tolerated. CT scan volumetry of patients who received CD133⁺ BMSC points to a liver ameliorating effect (2.2-fold increased daily mean proliferation rate) following PVE for the contra-lateral liver segments. A controlled trial to evaluate the effectiveness of this novel concept will be initiated.

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

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

Dendritic cell (DC) vaccination of glioma patients has recently emerged as a promising immunotherapy. The generation of tumor antigen-loaded DC, however, may be hampered in tumor patients either due to previous therapies or to the systemic effects of tumor cells themselves. Furthermore, *ex vivo* generation of DC must be suitable for clinical application and ideally be conforming with current Good Manufacturing Practices (cGMP). Dr. Sorg presented a protocol, which results in functional competence of immature and mature DC generated from CD14⁺ monocytes selected with the CliniMACS Plus Instrument. The protocol substituted autologous plasma-supplemented medium with serum-free medium and thus allows for an efficient, large-scale generation of functionally competent DC under cGMP conditions in a closed system. Dr. Sorg stated that this protocol for serum-free DC generation may be suitable even for patients whose plasma/serum may contain inhibitory factors, which may interfere with generation of monocyte-derived DC. To date, three patients have been vaccinated accordingly.



Satellite Symposium

World Congress of Cardiology, 2006

Autologous stem cell therapy in cardiac disease

The World Congress of Cardiology 2006, which took place from September 2 to 6 in Barcelona, Spain, also hosted the Satellite Symposium "Autologous stem cell therapy in cardiac disease".

The symposium was co-chaired by Wolfgang-Michael Franz, Associate Medical Director at the hospital of the LMU University, Munich, Germany, and by Gustav Steinhoff, Head of the Department of Cardiac Surgery at the University of Rostock, Germany.

The speakers discussed recent results of pilot trials, as well as phase I and II studies using CD133⁺ stem cells in different settings of cardiac diseases. Both intracoronary and intramyocardial delivery routes were used in the trials presented.

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.

Robert M. Graham,
University of New South Wales,
and Victor Chang Cardiac Research
Institute, Sydney, Australia

Experience with recombinant human granulocyte-colony stimulating factor (G-CSF) in no-option patients with end-stage chronic refractory ischemic heart disease.

The use of G-CSF in patients with ischemic heart disease remains under careful investigation. Dr. Graham presented results of a safety and efficacy trial including 20 patients with end-stage chronic heart disease. Patients had been exposed to exercise stress tests (EST) while receiving G-CSF, in order to induce ischemia and facilitate stem cell homing. After 3 months, a second round of mobilization / EST was performed, but in

addition, leukapheresis was applied to collect peripheral blood stem cells. Patients were then randomized double-blinded to receive either unselected cells or CD133⁺ stem cells by intracoronary infusion. Mobilization, as well as intracoronary cell infusion, was consistently uneventful. Dr. Graham presented significant improvements in angina, nitrate use, and EST performance after the first round of mobilization and a further improvement of those parameters after the second round of G-CSF combined with cell administration. Dr. Graham concluded that stem cell mobilization and intracoronary infusion appears to be safe and effective for the patients enrolled in this trial. No comparative results could be shown regarding the administration of unselected versus selected CD133⁺ cells, as the two treatment arms will remain blinded until data analysis is completed.

Rosaria Giordano,
"Cell Factory Franco Calori", Milan, Italy
Bone marrow or peripheral blood-derived stem cells for the treatment of AMI patients: follow-up on a controlled phase I/II study.

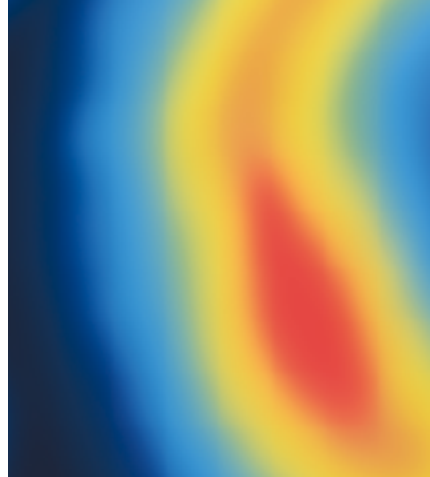
Dr. Giordano showed an interim analysis of 14 patients enrolled so far in a three-armed randomized controlled study based on intracoronary administration of CD133⁺ cells. The study is being conducted as a cooperative project of several hospitals in Milan. Acute myocardial infarction (AMI) patients without efficient reperfusion after primary percutaneous intervention (PCI) were randomly assigned to one of three groups: A) patients receiving bone marrow-derived CD133⁺ cells; B) patients receiving peripheral blood-derived CD133⁺ cells and C) patients receiving optimal therapy without cells. Patients of group B were mobilized by

administration of G-CSF (5 µg/kg/day for 4 days) starting from day 6–10 after AMI. A second PCI was performed in patients of group A and B for infusion of CD133⁺ cells. The median dose of CD133⁺ cells was about 10-fold higher in group B compared to group A (59×10⁶ vs 6×10⁶). Despite the lower cell dose infused, patients in the bone marrow group (A) showed a significant improvement of myocardial blood flow after 6 and 12 months evaluated by ¹³NH₃ PET Scan. Patients in group B and C showed a moderate decrease in myocardial blood flow over time. Analysis of changes in the ejection fraction also underlined the positive effect of bone marrow-derived CD133⁺ cells.

Dr. Giordano discussed the differences between bone marrow and peripheral blood-derived CD133⁺ stem cells which might explain different clinical efficiencies.

Danny Schoors
Department of Cardiology, University
Hospital in Brussels, Belgium
Evidence that intracoronary injected CD133⁺ peripheral blood stem cells (PBSCs) home to the myocardium in patients with chronic ischemic heart disease.

Dr. Schoors presented results from a pilot clinical trial investigating the homing of CD133⁺ cells to the myocardium of patients with chronic ischemic heart disease. Peripheral blood stem cells were mobilized with G-CSF and harvested by leukapheresis one day before intracoronary infusion of the purified and ¹¹¹Indium-labeled cells. A first cohort of three patients received a low dose of 5–10×10⁶ CD133⁺ cells. Using total body scans, only faint ¹¹¹Indium activity could be detected in the heart region for the first 2 hours after cell administration. Therefore a second cohort of five patients received a



higher dose (average 34.4×10^6) of cells, corresponding to a six-fold higher dose in radioactivity. For the high dose group, Dr. Schoors could show clear residual radioactivity at the level of the heart at 1–2, 12, and 36 hours after injection. 6.9–8.0% of total radioactivity was found in the heart region after 1–2 hours and 2.3–3.2% after 12 hours. Within this small patient group no significant increase in metabolic activity could be detected in the injected cardiac regions. On the other hand, magnetic resonance imaging showed a significant improvement in the overall left ventricular ejection fraction in one patient. No unexpected or serious adverse events were observed during stem cell mobilization, apheresis procedures, and coronary catheterization procedures.

Hans-Michael Klein,
Department of Thoracic and
Cardiovascular Surgery, University of
Düsseldorf, Germany
CABG and intramyocardial delivery of
CD133⁺ stem cells seems to improve
cardiac function long-term in the
majority of selected patients with end-
stage ischemic heart disease.

Dr. Klein summarized different approaches of stem cell therapy for myocardial regeneration in patients with end-stage heart failure. He presented clinical results of patients treated with four different modalities: 1) coronary bypass grafting (CABG) + stem cells; 2) CABG + transmural laser treatment (TMLR) + stem cells; 3) TMLR + stem cells; 4) sole stem cell injection. In all cases an 'IN-OR' (In Operating Room) stem cell selection procedure had been used, i.e., bone marrow harvest and magnetic CD133 selection are performed within the period of general anesthesia for the surgical procedure.

For 12 patients treated with CABG, TMLR, and stem cells, the mean ejection fraction (EF) improved from 26.7% preoperatively to 40.1% postoperatively. The same patient group showed a doubling of mean end-systolic wall thickness after treatment as evaluated by MRI. Dr. Klein reported also on major improvement for 10 patients treated with stem cells as sole therapy. The mean EF of 16% preoperatively is improved by 46.3% after a follow-up time of more than 12 months. Dr. Klein concluded that the intramyocardial injection of CD133⁺ stem cells is safe and leads to significant improvement of contractility in patients with end-stage cardiac disease.

Gustav Steinhoff,
Department of Cardiac Surgery,
University of Rostock, Germany
CABG and intramyocardial delivery
of CD133⁺ bone marrow stem cells for
chronic ischemic heart failure: Final
phase I and phase II results.

Dr. Steinhoff gave a summary on the treatment results of 55 patients with chronic ischemic heart failure, who have been treated in Rostock from 2001 to 2005. The feasibility and safety study in 15 CABG patients had shown that intramyocardial injection of autologous CD133⁺ bone marrow cells was not associated with cell-related complications. The left ventricular ejection fraction (LVEF) improved significantly from $39 \pm 9\%$ preoperatively to $50 \pm 8\%$ at 6 months postoperatively. The improvement was stable with $48 \pm 6\%$ measured at 18 months postoperatively. Thallium SPECT (single photon emission computed tomography)

perfusion scans revealed that perfusion was also improved significantly in the areas of interest. In a subsequent randomized, controlled, and prospective clinical trial in 40 patients, the positive results of the phase I trial could be confirmed. CABG plus intramyocardial injection of CD133⁺ bone marrow cells resulted in better LV ejection fraction and perfusion than CABG only. An inverse correlation for change in LVEF versus preoperative LVEF was found: patients with lower EF preoperatively had most benefit from the stem cell injection. Dr. Steinhoff also reported on a single patient who had been treated with CD133⁺ stem cells alone. The EF improved from 25% preoperatively to 41% at three months postoperatively. Dr. Steinhoff concluded that bone marrow stem cell delivery in CABG patients is safe and provides beneficial effects. He emphasized that randomized double-blinded clinical studies are indicated to prove long-lasting clinical advantages.

Frequently asked questions

Q.: I am unsure about which tubing set available from Miltenyi Biotec is recommended for which depletion application?

A.: For CD3 depletion or combined CD3/CD19 depletion you can utilize the Large Scale Tubing Set (LSTS) but also the Depletion Tubing Set (DTS). Depending on the number of labeled cells, the use of the DTS for CD3 depletion or CD3/CD19 depletion can reduce the processing time at the device up to 50% compared to LSTS.

For depletion using the CliniMACS CD19, CD8 or CD25 Reagents you should use the LSTS.

Q.: I have moved my CliniMACS Plus Instrument to another clean room. How can I be sure that the device is still working properly?

A.: Use the “Instrument Check” program to technically check all important device components. Starting from the basic screen press “5” to enter the Service Menu and then press “3” for the Instrument Check. Follow the instructions given on the screen. Prepare a 5 cm piece of tubing filled with liquid for the liquid sensor check. Please call your local representative; it may be necessary to do a IQ/OQ (installation and operational qualification of the CliniMACS® Plus Instrument).

Q.: My CliniMACS depletion process using the DTS was aborted due to an unexpected power loss. How can I rescue the cells?

A.: Cells that were already transferred into the Cell Collection Bag are depleted and can be collected. The remainder of the product that you find in the Cell Preparation Bag and the Reapplication

Bag needs to be pooled in order to be processed. If the volume is greater than 300 mL, reduce by centrifugation. The sample parameter input for the re-run has to be recalculated due to changed sample parameters (volume, cell concentration) before a new DTS is installed.

Q.: When connecting the CliniMACS Tubing Set with the CliniMACS PBS/EDTA Buffer under the laminar hood the buffer already entered the Tubing Set. Can I still use the tubing set?

A.: In a sterile environment let all buffer manually run back into the buffer bag and clamp the line correctly. Check that all other bags of the tubing set are free of fluid and according to the screen instructions on the CliniMACS instrument.

Q.: During a CliniMACS CD34 selection I forgot to open the clamp to the Cell Collection Bag. I only learned this when the process was finished and the Cell Collection Bag was still empty. Where are my target cells?

A.: The CD34⁺ cells are dispersed throughout the system. They typically do not, or only to a minor extent, end up in one of the other bags (e.g., Negative Fraction Bag or Buffer Waste Bag). The appropriate way to rescue the cells from the system would be to perform an “Emergency Run”. With this CliniMACS software program the system is rinsed and the trapped cells are recovered in a volume of 60–70 mL in the cell collection bag. For details refer to chapter “Trouble-shooting” in the CliniMACS Manual. After the procedure check the content of the cell collection bag for sufficient CD34⁺ cell recovery. Do not discard the tubing set possibly

containing remaining target cells until the results are known.

Q.: For logistical reasons it is not always possible for me to immediately analyze the sample taken after the CD3 labeling procedure. Do I really have to wait for the analysis in order to enter the sample parameters and start the depletion process?

A.: No, not necessarily. Determine the volume of the fraction to be attached to the CliniMACS Plus Instrument. Based on this volume and the total nucleated cell (TNC) count of the leukapheresis product, the actual cell concentration can easily be recalculated using the rule of proportion. The percentage of CD3-labeled cells can be taken from the result obtained from the leukapheresis product.

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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Preceding the 48th Annual Meeting of the American Society of Hematology, Orlando, Florida

Cell therapy: Present and future

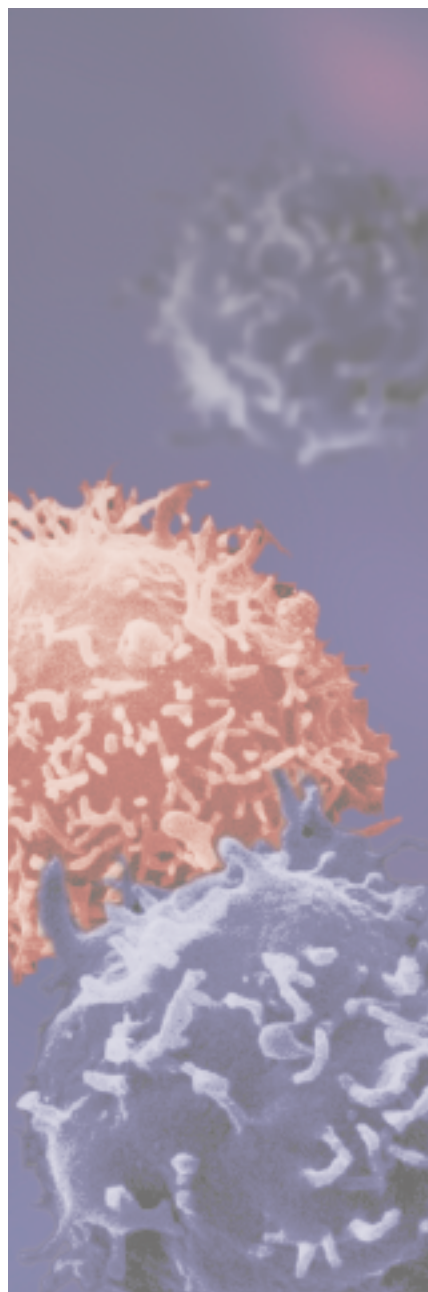
Friday, December 8, 2006

12:30–4:30 pm

Rosen Plaza Hotel,

Grand Ballroom

Chair: J. H. Frederik Falkenburg,
Leiden University Medical Center,
Leiden, The Netherlands



Depletion of regulatory T cells in patients with advanced renal cell cancer

Robert Hawkins, MB BS PhD,
Department of Medical Oncology,
Paterson Institute for Cancer Research,
Christie Hospital, Manchester, United
Kingdom

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

Nicolaus Kröger, MD, Department of
Transfusion Medicine/Transplantation
Immunology,
University Hospital Hamburg-
Eppendorf, University of Hamburg,
Hamburg, Germany

Haploidentical transplantation for children using CD3-depleted stem cell grafts

Gregory Hale, MD, St. Jude Children's
Research Hospital, Memphis, TN, USA

Allogeneic transplantation of CD8-depleted peripheral blood stem cells, clinical trial experience

Vincent Ho, MD, Department of Medical
Oncology, Dana Farber Institute,
Boston, MA, USA

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 CD133⁺ bone marrow stem cell transplantation in chronic heart failure—clinical study results in cardiac surgery

Gustav Steinhoff, MD, PhD, Department
of Cardiac Surgery, University of
Rostock, Rostock, Germany

CD34 selection in allogeneic transplantation

Ann Jakubowski, MD, PhD, Memorial
Sloan-Kettering Cancer Center, New
York, NY, USA

Upcoming meetings—meet us at the booth!

Date	Congress	Webpage
2006		
November 29– December 3	5th Intl. Congress on Autoimmunity, Sorrento, Italy	www.kenes.com/autoim2
December 9–12	ASH, 48th Annual Meeting, Orlando, Florida, USA	www.hematology.org/meeting



Date	Congress	Webpage
2007		
February 8–12	ASBMT 2007, Keystone, CO, USA	www.asbmt.org/
February 11–14	36th Annal Meeting, German Society for Thoracic and Cardiovascular Surgery, Hamburg, Germany	www.dgthg-jahrestagung.de/2007/en_index.php
March 11–13	XIV Convention Telethon, Italy	www.telethon.it
March 15–17	IGLD, Ulm, Germany	www.igld.de
March 22–23	4th Intl. Symposium on the Clinical Use of Cellular ProductsCellular Therapy, Regensburg, Germany	www.cellular-therapy.de
March 25–28	EBMT 33rd Annual Meeting, Lyon, France	www.akm.ch/ebmt2007/
March 28–30	British Transplant Society, MICC, Manchester, UK	www.bts2007.org.uk
April 12–14	73. Jahrestagung der DGK, Deutsche Gesellschaft für Kardiologie, Mannheim, Germany	www.ft2007.dgk.org
April 26–28	13th Annual International Symposium on Recent Advances in Stem Cell Transplantation, Heidelberg, Germany	
June	SIICA, Italian Society of Immunology and Clinical Allegology, Trieste, Italy	www.siica.it
June 24–27	ISCT 13th Annual Meeting, Sydney, Australia	www.celltherapysociety.org/



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ASH 2005 130-092-542	Abstract booklet, Miltenyi Biotec symposium "Cellular Therapy: Present and Future"	<input type="checkbox"/>
EBMT 2006 130-092-644	Abstract booklet, Miltenyi Biotec symposium "Innovative strategies for cellular therapy"	<input type="checkbox"/>
ISCT 2006 130-092-757	Abstract booklet, Miltenyi Biotec symposium "Novel routes in cellular therapy research"	<input type="checkbox"/>
WCC 2006 130-092-854	Abstract booklet, Miltenyi Biotec symposium "Autologous stem cell therapy for cardiac disease"	<input type="checkbox"/>
Childhood Leukemia 2006 130-092-645	Abstract booklet, Miltenyi Biotec symposium "Combined T/B cell depletion in allogeneic stem cell transplantation of children"	<input type="checkbox"/>
DGTHG 2006 130-092-593	Abstract booklet, Miltenyi Biotec symposium "Autologous stem cell treatment for myocardial repair"	<input type="checkbox"/>
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