

CliniMACS[®]

Newsletter

Vol. 7 No. 1/2007

Customer reports

- Cord blood transplantation for adults supported by co-infusion of selected CD34⁺ hematopoietic stem cells from third party donors
- Depletion of CD8-positive T cells from donor leukaphereses in a GMP procedure

CliniMACS[®] products

Meeting minutes satellite symposia

Upcoming satellite symposia

Conference calendar

In this issue

4 Customer reports

Cord blood transplantation for adults supported by co-infusion of selected CD34⁺ hematopoietic stem cells from third party donors

M. N. Fernández, C. Regidor, R. Cabrera, I. Sanjuan, R. Fores, S. Gil, J.A. García-Marco , E. Magro

10 Depletion of CD8-positive T cells from donor leukaphereses in a GMP procedure
R. G. Meyer, W. Herr

13 CliniMACS® products

Portfolio of CE-marked products for clinical-scale cell separation
Miltenyi Biotec cell culture bags CE

14 Meeting minutes

Satellite Symposium: ASH 2006

Satellite Symposium: EBMT 2007

Satellite Symposium: German Cardiac Society 2007

22 Upcoming satellite symposia

24 Frequently asked questions

26 Conference calendar

27 Fax reply form

CliniMACS Newsletter online: www.miltenyibiotec.com

The CliniMACS® System components (Instruments, Reagents, Tubing Sets, and PBS/EDTA Buffer) are manufactured and controlled under an ISO 13485 certified quality system. In Europe, the CliniMACS System components 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. MACS and CliniMACS are registered trademarks of Miltenyi Biotec GmbH.

The CliniMACS® Newsletter is published by Miltenyi Biotec GmbH.
Editor: Dr. Anke Friedetzky, e-mail: anke.friedetzky@miltenyibiotec.de
Editorial board: Dr. Dirk Balshüsemann, Heike Lahnor, Kirsten Langefeld
Graphics & Layout: Miltenyi Biotec GmbH
© 2007 Miltenyi Biotec GmbH, Friedrich-Ebert-Str. 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, e-mail: macs@miltenyibiotec.de

Editorial

Dear colleagues,

The past few years have seen a significant increase in the number of publications on cellular therapies, discussed at numerous meetings devoted to this fascinating subject. Many clinical studies involving cell-based approaches are currently being conducted worldwide.

On the website of the National Institutes of Health (www.nih.gov) information is provided about stem cells and cell-based therapies and their potential applications in a “myriad of diseases”. Listed among those are neurodegenerative conditions, stroke, spinal cord injury, heart disease, diabetes, autoimmune diseases, and cancer. The website also carefully and responsibly points out that many questions remain to be answered before the full scope of potential cellular therapies can be realized. Much research still needs to be conducted. In some areas, extensive clinical research is already underway; in others, it is still a theoretical prospect.

With this issue of the CliniMACS® Newsletter, we are again providing you with information about the wide range of potential uses of our CliniMACS® products. Over the years, with your support and collaboration, we have been able to establish the system in areas of clinical practice. In other fields we are exploring new clinical applications in close collaboration with researchers worldwide.

At Miltenyi Biotec, we believe that cellular therapies will generate important clinical progress in the future. Some of that will happen in areas where currently only limited therapeutic options can be offered to patients. It is here where we want to focus our efforts.

In order to further expand our clinical activities, Miltenyi Biotec has formed a Clinical Business Unit, dedicated to the development, marketing, and sale of clinical products globally. We firmly believe that the clear focus of this division will allow a comprehensive service to all our clinical partners. We also hope that this reorganization will help expedite developments and make therapeutic advances available to patients within a shorter time.

I joined Miltenyi Biotec last October as head of this Clinical Business Unit. Together with everyone in our company I am looking forward to working closely with you in an exciting and dynamic field of medicine.

With best personal regards,



Rainer Uhlenbusch, MD



Cord blood transplantation for adults supported by co-infusion of CD34⁺-selected hematopoietic stem cells from third party donors

M. N. Fernández, C. Regidor, R. Cabrera, I. Sanjuan, R. Fores, S. Gil, J.A. García-Marco and E. Magro
Hospital "Clínica Puerta de Hierro"

Facultad de Medicina, Univ. Autónoma Madrid, Spain

Introduction

Unrelated Cord Blood Transplant (UCBT) is now a well-established modality of allogeneic Hematopoietic Stem Cell (HSC) transplantation. Compared to Unrelated Voluntary Donor Transplant (UVDT), UCBT has the advantages of rapid availability, lower risk of transmission of viral diseases and lower incidence and severity of GVHD despite HLA mismatches. The disadvantage, when done according to conventional protocols, is the relatively late and low rate of engraftment, mainly in patients of high body weight. This is an important risk factor for serious infections during the post-transplant period of neutropenia, contributing to a relatively high rate of early transplant-related mortality (TRM).¹⁻⁵

We hypothesized that both post transplant neutropenia and risk of early neutropenia-related infections could be reduced after a strongly immunosuppressive-myeloablative conditioning by co-infusion of cord blood cells with a relatively low number of highly purified mobilized peripheral blood CD34⁺ cells from a third party donor (TPD), given the well-known capacity of these cells for prompt myeloid reconstitution. We rationalized that, if the number of infused TPD T cells was extremely low, there would be a very low risk of both GVHD and CB rejection by the TPD cells.

Patients and methods

Included in the study were 27 previously reported patients consecutively receiving "dual transplants" of a single CB unit and highly purified mobilized CD34⁺ cells from a TPD.⁶ Patients' demographic data and diagnoses are shown in table 1.

For most patients, the preparative regimen consisted of fractionated total body irradiation (TBI) of a total dose of 10 Gy with lungs shielded at 8 Gy, 120 mg/m² fludarabine (FDB), 120 mg/kg cyclophosphamide, and 30 mg/kg antithymocyte globulin (ATG). Busulfan (BUS) (8 mg/kg) substituted for TBI when this was contra-indicated (table 2).

The transplanted CB units were obtained from accredited CB banks and were selected with a maximum of two HLA A-B (serology) and DRB1 (allele) mismatches and pre-freezing TNC counts greater than 1.5×10⁷ TNC/kg of recipient body weight. The TPD cells were obtained from a haploidentical relative in 23 instances (mother in 4, other relatives in 19) and a more incompatible donor, related or unrelated, in 4. The TPD cells were obtained by standard apheresis procedures after mobilization with G-CSF for four days. Positive selection of CD34⁺ cells from the apheresis products was done using the CliniMACS[®] immunomagnetic method, aiming at final products containing less than 1×10⁴ CD3/kg and

no more than 3×10^6 CD34/kg. The final product was subjected to programmed freezing and stored in liquid nitrogen until use. Viability of the final product was assayed by trypan blue exclusion. HSC content was evaluated by cytofluorometry and growth of CFUs in methylcellulose-based culture media.

On the day of the transplant (day 0) the CB unit was given first immediately after thawing without washings. The TPD CD34⁺ cells were similarly thawed and infused afterwards.

Post transplant chimerism was evaluated in total nucleated cells, granulocytes and mononuclear cells from blood and bone marrow by HLA allele analysis using the RSCA method and/or by quantitative molecular genotype analysis using 15 markers of DNA STR (short tandem repeat).⁷⁻⁹

Table 1. Characteristics of patients

Total number		27
Gender distribution	Male	19
	Female	8
Age (years)	Median	29
	Range	16–60
Weight (kg)	Median	67
	Range	43–87
CMV serological status	Negative	3
	Positive	24
Toxoplasma serological status	Negative	11
	Positive	8
	Unknown	8
HBVs Ab	Positive	7
	Negative	20
Diagnosis	AML	6
	ALL	14
	AL (dendritic)	1
	CML	4
	NHL	2

Table 2. Preparative regimens and post-transplant immunosuppression and growth factors

Conditioning					
TBI (10 Gy)*	+ CTX (120 mg/kg)	+FDB (120 mg/m ²)	+ ATG (30 mg/kg)		17
TBI (12 Gy)*	+ CTX (120 mg/kg)		+ ATG (30 mg/kg)		3
TBI (10 Gy)*	+ CTX (120 mg/kg)	+ FDB (120 mg/m ²)			2
TBI (2 Gy)	+ CTX (120 mg/kg)	+ FDB (120 mg/m ²)	+ ATG (30 mg/kg)	+ AraC (2 g/m ²)	1
BUS (8 mg/kg)	+ CTX (120 mg/kg)	+ FDB (120 mg/m ²)	+ ATG (30 mg/kg)		4
(* lungs shielded at 8 Gy)					
Post transplant immunosuppression and growth factors					
GVHD Prophylaxis					27
	Cyclosporin A dosed to a serum level of 180–250 ng/mL				
	Prednisolone (1 mg/kg days, –1 to +10 to +14 and rapid tapering)				
G-CSF administration					
	From day +1				26
	From day +5				1
GVHD (grade II or higher) front line treatment					
	Methyl-Prednisolone 1–2 mg/kg/day				

Results

Transplanted products

Results of the infused TPD products after CliniMACS® CD34 selection are shown in table 3. Median dose of transplanted TPD CD34⁺ cells was $2.3 \times 10^6/\text{kg}$ (range 1.05–2.58) with a median purity of 98% (range 91–99.5). The median dose of CD3⁺ cells was $2.3 \times 10^3/\text{kg}$ (range 0.5–9.8) with a median CD3⁺ T cell depletion of 4.98 logs (range 3.47–5.47). Pre-freezing median TNC and CD34 cell dose were $2.37 \times 10^7/\text{kg}$ (range 1.31–3) and $0.11 \times 10^6/\text{kg}$ (range 0.035–0.370), respectively (table 4).

Engraftment and chimerism (table 5)

Post transplant sequential evaluation of chimerism both in peripheral blood and marrow cells showed initial predominance of TPD DNA, both in granulocytes and mononuclear circulating and marrow cells, followed by progressive replacement by cells from the CB transplant. This was not the case for the patients who received maternal HSC, which did not significantly engraft. For the whole

group of patients, time to ANC recovery above $0.5 \times 10^9/\text{L}$ was 10 days (range 9–36). After excluding the four patients who received maternal cells, the time to ANC above $0.5 \times 10^9/\text{L}$ was 9.5 days (range 9–17).

The estimated time to CB-ANC recovery was 22 days (range 13–55). For patients receiving non-maternal HSC CB-ANC recovery was 22 days (range 13–53). Final full CB chimerism was achieved by a total of 25 patients. Median time to full CB chimerism was 55 days (range 11–96) for the whole group and 58 days (range 19–96) for the patients who received non-maternal MHSC cells. Cumulative incidence of granulocyte and CB engraftment and chimerism are shown in figure 1.

Median time to sustained platelets higher than $20 \times 10^9/\text{L}$ and $50 \times 10^9/\text{L}$ were 33 and 57 days for the whole group and 30 and 50 days for the patients receiving non-maternal HSC (see figure 2).

Morbidity and mortality

One heavily pre-treated CML patient (including an auto-transplant) died because of graft failure. Two other heavily pre-treated patients died because of toxicity (VOD and MOF). No other patient had major toxic complications. None had severe mucositis, and requirements for parenteral alimentation was very limited.

No major neutropenia-related infections were observed during the period until ANC recovery. The most frequent severe infections after ANC recovery were CMV reactivations, their incidence being highest in the third month, decreasing thereafter, although continuing until near the end of the first year. Other infectious episodes were viral (mainly Polyoma, HZV, EBV, HHV-6 and HBV reactivations) and parasitic (Toxoplasma, Leishmania). Several episodes of non-serious Candida infections were also observed, but no case of infection by filamentous fungi occurred.

Table 3. Characteristics of TPD-infused products

HSC (CD34⁺ × 10⁶/kg)	Median	2.3
	Range	1.1–2.6
HSC Purity	Median	98%
	Range	91.0–99.5
CD3⁺ (× 10³/kg)	Median	2.3
	Range	0.5–9.8

Table 4. Characteristics of transplanted CB units

TNC (× 10⁷/kg)		(N=27)
	Median	2.37
	Range	1.31–3.73
CD34⁺ cells (× 10⁶/kg)		(N=24)
	Median	0.11
	Range	0.04–0.37

Nine patients (33%) had manifestations of aGVHD of grade II-IV initiated after intervals of 11 to 55 days from the transplant (table 6). Most of the patients with aGVHD responded to corticosteroid therapy. In four cases (14.8%) aGVHD reached grade III-IV, causing death in two cases. DNA analysis of skin biopsy material of aGVHD lesions showed never TPD DNA, which would suggest participation of the TPD cells in GVHD pathogenesis. Only four out of 20 patients at risk developed manifestations of cGVHD, which was limited in all (table 6).

All together, there were eight transplant-related deaths. Relapse occurred in three patients, two of which remained alive and in remission induced by chemotherapy at study closure. The other patient had a relapse of AML that proved refractory to chemotherapy.

Table 5. Engraftment

ANC recovery	
Number of deaths prior to engraftment	2
Time to ANC recovery (days)	
Median	10
Range	9–36
CB-ANC recovery	
Number of deaths prior to engraftment	2
Time to ANC recovery (days)	
Median	22
Range	13–55
Final full CB chimerism	
Number of deaths prior full CB chim.	2
Time to full CB chimerism (days)	
Median	55
Range	11–96
Platelet recovery	
Time to >20×10⁹/L recovery (days)	
Median	33
Range	13–96
Time to >50×10⁹/L recovery (days)	
Median	57
Range	14–240

Survival

Probability of four-year overall survival (OS) was 0.69 for the whole group of patients and 0.77 for the 23 patients receiving non-maternal TPD cells (figure 3).

Discussion

Our results using the strategy of “dual transplant” of a single unit CBT supported by co-infusion of highly T cell-depleted mobilized CD34⁺ cells from a TPD compare favorably to other HSC transplant procedures from unrelated donors, both UCBT and UVDT, in similarly high risk patients.^{1–6,10–14} The prompt recovery of the ANC, initially mainly derived from the TPD cells, appears to provide enough anti-microbial protection as to allow survival time for the relatively late engraftment of the CB progenitors, thus resulting in high rates of cumulative engraftment and full CB chimerism. Final effacement of the TPD cells seems to be the result of immune rejection by the CB-derived immune cells. On the other hand, the observed relatively low incidence and severity of GVHD suggests the

Table 6. Graft versus host disease

Acute GVHD (aGVHD)	N	(%)
Evaluable patients	27	
Grade:		
0	9	(33%)
I	11	(40%)
II	5	(19%)
III	3	(11%)
IV	1	(4%)
Chronic GVHD		
Evaluable patients	20	
Limited GVHD	4	(20%)
Extensive GVHD	0	(0%)
Time to aGVHD (days)		
Median	24	
Range	11–55	

induction of a certain degree of immunotolerance by the exposure of the transplanted CB cells to the TPD cells. The lack of engraftment of the maternal TPD cells was not related to non-inherited maternal antigens (NIMA). Recipient reactivity against minor histocompatibility antigens could be a possibility.¹⁵

The dual transplant strategy using TPD CD34⁺ cells results in high rates of CB engraftment and full chimerism. The main issue to further improve long-term outcomes of UCBT in adults is the development of procedures to enhance recovery of protective immunity. Further advances based on the strategy of using TPD cells are conceivable. We are in the process of assaying the co-infusion of TPD-mobilized selected CD133⁺ HSC. These might provide more potential than CD34⁺ selected HSC for platelet generation and/or enhancement of the CB engraftment and immune reconstitution, because a higher number of primitive lymphoid progenitors may be present in the CD133⁺ cell population. We are also evaluating the use of TPD *ex vivo* expanded marrow stromal cells (MSC) as potential enhancers of CB engraftment and as potential modulators of the immune response that could provide pre-emptive or therapeutic effects for GVHD and, indirectly, on immune reconstitution. Regulatory T cells selected as CD4⁺CD25⁺ is a cell population that is being investigated as potential inductor of immune tolerance of HSC transplants.¹⁶ It is a cell population that in case of UCBT could be obtained from a TPD since their modulating effect through direct cell interaction would not be HLA-restricted. On the other hand, NK cells from the TPD may deserve to be studied as a tool to induce both immunotolerance and anti-tumor effect.¹⁷ And there is also the possibility of using selected pathogen-specific T cells from a TPD to provide adoptive immunotherapeutic effects, a procedure that has been assayed with encouraging preliminary results (H. Einsele *et al.*, personal communication).

References

1. Laughlin, M.J. *et al.* (2004) *N. Engl. J. Med.* 351: 2265–2275.
2. Rocha, V. *et al.* (2004) *N. Engl. J. Med.* 351: 2275–2285.
3. Hamza, N.S. *et al.* (2004) *Br. J. Haematol.* 124: 488–498.
4. Rocha, V. *et al.* (2004) *Blood* 104: Abstract 2144.
5. Gluckman, E. *et al.* (2004) *Exp Hematol.* 32: 397–407.
6. Magro, E. *et al.* (2006) *Haematology* 91: 640–648.
7. Arguello, J.R. *et al.* (1998) *Nat. Genet.* 18: 192–194.
8. Schraml, E. *et al.* (2003) *Leukemia* 17: 224–227.
9. Thiede, C. *et al.* (2001) *Leukemia* 15: 293–306.
10. Barker, J.N. *et al.* (2003) *Blood* 102: 1915–1919.
11. Ooi, J. *et al.* (2003) *Blood* 101: 4711–4713.
12. Ooi, J. *et al.* (2004) *Blood* 103: 489–491.
13. Takahashi, S. *et al.* (2004) *Blood* 104: 3813–3820.
14. Barker J. *et al.* (2005) *Blood* 105: 1343–1347.
15. Mommaas, B. *et al.* (2005) *Blood* 105: 1823–1827.
16. Hoffman, P. *et al.* (2006) *BBMT* 12: 343–374.
17. Ruggeri, L. *et al.* (2002) *Science* 295: 2097–2100.

Figure 1-I

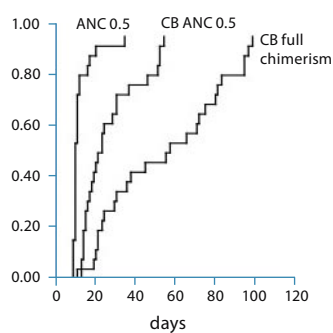


Figure 1-II

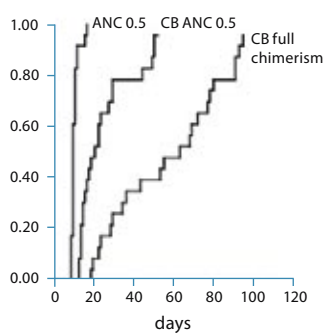


Figure 1. Cumulative incidences of neutrophil recovery (ANC > 0.5 × 10⁹/L) after transplantation

- I) All patients (N=27)
- II) Patients receiving non-maternal TPD cells (N=23)

Figure 2-I

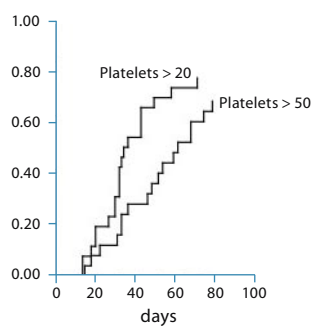


Figure 2-II

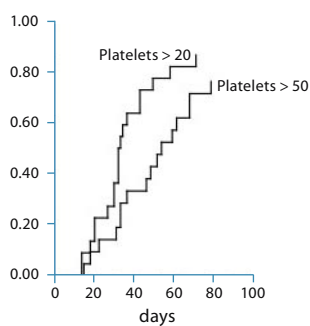


Figure 2. Probability of platelet recovery to 20 × 10⁹/L and to 50 × 10⁹/L

- I) All patients (N=27)
- II) Patients receiving non-maternal TPD cells (N=23)

Figure 3-I

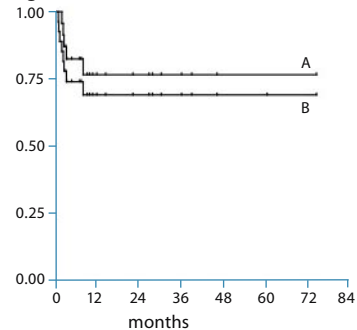


Figure 3-II

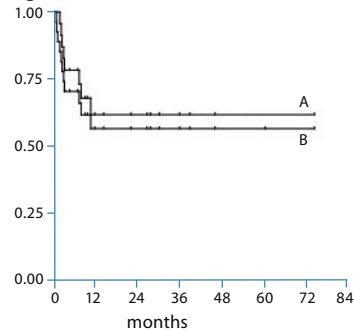


Figure 3. Kaplan-Meier survival curves

- I. Overall survival
 - A: Whole group of patients (N=27); four-year probability of survival, 0.69.
 - B: Patients receiving non-maternal TPD cells (N=23); four-year probability of survival, 0.77
- II. Disease free survival
 - A: Whole group of patients (N=27); four-year probability of survival, 0.56.
 - B: Patients receiving non-maternal TPD cells (N=23); four-year probability of survival, 0.61

Depletion of CD8-positive T cells from donor leukaphereses in a GMP procedure

R. G. Meyer and W. Herr
Department of Medicine III, Hematology and Oncology
Johannes Gutenberg University, Mainz, Germany

Introduction

In allogeneic hematopoietic stem cell transplantation (HSCT), T cell depletion (TCD) prevents severe Graft versus Host Disease (GVHD)¹. It is increasingly used for patients with a high GVHD risk profile defined by advanced age or HLA mismatch transplantation. A major limitation of TCD regimens is a severe and long-lasting posttransplant immunosuppression associated with increased rates of opportunistic infections and disease relapses². Donor lymphocyte infusions (DLI) are measures to treat both, but accompanying GVHD prevents their broader applicability beyond therapeutic settings^{3,4}. Depletion of CD8-positive T cells from DLI clearly reduces the incidence and severity of GVHD leaving considerable anti-tumor activity⁵⁻⁸. Thus, using CD8-depleted DLI prophylactically might be a strategy to promote immune reconstitution with lower risk of inducing GVHD.

Material and Methods

Clinical protocol

Patients and donors participated in a recently published phase I trial⁹ combining a reduced-intensity conditioning HSCT regimen with *in vivo* TCD by the CD52 antibody alemtuzumab¹⁰. After the immunosuppressive treatment was stopped, patients were eligible for prophylactic CD8-depleted DLI if they did not show signs of active GVHD.

CD8 depletion procedure

Leukaphereses were obtained from the original stem cell donors without prior G-CSF treatment. CD8-positive T cells were depleted from the leukapheresis products (LPs) by the use of the CliniMACS® Plus Instrument in a good manufacturing practice (GMP) procedure with permission of the local authorities. Briefly, the LP was diluted 1:3 in CliniMACS PBS/EDTA buffer and centrifuged at 300×g. After re-suspension in 95 mL buffer, 7.5 mL of clinical-grade CliniMACS CD8 Reagent was added. Subsequently, the resulting solution rotated for 30 minutes at room temperature on an orbital shaker at 25 rotations/min. The remaining unbound MicroBeads were removed by washing with buffer in a total volume of 600 mL and the pellet was diluted to a final concentration of 4×10⁸ cells/mL. The bag containing the diluted cells (“original fraction”) was connected to a CliniMACS Tubing Set LS. The depletion procedure was performed using the CliniMACS Plus Instrument and the “DEPLETION 2.1” program of the CliniMACS software (Rev. 2.31). The CD8-depleted target fraction was split into appropriate aliquots according to CD4⁺ T cells/kg body weight (bw) of the patient. Aliquots were cryopreserved in 10% DMSO following standard procedures and were stored in liquid nitrogen until use.

Quality controls

Standard procedures for sterile processing and the monitoring of facility and cell products according to GMP guidelines were implemented. As quality controls for the CD8 depletion procedure, cell counts and 5-color flow cytometry analyses were

performed on a Cytomics FC500 flow cytometer (Beckman Coulter, Krefeld, Germany) with samples obtained from the original leukapheresis, prior to connection to the tubing set (“original fraction”), the positive (“target”) fraction, and the negative fraction, respectively. All antibodies were obtained from Beckman Coulter. Dead cells were identified using propidium iodide (PI, Sigma-Aldrich, Munich, Germany) according to the manufacturer’s instruction. After CD8 depletion, remaining CD3/CD8-positive cells were detected using a gating strategy outlined in Figure 1.

Results and Conclusion

Results

In this report, we summarize the results of eight CD8 depletion procedures performed on leukapheresis products of six unrelated and two related donors (table 1). The clinical protocol⁹ was designed to apply dose-escalated numbers of CD8-depleted donor lymphocytes. The starting dose was 1×10^6 , followed by 3×10^6 , and 1×10^7 CD4-positive T cells per kg bw; this resulted in the need for at least 1.4×10^7 CD4-positive T cells per kg bw after depletion. We considered the potential loss

of cells during the procedure and the need for repetitive quality controls and therefore aimed at a total cell number of 3×10^7 CD4 T cells/kg bw at the start of the procedure. Except for one donor who had to terminate the donation prematurely, this number was exceeded within a single leukapheresis procedure (mean, 5.29×10^7). In six out of eight aphereses, we were able to additionally cryopreserve unmanipulated donor lymphocytes. The average cell numbers during the depletion process are summarized in table 1. The mean yield of CD4 cells was 68.3% (range, 48.1 to 75.2) and we were able to preserve all three intended dose levels. In addition, aliquots of 3×10^7 CD4 T cells/kg bw were stored in 4 patients.

CD8-positive T cells were removed from the leukapheresis products with a depletion efficiency of 2.5 to 6 log (median, 3.5) after a single round of depletion. The mean recovery of B cells, NK cells, and regulatory T cells (not shown) did not significantly differ from that of total lymphocytes (46.8%; range, 36.8% to 55.3%).

One separation procedure was performed with a prolonged labeling time for CliniMACS CD8 Reagent to 45 minutes instead of 30 minutes and

Table 1: Mean cell counts during the CD8 depletion procedure

Mean (min / max)	LP $\times 10^6$	Original fraction $\times 10^6$	Negative fraction $\times 10^6$	Target fraction $\times 10^6$	Yield %
Lymphocytes (total)	117.1	91.0	22.4	54.1	46.8
CD3 (total)	86.3	64.4	18.3	36.2	43.0
CD3 / CD4	52.9	41.7	1.96	34.5	68.3
CD3^{neg} / CD16 / CD56	11.2	8.8	2.38	4.97	47.6
CD3^{neg} / CD19	16.9	12.3	1.13	8.19	47.0
CD3 / CD8	29.6	19.5	16.6	0.02	0.07

The cell numbers are indicated as cells per kg bw of the recipient. LP, leukapheresis product.

an unintended rise in room temperature (up to 23 °C). As a result, the percentage of CD8-positive cells decreased to 17.4% of input cells already in the pre-column fraction, compared to a mean of 74.7% using standard procedure. Nevertheless, the total lymphocyte recovery (46.2%) and the CD8 depletion efficiency (3.5 log) in the target fraction remained in the range of the other procedures. The yield of B cells, however, was reduced to 20.6% compared to an average of 47.0%. This indicates that the procedure is robust and results in efficient elimination of CD8 T cells.

Conclusion

Our results show that the use of the clinical-grade CliniMACS CD8 Reagent in combination with the CliniMACS Plus Instrument allows the efficient depletion of CD8-positive T cells from leukaphereses under GMP conditions. We have already provided evidence of the safety and applicability of CD8-depleted DLI in a prophylactic setting following a reduced-intensity TCD

regimen⁹. Particularly, we have observed higher CD4 T cell counts, improved CMV T cell immunity and stabilization of complete hematopoietic donor chimerism after CD8-depleted DLI. Recent data have also suggested that low doses of CD8-depleted DLI can be safely administered after haploidentical HSCT¹¹. However, the real clinical impact of CD8-depleted DLI still remains to be demonstrated. Prospective randomized trials evaluating the effects of CD8-depleted DLI in prophylactic and therapeutic settings are currently under way.

References

1. Ho, V.T. and Soiffer, R.J. (2001) *Blood* 98: 3192–3204.
2. Chakraverty, R. *et al.* (2001) *Bone Marrow Transplant*. 28: 827–834.
3. Kolb, H.J. *et al.* (2004) *Blood* 103: 767–776.
4. Collins, R.H. Jr. *et al.* (1997) *J. Clin. Oncol.* 15: 433–444.
5. Champlin, R. *et al.* (1990) *Blood* 76: 418–423.
6. Giralt, S. *et al.* (1995) *Blood* 86: 4337–4343.
7. Shimoni, A. *et al.* (2001) *Biol. Blood Marrow Transplant.* 7: 568–575.
8. Alyea, E.P. *et al.* (1998) *Blood* 91: 3671–3680.
9. Meyer, R.G. *et al.* (2007) *Blood* 109: 374–382.
10. Kottaridis, P.D. *et al.* (2000) *Blood* 96: 2419–2425.
11. Corradini, P. (2006) *ASH Annual Meeting Abstracts* 108: 3138.

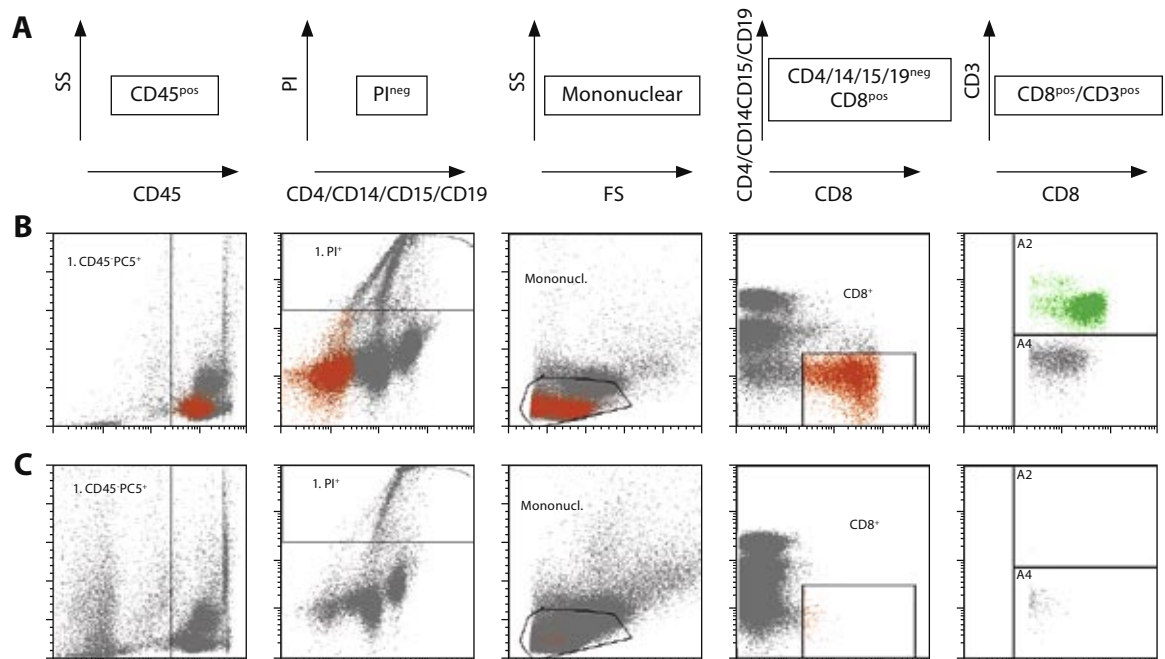


Figure 1:
Five-color flow cytometry analysis for the detection of CD8-positive T cells.

Total leukocytes were stained with CD45-PC5. Dead cells were excluded from the total leukocyte fraction by gating on PI-negative fraction. Of the latter, mononuclear cells were identified by forward and sideward scatter. In an attempt to identify small cell numbers of CD8-positive cells, non-CD8 cells were excluded by staining with PE-labeled CD4, CD14, CD15 and CD19 in a single step. CD8-positive T cells were detected in the remaining cells by staining with CD8-FITC and CD3-PC7. The gating strategy is outlined in panel A. Panels B and C display the original results of the leukapheresis and the target fraction of the donor of patient 10, respectively.

CliniMACS® Cell Selection System: 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		CE coming soon
CCS (Cytokine Capture System, IFN-gamma)	Antigen-specific T cells	CE

Miltenyi Biotec Cell Culture Bags are now released for clinical use within Europe. Outside Europe bags are available for research use only. Please contact us for further information.

Cell Expansion Bags

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

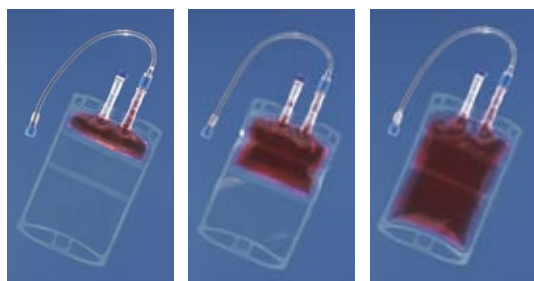


Figure 1: Cell Expansion Bag (tube) with two seals for expandable culture volumes.

Cell Differentiation Bags

(tube and 6 ports) are available for three different culture volumes (100 mL, 250 mL, 500 mL):



Figure 2: Cell Differentiation Bag (tube).

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

Satellite Symposium 2006, American Society of Hematology

Cell therapy: Present and future

The Friday Satellite Symposium "Cell therapy: Present and future" preceded the 48th Annual Meeting of the American Society of Hematology at Orlando, Florida, in December 2006, and was supported by an educational grant from Miltenyi Biotec.

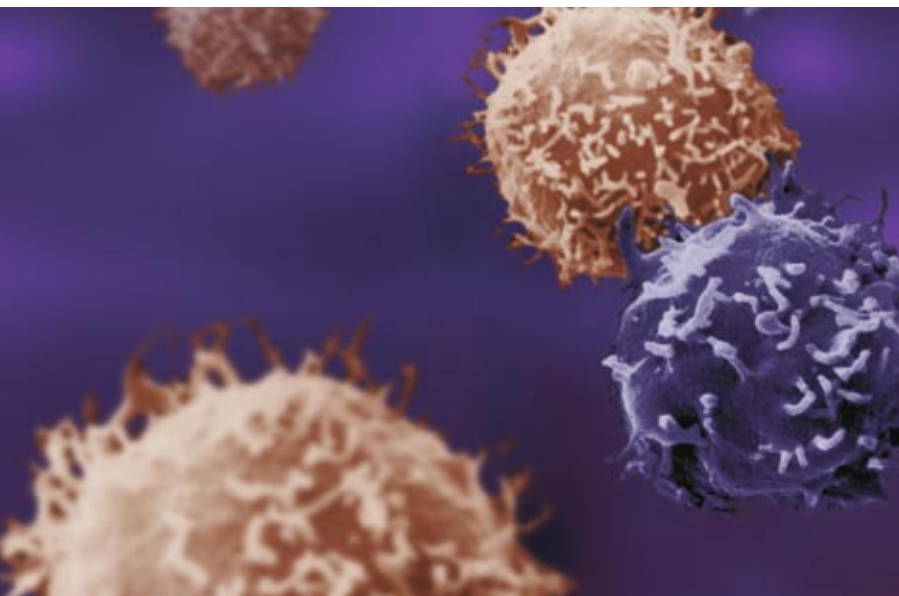
In his introduction, Dr. Frederik Falkenburg discussed how the field of *ex vivo* cellular therapy has evolved since hematopoietic stem cell transplantation has been used to rescue hematopoiesis following myeloablative regimen. Nowadays, sophisticated manipulation of cell populations may lead to broad applications of old and new therapeutic strategies. The symposium focused on novel protocols for cellular therapy in hematological, viral, and cardiovascular diseases.

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

Regulatory T cells in renal cell cancer: Adoptive transfer of CD25-depleted cells following pre-conditioning chemotherapy

Robert E. Hawkins, Eric Austin, Eyad Elkord, Richard Griffiths, Fiona Thistlethwaite, Peter Stern. University of Manchester Cancer Immunotherapy Group, Paterson Institute and Christie Hospital, Manchester, UK

In his talk, Dr. Hawkins highlighted the pivotal role of regulatory T cells (Tregs) in maintaining immune homeostasis, particularly in prevention of autoimmunity, allograft rejection, and downregulating the immune response following an infection. As Dr. Hawkins stated, elevated levels of Tregs have been reported in many tumors. His group has evaluated the presence of increased Treg numbers in the periphery and within the tumor in patients with renal cell carcinoma, a tumor conventionally thought of as "immunogenic". Dr. Hawkins presented preliminary data from six patients treated in a phase I study. Patients' leukapheresis samples were magnetically depleted of CD25⁺ cells, and the depleted cells were cryopreserved. Patients were then pre-conditioned by non-myeloablative chemotherapy with cyclophosphamide and Fludarabine. At completion of chemotherapy the depleted T cells were returned to the patients. The CD25 depletion was effective at reducing Tregs *ex vivo* (> 2 logs). The procedure was well tolerated with rapid recovery of hematopoiesis and lymphocytes.



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

Nicolaus Kröger¹, Andreas Sputtek², Michael Lioznov¹, Edeltraut Merle², Marie-Luise Reckhaus², Tatjana Zabelina¹, Boris Fehse¹, Axel Zander¹. Bone Marrow Transplantation¹ and Institute for Transfusion Medicine², University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Donor-lymphocyte infusion (DLI) has widely been used to prevent or treat relapses and infections after allogeneic stem cell transplantation, but unmanipulated DLI are associated with a risk of acute and chronic graft-versus-host disease. The goal of preclinical and clinical work is the promotion of the desired graft versus leukemia effect and the inhibition of GvHD. Dr. Kröger reported about investigations to establish an automated, efficient, rapid, and clinical-scale method for purifying human CD4⁺ cells.

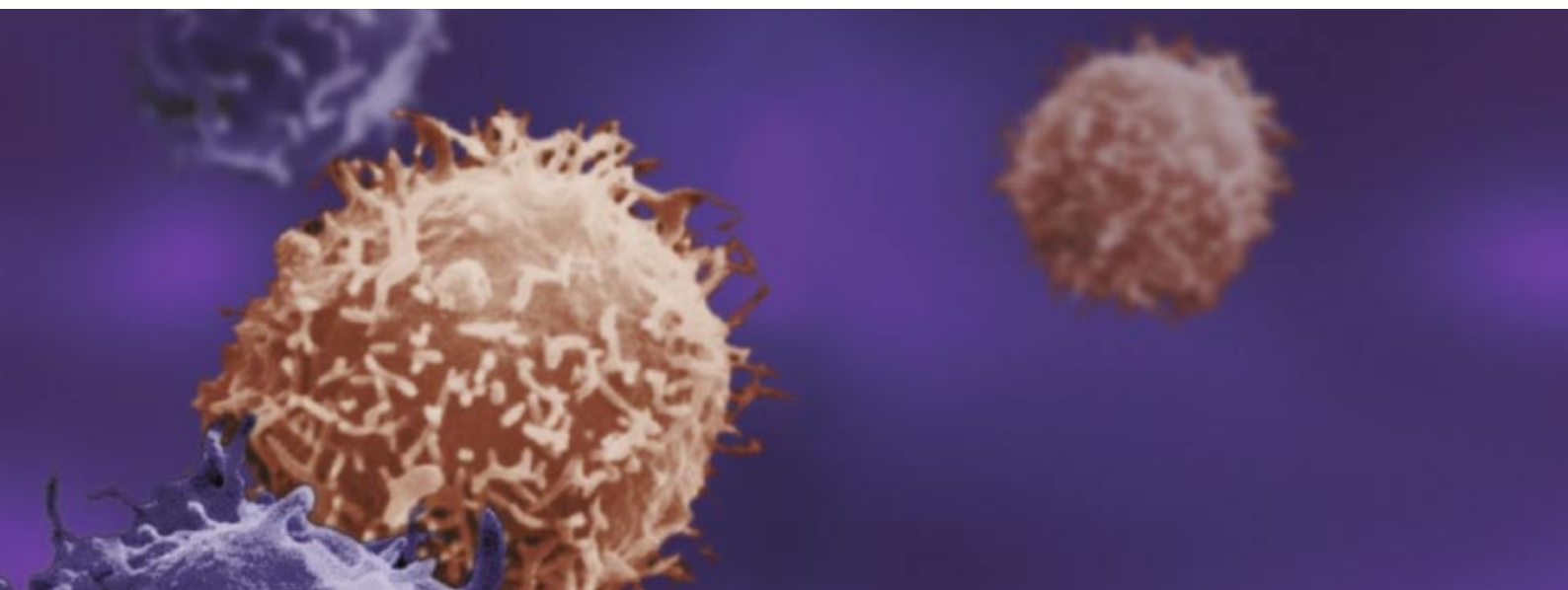
According to Dr. Kröger, in his study, administration of DLI selected for CD4⁺ cells resulted in a low incidence of chronic GVHD and no acute GVHD while retaining graft versus tumor effect or graft versus hematopoiesis effect.

Dr. Kröger aims to conduct further studies which focus on early administration after stem cell transplantation to improve immune recovery for lowering treatment-related infectious complications.

Haploidentical hematopoietic stem cell transplantation in children with hematologic malignancies

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

Dr. Hale discussed the options for haploidentical HSCT. The focus of the work in his institution lies on clinical trials that utilize mismatched family member donors for pediatric, adolescent, and young adult patients with both high-risk and chemotherapy-refractory malignancies. In their experience, patients with chemotherapy-refractory hematologic malignancies experience more rapid immune reconstitution and lower rates of viral reactivation following a reduced intensity-conditioning regimen than standard risk patients transplanted in remission following a standard myeloablative regimen. Dr. Hale summarized that haploidentical HSCT offers a unique opportunity for incorporating the anti-leukemic effects of



NK cells in KIR ligand–mismatched donor-recipient pairs. Nevertheless, optimizing the immunotherapeutic effect of grafts to employ less toxic conditioning regimens and identifying post-transplant immunomodulatory interventions is imperative to reduce disease recurrence rates and avoid regimen-related morbidity and mortality.

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

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

Dr. Ho reviewed recent studies on T cell depletion (TCD), a recognized method for effective graft versus host disease (GVHD) prophylaxis in allogeneic hematopoietic stem cell transplantation. He pointed out that TCD has not led to an improved overall survival compared with pharmacologic GVHD prophylaxis, because removal of donor T cells from the graft is also associated with loss of graft versus leukemia (GVL) activity, graft failure, and post-transplant lymphoproliferative disorders. In Dr. Ho's opinion, however, the advent of peripheral blood stem cells in allogeneic HSCT offers new options for a re-investigation of TCD as GVHD prophylaxis. In a current study, three log

CD8 T cell depletion of G-CSF mobilized PBSC to $< 10^5$ CD8 cells/kg was achieved. Despite extensive CD8 depletion, skin rash/GVHD was common (5 of 8 pts), but grade II-IV GVHD incidence (38%) appears comparable to non-TCD MUD transplants.

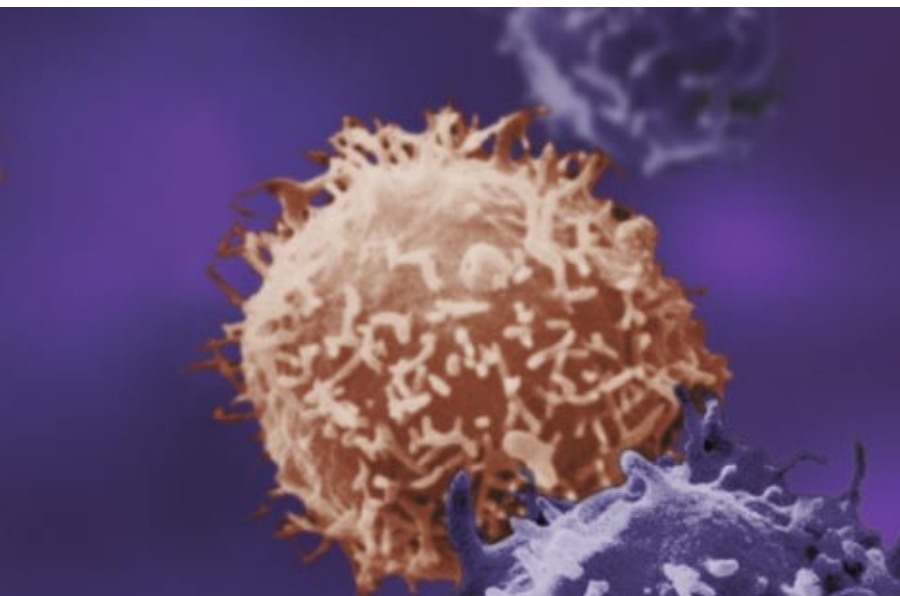
Further enrollment and longer follow-up is needed to assess the impact of CD8 depletion on cGVHD, relapse and long-term survival outcomes.

Adoptive immunotherapy after allogeneic stem cell transplantation with *in vitro* selected virus- or leukemia-reactive T cells

J. H. Frederik Falkenburg, Inge Jedema, Pauline Meij, Maarten Zandvliet, Roel Willemze, Department of Hematology, Leiden University Medical Center, Leiden, The Netherlands

Dr. Falkenburg suggested in his talk that a more effective control of the malignant disorder or the infectious complications might be accomplished by the administration of tumor-specific or virus-specific T cells. He presented the options for different selection procedures for antigen-specific, such as virus-specific, and tumor-reactive T cells. As illustrated by a recently initiated study cited by Dr. Falkenburg, he and others isolated virus-specific T cells by stimulation with virus-specific antigens. Due to their synchronized production of IFN- γ , these cells could then be isolated using the cytokine Capture System (IFN-gamma) and expanded *in vitro*. T cell populations with up to 90% of virus-specific T cells were achieved in the study. Their infusion resulted in successful treatment of patients with CMV reactivation after allogeneic SCT.

A similar approach was applied to the isolation of tumor-reactive T cells. Although the final purity of leukemia-reactive T cells was significantly lower than in the former approach, the enrichment of leukemia-reactive T cells for *in vivo* administration offers an option for treating residual hematological malignancies shortly after allogeneic SCT with a limited risk of GVHD.



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

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

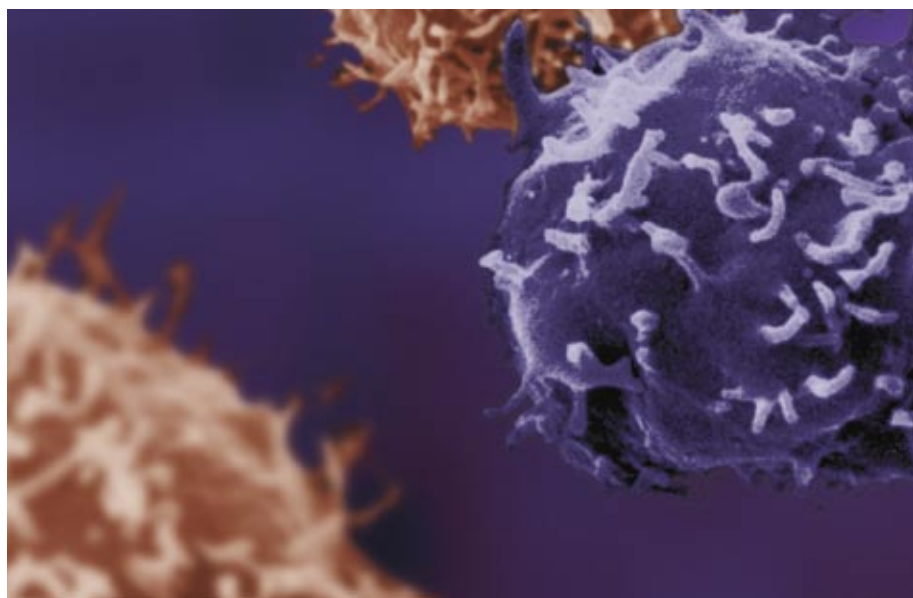
Dr. Steinhoff reported on two studies conducted to further investigate the still controversial potential of autologous human bone marrow progenitor cells as a therapeutic option for chronic ischemic heart disease. In the first study, a clinical phase I trial, his department assessed the feasibility and safety of intramyocardial CD133⁺ bone marrow cell injection together with coronary artery bypass surgery (CABG). With a subsequent phase II study the hypothesis was tested whether CABG plus injection of CD133⁺ cells results in better contractile function than CABG alone. Dr. Steinhoff concluded that in their studies intramyocardial bone marrow stem cell delivery in CABG patients was safe and met efficacy endpoints. Future studies are needed to show a clear clinical benefit.

CD34 selection in allogeneic transplantation

Ann Jakubowski, Nancy Collins, Memorial Sloan-Kettering Cancer Center, New York, NY, USA

Dr. Jakubowski first summarized the advantages and disadvantages of unmodified grafts compared to T cell-depleted (TCD), respectively. She described TCD transplantation as one approach to address the needs of a broader spectrum of patients, including those who are older and those who require transplantation across greater levels of HLA disparity when more compatible donors are not available. Over the past 5 years, Dr. Jakubowski and her colleagues have processed over 250 peripheral blood stem cell (PBSC) grafts as part of their ongoing program in TCD transplantation, using a device for clinical-scale magnetic cell separation followed by sheep red

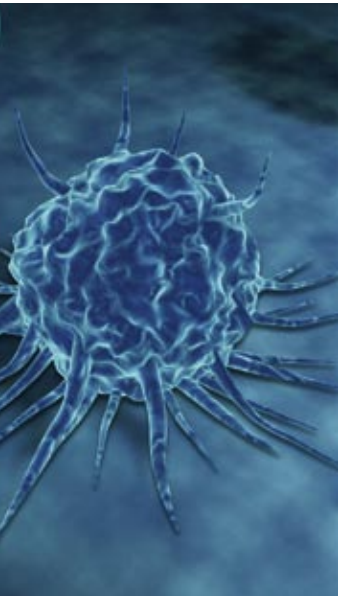
blood cell rosetting. Furthermore, results of more than 300 bone marrow (BM) grafts have been consistent throughout their experience since 1995; with a CD34⁺ cell content approximately 4-fold higher in PBSC grafts than in BM grafts. Dr. Jakubowski concluded that TCD using automated magnetic cell separation technology offers one method of dealing with the increased risk of graft versus host disease (GVHD), without sacrificing the antitumor effect of the allograft, and that this approach provides the opportunity for implementing targeted cell therapies.



Satellite Symposium EBMT 2007

The widening scope of cellular therapy

This year's Satellite Symposium, supported by an unrestricted educational grant from Miltenyi Biotec, preceding the 33rd annual meeting of the EBMT, took place on March 25, 2007, in Lyon, Paris.



In his introduction, chairman Professor Gorin, Hôpital Saint-Antoine, Department of Hematology and Cell Therapy, Paris, France, gave a historical overview on cellular therapy in the field of hematology. The session dealt with the most recent developments and trends in cellular therapy.

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

Haploidentical transplantation using post-transplant T cell immunotherapy to reconstitute anti-viral immunity

Helen Heslop, Baylor College of Medicine, The Methodist Hospital and Texas Children's Hospital, Center for Cell & Gene Therapy, Houston, USA

Dr. Heslop gave a very interesting multifaceted talk in which she presented the two approaches her group has investigated to reconstitute anti-viral immunity after haploidentical stem cell transplantation. The first approach is to adoptively transfer multivirus-specific T cells; expanded *ex vivo*, these donor cytotoxic T lymphocytes can restore immunity to CMV, EBV, and AdV simultaneously. Eleven patients in a phase I prophylaxis study were treated. Clinically relevant effects were shown against all three viruses were shown. The second approach she discussed was to infuse donor T cells from which alloreactive lymphocytes have been selectively depleted. In order to obtain an antileukemic effect, large cell doses need to be administered; thus, the safety of

such products needs to be increased. This was done by incorporation of a suicide gene, the inducible caspase 9, with CD19 as selectable marker, into the allodepleted cells. Thus, the destruction of the cells would be possible in case adverse effects, such as GVHD, occur.

Haploidentical transplantation in adults with CD3/CD19-depleted grafts and reduced-intensity conditioning

Christoph Faul, Medical University Hospital, Department of Oncology, Hematology, Immunology, Rheumatology and Pulmology, Tübingen, Germany

T and B cell depletion of the graft combined with a reduced intensity conditioning may allow for haploidentical hematopoietic stem cell transplantation (HCT) with lower toxicities and faster engraftment. CD3/CD19 depleted grafts not only contain CD34⁺ stem cells but also CD34-negative progenitors, graft facilitating cells, dendritic cells and NK cells. Dr. Faul presented data of thirty "high risk" patients treated who presented with refractory disease or relapse after preceding HCT. The CD3/CD19 depleted haploidentical grafts contained a median of 7.5×10^6 CD34⁺ cells and a median of 5.4×10^4 CD3⁺ T cells, and 7.5×10^7 CD56⁺ cells. Currently 15/30 patients are alive with a median follow-up of 151 days. From the data collected up to now Dr. Faul concluded that this regimen is promising in high-risk patients lacking a suitable donor. A prospective phase I/II study is ongoing.

Clinical-scale combined CD4 and CD8 depletion for infusion of donor innate lymphocyte infusions (DILI)

Volker Kunzmann, Julius Maximilians University of Würzburg, Medical Clinic and Policlinic II, Würzburg, Germany

Dr. Kunzmann gave an overview on TCR $\gamma\delta$ T cells, recognition of tumor cells by V γ 9V δ 2 T cells, their natural ligands, their anti-infectious activity and their possible use in cellular therapy. He discussed different clinical-scale strategies in order to obtain allogeneic innate lymphocytes, both natural killer cells and TCR $\gamma\delta$ T cells, as donor innate lymphocyte infusions (DILI). The best method available today, in his opinion, is the combined depletion of CD4⁺ and CD8⁺ T cells on the CliniMACS[®] Plus Instrument, thereby passively enriching for NK cells and TCR $\gamma\delta$ T cells. Dr. Kunzmann stated that clinical-scale depletion of CD4⁺ and CD8⁺ T cells is as efficient as CD3 depletion in reduction of alloreactive TCR $\alpha\beta$ T cells in the product. Finally, he also presented encouraging preliminary results from one patient with plasma cell leukemia who was in partial remission at time of DILI. The group is preparing a study protocol employing this strategy.

Intramyocardial stem cell transplantation for chronic ischemic heart failure—phase II results and the Berlin Cardio133 trial

Christof Stamm, German Heart Institute Berlin, Berlin, Germany

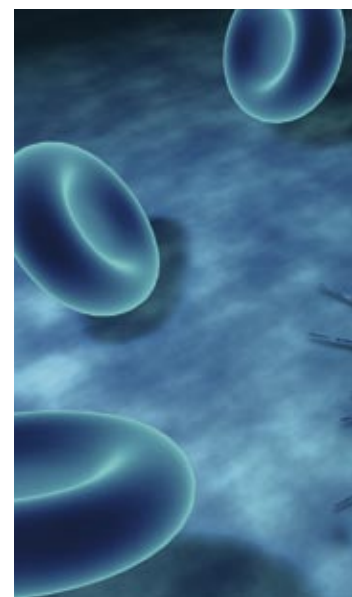
Based on their experience with patients suffering from end-stage heart failure, surgeons, physicians, and scientists at the German Heart Institute Berlin (DHZB) have begun to systematically evaluate the potential of cell-based regenerative medicine for heart disease. The presentation nicely summarized the previous experience with the intramyocardial application of unmodified bone marrow mononuclear cells in surgical patients, and

described the design and preliminary results of ongoing clinical studies using purified CD133⁺ bone marrow-derived cells. Although extensive experimental data support the concept of cardiac cellular therapy, neither the ideal source and cell type nor the quantity and mode of application in the clinical setting have been defined so far. Based on clinical data, Dr. Stamm concluded that cell therapy for myocardial regeneration seems to work in principle. Nevertheless, the underlying mechanisms are still under investigation and the field of cellular therapy needs more preclinical studies, as well as large controlled studies to determine efficacy of the treatment approaches.

CD34⁺-selected stem cell boosts for treatment of patients with poor graft function

Andrea Bacigalupo, Ospedale San Martino, Department of Hematology, Genova, Italy

Dr. Bacigalupo presented data that, as he pointed out, may be useful when discussing second stem cell donation for patients with poor graft function (PGF). As of today, it is uncertain whether a boost of donor marrow cells or blood cells is beneficial for patients developing PGF. The aim of the study presented was to compare patients with PGF and full donor chimerism, with or without receiving a boost of selected CD34⁺ donor stem cells. The 54 patients studied were divided into three groups; either receiving no further donor cell infusion, receiving a boost of unmanipulated marrow or blood cells, or receiving donor cells after CliniMACS[®] CD34 selection. The outcome showed that in patients with PGF, a boost of selected donor CD34⁺ PB is associated with a high chance of trilineage recovery, and a low risk of GVHD. Moreover, a boost of unmanipulated donor cells did not seem to offer a survival advantage over no infusion of cells and non-relapse related mortality is lower when using PB cells.



Satellite Symposium at the 2007 Annual Meeting of the German Cardiac Society

Kardiale Stammzelltherapie — Aktueller Entwicklungsstand (Translation: Stem Cell Therapy in Cardiac Disease – Where are we today?)

The symposium, hosted by Miltenyi Biotec, was intended to give an overview of current preclinical and clinical work regarding bone marrow stem cells in cardiac disease. Five leading investigators presented the work from their specific areas, followed by a podium discussion.
(73rd Annual Meeting of the German Cardiac Society in Mannheim, Germany, April 12–14, 2007)
Chairmen: W.-M. Franz and G. Steinhoff

Stammzellmobilisation und –aktivierung

(Stem cell mobilization and activation)
W.-M. Franz, Department of Internal Medicine and Interventional Cardiology at the Ludwig Maximilians University, Munich, Germany

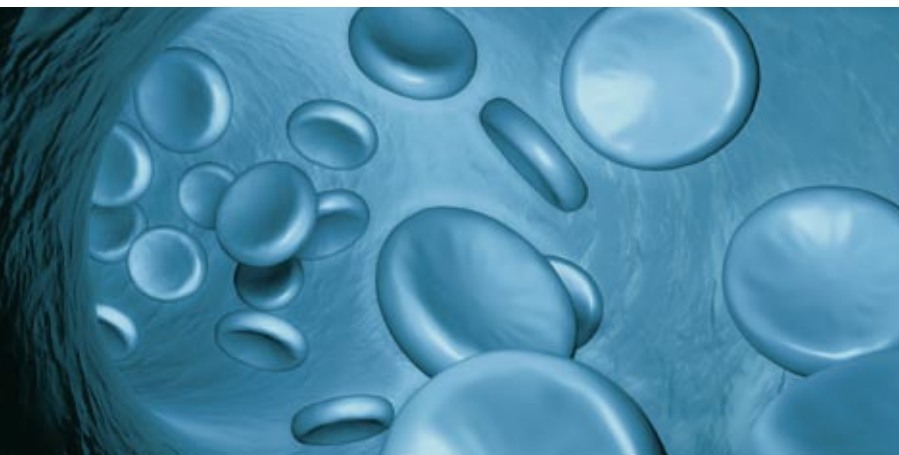
Prof. Franz reported about his work in murine infarction models, looking at the effects of G-CSF treatments. His group recently found that inhibition of CD26 (Dipeptidylpeptidase IV) in addition to G-CSF administration further substantially improved stem cell mobilization and homing. In accordance with that finding, cardiac function after infarction was also improved in CD26 inhibited mice. This approach might have potential for future improvement of cytokine-

based stem cell treatments in humans. Prof. Franz also presented results from the randomized double blinded G-CSF-STEMI trial. Ejection fraction as well as perfusion were improved in the G-CSF group compared to the placebo group though the effects did not reach statistical significance.

Intrakoronare Stammzelltherapie

(Intracoronary stem cell therapy)
S. Dimmeler, Department of Internal Medicine III, Molecular Cardiology, University of Frankfurt, Germany

Prof. Dimmeler presented a detailed analysis of results from the REPAIR-AMI trial after two years follow-up. This has been the largest randomized, blinded, placebo-controlled trial to date, evaluating the effect of intracoronary BMC (bone marrow mononuclear cells) infusion after acute myocardial infarction. A moderate but statistically significant improvement of ejection fraction and perfusion was found in the BMC group. The details of bone marrow processing and resulting stem cell properties were discussed in comparison to the ASTAMI trial, another randomized controlled trial which did not show improvement for the BMC treated group.



Intramyokardiale Stammzelltherapie

(Intramyocardial stem cell therapy)

G. Steinhoff, Department of Cardiac Surgery at the University of Rostock, Germany

Prof. Steinhoff reported the results of the phase I and subsequent phase II trials performed in Rostock, investigating the safety and beneficial effects of intramyocardial stem cell injections in CABG patients. CD133-positive stem cells had been selected using the CliniMACS® CD 133 Reagent System from bone marrow aspirates of the patients prior to surgery. No cell related complications were observed in long-term analysis up to five years after injection. The outcome was analyzed of 40 patients (20 cell treated, 20 control) from the phase II trial with a follow-up of six months. A statistically significant additional improvement of ejection fraction was achieved for the patients receiving cells in comparison to control patients treated with CABG alone (9.7% versus 3.4%). Based on these promising results a randomized controlled and blinded multicenter phase III trial is in preparation to further address the question of efficacy of stem cell treatment.

Stammzellen und Tissue engineering

(Stem cells and tissue engineering)

A. Haverich, Department of Thoracic and Cardiovascular Surgery at Hannover Medical School, Hannover, Germany

Prof. Haverich gave an overview of various concepts and strategies for stem cell therapy including tissue engineering in the cardiac field. One major driving force for the development of cell therapy and tissue engineering has been the dramatic lack of suitable donor organs for transplantation. Different types of adult stem cells have been evaluated over the last years and clinical trials exploring their efficacy in humans have created hopes and doubts as well. The potential for future clinical use of human embryonic stem cells was discussed in detail. The second part of the

presentation was focusing on recent work in tissue engineering for regeneration of cardiac tissue. Different animal models have shown that engineered cell-matrix constructs can be integrated into ischemic tissue and result in partial restoration of function.

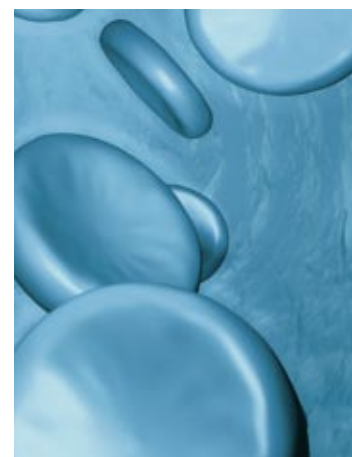
Stammzellen und Kunstherzunterstützung

(Stem cells and artificial heart support)

R. Hetzer, Department of Cardiothoracic and Vascular Surgery, German Heart Institute, Berlin, Germany

Prof. Hetzer discussed the experience at the German Heart Institute with implantation of left ventricular assist devices (LVAD) in heart failure patients. A number of patients with idiopathic dilated cardiomyopathy (IDCM) show myocardial recovery and improvement of cardiac function as a result of device support. Some of these patients can be weaned from the assist device and show stable cardiac function for several years. In a pilot trial to further improve the recovery process, a 14 year old male was treated with intramyocardial injection of autologous bone marrow mononuclear cells at the time of LVAD implantation. After 122 days, cardiac function had returned to normal values and has been stable for several years now. Nine more patients were treated following this approach but none of them showed a similar effect of cell treatment.

Following the presentations the current status of stem cell therapy in cardiac disease was discussed. It is now widely agreed that carefully designed, randomized, blinded, controlled trials are required to test the clinical efficacy of stem cell therapy in various indications. Three of the centers that presented in the symposium (Berlin, Hannover and Rostock) are now collaborating to start a randomized multicenter trial using CD133⁺ stem cells in CABG patients.





Program at ISCT Symposium in Sydney, Australia

New Prospects in Cellular Therapy

June 24, 2007, 3:00–5:00 pm

Chair: Jeff Miller, MD, University of Minnesota, Minneapolis, MN, USA

Introduction

Jeff Miller, MD, University of Minnesota, Minneapolis, MN, USA

Preemptive CD8⁺ T cell Depleted Donor Lymphocyte Infusion following Nonmyeloablative Stem Cell Transplantation (NMT) for Acute Myeloid Leukemia (AML) or High Risk Myelodysplastic Syndrome (MDS)

Chien-Shing Chen, MD, National University Hospital Singapore, Singapore

The Role of NK Cells and their Receptors in Cancer and Hematologic Malignancies

Jeff Miller, MD, University of Minnesota, Minneapolis, MN, USA

Hematopoietic Stem Cell Transplantation for Autoimmune Diseases

Daobin Zhou, MD, Peking Union Medical College Hospital, Beijing, China

Transplantation of Autologous Stem Cells in Peripheral Artery Disease (PAD)

Takayuki Asahara, MD, PhD, Kobe Institute of Biomedical Research and Innovation, Kobe, Japan

Panel discussion: New prospects in cellular therapy



Program at ESC Congress in Vienna, Austria

CD133 stem cells for cardiac disease—Multicenter and randomized trials after promising phase I and II results

September 1, 2007, 2:30–4:00 pm

Chair: Roland Hetzer, German Heart Institute Berlin, Germany

Jozef Bartunek, OLV Hospital Cardiovascular Center, Belgium

INSTEM multicenter trial—Intramyocardial application of stem cells in combination with transmyocardial Laser Revascularization (TMLR) in CABG Patients

Hans-Michael Klein, University of Duesseldorf, Clinic for Thoracic and Cardiovascular Surgery, Germany

SELECT-AMI—Selection of endothelial progenitor cells for coronary transplantation in Acute Myocardial Infarction

Jozef Bartunek, OLV Hospital Cardiovascular Center, Aalst, Belgium

Italian multicenter randomized trial using intramyocardial application of stem cells in combination with CABG

Giulio Pompilio, Department of Cardiovascular Surgery, Centro Cardiologico Monzino IRCCS, Milan, Italy

Autologous bone marrow MNC treatment in patients with LVAD and the CARDIO 133 randomized trial

Boris Nasser, German Heart Institute Berlin, Germany

PERFECT multicenter phase III trial—the next step based on the Rostock CD133 phase I and II results

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

Frequently asked questions

Please refer to the CliniMACS® user manual for complete instructions for use.

Q: The capacity of the standard tubing set (Ref. No. 161-01) is limited to 6×10^8 target cells. How is it then possible to process up to 5×10^9 CD56⁺ cells in a single process using program ENRICHMENT 1.1 with the standard tubing set?

A.: Selection program ENRICHMENT 1.1 is a so-called “staged loading” program. It automatically splits the product to be separated into portions if the number of target cells exceeds the tubing set capacity. Every portion is processed completely before the next is loaded to the separation column. This ensures maximum tubing set capacity for every loading step. To enable this individual sample processing, the user simply has to enter relevant sample parameters (cell concentration, percentage of labeled cells, and loading volume) into the software. The CliniMACS® Plus Instrument then automatically calculates the separation conditions and indicates if higher volumes of buffer and/or bags are required.

Q: What target cell fraction volume can we expect when selecting CD133⁺ cells from bone marrow for a cardiac application?

A.: For a CD133⁺ selection from bone marrow for cardiac application it is strictly recommended to use program “CD133 Selection 2” together with a CliniMACS Tubing Set LS. The typical volume for the final target cell fraction (Cell Collection Bag) is approx. 100 mL.

Q: In which bag do I find the CD8-depleted fraction?

A.: The cells of interest (labeled for enrichment and non-labeled for depletion) are always finally collected in the Cell Collection Bag. This is the left bag at the CliniMACS Plus Instrument.

Q: What is the appropriate tubing set for a CD8 depletion?

A.: The recommended combination is a CliniMACS Tubing Set LS together with selection program DEPLETION 2.1.

Q: What are suitable conditions for an overnight storage of CD133-selected bone marrow progenitor cells for cardiac applications?

A.: We recommend to keep the CD133-selected cells at a maximum cell concentration of 1×10^6 /mL in NaCl, supplemented with 10% autologous serum or HSA at 4 °C.

Q: Why are there different CliniMACS programs for the enrichment of CD34-positive and CD133-positive stem cells?

A: As the CD133 antigen of stem cells is less expressed than the CD34 antigen, the separation of CD133-positive cells requires slower processing in order to retain the target cells; this has to be taken into account when designing the programs.

Q: What types of cell culture bags are suitable for use with low-volume T cell cultures when aiming for expansion?

A: Cell Expansion Bags **CE** are perfect for this approach. Please note, that outside Europe bags are available for research use only. These bags are divided into compartments, but with easy-to-open seals for expandable culture volumes. The capacity of the first compartment is 15 mL. For increase of the culture volume up to 100 mL, the seals towards the consecutive compartments can easily be opened by transferring additional medium.

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.

Clinical Technical Support Team
E-mail macstec@miltenyibiotec.de
Phone +49 2204 8306 8484
Fax +49 2204 8306 89

Conference calendar 2007 —meet us at the booth!

Date	Congress	Webpage
2007		
Jun 24–27	ISCT 2007, Sydney, Australia	www.celltherapysociety.org/meeting/annual_meeting/abstracts.aspx
July 16–18	DC 2007, Bamberg, Germany	www.dc2007.eu/
Sep 1–5	ESC 2007, Vienna, Austria	www.escardio.org/congresses/esc_congress/esc2007
Sep 29–Oct 2	ISEH 2007—36th Annual Scientific Meeting, Hamburg, Germany	www.iseh.org/meetings/upcoming.com
Sep 28–Oct 3	ESOT—13rd Congress of the European Society for Organ Transplantation, Prague, Czech Republic	www.esot.org
Oct 4–6	5th Workshop on Haploidentical Stem Cell Transplantation, Catania, Italy	www.siematologia.it/index
Oct 5–9	DGHO, Basel, Switzerland	www.haematologie-onkologie-2007.ch
Oct 17–19	3rd World Congress of Regenerative Medicine, Leipzig, Germany	www.regmed.org
Dec 7–11	ASH, Atlanta, USA	www.hematology.org

Fax reply form

CliniMACS® Newsletter Vol. 7 No. 1/2007

Please mark below and fax to:

Miltenyi Biotec

Marketing Department, Attn.: Brigitte Borchert

Fax no. + 49 2204 85197

ASH 2006 130-092-980	Abstract booklet, Miltenyi Biotec symposium "Cell therapy: Present and future"	<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"/>
EBMT 2007 130-093-172	Abstract booklet, Miltenyi Biotec symposium "The widening scope of cellular therapy"	<input type="checkbox"/>
Annual Meeting German Society for Thoracic and Cardiovascular Surgery 2007 130-093-122	Abstract booklet, Miltenyi Biotec symposium "Stem cell therapy for myocardial repair"	<input type="checkbox"/>
ISCT 2007 130-093-252	Abstract booklet, Miltenyi Biotec symposium "New prospects in cellular therapy"	<input type="checkbox"/>
CliniMACS® CD8 Reagent Symposium Compilation Booklet 130-093-057	Novel strategies in clinical research implementing CliniMACS® CD8 depletion techniques	<input type="checkbox"/>
CliniMACS® Product Catalog 2006/2007 130-090-666.06		<input type="checkbox"/>
Baxter Product Info		<input type="checkbox"/>
Please check the box if you wish to receive further information. Your local Miltenyi Biotec representative would be available to visit your site and discuss our products in more detail.		<input type="checkbox"/>

My research focus is _____

Name, First name _____

Institute, Department _____

Street _____

City/ Postal code/ Country _____

Phone _____

Fax _____

E-mail _____

Subsidiaries

Germany/Austria/ Switzerland

Miltenyi Biotec GmbH
Friedrich-Ebert-Straße 68
51429 Bergisch Gladbach
Phone +49 2204 8306 0
Fax +49 2204 85197
macs@miltenyibiotec.de

USA/Canada

Miltenyi Biotec Inc.
12740 Earhart Avenue
Auburn, CA 95602
Phone 800 FOR MACS
Phone +1 530 888 8871
Fax +1 530 888 8925
macs@miltenyibiotec.com

Australia

Miltenyi Biotec Australia Pty.
Ltd.
Unit 16A, 2 Eden Park Drive
North Ryde NSW 2113
Phone +61 02 8877 7400
Fax +61 02 9889 5044
macs@miltenyibiotec.com.au

Benelux

Miltenyi Biotec B.V.
Postbus 85183
3508 AD Utrecht
Netherlands
Customer service,
Belgium
Phone (Belgium) 0800 94016
Fax (Belgium) 0800 99626
Customer service,
Luxembourg
Phone (Lux.) 800 24971
Fax (Lux.) 800 24984
Customer service,
Netherlands
Phone (NL) 0800 4020120
Fax (NL) 0800 4020100

China

Miltenyi Biotec Shanghai Office
Fareast International Plaza A
Rm. 2301, No. 319 Xianxia Rd.
Shanghai 200051, P.R.China
Phone +86 21 6235 1005
Fax +86 21 6235 0953
macs@miltenyibiotec.com.cn

France

Miltenyi Biotec
10 rue Mercœur
75011 Paris
Phone +33 1 56 98 16 16
Fax +33 1 56 98 16 17
macs@miltenyibiotec.fr

Italy

Miltenyi Biotec S.r.l.
Via Persicetana, 2/D
40012 Calderara di Reno (BO)
Phone +39 051 64 60 411
Fax +39 051 64 60 499
macs@miltenyibiotec.it

Japan

Miltenyi Biotec K.K.
Nittsu-Eitai Building 5F
16-10 Fuyuki, Koto-ku, Tokyo,
135-0041, Japan
Phone +81 3 5646 8910
Fax +81 3 5646 8911
macs@miltenyibiotec.jp

Singapore

Miltenyi Biotec Asia Pacific
Pte.Ltd.
7, Temasek Boulevard #16-06
Suntec Tower One
Singapore 038987
Phone +65 6238 8183
Fax +65 6238 0302
macs@miltenyibiotec.com.sg

Spain

Miltenyi Biotec S.L.
C/Luis Buñuel 2,
Ciudad de la Imagen
28223 Pozuelo de Alarcón
(Madrid)
Phone +34 91 512 1290
Fax +34 91 512 1291
macs@miltenyibiotec.es

United Kingdom

Miltenyi Biotec Ltd.
Almac House, Church Lane,
Bisley
Surrey GU24 9DR
Phone +44 1483 799 800
Fax +44 1483 799 811
macs@miltenyibiotec.co.uk

Distributors

Argentina

Lab Systems S.A.
Phone +54 11 4574 3447

Bahrain

Al Yamama
Phone +962 6 4655166

Brazil

Ambriex S/A
Phone +55 11 3511 1090

Bulgaria

Epsilon & Epsilon Medical S.A.
Phone +30 210 6996191

Chile

UB Ursula Biggemann y Cia Ltda.
Phone +56 2 334 9282

Colombia

Equimed Ltda.
Phone +57 1 285 5053

Croatia

MEDIVA d.o.o.
Phone +385 1 6191 580

Cyprus

Epsilon & Epsilon Medical S.A.
Phone +30 210 6996191

Czech Republic

BIOHEM spol. s.r.o.
Phone +421 32 6524998

Denmark

Fisher Scientific *
Phone +45 70 279920

Egypt

Al Yamama
Phone +962 6 4655166

Estonia

AS Oriola
Phone +372 65 15 159

Finland

Oriola OY Prolab *
Phone +358 10 429 4927

Greece

Epsilon & Epsilon Medical S.A.
Phone +30 210 6996191

Hong Kong

Miltenyi Biotec Shanghai Office
Phone +86 21 6235 1005

Hungary

Frank Diagnosztika Kft.
Phone +361 250 1813

India

Labmate (Asia) Pvt., Ltd. *
Phone +91 44 2220 0066
N.W.Overseas (Pvt) LTD**
Phone +91 172 272 2045

Israel

Almog Diagnostic & Medical
Equipment
Phone +972 3 977 33 90

Jordan

Al Yamama
Phone +962 6 4655166

Kuwait

Tri_Alpha Company
Phone +965 24 3 1344

Latvia

SIA Oriola
Phone +371 780 2450

Lebanon

Al Yamama
Phone +962 6 4655166

Lithuania

UAB Oriola
Phone +370 526 88453

Malaysia

Miltenyi Biotec
Asia Pacific Pte Ltd.
Phone +65 6238 8183

Mexico

Uniparts S.A.
Phone +52 55 5281 4718

New Zealand

Miltenyi Biotec
Australia Pty. Ltd.
Phone: +61 02 8877 7400

Northern Ireland

Ulster Anaesthetics Ltd. *
Phone +44 2890 448800

Norway

AH Diagnostics as *
Phone +47 2323 3260

Oman

Al Yamama
Phone +962 6 4655166

Pakistan

T & A Scientific *
Phone +92 21 4555 351

Peru

Inmunochem
Phone +51 1 440 9678

Poland

MEDIANUS Sp. zo.o.
Phone +48 12 665 3131

Portugal

Citomed Lda.
Phone +351 218 421 430

Qatar

Al Yamama
Phone +962 6 4655166

Romania

Novaintermed SRL
Phone +40 31 401 10 90

Russia

Pocard
Phone: +7 495 414 68 15

Saudi Arabia

Pharma Co. Ltd.
Phone +966 1 4816 330

Slovak Republic

BIOHEM spol. s.r.o.
Phone +421 32 6524998

South Africa

Biocom biotech
Phone +27 12 654 4614

South Korea

Medilab Korea Co. Ltd.
Phone +82 2 424 63 67

Sweden

GTF Sweden *
Phone +46 31 680 490

Taiwan

J & H Technology Co.Ltd. *
Phone +886 2 879 127 69
Miltenyi Biotec Asia Pacific Pte
Ltd. **
Phone +65 6238 8183

Thailand

Biomed Diagnostic (Thailand)
Co. Ltd. *
Phone +66 2 4460 755
Venus Technology Co., Ltd **
Phone +66 2 9356 761

Turkey

MAK Sağlık Ürünleri
Pocard
İth. İhr. Tic. San.İtd.Şti.
Phone +90 216 360 62 74

United Arab Emirates

Al Yamama
Phone +962 6 4655166

* Research products only

** Clinical products only

www.miltenyibiotec.com

The CliniMACS® System components (Instruments, Reagents, Tubing Sets, and PBS/EDTA Buffer) are manufactured and controlled under an ISO 13485 certified quality system. In Europe, the CliniMACS System components 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. MACS and CliniMACS are registered trademarks of Miltenyi Biotec GmbH.

With compliments from: