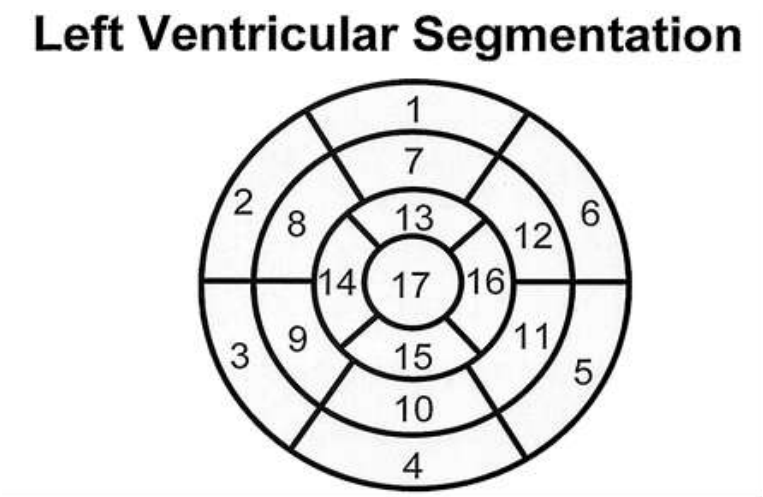
Supplement Figure 1

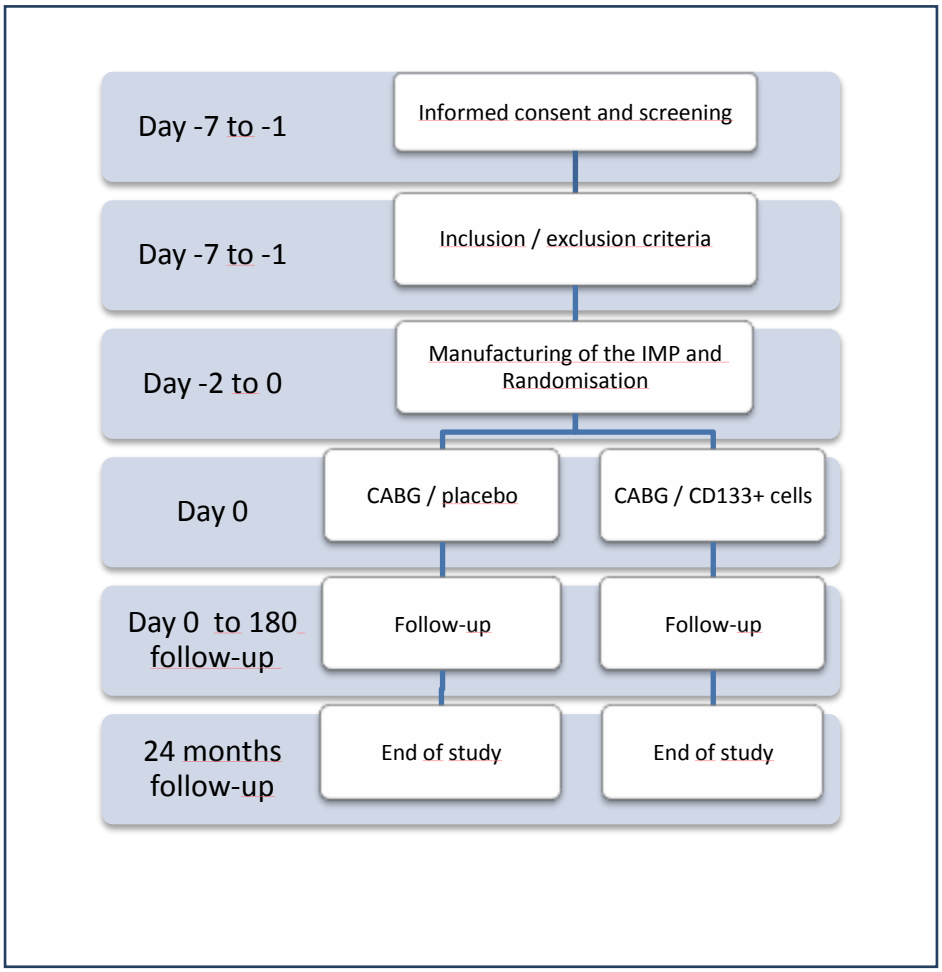


1. basal anterior	7. mid anterior	13. apical anterior
2. basal anteroseptal	8. mid anteroseptal	14. apical septal
3. basal inferoseptal	9. mid inferoseptal	15. apical inferior
4. basal inferior	10. mid inferior	16. apical lateral
5. basal inferolateral	11. mid inferolateral	17. apex
6. basal anterolateral	12. mid anterolateral	

Supplement Figure 1: Left ventricular segmentation for therapy targeting and segmental analysis

Left ventricular segments were used for definition of target tissue (MRI at rest and stress), intraoperative target zone for treatment documentation and outcome parameters (MRI at rest and stress). Evaluation of treatment efficacy was based upon assessment of the LVEF at 6 months postoperatively versus baseline measured by MRI at rest and was the primary efficacy end-point. Cardiac MRI scan parameters recorded were: 1. LV mass (g/m^2), body surface (m^2), weight (kg), height (cm); 2. Left ventricular end diastolic volume (LVEDV), LVESV, LVEF (%), Scar tissue total (g), Non-viable tissue (g); 3. Ventricle function and wall motion (quality) for segments 1-17 (1=hyperkinetic, 0=normokinetic, -1=hypokinetic, -2=akinetic, -3=dyskinetic); 4. Wall motion thickening (%); 5. Regional muscle mass (g); 6. Perfusion at stress and at rest for segments 1-17; 0=normal, 1=reduced subendocardial, 2=reduced transmural; 7. Vitality/late enhancement (LE) for segments 1-17; LE volume (% LV mass), transmurality LE (%); 8. Total assessment vitality for segments 1-17; 0=normal, -1=stunned, -2=hibernating, -3=infarct; 9. Unwanted tissue changes; no/yes, if yes, describe; 10. Pericardial effusion (no, few, moderate, much); 11. Pleural effusion (no, few, moderate, much); 12. Thrombus no/y

Supplement Figure 2: Flow-chart PERFECT trial



Supplement Table 1: Stress Perfusion measured by MRI between groups

	Placebo	p	P^A	CD133+	p	P^A	Responder	p	P^A	Non-responder	p	p^A
	N,Mean,SD minimum maximum			N,Mean,SD minimum maximum			N,Mean,SD minimum maximum			N,Mean,SD minimum maximum		
Perfusion Stress Perfusion score (mean Segment 1-17) (MRI) 0	24, 0·80±0·33 0·2-1·4 Median 0·81	0·084	0·065	27, 0·86±0·42 0-1·6 Median 1·0	0·065	0·006	32, 0·78±0·38 0-1·4 Median: 0·81	0·011	0·004	19, 0·90±0·36 0.3-1·6 Median: 1·0	0·432	0·101
Perfusion Stress Perfusion score (mean Segment 1-17) (MRI) 10d	13, 0·68±0·35 0·1-1·4 Median 0·63	0·667		13, 0·53±0·31 0-1 Median 0·5	0·730		17, 0·54±0·35, 0·0-1·4 Median 0·50	0·841		9, 0·74±0·0·42 0·3-1·2 Median 0·75	0·523	
Perfusion Stress Perfusion score (mean Segment 1-17) (MRI) 180d	24, 0·65±0·33 0-1·1 Median 0·66			27, 0·63±0·49 0-1·7 Median 0·44			32, 0·58±0·39 0-1·6 Median: 0·47			19, 0·73±0·51 0-1·6 Median: 0·81		
Perfusion Stress Perfusion score in injected segments (MRI) 0	24, 1.0±0·5 0-1·8 Median 1·1		0·045	26, 1·05±0·60 0-2 Median 1·1		0·006	31, 0·95±0·48 0-1·7 Median: 1.0		0·009	19, 1·18±0·65 0-2 Median: 1·3		0·034
Perfusion Stress Perfusion score in injected segments (MRI) 180d	24, 0·79±0·56 0-2 Median 0·73			26, 0·77 ±0·66 0-2 Median 0·67			31, 0·71±0·52 0-1.8 Median: 0·67			19, 0·89±0·72 0-2 Median: 0·80		
Perfusion Stress Perfusion score in non-injected segments (MRI) 0	24, 0·63±0·41 0.1-1.6 Median 0·57		0·140	26, 0·73±0·44 0-1·5 Median 0·76		0·057	31, 0·64±0·45 0-1·7 Median: 0·64		0·017	19, 0·73±0·38 0.2-1·5 Median: 0·73		0·383
Perfusion Stress Perfusion score in non-injected segments (MRI) 180d	24, 0·53±0·29 0.1-1·1 Median 0·53			26 0·56±0·50 0-1·5 Median 0·39			31, 0·48±0·40 0-1·5 Median: 0·36			19, 0·65±0·41 0-1·5 Median: 0·67		

SupplementTable 1: Stress Perfusion measured by MRI between groups
Statistical method: Wilcoxon-Test
p-value between 0 days / 10 days and 10 days / 6 months, p^A -value between 0 days and 6 months
Perfusion score: 0- normal perfusion, 1 – hypoperfusion, 2 – strong reduced perfusion

Supplement Table 2: Summary of adverse events during the main trial phase

	$CD133^+$	Placebo	Total	p-value [1]
pts with at least one adverse event (AE)	37 (100%)	40 (100%)	77 (100%)	0·382
number of AE	282	311	593	…
Patients (pts) with at least one serious adverse event (SAE)	19 (51·4%)	15 (37·5%)	34 (44·2%)	0·256
number of SAEs	25	24	49	…
number of Deaths	-	-	-	…
pts with at least one major adverse cardiac event (MACE)	1 (2·7%)	1 (2·5%)	2 (2·6%)	1·000
number of MACEs	1	1	2	…
pts with at least one adverse event s of special interest (AESI)	32 (86·5%)	31 (77·5%)	63 (81·8%)	0·382
number of AESIs	67	68	135	…
pts with at least one AE that was at least possibly related	2 (5·4%)	7 (17·5%)	9 (11·7%)	0·156
number of AEs that were at least possibly related	6	13	19	…
pts with at least one SAE that was at least possibly related	-	2 (5·0%)	2 (2·6%)	0·494
number of SAEs that were at least possibly related	-	2	2	…
pts with at least one AE that were not or unlikely related	37 (100%)	39 (97·5%)	76 (98·7%)	1·000
number of AEs that were not or unlikely related	276	298	574	…
pts with at least one SAE that was not or unlikely related	19 (51·4%)	14 (35·0%)	33 (42·9%)	0·172
number of SAEs that were not or unlikely related	25	22	47	…
pts with at least one AE leading to withdrawal	1 (2·7%)	-	1 (1·3%)	0·481
number of AEs leading to withdrawal	1	-	1	…

Supplement Table 2: For patients (pts) denominator for percentages is column N.
Note: For severity, relation and outcome denominator for percentages is total number of AEs.
[1] Group comparison using Fishers Exact Test.
Multiple occurrences of the same adverse event in one individual counted only once
* For multiple occurrences of the same adverse event maximal intensity is displayed
** For multiple occurrences of the same adverse event 'worst case' relation is displayed
*** For multiple occurrences of the same adverse event outcome of latest AE occurrence is displayed

Source: P132_perfect - AE01T.sas [SVN:29690] Data Extract: 15JUL2016 Generation Date : 23SEP2016 12:45

Supplement Table 3: Serious Adverse Events during Main Trial Phase by Treatment Group and System Organ Class

	Placebo SAEs / Subjects (%)	CD133+ SAEs / Subjects (%)	Total SAEs / Subjects (%)	p-value [1]
All body systems	24 / 15 (38%)	25 / 19 (51%)	49 / 34 (44%)	0·256
Cardiac disorders	8 / 7 (18%)	11 / 10 (27%)	19 / 17 (22%)	0·412
Infections and infestations	7 / 6 (15%)	3 / 3 (8%)	10 / 9 (12%)	0·484
Respiratory, thoracic and mediastinal disorders	1 / 1 (3%)	3 / 3 (8%)	4 / 4 (5%)	0·346
General disorders and administration site conditions	-	3 / 3 (8%)	3 / 3 (4%)	0·106
Injury, poisoning and procedural complications	1 / 1 (3%)	1 / 1 (3%)	2 / 2 (3%)	1·000
Nervous system disorders	-	2 / 2 (5%)	2 / 2 (3%)	0·228
Renal and urinary disorders	1 / 1 (3%)	1 / 1 (3%)	2 / 2 (3%)	1·000
Blood and lymphatic system disorders	1 / 1 (3%)	-	1 / 1 (1%)	1·000
Eye disorders	1 / 1 (3%)	-	1 / 1 (1%)	1·000
Gastrointestinal disorders	1 / 1 (3%)	-	1 / 1 (1%)	1·000
Reproductive system and breast disorders	1 / 1 (3%)	-	1 / 1 (1%)	1·000
Skin and subcutaneous tissue disorders	1 / 1 (3%)	-	1 / 1 (1%)	1·000
Surgical and medical procedures	-	1 / 1 (3%)	1 / 1 (1%)	0·481
Vascular disorders	1 / 1 (3%)	-	1 / 1 (1%)	1·000

Supplement Table : Note: Denominator for percentages is column N. Multiple occurrences of the same adverse event in one individual counted only once

Investigator Term for Adverse Events encoded using MedDRA version 15.0 [1] hGroup comparison using Fishers Exact Test. Source: P132_perfect - AE0301T.sas [SVN:29329] Data Extract: 15JUL2016 Generation Date : 09AUG2016 11

Supplement Table 4 Classification and outcome of the adverse events (AE) during the main trial phase

	CD133+	Placebo	Total	p-value [1]
Severity*				
no AE	-	12 (3·9%)	12 (2·0%)	5·087E-04
Asymptomatic	77 (27·3%)	71 (22·8%)	148 (25·0%)	0·218
symptomatic, no treatment	71 (25·2%)	72 (23·2%)	143 (24·1%)	0·566
symptomatic, specific treatment	128 (45·4%)	154 (49·5%)	282 (47·6%)	0·324
life threatening	6 (2·1%)	2 (0·6%)	8 (1·3%)	0·159
N	282	311	593	…
Relation**				
not related	245 (86·9%)	264 (84·9%)	509 (85·8%)	0·556
Unlikely	31 (11·0%)	34 (10·9%)	65 (11·0%)	1·000
Possible	6 (2·1%)	13 (4·2%)	19 (3·2%)	0·170
N	282	311	593	…
Outcome***				
recovered without sequelae	210 (74·5%)	237 (76·2%)	447 (75·4%)	0·634
recovered with sequelae	10 (3·5%)	10 (3·2%)	20 (3·4%)	0·825
Persisting	38 (13·5%)	47 (15·1%)	85 (14·3%)	0·639
Unknown	24 (8·5%)	17 (5·5%)	41 (6·9%)	0·149
N	282	311	593	…

Supplement Table 4: Note: For patients denominator for percentages is column N.
Note: For severity, relation and outcome denominator for percentages is total number of AEs.
[1] Group comparison using Fishers Exact Test.
Multiple occurrences of the same adverse event in one individual counted only once
* For multiple occurrences of the same adverse event maximal intensity is displayed
** For multiple occurrences of the same adverse event 'worst case' relation is displayed
*** For multiple occurrences of the same adverse event outcome of latest AE occurrence is displayed .
Source: P132_perfect - AE01T.sas [SVN:29690] Data Extract: 15JUL2016 Generation Date : 23SEP2016 12:45

Supplement Table 5: Classification of the AEs during the main trial phase per severity, relationship to treatment and outcome by treatment group

AESI (according to SAP Section)	Coded (MedDRA version 15·0)	Placebo	CD133+	Total	p-value [1]
AV block I, II or III		2 (6%)	3 (8%)	5 (7%)	…
	Atrioventricular block	-	2 (5%)	2 (3%)	0·228
	Atrioventricular block first degree	1 (3%)	1 (3%)	2 (3%)	1·000
	Atrioventricular block complete	1 (3%)	-	1 (1%)	1·000
Prolonged QT interval		3 (8%)	1 (3%)	4 (5%)	…
	Electrocardiogram QT prolonged	3 (8%)	1 (3%)	4 (5%)	0·616
Sinus bradycardia		4 (10%)	1 (3%)	5 (6%)	…
	Bradycardia	4 (10%)	1 (3%)	5 (6%)	0·360
Supraventricular arrhythmia		16 (41%)	19 (52%)	35 (45%)	…
	Atrial fibrillation	9 (23%)	9 (24%)	18 (23%)	1·000
	Supraventricular tachyarrhythmia	2 (5%)	3 (8%)	5 (6%)	0·667
	Arrhythmia supraventricular	2 (5%)	1 (3%)	3 (4%)	1·000
	Supraventricular extrasystoles	2 (5%)	1 (3%)	3 (4%)	1·000
	Supraventricular tachycardia	-	3 (8%)	3 (4%)	0·106
	Atrial flutter	1 (3%)	1 (3%)	2 (3%)	1·000
	Sinus tachycardia	-	1 (3%)	1 (1%)	0·481
Ventricular arrhythmia		8 (21%)	6 (16%)	14 (17%)	…
	Ventricular arrhythmia	2 (5%)	2 (5%)	4 (5%)	1·000
	Ventricular extrasystoles	2 (5%)	2 (5%)	4 (5%)	1·000
	Ventricular tachycardia	3 (8%)	-	3 (4%)	0·241
	Tachyarrhythmia	1 (3%)	-	1 (1%)	1·000
	Ventricular fibrillation	-	1 (3%)	1 (1%)	0·481
	Ventricular flutter	-	1 (3%)	1 (1%)	0·481
Vasovagal syncope		1 (3%)	1 (3%)	2 (3%)	…
	Syncope	1 (3%)	1 (3%)	2 (3%)	1·000
Left ventricular failure		2 (6%)	3 (8%)	5 (7%)	…
	Cardiovascular insufficiency	1 (3%)	1 (3%)	2 (3%)	1·000
	Cardiac failure	1 (3%)	2 (5%)	3 (4%)	0·606
Myocardial ischemia		1 (3%)	2 (5%)	3 (4%)	…
	Acute myocardial infarction	1 (3%)	-	1 (1%)	1·000
	Angina pectoris	-	2 (5%)	2 (3%)	0·228
Cerebral ischemia		-	2 (6%)	2 (6%)	…
	Cerebral infarction	-	1 (3%)	1 (1%)	0·481
	Cerebrovascular accident	-	1 (3%)	1 (1%)	0·481
Myocarditis		-	-	-	-
Pericardial effusion		8 (20%)	6 (16%)	14 (18%)	…
	Pericardial effusion	8 (20%)	6 16%)	14 (18%)	0·772
Pericarditis		-	-	-	-

Supplement Table 5: Classification of the AEs during the main trial phase per severity, relationship to treatment and outcome by treatment group

AESI (according to SAP Section)	Coded (MedDRA version 15·0)	Placebo	CD133+	Total	p-value [1]
Deep sternal wound infection		-	-	-	-
Other arrhythmias		4 (11%)	3 (8%)	7 (9%)	…
	Cardiac arrest	1 (3%)	-	1 (1%)	1·000
	Bradyarrhythmia	1 (3%)	-	1 (1%)	1·000
	Tachycardia	2 (5%)	1 (3%)	3 (4%)	1·000
	Arrhythmia	-	2 (5%)	2 (3%)	0·228

Supplement Table 5: Multiple occurrences of the same adverse event in one individual counted only once· Denominator for percentages is column N· [1] Group comparison using Fishers Exact Test· Note: For patients denominator for percentages is column N·
Note: For severity, relation and outcome denominator for percentages is total number of AEs·
[1] Group comparison using Fishers Exact Test·
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Supplement Table 6: Responder vs. NonResponder Analysis:

Adverse events class	Non-responders (n=23) AEs / Subjects (%)	Responders (n=35) AEs / Subjects (%)	Total SAEs / Subjects (%)	p-value [1]
All cardiac disorders	15 (65%)	25 (71%)	40 (69%)	0·773
Atrial fibrilation	9 (39%)	6 (17%)	15 (26%)	0·074
Ventricular arrhythmia	3 (13%)	0	3 (5%)	0·057
Cardiac failure	3 (13%)	1 (3%)	4 (7%)	0·290
Infections and infestations	8 (35%)	7 (20%)	15 (26%)	0·235
Lung infection	4 (17%)	0	4 (7%)	0·021
Injury, poisoning and procedural complications	11 (48%)	12 (34%)	23 (40%)	0·412
Nervous system disorders	7 (30%)	4 (11%)	11 (19%)	0·093
Renal failure	1 (4%)	4 (11%)	5 (9%)	0·639
Blood and lymphatic system disorders	7 (30%)	6 (17%)	13 (22%)	0·336
Vascular disorders	5 (22%)	9 (26%)	14 (24%)	1·000

Denominator for percentages is column N. Multiple occurrences of the same adverse event in one individual counted only once. Investigator Term for Adverse Events encoded using MedDRA version 15.0 [1] Group comparison using Fishers Exact Test. Source: P132_perfect - AE0301T.sas [SVN:29329] Data Extract: 15JUL2016 Generation Date : 09AUG2016 11:30

CLINICAL STUDY REPORT OF PERFECT 001

Intramyocardial transplantation of bone marrow stem cells for improvement of post-infarct myocardial regeneration in addition to CABG surgery: a controlled, prospective, randomized, double blinded multicentre trial (PERFECT trial)

Development Phase:	III
Name of Investigational Drug:	$CD133^+$ autologous bone marrow stem cells
Sponsor:	Miltenyi Biotec GmbH Friedrich-Ebert-Straße 68 51429 Bergisch Gladbach Germany
Sponsor Study Number:	PERFECT 001 (M-2006-144)
IND/NDA number:	
EudraCT number:	2006-006404-11 (DRKS, Number: DRKS00000213)
Date of first visit:	20 August 2009
Date of last visit:	10 March 2016
Date of database snapshot:	15 July 2016
Principal Investigator:	Professor Dr. med. Gustav Steinhoff Universität Rostock Direktor Klinik und Poliklinik für Herzchirurgie, Schillingallee 35 D-18057 Rostock Phone: +49 (381) 494-6100 E-mail: gustav.steinhoff@med.uni-rostock.de
Author(s):	Claudia Frumento PhD, Medical Writer Professor Dr. Günther Kundt, Statistician Uta Mehdorn, Statistician
Version identification:	Final 1.0 - 08 March 2017

The study was conducted in accordance with the Helsinki Declaration and Good Clinical Practice. Independent Ethics Committee approval and written informed consent were obtained before starting the study.

SIGNATURE PAGE [SPONSOR]

CLINICAL STUDY

REPORT OF STUDY: PERFECT 001 (M-2006-144)

STUDY TITLE: Intramyocardial transplantation of bone marrow stem cells for improvement of post-infarct myocardial regeneration in addition to CABG surgery: a controlled, prospective, randomized, double blinded multicentre trial (PERFECT trial)

AUTHORS: Claudia Frumento PhD

Professor Dr. Günther Kundt, Statistician

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APPROVAL PAGE OF THE PRINCIPAL INVESTIGATOR

CLINICAL STUDY

REPORT OF STUDY: PERFECT 001 (M-2006-144)

STUDY TITLE: Intramyocardial transplantation of bone marrow stem cells for improvement of post-infarct myocardial regeneration in addition to CABG surgery: a controlled, prospective, randomized, double blinded multicentre trial (PERFECT trial)

AUTHORS: Claudia Frumento PhD

Professor Dr. Günther Kundt, Statistician

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I have read this report and confirm that to the best of my knowledge it accurately describes the conduct and results of the study.

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Signature:

Date:

SYNOPSIS

NAME OF SPONSOR Miltenyi Biotec GmbH	**INDIVIDUAL STUDY TABLE REFERRING TO PART OF THE DOSSIER** Volume: Page:	**(FOR NATIONAL AUTHORITY USE ONLY)**
NAME OF FINISHED PRODUCT CD133+ autologous bone marrow derived cells IMPD 1.1.1		
NAME OF ACTIVE INGREDIENT CD133+ autologous bone marrow derived cells		

TITLE OF STUDY

Intramyocardial transplantation of bone marrow stem cells for improvement of post-infarct myocardial regeneration in addition to Coronary Artery Bypass Graft (CABG) surgery: a controlled, prospective, randomized, double blinded multicentre trial (PERFECT trial)

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CD133+ autologous bone marrow derived cells		

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PUBLICATION (REFERENCE)

Validating intramyocardial bone marrow stem cell therapy in combination with coronary artery bypass grafting, the PERFECT Phase III randomized multicentre trial: study protocol for a randomized controlled trial. Donndorf P, Kaminski A, Tiedemann G, Kundt G, Steinhoff G. 2012 Jul 2;13:99. doi: 10.1186/1745-6215-13-99.

STUDY PERIOD	**PHASE OF DEVELOPMENT**
Screening date of first patient in: 20 August 2009 Date of last patient completed: 10 March 2016 The study was prematurely terminated due to very slow patient recruitment	Phase III

NAME OF SPONSOR	**INDIVIDUAL STUDY TABLE**	**(FOR NATIONAL AUTHORITY USE ONLY)**
Miltenyi Biotec GmbH	**REFERRING TO PART**	
NAME OF FINISHED PRODUCT	**OF THE DOSSIER**	
CD133+ autologous bone marrow derived cells IMPD 1.1.1	Volume:	
NAME OF ACTIVE INGREDIENT	Page:	
CD133+ autologous bone marrow derived cells		

OBJECTIVES

Primary objective

Determine whether injection of autologously derived bone marrow stem cells yielded a functional benefit in addition to the coronary artery bypass graft (CABG) operation as determined by left ventricular heart function (left ventricular ejection fraction [LVEF] determined with magnetic resonance imaging [MRI]).

Secondary Objectives

Determine the effects of an injection of autologously-derived bone marrow stem cells on physical exercise capacity, cardiac function, safety and Quality of Life (QoL).

METHODOLOGY

Controlled, prospective, randomized, double blinded multicentre trial

NUMBER OF PATIENTS (PLANNED AND ANALYSED)

Analysed for safety: The safety population comprised all patients randomized into the study and treated. Safety evaluations were performed on the safety population (SAS). All comparisons were executed per group, to which the patients were randomized.

Analysed for efficacy: The “Full Analysis Set” (FAS) following the principle of intent-to-treat (ITT) included every patient randomized and compare the patients per group to which they were randomly allocated, regardless of patients' compliance, or withdrawal from the study. Confirmatory analyses on primary efficacy end-point was to be performed on the full analysis set (FAS) patients. This intention to treat (ITT) analysis was to be considered as the primary one.

The “Per Protocol Set” (PPS) was defined as a subset of the FAS/ITT patients who were compliant with the study protocol. The PPS consisted of all patients from the FAS/ITT group without any major protocol violation. A secondary efficacy analysis of the primary endpoint was performed based upon the PPS, to assess the sensitivity of the analysis to the choice of analysis population.

Planned number of patients: 142

Screened number of patients: 119

Randomized number of patients: 82

MAIN CRITERIA FOR INCLUSION

Inclusion criteria

- Coronary artery disease after myocardial infarction with indication for CABG surgery
- Currently reduced global LVEF assessed at site by cardiac MRI at rest (25% ≤ LVEF ≤ 50%)
- Presence of a localized akinetic/hypokinetic/hypoperfused area of LV myocardium for defining the target area
- Informed consent of the patient
- 18 years ≤ Age < 80 years
- Not pregnant and not planning to become pregnant during the study. Females with childbearing potential had to provide a negative pregnancy test within 1-7 days before OP and had to be using oral or injectable contraception (non-childbearing potential is defined as post-menopausal for at least 1 year or surgical sterilization or hysterectomy at least 3 months before study start).

NAME OF SPONSOR	INDIVIDUAL STUDY TABLE REFERRING TO PART OF THE DOSSIER	(FOR NATIONAL AUTHORITY USE ONLY)
Miltenyi Biotec GmbH	Volume:	
NAME OF FINISHED PRODUCT CD133+ autologous bone marrow derived cells IMPD 1.1.1	Page:	
NAME OF ACTIVE INGREDIENT CD133+ autologous bone marrow derived cells		

Exclusion criteria

- Emergency operation
- Presence of any moderate-severe valve heart disease requiring concomitant valve replacement or reconstruction
- Medical History of recent resuscitation in combination with ventricular arrhythmia classified by LOWN ≥ class II
- Acute myocardial infarction within last 2 weeks
- Debilitating other disease: Degenerative neurologic disorders, psychiatric disease, terminal renal failure requiring dialysis, previous organ transplantation, active malignant neoplasia, or any other serious medical condition that, in the opinion of the Investigator is likely to alter the patient's course of recovery or the evaluation of the study medication's safety
- Impaired ability to comprehend the study information
- Absence of written informed consent
- Treatment with any investigational drug within the previous 30 days
- Apparent infection (c-reactive protein [CRP] ≥ 20 mg/L, fever ≥ 38.5° C)
- Contraindication for MRI scan
- Immune compromise including Anti human immunodeficiency virus (HIV) 1/2, HBsAg, Anti-HBc-IgG, Anti hepatitis C virus (HCV), Treponema pallidum
- Pregnant or breast feeding
- Childbearing potential with unreliable birth control methods
- Have previously been enrolled in this study, respectively phase I and phase II
- Known hypersensitivity or sensitization against murine products and human-anti-mouse-antibody-titer ≥ 1:1000
- Contraindication to bone marrow aspiration
- Known hypersensitivity against iron dextran

INVESTIGATIONAL DRUG, DOSE AND MODE OF ADMINISTRATION, BATCH NUMBER

0.5-5x10^6 CD133+ in 5 mL saline and serum suspension, injected intramyocardially during CABG surgery.

The 5 mL suspension were distributed in 15 individual 1 mL syringes (26 Gauge needle) of 0.3 mL aliquots (in total 5 mL, including up to 0.5 mL rest in syringes) and were applied within 3 minutes in the region of interest (infarction border zone) at the end of bypass surgery. No more than one injection per square centimetre could be injected.

REFERENCE DRUG, DOSE AND MODE OF ADMINISTRATION, BATCH NUMBER

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Miltenyi Biotec GmbH	**REFERRING TO PART**	
NAME OF FINISHED PRODUCT	**OF THE DOSSIER**	
CD133+ autologous bone marrow derived cells IMPD 1.1.1	Volume:	
NAME OF ACTIVE INGREDIENT	Page:	
CD133+ autologous bone marrow derived cells		

DURATION OF TREATMENT

Not defined

CRITERIA FOR EVALUATION – SAFETY ENDPOINTS

- Major adverse cardiovascular events (MACE - cardiac death, myocardial infarction, secondary intervention/reoperation, ventricular arrhythmia).
- Adverse Events of Specific Interest (AESI): AV-block (I, II or III), prolonged QT interval, sinus bradycardia, supraventricular arrhythmia, ventricular arrhythmia, vasovagal syncope, left ventricular failure, myocardial ischemia, cerebral ischemia, myocarditis, pericardial Effusion, pericarditis, deep sternal wound infection (or wound infection at the site of graft sampling) coded as “deep postoperative wound infection” (Meddra LLT 10074392).
- Serious Adverse Events (SAEs)and Adverse Events (AEs).

CRITERIA FOR EVALUATION – EFFICACY ENDPOINTS

Primary endpoint: LVEF at 6 months postoperatively, measured by MRI at rest and change in LVEF at 6 months post-OP compared with preoperatively (screening) and early postoperatively (discharge) as assessed by MRI. Cardiac MRI was established as the gold standard for determination of LV function (LVEF and LV volumes).

Secondary endpoints:

- Change in LVEF at 6 months post-OP compared with preoperatively (screening) and early postoperatively (discharge) as assessed by echocardiography.
- Change in LV dimensions (left ventricular end systolic dimension [LVESD], [LVEDD]) at 6-month post-OP compared with preoperatively (screening) and early postoperatively (discharge) as assessed by echocardiography.
- Change in physical exercise capacity determined by 6-minute walk test at 6 months post-OP compared with preoperatively (screening) and early postoperatively (discharge).
- Change in New York Heart Association (NYHA) and CCS class at 6 months post-OP compared with preoperatively (screening) and early postoperatively (discharge).
- MACE (cardiac death, myocardial infarction, secondary intervention/reoperation, ventricular arrhythmia).
- QoL-score at 6 months post-OP compared with preoperatively (screening) and 3 months (telephone).

STATISTICAL METHODS

Interim Analysis (IA)

An interim analysis was performed on the first 70 patients randomized, and followed-up for at least 6 months.

Main Analysis (MA)

When all patients included into the trial (planned: 142) had had their 6 Month Follow-up visit the main analysis was to be performed.

In case of stopping for futility patient recruitment was to be stopped. All patients included so far were to be followed up for safety evaluation as foreseen by the protocol. All data analysis as foreseen in MA except the

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CD133+ autologous bone marrow derived cells IMPD 1.1.1	Volume:	
NAME OF ACTIVE INGREDIENT	Page:	
CD133+ autologous bone marrow derived cells		

confirmatory statistical test were to be performed based on the data of those patients enrolled so far at the time point when all patients enrolled so far were followed for at least 6 months.

Safety-Follow-up-Analysis (SFUA)

A Safety-Follow-up-Analysis was to be performed at the time point when the last patient was followed up for additional 18 months and her/his 24-month visit was performed and data are included into the database.

In case of stopping the trial the SFUA was to be performed when all patients enrolled so far had their 24-month safety follow-up visit.

Sample Size

Sample size determination was done under the assumption of a two-sided type I error (α) at 5% and a type II error (β) at 10% (i.e. a power at 90%).The scenario of a difference in LVEF at month 6 post- operatively between the two treatment arms of about 4 to 5 was considered as a clinical relevant difference. With a difference of 4.5 and a standard deviation of 7.5, at least n=60 patients per group were considered necessary and with an additional 15% drop-out rate a total of at least 142 patients were to be randomized.

SAFETY RESULTS

Summary of Adverse Events

- The occurrence rate of MACE was very low when compared with the occurrence reported in the literature for patients undergoing CABG surgery (26.9%). With a p-value of 1.000, there was no difference in the occurrence of MACE between the two groups of patients (1 MACE in the placebo group and 1 MACE in the CD133+ group).
- In the AESIs analysis no statistically significant differences could be observed between both treatment groups indicating that the AESIs were related either with the CABG surgery or the underlying disease (67 AESIs in the placebo group and 68 AESIs in the treatment group);
- During the main trial phase, there were 49 SAEs, 25 (15 patients) in the placebo group and 24 (19 patients) in the CD133+ group. There were no statistical differences observed between the placebo and the CD 133+ group neither overall nor in any of the system organ classes. The most common SAEs were cardiac disorders followed by infections and infestations and respiratory, thoracic and mediastinal disorders. There were no SAEs considered related with the treatment in the CD133+ group.
- In total, there were 619 AEs during the study. Twenty-six AEs during the screening phase and 593 AEs during the main trial phase. There were no statistical differences between the placebo and the CD133+ group, neither overall nor in any of the categories in which the patients or AEs were classified.
- All patients experienced at least one AE during the main trial phase. Overall, there were 19 AEs and two SAEs that were at least possibly related. There were no deaths during the screening nor the main trial phase.

EFFICACY RESULTS

Primary Efficacy

With p-values of 0,8130 and 0,4454 for Visit III and Visit V respectively in the FAS/ITT and p-values of 0,6771 and 0,4261 for Visit III and Visit V respectively in the PPS, the difference between the treatments groups is not statistically significant. The probability of not having detected a possible positive effect of the therapy (injection of CD133+ in the myocardium) on the improvement of LVEF is very small.

Though there were no statistical tests performed with the following endpoints, the following was observed:

- Unadjusted values of LVEF measured by MRI revealed a larger increase in the patients treated with CD133+. In the FS/ITT, baseline LVEF for the placebo group was 35,1 compared to 42.5 at Visit V and for the treatment group it was 32.7 at baseline versus 44.1 at Visit V.
- Mean values of scar tissue in the FAS/ITT measured by MRI were less in the CD133+ group than in the placebo group (27.0 versus 37.3) and in the PPS were also less in the CD133+ group than in the placebo group (27.9 versus 34.8).
- Mean values of non-viable tissue in the FAS/ITT measured by MRI were less in the CD133+ group than in the placebo group (20.0 versus 30.2) and in the PPS were also less in the CD133+ group than in the placebo group (20.7 versus 28.0).

Secondary Efficacy

The secondary efficacy analysis was only a descriptive analysis of the variables and no tests were done regarding possible differences between the placebo and the active treatment group. The following was observed:

- The poor quality of the echocardiographies did not allow any conclusions nor a comparison with the MRI results. Since the two previous studies used the LVEF measured with echocardiography as a primary endpoint, it was decided to use this same method for the secondary endpoints to be able to compare with the previous studies.
- The 6MWT showed the following changes (Visit V-Visit 1) in the mean values: in the FAS/ITT (49.3 for the placebo group and 59.4 in the treatment group. In the PPS, these changes were 50.7 for the placebo group and 56.1 in the treatment group.
- Minimal changes in NYHA class and CCS were observed after surgery plus placebo or active treatment. Mean difference of the NYHA in the placebo and treatment group was -0.7 in the FAS/ITT and in the PPS the mean differences were -0.7 in the placebo group and -0.8 in the treatment group. Mean difference of the CCS in the placebo group was -1.4 in the treatment group was -1.0 in the FAS/ITT and in the PPS the mean differences were -1.4 in the placebo group and -0.9 in the treatment group.
- The occurrence rate of MACE was very low when compared with the occurrence reported in the literature for patients undergoing CABG surgery (26.9%). With a p-value of 1, there was no difference in the occurrence of MACE between the two groups of patients (1 MACE in the placebo group and 1 MACE in the CD133+ group). There were no changes in the EQ-5D - mobility index
- The EQ-5D VAS showed some changes in the mean value: in the FAS/ITT (6.1 for the placebo group and 11.1 in the treatment group. In the PPS, these changes were 4.4 for the placebo group and 16.4 in the treatment group. It should be noted that the increase of the index indicates an improvement of the condition.
- The MLHF-Q total score showed changes in the mean value: in the FAS/ITT (-14.7 for the placebo group and -8.6 in the treatment group. In the PPS, these changes were -16.1 for the placebo group and -10.1 in the treatment group. It should be noted that a negative change in the index indicates an improvement of the condition.

CONCLUSIONS

The procedure was demonstrated to be safe, showing a low incidence of SAEs and MACEs when compared to the SAEs and MACEs in other trials.

Overall, the LVEF increase in the total population (placebo and treatment group) was clinically significant (FAS/ITT: 9,5%; PPS: 9,6%), however the study could not demonstrate a positive effect of the CD133 injection in the LVEF 6 months after surgery.

VERSION IDENTIFICATION FINAL 1.0 – 08 MARCH 2017

Appendix 2: Design and course of the PERFECT trial (ClinicalTrials.gov identifier: NCT00950274)

Inclusion criteria:

Coronary artery disease after myocardial infarction with indication for CABG surgery
Currently reduced global LVEF assessed at site by cardiac MRI at rest (25% ≤ LVEF ≤ 50%)
Presence of a localized akinetic/hypokinetic/hypoperfused area of LV myocardium for defining the target area
Informed consent of the patient
18 years ≤ Age < 80 years
Are not pregnant and do not plan to become pregnant during the study..

Exclusion criteria:

Emergency operation
Presence of any moderate-severe valvular heart disease requiring concomitant valve replacement or reconstruction
Medical History of recent resuscitation in combination with ventricular arrhythmia classified by LOWN ≥ class II
Acute myocardial infarction within last 2 weeks
Debilitating other disease: Degenerative neurologic disorders, psychiatric disease, terminal renal failure requiring dialysis, previous organ transplantation, active malignant neoplasia, or any other serious medical condition that, in the opinion of the Investigator is likely to alter the patient's course of recovery or the evaluation of the study medication's safety
Impaired ability to comprehend the study information
Absent informed written consent
Treatment with any investigational drug within the previous 30 days

Apparent infection (c-reactive protein [CRP] ≥ 20 mg/L, fever ≥ 38.5° C)
Contraindication for MRI scan
Immune compromise including active infection with Hepatitis B, C, HIV virus or seropositivity for Treponema pallidum

Pregnant or breast feeding

Childbearing potential with unreliable birth control methods
Have previously been enrolled in this study, respectively phase I and phase II
Known hypersensitivity or sensitization against murine products and human-anti-mouse-antibody-titer ≥ 1:1000
Contraindication to bone marrow aspiration
Known hypersensitivity against iron dextran

Prespecified outcomes

Primary Outcome Measures:

Left ventricular ejection fraction at rest, measured by MRI [Time Frame: 6 months]
[Designated as safety issue: No]

Secondary Outcome Measures:

Change in LVEF as assessed by MRI and echocardiography [Time Frame: early postoperatively and 6 months]
[Designated as safety issue: No]

Regional contractility in the AOI / Change in LV dimensions[1] (left ventricular end systolic diameter [LVESD], left ventricular end diastolic diameter [LVEDD]) as assessed by echocardiography [Time Frame: early postoperatively (discharge), 6 months] [Designated as safety issue: No]

Physical exercise capacity determined by 6 minute walk test [Time Frame: early postoperatively (discharge), 6 months] [Designated as safety issue: No]

NYHA and CCS class [Time Frame: early postoperatively (discharge), 6 months]
[Designated as safety issue: No]

MACE (cardiac death, myocardial infarction, secondary intervention/reoperation, ventricular arrhythmia)
[Time Frame: 6 months] [Designated as safety issue: Yes]

QoL-score[2]: Minnesota Living with Heart Failure Questionnaire, SF36 Questionnaire, EQ-5D Questionnaire
[Time Frame: 3 months, 6 months post-OP] [Designated as safety issue: No]

Recruitment period
Enrollment: 82
Study Start Date: July 2009
First-patient-in: October 2009
Last-patient-completed: March 10, 2016
Estimated study completion date: March 2018
Primary completion date: March 8, 2017 (final data collection date for primary outcome measure)

Interim analysis
An interim efficacy analysis was performed on the FAS/ITT analysis population. Certain demographic and baseline parameters were presented in summary or frequency tables as specified below as well as reasons for exclusion from SAS. Besides the evaluation of the primary endpoint the following secondary endpoints were evaluated based on the FAS/ITT population:

- Change in LVEF at 6 months post-OP compared with preoperatively (screening) assessed by echocardiography
- Change in physical exercise capacity determined by 6-minute walk test at 6 months compared with preoperatively
- Change in NYHA and CCS class at 6 months post-OP compared with preoperatively
- MACE (cardiac death, myocardial infarction, secondary intervention/reoperation, ventricular arrhythmia).

Results of the interim analysis
In total, 35 patients were treated with the test product and 35 patients were treated with placebo.
The interim primary efficacy analysis (LVEF at Visit V) indicated that there was a statistically significant difference between the treatment group and the placebo group (mean LVEFs 40.44 vs. 44.94; p=0.026).
The interim efficacy analysis of the secondary endpoints LVEF-, 6MWT-, NYHA- and CCS-change did not show remarkable group differences.
It was decided to continue the study until completion of enrolment.

Criteria for stop of patient recruitment
Patient recruitment was stopped on 12th November 2015 when 82 (40 in the test product group, 40 in the placebo group, and 2 randomized but no treated) instead of the planned 142 evaluable patients were included in the trial. Due to this lower number of patients several changes to the planned analysis were considered.
The most relevant change was:
The trial was conducted at six study sites. Since there were more than 20 patients recruited in only two centres and the other centres recruited less than 10 patients each, it was decided not do a per centre analysis.

Appendix 3: List of biomarkers, analytical methods and units.

Bio-marker	**Bio-logical sample**	**Time points**	**Method / Technology / Assay**	**Additional Method details / crucial points**	**Analysis / formula / standardization**	**Unit**	**Reference**
Total and living cell number of $CD133^+$	BM $CD133^+$ SC	ASS I	Magnetic isolation 1	Before cell insolation an aliquot of filtered undiluted BM was taken for quality control analysis via flow cytometric measurements (see below) to determine numbers of living cells in the source material. The isolation process was performed as described (Skorska *et al*., 2017).	Total number of nucleated cells was determined using 3% Acetic Acid with Methylene Blue (STEMCELL ^{TM} Technologies Inc.). Living cells in MACS-purified $CD133^+$ SC suspension was determined using trypan blue dye(0.4 %, Sigma-Aldrich) and hemocytometer chamber (Marienfeld-Superior)		Skorska *et al*., 2017
Viability	BM $CD133^+$ SC	ASS I	Flow cytometry: Quality Control (QC) 2	Compensation was established using single stained controls and gating was performed with matched isotype/fluorescence minus one (FMO) controls. An adapted Boolean gating strategy for $CD133^+$ cells was arranged on the basis of the International Society of Hematotherapy and Graft Engineering (ISHAGE) guidelines for $CD34^+$ cell analysis.		[%]	ISHAGE Guidelines (Sutherland *et al.*, 1996), internal Boolean gating strategy (Mueller *et al.*, 2016, Skorska *et al.*, 2017)

Frequency of the viable $CD45^{dim}$ $CD34^{+}CD133^{+}$ cells (=target cells)				Following exclusion of dead $CD45^{+}$ leukocytes (R1 to R2), region containing positive CD34 cells with low expression of CD45 antigen ($CD45^{dim}$) and with blast morphology (FSC/SSC plot) were chosen. Further gating strategy for enumeration of the $CD133^{+}$ target cells was made in the same manner as for the interim $CD34^{+}$ analysis. Frequency of the target cells among all leukocytes: Frequency of the target cells among all viable leukocytes:	$= \frac{(\text{viable } CD45^{+}\text{events(R2)})}{(\ CD45^{+}\text{events(R1)})} \times 100\ \%$ $= \frac{(\text{viable } CD45^{dim}CD34^{+}CD133^{+}\ (R6))}{(R1)} \times 100\ \%$ $= \frac{(R6)}{(R2)} \times 100\ \%$		
Frequency of the viable $CD45^{dim}$ $CD34^{+}$ ($CD133^{-}$) cells	un-diluted filtered BM	ASS I		Frequency of $CD133^{+}$ SC among all leukocytes: Frequency of the target cells among all viable leukocytes:	$= \frac{(\text{viable } CD45^{dim}CD34^{+}CD133^{-}\ )}{(R1)} \times 100\ \%$ $= \frac{(\text{viable } CD45^{dim}CD34^{+}CD133^{-})}{(R2)} \times 100\ \%$	[%]	
Frequency of the viable $CD45^{dim}$ $CD133^{+}$ cells				Frequency of $CD133^{+}$ SC among all leukocytes: Frequency of the target cells among all viable leukocytes:	$= \frac{(\text{viable } CD45^{dim}CD133^{+}\ (=\ R6\ IHG\ 133))}{(R1)} \times 100\ \%$ $= \frac{(R6\ IHG\ 133)}{(R2)} \times 100\ \%$		

Frequency of the viable $CD45^{dim}$ $CD34^{-}$ ($CD133^{+}$) cells				Frequency of $CD133^{+}$ SC among all leukocytes: Frequency of the target cells among all viable leukocytes:	$= \frac{(\text{viable } CD45^{dim}CD34^{-}CD133^{+})}{(R1)} \times 100\ \%$ $= \frac{(\text{viable } CD45^{dim}CD34^{-}CD133^{+})}{(R2)} \times 100\ \%$		
$CD34^{+}$ $CD133^{+}$ ($CD117^{+}$)	BM $CD133^{+}$ SC	ASS I	Flow cytometry Stemness characterization (2x7-fold staining) 3	Frequency of each subset was determined within $CD45^{dim}$ $CD34^{+}CD133^{+}$ (=R6) region in accordance with an internal adapted protocol (ISHAGE). Frequency of viable $CD117^{+}$ cells was determined within $CD45^{dim}$ $CD34^{+}CD133^{+}$ (R6) region in accordance with ISHAGE guidelines	$= \frac{(\text{viable } CD117^{+}\text{events})}{(\text{ viable } CD45^{dim}CD34^{+}CD133^{+}\ (R6))} \times 100\ \%$	[%]	Laupheimer *et al.*, 2014; Lux *et al.*, 2015
$CD34^{+}$ $CD133^{+}$ ($CD309^{+}$)				Frequency of viable $CD309^{+}$ cells was determined within R6 region in accordance with ISHAGE guidelines	$= \frac{(\text{viable } CD309^{+}\text{events})}{(\text{ R6})} \times 100\ \%$		
$CD34^{+}$ $CD133^{+}$ ($CD184^{+}$)				Frequency of viable $CD184^{+}$ cells was determined within R6 region in accordance with ISHAGE guidelines	$= \frac{(\text{viable } CD184^{+}\text{events})}{(\text{ R6})} \times 100\ \%$	[%]	
$CD34^{+}$ $CD133^{+}$ ($CD117^{+}$ $CD309^{+}$)				Frequency of viable $CD117^{+}CD309^{+}$ was determined within R6 region in accordance with ISHAGE guidelines	$= \frac{(\text{viable } CD117^{+}CD309^{+}\text{events})}{(\text{ R6 })} \times 100\ \%$		
$CD34^{+}$ $CD133^{+}$ ($CD117^{+}$ $CD184^{+}$)	BM $CD133^{+}$ SC	ASS I		Frequency of viable $CD117^{+}CD184^{+}$ was determined within R6 region in accordance with ISHAGE guidelines	$= \frac{(\text{viable } CD117^{+}CD184^{+}\text{events})}{(\text{ R6 })} \times 100\ \%$		
$CD34^{+}$ $CD133^{+}$ ($CD14^{+}$)				Frequency of viable $CD14^{+}$ was determined within R6 region in accordance with ISHAGE guidelines	$= \frac{(\text{viable } CD14^{+}\text{events})}{(\text{R6 })} \times 100\ \%$		
Total cell count of	PB	ASS I-III	Density gradient centrifugation		Total number of living MNC was determined using Neubauer chamber and trypan blue dye.	[1/	Donndorf *et al.*, 2015

living MNC			4			mL] [n]	
Subpopu-lations	MNC PB	ASS I-III	Flow cytometry staining Phenotypic characterization of endothelial progenitor (EPC) and circulating endothelial (CEC) cell subpopulations 5	Beside the Cytometer Setup & Tracking (CS&T) beads used for a daily cytometer performance, using a single staining of 0.5×10^5 mononuclear cells (MNC) the compensation for each patient was optimal adjusted. At each time point 1×10^6 cells were applied for multiple color staining (in each case for EPC and CEC probe) and for unstained probe. Generally, the optimal setting for gating was performed on the basis of FMO staining. The list of antibodies added to the respective multicolor staining either of EPC or CEC panel is shown separately below. CAVE: Number of events of each subpopulation was taken from the flow cytometric analysis performed in accordance with ISHAGE Guidelines; for calculation: total cell number of viable MNC was obtained using trypan blue dye and hemocytometer chamber and normalized per mL of peripheral blood was used (double-platform method).	Two different interpretations of the raw data were conducted: 1) the gating strategy was done as for BM (please see the 7-fold staining description above) in accordance with ISHAGE guidelines and the relative frequencies were generated, 2) frequencies of only single subpopulations were generated within viable $CD45^+$ region. List of subpopulations determined using flow cytometry and the respective formula used for calculation their concentrations per mL of peripheral blood applied are shown below. Concentrations of the respective subpopulations were generated as in formula shown. The fixed formula defined as “A” and “B” are: $$A = \frac{\text{total cell number of mononucelar cells determined with Trypan blue}}{\text{total volume of peripheral blood [mL]}}$$ $$B = \frac{'\text{living events}'}{\text{A}}$$		

$CD34^+$	MNC PB	ASS I-III	Flow cytometry Panel: Endothelial progenitor cells (EPC) 6	CAVE: Number of events was taken from flow cytometric analysis (accordingly with ISHAGE) Concentration of $CD34^+$ cell subpopulation per 1 mL peripheral blood. Frequency of $CD34^+$ cell within the living cells.	$\frac{(\#\text{viable } CD45^{dim}CD34^+ \text{ backgate events})}{B}$ $\frac{(\text{viable } CD45^{dim}CD34^+ \text{ backgate events})}{(\text{living events})} \times 100\ \%$	[1 / mL PB] [%]	
$CD34^+$ $CD133^+$				Concentration of $CD133^+$ cell subpopulation per 1mL peripheral blood. Frequency of $CD34^+$ $CD133^+$ cell subpopulation within the living cells.	$\frac{\#\text{viable } CD45^{dim}CD34^+CD133^+ \text{ backgate events}}{B}$ $\frac{(\#\text{viable } CD45^{dim}CD34^+CD133^+ \text{ backgate events})}{(\text{living events})} \times 100\ \%$		
$CD117^+$	MNC PB	ASS I-III		Concentration of $CD117^+$ cell subpopulation per 1mL peripheral blood. Frequency of $CD117^+$ cell subpopulation within the living cells.	$\frac{\#\text{viable } CD45^{dim}CD117^+ \text{ backgate events}}{B}$ $\frac{(\#\text{viable } CD45^{dim}CD34^+CD133^+ \text{ backgate events})}{(\#\text{living events})} \times 100\ \%$	[1 / mL PB] [%]	
CD45_PB_ EPC_ IHG	MNC PB	ASS I-III		Frequency of viable $CD45^+$ cell sub-population estimated within the viable MNC (data taken from analysis made on the basis of the ISHAGE guidelines)	$\frac{(\text{viable } CD45^+ \text{ events})}{(\text{living events})} \times 100\ \%$	[%]	
Liv_PB_ EPC_IHG				Frequency of all viable cells estimated within all MNC (data taken from analysis made on the basis of the ISHAGE guidelines)	$\frac{(\#\text{living events})}{(\#\text{all events})} \times 100\ \%$		
CD34_133 _184_PB_ EPC _IHG				Frequency of the $CD184^+$ cell subpopulation within viable $CD34^+CD133^+$ region (estimated according to the ISHAGE rules)	$\frac{(\#\text{ viable } CD45^{dim}\ CD34^+CD133^+(CD184^+)\text{events})}{(\#\text{ living events})} \times 100\ \%$		

CD34_133_117_PB_EPC _IHG				Frequency of the $CD117^+$ cell subpopulation within viable $CD34^+CD133^+$ region (estimated according to the ISHAGE rules)	$\frac{(\#\ \text{viable}\ CD45^{dim}\ CD34^+CD133^+(CD117^+)\text{events})}{(\#\text{living events})} \times 100\ \%$		
CD34_133_309_PB_EPC_IHG				Frequency of the $CD309^+$ cell subpopulation within viable $CD34^+CD133^+$ region (estimated according to the ISHAGE rules)	$\frac{(\#\ \text{viable}\ CD45^{dim}\ CD34^+CD133^+(CD309^+)\text{events})}{(\#\text{living events})} \times 100\ \%$		
CD34_PB_EPC_ IHG				Frequency of the viable $CD34^+$ region (estimated according to the ISHAGE rules)	$\frac{(\#\ \text{viable}\ CD45^{dim}\ CD34^+\text{events})}{(\#\text{living events})} \times 100\ \%$		
CD34_184_PB_EPC_IHG				Frequency of the $CD184^+$ cell subpopulation within viable $CD34^+$ region (estimated according to the ISHAGE rules)	$\frac{(\#\text{viable}\ CD45^{dim}\ CD34^+\ (CD184^+)\text{events})}{(\#\text{living events})} \times 100\ \%$		
CD34_117_PB_EPC_IHG				Frequency of the $CD117^+$ cell subpopulation within viable $CD34^+$ region (estimated according to the ISHAGE rules)	$\frac{(\#\ \text{viable}\ CD45^{dim}\ CD34^+(CD117^+)\text{events})}{(\#\text{living events})} \times 100\ \%$		
CD34_309_PB_EPC_IHG				Frequency of the $CD309^+$ cell subpopulation within viable $CD34^+$ region (estimated according to the ISHAGE rules)	$\frac{(\#\ \text{viable}\ CD45^{dim}\ CD34^+(CD309^+)\text{events})}{(\#\text{living events})} \times 100\ \%$		
CD117_PB_EPC_IHG	MNC PB	ASS I-III		Frequency of the viable $CD117^+$ region (estimated according to the ISHAGE rules)	$\frac{(\#\ \text{viable}\ CD45^{dim}\ CD117^+\text{events})}{(\#\text{living events})} \times 100\ \%$	[%]	
CD117_184_PB_EPC_IHG				Frequency of the $CD184^+$ cell subpopulation within viable $CD117^+$ region (estimated according to the ISHAGE rules)	$\frac{(\#\ \text{viable}\ CD45^{dim}\ CD117^+(CD184^+)\text{events})}{(\#\text{living events})} \times 100\ \%$		

CD117_34_PB_EPC_IHG				Frequency of the $CD34^+$ cell subpopulation within viable $CD117^+$ region (estimated according to the ISHAGE rules)	$\frac{(\#\ \text{viable}\ CD45^{dim}\ CD117^+(CD34^+)\text{events})}{(\#\text{living events})} \times 100\ \%$		
CD117_309_PB_EPC_IHG				Frequency of the $CD309^+$ cell subpopulation within viable $CD117^+$ region (estimated according to the ISHAGE rules)	$\frac{(\#\ \text{viable}\ CD45^{dim}\ CD117^+(CD309^+)\text{events})}{(\#\text{living events})} \times 100\ \%$		
CD133_PB_EPC_IHG				Frequency of the viable $CD133^+$ cells (estimated according to the ISHAGE rules)	$\frac{(\#\ \text{viable}\ CD45^{dim}\ CD133^+\text{events})}{(\#\text{living events})} \times 100\ \%$		
CD34N_CD133_PB_EPC_IHG				Frequency of the viable $CD133^+$ cells (estimated according to the ISHAGE rules) within $CD45^{dim}$ $CD34^-$ region	$\frac{(\#\ \text{viable}\ CD45^{dim}\ CD34^-(CD133^+)\text{events})}{(\#\text{living events})} \times 100\ \%$		
CD34_CD133N_PB_EPC _IHG				Frequency of the viable cells lacking CD133 antigen (estimated according to the ISHAGE rules) within $CD45^{dim}$ $CD34^-$ region	$\frac{(\#\ \text{viable}\ CD45^{dim}\ CD34^+(CD133^-)\text{events})}{(\#\text{living events})} \times 100\ \%$		
CD133_184_PB_EPC_IHG				Frequency of the $CD184^+$ cell subpopulation within viable $CD133^+$ region (estimated according to the ISHAGE rules)	$\frac{(\#\ \text{viable}\ CD45^{dim}\ CD133^+(CD184^+)\text{events})}{(\#\text{living events})} \times 100\ \%$		
CD133_309_PB_EPC_IHG				Frequency of the $CD309^+$ cell subpopulation within viable $CD133^+$ region (estimated according to the ISHAGE rules)	$\frac{(\#\ \text{viable}\ CD45^{dim}\ CD133^+(CD309^+)\text{events})}{(\#\text{living events})} \times 100\ \%$		
CD133_117_PB_EPC_IHG				Frequency of the $CD117^+$ cell subpopulation within viable $CD133^+$ region (estimated according to the ISHAGE rules)	$\frac{(\#\ \text{viable}\ CD45^{dim}\ CD133^+(CD117^+)\text{events})}{(\#\text{living events})} \times 100\ \%$		
CD146_PB_CEC	MNC PB	ASS I-III	Flow cytometry	Frequency of the viable $CD146^+$ region within viable $CD45^+$ cells	$\frac{(\#\ \text{viable}\ CD45^+\ CD146^+\text{events})}{(\#\text{living events})} \times 100\ \%$	[%]	

CD105_PB_CEC			Panel: Circulating endothelial cells (CEC) 7	Frequency of the viable $CD105^+$ region within viable $CD45^+$ cells	$\frac{(\#\ \text{viable}\ CD45^+\ CD105^+\text{events})}{(\#\text{living events})} \times 100\ \%$		
CD31_PB_CEC				Frequency of the viable $CD31^+$ region	$\frac{(\#\ \text{viable}\ CD45^+\ CD31^+\text{events})}{(\#\text{living events})} \times 100\ \%$		
CD45N_105_34_146_31_PB_CEC				Frequency of the viable $CD45^-$ $CD105^+$ $CD34^+$ $CD146^+$ $CD31^+$ cells	$\frac{(\#\ \text{viable}\ CD45^-\ CD105^+CD34^+CD146^+CD31^+\text{events})}{(\#\text{living events})} \times 100\ \%$		
CD31_105_PB_CEC				Frequency of the $CD105^+$ cell subpopulation within viable $CD45^+CD31^+$ region	$\frac{(\#\ \text{viable}\ CD45^+\ CD31^+\ (CD105^+)\text{events})}{(\#\text{living events})} \times 100\ \%$		
CD31_14N_PB_CEC				Frequency of the $CD14^-$ cell subpopulation within viable $CD45^+$ $CD31^+$ region	$\frac{(\#\ \text{viable}\ CD45^+\ CD31^+(CD14^-)\text{events})}{(\#\text{living events})} \times 100\ \%$		
CD45_133_146_PB_CEC				Frequency of the $CD146^+$ cell subpopulation within viable $CD45^+$ $CD133^+$ region	$\frac{(\#\ \text{viable}\ \ CD45^+\ CD133^+(\ CD146^+)\text{events})}{(\#\text{living events})} \times 100\ \%$		
CD45_133_105_PB_CEC				Frequency of the $CD105^+$ cell subpopulation within viable $CD45^+$ $CD133^+$ region	$\frac{(\#\ \text{viable}\ \ CD45^+\ CD133^+(\ CD105^+)\text{events})}{(\#\text{living events})} \times 100\ \%$		
CD45_133_31_PB_CEC				Frequency of the $CD31^+$ cell subpopulation within viable $CD45^+$ $CD133^+$ region	$\frac{(\#\ \text{viable}\ \ CD45^+\ CD133^+\ (CD31^+)\text{events})}{(\#\text{living events})} \times 100\ \%$		
CD45_133_146_105_PB _CEC				Frequency of the $CD146^+$ $CD105^+$ cell subpopulation within viable $CD45^+$ $CD133^+$ region	$\frac{(\#\ \text{viable}\ CD45^+\ CD133^+\ (CD146^+CD105^+)\text{events})}{(\#\text{living events})} \times 100\ \%$		
CD45_133_146_31_PB_CEC				Frequency of the $CD146^+$ $CD31^+$ cell subpopulation within viable $CD45^+$ $CD133^+$ region	$\frac{(\#\text{viable}\ \ CD45^+\ CD133^+\ (CD146^+CD31^+)\ \text{events})}{(\#\text{living events})} \times 100\ \%$		

CD45_133_31_105_PB_CEC	MNC PB	ASS I-III		Frequency of the $CD31^+$ $CD105^+$ cell subpopulation within viable $CD45^+$ $CD133^+$ region	$\frac{(\#\ \text{viable}\ \ CD45^+\ CD133^+(\ CD31^+CD105^+)\text{events})}{(\#\text{living events})} \times 100\ \%$	[%]	
CD45_133_34_146_PB_CEC				Frequency of the $CD146^+$ cell subpopulation within viable $CD45^+$ $CD133^+$ $CD34^+$ region	$\frac{(\#\text{viable}\ \ CD45^+\ CD133^+\ CD34^+\ (CD146^+)\text{events})}{(\#\text{living events})} \times 100\ \%$		
CD45_133_34_105_PB_CEC				Frequency of the $CD105^+$ cell subpopulation within viable $CD45^+$ $CD133^+$ $CD34^+$ region	$\frac{(\#\text{viable}\ \ CD45^+\ CD133^+\ CD34^+(\ CD105^+)\text{events})}{(\#\text{living events})} \times 100\ \%$		
CD45_133_34_146_105_PB_CEC				Frequency of the $CD146^+$ $CD105^+$ cell subpopulation within viable $CD45^+$ $CD133^+$ $CD34^+$ region	$\frac{(\#\text{viable}\ CD45^+\ CD133^+\ CD34^+(\ CD146^+\ CD105^+)\text{events})}{(\#\text{living events})} \times 100\ \%$		
CD45_133_34_146_105_31_PB_CEC				Frequency of the $CD146^+$ $CD105^+$ $CD31^+$ cell subpopulation within viable $CD45^+$ $CD133^+$ $CD34^+$ region	$\frac{(\#\text{viable}\ CD45^+\ CD133^+\ CD34^+\ (CD146^+\ CD105^+\ CD31^+)\text{events})}{(\#\text{living events})} \times 100\ \%$		
CD45_133_146_105_31_PB_CEC				Frequency of the $CD31^+$ cell subpopulation within viable $CD45^+$ $CD133^+$ $CD146^+$ $CD105^+$ region	$\frac{(\#\text{viable}\ \ CD45^+\ CD133^+\ \ (CD146^+\ CD105^+\ CD31^+)\text{events})}{(\#\text{living events})} \times 100\ \%$		
CD45_133_PB_CEC				Frequency of the $CD133^+$ cell subpopulation within viable $CD45^+$ region	$\frac{(\#\text{viable}\ \ CD45^+\ CD133^+\ \text{events})}{(\#\text{living events})} \times 100\ \%$		
Total CFU / 10^3 $CD133^+$ cells	CD133 SC	ASS I	*In vitro* Endothelial differentiation capacity, Colony-forming unit endothelial cell (CFU-EC) assay *8*		Adherent and non-adherent colonies per dish were counted after 21 days using light microscope (Leica DMIL) and 5× objective. Averages of each CFU were generated. Total colony number (CFU) per 10^3 $CD133^+$ cells was calculated.	[n]	Ong *et al.*, 2010, Lux *et al.*, 2015 (refer for detailed medium composition)

Number of colonies per volume of used peripheral blood	MNC	ASS I-III	*In vitro* Endothelial differentiation capacity, Colony-forming unit (CFU-Hill) assay *9*		At day 5, positive colonies defined as a central core of "round cells" with more elongated "sprouting" cells at the periphery were counted per 1×10^6 cells and normalized to the full blood volume (mL).	[n / mL]	Hill *et al.*, 2003, Donndorf *et al.*, 2015
Number of nuclei per mm^2 of the plug area	$CD133^+$ or MNC	ASS I (CD 133) ASS I-III (MNC)	*In vivo* Angiogenesis assay, Matrigel™ plug assay, Morphology (H&E) *10*	The federal animal care committee of LALLF Mecklenburg-Vorpommern (Germany) approved the study protocol (approval number LALLF M-V/TSD/7221.3-1.1-001/12).	Total nuclei number and $CD31^+$ cells were counted by blinded investigators within the plug area and presented per 1 mm^2 area. Additionally, numbers of newly formed vessels were counted and normalized per 1 mm^2 plug area as well as ratio of the capillaries area to the entire plug area was calculated.	[n / mm^2]	
Number of $CD31^+$ cells per mm^2 of the plug area						[n / mm^2]	
Number of capillaries per mm^2 of the plug area						[n / mm^2]	
Capillary area per mm^2 field of view (the entire plug area)						[mm^2 / mm^2] mm^2	
VEGF, IP-10, IL-6	Serum	ASS I-III	Cytometric Bead Array (CBA) using flow cytometer 11	In case of internal patients (Rostock) sera were processed after whole blood donation (centrifugation, storage of aliquots -80 °C). Due to longer transporta-tion the samples from external patients were processed at the day after donation.	Concentrations for each soluble protein in test samples were generated from the standard curves created on the basis of mean fluorescence intensities (MFI) versus concentration of the specific standard sample. The standard curve with the highest R^2 was chosen. Various soluble proteins were multiplexed. Therefore the MFI of the 0 pg/mL standard point was defined as a background. On the basis of the manufacturer's information following values for limit of the detection when MFI at 0 pg/mL defined as a negative control were provided: VEGF: 4.5 pg/mL; IP-10: 0.5 pg/mL, IL-6: 1.6 pg/mL. IL-8:	[pg / mL]	

					69.9 fg/mL and IL-10: 13.7 fg/mL. The automatically generated concentrations with the value equal null or at lower level than the sensitivity of the assay allowed were considered as those in not detectable range.		
IL-8, IL-10	Serum	ASS I-III				[fg / mL]	
TNF, SDF-1, SCF	Serum	ASS I-III	Enzyme-linked immunosorbend assay (ELISA) 12		Analysis was performed according to manufacturer's instruction (R&D Systems GmbH).	[pg / mL]	Kleiner *et al.* 2013
IGF-1, IGFBP-2, IGFBP-3						[ng / mL]	Balcells *et al.*, 2001, Duron *et al.*, 2014
Erythro-poietin (EPO)						[mIU / mL]	Chatterjee *et al.*, 2000, Wenzel *et al.*, 2011
Vitronectin						[µg / mL]	Boyd *et al.*, 1993, Aslan *et al.*, 2014
Anti-CMV_IgG	Serum, plasma	ASS I and III	ELISA 13		Analyses were performed by the accredited laboratory (DIN EN ISO 15189) Institute for Medical Microbiology Virology and Hygiene, Rostock University Medical Center	[U / mL]	
Anti-EBV_VCA_IgG						[U / mL]	
Anti-EBV_EA_IgG						[U / mL]	
Anti-EBV_EBNA_IgG						[U / mL]	
Anti-Parvo						[IU /	Rohayem

_IgG						mL]	*et al*., 2001, Knöll *et al*., 2002
Anti-CMV_IgM						-	
Anti-EBV_VCA_IgM						[U / mL]	
Anti-Parvo_IgM						[U / mL]	
CMV_DNA	Serum, plasma	ASS I and III	Real Time-Polymerase chain reaction (RT-PCR) 14			[Copies / mL]	Lundberg *et al*., 2006
Parvo_DNA							
SH2B3	Whole blood (EDTA)	ASS I	RT-PCR 15		Measurement of RNA Integrity Number (RIN) using the Agilent 2100 Bioanalyzer. Samples with RIN ≥7 were used for further experiments. Measurement of RNA concentration and purity using the NanoDrop 1000. The two endogenous normalization controls POLR2A TaqMan® Gene Expression Assay (Hs00172187_m1, Thermo Fisher Scientific) and GAPDH (4326317E, Thermo Fisher Scientific) were used for ΔΔCT method.		

Appendix 3: List of biomarkers, analytical methods and units.

Bone marrow (BM) and peripheral blood (PB) samples were analyzed using the following methods; SC (stem cells). ASS I = pre OP, ASS IIa = 1d post OP, ASS IIb = 3d post OP, ASS III = 10 d.

[1] MACS® technology

[2] Samples were characterized regarding their hematopoietic phenotype (CD45, CD133, CD34) and using the antibodies: anti-CD133-phycoerythrin (PE) (293C2), anti-CD34-fluorescein isothiocyanate (FITC) (AC136) (both mouse anti-human, Miltenyi Biotec GmbH), and anti-CD45-allophycyanin-H7 (APC-H7) (HI30)(mouse anti-human, Becton Dicinson (BD) Biosciences). 10 µL of un-diluted BM and 10^5 of purified CD133+ cell samples, respectively were suspended in MACS® buffer (PBS containing 2 mM EDTA, 0.5 % bovine serum albumin (BSA, Sigma-Aldrich Chemie GmbH)). FcR blocking reagent (Miltenyi Biotec GmbH) was added to reduce unspecific bindings. Subsequently, cells were incubated with antibodies for 10 min in the dark at 4 °C. To distinguish viable from dead cells, 7-Amino-Actinomycin (7-AAD) staining solution (BD) was used. Whereas to BM probe 1 mL of red blood lysis buffer (eBioscience) was added, CD133 samples were incubated with 1 mL cold PBS. After 10 min incubation on ice, probes were washed once at 300×g/ 4 °C for 10 min, resuspended in 200 µL PBS and measured with BD LSRII flow cytometer (BD).The analysis was performed using BD FACS Diva Software (version 6.1.2, BD).

[3] Two samples from batch of the purified BM $CD133^+$ containing each 3×10^4 cells were taken for stemness characterization. After addition of the fixed amount of antibodies, the remaining volume to up volume of 50 µL was filled with MACS® buffer. After the incubation time, cells were washed once and acquired on flow cytometer. Following antibodies were always added to both samples: anti-CD34-FITC (AC136) (Miltenyi Biotec GmbH), anti-CD133-PE (293C2) (Miltenyi Biotec GmbH), anti-CD117-PE-Cy7 (104D2) (BD), anti-CD45-AmCyan (HI30) (BD), and anti-CD14-PacificBlue (MϕP9) (BD). For APC-channel either anti-CD184 (CXCR4) (12G5)(BD) or anti-CD309 (VEGFR2/KDR) (ES8-20E6) (Miltenyi Biotec GmbH) was added. To distinguish living cells from dead cells, 7-AAD dye was utilized. As for QC the analysis of CD34+CD133+SC was performed in accordance with an internal adapted ISHAGE protocol. Additionally, dependent on probe staining, following sub-populations were characterized within this region: a) $CD117^+$, b) $CD309^+$, c) $CD184^+$, d) $CD117^+CD309^+$, e) $CD117^+CD184^+$ and f) $CD14^+$

4 Density gradient centrifugation was performed to isolate MNC from PB.

5 Cells were suspended in cold PBS, FcR blocking reagent (Miltenyi Biotec GmbH) and appropriate antibodies (see below) were added. To distinguish dead from living cells LIVE/DEAD™ Fixable Near-IR Dead Cell Stain Kit (Thermo Fisher Scientific GmbH) was used. Following incubation at 4 °C for 30 min, cells were washed once with PBS at 300×g for 10 min at 4 °C, suspended in 300 µL PBS and acquired immediately on BD FACS LSRII flow cytometer (BD). The analysis was performed using FACS Diva Software (BD). For calculation of concentration of the respective subpopulations total cell number of viable MNC was obtained from counting method using trypan blue dye.

6 Antibodies/dyes used for multicolor staining (8-fold) to enumerate various endothelial progenitor cell (EPC) subset within mononuclear cells isolated from peripheral blood: a) anti-CD34-FITC (AC136); Mouse isotype control: IgG_{2a} (Miltenyi Biotec GmbH), b) anti-CD133/2-PE (293C3); Mouse isotype control: IgG_{2b} (Miltenyi Biotec GmbH), c) anti-CD184-PE-Cy5 (12G5); Mouse isotype control: IgG_{2a}, κ (BD), d) anti-CD117-PE-Cy7 (104D2); Mouse isotype control: IgG_1, κ (BD), e) anti-CD309-APC (ES8-20E6); Mouse isotype control: IgG_1, κ (Miltenyi Biotec GmbH), f) near-IR fluorescence dye-APC-Cy7 (Thermo Fisher Scientific GmbH), g) anti-CD14- Pacific Blue (V450) (MϕP9); Mouse isotype control: IgG_{2b}, κ, (BD), and h) anti-CD45-AmCyan (V500) (HI30); Mouse isotype control: IgG_1, κ (BD)

7 List of antibodies/dyes used for multicolor staining (8-fold) to enumerate various circulating endothelial cell (EPC) subsets within mononuclear cells isolated from PB: a) anti-CD31-FITC (WM59) Mouse isotype control: IgG1, κ (BD), b) anti-CD133- PE (293C3) Mouse isotype control: IgG2b (Miltenyi Biotec GmbH), c) anti-CD146- Biotin; Mouse isotype control: IgG1 (both (Miltenyi Biotec GmbH) and secondary antibody streptavidin- PerCP-Cy5.5 (BD), d) anti-CD34- PE-Cy7 (8G12) Mouse isotype control: IgG1, κ (BD), e) anti-CD105- Alexa Fluor-647 (APC) (SN6) Mouse isotype control: IgG1 (AbD Serotec), f) near-IR fluorescence dye-APC-Cy7 (Thermo Fisher Scientific GmbH), g) anti-CD14- Pacific Blue (V450) (MϕP9) Mouse isotype control: IgG2b, κ, (BD), and h) anti-CD45- AmCyan (V500) (HI30) Mouse isotype control: IgG1, κ (BD)

8 1×10^3 $CD133^+$ cells were plated in triplicates on a 35 mm dish in supplemented MethoCult SF H4236 (both from StemCell Technologies Inc.) and incubated under standardized conditions 37 °C, 5 % CO_2 at humidity > 80% for 21 days.

9 CFU-Hill assays were performed in triplicates in accordance with Hill *et al.* (2003) and following the manufacturer's instructions using CFU-Hill Liquid medium (StemCell Technologies Inc.). In brief, 5×10^6 MNC were plated per one 35mm fibronectin-coated dish. After three days, non-adherent cells were collected, counted using methylene blue dye (Stem Cell technologies Inc.) and re-plated at the density 1×10^6 cells per fibronectin -coated 24 well plate.

10 Severe Combined Immunodeficiency mice (CB17-Prkdcscid/J) were purchased from Charles River Laboratories. SCID mice (female, 22±2 g) were randomly assigned to five groups with MatrigelTM (BD Matrigel Matrix Growth Factor Reduced, Phenol Red-Free, (BD)) injection containing either 1×105 of CD133+ SC manually isolated from iliac crest biopsy before CABG therapy (133pre, n=(13/8 [H/E]) or MNC isolated from PB (PBMNC) withdrawn before CABG therapy (MNCpre, n=13/14 [H/E]), PBMNC applied 24 h post CABG therapy (MNC24h, n=12/11 [H/E]), PBMNC applicated 72 h post CABG therapy (MNC72h, n=15/12 [H/E]) and PBMNC applied 10 d post CABG therapy (MNC10d, n=14/10 [H/E]). Mice were anesthetized with intraperitoneal injection of Ketamin/Xylazin (75/25 mg/kg). Matrigel™ was injected subcutaneously in abdominal wall through 1 mL syringe with 26 G needle (B.

Braun). Mice were sacrificed 14 d post injection by cervical dislocation and Matrigel™ plug was carefully removed. The plug with cells was harvested together with abdominal muscle and skin, fixed in 4 % neutral bufferd formalin (FormaFix, Grimm MED. Logistik GmbH), embedded in paraffin. Formalin-fixed paraffin embedded plugs were pseudonymized and sectioned at 10 µm thickness using Mikrotom Hyrax M55 (Carl Zeiss GmbH). Following deparaffinization and dehydratation steps, some sections were stained with Hematoxylin Meyer and 0.25 % Eosin (both Sigma-Aldrich Chemie GmbH) for further morphological examination (nuclei, vessel-like structures), otherwise, some sections were boiled in 10 mM citrate buffer for 10 min for antigen retrieval, blocked with DAKO Serum-free block (DAKO Cytomation) 1h at RT, further stained with primary polyclonal goat anti-CD31 antibody (1.25, sc-1506; Santa Cruz Technologies Inc.) overnight at 4 °C and visualized with donkey anti-goat Alexa Fluor 488 (1:250, Thermo Fisher Scientific GmbH) for 2 h at 37 °C. Plugs were counterstained with 5 µM TO-PRO-3 Iodide (Thermo Fisher Scientific GmbH) for nuclei. Random images within plug area were taken with ELYRA PS.1 confocal scanning microscope using 40 × objectives and analyzed with ZEN 2011 software (blue edition, both from Carl Zeiss).

11 Estimation of cytokine levels in serum: Concentrations of VEGF, IL-6 and IP-10 in patient's sera samples were measured using BD™ Cytometric Bead Array (CBA) Human Soluble Protein Master Buffer Kit (BD) according to the manufacturer's instruction. These three BD™ CBA Soluble Protein Flex Set Assays (BD) composed of standards, capture beads and PE-detections reagents were multiplexed. Frozen sera samples were thawed and diluted 1/4 in Assay Diluent. Lyophilized standards were diluted appropriately giving an assay range for each soluble protein at minimum of 10 pg/mL until 'TOP' 2,500 pg/mL. Following one hour incubation step to ensure binding of capture beads with recognized analytes in samples and standards, visualisation of formed complexes was ensured by addition of PE-conjugated detection reagent for another 2 h. Triplicates either of test samples or standards were measured using BD LSRII flow cytometer (BD) equipped with BD FACS Diva software. The transferred raw data (FCS 2.0) were further analysed by FCAP Array™ software 3.0 (BD). Concentrations (fg/mL) of IL-8 and IL-10 in patient's sera samples, collected at certain time points, were measured by BD™ CBA Human Enhanced Sensitivity Master Buffer Kit according to the manufacturer's instruction. Both BD™ CBA Soluble Protein Enhanced Sensitivity Flex Sets (BD) composed of standards, capture beads and detection reagents (Part A) were multiplexed. Frozen sera were thawed and diluted at 1/3 with Assay Diluent. Lyophilized standards provided in the kit were diluted appropriately giving an assay range for each soluble protein at minimum of 274 fg/mL until 'TOP' 200,000 fg/mL. Samples were incubated with capture beads (2 h) followed by another incubation steps with detection reagents A and B (each 2 h).

12 ELISA were conducted in triplicates. OD values were generated at 450 nm wavelength using Tecan Infinite® M200 device with Tecan i-control, 1.9.17.0 software (both from Tecan Group Ltd.). Frozen sera samples were thawed and prepared following the manufacturers´ instructions (R&D Systems GmbH). Created standard curves were used to determine each protein concentration. For adequate ELISA performance patient samples were diluted considering detection limits provided by the manufacturer, literature ranges and internal laboratory experience: TNF, SDF-1 and EPO: un-diluted, IGF-1 and IGFBP-3 1:100, IGFBP-2 1:50, Vitronectin 1:10 and SCF 1:3.

13 Native samples of PB serum or plasma were used for semi-quantitative Immunoassays using the Immunomat™ system (Institute Virion\Serion GmbH).

14 Native samples of peripheral blood (EDTA blood) were used for quantitative Real Time-PCR using LightCycler 480 II (Roche Deutschland Holding GmbH).

15 Isolation of RNA from 1 ml whole blood aliquots (stored at -80 °C) was performed using the GeneJET Stabilized and Fresh Whole Blood RNA Kit (Thermo Fisher Scientific). Reverse transcription was performed using the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific). RT-PCR was performed using the StepOnePlus RT PCR System (Applied Biosystems™). cDNA (30 ng for each reaction), TaqMan® Universal PCR Master Mix (Thermo Fisher Scientific) and SH2B3 TaqMan® Gene Expression Assay (Hs01081959_g1, Thermo Fisher Scientific) were used. Three technical replicates were performed. To calculate the relative expression ratio of SH2B3 the ΔΔCT method was applied.

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