PS analyses, the authors found that the results between these two techniques were not materially different in most of these studies. Even if the results were different between the two approaches, one could not necessarily assume that the PS analysis yielded the ‘true’ answer, at least not without specifying underlying assumptions.

In general, the approach of using traditional multivariate regression adjustment is preferable if the sample size is sufficiently large and the outcome of interest is not rare. Only if the outcome is rare relative to the number of confounders and the number of study subjects in the smaller exposure group is sufficiently large to warrant multivariable PS estimation, then this statistical technique has a legitimate role to potentially reduce bias and expand the possibilities in observational outcomes research [6]. Only then, the use of PS can be regarded a substantial help, not just hype.

Conflict of interest statement. None declared.

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Stem cell therapy of cardiac disease: an update

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Keywords: angiogenesis; cardiac repair; perfusion; stem cells

Despite the development of therapeutic strategies and electrophysiological and surgical treatment regimens, heart failure remains one of the major causes of mortality in the Western world. Heart failure can develop as a consequence of myocardial infarction despite revascularization procedures. Ischaemia-induced death of cardiac myocytes results in scar formation and reduced contractility of the ventricle. Various experimental studies provided evidence that the infusion or injection of stem or progenitor cells may reduce scar formation and fibrosis. Moreover, predominantly bone marrow-derived cells were shown to augment blood flow, thereby providing a novel therapeutic option for the prevention and/or treatment of heart failure.

This review article intends to summarize the current experimental and clinical evidence for a potential role for stem cells in cardiac regeneration.

Which cells?

In animal models, several stem and progenitor cells showed a potential to improve cardiac regeneration (Figure 1). Human embryonic stem (ES) cells have the capacity to generate all cell types of an organism and can give rise to cardiac myocytes [1]. However, at present, there are several challenges to be overcome before ES cells can be clinically applied. One of the most prominent problems is the tumorigenicity of these cells; another challenge is that ES cells may be rejected by the immune system. Although ES cells are by nature more versatile than their adult counterparts, increasing evidence suggests that stem and progenitor cells of the
adult organism also have the capacity to give rise to several lineages and may be suitable for regenerative medicine [2]. The best established source for adult stem cells is the bone marrow. It contains different cell types. The haematopoietic stem cells (HSCs) are characterized by the marker protein CD34 or the more immature marker protein CD133, and have been used for clinical bone marrow transplantation for many years. A specific subset of enriched HSCs are the ‘side population’ (SP) cells [3]. Mesenchymal stem cells can also be isolated from bone marrow [4]. Verfaillie et al. [5] succeeded in isolating multipotent adult progenitor cells (MAPCs), which differentiate, at the single cell level, not only into mesenchymal cells, but also into cells with characteristics of the visceral mesoderm, neuroectoderm and endoderm in vitro. Other sources of adult stem cells include the fat tissue, which contains HSCs, and mesenchymal stem cells [6]. Finally, peripheral blood-derived progenitor cells can be used for cell transplantation. The blood contains low concentrations of HSCs and endothelial progenitor cells (EPCs). EPCs can be isolated from the peripheral blood and they express endothelial marker proteins [7–9]. Some evidence also suggests that peripheral blood may contain a small number of pluripotent cells as well, which can differentiate in vitro into other lineages [10,11]. Very interesting recent studies showed further that the heart itself contains tissue resident stem cells [12,13]. Cardiac stem cells were isolated using antibodies directed against the surface marker c-Kit [12] or Sca-1 [13]. Both cells expressed high levels of telomerase as a functional stem cell marker and were shown to differentiate into cardiac myocytes. c-Kit-positive cells additionally give rise to various other lineages [12]. Although it is not yet clear whether these cells are bone marrow-derived cells, which remained in local niches in the heart, or represent a novel entity, the mobilization and attraction of tissue resident cardiac stem cells could be an extremely attractive option for future cell therapy.

**Functions of stem/progenitor cells to improve cardiac function**

Stem or progenitor cells may contribute to cardiac regeneration in several ways. These include a differentiation in the endothelial and cardiac lineages as well as a possible influence on the local environment by the release of paracrine factors.

**Differentiation and/or fusion**

Injection of various bone marrow-derived stem cells after myocardial infarction leads to an engraftment of these cells in the border zone of the infarcts [3,14]. When dyes or genetic markers were used to track the cells after engraftment, the injected cells partly showed the expression of cardiac and/or endothelial marker proteins [3,14,15]. These initial results suggested that cells might differentiate into the cardiac lineage and thereby contribute to cardiac regeneration. However, meanwhile, several other studies suggested that the expression of cardiac marker proteins was not the result of a differentiation process, but rather reflected cell–cell fusion. Such events were reported initially for ES cells, and now have been confirmed with HSCs [17]. Interestingly, a recent study showed that murine cardiac stem cells can fuse and differentiate to a similar extent when injected after myocardial infarction [13]. The overall contribution of differentiation vs fusion to
cardiac regeneration by the different human cell types investigated thus far is unclear.

**Improvement of neovascularization**

It has been shown by various groups that HSCs can differentiate to endothelial cells and increase the formation of new capillaries [7,8]. Further studies showed that these EPCs could be detected in the circulating blood by fluorescence-activated cell sorting (FACS) analysis (CD133/KDR double-positive cell). Various cultivation methods were used to isolate these endothelial precursor cells from the peripheral blood. The isolated cells showed expression of endothelial marker proteins including VE-cadherin, von Willebrandt factor, KDR and endothelial nitric oxide synthase (eNOS). Different culture techniques result in the enrichment of slightly different cell phenotypes; however, overall, it is clear that endothelial progenitor cells can be isolated and cultivated from peripheral blood. Most importantly, infusion of these *ex vivo* expanded EPCs augmented blood flow and heart function in animal models after hind limb ischaemia or myocardial infarction [9,18,19]. Similar results were achieved when HSCs were used [14]. The therapeutic implication is underscored further by the finding that wild-type stem cell infusion is capable of rescuing a dysfunctional neovascularization as seen in eNOS-deficient animals [20]. Likewise, young adult bone marrow-derived progenitor cells were shown to improve ageing-impaired cardiac angiogenic function [21].

**Paracrine effects**

Bone marrow-derived stem and progenitor cells home to sites of ischaemia. This may allow the local release of factors acting in a paracrine manner on the surrounding ischaemic tissue. Bone marrow-derived mononuclear cells (BMCs) release angiogenic growth factors such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and angiopoietins, thereby enhancing the local angiogenic response [22]. Isolated human EPCs also express various growth factors which can enhance cardiac myocyte survival and improve angiogenesis (personal communication). Locally released paracrine factors may additionally act by promoting arteriogenesis.

**Results from clinical studies**

In the clinical setting, the effect of bone marrow-derived cells or peripheral blood-derived EPCs has also been assessed (Tables 1 and 2). Intracoronary infusion was used as a delivery route in patients after myocardial infarction. Thereby, injection of BMCs

### Table 1. Stem/progenitor cell therapy in patients with acute myocardial infarction

<table>
<thead>
<tr>
<th>Reference</th>
<th>Cell type</th>
<th>Patients</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intracoronary application</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strauer <em>et al.</em> [23]</td>
<td>BMC (40 ml) vs control</td>
<td>$n = 10$</td>
<td>Hypokinetic area ↓ (LVA)</td>
</tr>
<tr>
<td>(AHA, 2003)¹ª</td>
<td></td>
<td></td>
<td>Contractility infarct region ↑</td>
</tr>
<tr>
<td>TOPCARE-AMI [24,25]</td>
<td>BMC (50 ml) vs CPC (250 ml)</td>
<td>$n = 26$</td>
<td>End systolic volume ↓</td>
</tr>
<tr>
<td>(AHA, 2003)¹ª</td>
<td></td>
<td></td>
<td>Perfusion ↑ (TI szinti)</td>
</tr>
<tr>
<td>BOOST [26]</td>
<td>BMC vs control</td>
<td>$n = 30$</td>
<td>Global and regional EF ↑</td>
</tr>
<tr>
<td>(AHA, 2003)¹ª</td>
<td>prospective, randomized</td>
<td></td>
<td>End systolic volume ↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Viability ↑ (PET, MRI)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Coronary flow reserve ↑ (i.c. Doppler)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>EF ↑ (MRI)</td>
</tr>
</tbody>
</table>

¹ªUpdated patient numbers reported at the Scientific Sessions of the AHA, 2003.

### Table 2. Stem/progenitor cell therapy in patients with chronic ischaemic heart failure

<table>
<thead>
<tr>
<th>Reference</th>
<th>Cell type</th>
<th>Application</th>
<th>Patients</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surgical application</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stamm <em>et al.</em> [27]</td>
<td>CD133⁺ BMC (85–195 ml)</td>
<td>Injection during bypass surgery</td>
<td>$n = 6$</td>
<td>Feasible</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>EF ↑ in 4 patients (LVA)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Perfusion ↑ in 5 patients (SPECT)</td>
</tr>
<tr>
<td>Percutaneous application</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tse <em>et al.</em> [29]</td>
<td>BMC (50 ml)</td>
<td>i.m./NOGA</td>
<td>$n = 8$</td>
<td>Feasible</td>
</tr>
<tr>
<td>Fuchs <em>et al.</em> [30]</td>
<td>BMC</td>
<td>i.m./NOGA</td>
<td>$n = 10$</td>
<td>Wall motion and thickening ↑</td>
</tr>
<tr>
<td>Perin <em>et al.</em> [28]</td>
<td>BMC vs control</td>
<td>i.m./NOGA</td>
<td>$n = 14$</td>
<td>Feasible</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>EF ↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>End systolic volume ↓</td>
</tr>
</tbody>
</table>
improved heart function in the studies reported so far. Strauer et al. [23] infused BMCs, which were isolated by Ficoll gradient centrifugation and cultivated overnight. BMCs (mean number 2.8 × 10⁶) were infused 5–9 days after acute myocardial infarction. In comparison with 10 non-randomized control patients, who did not undergo cell therapy or additional catheterization, BMC infusion enhanced regional infarct region perfusion as assessed by thallium scintigraphy. Moreover, stroke volume, end systolic volume and regional contractility indices were improved after cell therapy.

In the TOPCARE-AMI trial, patients were randomized to receive either BMCs or EPCs. BMCs (50 ml aspirate) were isolated by Ficoll gradient centrifugation and immediately infused after the preparation. EPCs were ex vivo expanded out of peripheral blood mononuclear cells for 3 days. BMCs or EPCs were infused after a mean of 5 days after myocardial infarction. BMCs and EPCs significantly improved global ejection fraction as assessed by left ventricular angiography compared with a non-randomized control patient collective [24]. Functional improvement and reduction of end systolic volumes were confirmed by magnetic resonance imaging (MRI) in a patient subcollective [25]. Cell therapy also increased coronary flow reserve which indicates an enhancement of neovascularization. The effects of EPCs and BMCs were comparable, suggesting that both cell types have the capacity to augment cardiac regeneration.

Furthermore, a first randomized trial was presented recently by Wollert et al. [26]. In this study, 60 patients with acute myocardial infarction were randomized to receive intracoronary infusion of BMCs or were not treated (no recatheterization, no placebo). The authors reported an increase in ejection fraction and a reduction of end systolic volumes in the group of patients receiving BMCs [26]. Intramuscular injection of bone marrow-derived stem/progenitor cells was used as route of application in patients with chronic ischaemic cardiomyopathy. Direct injection of isolated HSCs, which express CD133⁺ (1–1.6 × 10⁶ cells), was performed during bypass surgery in six patients [27]. In three other studies, BMCs were injected by catheter-based transendocardial delivery. Perin et al. [28] and Tse et al. [29] used the NOGA catheter system, where injection is guided by electromechanical mapping. Perin et al. showed significant improvement of ejection fraction and end systolic volume in 14 patients with chronic ischaemic heart failure, who had been treated with BMCs, as compared with seven controls. Perfusion was determined by SPECT and was significantly improved in BMC-treated patients. Tse et al. [29] treated eight patients with ischaemic heart disease and reported improvement of wall motion and wall thickening as well as a decrease in hypoperfusion 90 days after cell therapy. Finally, Fuchs et al. [30] tested the effect of unfractionated total bone marrow in 10 no-option patients with advanced coronary artery disease. Canadian Cardiovascular Society angina score and stress-induced ischaemia were significantly improved after 3 months follow-up.

All published studies at present are limited by small patient numbers and by the design as pilot safety and feasibility studies, which precludes a placebo-controlled design. However, indices of improved function were obtained in most of the studies [23, 24, 26, 28]. Finally, a randomized, placebo-controlled study in peripheral leg ischaemia (TACT-study) showed improvement in peripheral leg ischaemia by BMC infusion [31]. This supports the concept that cell therapy may augment neovascularization leading to improved oxygen supply to ischaemic tissue [31].

The question of whether the infused cells in the heart preferentially act via improving tissue perfusion or also regenerate cardiac myocytes remains unresolved. Obviously, stem or progenitor cells may exert synergistic effects by enhancing both neovascularization and cardiac regeneration. Additional release of paracrine mediators by incorporated stem or progenitor cells may amplify the response by attracting circulating progenitor cells and/or tissue resident stem cells.

Conflict of interest statement. None declared.

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**FTY720—the first compound of a new promising class of immunosuppressive drugs**

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**Keywords:** FTY720; kidney transplantation; immunosuppression

Immunosuppressive regimens containing cyclosporin or tacrolimus have reduced acute kidney graft rejections to an incidence as low as 10–30%, but acute rejections remain one of the strongest predictors for long-term kidney graft function [1–3]. Even though an increased dosage of calcineurin inhibitors can easily suppress rejection episodes, the risk of chronic transplant nephropathy, cardiovascular diseases and