



Miltenyi Biotec

CliniMACS® Newsletter

Vol. 11 No. 1/2011

Customer reports



- Generation of tumor-infiltrating lymphocyte cultures for adoptive therapy
- Clinical-grade purification and expansion of CD56⁺CD3⁻ NK cells
- T cell activation and expansion with GMP-grade CD3/CD28 antibodies



Products and applications

- New MACS® GMP CD3 and CD28 antibodies
- Cell Culture Bags



Meeting minutes

- Stem Cell Meeting Cologne 2010
- DC2010: Forum on Vaccine Science



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Editorial



Dear Friends and Colleagues,

One of our core competencies as an organization is to provide tools for your use in order to translate your research into clinical practice. Academic and investigator-driven research is a key innovator when it comes to trying and establishing new therapies to help patients. Oftentimes such progress is hampered by the inability to transform research ideas into clinical treatment regimens because of the lack of adequate methods or components that fulfill necessary regulatory requirements.

The reports in this issue highlight some of our collaborative efforts to refine techniques and develop products to enable translational research and to automate and standardize the manufacture of cellular therapies for clinical use. New and existing regulations set the bar higher and higher. Standards for the quality of materials used in the manufacture of cell therapies are rising. A big focus is set on the ability to generate reproducible cellular products, which makes the translation of personalized autologous or allogeneic therapies even more challenging.

The first report by Parker *et al.* highlights the strength of automation to standardize and speed up production of a tumor-infiltrating lymphocyte therapy in patients with metastatic melanoma. The use of the gentleMACS™ Dissociator in this case can support the preparation of tumor material to generate viable lymphocytes for reinfusion after expansion to attack the metastasized tumor. Incorporating an automated dissociator helps reduce variability due to slight differences

in manual methods of dissection or handling and therefore reduces potential differences and variability in the potency of cellular therapies.

Koehl *et al.* utilized the CliniMACS® Cell Separation System for the generation of a highly pure NK cell product through depletion of CD3⁺ cells and the further enrichment of CD56⁺ NK cells. NK cells are coming more and more into focus due to their ability to kill tumor cells and fight infections in patients after hematopoietic stem cell transplantation. Since several cell populations can be found within a leukapheresis, with some that might not be beneficial for a recipient, cell separation provides the ability to remove such unwanted cells. The report by Koehl *et al.* highlights the ability of the CliniMACS System to highly purify NK cell populations in order to enable the best starting material for expansion purposes and later treatment.

In the following report by Nabil Ahmed, the added value of improved cell culture expansion methods is shown through the use of GMP-grade CD3 and CD28 antibodies. Faster expansion rates were observed that do lead to a shorter production time of the cellular product. This could speed up the time to treatment for the patient and reduce GMP-related costs.

All the reports in this issue, as well as the product information on some of our new developments in our GMP antibody and cell culture bag portfolio, show the advances made in the field of cellular therapies to translate these promising approaches into the clinic and make them widely available through step-by-step improvement and simplification.

You will find summary notes on the Stem Cell Meeting Cologne 2010 and the DC2010: Forum on Vaccine Science with some of the highlights of those expert meetings. We hope you enjoy reading this newsletter and find inspiration for your research, tackling some of the most devastating diseases today.

With best regards,

Kai Pinkernell
Miltenyi Biotec GmbH

gentleMACS™ Dissociation of melanoma tumors for the generation of tumor-infiltrating lymphocyte cultures for adoptive cell therapy

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Introduction

The incidence of metastatic melanoma has increased over the past three decades and currently there are only two treatment options that have received approval by the US Food and Drug Administration (FDA): application of interleukin-2 or dacarbazine result in response rates of 13–16% and 15%, respectively^{1,2}.

Adoptive cell therapy (ACT) combining lymphodepletion with the application of expanded autologous tumor-infiltrating lymphocytes (TIL) has demonstrated promising results in several non-randomized trials^{3,4}. Current efforts are mainly directed towards simplification of the procedures for individualized patient-specific TIL infusions, and ultimately effective ACT of advanced melanoma that could be applied by laboratories in multiple centers. In a first clinical trial CD8⁺ young TIL, enriched with the CliniMACS® System, were administered to melanoma patients following lymphodepletion⁵.

Key steps during the preparation of young TIL are the dissociation of fresh tumor tissue, TIL enrichment and rapid expansion⁶. In this report we present a new tissue dissociation procedure. The primary method for initiation of TIL has traditionally been the overnight digestion of fresh tumor tissues in a triple enzyme medium. This method is open, uses non-FDA-approved enzymes (hyaluronidase) and is operator-dependent. The gentleMACS™ Dissociator from Miltenyi Biotec is a mechanical tissue dissociation device, which potentially simplifies and standardizes tissue dissociation for the generation of TIL. The gentleMACS Dissociator works by disrupting the extracellular matrix and cell adhesion components without harming the integrity of the

cell membrane. This is achieved by a combination of varying enzyme mixes, mechanical forces, incubation periods, and temperatures. The automation of all mechanical steps, using the gentleMACS Dissociator, has led to reproducible results with reduced overall processing times. It is a closed system, utilizes single-use, disposable supplies, has rapid processing times, and can be standardized.

We examined the gentleMACS Dissociator for generation of TIL cultures for research and clinical use. Our early experiments using the instrument explored multiple variables. Qualitative and quantitative aspects of tissue processing were optimized, including cell yield and viability, flexibility for diverse clinical samples, technical simplicity, and regulatory compliance. Some variables considered included gentleMACS Program settings, inclusion of enzymes, the composition of enzymes, incubation times, and sample size.

This analysis resulted in a standard operating procedure (SOP) and the identification of a minimum number of variables to be optimized.

We next focused on optimizing an SOP for the gentleMACS Dissociator and comparing it with the current clinical SOP to process fresh tumor for the generation of TIL. Multiple head-to-head comparisons were performed to evaluate:

- i) the initial dissociation product for the total cell yield, lymphocyte/tumor cell ratio, and percentage of viable cells;
- ii) the resulting TIL cultures for initial expansion, phenotype, and cell function;
- iii) clinical-scale expansion and clinical efficacy.

Materials and methods

Dissociation of fresh tumor tissues

All samples collected and used were derived from patients who signed an informed consent approved by the institutional review board of the NIH. All patients receiving treatment on this study were treated as part of a clinical protocol.⁶

On the day of tumor resection, the specimen was received in the laboratory immediately following surgery. The specimen was bathed in sterile saline in a sterile container. Viable tumor tissue was dissected away from the majority of non-viable tissue and healthy (non-tumor) tissue in a laminar flow hood using a scalpel and sterile forceps. Research samples were collected during this dissection, including procurement of tissue for confirmation of diagnosis by pathology. Other research specimens included flash frozen

tissue for RNA isolation, tissue cryopreserved in OCT for histopathology, an FNA sample for cytopathology, and separate samples for tumor tissue culture lines. The tumor sample was weighed and kept in a 50-mL centrifuge tube containing a small amount of sterile HBSS if processed the same day, or in 10-mL sterile Complete Medium (CM) at 4 °C if it was to be processed after overnight storage. The complete medium for culturing TIL was prepared by supplementing RPMI 1640 with 10% human serum (heat-inactivated at 56 °C for 30 minutes), and with final concentrations of penicillin G (100 units/mL), streptomycin (100 µg/mL), gentamicin (50 µg/mL), Hepes (25 mM), and 2-mercaptoethanol (5.5×10^{-5} M). Selected antibiotics for relevant allergies were omitted.

The tissue specimen was placed on a sterile cutting surface (cutting board or an open Petri dish). Using sterile scalpel and forceps the specimen was cut into small (3–5 mm) fragments. Cut

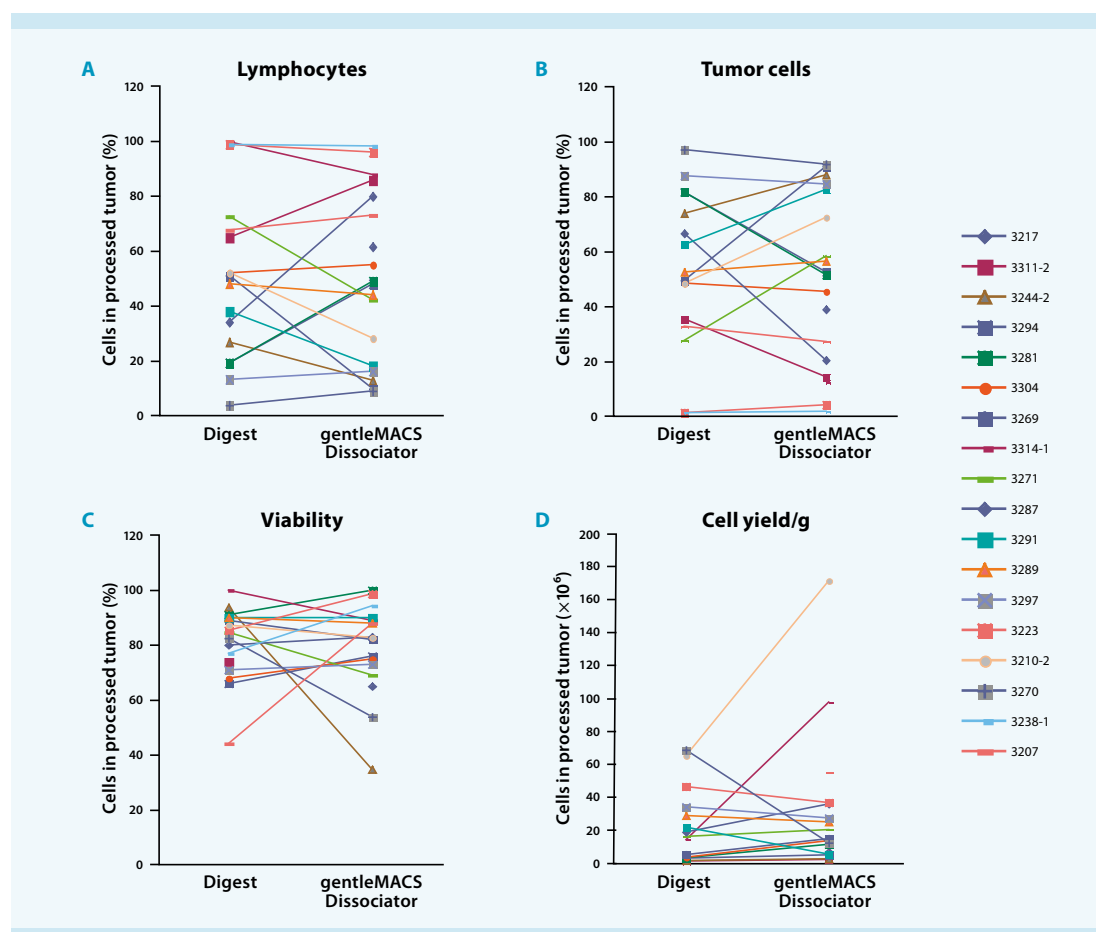


Figure 1: Comparison of the gentleMACS Procedure with the overnight digestion procedure with regard to cell numbers, viability, and cell yield. For comparison, samples from 18 patients were processed using the gentleMACS Dissociator or overnight digestion. Relative cell numbers of TIL (A) and tumor cells (B) as well as viability (C) and cell yield (D) were determined as described in Materials and methods.

fragments were transferred into a gentleMACS C Tube. Enzyme medium (EM) was added to the tube (5 mL for 0.2–2 g of tissue and 10 mL for 2–5 g) and the tube was securely closed by turning the cap until it clicks. EM was used to help disperse tumor cells from the surgical specimen during the short incubation periods between the gentleMACS Program runs. The enzyme-containing medium, RPMI 1640, did not contain serum. It had the following added components (final concentrations): penicillin G (100 units/mL), streptomycin (100 µg/mL), gentamicin (50 µg/mL), Fungizone® (1.25 µg/mL), Collagenase (1 mg/mL) and Pulmozyme® (~30 units/mL). The enzyme stocks were dry powders stored at -20 °C. The C Tubes were installed vertically and cap-side down into the gentleMACS Sleeve. Proper installation ensured that the C Tubes were held in position against rotational and axial forces. Pre-defined programs are provided by the internal gentleMACS Dissociator memory. The programs vary in intensity, so an appropriate program was selected depending on the texture of the tissue to be processed. Softer tissues required a more gentle

rotation (lower speed) and harder tissues required a more vigorous, longer rotation. For the human tumor tissues received by this lab, we have found the following series of programs and incubations to be optimal:

- **h_tumor_01.01 program**
- 30 minutes incubation at 37 °C
- **h_tumor_02.01 program**
- 30 minutes incubation at 37 °C
- **h_tumor_03.01 program**

After the first program had run (h_tumor_01.01), the C Tube was removed from the gentleMACS Dissociator and placed in a 37 °C incubator for 30 minutes. Subsequently, the C Tube was installed into the gentleMACS Dissociator for its second program run (h_tumor_02.01). After another 30 minutes incubation step the third program run (h_tumor_03.01) was performed. After the final program run, the tissue appeared mostly dissociated. If significant chunks of tissue were macroscopically visible, one or two additional

		Melanoma cell line			
		A2-		A2+	
	None	888 A1, 24	938 A1, 24	526 A2, 3	624 A2, 3
TIL 3207 (A1, A30) Std. Digest d22	322	247	390	45	
TIL 3207 (A1, A30) gentleMACS d23	78	443	861	116	
TIL 3211 (A1, A3) Std. Digest d24	29	23	18	20	33
TIL 3211 (A1, A3) gentleMACS d25	40	253	119	69	150
TIL 3212 (A1, A2) Std. Digest d19	46	82	658	488	322
TIL 3212 (A1, A2) gentleMACS d20	23	191	482	328	284
TIL 3210-2 (A1, A30) Std. Digest d28	45	34	33	20	33
TIL 3207 (A1, A30) gentleMACS d28	6	28	23	11	21
TIL 3223 (A2) Std. Digest d15	19	93	154	>32890	>17980
TIL 3223 (A2) gentleMACS d16	14	71	112	>38210	>27440
TIL 3217 (A24, A68) Std. Digest d21	15	332	180	87	289
TIL 3217 (A24, A68) gentleMACS d22	11	206	134	37	131

Table 1: Function of TIL generated by using the gentleMACS Dissociator or overnight digestion. Function of TIL was evaluated by coculturing TIL with melanoma cell lines or primary melanoma cells (FrTu) and analysis of IFN-γ release by TIL.

gentleMACS Dissociation runs were applied to the tissue, with or without one additional 30-minute incubation step at 37 °C.

The dissociated tissue from the C Tube was filtered through an autoclaved wire mesh placed in an autoclaved funnel on top of a 250mL centrifuge tube. The wire mesh was rinsed with sterile HBSS and the tube was filled with additional sterile HBSS. The dissociated tissue was then washed once by centrifuging 10 minutes at 1,500 rpm. The supernatant was aspirated and the pellet resuspended in a known volume of HBSS.

Processing dissociated single-cell tissue samples and generation of TIL cultures

The resulting single-cell suspension was counted using a hemocytometer with trypan blue exclusion to analyze cell yield. Total numbers of lymphocytes, tumor cells, and red blood cells (RBC), as well as overall cell viability were calculated. If the cell suspension had extensive cellular debris, or the RBC:nucleated cell ratio exceeded 6:1, or the overall viability was lower than 50 %, the sample

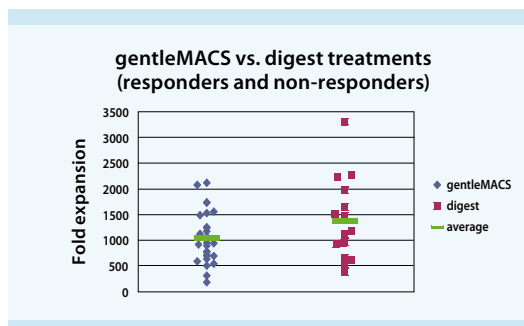


Figure 3: Clinical-scale expansion of TIL generated by using the gentleMACS Dissociator or overnight digestion. TIL generated by the gentleMACS Procedure or the standard digest were expanded to clinically relevant cell numbers. The analysis included both TIL that resulted in an objective clinical response and TIL that did not induce a response.

Fisher's exact test:	OR	NR
gentleMACS Dissociator	10 (38 %)	16
digest	7 (41 %)	10

Figure 4: Efficacy of TIL generated by using the gentleMACS Dissociator or overnight digestion. Fisher's exact test was performed on the percentage of objective responses (OR) to compare efficacies of TIL generated by the gentleMACS Procedure or overnight digestion (p= 0.69).

FrTu							
3104	3210	3003	3210-2	3217	3089	3010-2	3257-1
A11, 26	A2, 3	A1,2	A2, 30	A24, 68	A1, 0201	A 30, 33	A 24, 32
46	61						
59	67						
88		353					
97		322					
44			141				
97			315				
				424	151	93	
				298	69	92	
							17
							133

Numbers indicate IFN-γ concentrations in pg/mL. Bold numbers indicate results that were significantly different from the controls that included no tumor cells.

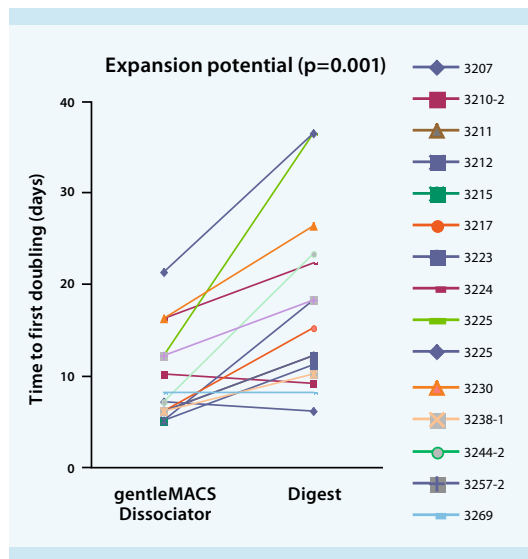


Figure 2: Expansion potential of TIL generated by using the gentleMACS Dissociator or overnight digestion. For comparison, samples from 15 patients were processed using the gentleMACS Dissociator or overnight digestion. Expansion of TIL was measured as described in Materials and methods.

was further processed over a Ficoll® gradient. If the cell suspension did not need to be cleaned up with a Ficoll gradient, then the final wash was completed as follows: the cells were centrifuged (10 minutes, 1,500 rpm), and resuspended in CM. Cultures were set up in 24-well tissue culture plates at 0.5×10^6 total live cells/mL (2 mL/well) in CM containing 10% human AB serum and IL-2 (6000 IU/mL). Remaining cells were frozen at

10^7 live cells/vial. TIL were cultured as previously described.⁷

TIL cultures were monitored daily for expansion, which was determined by microscopically counting viable cells using trypan blue exclusion and a hemocytometer.

Functional analysis of TIL

Selected TIL were evaluated for tumor recognition by interferon- γ (IFN- γ) release assay as previously described.⁷

Clinical-scale expansion and therapeutic administration

Successfully generated TIL were used to treat patients in ongoing surgery branch clinical protocols. These TIL were rapidly expanded and administered to metastatic melanoma patients.⁶

Statistical comparisons

Head-to-head comparisons were tested with a paired t-test. Populations were compared using a student's 2-sided t-test assuming unequal variance. Fisher's exact test was used to evaluate responses vs. source of TIL. All responses were considered significant for $P < 0.05$. Patients treated between May 2009 and May 2010 (inclusive) under Surgery Branch, NCI Protocols #07-C-0176, were included in this analysis.

Results

The initial gentleMACS Dissociation product, when compared to that of the current clinical method of overnight enzymatic digestion, showed no significant difference in the cellular composition, the yield of viable cells, or the overall viability (fig. 1).

For both methods, the initial dissociation products could be efficiently expanded in culture and were functional *in vitro* and *in vivo*. There was similar TIL generation and TIL attributes in most respects. Results do show, however, that the resulting TIL cultures from the standardized, semi-automated protocol using the gentleMACS Dissociator expanded significantly faster than those from manually dissociated tumors (fig. 2). Further analysis of these expanded TIL showed full functionality of the cells *in vitro*. There were few evaluable samples, but there was no obvious difference in function between cells obtained through the gentleMACS Procedure or standard digest (table 1).

There was no significant difference in lymphocyte subsets of the resulting TIL. 12 of 16 TIL cultures derived from the gentleMACS Protocol had more CD8⁺ cells than those of a standard digest, but this trend was not statistically significant. There was also a slight trend towards more CD4⁺ cells in the TIL of a standard digest (data not shown).

The optimized gentleMACS Dissociation programs were compared with the standard digest method for clinical expansion and efficacy. The cultures derived from the gentleMACS Protocol expanded well (fig. 3) and caused tumor regression in patients when used in melanoma immunotherapy protocols (fig. 4).

Conclusion

An optimal SOP for the preparation of TIL using the gentleMACS Dissociator was established. The gentleMACS Dissociator from Miltenyi Biotec provides a semi-automated protocol for the dissociation of melanoma tissue leading to single-cell suspensions with yields and viabilities comparable to that of overnight enzymatic digestion. Both methods provide similar initial TIL/tumor cell ratios, viability, and total cell yield for most tumor tissue samples. However, TIL from gentleMACS Dissociations showed significantly enhanced initial expansion in culture.

The gentleMACS Procedure is faster, more standardized, and more efficient for tumor samples up to 5 grams, whereas we have found that the standard digest is more convenient for processing larger tumors. Samples prepared using the gentleMACS Dissociator were efficiently expanded to clinically relevant cell numbers ($\sim 5.0 \times 10^{10}$) and were able to mediate tumor regression in advanced melanoma patients.

Based on our results, we have incorporated the gentleMACS Dissociator into our clinical SOPs and use it interchangeably with the current overnight enzymatic digestion for the generation of TIL for adoptive cell therapy trials.

The authors would like to acknowledge Michelle Langhan, Kate Hogan, Tom Shelton, and John Wunderlich of the Surgery Branch, NCI for their expert technical help with all aspects of this project.

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Clinical-grade purification and expansion of CD56⁺CD3⁻ NK cells for adoptive immunotherapy of solid tumors and leukemia

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Introduction

Cell therapy may represent a promising treatment option for patients suffering from leukemia and tumors who have a high risk for relapse after allogeneic, especially haploidentical, stem cell transplantation (SCT). While established T cell therapies, such as donor lymphocyte infusions, are associated with the risk of graft-versus-host disease (GvHD), natural killer (NK) cells may mediate graft-versus-leukemia/tumor effects without induction of GvHD. Therefore, immunotherapy with highly purified NK cells in recipients of haploidentical SCT could serve as an attractive alternative cell therapy^{1,2}.

Human NK cells are lymphocytes of the innate immune system involved in the early defense against infectious pathogens and against MHC class-I negative or low-expressing malignant targets without the requirement for prior immune sensitization of the host^{3,4}. They reside mainly in the marrow, spleen, and peripheral blood, where they account for approximately 2–18% of the peripheral blood lymphocytes. NK cells are usually the first lymphoid subpopulation to recover after SCT⁵. Phenotypically they can be defined by the expression of CD56, an isoform of the neural cell adhesion molecule and the lack of the CD3 antigen on the surface. Further characterization allows the major CD56^{dim}CD16⁺ (around 90%) to be distinguished from the minor CD56^{bright}CD16⁻ NK subpopulation. The immunoregulatory CD56^{bright} NK cell subsets express the high-affinity interleukin-2 (IL-2) receptor, which enables them to proliferate in response to IL-2 and to produce high amounts of cytokines, such as IFN- γ ,

TNF- α , TNF- β , GM-CSF, IL-10, and IL-13. The CD56^{dim} NK cells are essentially cytotoxic cells that express low levels of the IL-2 receptor. NK cells are able to lyse targets by releasing cytotoxic granules containing perforin and granzymes and using antibody-dependent cellular cytotoxicity pathways via membrane receptor binding to the Fc portion of IgG antibody, and by the induction of apoptosis through molecules of the TNF super family (Fas/CD95, TRAIL).

Killing activity of NK cells is regulated by a set of surface receptors that either induce or inhibit the cytotoxic response^{3,6,7}. Activation of NK cells is facilitated by the engagement of activating surface receptors through interaction with stimulatory ligands expressed by malignant cells. These immune recognition receptors include NKG2D, the natural cytotoxicity receptors (NCR) NKp30, NKp44, and NKp46, CD16, NKp80, DNAM-1, and 2B4 (CD244)^{6,8}. Activation with cytokines, such as IL-2, leads to a strong up-regulation of the NCRs and NKG2D and this correlates with increased NK cell cytotoxicity against malignant cells⁹. Inhibitory receptors comprise both killer cell immunoglobulin-like receptors (KIRs) and the heterodimeric C-type lectine receptor CD94-NKG2A/B¹⁰. In addition, several activating KIR and the C-type lectin receptor CD94-NKG2C/E/F are known³. A number of studies have demonstrated NK cell-based killing of many different mouse and human tumors and leukemias and have led to the initiation of the first clinical phase I/II trials using allogeneic NK cells for treatment of cancer.

Materials and methods

Clinical-scale NK cell enrichment

Protocols for the enrichment of NK cells from non-stimulated leukapheresis products using good manufacturing practice (GMP) procedures have already been established (table 1; fig. 1). The aim of these procedures is to obtain a highly purified NK cell product with minimal T cell contamination and conserved NK cell cytotoxicity. NK cell enrichment usually consists of one or two rounds of CD3⁺ cell depletion with subsequent CD56⁺ cell enrichment^{11,12}.

In our procedure, after steady-state leukapheresis of unstimulated donors, the cells were washed twice for platelet reduction with CliniMACS[®] PBS/EDTA Buffer (Miltenyi Biotec, Bergisch Gladbach, Germany) supplemented with 0.4% human serum albumin (Red Cross Blood Donor Service, Baden-Württemberg-Hessen, Germany). Thereafter, 5 mL of Intraglobin (Biotest, Dreieich, Germany) were added and incubated for five minutes to reduce non-specific antibody binding. Cells were labeled for 30 minutes with CliniMACS CD3 Reagent (Miltenyi Biotec), using one vial of reagent in case of total nucleated cell (TNC) numbers up to 40×10⁹ or

CD3⁺ cell numbers up to 15×10⁹ and two vials in case of TNC numbers up to 80×10⁹ or CD3⁺ cell numbers up to 30×10⁹. After washing twice, CD3⁺ cells were depleted with the CliniMACS Plus Instrument using the separation program “DEPLETION 2.1”. If necessary, the T cell depletion step was repeated to further remove residual T cells. Thereafter, the T cell-depleted harvests were concentrated and labeled with CliniMACS CD56 Reagent (Miltenyi Biotec) for 30 minutes (one vial CD56 Reagent for TNC numbers up to 40×10⁹ and CD56⁺ cell numbers up to 10×10⁹). After washing, CD56⁺CD3⁻ NK cells were enriched using the separation program “ENRICHMENT 1.1”. All steps were performed in a closed system observing GMP. The study protocol was approved by the local ethics committee in Frankfurt and Basel and informed consent of the donors has been obtained.

Expansion and activation of NK cells

The purified CD56⁺CD3⁻ NK cells were suspended and seeded at a concentration of 1–2×10⁶ cells/mL in X-VIVO[™] 10 media (BioWhittaker, Verviers, Belgium) supplemented with 5% heat-inactivated human fresh frozen plasma and 1,000 U/mL rhIL-2 (Proleukin[®], Novartis, Germany) under

Clinical phase I/II study with allogeneic NK cells post haploidentical SCT: Patients with high-risk leukemia and malignant tumors

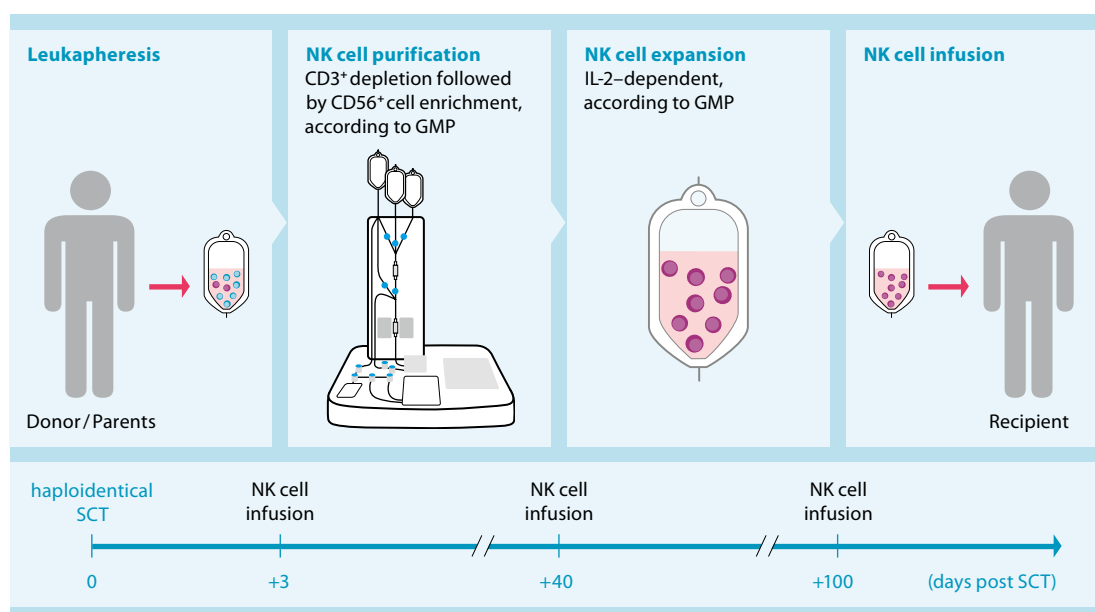


Figure 1: Purification, *ex vivo* expansion, and infusion of donor NK cells. In an ongoing clinical phase I/II trial patients receive freshly isolated NK cells on day (+3), +40, +100 or IL-2-activated NK cells on day +40 and +100 post haploidentical SCT.

GMP-compliant conditions (fig.1). In an early phase of the study, cells had been expanded and activated using both 175 cm² culture flasks (Nunc, Wiesbaden, Germany) and VueLife™ cell culture bags (CellGenix, Freiburg, Germany); for the ongoing phase I/II trial only VueLife cell culture bags were used¹¹. Fresh medium was added every three days and samples for monitoring cell content and viability were taken directly after leukapheresis, after each depletion/enrichment step, and every second day during stimulation. Phenotyping and evaluation for cytotoxicity was performed by flow cytometry. After 10 days, stimulated NK cells were administered to the patients or cryopreserved in X-VIVO™ 10 medium supplemented with 10% DMSO.

Phenotyping and functional characterization of the product for quality control

The absolute number of CD56⁺CD3⁻ NK cells and the number of residual T cells were determined by flow cytometry performed on a four- or a five-color flow cytometer (Epics XL or FC 500, Beckman Coulter, Krefeld, Germany) in a single-platform technique. The gating strategy was based on the ISHAGE single-platform stem cell enumeration method using low scatter, high expression of CD3 and CD45 antigens, CD16 and CD56 expression and 7-AAD staining, in a no-wash preparation with counting beads. Our previously described four-color panels¹³ were extended to the following five-color panels: CD45-FITC/

CD56-PE/CD3-ECD/7-AAD/CD16-PC7 and 45-FITC/CD3-PE/CD14-ECD/7AAD/CD56-PC7. Samples were prepared in triplicate and 45-FITC/IgG1-PE/CD14-ECD/7AAD/CD56-PC7 served as a control. In addition, cells were labeled with appropriate combinations of fluorochrome-conjugated antibodies (MAb) to monitor NK cell subsets, activating and inhibitory NK cell receptors, and activation status. MAbs used were CD16 (clone 3G8), CD45 (clone J.33), CD56 (clone N901), HLA-DR (clone Immu-357), CD69 (clone TP1.55.3), CD158a,h (KIR2DL1/S1, clone EB6B), CD158b1/b2,j (KIR2DL2/3/S2, clone GL183), CD158e1/e2 (KIRp70, KIR3DL1/S1, clone Z27.3.7), CD158i (KARp50.3, KIR2DS4, clone FES172), CD337 (NKp30, clone Z25), CD336 (NKp44, clone Z231), CD335 (NKp46, clone BAB281) and CD314 (NKG2D, clone ON72), all supplied by Beckman Coulter (Marseille, France), and CD3 (clone SK7) supplied by BD Bioscience.

The cytotoxicity of the highly enriched NK cells before and after IL-2 stimulation was tested against the MHC class I-negative cell line K562 or against the patient's individual leukemic cells using an antibody-based flow cytometric assay as described previously^{14,15}. NK cells and leukemic cells were co-cultured for four hours at effector-to-target ratios between 0.5:1 and 10:1. Absolute cell counts were determined using Flow-Count® beads. Cytotoxicity was defined as the loss of viable target cells relative to the control. In other studies NK cell functionality was measured by a CD107a degranulation assay^{16,17}.

Author	Method	Donors (n)	NK cell purity (%)	Recovery (%)	Log T cell depletion
¹⁸ Lang <i>et al.</i> (2002)	CD56 enrichment, followed by CD3 depletion	4	98.6 (m)	42.0 (m)	3.6×10 ⁴ -fold
¹⁹ McKenna <i>et al.</i> (2007)	CD3 depletion	36	37.7 (x)	78.8	2.7
¹⁹ McKenna <i>et al.</i> (2007)	CD3 depletion, followed by CD56 enrichment	13	89.7 (x)	19.4	4.3
²⁰ Koehl <i>et al.</i> (2004)	CD3 depletion, followed by CD56 enrichment	6	95.0 (m)	37.0 (m)	4.5 (m)
¹¹ Koehl <i>et al.</i> (2005)	CD3 depletion twice, followed by CD56 enrichment	15	94.9 (m)	33.0 (m)	5.0 (m)
²¹ Iyengar <i>et al.</i> (2003)	CD3 depletion, followed by CD56 enrichment	12	91.0 (m)	48.7 (m)	5.3 (m)
²² Uharek <i>et al.</i> (2003)	CD34 neg fraction: CD3 depletion, CD56 enrichment	7	75.0 (m)	42.0 (m)	4.0
²³ Passweg <i>et al.</i> (2004)	CD3 depletion, followed by CD56 enrichment	6	97.3 (m)	35.5 (m)	3.6
¹² Meyer-Monard <i>et al.</i> (2009)	CD3 depletion, followed by CD56 enrichment	24	94.5 (m)	58.0 (m)	4.2 (m)
²⁴ Rizzieri <i>et al.</i> (2010)	CD56 enrichment	51	96.5	80	

m: median; x: mean

Table 1: Clinical-grade NK cell enrichment using the CliniMACS® System.

Results and discussion

Technical aspects—clinical-scale NK cell product manufacture and NK cell activation

A number of studies have shown that clinical-scale NK cell product manufacture from non-stimulated leukapheresis products, using a CD3⁺ cell depletion step followed by CD56⁺ cell enrichment, leads to highly purified CD56⁺CD3⁻ NK cell products with a purity ranging from 89.7–98.6% (table 1)^{11,12,18–24}. Purity of NK cell products was lower if the starting product was the negative fraction after CD34⁺ cell selection²². The high NK cell purity and extensive T cell depletion is at the expense of a considerable loss of NK cells during isolation. The final recovery of CD3⁻CD56⁺ NK cells ranged between 19.4% and 58%. Overnight storage of the leukapheresis product led to a greater loss of NK cells during the NK cell enrichment process compared to processing of fresh harvests¹². A much higher NK cell recovery was obtained by using only a CD3⁺ cell depletion step, without further CD56⁺ cell enrichment. However, such a product was associated with low purity and less T cell depletion¹⁹. Similarly, the final T cell number was much higher if a CD56⁺ cell selection was used alone, but this kind of purification was associated with an increased NK cell recovery and purity²⁴. In contrast, the two-step NK cell product manufacture described here led to efficient T cell depletion of 3.6 to 5.3 orders of magnitude (table 1), and this could be further increased by performing the CD3⁺ cell depletion step twice¹¹. A residual T cell contamination between 0.01 and 0.09% in the final product allows the infusion of NK cell products of more than 1.0×10^7 CD56⁺CD3⁻ NK cells/kg BW with less than 5.0×10^4 CD3⁺ cells/kg BW and often less than 2.5×10^4 CD3⁺ T cells/kg BW¹.

The objective of NK cell purification is not only to remove potentially unwanted T cells but also to enable activation and expansion of the NK cells. Indeed, enriched NK cells can be infused without any additional manipulation, or after overnight culture in high-dose IL-2. They can also be expanded in IL-2 or other cytokines, such as IL-15, alone or in combination for two to several weeks in cell culture bags or in a bioreactor^{11,25}. Similarly, it is possible to expand single KIR⁺NK cells²⁶. *In vitro* expansion has two aims, to activate the selected CD56⁺CD3⁻ cells, and to increase the total

number of NK cells. Using CD69 as an activation marker, activation of NK cells was found to occur within 1–3 days of incubation with IL-2^{9,20}. When enriched CD56⁺CD3⁻ NK cells were cultured with IL-2, a significant expansion was observed although there was a lag of 3–5 days before the NK cells started to proliferate¹¹. On day five, expansion occurred and led to a two- to tenfold increase of CD56⁺CD3⁻ NK cells after 10–14 days. Although NK cells were viable immediately after purification (>90%), the vital NK cell count decreased by 30–50% during the first three to five days following IL-2 stimulation. Afterwards, cell viability recovered to >98%, and by day 10–14, a maximal NK cell expansion was obtained. No overgrowth of the remaining T cells was observed during expansion and activation with IL-2. With a protocol that enables the generation of NK cells on a clinical scale, using a closed system that conforms to GMP guidelines, the expanded NK cells were highly cytotoxic against different leukemic and tumor target cells^{9,20}. Importantly, no non-specific activation against normal allogeneic lymphocytes occurred¹⁸. In addition we were able to demonstrate that IL-2 stimulation led to up-regulation of all natural cytotoxicity receptors (NCRs) and the activating receptor NKG2D, which might explain the observed increased cytotoxicity against MHC-I^{negative} targets⁹. There is evidence that a combination of cytokines, such as IL-2, IL-12, IL-15, and IL-21, may further increase cytotoxic activity of NK cells. In addition to NK cell enrichment from leukapheresis products as summarized in table 1, NK cells can also be generated from cord blood²⁷.

Clinical-scale collection, enrichment, activation, and expansion of purified NK cells are feasible. Most of the technical aspects for adoptive NK cell therapy have been developed for clinical applications. However these laboratory procedures are time-consuming and expensive, need particular skills, and must be performed according to a GMP-compliant protocol.

Clinical studies using freshly purified or IL-2-activated NK cells

Previous trials and ongoing clinical phase I/II studies have shown the feasibility of using freshly purified or IL-2-activated donor NK cells for the treatment of high-risk patients suffering from

leukemia or tumors in both non-transplant settings and after haploidentical SCT as an additional immunotherapy^{20,23,24,28,29}. NK cell products were infused as a single dose rate or as multiple applications with doses between 0.2×10^7 /kg and 8.1×10^7 CD56⁺CD3⁻ NK cells/kg BW, mostly with less than 2.5×10^4 CD3⁺ T cells/kg BW^{1,9,24,29}. These first immunotherapy trials show that NK cells can be administered without immediate adverse events, that they are well-tolerated by the patients and do not induce GvHD > grade II. However, some cases of GvHD have been observed after NK cell infusion. In some instances this has been associated with a less efficient T cell depletion. Whether GvHD is attributable to contamination by T cells or is due to the effects of NK cells cannot be determined on the basis of this clinical data. The fact that (at least in some cases of GvHD) the T cell content was higher than in cases without GvHD, seems to favor a T cell effect. With regard to NK cell efficiency, Rubnitz *et al.*²⁹ recently reported that NK cell administration to 10 pediatric patients with AML in first complete remission led to a two-year event-free survival of 100%, with all patients still in complete remission. An earlier study demonstrated that patients with AML had a lower rate of leukemia relapse compared to the expected rate, a lower rate of graft rejection, and a paradoxical reduction in GvHD post-haploidentical SCT, when the NK cells possessed inhibitory KIRs for which the recipient had no ligand³⁰. We could show an increased cytotoxic activity of stimulated NK cells against high-risk neuroblastoma (NB) due to IL-2-mediated up-regulation of the activating receptors NKp30, NKp44, NKp46, and NKG2D⁹. However, we have also been able to demonstrate tumor escape from immune surveillance by release of soluble MICA (ligand MHC class I-chain-related gene A) compromising NKG2D-dependent NK cell cytotoxicity in patients with NB. Elevated sMICA levels in patients' plasma correlated significantly with impaired NK-cell-mediated cytotoxicity of the infused donor NK cells³¹.

Future perspectives

Future studies should improve NK cell immunotherapy by increasing the understanding of the conditions leading to tumor cell kill by NK cells, by increasing the cytotoxicity of NK cells against various malignancies, and by optimizing the

schedule of the NK administration based on results of ongoing phase I/II studies. Given the plausible benefit of IL-2-stimulated NK cells compared to freshly isolated, resting NK cells with regard to cytotoxicity, it may be possible to increase cytotoxicity by activation with cytokine combinations like IL-2/IL-15 or by cross-talk with dendritic cells (DCs). Additional investigation is necessary to develop strategies to overcome tumor immune escape mechanisms. Options may encompass development of MAb against sMICA, genetic engineering of NK cells by introduction of chimeric receptors for tumor retargeting, or enhancing tumor cell recognition by using small interfering RNA to silence inhibitory receptors. Open issues in clinical studies also include NK cell dose rate, time schedule, appropriate selection of donor/recipients, and also the types of tumors to be considered for treatment, because it is already known that certain types of malignant cells may be more responsive to NK cell therapy than others. Ultimately expansion of tumor-reactive NK cells within the patient might prove to be feasible. It is possible to transfuse NK cells simultaneously with the transplants, and the first clinical trials indicate that an early NK application post-SCT may be most effective in attacking minimal residual disease.

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GMP-grade CD28 monoclonal antibody induces more robust expression of retroviral transgene while preserving a favorable T cell phenotype

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Background

The presence of a CD28 costimulatory signal in combination with CD3 activation represents a more physiological way in which T lymphocytes can be stimulated to become T lymphoblasts. This could result in more robust T cell activation, proliferation, and consequently more efficient expression of a retroviral transgene, while simultaneously preserving a favorable differentiation phenotype.

Methodology

Peripheral blood mononuclear cells (PBMC) were isolated from 3 donors by Lymphoprep gradient centrifugation.

5×10^5 PBMC per well of a 24-well plate were activated with CD3 (Ortho Biotech, referred to as “OB”) or CD3 +/- CD28 (Miltenyi Biotec, Bergisch Gladbach, Germany, referred to as “MB”) at a final concentration of 1 $\mu\text{g}/\text{mL}$ each. On day two, recombinant human IL-2 (Chiron, Emeryville, CA) was added at a final concentration of 50 units/mL, and on day three cells were harvested for retroviral transduction. For transduction, we pre-coated a non-tissue, culture-treated, 24-well plate with a recombinant fibronectin fragment (FN CH-296; Retronectin; Takara Shuzo, Otsu, Japan). Wells were washed with phosphate-buffered saline (PBS; Sigma, St. Louis, MO) and incubated twice for 30 minutes with a high-titer retrovirus supernatant, encoding a CD19-specific Chimeric Antigen Receptor (CAR). Subsequently, 3×10^5 T cells per well were transduced with retrovirus in the presence of 50 units of IL-2 per milliliter. After 48–72 hours, cells were removed and expanded in the presence of 50–100 units of IL-2 per milliliter for 10–15 days prior to use. Conditions were compared for proliferation,

percentage and density of T cell transduction with CAR as well as effector memory T cell markers.

The above outlined methodology is the current approach to generate CAR-transduced activated T lymphocytes at the CAGT for clinical use.¹

Results

In all three donors, there was an advantage to using the combination of CD3 and CD28 Ab in T cell proliferation (fig. 1). Consequently, a significantly higher degree of transduction of T cells (as evidenced by a higher percentage of T cells transduced, fig. 2) and density of T cell transduction (as evidenced by consistently higher mean fluorescence intensity [MFI] values, fig. 3) were observed under these conditions. The use of CD3 and CD28 Ab together was associated with an effector memory phenotype pattern that was similar to that of CD3 alone (fig. 4).

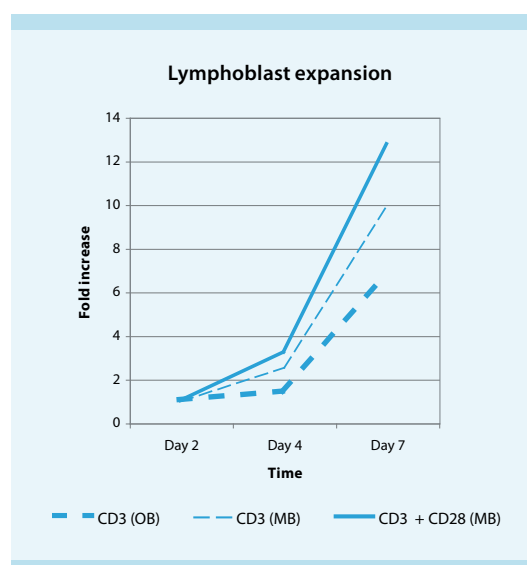


Figure 1: Mean expansion rate of CD19-CAR transduced T cells from three different donors after activation with the three different antibodies.

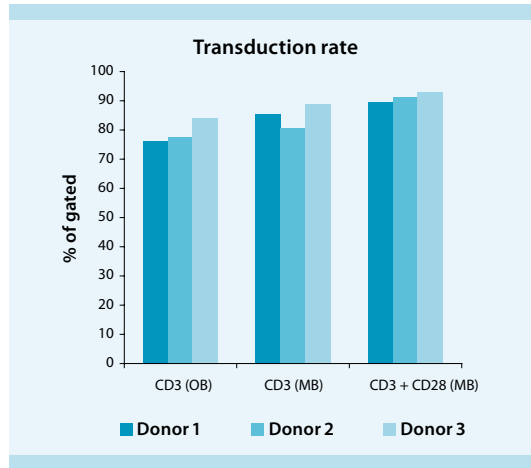


Figure 2: Individual transduction rate for each donor

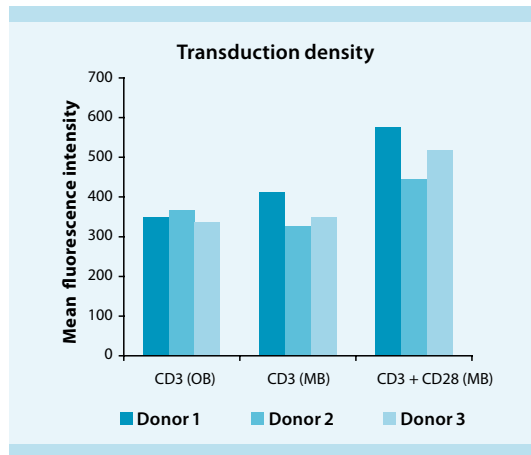


Figure 3: Density of T cell transduction for each donor

Conclusion

The noted improvement in expansion could reduce production time by approximately 5 days, saving precious time for patients with progressive tumors as well as presumably saving reagents and technician time by achieving clinically relevant cell numbers early. The improved transduction rate and density could theoretically predict better T cell activation upon encountering target molecules, a fact that is particularly important in the context where target molecules are expressed only modestly on the cell surface. Lastly, the presence of a similar effector memory phenotype indicates that the improvement in activation and expansion is not associated with a more exhausted T cell phenotype.

Researchers at the CAGT have adapted the protocol to generate CAR-transduced activated T lymphocytes for clinical use to include this GMP-grade CD28 monoclonal antibody.

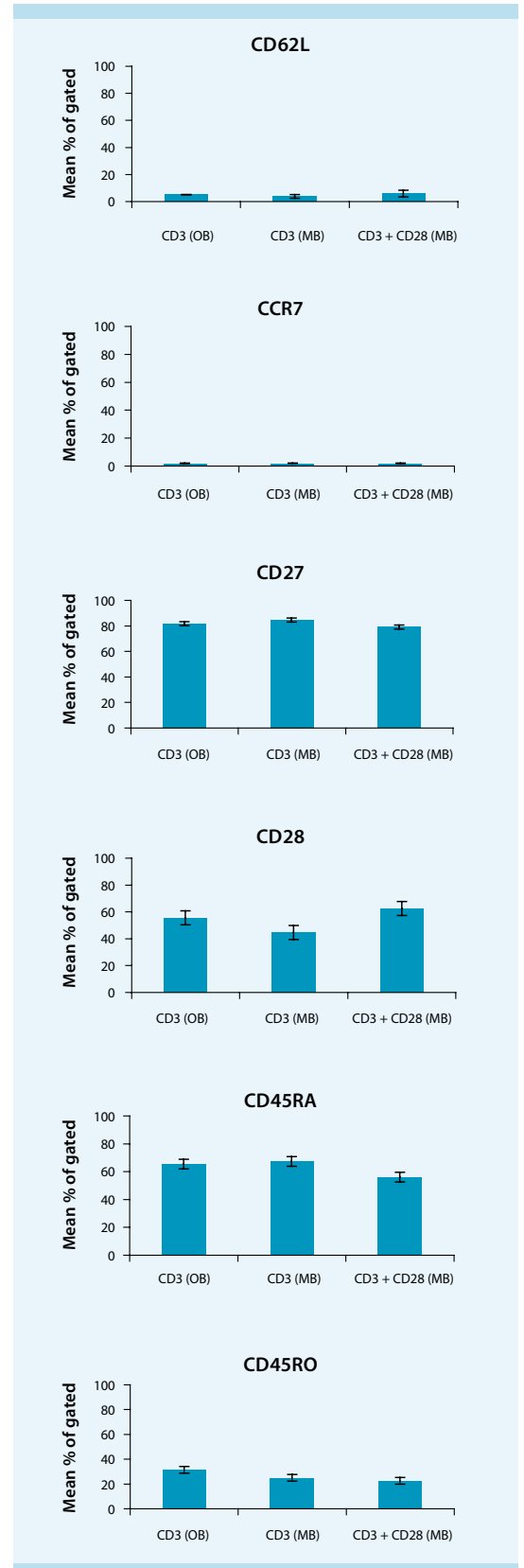


Figure 4: Expression of effector memory T cell markers (mean percentage of CD3-gated cells from three different donors)

Reference

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New tools for *ex vivo* T cell stimulation: Miltenyi Biotec is now offering GMP-grade soluble antibodies

MACS® GMP Antibodies

GMP-grade soluble antibodies are essential for powerful *in vitro* T cell stimulation protocols. Miltenyi Biotec introduces two antibodies optimized for high quality T cell cultures: MACS GMP CD3 pure and MACS GMP CD28 pure.*

Optimized T cell expansion for clinical-grade cell cultures

Stimulation of the CD3 part of the T cell receptor-CD3 complex with agonistic antibodies induces T cell activation and expansion. To amplify the response, activation via CD3 is often combined with additional stimulation of CD28. Combined stimulation with MACS GMP CD3 pure and MACS GMP CD28 pure markedly increases T cell expansion as compared to stimulation with CD3 alone (fig. 1). The two signals provide a physiological stimulus and optimize T cell expansion for clinical-grade cell cultures.

Consistent, high quality

All MACS GMP Products, including our GMP-grade antibodies, are manufactured in our state-of-the-art GMP facility in Teterow, Germany, in standardized and strictly controlled industrial processing steps. They are designed following the recommendations of the United States Pharmacopeia, chapter <1043> on ancillary materials for cell-, gene-, and tissue-engineered products. Consequently, they are free of animal- and human-derived contaminants, and product specifications are confirmed by batch-specific certificates of analysis to ensure consistency of

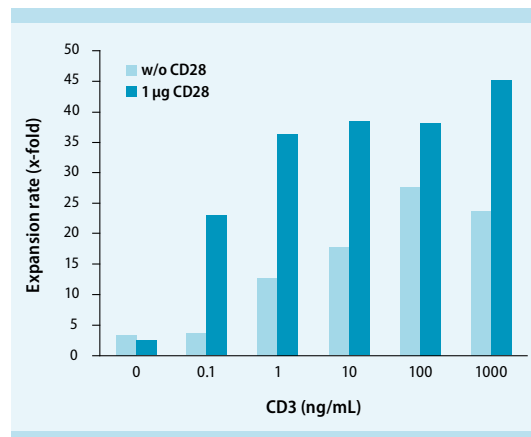


Figure 1: Optimized T cell expansion by a combination of MACS GMP CD3 pure and MACS GMP CD28 pure. T cells were co-cultured with APC (T cell-depleted PBMC) at a ratio of 1:10 for 7 days. MACS GMP CD3 pure and MACS GMP CD28 pure antibodies were added at the indicated concentrations.



Figure 2: The Miltenyi Biotec GMP facility allows standardized and strictly controlled industrial processing.

quality. By using GMP-compliant manufacturing processes, the products are then ideally suited for research use and *in vitro* cell processing in a clinical research setting.

*) MACS GMP Products are for research use and *ex vivo* cell culture processing only, and are not intended for human *in vivo* applications. For regulatory status in the USA, please contact your local representative.

Harmonization and expansion of the Cell Culture Bag portfolio

The complete portfolio of Miltenyi Biotec Cell Culture Bags will be simplified and harmonized. With the release of new production lots in May/June 2011, the Cell Differentiation and Expansion Bags for research use and the CE-marked Cell Differentiation and Expansion Bags will become one product group: MACS® GMP Cell Culture Bags.

The features of the bags, including volumes, connectors, and bag material, will remain unchanged. We guarantee the high product quality that you can expect from Miltenyi Biotec. MACS GMP Cell Culture Bags will be available worldwide. Please note that new order numbers will be effective as of August 2011.

The gas-permeable and transparent bags are intended for the *in vitro* cultivation, differentiation, or expansion of human cells from heterogenous hematologic cell populations, e.g., expansion of T cells or generation of dendritic cells.



The Cell Culture Bag portfolio will soon be expanded by the addition of Cell Differentiation Bags – 1000 and – 3000, with maximum culture volumes of 1000 mL and 3000 mL, respectively.

Product	Content	Capacity	Regulatory status	Availability	Order no.
MACS GMP Cell Differentiation Bag – 100	5 bags	100 mL	*)	Worldwide ¹⁾	170-076-400
MACS GMP Cell Differentiation Bag – 250	5 bags	250 mL	*)	Worldwide ¹⁾	170-076-401
MACS GMP Cell Differentiation Bag – 500	5 bags	500 mL	*)	Worldwide ¹⁾	170-076-402
MACS GMP Cell Expansion Bag	5 bags	up to 100mL	*)	Worldwide ¹⁾	170-076-403
MACS GMP Cell Differentiation Bag – 1000	2 bags	1000 mL	*)	Worldwide ¹⁾	Coming soon 170-076-404
MACS GMP Cell Differentiation Bag – 3000	2 bags	3000 mL	*)	Worldwide ¹⁾	Coming soon 170-076-405

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1) For availability in your country please contact your local representative.

Meeting minutes

2010

Stem Cell Meeting Cologne 2010
DC 2010: Forum on Vaccine Science



Stem Cell Meeting Cologne 2010

Adult, embryonic and induced pluripotent stem cells for tissue regeneration

September 17–18, 2010

Day 1: Basic research and pre-clinical aspects

Day 2: Clinical study concepts and patient cohorts



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This vibrant meeting focused on some of the most recent developments in basic stem cell research, pre-clinical aspects, and clinical study concepts and patient cohorts. The presentations on basic and pre-clinical research discussed numerous studies on ES and iPS cells, stem cells and differentiation, and adult stem cells.

Here are a few highlights of the symposium's second day on "Clinical study concepts and patient cohorts".

Wolfgang-Michael Franz gave an overview of stem cell therapy in the field of cardiovascular disease and discussed the treatment of patients

with chronic heart disease, acute myocardial infarction, and cardiac insufficiency. He pointed out that worldwide there are approximately 200 clinical trials studying the role of stem cell therapy in myocardial infarction and heart failure.

The first session of the "Clinical study concepts and patient cohorts" symposium focused on the role of stem cells in clinical settings.

Andreas M. Zeiher showed promising results of the REPAIR-AMI trial, which was initiated 5 years ago. The trial was based on the premise that since reduced vascularization at the infarct border zone contributes to cell death and adverse

remodeling, reperfusion of the infarcted tissue, accompanied by infusion of enriched stem cells into the border zone, might support the recovery of cardiac function.

Alexander Kaminski reported on the first phase III trial (PERFECT) on intramyocardial transplantation of autologous stem cells in addition to bypass surgery. The trial postulates that the use of bone marrow-derived CD133⁺ cells as an adjunct to coronary artery bypass grafting will improve recovery of the myocardium following infarction.

Eric Duckers outlined the double-blind APOLLO study where post-infarction patients were treated with adipose tissue-derived mesenchymal stromal cells with the premise that these cells will improve restoration of cardiac function.

Hans-Michael Klein showed first results of the INSTEM trial aiming at evaluating safety and feasibility of the perioperative isolation and subsequent myocardial transplantation of CD133⁺ cells in combination with coronary bypass surgery and transmyocardial laser revascularization.

Pilar Jimenéz-Quevedo elaborated on the PROGENITOR trial, a randomized, blinded, multicenter, controlled study to assess safety and feasibility of the transendocardial injection of peripheral blood-derived CD133⁺ cells in patients with refractory angina.

Rachel Ruckdeschel Smith explicated the CADUCEUS trial that examines the safety and preliminary efficacy of autologous cardiosphere-derived stem cells to reverse ventricular dysfunction in patients with recent myocardial infarction.

Klaus Wagner reported on the development of a current therapy program employing CD133⁺ stem cells for the treatment of end-stage peripheral vascular disease. A trial is currently in the preparation stage aimed at showing the safety

and feasibility of treatment with isolated bone marrow-derived CD133⁺ endothelial progenitor cells.

Consuelo del Cañizo showed first results of a non-blinded, randomized study on the safety and efficacy of autologous peripheral blood-derived CD133⁺ cells in the treatment of patients with critical limb ischemia.

Jan Schulte am Esch presented data from a study on the use of CD133⁺ bone marrow-derived cells for liver regeneration upon liver resection. The treatment is based on the evidence that hepatic stem cells provide a stimulus for liver regeneration. A controlled multicenter trial is currently being designed to test this novel form of treatment more rigorously.

In the second session of the “Clinical study concepts and patient cohorts” symposium various underlying mechanisms in stem cell therapy were presented.

Johannes Waltenberger explicated that diabetes mellitus impairs CD133⁺ progenitor function after myocardial infarction, which potentially affects responses to progenitor cell transplantation.

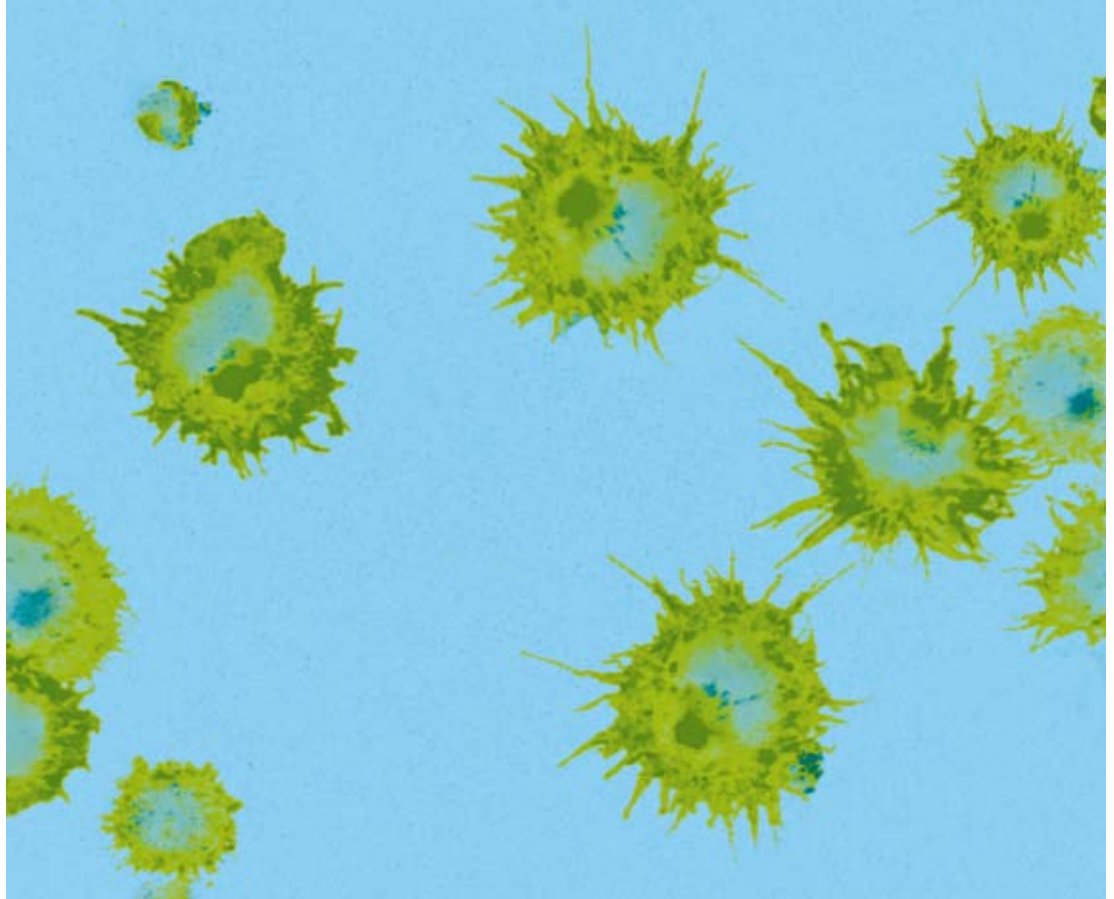
Wolfgang-Michael Franz presented first results of the multicenter, randomized, placebo-controlled SITAGRAMI trial in acute myocardial patients. The trial examines the efficacy and safety of a combined administration of G-CSF (to mobilize stem cells) and sitagliptin (to stimulate homing by inhibiting the cleavage of SDF-1 α) after acute myocardial infarction.

Stefanie Dimmeler elaborated on a potential cell therapeutic approach to cardiac disease by augmenting functionality of autologous bone marrow cells. Cardiac repair by bone marrow-derived cells is currently limited due to impaired homing and survival of progenitor cells. However, modulation of microRNA expression in bone marrow-derived cells holds potential to enhance cell function and homing.

DC2010: Forum on Vaccine Science

September 26–30, Lugano, Switzerland

The 11th International Symposium on Dendritic Cells in Fundamental and Clinical Immunology has seen numerous highlights. Here are a few of them.



Tumor immunology and vaccines

Ira Mellman from Genentech Inc. provided an overview of some important developments in cancer therapy that were achieved in 2010. Positive results in a large prostate cancer trial using the presumptive dendritic cell-based vaccine Provenge (sipuleucel-T) led to the first US-FDA approval for an active immunotherapy. Ipilimumab (an anti-CTLA4 antibody) treatment showed promising results in advanced, metastatic melanoma patients. Both therapies showed increases in overall survival.

While these developments help to establish proof of principle in humans, a realistic view reveals that the benefits are modest: The ipilimumab trial

showed a twofold increase in overall survival and some complete remission. However, there are also serious on-target toxicities leading to grade 3–4 adverse events, enterocolitis or hypophysitis, in 23% of the patients.

The mechanisms of action are not resolved; in the case of Provenge it is not even clear whether it functions as a vaccine. Effective cancer immunotherapy seems like a reachable goal, although a lot more investigation will be required. Based on common experience with other targeted therapies, immunotherapy will most likely have to be developed in combination with non-immunological agents.

Wilms' tumor 1 antigen–targeted dendritic cell

Active immunization by tumor antigen-loaded dendritic cells (DCs) represents a promising approach towards adjuvant cancer treatment to eliminate or control residual disease. In most DC trials, however, end-stage cancer patients with high tumor loads were treated.

Viggo van Tendeloo from the University of Antwerp, Belgium, and collaborators performed a phase I/II trial in ten acute myeloid leukemia (AML) patients investigating the effect of autologous DC vaccination. As an immunotherapeutic target, they selected Wilms' tumor 1 protein (WT1), which is a virtually universal tumor antigen. Its role in leukemogenesis is established and it possesses highly immunogenic characteristics.

DCs were matured with TNF- α /PGE₂ and mature DCs (mDCs) were electroporated in the presence

of WT1 mRNA. The DC vaccine was injected intradermally biweekly four times. Until now, 17 patients in remission with a high risk for relapse were treated. In five patients the AML-associated tumor marker returned to normal upon vaccination. Moreover, two patients in partial remission after chemotherapy went into complete remission. Van Tendeloo *et al.* monitored various immunological parameters upon vaccination, which correlated with the clinical responses: WT-1–specific IFN- γ –producing CD8⁺ T cells increased, IL-2 was up-regulated, and NK cells were elevated. There was no correlation between Treg cells or IFN- α ⁺ T cells and the clinical response.

Future approaches will include tagging mRNA with LAMP sequences in order to target tumor antigen to MHC II. This will allow the induction of a CD4⁺ long-term memory response.

Plasmacytoid dendritic cells for immunotherapy in melanoma patients

Jolanda de Vries from Radboud University Nijmegen, The Netherlands, reported the results of the first phase I clinical trial using plasmacytoid dendritic cells (PDCs) for DC-based immunotherapy of melanoma patients. PDCs represent a highly specialized naturally occurring DC subset and are main producers of type I interferons (IFN) in response to viral infections. For vaccination, PDCs were isolated directly from blood in a closed isolation system (CliniMACS® System). After overnight culture, cells were activated by the commonly used TBE (tick-borne encephalitis)–preventative vaccine and loaded with tumor peptides. After six more hours of culture, cells were used for vaccination.

Fifteen stage IV melanoma patients received three intranodal injections of 3 \times 10⁶ PDCs each at two-week intervals.

The results indicate that vaccination with peptide-loaded, TBE-activated PDCs is feasible and that even small numbers of PDCs resulted in TBE-specific T cell proliferation and antibody responses. In two out of 15 patients tumor antigen–specific T cells were detected in delayed-type hypersensitivity reactions after only one round of vaccination, without vaccine-related toxicity. These results break new ground for trials, in which DC subsets are used to optimize DC vaccination.

DNGR-1⁺BDCA3⁺ leukocytes as putative equivalents of mouse CD8 α ⁺ dendritic cells

Lionel Poulin from C. Reis e Sousa's laboratory at the London Research Institute, UK, presented data on the characterization of a human dendritic cell (DC) population, which expresses DNGR-1 (CLEC9A) and high levels of CD141 (BDCA-3). These DCs resemble mouse CD8 α ⁺ DCs in phenotype and function. Like mouse CD8 α ⁺ DCs, human DNGR-1⁺CD141 (BDCA-3)^{high} DCs are TLR3-pos-

itive, TLR7-negative, and (unlike CD8 α ⁺ DCs) TLR9-negative. Upon stimulation with poly(I:C) and TLR8 agonists the cells produce IL-12. This DC subset efficiently takes up material derived from dead cells and cross-presents exogenous antigens to CD8⁺ T cells. The characterization of human DNGR-1⁺CD141 (BDCA-3)^{high} DCs paves the way for utilizing this subset in immunotherapy.

FAQs

Why does Miltenyi Biotec offer MACS® Cytokines at different quality levels? What are the differences between the different grades?

MACS® Cytokines are available at four different quality levels in order to suit the specific requirements of different types of research. MACS Cytokine grades are suitable for the following applications:

Research grade is the standard quality for all our products. Research-grade cytokines are sterile-filtered and pass strict quality control measures. Generally, endotoxin levels are below 0.1 ng/μg (<1 EU/μg) and purities are higher than 95%. Their biological activity has usually been tested employing appropriate bioassays, and minimum activity levels are assured. The ED50 value is also provided.

Cell culture grade cytokines and growth factors are lyophilized with DMEM/F12 and fetal bovine serum or human serum albumin. These cytokines

are best-suited for applications where direct reconstitution in water is preferable, e.g., where small volumes and/or tests in 96-well plates are involved. Selected MACS Cytokines can also be produced at premium-to-GMP grades, with premium grade offering the convenience of determined lot-specific biological activities to eliminate laborious pre-testing.

Premium-grade cytokines share major characteristics with MACS GMP Cytokines for relevant pre-clinical studies.

MACS GMP Cytokines boast both superior quality and GMP compliance attributes: They are manufactured and tested under a certified ISO 9001 quality system and compliant with relevant GMP guidelines.

MACS GMP Cytokines are designed in accordance with the recommendations of USP <1043> on ancillary materials; They are free of all human- and animal-derived components.



Where can I find more information on the biological activities of MACS GMP-grade Cytokine?

The activity of GMP-grade cytokines is determined for each lot, and can be found in the lot-specific Certificate of Analysis with which they are delivered.

Which GMP-grade antigens are available for use with the Cytokine Capture System?

The following GMP-grade antigens may be used with the Cytokine Capture System:

- MACS GMP HCMV pp65 – Recombinant protein
- MACS GMP PepTivator® HCMV pp65
- MACS GMP PepTivator AdV5 Hexon
- MACS GMP PepTivator EBV LMP2a
- MACS GMP PepTivator WT1

What are the main characteristics of Miltenyi Biotec Cell Culture Bags?

Miltenyi Biotec Cell Culture Bags are individually packed, sterile, and make use of non-pyrogenic fluid paths. They are transparent for easy monitoring of cell culturing processes by microscopy, and they are gas-permeable.

I have completed a CliniMACS® Separation and switched off the machine without copying down the process code. Is it still possible to retrieve it?

Yes, the CliniMACS® Plus Instrument automatically stores the process codes for the last 15 separations performed on it. To recall them, simply switch on the device and press “5”, and then “2”, instead of “Enter” when the starting screen is displayed. The most recent process code will be at the very top of the resulting list.



We would greatly appreciate your feedback.

Please e-mail us at macs@miltenyibiotec.de

Conference calendar

Meet us at the booth!

Date	Congress	Webpage
September 4–7, 2011	ESOT – European Society for Organ Transplantation Congress, Glasgow, UK	www.esot.org/Congresses/Glasgow/
September 8–11, 2011	COSTEM – International Congress on Controversies in Stem Cell Transplantation and Cellular Therapies, Berlin, Germany	www.comtecmed.com/costem
September 12–15, 2011	Stem Cells USA & Regenerative Medicine Congress 2011, Boston, MA, USA	www.terrapinn.com/2011/stem-cells-usa-and-regenerative-medicine-congress/
September 30 – October 4, 2011	DGHO, ÖGHO, SGH und SGMO – Gemeinsame Jahrestagung der Deutschen, Österreichischen und Schweizerischen Gesellschaften für Hämatologie und Onkologie, Basel, Switzerland	www.haematologie-onkologie-2011.ch/
October 22–25, 2011	AABB – American Association of Blood Banks Annual Meeting, San Diego, CA, USA	www.aabb.org/events/annualmeeting/
October 29–30, 2011	Advanced EFIS-EJI Course on: Innovative strategies to prevent transplant rejection, Sorrento, Italy	www.ceppellini.it/
November 3–6, 2011	LC 2011 – 12th International Workshop on Langerhans Cells, Innsbruck, Austria	www.lc2011.org/
November 3–6, 2011	iSBTC/SITC – Society for Immunotherapy of Cancer, 26th Annual Meeting, Bethesda, MD, USA	www.sitcancer.org/meetings/am11/
November 10–12, 2011	WBMT – Scientific Symposium of the Worldwide Network for Blood & Marrow Transplantation, Hanoi, Vietnam	www.wbmt.org/
December 10–13, 2011	ASH – American Society of Hematology Annual Meeting, San Diego, CA, USA	www.hematology.org/Meetings/Annual-Meeting/

Fax reply form

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Please mark below and fax to: Miltenyi Biotec, Marketing Department, Attn.: Brigitte Borchert
Fax no. +49 2204 85197

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Clinical Product Catalog 2011/2012 130-090-666.10		<input type="checkbox"/>
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