CliniMACS®

Newsletter Vol. 4 No. 1/2004

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Rapid generation of CMV-specific T cell lines for adoptive transfer into allogeneic stem cell transplant recipients

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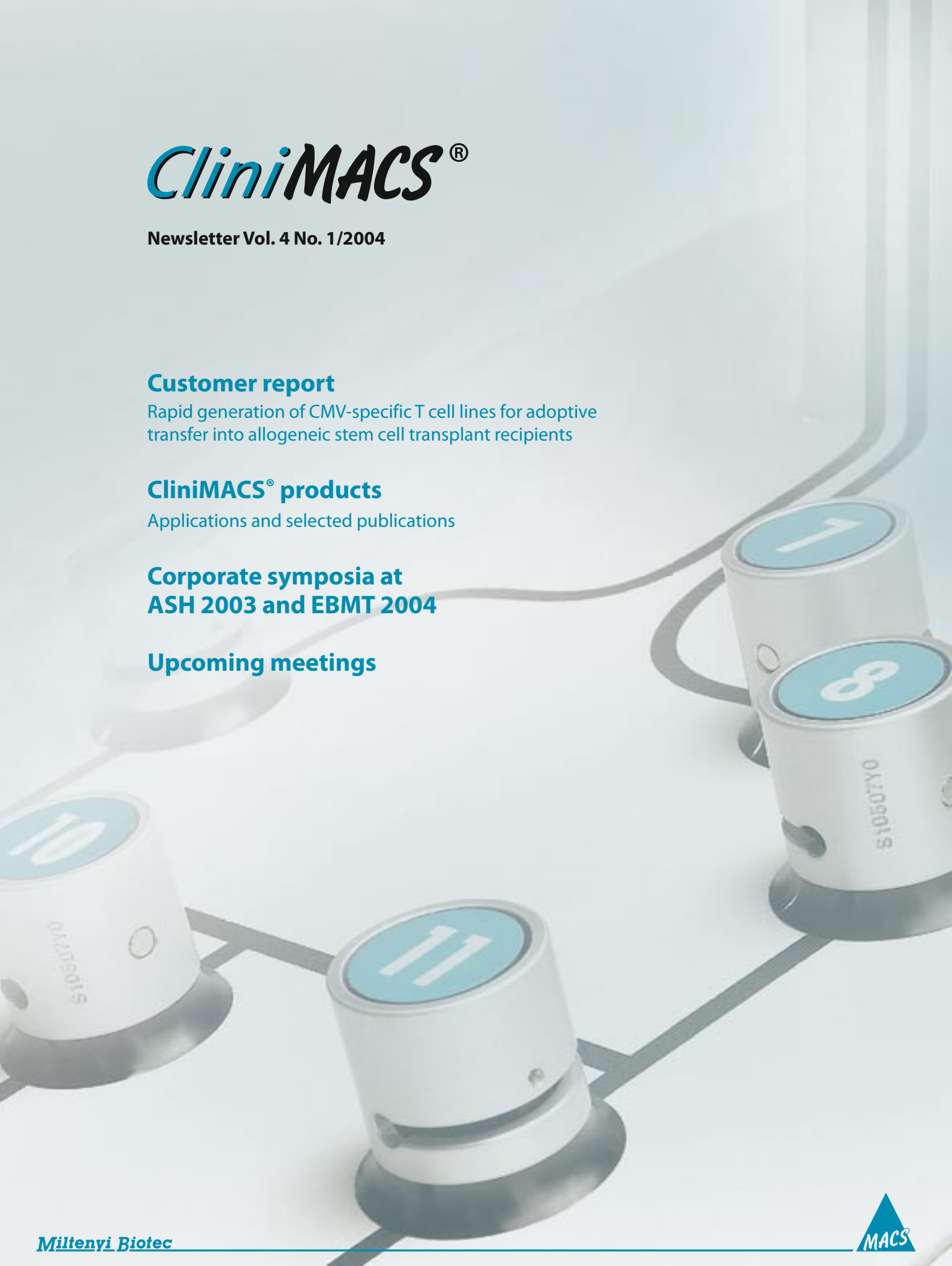

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Dear CliniMACS® System user,

It is a pleasure to welcome you to our new CliniMACS Newsletter. Recent years have seen a dramatic increase in activity in the immunotherapy field, with many new therapies now coming to trial that would have been unimaginable even a few years ago. As we develop our ever-expanding range of tools for immunotherapy, we at Miltenyi Biotec have been privileged to work with many of the leading researchers in the field. Through this synergy we all can bring new ideas and therapeutic approaches through development and validation as quickly as possible.

This newsletter aims to keep our CliniMACS community abreast of the latest developments in our technology, and to highlight new and exciting work by our collaborators.

In this first edition, we present a mixture of company and customer reports. The CliniMACS family of reagents is growing all the time, and here we update you on our latest technology for graft engineering and immunotherapy, as well as the new and exciting potential application of stem cells in cardiac repair. We are happy to have a customer report on the very topical subject of virus-specific T cells for adoptive transfer into allogeneic stem cell transplant recipients.

One of the best forums for us all to share new results is through our satellite symposia at major conferences. In this issue we summarize the latest presentations from ASH and EBMT – please use the fax-back forms in this newsletter to get full information on any of the presentations which are of particular interest to you. Further conferences this year where we can meet and discuss your requirements are also listed. We will also regularly keep you updated on technical issues, such as the frequently asked questions and procedures for ordering new reagents.

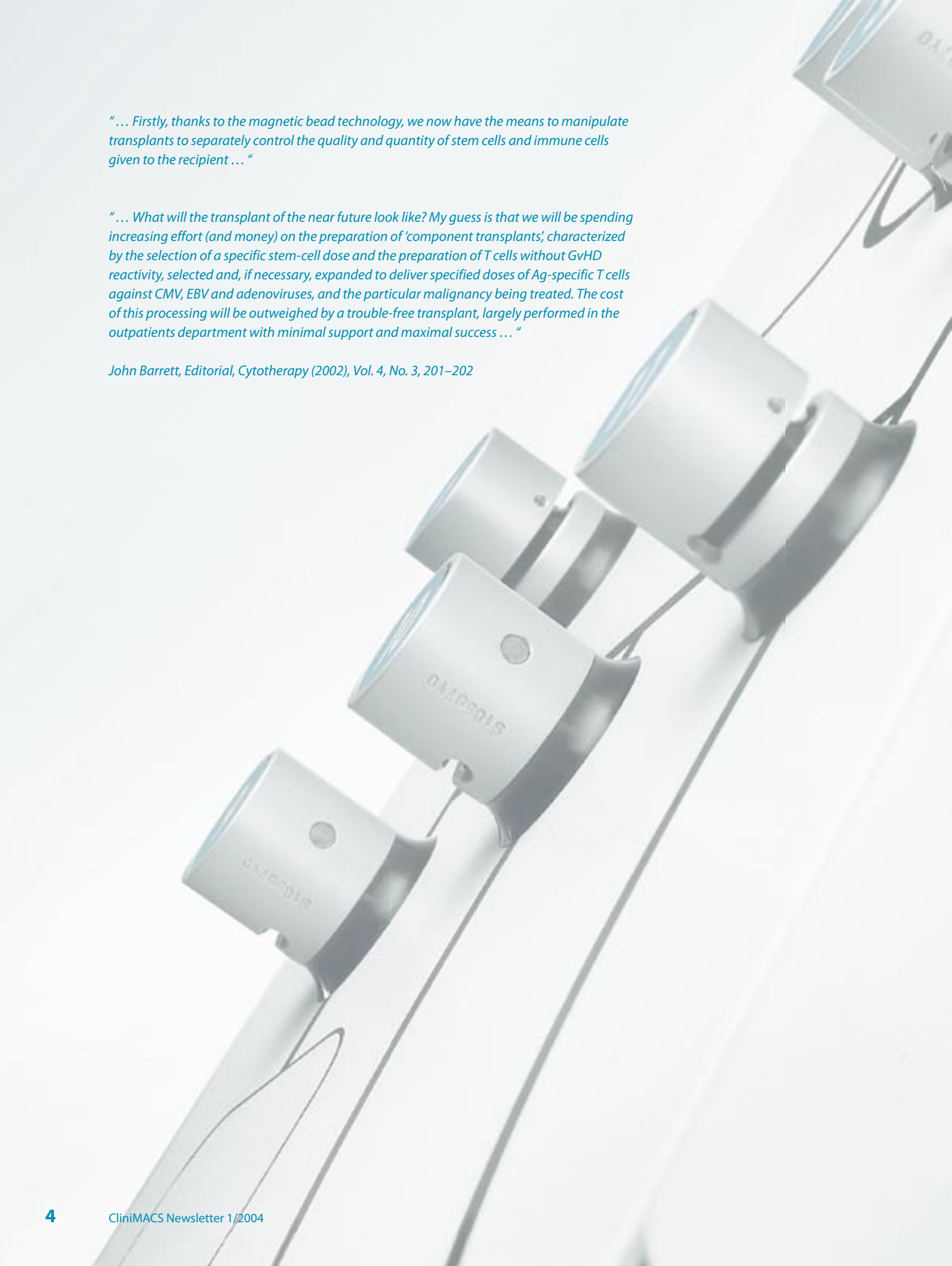
Finally, I would like to urge you to contact us if you feel that there is a current "hot topic" that should be discussed in this forum. Suggestions for features for future issues are always welcome. We hope that you enjoy this issue and look forward to our future work together.

Dr. John Campbell
Project Leader, R&D
Clinical Immunology Manager, UK

"… Firstly, thanks to the magnetic bead technology, we now have the means to manipulate transplants to separately control the quality and quantity of stem cells and immune cells given to the recipient … "

"… What will the transplant of the near future look like? My guess is that we will be spending increasing effort (and money) on the preparation of 'component transplants', characterized by the selection of a specific stem-cell dose and the preparation of T cells without GvHD reactivity, selected and, if necessary, expanded to deliver specified doses of Ag-specific T cells against CMV, EBV and adenoviruses, and the particular malignancy being treated. The cost of this processing will be outweighed by a trouble-free transplant, largely performed in the outpatients department with minimal support and maximal success … "

John Barrett, Editorial, Cytotherapy (2002), Vol. 4, No. 3, 201–202



Rapid generation of CMV-specific T cell lines for adoptive transfer into allogeneic stem cell transplant recipients

Georg Rauser, Hermann Einsele, Gabriele Kuntz, and Max S. Topp
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Introduction

Despite the introduction of new antiviral prevention and treatment strategies, patients undergoing allogeneic stem cell transplantation (SCT) are still at high risk for developing cytomegalovirus (CMV)-associated disease[1]. CMV-specific T cell immunity can be transferred to allogeneic SCT recipients by infusion of *ex vivo* generated donor-derived CMV-specific T cells[2].
Currently employed approaches to generate virus-specific T lymphocytes are time-consuming, require leukapheresis of donor, use replicative competent virus, or isolate either $CD4^+$ or $CD8^+$ virus-specific T cells[2–7]. By using a new isolation strategy we were able to generate sufficient numbers of functional $CD4^+$ and $CD8^+$ CMV-specific T cells for adoptive transfer from 8/8 CMV-seropositive donors from one single 500 mL blood donation in 10 days utilizing only autologous cellular and humoral components.

Material and methods

Isolation and expansion of CMV-specific T cells

PBMC were isolated by Ficoll/Paque density gradient centrifugation and incubated overnight with HLA-restricted CMV peptides (HLA-A*0201 and/or HLA-B*0702) and CMV lysate at 37 °C. Magnetic labeling of IFN-γ-secreting cells was performed similarly as described previously[8] using the IFN-γ secretion assay (Miltenyi Biotec). Magnetically labeled cells were selected with the fully automated CliniMACS device (Miltenyi Biotec) according to the manufacturer's instructions. Isolated cells were expanded for up to 11 days in presence of irradiated autologous PBMC in CTL medium (RPMI 1640 / 10% autologous heat-inactivated serum / Antibiotics) containing 50 IU rhIL-2/mL.

Tetramer staining

Frequency of peptide-specific $CD3^+CD8^+$ T cells was assessed by staining with PE-labeled tetrameric peptide complexes (Proimmune).

Intracellular IFN-γ staining

PBMC were stimulated for 6 hours with CMV peptide or CMV lysate, T cell lines were stimulated with autologous lysate-/peptide-loaded DC, both in the presence of the costimulatory mAbs CD28 and CD49d (Becton Dickinson). Brefeldin A (Sigma) was added for the last 5 h of the incubation.

CFSE staining

Cells were labeled with CFSE (Molecular Probes) and plated with mature, autologous DC preincubated with CMV lysate. 5 IU/mL rhIL-2 was added at day 1. After 7 days, cells were harvested, counted and stained with anti-CD8 PE and anti-CD4 PerCP (Becton Dickinson).

Cytotoxicity assay

Standard 4-hour ^{51}Cr release assay was performed with HLA-A*0201$^+$ CMV-infected and non-infected fibroblasts as target cells.

Mixed lymphocyte reaction

Purified $CD4^+$ T cells derived either from the starting fraction or after selection and expansion, were cultured with third party mature DC for 5 days. DNA synthesis was assayed by adding $[^3H]$thymidine (Amersham) during the last 18 hours of culture.

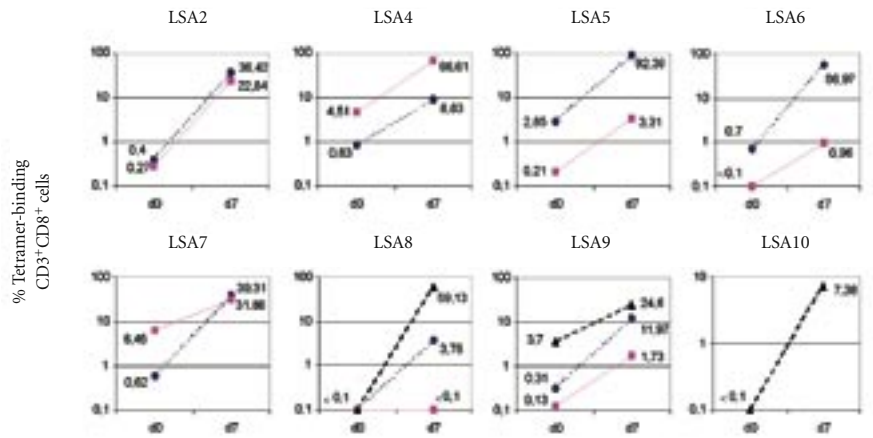

Figure 1: Enrichment of tetramer-specific $CD8^+$ T cells. The diagrams show the percentages of tetramer-binding $CD3^+CD8^+$ lymphocytes from all 8 donors before enrichment (d0) and at day 7 after enrichment and expansion (d7). Tetramers were complexed with the peptides NLVPMVATV (HLA-A*0201/pp65 (…●…)), VLEETSVML (HLA-A*0201/IE (_■_)) and TPRVTGGGAM (HLA-B*07/pp65 (--▲--)), respectively.

Results

Generation of combined CD4+ and CD8+ CMV-specific T cell lines

On average 3.0×10^6 (2×10^5–1×10^7, n=8) cells were isolated from 2×10^8–4×10^8 PBMC and could be expanded *ex vivo* after 7 days to 4.6×10^7 cells (7×10^6–2.4×10^8, n=8) and after 10/11 days to 4.6×10^8 cells (8.4×10^7–2.2×10^9, n=7).

Frequency of CMV-specific CD8+ T cells as determined by tetramer staining

Figure 1 shows the frequency of tetramer-binding $CD3^+CD8^+$ T cells from all 8 donors before enrichment (d0) and at day 7 post enrichment and expansion (d7). Remarkably, in 3 donors, tetramer-positive T cells could not be detected for some of the evaluated CMV epitopes (frequency less than 0.1% in the starting fraction) but could be readily visualized after enrichment and expansion of the T cells.

Functional enumeration of CMV-specific CD4+ and CD8+ T cells by intracellular IFN-γ staining

To determine the frequency and functionality of antigen-specific T cells, IFN-γ staining of PBMCs and enriched cells after 7 days of expansion was performed after activation with either CMV lysate or CMV/MHC-I peptides. Based on the percentage of IFN-γ-producing T cells and the cell counts the absolute numbers of CMV-specific T cells was calculated in the starting fraction and after isolation plus 10 day expansion. The absolute numbers of CMV-specific $CD4^+$ T cells increased on average from 7.3×10^5 to 9.0×10^7 (n=8) and the absolute numbers of CMV-specific $CD8^+$ T cells increased on average from 2.1×10^6 to 4.4×10^7 (n=8).

Generated CD4+ and CD8+ CMV-specific T cell lines appropriately divide after restimulation

Analysis of CFSE staining on day 7 demonstrated, as depicted for one representative donor in figure 2, proliferative capacity of the generated $CD4^+$ and $CD8^+$ T cells after restimulation with CMV antigen.

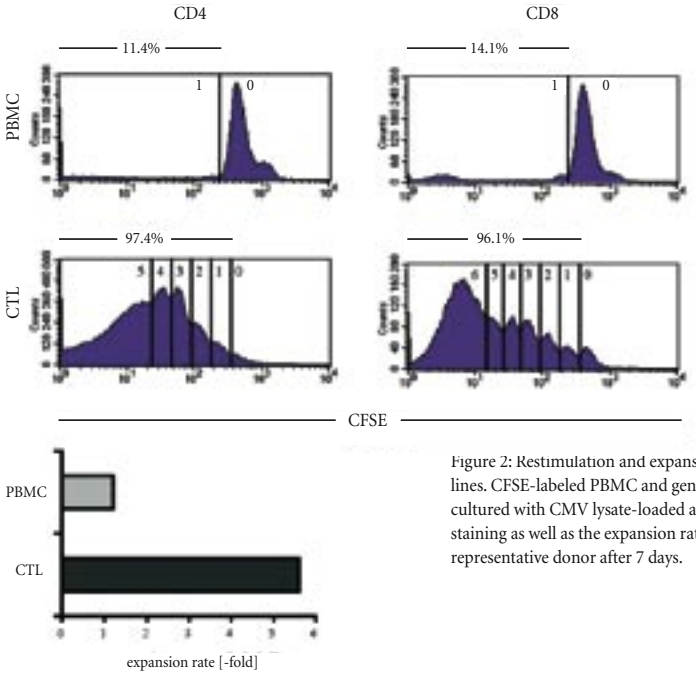

Figure 2: Restimulation and expansion of generated T cell lines. CFSE-labeled PBMC and generated T cells were cultured with CMV lysate-loaded autologous DC. The CFSE staining as well as the expansion rate is shown with one representative donor after 7 days.

Ex vivo generated CMV-specific T cell lines specifically lyse CMV-infected targets.

As shown in figure 3, CMV-infected fibroblasts were lysed specifically by 3/3 CMV-specific T cell lines derived from HLA-A^*0201^+ donors whereas the uninfected fibroblasts were not lysed.

Reduced alloreactivity of the generated CMV-specific T cells

In 3/3 donors $CD4^+$ T cells derived from the enriched and expanded cultures showed a reduction of more than 95% in alloreactivity when compared to the $CD4^+$ T cells selected from the starting cell fraction.

Conclusion

The developed culture system allows rapid generation of allo-depleted and highly enriched combined $CD4^+$ and $CD8^+$ CMV-specific T cells under good manufacturing practice conditions.

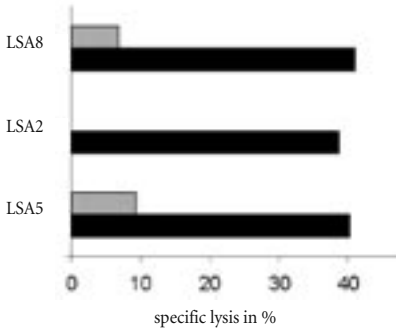

Figure 3: Efficient lysis of CMV-infected targets by generated CMV-specific T cell lines. Generated T cells lysed CMV-infected HLA-A^*0201 fibroblasts (black bars), whereas non-infected fibroblasts were not lysed (grey bars; n=3). The displayed data represent chromium release assays performed at an effector-to-target ratio of 30:1.

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CliniMACS® products for graft engineering

CD34 Selection:
the most efficient tool for passive T and B cell depletion

Since its introduction, CliniMACS® CD34 selection technology has continuously demonstrated its excellent performance, providing progenitor cells with high purity and yield in conjunction with an excellent passive depletion of T cells and non-CD34$^+$ cells of up to 4 to 5 log scales.

The concomitant T and B cell depletion using positive CD34 selection reduces the incidence of graft versus host disease (GVHD) and EBV-induced lymphoproliferative disease in allogeneic transplantation settings.

Recently, results from a notable number of studies on the use of CD34 selection in allogeneic transplantation of non-malignant diseases have been published. Gaipa *et al.*, reported on the feasibility of T cell-depleted bone marrow transplantation from unrelated donors in inherited metabolic storage diseases like globoid cell leukodistrophy and mucopolysaccharidosis[1]. All patients engrafted with no signs of GVHD. There are studies on transplantation of mega doses of CD34 cells in conjunction with a fixed dose of T cells without additional immune suppression in nonmalignant diseases[2,3]. Diseases comprised thalassemia, immunodeficiency and metabolic disorders as well as adrenoleucodystrophy and familial hemophagocytic syndrome. Neither GVHD nor severe infections occurred during and post transplantation. Outcome so far has been very favorable. Lang *et al.* reported on 25 pediatric patients with nonmalignant diseases (anemia, immunodeficiencies, inborn errors, and other) receiving CD34-selected grafts from matched related or unrelated as well as mismatched related donors[4]. Only one patient failed to engraft, acute GVHD was never higher than grade I and only two patients developed limited chronic GVHD. Similar results have recently been published for refractory severe aplastic anemia[5].

For malignant diseases, a new and effective BMT regimen for treatment of CML patients, combining low toxicity peripheral blood stem cell transplantation (PBSCT) with CD34 selection from HLA-identical donors and a fixed dose of T cells (10^5 T cells/kg) has been reported[6]. The rate of engraftment in the 31 patients was high (94%) and the incidence of post transplantation infections and GVHD was very low. All patients were in hematological remission after stem cell transplantation. In cases with persistence of or conversion to bcr/abl as a marker for molecular relapse (13 cases), donor lymphocyte infusions (DLI) were given at a median of 9 months post transplant with no significant morbidity or mortality. Nine patients converted to PCR negativity, for two patients it was too early to evaluate. With a median follow-up of almost two years the authors suggest that CML is potentially curable using T cell-depleted grafts without post-transplant GVHD prophylaxis and late DLI[6].

Megadoses of T cell-depleted stem cells also seem to be advantageous in promoting engraftment across HLA barriers in haploidentical transplantation. Aversa *et al.*, presented an update of the Perugia series of haploidentical transplants of acute leukemias[7]. Patient population included 55 AML and 31 ALL patients. Most patients were in relapse or bad risk CR. Primary engraftment was 92%, secondary engraftment 99%. Acute GVHD >II and chronic GVHD were very rare (2% and 5%, respectively). By omitting G-CSF post-transplant, immunological reconstitution improved substantially and infection-related deaths could be reduced by almost 50 %. The authors conclude that mismatched transplants should be offered to high-risk acute leukemia patients without HLA-identical donor as a viable option in the early stages of disease[7]. A similar study with CD34-selected stem cells in haploidentical stem cell transplantation of adults was undertaken by Bethge *et al.*, and comprised acute leukemias as well as several CML, NHL and MDS patients. Engraftment and full donor chimerism was achieved in all patients. Incidence of GVHD and treatment related mortality were low[8].

T cell depletion (TCD) also improved the outcome in pediatric matched unrelated donor bone marrow transplantation. Chamberlain and colleagues presented data on bone marrow transplantation of 68 children with leukemias, myelodisplasias, aplastic anemias and other diseases[9]. GVHD prophylaxis consisted of passive T cell depletion plus cyclosporin A in 46 cases and of CSA/Methotrexate only in 22 cases. There was significantly less grade II-IV acute GVHD and extensive cGVHD in the TCD group. The TCD regimen could reduce deaths associated with infection/ interstitial pneumonitis and improved event-free survival (EFS) as well as overall survival (OS). OS was 55% at a mean follow-up of 59 months in the TCD group, compared to 25% with a mean follow-up of 46 months ($p = 0.01$) in the unmanipulated group. Alike results were presented by Lang *et al.*. They compared 30 pediatric patients with high-risk acute leukemias transplanted with CD34- or CD133-selected stem cells from mismatched related donors (1–3 loci) without additional immune suppression. A group of 18 patients received unmanipulated grafts from matched unrelated donors[10]. The rate of sustained engraftment was slightly lower in the mismatched group. All patients with graft failure were rescued by reconditioning regimen. Another important finding was that profound T cell depletion by CD34 or CD133 selection did minimize GVHD but did not result in significantly

increased relapse rates. CD34$^+$ selection did not influence the risk of CMV infection after allogeneic stem cell transplantation[11]. Here, 51 adult patients received a CD34-selected graft with a fixed dose of 0.3×10^6/kg and were compared to 19 adult patients receiving unmanipulated grafts. Probability of CMV infection and CMV disease was comparable in both groups.

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To purge or not to purge?

Autologous transplantation is associated with the potential risk of reinfusing malignant cells with the graft. There is considerable debate on the value of purging, i.e. tumor cell removal in this setting. Whereas a randomized trial using CD34 selection in multiple myeloma did not show any improvement in disease-free or overall survival[1], other patients such as neuroblastoma or lymphoma patients seem to benefit from this approach[2,3]. In this regard, the results of a recently published comparison study sponsored by IBMTR/EBMT between syngeneic, allogeneic (T cell depleted or T cell replete) and autologous (purged and unpurged) stem cell transplantation for NHL are interesting. The authors suggested that syngeneic transplants are representative of uncontaminated autografts. By comparing syngeneic with purged or unpurged autologous grafts, theoretical evidence was presented regarding the effect that contaminating tumor cells may have on relapse and survival. Results of this phase III trial with data of 2483 autologous transplants showed that the approach of purging in low-grade NHL patients was associated with a significantly better disease-free survival and overall survival compared to unpurged autografting[4].

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CD133 – the key marker for transplantation of very early stem cells

A subset of CD34$^+$ cells in bone marrow, peripheral blood, and cord blood with primitive phenotypic characteristics express CD133. Antibodies specific for CD133 stain 35–75% of the CD34$^+$ population, depending on the source of stem cells[1]. Transplantation of an isolated CD133$^+$CD34$^-$ non-adherent stem cell fraction into immunodeficient NOD/SCID mice induced high myeloid and lymphoid multilineage engraftment, suggesting that these cells are highly enriched in SCID-repopulating cells[2]. It was also shown that only the CD133$^+$ subset of bone marrow was able to generate all categories of megakaryocyte colony forming units (CFU-Mk) *in vitro*, a subset relevant to platelet development[3,4]. This may explain the results of a recent study in which patients received additional CD133$^+$ stem cells from haploidentical donors and subsequently had a more rapid platelet recovery. The procedure effectively reduced the need for platelet transfusions compared to a historical group of patients transplanted with CD34$^+$ selected grafts alone[5]. Supporting this finding is a recently published case report of a Wiskott-Aldrich patient with sustained low platelet counts after haploidentical transplantation 10 years ago. Platelet counts normalized after receiving a stem cell boost, consisting of CD133$^+$ selected progenitors from the same haploidentical donor[6]. Rapid and stable multilineage engraftment using CD133$^+$ cells has also been demonstrated in the HLA-matched sibling setting[7].

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A new method for combined T and B cell depletion

Recently, a new method for graft engineering created high interest in the transplantation community: the immunomagnetic bulk depletion of T cells alone or together with B cells using CliniMACS CD3 and CD19 MicroBeads. A special depletion program on the CliniMACS^{plus} device generates the greatest degree of depletion of these cell types. The first results of T/B cell depletion from bone marrow were published at last year's ISCT meeting, showing a median of 3.5 log T cell depletion and 74 % CD34 and NK cell recovery[1]. At the most recent ASH meeting, data from St. Jude Children's Research Hospital (Memphis, TN) indicated that this method is feasible and safe for mobilized leukapheresis products as well. Results were comparable to those achieved from bone marrow with a mean T and B cell depletion of 3.4 and 2.2 logs, respectively. The mean recovery of CD3- and CD19-negative mononuclear cells and CD34$^+$ stem cells after depletion was 70%. *In vitro* colony assays and *in vivo* NOD/SCID repopulation assays showed no negative effect on the functionality of the hematopoietic stem cells[2]. These results were published in Cytotherapy[3]. Another abstract at ASH reported on initial clinical experience with CliniMACS CD3 depleted mobilized leukapheresis products in the pediatric setting[4]. The authors showed the feasibility and safety of this approach and concluded that haploidentical transplantation of T cell-depleted PBSC after reduced conditioning might be a therapeutic option for highly pretreated patients with advanced malignant disease.

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Potential of CD133$^+$ Hematopoietic Stem Cell in Cardiac Repair

Ischemic heart disease accounts for 50% of all cardiovascular-related deaths and is the leading cause of congestive heart failure. Medical therapy, cardiac assist devices and surgical procedures including heart transplantation have limited efficiency and availability. While current management of this condition is primarily palliative treatment, stem cell transplantation may represent a new therapeutic option for patients with cardiac diseases.

Recent studies have suggested that marrow and blood hematopoietic stem cells may contribute to nonhematopoietic tissue repair in multiple organ systems. In animal models and first clinical trials, stem cells have been used to treat patients with ischemic and refractory peripheral vascular or coronary artery disease[1–3].

CD133, a hematopoietic stem cell marker, describes a population with a fairly primitive phenotype. The potential to differentiate into cell types of nonhematopoietic origin clearly distinguishes CD133$^+$ stem cells from other stem cell markers such as CD117 or CD34[4, 5]. Recently, results from an athymic nude rat model were presented at the European Society of Cardiology (2003), demonstrating that CD133$^+$ stem cells play a potential role in therapeutic trials of neoangiogenesis in ischemic vascular disease. In addition to the development of endothelial and vascular smooth muscle cells, CD133$^+$ stem cells may also undergo transdifferentiation into cardiac myocytes[6].

Stamm and colleagues (2003) used autologous CD133-positive stem cells in a phase I study. Here, the cells were injected intramyocardially during cardiac bypass surgery. The procedure was proven to be safe without complications. Early results suggest that the injection of CD133-selected stem cells leads to superior left ventricular function and improvement of the infarct tissue perfusion[7]. Results from the completed phase I study with 15 patients were presented at ASH (2003). The investigators have recently initiated a prospective, randomized, controlled phase II trial to evaluate functional cardiac parameters in patients undergoing bypass surgery plus CD133$^+$ stem cell injection versus bypass surgery alone. Initial results from this trial support the observations from the phase I trial.

In another cardiology surgical trial, G-CSF-mobilized peripheral blood CD133$^+$ stem cells have been used to treat patients not amenable to conventional revascularization procedures. These patients have been treated without any adverse events attributable to the mobilization regimen or the CD133$^+$ stem cells[8].

CD133$^+$ stem cells could become one of the most exciting advanvcements in the treatment of cardiovascular dieseases, such as myocardial infarction and heart failure in the future.

Miltenyi Biotec's experiences in magnetic purification of various cell types can be easily integrated into these new and innovative applications. The selection of CD133$^+$ stem cells using the CliniMACS® ^{plus} device leads to a defined stem cell population with high purity.

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T cells in cellular therapy

T cells play critical roles in the regulation of immune responses, and are responsible for mediating both beneficial and pathogenic immune responses. The explosion in activity in the field of immunotherapy, together with the increased funding for respective clinical trials, has led to strengthened interest in methods that accurately assess T cell function and allow for engineering and the effective use of T cells.

Allogeneic hematopoietic stem cell transplantation (HSCT) is an important therapeutic option for a number of malignant and nonmalignant disorders. Allogeneic transplant is gaining more and more popularity, as graft engineering is enabling a strong graft-versus-leukemia (GVL) effect by employing alloimmune effector lymphocytes to eliminate tumor cells. When leukemia relapses after allogeneic SCT, donor leukocyte infusions (DLI) can induce sustained remissions in patients. Based on the first encouraging results of DLI in chronic myeloid leukemia (CML), a number of centers have implemented studies of DLI in conjunction with allogeneic SCT for a variety of hematological malignancies. The reduction in relapse attributed to donor T cells according to the EBMT-95 survey is greatest for CML (durable remission in 50–88%), high in multiple myeloma (MMY) with 29% of patients responding to the therapy, intermediate for acute myeloid leukemia (AML) (response rates of about 20%) and lowest for acute lymphoblastic leukemia (ALL) (response rates ≤15%). On the other hand the complication of graft versus host disease (GVHD) is a common occurrence after allogeneic transplantations. Recently, the use of non-myeloablative regimen protocols, also known as reduced intensity conditioning (RIC), is increasing. RIC is being used both with and without subsequent DLI. RIC protocols have allowed for the use of transplants in elderly, or medically infirm patients with increased safety. Further, the use of RIC may also permit innovative approaches to SCT, including tandem autologous transplantation.

Both graft-versus-leukemia (GVL) and graft-versus-host responses are in large part conveyed by donor T lymphocytes contained in the graft as part of the unfolding adaptive immune response. New immunotherapy strategies have evolved, designed to boost GVL and reduce GVHD. The GVL effect is thought to be the main reason that allogeneic HSCT for hematological malignancies results in lower relapse rates than autologous SCT, given identical conditioning regimen.

Efforts are currently underway so that in the future, GVL can be separated from GVHD. Through transfer of T cell-depleted DLI, or selected T cells that recognize leukemia-specific antigens or minor histocompatibility antigens (mHA), the benefits of GVL may be maintained without inducing GVHD.

Another T cell subset, the regulatory T cells, is of high interest. Regulatory T cells can help modulate immune reactions by e.g. augmenting tumor-specific reactions of $CD8^+$ T cells otherwise suppressed by escape mechanisms of the tumor. Moreover, they might have the capacity to suppress auto-reactive T cells in autoimmune diseases.

Miltenyi Biotec has broadened its product portfolio for T cell products.

The CliniMACS® platform, together with the new T cell products, allows positive selection and depletion of different T cell subsets thus providing the basis for a variety of different applications.

CD3

The CD3/T cell receptor complex is expressed during thymopoiesis and on mature T cells in the periphery. CliniMACS CD3 MicroBeads are suitable for bulk depletion of T cells from unmobilized as well as mobilized leukapheresis products. CliniMACS CD3 MicroBeads can also be combined with CliniMACS CD19 MicroBeads to simultaneously deplete T and B cells from hematopoietic stem cell sources. The rationale for this application in a clinical setting is the depletion of T cells to prevent GVHD[1,2]. B cells would be depleted from the graft to minimize the risk of developing EBV-induced lymphoproliferative diseases (PTLD)[3,4]. This approach keeps stem and progenitor cells untouched and more importantly, leaves immune effector cells like NK cells in the graft.

CD3 MicroBeads are also an important tool for the enrichment of NK cells. Preceding CD56 selection, CD3 MicroBeads can be employed for depleting the $CD3^+CD56^+$ NKT cell fraction, enabling subsequent enrichment of $CD3^-CD56^+$ NK cells.

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4. Meijer, E. *et al.* (2002) Increased incidence of EBV-associated lymphoproliferative disorders after allogeneic stem cell transplantation from matched unrelated donors due to a change of T cell depletion technique. Bone Marrow Transplant. 29: 335–339.

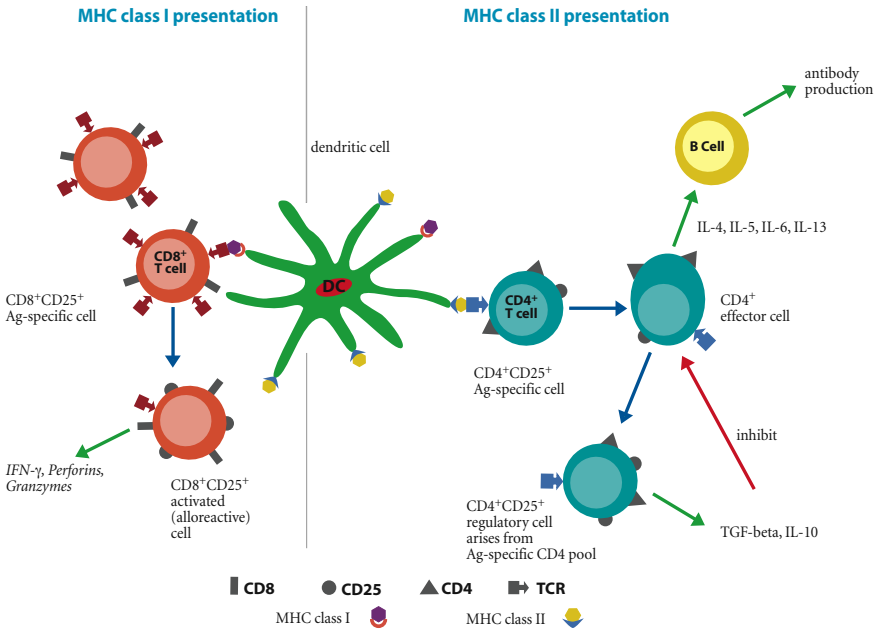
CD8

CD8, the co-receptor for MHC class I molecules, is expressed strongly on cytotoxic T cells and dimly on a subset of NK cells. CliniMACS® CD8 MicroBeads allow positive or negative selection of CD8 T cells.

CD8 T cell depletion is increasingly performed in the allogeneic transplantation setting where $CD8^+$ T cells are removed from a graft or donor lymphocyte infusion for the purpose of preventing GVHD[1]. $CD8^+$ cells can be depleted from DLI without affecting the GVL effect, while concurrently diminishing the GVHD. This is true for standard high-dose myeloablative regimen and also in the rapidly growing field of reduced intensity conditioning (RIC).

Also in some autologous settings CD8 depletion may be advantageous, e.g. T cell-induced autoimmune diseases.

Enrichment of $CD8^+$ T cells can be performed if the generation of CD8 T cell lines for antigen-specific treatment is desired[3,4].



Selective depletion of CD8+ T cells leaves all other cell types intact. Therefore, CliniMACS CD8 MicroBeads can also be used for the depletion of CD8+ T cells in settings in which CD4+ T cells are the targets. In the HIV setting, gene therapeutic protocols are under investigation in which CD4+ T cells shall be genetically modified. The depletion of CD8+ T cells hopefully promotes increased transduction efficiencies of CD4+ T cells[2]. CD8 depletion can also be a component in enrichment strategies for regulatory T cells where the depletion of unwanted cell types (CD8 cells expressing CD25) precedes CD25 enrichment.

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CD25

CD25 is the low-affinity interleukin-2 receptor alpha chain (IL-2Rα) and is expressed on activated T cells, B cells, monocytes, and on regulatory T cells.

CliniMACS® CD25 MicroBeads are suitable for positive selection or depletion of activated T cells. In conjunction with e.g. CliniMACS CD8 or CliniMACS CD19, the product is being used in different protocols for the enrichment of regulatory T cells.

CD25 T cell depletion is increasingly performed in the allogeneic transplantation setting where alloreactive CD25+ T cells are removed from a graft or DLI for the purpose of preventing GVHD[1]. Those approaches are performed, depending on protocol, with CD25 as sole reagent or in combination with other markers targeting activated T cells like CD69[2].

Also the use of CD25 IT (Immunotoxins) to deplete alloreactive T cells has been described[2]. Improving immunity after haplo-identical allo-SCT with allodepleted donor T cells also is a promising application.

Several groups perform enrichment of CD25+ T cells for the generation of antigen-specific cell lines starting from *in vitro* cultures after stimulation with a specific antigen.

CD25 also is the key antigen on a subset of CD4+ T cells, the regulatory T cells, which are in the center of interest right now. Regulatory T cells are important in the induction and maintenance of self-tolerance. Depletion of regulatory T cells can help augment anti-tumor reactions by CTLs. The cells also play a crucial role in decreasing autoimmune responses[3]. Recent description and improved understanding of regulatory T cells has opened up the possibility of modulating GVHD alloresponses through the addition of regulatory T cells.

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Immune reconstitution without graft-versus-host disease after haemopoietic stem cell transplantation: a phase 1/2 study.
Lancet 360: 130–137.
The authors present the results of a study designed at eliminating GVHD in the haploidentical setting by use of allodepleted T cells between days 15 and 47 in 15 pediatric patients who received HSCT on day 0. By use of an anti CD25-Immunotoxin, residual anti-host alloreactivity was less than 1%. The findings showed that *ex vivo* selective depletion of alloreactive T cells is efficient and feasible. To reliably erase alloreactivity the authors state that they will establish T cell depletion based on anti-CD25 and magnetic beads.

Edinger, M., Hoffmann, P., Ermann, J., Drago, K., Fathman, C.G., Strober, S., Negrin, R.S. (2003)
$CD4^+CD25^+$ regulatory T cells preserve graft-versus-tumor activity while inhibiting graft-versus-host disease after bone marrow transplantation.
Nat. Med. 9(9): 1144–1150.
The work by Edinger and colleagues showed that in the mouse model $CD4^+CD25^+$ T cells are potent regulatory cells that can separate GVHD from GVT activity mediated by conventional donor T cells. In mice suffering from leukemia or lymphoma, $CD4^+CD25^+$ regulatory T cells suppressed the early expansion of alloreactive donor T cells, their interleukin-2-receptor (IL-2R) α-chain expression, and their capacity to induce GVHD without abrogating their GVT effector function. These results hold a great promise for successful transplantations from mismatched donors, thereby extending the applicability of BMT.

Antigen-specific/IFN-γ-secreting cells

IFN-γ is predominantly secreted by activated $CD4^+$ and $CD8^+$ T cells, but also by activated NK cells.

The CliniMACS® Cytokine Capture System (IFN-gamma) allows for the simultaneous isolation of viable Ag-specific $CD4^+$ and $CD8^+$ cells for immunotherapy, which may be responsible for better establishment of immune responses than infusion of $CD8^+$ cells alone[1]. The application of $CD4^+$ T cells together with $CD8^+$ T cells is crucial for sustained antigen-specific immune reaction.

Antigen-specific T cells have become a new focus in T cell research and opportunities for utilizing such cells for clinical applications are currently being exploited. Highest interest is in the area of virology, i.e. enrichment of CMV-, EBV-, or Adenovirus-specific T cells[2,3]. Enrichment of functional antigen-specific T cells could be very useful for treatment and/or prevention of infections after allogeneic HSCT, RIC SCT, and also after solid organ transplantation. The adoptive transfer of donor T cells specific for antigens expressed by cytomegalovirus (CMV) or Epstein Barr virus (EBV) has been shown to restore CMV- and EBV-specific immunity after allogeneic HSCT without causing GVHD.

Also for oncological applications the assay is of high value. In contrast to the virology setting the main challenge in the oncological setting is the identification of the most immunogenic antigen. Many studies are under way addressing the question of the most suitable antigens for a given malignant disease.

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Detection and enrichment of antigen-specific $CD4^+$ and $CD8^+$ T cells based on cytokine secretion.
Methods. 31(2): 150–159.
This publication is an excellent work of reference for any user who aims at learning more about the principle and the detailed use of the CliniMACS Cytokine Capture System.

Einsele, H. (2003)
Antigen-specific T cells for the treatment of infections after transplantation.
Hematol. J. 4(1):10–17.
This review gives a very interesting and broad overview on the current technologies in cellular immunotherapy for the treatment of infections in immunocompromised hosts. Insights derived from animal model and human studies increased the interest in the use of specific T cells as adoptive immunotherapy for infections and malignant diseases. The review covers the use of alpha/beta$^+$ T cells to restore responses considered essential for protective immunity to cytomegalovirus and Epstein-Barr virus, the role of adoptive immunotherapy in the prevention and treatment of adenovirus and invasive fungal infection, and also sheds light on the use of genetically modified T cells.

Rauser, G., Einsele, H., Sinzger, C., Wernet, D., Kuntz, G., Assenmacher, M., Campbell, J.D.M., Topp, M.S. (2003)
Rapid generation of combined CMV-specific $CD4^+$ and $CD8^+$ T cell lines for adoptive transfer into allogeneic stem cell transplant recipients.
Blood, Dec 11 [Epub ahead of print]
The very interesting publication by Dr. Rauser and colleagues describes a culture system allowing for rapid generation of allo-depleted and highly enriched combined $CD4^+$ and $CD8^+$ CMV-specific T cells under good manufacturing practice conditions by use of the interferon-γ secretion assay.
Also see the customer report by Georg Rauser *et al.* on p. 5.

Feuchtinger, T., Lang, P., Hamprecht, K., Schumm, M., Greil, J., Jahn, G., Niethammer, D., Einsele, H. (2004)
Isolation and expansion of human adenovirus-specific $CD4^+$ and $CD8^+$ T cells according to IFN-γ secretion for adjuvant immunotherapy.
Experimental Hematology: 282–289.
The aim of the study was to isolate and expand donor-derived human adenovirus (ADV)-specific T lymphocytes for adoptive transfer of sufficient cell numbers to restore protective immunity after allogeneic stem cell transplantation. For this purpose the generation of ADV-specific T cells in a simple and rapid clinical-grade protocol was established, using the IFN-γ secretion assay with short expansion times. IL-2 and autologous feeder cell stimulation led to sufficient numbers of ADV-specific $CD4^+$ and $CD8^+$ T cells.

CD3/CD56 selection for natural killer cell products

Cancer cells and virus-infected cells are among the targets of natural killer (NK) cells[1,2]. Once NK cells are activated by IL-2 they are able to effectively lyse cell lines that previously were insensitive to NK-mediated lysis. Other findings point to antigen-specific T cells besides NK cells as an important part of tumor immunity[3,4,5].

Escudier and colleagues[6] infused lymphokine-activated NK cells into ten pre-selected patients with metastatic renal cell carcinoma and achieved four complete remissions.

Killer cell Immunoglobulin-like Receptors (KIR) on NK cells are involved in recognition of MHC class I molecules on target cells. More recently, some retrospective analyses of KIR-Ligand repertoire mismatches in allogeneic stem cell transplantation demonstrated a significantly reduced probability of relapse and a significantly improved event-free survival for AML patients in the presence of "alloreactive" NK cells.[7,8]

Over the last years investigators have selected haploidentical NK cells for infusion into patients with myeloid malignancies in order to rescue a decreasing chimerism or a relapse arising after reaching minimal residual disease (MRD). As reported by Passweg *et al.* (EBMT 2003) the NK cells were well tolerated with no side effects reported. Uharek *et al.* described some cases of mild GVHD grade I/II in his study (DGHO 2003). In the latter study, cells were infused after IL-2 activation *in vitro*. The findings led to the conclusion that NK cell infusion is feasible and safe for hematological malignancies investigated. Both groups used the CliniMACS® ^{plus} Instrument and a two-step selection procedure for isolation of NK cells starting from leukapheresis products. The first step consisted of a depletion of $CD3^+$ cells followed by enrichment of $CD56^+$ cells. The final product selected from non-mobilized donors displayed a purity of more than 90% NK cells and a 4.5 to 5 log T cell depletion.

CD56

In the hematopoietic system CD56 is restricted to NK cells, a subpopulation of T cells (referred to as NKT cells) and a small subpopulation of monocytes. NKT cells mediate non-MHC-restricted cytotoxicity and display regulatory functions in autoimmune disease. The role of the monocyte subpopulation is currently unknown.

NK cells can easily be selected by use of the CliniMACS® ^{plus} Instrument. The combined use of CliniMACS CD3 and CliniMACS CD56 MicroBeads leaves the investigator with a highly enriched population of $CD56^+CD3^-$ NK cells.

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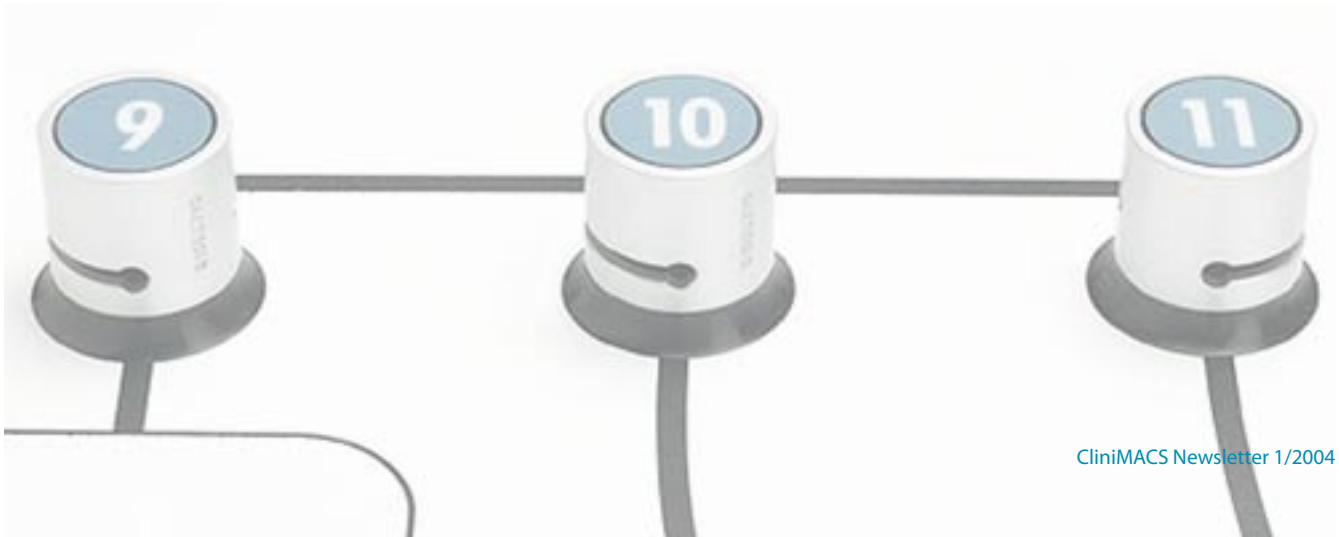
What is a natural killer cell?
Nat. Immunol. 3(1): 6–8.
Short summary of main features of NK cells including recent advances in this field. This review points a way from basic research to clinical application.

Leung, W., Iyengar, R., Turner, V., Lang, P., Bader, P., Conn, P., Niethammer, D., Handgretinger R. (2004)
Determinants of antileukemia effects of allogeneic NK cells.
J. Immunol. 172(1): 644–650.
The paper discusses the question whether KIR and HLA molecules are intra-individual mirror images. This paper promotes antibody based KIR-Typing for clinical application in hematopoietic stem cell transplantation.

Iyengar, R., Handgretinger, R., Babarin-Dorner, A., Leimig, T., Otto, M., Geiger, T., Holladay, M., Houston, J., Leung, W. (2003)
Purification of human natural killer cells using a clinical-scale immunomagnetic method.
Cytotherapy (2003) 5(6): 479–784.
This paper gives a detailed description of the two-step selection method for enrichment of $CD3^-$ $CD56^+$ natural killer cells using the CliniMACS® ^{plus} Instrument.



Novel strategies for isolation of dendritic cell subsets

Dendritic cells (DC) are considered the most potent antigen-presenting cells. These cells have the unique ability to generate primary immune responses to antigens and play a key function in regulation of immune responses and maintenance of immunological memory.

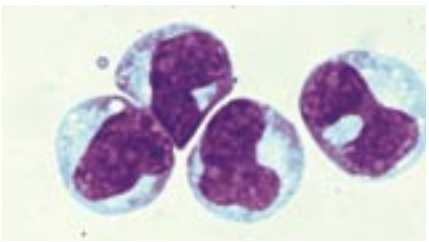


Figure 1: Plasmacytoid blood dendritic cells isolated by MACS Technology and May-Grünwald/Giemsa-stained

These features make DC a very attractive tool for immunotherapeutic approaches in malignant diseases, e.g. melanoma, renal cell carcinoma, and prostate cancer.

In the past, three main sources for the generation of DC have been used in clinical trials: DC derived from $CD14^+$ monocytes (MoDC), $CD34^+$ progenitor cells, and peripheral blood DC precursors. However, DC generated primarily from $CD14^+$ monocytes have been used in vaccine-based clinical studies[1].

Clinical scale separation of $CD14^+$ monocytes is routinely performed using the CliniMACS® system resulting in highly pure cells for subsequent *in vitro* generation of monocyte-derived DC[2]. Another approach is the isolation of $CD34^+$ expressing cells as progenitors for the subsequent generation of DC. The isolation of immature DC from peripheral blood is performed by depletion of unwanted cells such as monocytes, B cells and T cells. To overcome the low frequency of the DC precursors (<1%) in peripheral blood, Flt3 ligand stimulation was commonly used *in vivo*, resulting in expanded numbers of DC.

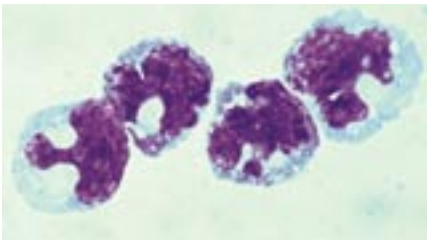


Figure 2: $CD1c^+$ blood dendritic cells, May-Grünwald Giemsa-stained.

Recently, Miltenyi Biotec extended their clinical portfolio to offer antibodies against blood dendritic cell antigens (BDCA). CD1c (anti-BDCA-1) recognizes myeloid dendritic cells (MDC1) and anti-BDCA-4 plasmacytoid DC (PDC)[3]. These DC subpopulations can be enriched from leukapheresis products without Flt3 ligand treatment prior to separation. These unique reagents allow evaluation of MDC1 and PDC cells in clinical trial applications for different diseases.

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Plasmacytoid dendritic cells, antigen and CpG-C license human B cells for plasma cell differentiation and immunoglobulin production in the absence of T cell help.
Blood. Dec 30 [Epub ahead of print]
The authors report on a T cell independent pathway of plasma cell development that involves costimulation of B cells with plasmacytoid dendritic cells, a subset of dendritic cells specialized on antiviral immune response.

Jefford, M., Schnurr, M., Toy, T., Masterman, K.A., Shin, A., Beecroft, T., Tai, T.Y., Shortman, K., Shackleton, M., Davis, I.D., Parente, P., Luft, T., Chen, W., Cebon, J., Maraskovsky, E. (2003)
Functional comparison of DC generated *in vivo* with Flt3 ligand or in vitro from blood monocytes: differential regulation of function by specific classes of physiologic stimuli.
Blood 102(5): 1753–1763.
This work describes the functional analysis of FL (Flt3 ligand)-expanded $CD1c^+$ peripheral blood DCs (PBDC) isolated from patients with malignant melanoma compared to autologous MoDC. The group found major differences between the responses of MoDC and $CD1c^+$ PBDC toward three different classes of physiologic stimuli with respect to migratory function, cytokine production, and regulation of T cell function.

Minutes from the Miltenyi Biotec Satellite Symposium ASH 2003

Cellular therapy – from graft engineering to immunotherapy

The 45th Annual Meeting of the American Society of Hematology, which took place from December 4 to 9, 2003, in San Diego, CA, also hosted the Satellite Symposium "Cellular Therapy – From Graft Engineering to Immunotherapy" organized by Miltenyi Biotec.

Miltenyi Biotec was honored that Prof. Robert Negrin, Professor at Stanford University School of Medicine, agreed to chair the session. Seven speakers presented a diverse and interesting program showing new trends in cellular transplantation made possible through cellular therapy and graft engineering. Current applications of cellular therapy and the clinical potential of T, NK, dendritic and stem cell populations to treat such diverse clinical problems as cancer relapse, viral infections, graft rejection, graft versus host disease and organ damage were addressed in the presentations, both from theoretical and practical viewpoints. The almost 600 attendees helped to create a forum in which fruitful discussions developed.

We have prepared a booklet containing the summaries of the presentations for you, which we will be happy to send out upon request. You will find the fax-back form on the inside of the back cover.

Roberto M. Lemoli, Institute of Hematology and Medical Oncology, University of Bologna, Italy

Positive selection of CD14$^+$ monocytes as a platform for anti-idiotype vaccination for myeloma and lymphoma patients

Previously, Dr. Lemoli and colleagues proved that positively selected peripheral blood CD14$^+$ monocytes from multiple myeloma (MM) patients can be induced to differentiate into fully functional, mature, CD83$^+$ dendritic cells (DC) for subsequent vaccination against lymphoma or myeloma cells. During this symposium, the first clinical results in MM patients vaccinated with idiotype-pulsed dendritic cells were presented. In summary, positive selection of CD14$^+$ monocytes using the CliniMACS® ^{plus} Instrument generates mature and functional DC suitable for clinical trials. In addition, cryopreservation does not affect the phenotype and function of pre-loaded DC. Intravenous and subcutaneous injection of cryopreserved DC pulsed with tumor idiotypes was shown to be a safe procedure and can induce T cell tumor-specific responses.

Hermann Einsele, Department of Internal Medicine II, University of Tübingen, Germany

Adoptive immunotherapy of immunotherapy of CMV and EBV infections

In his presentation, Dr. Einsele gave an informative insight into the recently focused interest in the use of antigen-specific T cells as adoptive immunotherapy for infections and malignant diseases. Until now, strategies for adoptive T cell immunotherapy for transplant recipients were limited to the use of virus-specific $\alpha\beta^+$ T cells. Recently, new techniques such as the use of peptide and protein pulsed dendritic cells and the Tetramer technology, as well as the Cytokine Capture System have been developed. CMV-specific cells can be highly enriched and expanded *in vitro*. Transfer of these cells into patients after allogeneic transplantation has been shown to be safe, while the enriched CD4$^+$ T cells transferred with CD8$^+$ T cells support the persistence of the anti-viral reaction. Transfer of CMV-specific CD4$^+$ T cells was shown to induce CMV-specific CD8$^+$ T cell responses and reduction of CMV DNA load. The enrichment of polyclonal CD4$^+$ and CD8$^+$ T cell lines for EBV reactivity has been used for the treatment and prevention of EBV-LPD after T cell-depleted HCT. The clinical potential of antigen-specific T cells for CMV and EBV in the use for treatment of viral infections after allogeneic stem cell transplantation is very promising.

Jakob R. Passweg, Bone Marrow Transplant and Leukemia Unit, Department of Hematology/Oncology, Kantonsspital Basel, Switzerland

Large-scale purification of NK cells from healthy donors. Benefit of KIR-mismatched NK cells in allogeneic transplantations to induce full chimerism and to prevent relapse – early clinical experience

Donor lymphocyte infusions (DLI) are used to treat relapse or consolidate donor chimerism in HLA-compatible hematopoietic stem cell transplantation. DLI is not recommended in the haploidentical transplantation setting, due to risk of graft-versus-host disease. The presented pilot study shows that the selection of T cell depleted NK donor cells is technically feasible, and that large numbers of purified NK cells may be obtained. Furthermore, it was shown that NK donor lymphocyte infusions from haploidentical donors were well tolerated, and may revert impending rejection.

Martin Bornhäuser, Department of Hematology and Oncology, University Hospital Dresden, Germany

Engraftment kinetics of CD133-selected allogeneic hematopoietic progenitor cells

Ongoing studies have demonstrated that cells expressing the stem cell marker CD133 may have long-term repopulating capacity. Dr. Bornhäuser reported on their first clinical experience using CD133-selected stem cells in the allogeneic HLA matched stem cell transplantation. The presented phase I study provided evidence that allogeneic CD133 cells from G-CSF stimulated peripheral blood of healthy donors, enriched with the CliniMACS system, can be used as an alternative source of hematopoietic cells. Rapid and durable engraftment was seen in recipients with hematological malignancies. Significant numbers of myeloid and plasmacytoid blood dendritic cells of donor origin could be detected as early as 28 days after transplantation. The low number of T cells infused with the graft corresponded with a very low incidence of acute GVHD.

Rupert Handgretinger, Division of Stem Cell Transplantation, St. Jude Children's Research Hospital, Memphis, TN, USA

The role of graft engineering in stem cell transplantation

Dr. Handgretinger discussed recent advances in graft engineering technologies allowing the depletion of T and B lymphocytes from peripheral blood stem cells. In contrast to $CD34^+$ enrichment, which passively depletes all other cell populations, CD3 depletion removes unwanted T cells, leaving stem cells, CD34-ngative cells such as natural killer cells, monocytes, dendritic cells or $CD34^-$ stem cells in the graft. In this presentation, the first data of a randomized clinical study in children with malignant diseases comparing $CD34^+$ positive selection and CD3 depletion of mobilized peripheral stem cell grafts obtained from haploidentical donors were shown. The potential future benefit of CD3 depletion versus CD34 enrichment in different allogeneic transplantation settings, especially with regard to alloreactive natural killer cells, was debated.

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

CD8-depleted stem cell transplantation in the HLA identical setting

The pros and cons of removing T lymphocytes from donor grafts, as well as on the most appropriate way of tailoring DLI, is still ongoing. Assuring graft versus leukemia (GVL) effect, while concurrently preventing graft versus host disease, graft failure, immune deficiency and disease relapse are the cornerstones of cellular therapy in the allogeneic setting. A randomized trial with patients receiving either CD8-depleted or unmanipulated bone marrow suggested that CD8-depletion reduced the incidence of GVHD without jeopardizing the important GVL effect. First data from patients receiving CD8- depleted donor lymphocyte infusions showed a significant reduction in the incidence of GVHD as compared to patients receiving a replete DLI containing similar numbers of $CD4^+$ cells. The relapse rate and conversion to full donor chimerism was no different when comparing the two patient populations. Dr. Soiffer and colleagues concluded that CD8 depletion may be an effective way to prevent GVHD following PBSC transplantation in the HLA matched related donor graft setting.

Christof Stamm, Department of Cardiac Surgery, University of Rostock, Germany

Cardiac Regeneration after intracardiac bone marrow stem cell transplantation

One of the most promising areas of medicine today is the use of stem cells in the regeneration of functionally impaired, infarcted myocardium. Several experimental studies are ongoing to demonstrate the safety of $CD133^+$ stem cell injection directly into heart or other vascular tissue. Dr. Stamm presented results of a trial, which investigated the injection of CD133-selected stem cells into the heart muscle during coronary artery bypass surgery (CABG). $CD133^+$ stem cells are characterized as multipotent stem cells with a high degree of plasticity. Results of the phase I trial suggest that injection of autologous bone marrow-derived $CD133^+$ stem cells for myocardial regeneration is feasible and safe. The results from the first cohort of patients in a phase II trial show evidence that CABG and stem cell injection may result in better post-operational left ventricular function than CABG alone.

Minutes from the Miltenyi Biotec Satellite Symposium EBMT 2004

Novel strategies in graft engineering and cellular therapy

This year's Satellite Symposium at EBMT, organized by Miltenyi Biotec, stimulated lively discussions on novel therapy options for the treatment of autoimmune, viral, cardiovascular, and malignant diseases. Prof. Alvaro Urbano-Ispizua, Head of Research Unit of Cell Therapy and Stem Cell Transplantation, Department of Hematology, Barcelona, chaired the very enlightening symposium, which took place on March 28 at Barcelona, Spain. Investigators reported on immunotherapeutic strategies using natural killer cells and antigen-specific T cells. Graft engineering in haploidentical allogeneic stem cell transplantation was discussed with regard to feasibility, clinical use and benefit. Allogeneic HSCT approaches in autoimmune diseases and the benefit of multipotent stem cells to regenerate damaged heart tissue were also addressed.

We have prepared a booklet containing the summaries of the presentations for you, which we will be happy to send out upon request. You will find the fax-back form on the inside of the back cover.

Max S. Topp, Department of Hematology and Oncology, University of Tübingen, Germany

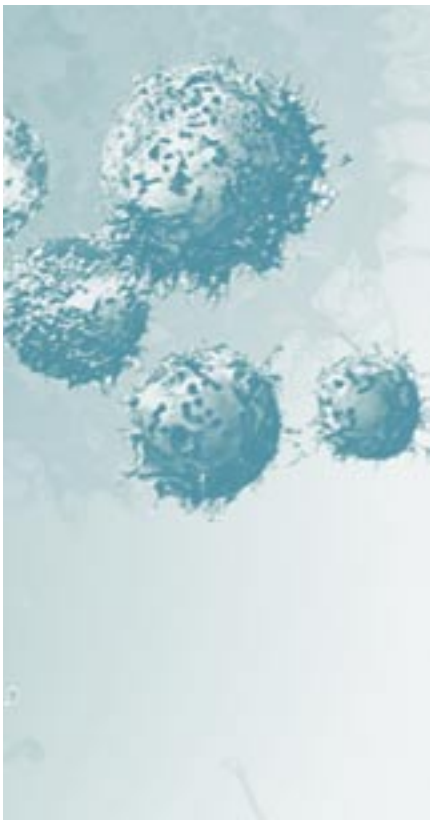
Adoptive immunotherapy of CMV and EBV infections

The first evaluation of adoptive immunotherapy by Riddell and colleagues in humans with virus-specific T cells was performed in allogeneic hematopoietic cell transplantation (HCT) and focused on safety and immunomodulatory properties of administering $CD8^+$ CMV-specific cytotoxic T cell clones. In a separate study by Einsele and colleagues, CMV-specific $CD4^+$ T cells, generated by repetitive stimulation with CMV-Ag loaded monocytes, resulted in a clearance of established CMV-viremia. Dr. Topp discussed the clinical potential of antigen-specific cytotoxic T lymphocytes generated by use of techniques such as pulsed dendritic cells, Tetramer technology, and Cytokine Secretion Assay for treatment of viral infections after allogeneic stem cell transplantation.

Jakob R. Passweg, Bone Marrow Transplant and Leukemia Unit, Kantonsspital Basel, Switzerland

Purified donor NK lymphocyte infusion to consolidate engraftment after haploidentical stem cell transplantation

Dr. Passweg presented encouraging results of a pilot study initiated to test feasibility of natural killer (NK) cell lymphocyte infusion (NK-DLI) in patients with incomplete engraftment or minimal residual disease after haploidentical hematopoietic stem cell transplantation (HSCT). Five patients with high-risk myeloid malignancies were included. A two-step *ex vivo* procedure was employed to immunomagnetically purify NK cells; in a first step T cells were depleted, followed by NK cell enrichment. Every patient received $>10^7$ NK cells/kg body weight and $<10^5$ T cells/kg body weight. Infusions were well tolerated, no graft versus host disease developed. Benefit of KIR-mismatched NK cells in allogeneic transplantations to induce full donor chimerism and to prevent relapse were discussed.



Johann Greil, Department of Pediatrics, University of Tübingen, Germany

Allogeneic transplantation of engineered selected peripheral blood stem cells from haploidentical donors

Preclinical studies suggest that $CD133^+$ stem cells may have a greater potential for engraftment than $CD34^+$ stem cells. In addition, cells including $CD34^-$ progenitors and effector cells other than T cells may have an influence on engraftment and relapse probability. Dr. Greil reported on first clinical experiences with $CD133^+$ stem cells in allogeneic HLA mismatched transplantations in pediatric patients and assessed the feasibility of T and B cell depletion by use of CD3 and CD19 CliniMACS® MicroBeads in this setting. All patients had sustained engraftment; no GVHD >grade I and no transplant related toxicity occurred. Moreover, the data showed that by addition of positively selected $CD133^+$ stem cells, thrombocyte recovery improved remarkably and that CD3/CD19 depletion in the haploidentical setting was feasible. The simultaneous depletion of CD3 and CD19 positive cells leaving NK cells in the graft might also be advantageous in a KIR-mismatched situation between donor and host in allogeneic transplantations. Thus, both methods represent a new approach for graft engineering in mismatched transplantations.

Marc Vanderheyden, Cardiovascular Center, OLV Hospital Aalst, Belgium

Bone marrow stem cells for cardiac repair

Early experience with bone marrow stem cells for cardiac repair is encouraging. In this regard, $CD133^+$ hematopoietic stem cells were suggested to represent a population of cells with high pluripotent capacity and high engraftment rates. Hopes are that $CD133^+$ cells may help to regenerate damaged heart tissue after myocardial infarction. In his presentation, Dr. Vanderheyden reported on the preliminary experience with the intracoronary injection of selected $CD133^+$ cells in patients with acute myocardial infarction or chronic ischemia. $CD133^+$ cells were selected from bone marrow and injected into the culprit vessel in the course of the intervention. Cell infusion-related serious adverse events were not reported. A marked improvement in myocardial perfusion and in ventricular pump function was observed. Remarkably, a marked increase in the mass of metabolically active myocardium surrounding the original infarct site was measured. In a historic control group with patients not receiving stem cells, such improvement was not observed. Out of 11 patients, one presented with progressive narrowing of the lumen distal to the implanted stent, which may represent natural disease progression. The safety and efficacy of the treatment will now be investigated in a prospective randomized trial involving 40 patients.

Richard K. Burt, Northwestern University, Division of Immunotherapy, Chicago, IL, USA

Hematopoietic stem cell transplantation for autoimmune diseases

Autologous stem cell transplantation is a promising treatment for severe autoimmune diseases refractory to conventional therapy. However, relapse occurs and therefore it remains uncertain if autologous hematopoietic stem cell transplantation (HSCT) will result in a medication-free cure. Allogeneic HSCT is more likely to cure, but the risk of graft versus host disease (GVHD) is a major problem that needs to be addressed. Dr. Burt discussed immunosuppressive regimens and CD34-selection in rheumatoid arthritis and scleroderma as an approach to eliminate or effectively reduce the level of autoimmune T and B cells thereby 'resetting' the immune system, while at the same time minimizing GVHD.

Frequently asked questions

Q Which type of Tubing Set (TS) should be used for CD3 depletion of an unmobilized apheresis?

A We always recommend using the large scale tubing set (LSTS) due to the significantly shorter processing time.
(Example: unmobilized apheresis containing 2×10^{10} TNC and 40% $CD3^+$ T cells: CD3 depletion with LSTS in about 2 hours.
CD3 depletion with standard TS takes about 6 hours.
The use of the upcoming depletion TS will further reduce depletion processing time by 50% compared to LSTS)

Q How many $CD56^+$ cells can I enrich when using a maximum of 1 L of buffer?

A It is possible to enrich up to 2×10^9 $CD56^+$ cells using the software program ENRICHMENT 1.1 and the standard Tubing Set with 1L of buffer (up to 5×10^9 with 2 liters of buffer)

Q Is it allowed to directly reinfuse CD133-selected cells after selection (suspended in CliniMACS PBS/EDTA Buffer)?

A No, the CliniMACS PBS/EDTA Buffer is not approved for *in vivo* use. You must wash and resuspend the $CD133^+$ cells in solutions approved for *in vivo* use, e.g. isotonic NaCl, PBS Buffer, Ringer Lactate Solution, Plasmalyte.

Q What is the maximum shelf life of CliniMACS CD34 Reagent, CliniMACS Tubing Set and CliniMACS PBS/EDTA Buffer?

A Maximum shelf life:
CliniMACS CD34 Reagent: 5 months
CliniMACS Tubing Set and TSLS: 3 years
CliniMACS PBS/EDTA Buffer: 2 years

Q How much CliniMACS Buffer do I need for a large scale CD34 selection (CD34 selection 2 and LS Tubing Set)?

A For the whole process you will need 3 bags of CliniMACS Buffer, the same as for a standard scale CD34 separation. For the CD34 selection 2 itself, 1 L of CliniMACS Buffer is sufficient.

Q Is there a minimum cell number defined for the depletion program?

A There is no lower process limitation in TNC number, but the device does not allow entering values $<2\times10^7$ cells/mL.

Q Is there a different maximum TNC and target cell number for the large tubing set in stem cell selection?

A Capacities for LSTS for CD34 and CD133 selection are: 12×10^{10} TNC, 12×10^8 target cells.

The 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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Baxter and Miltenyi Biotec sign exclusive distribution agreement for cellular therapy products

In September 2003 Baxter Healthcare Corporation and Miltenyi Biotec announced that they signed an agreement whereby Miltenyi Biotec will have exclusive rights to distribute, market, and sell Baxter's cellular therapy products worldwide, with the exception of China, Japan, Korea, and Taiwan. Baxter will continue to manufacture these products.

Included in the agreement are the Isolex 300i Magnetic Cell Selection System v2.5, the CytoMate Cell Processing System, as well as Cryocyte Preservation and Lifecell Culture Bags.

Beginning December 2003, Miltenyi Biotec assumed responsibility for orders for Baxter products in the US and EU.

CytoMate

Isolex 300i

"We believe that this distribution agreement with Miltenyi is the best way to keep cell selection, preservation and expansion products available to customers worldwide," said Jim Utts, General Manager of Baxter Oncology. "Miltenyi Biotec's dedicated focus on cellular therapies and their global sales force will ensure that our customer needs are met."

"We are very pleased with the agreement we have signed with Baxter," said Stefan Miltenyi, Managing Director of Miltenyi Biotec. "Not only do these products represent a significant enhancement to our current portfolio, we also gain immediate access to the United States, one of the key growth market for us. We plan on leveraging our distribution infrastructure to continue to provide customers with the quality products and services they require."

For more information, contact Miltenyi Biotec, visit our website or contact our technical support team.

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Upcoming meetings – Meet us at the booth!

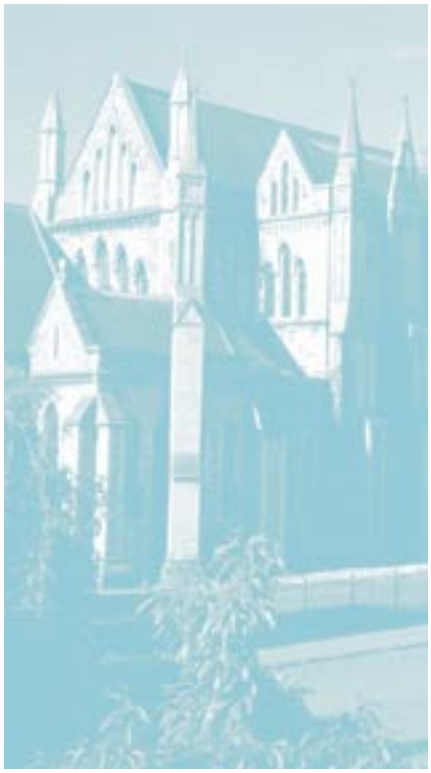
Date	Congress	Webpage
April 15–17	Jahrestagung der Dt. Gesellschaft für Kardiologie, Mannheim, Germany	www.dgkardio.de
April 21–24	Intl. Symp. On Recent Advances in Hematopoietic Stem Cell Transplantation, Heidelberg, Germany	www.cytonet.de
April 24–28	20th Intl. NK Cell Workshop, 8th Annual Meeting Society for Natural Immunity, Nordwijkerhout, NL	www.NK2004.org
April 28–30	British Transplantation Society Annual Congress, Birmingham, UK	www.bts2004.org.uk
April 28–May 1	Society for Investigative Dermatology, Providence, RI, USA	www.sidnet.org/public/articles/SID_Exhibitor_Prospectus.pdf
May 7–10	ISCT, 10th Annual Meeting, Dublin, Ireland	celltherapy.org/Dublin2004
May 23–25	NOPHO, Nordic Society of Pediatric Hematology and Oncology, Odense, Denmark	www.nopho.org
May 24–27	British Cardiac Society Annual Scientific Conference, Manchester, UK	http://www.bcs.com
June 3–4	Canadian Bone Marrow Transplant Group, London, Ontario, Canada	www.cbmt.org/en/index.cfm?dsp=ecbi
June 10–13	Intl. Society for Stem Cell Research, Boston, MA, USA	www.issct.org/meetings/index.htm
June 18–20	EBMT Working Party on Paediatric Diseases, Nordwijkerhout, NL	www.congresscare.com/2004EBMT.htm
July 17–20	ISEH, 33rd Annual Meeting of the Intl. Society for Experimental Hematology, New Orleans, USA	www.iseh.org
July 18–23	FOCIS, Montreal, Canada	www.focisnet.org/
Aug 28–Sep 1	ESC European Society of Cardiology, Munich, Germany	www.escardio.org/publications/News/2003/11/congress/htm
Oct 2–6	DGHO, Innsbruck, Austria	www.dgho.de

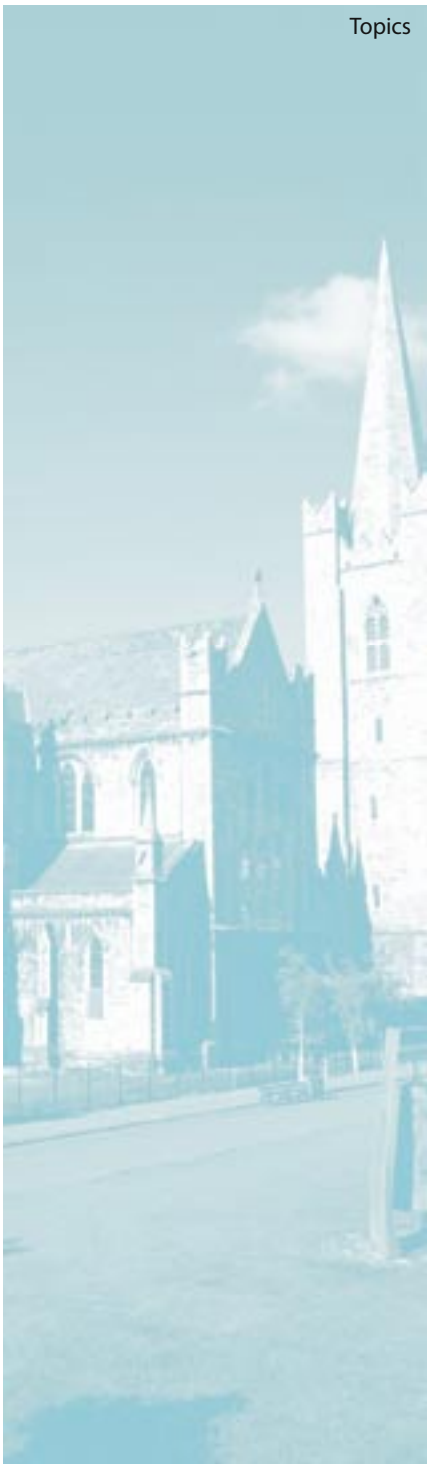
Miltenyi Biotec Satellite Symposium ISCT 2004

Innovative approaches for graft modulation and tissue repair

Friday, May 7, 2004
7:30 pm – 9:30 pm

Chair: Gordon Cook, MD, St James's University Hospital, Blood and Bone Marrow Transplantation Center, Leeds, United Kingdom





Topics

Megadose of NK cells for pediatric patients following haploidentical stem cell transplantation
Ulrike Koehl, MD, University Hospital Frankfurt, Pediatric Hematology and Oncology, Frankfurt, Germany

Transplantation of positively selected or T and B cell depleted grafts from alternative donors
Peter Lang, MD, University of Tübingen, Children's Hospital, Tübingen, Germany

Selective T cell depletion and graft-versus-host disease: Efficiency of a clinical protocol for CD8$^+$ T cell depletion
Gordon Cook, MD, St James's University Hospital, Blood and Bone Marrow Transplantation Center, Leeds, United Kingdom

Cord-blood derived CD133$^+$ stem cells for myocardial tissue repair
Jonathan Leor, MD, Neufeld Cardiac Research Institute, Sheba Medical Center, Tel-Aviv University, Tel-Hashomer, Israel

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ISCT 2004	Abstract booklet Miltenyi Biotec symposium "Innovative approaches for graft modulation and tissue repair"	❑
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