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Research Paper

Cardiac Function Improvement and Bone Marrow Response – Outcome Analysis of the Randomized PERFECT Phase III Clinical Trial of Intramyocardial CD133$^+$ Application After Myocardial Infarction

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ABSTRACT

Objective: The phase III clinical trial PERFECT was designed to assess clinical safety and efficacy of intramyocardial CD133$^+$ bone marrow stem cell treatment combined with CABG for induction of cardiac repair.
Design: Multicentre, double-blinded, randomised placebo controlled trial.
Setting: The study was conducted across six centres in Germany October 2009 through March 2016 and stopped due slow recruitment after positive interim analysis in March 2015.
Participants: Post-infarction patients with chronic ischemia and reduced LVEF (25–50%). Interventions: Eighty-two patients were randomised to two groups receiving intramyocardial application of 5 ml placebo or a suspension of 0.5–5 × 10^6 CD133$^+$.
Outcome: Primary endpoint was delta (Δ) LVEF at 180 days (d) compared to baseline measured in MRI.
Findings (prespecified): Safety ($n = 77$): 180 d survival was 100%, MACE $n = 2$, SAE $n = 49$, without difference between placebo and CD133$^+$. Efficacy ($n = 58$): The LVEF improved from baseline LVEF 33.5% by +9.6% at 180 d, $p = 0.001$ ($n = 58$). Treatment groups were not different in ΔLVEF (ANCOVA: Placebo +8.8% vs. CD133$^+$ +10.4%, ΔCD133$^+$ vs placebo +2.6%, $p = 0.4$).
Findings (post hoc): Responders (R) classified by ΔLVEF ≥ 5% after 180 d were 60% of the patients (35/58) in both treatment groups. ΔLVEF in ANCOVA was +17.1% in (R) vs. non-responders (NR) (ΔLVEF 0%, $n = 23$). NR were characterized by a preoperative response signature in peripheral blood with reduced CD133$^+$ EPC (RvsNR: $p = 0.005$) and thrombocytes ($p = 0.004$) in contrast to increased Erythropoeitin ($p = 0.02$), and SH2B3 mRNA expression ($p = 0.073$). Actuarial computed mean survival time was 76.9 ± 3.32 months (R) vs. +72.3 ± 5.0 months (NR), HR 0.3 [CI 0.07–1.2]; $p = 0.067$.Using a machine learning 20 biomarker response parameters were identified allowing preoperative discrimination with an accuracy of 80% (R) and 84% (NR) after 10-fold cross-validation.

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Interpretation: The PERFECT trial analysis demonstrates that the regulation of induced cardiac repair is linked to the circulating pool of CD133+ EPC and thrombocytes, associated with SH2B3 gene expression. Based on these findings, responders to cardiac functional improvement may be identified by a peripheral blood biomarker signature.
TRIAL REGISTRATION: ClinicalTrials.gov NCT00950274.

Research in Context

Evidence Before This Study

Intramyocardial $CD133^+$ purified autologous bone marrow stem cell (BMSC) transplantation has been investigated as an adjunctive strategy to coronary artery bypass graft (CABG) revascularization in order to improve left ventricular heart function following deterioration of left ventricular ejection fraction (LVEF) after acute myocardial ST-segment elevation infarction (STEMI), and coronary artery 3-vessel disease sequentially treated by acute PCI and secondary CABG revascularization. Previous safety and efficacy (phase I, IIa, IIb) trials have demonstrated clinical safety and some evidence of therapeutic efficacy of adjunctive $CD133^+$ BMSC treatment adjunctive to CABG coronary revascularization. The randomised double-blinded placebo controlled PERFECT-trial was designed to assess clinical safety and efficacy in a, ICH-GCP complaint study setting. Post hoc biomarker and subgroup analyses were performed to identify $CD133^+$ bone marrow stem cell related cardiac repair mechanisms related to interventional $CD133^+$ BMSC transplantation.

Added Value of This Study

The study demonstrates the central regulatory importance of $CD^{133,34+}$ EPC response for angiogenesis, suppression of response by SH2B3, impact for cardiac tissue repair, selection of responding patients, and monitoring of angiogenesis response by combined diagnostic factors using machine learning.

Implications of All the Available Evidence

The described mechanism of suppression bone marrow $CD133^+$ angiogenesis response may have a pivotal role in cardiovascular tissue repair. Selection of patients by specific diagnostic peripheral blood biomarkers appears to be feasible and may lead to tailored therapy in cardiovascular disease. The lack of vascular repair by reduced blood angiogenesis may be a decisive determinant for cardiovascular disease and impaired tissue repair.

1. Introduction

Reparative therapies using stem cells for the repair of heart tissue have been at the forefront of preclinical and clinical development during the past 16 years (Fisher et al., 2016). Among the different approaches, the direct implantation of bone marrow- derived cells into heart tissue still attracts the most dedicated clinical developmental attention since the first-in-man application in 2001 and several promising clinical pilot trials (Stamm et al., 2003; Tse et al., 2003; Stamm et al., 2007). Yet, in these trials, clinically relevant improvements of LVEF as well as non-responsive patients were observed both in treatment and placebo groups (Henry et al., 2016; Nasseri et al., 2014; Bartunek et al., 2016). This has raised the question of induction of reparative mechanisms independent of stem cell application and potential suppressive factors of vascular repair associated with $CD34^+$ Endothelial Progenitor Cells (EPC) (Werner et al., 2005; Taylor et al., 2016; Bhatnagar et al., 2016; Contreras et al., 2017).

In light of this uncertainty, we have attempted to investigate the mechanism of cardiac repair and the role of bone marrow CD133+ EPC regulated angiogenesis using the results of the clinical PERFECT trial and its data recorded (Donndorf et al., 2012). Extensive additional laboratory analyses was carried out to delineate the underlying mechanisms and to develop diagnostic approaches for identifying patient (non)responsiveness to stem cell therapies by analyzing the following clinical features: 1. Baseline characteristics of treatment responders vs. non-responders; 2. Mechanism of action for cardiac regeneration and diagnostic access; 3. Relevance of LVEF endpoint for long term survival.

2. Methods

2.1. Trial Design

The PERFECT trial was a randomised, multicenter, placebo-controlled, double-blinded phase III study investigating the effects of intramyocardial $CD133^+$ BMSC treatment in combination with coronary artery bypass graft revascularization (CABG) for post infarction myocardial ischemia (Donndorf et al., 2012). The trial performed according to ICH-GCP was listed under the EudraCT number 2006-006404-11, DRKS number DRKS00000213, and approved by the committee of the University Medicine Rostock (FK 2007-07) and all trial sites in Germany (Supplement Appendix 1). Regulatory approval was given by the Paul-Ehrlich-Institute, Langen, Germany. The trial was registered at ClinicalTrials.gov identifier: NCT00950274. Characteristics of trial design, changes to trial design, outcomes, interim analysis, and recruitment period are depicted in Appendix 2 (Supplement) and the Clinical Trial Report (Appendix 1).

Inclusion criteria of the PERFECT trial (Supplement Appendices 1 and 2) were (a) coronary artery disease after myocardial infarction with the indication for CABG surgery, (b) reduced LVEF (25–50%) and (c) presence of a localized kinetic/hypokinetic/hypoperfused area of LV myocardium defining the SC target area (Supplement Fig. 1). According to the trial flow chart (Supplement Fig. 2) assessments were performed preoperative and at days 1, 3, 10, 90, and 180 post operation. In addition, safety (MACE) follow up was performed at 24 months post-treatment.

2.2. Participants and Study Settings

A total of 119 patients were screened in 6 centres in Germany (Fig. 1). All patients signed the informed consent form and were included in the study. Eighty-two (82) patients were randomised to active treatment or placebo. The allocation of patients to the different analysis sets is shown in Fig. 1. Initially, we evaluated the basic patient characteristics of the randomised patient groups for safety set (SAS) analysis ($n = 77$) and per-protocol set (PPS) efficacy analysis ($n = 58$) respectively for subanalysis of MRI early/late, primary endpoint responder/non-responder, biomarkers, preoperative cardiac disease state, age, sex, concomitant diseases, taking medications, operative procedures and postoperative course (Table 1).

2.3. Cell Preparation and Manufacturing

All patients enrolled in the study underwent bone marrow aspiration (mean 166 ± 20 ml) and withdrawal of 20 ml blood one to two

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