



Myocardial regeneration – autologous stem cell therapy

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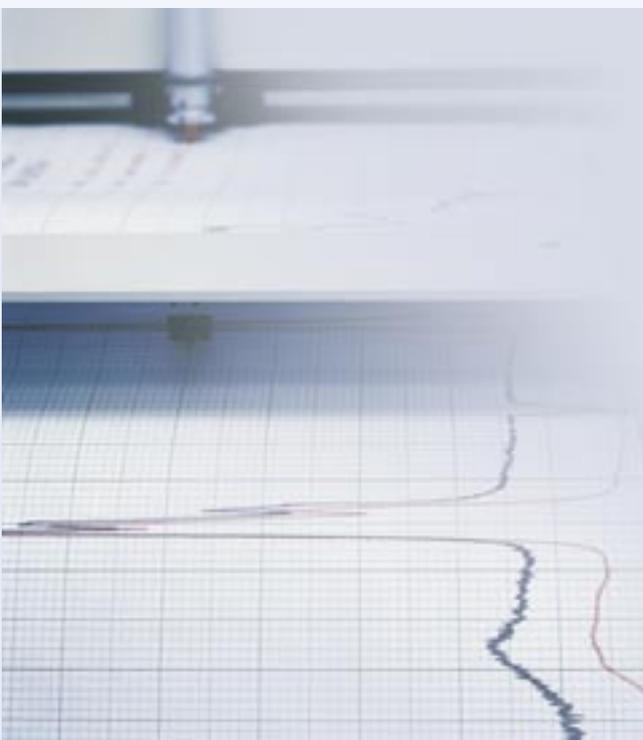
Main patient cohort

- ▶ Patients after recent acute myocardial infarction
- ▶ Patients with chronic ischemic heart disease
- ▶ Patients with end-stage heart failure

Introduction

Despite many recent advances in medical therapy, interventional and surgical techniques, ischemic heart disease and congestive heart failure remain a major cause of morbidity and mortality in the U.S. and other countries. It has been apparent for the last decade that heart disease is not only the leading cause of death, disability, and health care expenses in the U.S., but also the leading cause of death worldwide.

- ▶ **In Europe, coronary heart disease is the most common cause of death, accounting for nearly 2 million deaths each year.**
- ▶ **The death rates from coronary heart disease have decreased in North America and many Western European countries due to improved prevention, diagnosis and treatment; nevertheless, cardiovascular disease still remains the number one health threat.**
- ▶ **The WHO predicts 11.1 million deaths from coronary heart disease in 2020.**



Given the predicted change in population demographics, an aging population will undoubtedly lead to a concomitant increase in the number of people with chronic diseases, including coronary artery disease, heart failure and stroke (fig. 1). In order to meet this demand, it is estimated that the cost to health care will dramatically increase, having an influence on the quality of life, especially of elderly patients.

In spite of the fact that the post-myocardial infarction survival rate has improved with recent medical advances, reduced heart function attributed to irreversible loss of viable cardiomyocytes is still a major clinical problem. This loss of viable tissue results in the formation of scar tissue, and subsequently, in left ventricular remodeling, and progression of congestive heart failure. The initial stage of heart failure is managed with medical therapy; however, treatment options for end-stage heart failure patients are very limited.

Cellular therapy is a promising approach to treat injured tissue and has good potential to improve cardiac function. The exact mechanism underlying this positive outcome has not yet been elucidated and, moreover, is still intensely debated. Nevertheless, the concept of cell therapy has already been introduced into the clinical setting.

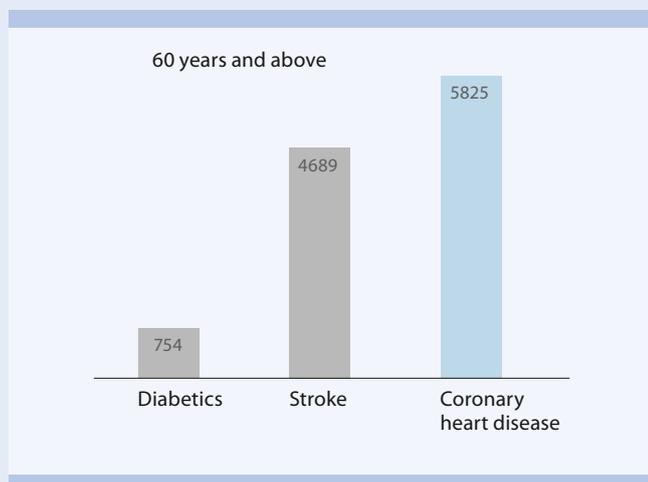


Figure 1: Mortality worldwide from coronary heart disease compared with other causes in 2002. (Number of deaths x 1000; adapted from The Atlas of Heart Disease and Stroke, 2003)

Cell therapy using adult, autologous stem cells

An increasing body of evidence indicate that adult stem cells provide an additional therapeutic option for the treatment of patients suffering from acute myocardial infarction, chronic ischemic heart diseases, and with end-stage heart failure.^{1,2} Within the area of adult stem cells, the marker CD133 (antigen AC133) has become the focus of preclinical and clinical investigations.

► **CD133⁺ cells have been associated with cardiovascular regeneration e.g.:**

- **Myocardial recovery following MI⁵**
- **Vasoregeneration⁶**
- **Implication in postinfarction remodeling of the myocardium⁷**

Expanding the therapeutic horizon—the cellular properties of CD133⁺ cells:

- **CD133 is an important stem cell marker for the identification and isolation of primitive progenitor cells from hematopoietic tissue.³**
- **CD133⁺ cells show pluripotency, i.e., can differentiate into cells comprising all three germ layers⁴**

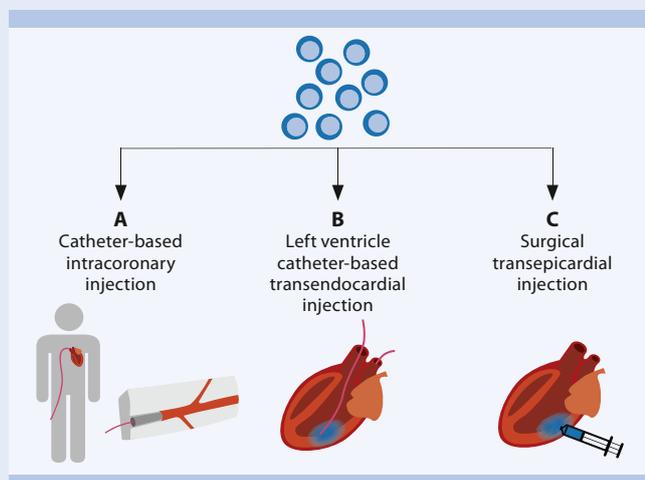


Figure 2: Scheme of different delivery routes for CD133⁺ cells. (A) intracoronary administration of cells, (B) catheter-based, direct intramyocardial injection of cells through the endocardium (guided by fluoroscopy and/or electromechanical mapping), (C) direct intramyocardial injection through the epicardium during open-chest cardiac surgery

Clinical study results

There are different routes to deliver cells to an area of interest. In interventional applications cells are either transferred through a catheter into the infarct-related artery (intracoronary delivery), or directly into the heart muscle by using a catheter-guided mapping system (transendocardial delivery). In surgical applications the intramyocardial delivery of cells directly into the heart muscle by using needle injections (transepicardial delivery) is also applied (fig. 2).

1. A transepicardial method was used to deliver CD133⁺ cells into patients with chronic ischemic heart disease.⁸ This was performed in conjunction with a standard coronary artery bypass grafting (CABG). The results suggested a clear safety profile and tendencies towards:

- ▶ **Enhanced left ventricular ejection fraction (LVEF) and a reduction in the left ventricular end-diastolic volume (LVEDV).**
- ▶ **Improved perfusion at the site of CD133⁺ stem cell injections at 1 year follow-up (fig. 3).^{8,9}**

These positive findings provided the basis for a follow-up phase II clinical trial involving 40 patients.¹⁰

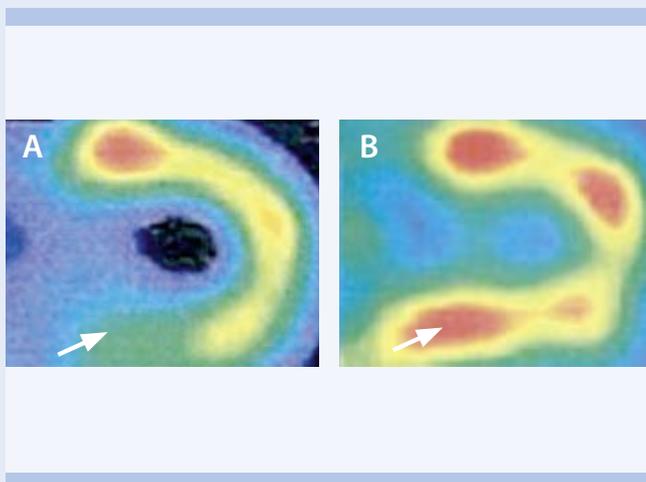


Figure 3: Representative single-photon emission computer tomographic scans of a patient treated with CABG and CD133⁺ cells, (A) preoperative, (B) one year follow-up. (Courtesy of Prof. Steinhoff, Rostock, Germany)

As compared to the pre-operative baseline:

- ▶ **The mean LVEF in the cellular therapy group showed a significant improvement at 6 months follow-up (fig. 4).**
- ▶ **No significant improvement was observed in the control CABG only group (fig. 4).**
- ▶ **35 patients treated with CD133⁺ cells demonstrated a consistent improvement in cardiac function (up to 27% LVEF; mean increase of LVEF of 10% for both, phase I and II study patients).**

Based on these promising findings, the PERFECT study, a multicentric phase III clinical trial, is currently underway. Moreover, similar investigations are also being performed; for example, the CARDIO133 study: a double-blinded, randomized, placebo controlled 60 patient study (<http://clinicaltrials.gov/ct2/show/NCT00462774>).

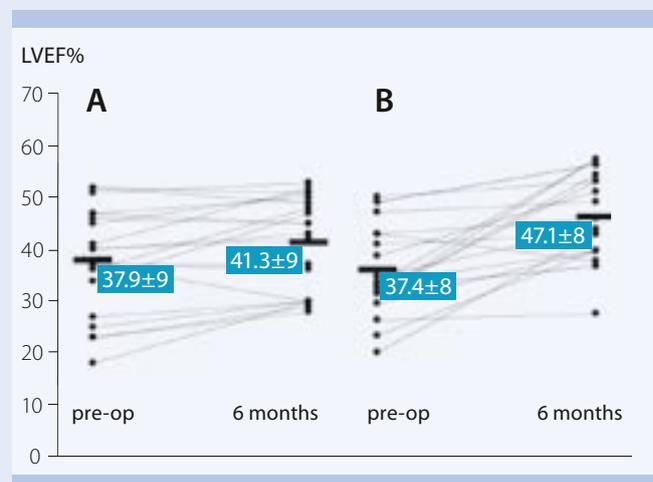


Figure 4: Left ventricular ejection fraction (LVEF) of patients undergoing CABG alone (A) and patients who underwent CABG with CD133⁺ cell injection (B). (Courtesy of Prof. Steinhoff, Rostock, Germany)

2. Transepical injection of enriched CD133⁺ bone marrow-derived cells has been used in combination with transmyocardial laser revascularization (TMLR) and CABG.¹¹

- ▶ **Innovative technique whereby autologous bone marrow-derived CD133⁺ cells are enriched in the operation room whilst the patient is undergoing TMLR/CABG treatment.**
- ▶ **Enriched CD133⁺ cells are injected transepically in a predefined manner around the laser channels.**
- ▶ **Since the original study¹¹, the TMLR/CD133⁺ procedure has been used to treat over 50 patients.^{12,13}**

As compared to the pre-operative baseline^{13,14}:

- ▶ **The mean LVEF in the TMLR/CABG/CD133⁺ cell therapy group showed a significant improvement at 12 months follow-up (fig. 5).**
- ▶ **Improved end-systolic left ventricular wall thickness (fig. 6).**
- ▶ **Notably, in patients treated exclusively with CD133⁺ cells, a sustained improvement in LVEF was demonstrated (fig. 5).**

The INSTEM trial, a multicenter, non-randomized, phase II study has already been started to further investigate the outcome of CD133⁺/TMLR/CABG combinatory therapy for ischemic heart disease.

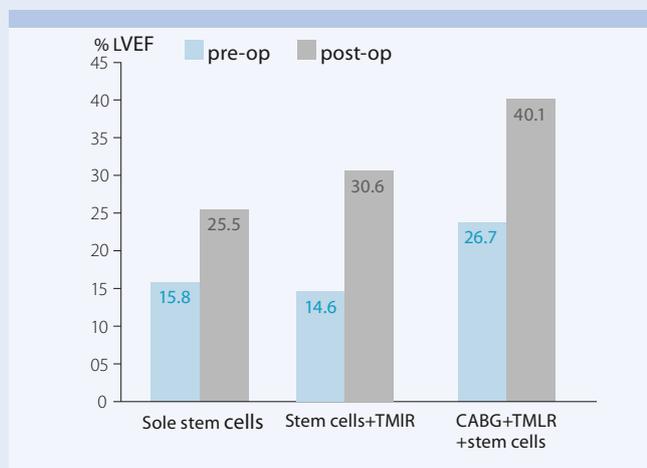


Figure 5: Mean LVEF of patients treated with CD133⁺ cells alone, TMLR and CD133⁺ cells, and a combination of laser treatment, stem cells and CABG at 12 months follow-up. (Courtesy of Prof. Klein, Düsseldorf, Germany)

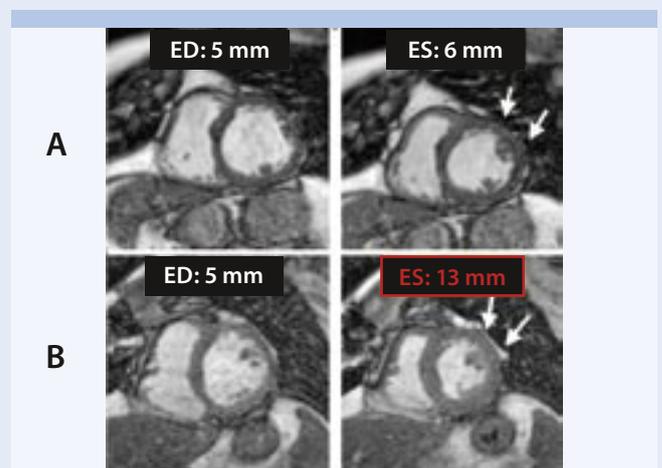


Figure 6: Improved wall thickness of a representative magnetic resonance image (MRI) of a patient treated with TMLR, CD133⁺ cells and CABG at 12 months follow-up. A) preoperative, B) postoperative, ED= end-diastolic, ES= end-systolic. (Courtesy of Prof. Klein, Düsseldorf, Germany)

3. Intracoronary injection of CD133⁺ cells through a catheter was used to treat patients suffering from acute myocardial infarction (AMI) in conjunction with interventional revascularization methods.^{15,16} The feasibility of this procedure was demonstrated in a 35 patient study (table 1). The intracoronary administration of CD133⁺ cells was shown to be feasible, but was associated with a trend towards increased incidence of reocclusion/restenosis events. However, no increase in the incidence of ventricular arrhythmia was noted. Nevertheless, enriched CD133⁺ cells appeared to contribute to functional recovery after myocardial infarction at 4-months follow-up.

To further investigate the observed trends, the SELECT-AMI trial, a multicenter, randomized, double-blinded study started in 2008 (<http://clinicaltrials.gov/ct2/show/NCT00529932>). In addition, another study was initiated to investigate the benefit of CD133⁺ cellular therapy in AMI patients (<http://clinicaltrials.gov/ct2/show/NCT00400959>).

Furthermore, studies using a catheter-guided mapping system and the transendocardial delivery of CD133⁺ cells are forthcoming.

	Group 1 (n=19)		Group 2 (n=16)	
	Baseline	Follow-Up	Baseline	Follow-Up
Heart rate, bpm	73 ± 3	69 ± 3	74 ± 3	70 ± 3
LVEDVI, mL/m	91 ± 7	99 ± 7	91 ± 7	103 ± 9*
LVEF, %	45.0 ± 2.5	52.1 ± 3.5*	44.3 ± 3.1	48.6 ± 3.6
LVSP, mm Hg	111 ± 4	123 ± 4	120 ± 7	121 ± 7
LVSP/LVESVI, (mm Hg/mL m ⁻¹)	1.53 ± 0.3	2.09 ± 0.5*	1.36 ± 0.2	1.45 ± 0.2
Chordae short, %	11.5 ± 1.0	16.1 ± 1.3*	11.1 ± 1.1	12.7 ± 1.3
MIBI defect, %	28.0 ± 4.1	22.5 ± 4.1*	25.0 ± 3.4	22.6 ± 4.1

Table 1: Effect of intracoronary infusion of CD133⁺ cells on left ventricular function and perfusion. Group 1= CD133⁺ cells, Group 2 = control, LVEDVI= left ventricular end-diastolic volume index, LVEF= left ventricular ejection fraction, LVSP= left ventricular systolic pressure, LVESVI= left ventricular end-systolic volume index. * p<0.05 vs. baseline. (Courtesy of Prof. Bartunek, Aalst, Belgium)

Methodology

The enrichment of CD133⁺ cells can be performed from bone marrow aspirates (mostly 100–200 mL; fig. 6) or mobilized leukapheresis products. Nevertheless, bone marrow–derived CD133⁺ cells are preferentially employed in the majority of clinical protocols. Currently, in clinical studies investigating cardiovascular therapies, a dose of $\geq 1 \times 10^6$ CD133⁺ stem cells is typical. The principle of the method is based on the MACS[®] Technology (fig. 6):

- ▶ **Superparamagnetic particles bound to antigen-specific antibodies (here anti-CD133 antibodies).**
- ▶ **Only 50 nm in size.**
- ▶ **Composed of a biodegradable matrix.**
- ▶ **Not necessary to remove particles after separation.****
- ▶ **High-gradient magnetic fields ensure particle-labeled cells (target cells) are retained in the column.**

- ▶ **Non-target cells flow through the column.**
- ▶ **Labeled CD133⁺ cells are released from the column after magnetic field has been removed.**

Flow cytometry analysis is an important part of the overall procedure. The recommended antibodies and gating strategies are described in a special protocol and are available on request.

** Sensitive patients may develop human anti-mouse antibodies (HAMA). To date, no cases of HAMA were observed in heart-related clinical studies.¹⁵

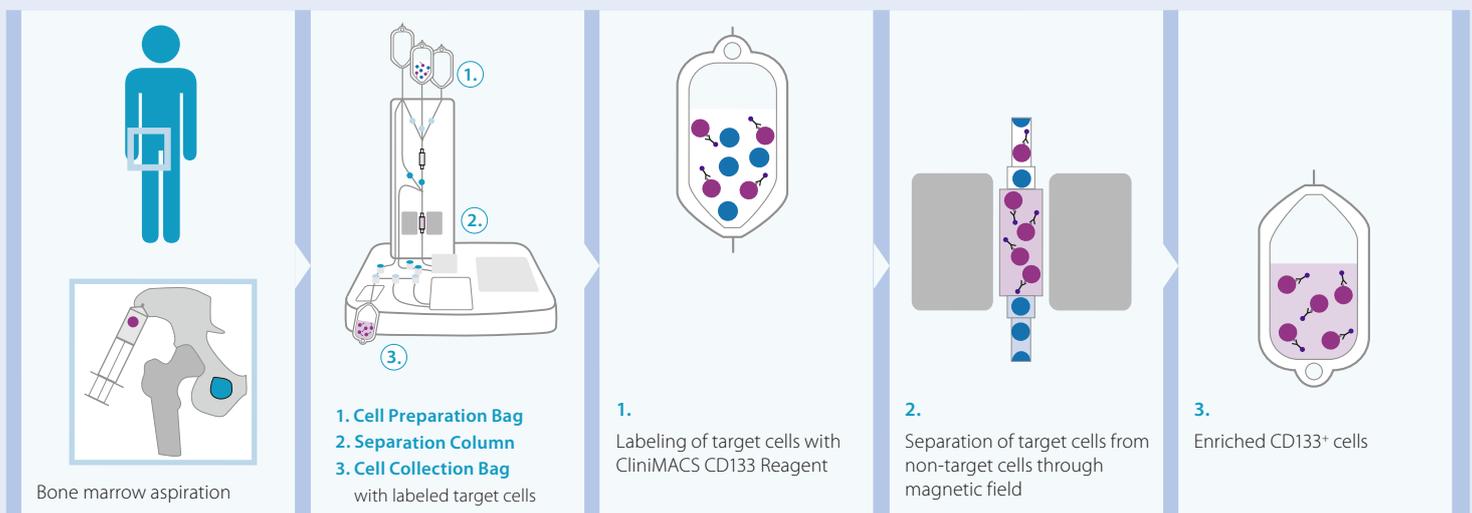


Figure 6: Schematic view of an enrichment procedure using the CliniMACS CD133

- Magnetically labeled target cell
- Non labeled cell (non-target cell)
- Y Antibody
- Y MicroBead + antibody

Logistics

There are different models of how the enrichment procedure can be integrated into daily clinical practice. These may be classified into three main approaches:

- ▶ **Intraoperative: CliniMACS® Technology employed in the operation room during the procedure.**
- ▶ **Extraoperative: Before the surgical procedure, CD133⁺ cells are enriched using local cell processing centers (e.g., blood banks, hematology facilities), which deliver the final cellular product to the treating physician.**
- ▶ **For certain areas, centralized cell processing service is available and can be arranged by Miltenyi Biotec.**

Logistics regarding the integration of the CliniMACS® Technology are dependent on country-specific requirements and should be discussed individually.

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Miltenyi Biotec

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

www.miltenyibiotec.com

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

**Miltenyi Biotec
Australia Pty. Ltd.**
Phone +61 02 8877 7400
macs@miltenyibiotec.com.au

Miltenyi Biotec B.V. (Benelux)
macs@miltenyibiotec.nl
Customer service Netherlands
Phone 0800 4020120
Customer service Belgium
Phone 0800 94016
Customer service Luxembourg
Phone 800 24971

**Miltenyi Biotec Trading
(Shanghai) Co., Ltd. (P.R. China)**
Phone +86 21 6235 1005
macs@miltenyibiotec.com.cn

Miltenyi Biotec SAS (France)
Phone +33 1 56 98 16 16
macs@miltenyibiotec.fr

Miltenyi Biotec S.r.l. (Italy)
Phone +39 051 646 0411
macs@miltenyibiotec.it

Miltenyi Biotec K.K. (Japan)
Phone +81 3 5646 8910
macs@miltenyibiotec.jp

**Miltenyi Biotec Asia Pacific
Pte. Ltd. (Singapore)**
Phone +65 6238 8183
macs@miltenyibiotec.com.sg

Miltenyi Biotec S.L. (Spain)
Phone +34 91 512 12 90
macs@miltenyibiotec.es

Miltenyi Biotec Ltd. (UK)
Phone +44 1483 799 800
macs@miltenyibiotec.co.uk