REVIEW

Translational medicine

Stem and progenitor cell-based therapy in ischaemic heart disease: promise, uncertainties, and challenges

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In the absence of effective endogenous repair mechanisms after cardiac injury, cell-based therapies have rapidly emerged as a potential novel therapeutic approach in ischaemic heart disease. After the initial characterization of putative endothelial progenitor cells and their potential to promote cardiac neovascularization and to attenuate ischaemic injury, a decade of intense research has examined several novel approaches to promote cardiac repair in adult life. A variety of adult stem and progenitor cells from different sources have been examined for their potential to promote cardiac repair and regeneration. Although early, small-scale clinical studies underscored the potential effects of cell-based therapy largely by using bone marrow (BM)-derived cells, subsequent randomized-controlled trials have revealed mixed results that might relate, at least in part, to differences in study design and techniques, e.g. differences in patient population, cell sources and preparation, and endpoint selection. Recent meta-analyses have supported the notion that administration of BM-derived cells may improve cardiac function on top of standard therapy. At this stage, further optimization of cell-based therapy is urgently needed, and finally, large-scale clinical trials are required to eventually proof its clinical efficacy with respect to outcomes, i.e. morbidity and mortality. Despite all promises, pending uncertainties and practical limitations attenuate the therapeutic use of stem/progenitor cells for ischaemic heart disease. To advance the field forward, several important aspects need to be addressed in carefully designed studies: comparative studies may allow to discriminate superior cell populations, timing, dosing, priming of cells, and delivery mode for different applications. In order to predict benefit, influencing factors need to be identified with the aim to focus resources and efforts. Local retention and fate of cells in the therapeutic target zone must be improved. Further understanding of regenerative mechanisms will enable optimization at all levels. In this context, cell priming, bionanotechnology, and tissue engineering are emerging tools and may merge into a combined biological approach of ischaemic tissue repair.

Keywords   Stem and progenitor cells • Bionanotechnology • Cell-based therapy • Ischaemic cardiomyopathy • Ischaemic heart disease • Myocardial infarction

Background

Treatment of acute myocardial infarction (AMI) and ischaemic cardiomyopathy includes rapid revascularization to limit ischaemic damage and consecutive left ventricular (LV) dysfunction and remodelling, and optimized secondary prevention strategies aiming to attenuate progression of cardiac dysfunction and vascular disease. As a result, the prevalence of heart failure from post-ischaemic cardiac dysfunction rather increases[1] causing a substantial morbidity.[2] Furthermore, despite modern medical therapy, there is a substantial number of patients with ischaemic heart disease refractory to current therapeutic approaches lacking further treatment options, i.e. patients with angina pectoris and no option of interventional or surgical revascularization.

After the initial description of putative endothelial progenitor cells (EPCs) more than a decade ago,[3] extensive research in the field of

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regenerative medicine has undermined the long-standing dogma that some differentiated organs such as the heart cannot be repaired in post natal life. We are standing on the merge to the era of biological repair in ischaemic cardiovascular disease after the potential of cardiac repair by a variety of stem and progenitor cell populations has been revealed in pre-clinical and early clinical studies.

In the first part of this review, we recapitulate the *status quo* of adult cell-based therapy in ischaemic heart disease with a clear focus on randomized-controlled clinical trials where available. In addition, we chose to include smaller-size, uncontrolled clinical studies where randomized-controlled data are not available and interesting insights are suggested. Due to space limitations, we were unfortunately not able to include all clinical studies. In the second part, we critically reflect limitations, uncertainties, and challenges of current approaches before finally discussing potential roadmaps of future developments in the field of cell-based cardiac repair. For a comprehensive review of stem and progenitor cell biology, the reader is referred to other in-depth reviews.[4−8]

Clinical experience from cell-based therapy

By definition, stem cells are capable to self-renew and to generate progenitor cells that continue to differentiate into lineage-committed mature cells. Progenitor cells, hence, are more lineage-determined, and therefore carry a more limited differentiation potential and may proliferate for a finite number of divisions and lack a self-renewal capacity. In this nomenclature, CD133 is a marker of premature, rather undifferentiated, barely lineage-committed stem and progenitor cells that is lost early during differentiation, whereas expression of CD34 is maintained to later stages.

The therapeutic use of unselected bone marrow cells that contain stem and progenitor cells initially gained most momentum and has been evaluated farthest in the clinic setting. More recently, other adult stem and progenitor cells, such as circulating stem and progenitor cells, resident cardiac stem cells, and mesenchymal stem cells (MSCs) are being used in translational studies for clinical applications (*Figure 1*). Skeletal myoblasts (SMs) constitute another cell category that was considered suitable for cardiac repair. Each cell population seems to carry its own profile of advantages, disadvantages, practical limitations, and translational practicability.[9]

In the field of ischaemic heart disease, stem and progenitor cell-based therapies have been applied after AMI, after remote (chronic) myocardial infarction (CMI) with cardiac dysfunction and/or in ischaemic cardiomyopathy, and for patients with intractable (chronic) angina pectoris that is not amenable to revascularization and is refractory to medical therapy. Dependent on the targeted entity, different delivery routes have been used for cell transfer.
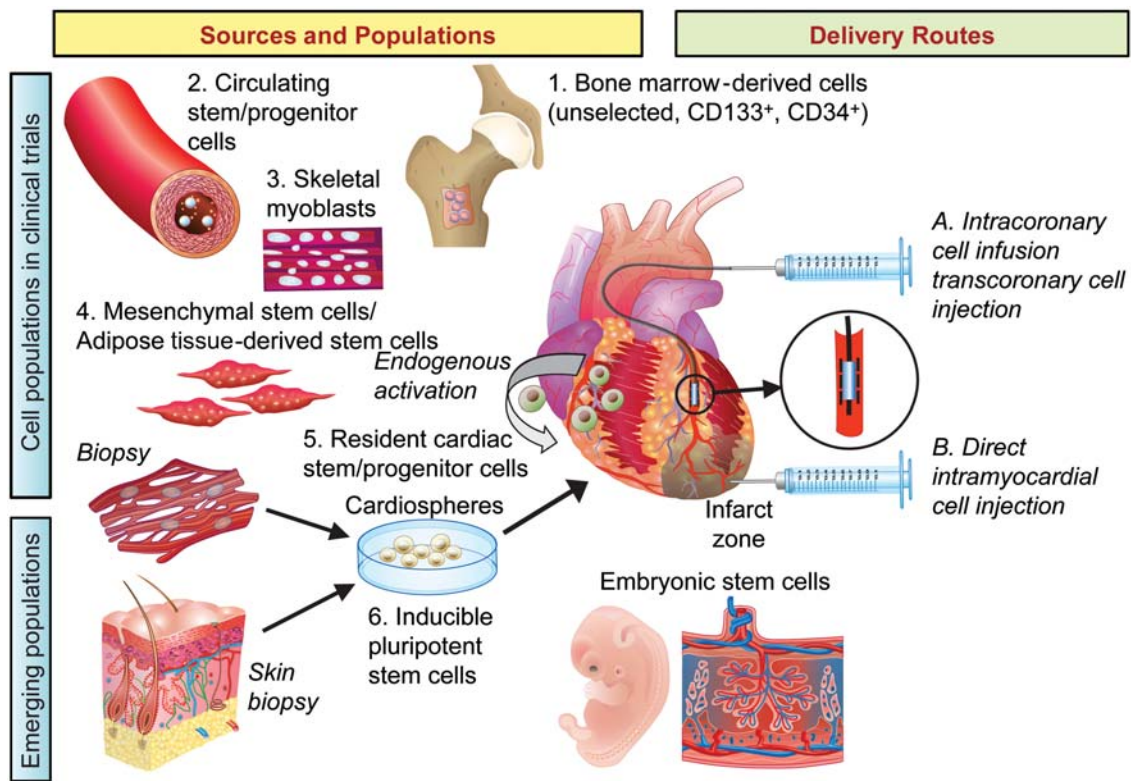

Figure 1 Clinically examined as well as emerging stem and progenitor containing cell populations, and their delivery routes for the treatment of ischaemic heart disease.

Delivery routes for stem and progenitor cell-based therapies

Conceptually, the goal is to safely deliver an optimal number of effective cells as selective as possible to the therapeutic target zone via a minimally invasive route. So far, cells have been transplanted into the heart overly via intracoronary infusion or intramyocardial injection (*Figure 1*).

Adapting the elegant, minimal-invasive technique lent from interventional cardiology for transvascular approaches, stem cells can be homogenously distributed via intracoronary infusion. To reduce spill-back and, in turn, optimize the contact time of cells and coronary vessel wall, cells are infused in block-balloon technique.[10] This approach is only feasible in patients with a patent target vessel and, therefore, no option for non-revascularized areas. In addition, its efficiency is impaired by a complex multistep process of vessel adhesion and transmigration to allow infused stem cells to invade the tissue. Then, homing of invaded cells is also dependent on chemoattraction towards factors secreted from the ischaemic tissue.[11] In line with this concept, cardiac homing of early EPCs after intracoronary infusion was increased in patients with an acute compared with chronic MI.[12] As a side note, although intravenous infusion of stem/progenitor cells initially appeared effective,[13] low target zone cell concentration due to off-target homing[14] and biological redistribution[15] strongly challenged this transvascular strategy which cannot be favoured anymore.

The more invasive intramyocardial cell injection, on the other hand, overcomes some of these hurdles and appears particularly suitable in the case of non-revascularized infarct-related vessels and/or ischaemic cardiomyopathy. Cells can be injected into the myocardium from the epimyocardial side, commonly during open-heart surgery, or endomyocardial side by means of needle-tipped delivery catheters. Detouring the bloodstream has also been particularly attractive for large cell types such as MSCs and SMs. On the downside, injection-related puncture of the viable or necrotic myocardium comes with some risk, i.e. of ventricular perforation. Further, injections lead to inhomogeneous distribution of cell clusters in malperfused tissue. In this scenario, an elegant and now commonly used catheter-based option is to guide the endomyocardial injection by electromechanical mapping (EMM, e.g. NOGA mapping). This technique enables to focus the cells to ischaemic but viable (hibernating) myocardium.

For the future advance, however, it has to be kept in mind from the clinician's perspective that the more invasive the technique and the higher percentage of co-morbidities introduced, the higher the procedural risk in this group of commonly elderly, multimorbid patients, who will be eligible for cell-based therapies.

Unselected bone marrow-derived cells

The use of unselected BM-derived mononuclear cells (BMCs) is clearly the most examined cardiac cell-based therapy in clinical studies (*Figure 2*) with a clinical follow-up experience up to 5 years.[16] To some extent, this development is the result of the pragmatic attractiveness of BMCs: (i) BMCs are rather easy to harvest, (ii) yielded cell numbers do not limit clinical applications, (iii) while it remains
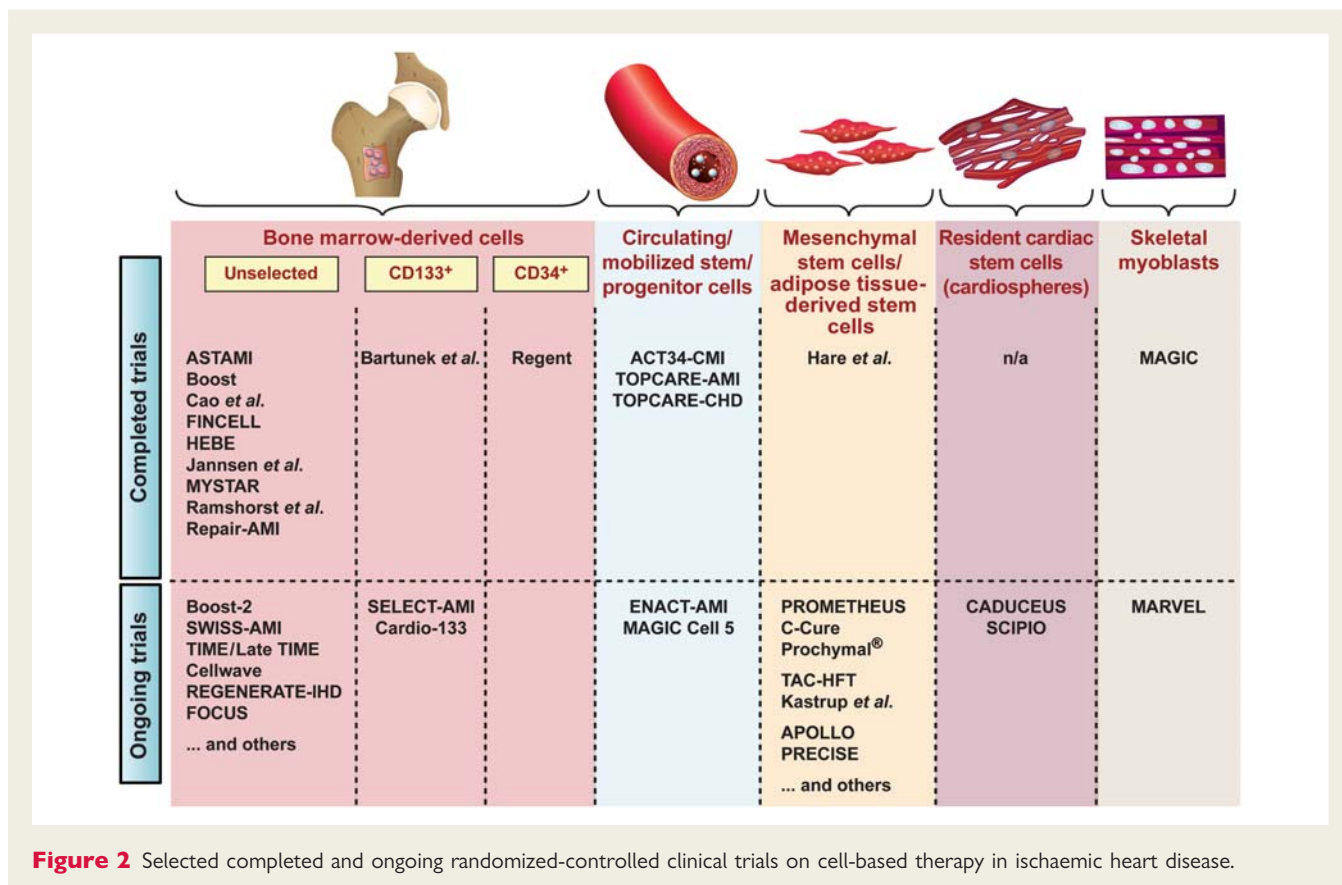

	Bone marrow-derived cells			Circulating/ mobilized stem/ progenitor cells	Mesenchymal stem cells/ adipose tissue-derived stem cells	Resident cardiac stem cells (cardiospheres)	Skeletal myoblasts
	Unselected	**CD133+**	**CD34+**				
Completed trials	ASTAMI Boost Cao et al. FINCELL HEBE Jannsen et al. MYSTAR Ramshorst et al. Repair-AMI	Bartunek et al.	Regent	ACT34-CMI TOPCARE-AMI TOPCARE-CHD	Hare et al.	n/a	MAGIC
Ongoing trials	Boost-2 SWISS-AMI TIME/Late TIME Cellwave REGENERATE-IHD FOCUS ... and others	SELECT-AMI Cardio-133		ENACT-AMI MAGIC Cell 5	PROMETHEUS C-Cure Prochymal® TAC-HFT Kastrup et al. APOLLO PRECISE ... and others	CADUCEUS SCIPIO	MARVEL

Figure 2 Selected completed and ongoing randomized-controlled clinical trials on cell-based therapy in ischaemic heart disease.

unknown which cell types are more potent or have particular potent repair properties, BMCs contain an 'un-narrowed' composition of cells including fractions of stem and progenitor cells, and (iv) their preparation does not need prolonged *ex vivo* manipulation.

Acute myocardial infarction

After early-phase clinical studies had suggested the safety and feasibility of intracoronary BMC infusion after AMI,[10,17−19] several mid-sized, randomized, partly placebo-controlled trials have generated mixed results. The randomized-controlled REPAIR-AMI and BOOST trials showed an improvement of global LV ejection fraction (LV-EF) without significant changes of LV end-diastolic volumes 4−6 months after cell transfer.[20,21] A REPAIR-AMI sub-study revealed that the increase in LV-EF did not occur at the expense of increases in end-systolic or end-diastolic volumes.[22] Two other landmark studies, on the other hand, did not observe a significant improvement in LV function or dimensions at 4- to 6-month follow-up,[23,24] although Janssens *et al.*[23] observed a reduction in infarct size 4 months after intracoronary cell transfer in a study with very early BMC administration (i.e. within 24 h). Although definite reasons for these mixed results remain elusive, differences in study protocol and design, including time from reperfusion to cell injection, type, number, and isolation technique of cells, or follow-up design have been discussed. Whereas BMCs were infused within the first 7 post-infarct days in most trials, in Janssen's trial, cells were injected within the 24 h after AMI.[23] In the ASTAMI trial, magnetic resonance imaging (MRI) was not performed until 2−3 weeks after cell transfer, whereas only echocardiography was done at baseline. Further, cells by contrast were prepared differently following the Lymphoprep technique.[24] Subsequently, follow-up data on the REPAIR-AMI and BOOST collectives have become available. In REPAIR-AMI, the improvement of LV function was sustained after 12 months[20] and was associated with a significant reduction in major adverse cardiovascular events after AMI over this period;[25] an observation that carried on until 2 years of follow-up.[26] Hence, in BOOST, the difference in LV-EF between the groups was no longer significant after 18 months,[27] although an echocardiographic substudy revealed a persistent enhanced diastolic function from BMC application.[28] Recently, 5-year follow-up data from the BOOST collective did not show a sustained benefit on systolic and diastolic LV function after a single BMC infusion.[16] Interestingly, subgroup analyses suggested that patients with a more severely impaired LV function may have a benefit from BMC administration, whereas patients with a rather preserved LV function post-MI may not benefit.

In the controlled but non-randomized BALANCE study,[29] haemodynamics, measures of LV function and geometry, contractility, infarct size, exercise capacity, and, of note, mortality remained improved up to 5 years after BMC infusion when compared with a matched, non-randomized control group. However, these results should be interpreted with caution, given the non-randomized design of the study. Another randomized study from China with a long-term follow-up has recently suggested that BMC administration may lead to an improved LVEF after 4 years.[30] Notably, the results of the recently published REGENT trial are discussed below, because one study arm received selected CD34+/KDR+ BMCs.

Chronic myocardial infarction

In patients with CMI, less experience from clinical studies is available. The early-phase non-randomized IACT study by Strauer *et al.*[31] suggested that intracoronary BMC transfer more than 5 months after MI may result in smaller infarct size, better LV-EF, and wall movement velocity associated with signs of higher cardiac metabolism in the infarcted myocardium. This application has been revisited by a carefully designed cross-over study where intracoronary BMC transfer more than 3 months after MI led to a significant improvement of LV-EF related to enhanced regional contractility in the area of cell application.[32] Of note, this effect was also visible even in patients who crossed-over from control or treatment with circulating progenitor cells.[32]

Strauer *et al.*[33] have recently reported the long-term follow-up data on the intracoronary application of BMCs in patients with chronic heart failure (CHF) due to ischaemic cardiomyopathy from the non-randomized STAR study. Over a 5-year follow-up, intracoronary BMC administration was not associated with adverse events. The authors reported an improved LV performance, quality of life, and survival in patients with CHF who agreed to BMC application when compared with the control group with a similar LV-EF, who did not agree to BMC treatment.[33] However, due to the non-randomized design of the study, these results need to be interpreted with caution.

Refractory myocardial ischaemia

Another set of early-phase studies has addressed the effect of BMCs in patients with refractory myocardial ischaemia lacking options for revascularization. These studies jointly suggest that BM-derived cell injection via transepicardial (e.g. during bypass surgery) or transendocardial (e.g. guided via EEM) may improve subjective endpoints such as angina frequency or heart failure symptoms, and/or measures of global/regional wall motion and perfusion.[34−38] In the randomized-controlled, double-blind trial conducted by van Ramshorst *et al.*,[39] intramyocardial application of BMCs resulted in a modest but significant improvement of myocardial perfusion as assessed by SPECT, angina severity, and quality of life during a 3-month follow-up in patients with severe angina (classes III−IV), despite optimal medical therapy, ineligible for myocardial revascularization, and evidence of myocardial ischaemia at baseline. Similarly, 6 months after direct injection of autologous BMCs in patients with severe refractory angina, a significant improvement of exercise time, LV function, and NYHA functional class was observed in the PROTECT-CAD trial.[40] The data of the ACT34-CMI trial in a similar patient population are discussed below.

Cumulatively, the studies discussed above suggest feasibility and some studies suggest efficacy of BMC-based therapy in acute/chronic MI as well as chronic refractory angina. Recent meta-analyses that summarized more than 1000 patients showed a significant improvement in LV-EF after BMC therapy on top off standard treatment.[41−43] It needs to be acknowledged, however, that LV-EF might not have been the ideal endpoint to clearly detect efficacy (also see Endpoint selection). Overseeing safety data from up to 5 years, no sustained safety issues associated with the use of unselected BMCs have been observed after cardiac transplantation in clinical studies.

Circulating/mobilized CD133^{+} and CD34^{+} stem and progenitor cells

CD133 and CD34 are the most commonly used single markers for the enrichment of haematopoietic stem cells (HSCs). The observation that selection of certain cell populations, i.e. CD34^{+} cells from total mononuclear circulating cells, may augment their potency for cardiac repair has raised the interest in using selected cell populations as opposed to unselected BMCs.[44] Baseline levels of circulating stem/progenitor cells are known to be low, which restricts their therapeutic use. To leverage their therapeutic potential, these cell types can be pharmaceutically mobilized from the BM into the circulation in order to be isolated and enriched (i.e. leukapheresis). Driven by the history of the field, EPCs, a potential progeny of HSCs, have been in the focus (for review see[45,46]).

CD133^{+} cells

CD133^{+} cells are more immature and less lineage-determined than CD34^{+} cells. Intracoronary infusion of selected CD133^{+} cells after recent AMI has been evaluated in a small-scale, non-randomized clinical study that revealed improved LV-EF paralleled by a reduction in myocardial perfusion defect after 4 months.[47] However, more coronary events, such as stent occlusion and in-stent restenosis, were observed after CD133^{+} cell transfer. The authors further reported a time-dependent adverse remodelling of the infarct-related artery with accelerated luminal loss and a reduced conductance after CD133^{+} cell transfer.[48] However, when CD133^{+} cells were injected locally during bypass surgery in patients with recent AMI, no safety concerns became evident in a small, non-randomized study, whereas regional wall motion was improved associated with better myocardial perfusion and viability.[49] Transepicardial injection of CD133^{+} cells into the border zone of MI during operative revascularization in patients who had previous MI was reported by Stamm et al.[50,51] in a small, uncontrolled study. Since no adverse events were reported, treatment was considered safe and feasible. The conclusion that this treatment resulted in improved LV function, however, is limited by the lack of a control group.[50,51]

CD34^{+} cells

CD34^{+} cells contain more endothelial lineage-determined cells than CD133^{+} cells and are therefore considered as a cell population enriched for 'early' EPCs. In the randomized-controlled REGENT trial, unselected and CD34^{+}/CXCR4^{+}-selected BMCs were examined for their effects in LV function after MI in patients with reduced LV function (EF 40%).[52] After 6 months, LV-EF increased by 3% in patients treated with unselected BMCs, 3% in patients receiving CD34^{+}/CXCR4^{+}-selected BMCs, and remained unchanged in the control group. There were, however, no significant differences in absolute changes of LV-EF between the groups.[52] There was a trend in favour of BMC efficacy in individuals with most severely impaired LV dysfunction, as has been observed in several other studies using BMCs after acute MI.[53] A potential limitation of this trial was that MRI measurements of LV function were only completed in a subgroup of patients.

We and others have evaluated intramyocardial injection of CD34^{+} progenitors in coronary artery disease (CAD) patients with chronic angina. Our Phase I/IIa pilot study suggested safety and feasibility as well as a positive trend of bioactivity as evidenced by SPECT perfusion imaging 6 months after CD34^{+} progenitor cells were injected into the hibernating myocardium in patients with refractory chronic angina; transendocardial delivery was guided by EMM (NOGA mapping) to identify ischaemic but still viable myocardium.[54] These trends were further substantiated by our subsequent Phase IIb randomized-controlled, multicentre ACT34-CMI trial.[55] In ongoing studies, this concept is under evaluation for patients critical limb ischaemia (clinicaltrials.gov: NCT00616980). So far, no safety issues are evident with the use of CD34^{+} cells. Whether further selection using the combination of different markers augments cellular repair capacity has to be determined in the future.

Mesenchymal stem cells

Mesenchymal stem cells or stromal cells constitute another potential option for stem/progenitor cell-based therapy, and their use is also evaluated as a potential allogeneic approach based on their immunomodulatory properties. Mesenchymal stem cells are stromal cells present in various tissues, such as BM and adipose tissue. Reflecting their paracrine activity, they exert anti-inflammatory and anti-apoptotic effects.[56] Local immunosuppressive action has fuelled hope of an allogeneic MSC transplantation as an 'of-the-shelf' cell-based therapy to surround practical limitations of cell resources and ex vivo culture often required in the autologous setting.

The regenerative capacity of MSCs in general and the controversially discussed aspect of immune privilege[57,58] of allogeneic MSCs needs to be evaluated in men. Autologous and allogeneic MSC transfer is currently under investigation; however, clinical data are scarce. In a randomized-controlled, double-blinded Phase I study, intravenous application of allogeneic MSCs after acute MI did not raise safety concerns and as assessed by a global symptom score might be efficacious over a period of 6 months.[59] In early studies, MSCs were injected intravenously; however, the pulmonary passage of these large cells may be problematic.[60] Since these positive efficacy data stand in contrast to negative experience from intravenous BMC applications, this set of data should still be considered with caution. Subsequently, intracoronary application of MSCs after AMI has been evaluated in two non-randomized early-phase studies by Chen et al. A high-dose of BM-derived MSCs (6×10^{10}) resulted in a significant improvement of LV-EF in several modalities,[61] whereas a lower dose (5×10^6) did not improve LV function in chronic ischaemic cardiomyopathy in a subsequent study by the same authors.[62] Still, MSC treatment resulted in an improved exercise capability and heart failure symptomatology after 3 months.

Adipose tissue can also serve as a source of MSCs, i.e. adipose tissue-derived stem cells (ADSCs). Two clinical trials—APOLLO and PRECISE—are exploring the safety, feasibility, and efficacy of freshly isolated ADSCs with the Cellution[TM] system (Cytori Therapeutic Inc.) in patients with either AMI or CMI.

Cardiac-derived cardiovascular stem and progenitor cells

Until recently, our dogma was that the fully differentiated heart has no capability for cell turnover and self-repair. In their elegant observation, Bergmann et al.[63] showed evidence for in-men cardiomyocyte renewal at a rate of 1% per year in younger adults and 0.5% in the elderly. In post-natal hearts, various subtypes of tissue-resident cardiac stem and progenitor cells (CSCs) classified by surface antigens and transcription markers have been reported, although it is undetermined whether these subtypes have clearly distinct phenotypes. Cardiac stem and progenitor cells, which have been suggested to be capable of creating cardiomyocytes and all surrounding cell types, are a promising candidate-at least in theory-to provide contractility and vascularization.[64] In the light of the fact that their genuine number is low, CSCs isolated from endomyocardial biopsies have successfully been expanded ex vivo to leverage this therapeutic concept.[65] Dr Marban's group has proposed a population of potential clinical relevance that has been identified by expanding CSCs from self-adherent clusters (cardiospheres) under certain conditions, i.e. cardiosphere-derived stem cells (CDCs).[66,67] There is still some controversy on the cardiomyogenic potential of cardiospheres.[68,69] Dr Field's group has recently suggested by using genetic cell tracking that there are temporal limitations for the ability of cardiac-resident c-kit$^+$ cells to acquire a cardiomyogenic phenotype, i.e. that the cardiomyogenic population is present in neonatal hearts but largely lost in adult mouse hearts and suggested that elucidation of the underlying molecular mechanisms may permit a more robust cardiomyogenic induction in adult-derived cardiac c-kit$^+$ cells.[70]

Early-stage clinical studies currently address safety and feasibility of cardiosphere-derived stem and progenitor cell transplantation. In the SCIPIO trial (clinicaltrials.gov: NCT00474461), patients with ischaemic cardiomyopathy from CMI who undergo bypass surgery received an intracoronary infusion of autologous CSCs isolated from their right atrial appendages. Further, patients with ischaemic LV dysfunction from recent MI receive an intracoronary injection of CDCs in the ongoing Phase I randomized dose-escalation CADUCEUS trial (clinicaltrials.gov: NCT0089336).

Skeletal myoblasts

The concept of resident stem cells (i.e. satellite cells), which are quiescent under normal conditions, contribute to new myocytes in response to injury is rather well established for skeletal musculature. Skeletal myoblasts can be conveniently isolated from muscle biopsies and ex vivo expanded for therapeutic use. After pre-clinical evidence showed their repair effects in ischaemic myocardium,[71,72] SMs have been thought to transdifferentiate into cardiomyocytes. In the meanwhile, pre-clinical data indicate that SMs maintain a skeletal muscle fate,[73] infrequently fuse with cardiomyocytes,[74] and hardly couple electromechanically with the host myocardium.[75]

The clinical value of SMs is uncertain. Initially, several uncontrolled early-phase studies generated promising results for the therapeutic utility of SMs in CHF, suggesting an improved global and regional contractility and/or viability in the infarct zone. In most studies, SMs were overly injected via a transepicardial route during graft surgery,[76–78] whereas only one study reported the transendocardial injection of myoblasts as safe and feasible.[79] The most comprehensive clinical evaluation of SMs, so far, has been the multicentre, randomized-controlled, double-blind Phase II MAGIC trial.[80] Herein, autologous, for 3 weeks ex vivo expanded SMs were directly administered around the scar tissue in patients with ischaemic cardiomyopathy who underwent bypass surgery. After 6 months, primary endpoints, namely global and regional LV function assessed by echocardiography, were not significantly changed. Notably, the authors reported a significant reduction in LV volumes in the high-dose group. The longest clinical experience with SM transfer, however, has been published by Dib et al. In their non-randomized, uncontrolled Phase I study, patients undergoing surgery for revascularization or implantable cardioverter-defibrillator implantation showed an increase in LV-EF and viability, whereas no adverse events were observed up to 4 years transepicardial injection of autologous SMs.[81]

Fuelled by an earlier report from Menasche et al.[76] where the use of myoblasts was associated with arrhythmic events in 40% of the patients, SMs have been considered to be pro-arrhythmogenic. In the subsequent MAGIC trial, no significant increase in arrhythmic events was reported, although it must be acknowledged that there was a trend towards more arrhythmias in SM-treated patients despite treatment with amiodarone a priori.[80] Hence, none of the other early phase above nor the 4-year surveillance by Dib et al.[81] has added evidence for SM-induced sudden death. Chachques et al. hypothesized that the arrhythmicity of SMs may be conditioned by the bovine serum used during ex vivo expansion for 3 weeks (cellular cardiomyoplasty) that may constitute an antigenic and thereby inflammatory substrate. A strictly autologous preparation using the corresponding donor serum in this rather small-sampled study did not lead to any arrhythmias over 1–2 years of follow-up.[82] As the majority of the above-mentioned, early-phase studies did not report arrhythmic events, it remains vague whether this particular procedure stabilizes SMs electrically. There is still debate about the potential pro-arrhythmicity of different adult stem/progenitor cells, in particular the SMs.[83,84] Further, in contrast to earlier hesitations to infuse SMs intracoronary in the light of a potential risk for microembolization due to their cell size, there were no adverse events following transcoronary-venous SMs delivery observed in the POZNAN trial.[85] The potential risk of arrhythmias and sudden death needs, however, ultimate clarification for this cell type.

Endogenous mobilization of stem/progenitor cells

In contrast to exogenous cell transfer requiring invasive delivery, adult stem/progenitor cells can be mobilized from the BM by systemic administration of certain cytokines [e.g. granulocyte colony-stimulating factor (G-CSF)] to augment circulating levels of these cells and, thereby, enhance ischaemic tissue repair via circulating stem/progenitor cells. This concept has been transferred from the haematologists who isolate HSCs from the bloodstream before ablation of the BM in stem cell transplantation.

Earlier, the authors of the much debated MAGIC trial reported that G-CSF mobilization after MI was associated with an unexpected higher rate of in-stent restenosis at the culprit lesion, whereas intracoronary infusion of peripheral blood stem cells appeared safe and potentially efficacious; because of this safety issue, the study was discontinued.[86] Subsequently, in a controlled, non-randomized study by Ince et al.,[87] G-CSF mobilization of CD34+ BMCs shortly after angioplasty in AMI improved LV function and metabolic activity and attenuated LV remodelling up to 12 months without accentuation of the restenosis rate or late adverse events. In the same line, Valgimigli et al.[88] showed an unremarkable safety profile for G-CSF in patients with AMI, although LV perfusion or function was unchanged 6 months after treatment. Furthermore, G-CSF did not improve LV wall motion or perfusion over the period of 1 month in patients with stable CAD.[89] Of note, there was a trend towards more ischaemia, and in 2 out of 16 patients reinfarction occurred.[89] Early clinical experience with stem cell mobilization by G-CSF from 10 trials, including 445 patients with AMI, was recently summarized in a meta-analysis by Zohlnhöfer et al. Herein, the authors concluded that cumulatively the data do not support efficacy of endogenous stem cell mobilization by G-CSF.[90] Without evidence for efficacy on the one side, and a potentially questionable safety profile on the other (i.e. with respect to restenosis), large-scale trials, which would be needed to clarify the clinical potential, could not be advocated. Interestingly, very early G-CSF administration (<12 h) has recently been reported to attenuate ventricular remodelling after large, anterior MI, whereas systolic function and myocardial perfusion were unchanged in the STEM-AMI collective.[91]

In recent years, pharmaceutical compounds emerged to modulate well-defined cascades of stem/progenitor cell biology and homing. Continuing the concept of endogenous stem cell mobilization, Zaruba et al.[92] proposed a combined strategy of G-CSF with dipeptidylpeptidase IV (DPP-IV) inhibition to improve cardiac homing of mobilized stem/progenitor cells, which is currently under clinical investigation in the SITAGRAMI trial in patients after AMI. This concept proposes that inhibition of DPP-IV that is cleaving one of the main stem cell attracting chemokines, SDF-1α, will improve homing of mobilized stem/progenitor cells. More recent results from our group and others described the CXCL12/CXCR4 axis, the SDF-1α pathway, as a relevant regulator of endogenous cell mobilization.[93] In pre-clinical studies, the CXCR4-antagonists Plerixafor (known as Mobozil or AMD3100) mobilizes stem/progenitor cells from the BM, increases BM-derived cell incorporation in the ischaemic border zone, and improves cardiac function post-MI.[93] Because this agonist has clinically been used as HIV therapeutic without relevant side effects for years, its safety profile appears favourable. To our knowledge, no clinical data are available on any of these pharmaceutical strategies to therapeutically modulate endogenous stem/progenitor cell trafficking.

Emerging stem cell types with a potential for cardiomyogenic differentiation

Embryonic stem cells
Embryonic stem cells (ESCs) posses several features that are conceptually attractive for cardiac repair. Embryonic stem cells are pluripotent, which means that they have the ability to differentiate into all cell lineages.[94,95] On the other hand, there are substantial ethical and regulatory concerns with their retrieval. The limited availability further hinders its therapeutic utility. In addition, there is a risk for malignancies (i.e. intra- and extracardiac teratoma or other tumour formation) associated with the use of ESCs,[96,97] as it has recently become evident in a patient who developed a likely donor-derived multifocal brain tumour after treatment with foetal neural stem cells.[97] At the same time, reliable modalities to regulate and control differentiation in a targeted and controllable fashion are challenging, although several approaches to limit tumour formation have been explored, including strategies to enhance cardiopoietic programming of ESCs.[98]

Furthermore, following allogeneic application, ESCs may trigger an immune response in the recipient. Finally, it remains elusive whether human ESCs efficiently structurally engraft and electromechanically integrate into ischaemic myocardium. The above-described limitations are some of the hurdles that currently hinder the transition of ESC-based therapy into clinical translation.

Inducible pluripotent cells
In 2006, Takahashi and Yamanaka[99] reported that differentiated murine fibroblasts could be reprogrammed into stem cells with the capacity to form all three germ layers and termed these cells induced pluripotent stem cells (iPS). Nuclear reprogramming with ectopic stemness factors has opened the opportunity to generate autologous patient-derived iPS from adult somatic cells.[99,100] The ability of both mouse and human iPS to differentiate in functional cardiomyocytes has recently been demonstrated.[101,102]

The study of iPS from selected cohorts of patients is an innovative way to uncover molecular mechanisms of disease, such as nicely illustrated in the study of Moretti et al.,[103] who generated pluripotent stem cells from dermal fibroblasts of patients with long-QT syndrome type-1 and subsequently induced their differentiation into functional cardiac myocytes that recapitulated the electrophysiological features of the disorder.[104]

Functionally, intramyocardially injected undifferentiated iPS, but not parental fibroblasts, engrafted and improved contractile function of infarcted myocardium while attenuating adverse remodelling in immunocompetent mice.[105] Only in immunocompetent mice, the environment after cardiac transplantation was permissive for differentiation, whereas in immunodeficient mice, tumour development was observed, which highlights the importance of immune surveillance to prevent tumour growth.[105] New technologies such as small molecule screen and epigenetic modifications most probably will establish further potentially more safe options for the generation of iPS. It will, however, be crucial to validate the epigenetic and phenotypic stability in the reprogrammed state. Also, reprogramming pluripotency in somatic cells comes with the risk of tumorigenicity as described above. Furthermore, iPS become immunologically relevant while they differentiate with up-regulation of histocompatibility antigens. This aspect would narrow the range of applications to the autologous setting.[106] As described above, several important hurdles have to be resolved before a clinical translation of iPS-based therapies can be considered, including safety issues to prevent tumour growth that may require determination of the appropriate

differentiation of iPS-derived cells and removal of residual undifferentiated cells before transplantation.[107] In addition, upscaling for an effective iPS-based cell generation will be required. Several important considerations in the proposed mechanisms of cell-based therapy for cardiac repair and its further development are described in the Supplementary material online and depicted in Figures 3–5.

Future directions of cell-based therapy: a roadmap

Various strategies have been proposed to support stem cells in the hostile environment of ischaemic tissue characterized by ischaemia, acidosis, inflammation, and oxidative stress. Beyond the modulation of cell homing, viability, engraftment, and retention, it becomes more and more clear that true repair of damaged myocardium will need more than a single-dose administration of cells.

Priming of stem and progenitor cells to enhance their therapeutic efficacy

The concept to pre-treat or modify stem/progenitor cells before application (priming) and thereby enhance their therapeutic potency has evolved from earlier pre-clinical observations.[108–114] These strategies basically target any function step that influences cell fate from the application on: adhesion/transmigration,

homing, migration, engraftment, survival, cell–cell interaction, repair capacity, differentiation, and retention. Potential tools for modification include drugs, small molecules, naked and vector-facilitated plasmids, and epigenetic reprogramming (for more details see[115,116]). Priming of dysfunctional autologous cells from cardiovascular patients via any of these tools may allow for a 'resetting of impaired biopotency'.

Among multiple targets stemming from pre-clinical evaluation, the following examples are under clinical investigation: we and others have identified a reduced endothelial NO synthase-dependent NO production as an important mechanism limiting the functional repair capacity of endogenous progenitor cells in patients with diabetes or hypertension.[108,117] In the randomized, placebo-controlled ENACT-AMI trial, the therapeutic use of eNOS-overexpressing EPCs is presently studied in patients with large MI (clinicaltrials.gov: NCT00936819).[118] As another example of modified stem/progenitor cells, in the MESAMI II trial (clinicaltrials.gov: registration in process) designed by Roncalli and colleagues, patients with no-option chronic ischaemic cardiomyopathy will receive an intramyocardial injection of MSCs pre-treated with melatonin. This concept is based on pre-clinical data showing that pre-treatment with the pineal hormone increases survival, paracrine activity, and therapeutic efficiency of MSCs in a rodent ischaemia model.[119] Another way to boost paracrine and differential cell functions is to pre-stimulate cells before application.[120,121] This concept is currently translated into patients with
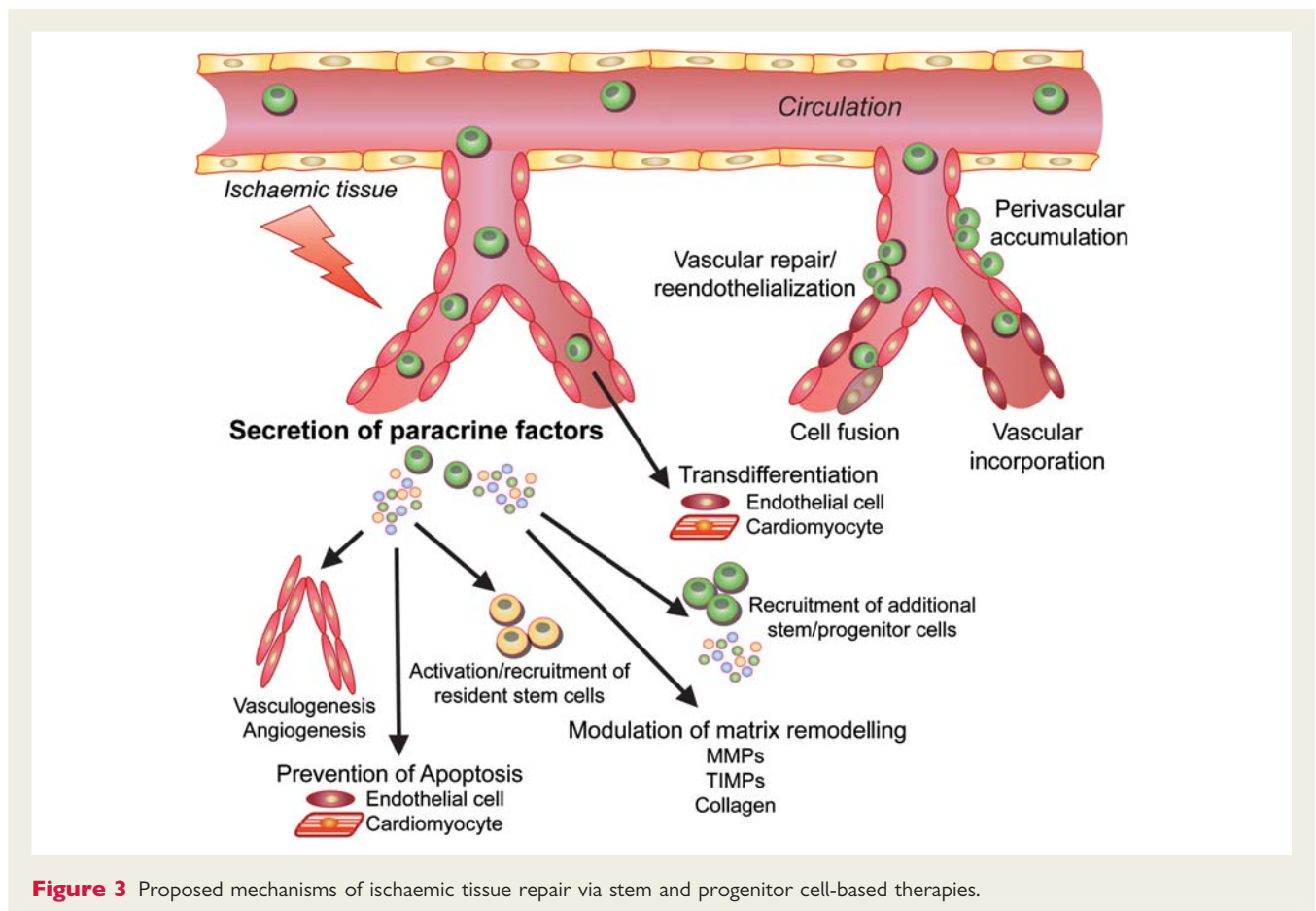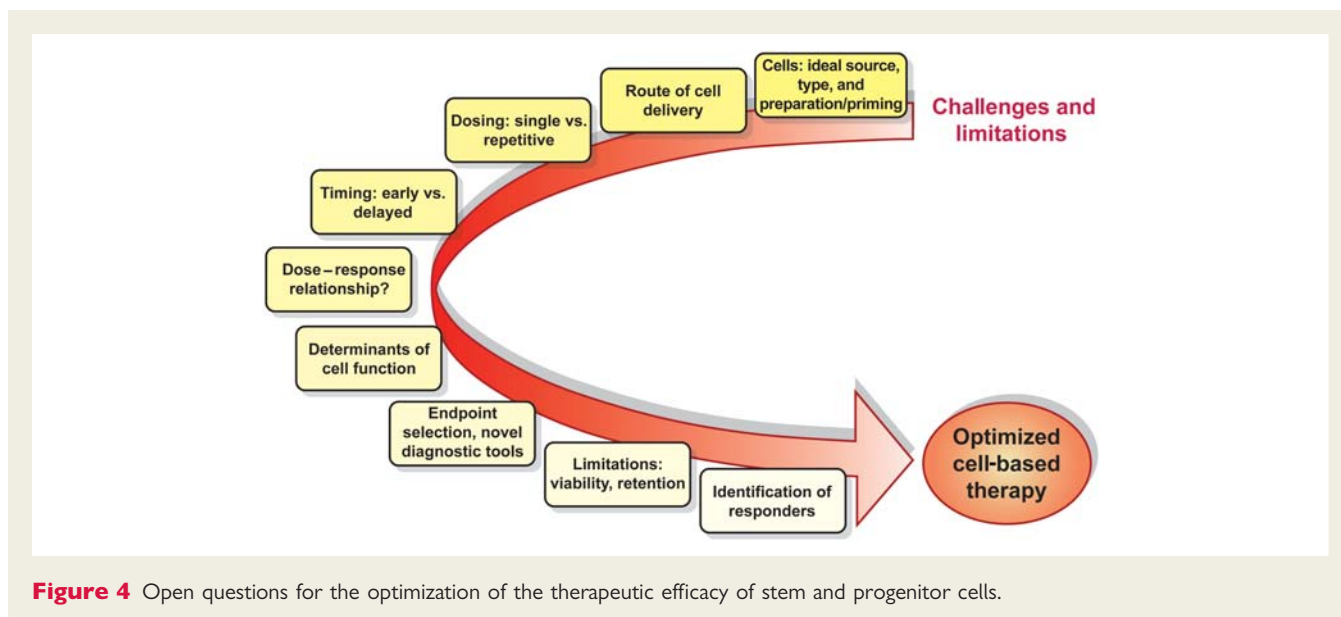

Figure 3 Proposed mechanisms of ischaemic tissue repair via stem and progenitor cell-based therapies.

Figure 4 Open questions for the optimization of the therapeutic efficacy of stem and progenitor cells.

heart failure from ischaemic cardiomyopathy by pre-incubation of BM-derived MSCs with growth factors to guide their transition into cardiopoietic cells (C-Cure trial, clinicaltrials.gov: NCT00810238).

As no clinical study of primed stem cells has been completed yet, it is going to be interesting whether these strategies will hold promise in clinical applications. Many more targeted cell modifications can be expected to be in transition from bench to bedside at present.

Bionanotechnology to support cell-based therapies

In addition to the harmful biochemical milieu, loss of tissue architecture and loss of matrix support within the infarcted myocardium detrimentally affect cell–cell and cell–matrix interaction and, in turn, regulation of dependent pathways that are functionally inevitable for the homeostasis of transplanted cells. The rapidly evolving field of bionanotechnology allows to specifically design biomaterials to support transplanted cells within this ischaemic environment.[122] Herein, the structure, dimensions, and shape of constructs are pivotal to better mimic the native architecture of extracellular matrix. An optimal biomaterial to support cell therapy should provide a three-dimensional environment to enhance biomechanical properties of extracellular matrix; a purpose for which controlled organization at nano-scale is needed (for more details see[123]). In some biomatrices, bioactive signals can be incorporated to specifically modulate stem cell biology while supporting them structurally.[124]

The strategy to support cell transfer has rapidly gained attention triggered by exciting pre-clinical data. In murine models, nanofibres that self-assemble into a matrix recruited endogenous progenitors to the myocardium and supported transplantation of cardiomyocytes providing a microenvironment.[125] In principle, biomaterials can be custom-designed to optimally fit the organ-specific microenvironment.[126] Furthermore, bioactive signals can be incorporated in some biomaterials to additionally enhance cell survival, retention, proliferation, and differentiation. In this context,

Padin-Iruegas et al.[127] reported that an insulin-like growth factor carrying nanofibre enhances CSC-dependent repair of cardiac injury.[128] In our hands, the combination of a linage-specific optimized, self-assembling nanofibre enhances the potency of cell-therapy in ischaemic tissue repair.[129] Also, bioactive sequences of biologically attractive paracrine factors, e.g. SDF-1, can more effectively be presented via biomaterials with the aim to recruit endogenous into or support exogenously applied cells in ischaemic myocardium.[130]

Although emerging results for the role of bionanomaterials in cell-based ischaemic tissue repair are promising, there has not been precedence in humans. Before any clinical translation, several aspects such as dosing, dynamics and kinetics of bioactivity, biodegradability, occurrence and excretion of breakdown products, and immune compatibility have to be carefully addressed.

Tissue engineering

Tissue engineering has now been advanced for several years. In particular aiming at replacement of the myocardium, the breakthrough in this field has been hindered by several challenges. While cell seeding of construct backbones has worked well for proliferative and hypoxia-tolerant cells, it has been difficult to achieve tissue-like densities with cells with low proliferation rate such as cardiomyocytes,[122] although successful generation and transplantation of engineered heart tissue grafts has been reported in small animal models.[131] Achieving sufficient perfusion of larger constructs is challenging. Passive diffusion is generally tolerated up to a distance of $<0.1-0.2$ mm. In the case of longer distances, dysfunctionality and necrosis occur as a failure of sufficient diffusion.[132] At present, bigger, more complex constructs such as myocardium (1.0–1.5 cm) cannot be maintained by simple diffusion and need anastomosis to a vascular network. Since generation of a stable and functional construct microenvironment remains critical, stem and progenitor cells display a strong potential to accelerate tissue engineering for the purpose of seeding but also for the creation of a vascular networks.[133]
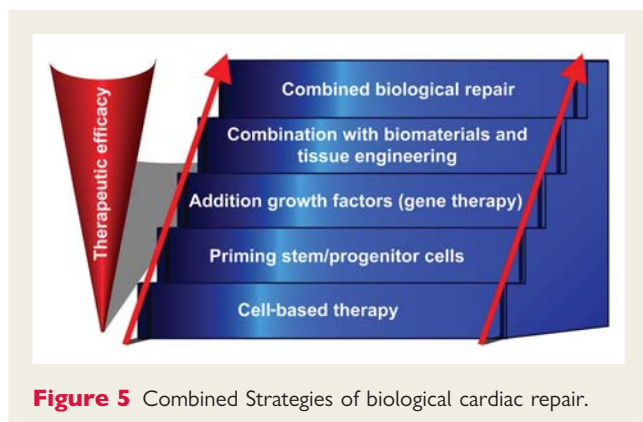
Figure 5 Combined Strategies of biological cardiac repair.

Combined strategies of biological repair

As stated above, single-dose transfer of a single cell type into a deranged environment will likely not be sufficient to 'regenerate' a complex tissue such as the heart. More recent insights suggest that combined strategies of biological repair more closely resemble native biological mechanisms and, thus, may be more potent than current one-stop, single-cell strategies (*Figure 5*). Conceptually, the combination of different types of stem/progenitor cells with stromal cells is functionally very attractive aiming at synergisms. Different cell (sub-) populations may exert different regenerative effects. Unselected and selected cells, for example, showed different spatial patterns of homing.[15] Melero-Martin *et al.*[133] recently described striking evidence for the synergistic interaction of EPCs and MSCs, which contain relevant pericytes, to create a substantial and functional vascular network in a Matrigel assay that was stable for up to 4 weeks.

To add yet another level of complexity, supplemental tools that are aimed to improve efficacy of cell transfer have become available. Engraftment of cardiac-derived stem cells (CSCs) after intramyocardial application, for example, is improved after sealing the injection site with fibrin glue to attenuate the known leakage of cell suspension.[134] Low-energy shock wave treatment of the target tissue has been shown to enhance recruitment of circulating EPCs by enhancing local expression of chemoattracting growth factors in mice.[135] This technology is thought to prepare the ischaemic target zone for cell transfer and thereby indirectly augment cell homing and retention. In the ongoing Cellwave study (clinicaltrials.gov: NCT00326989), extracorporal shock wave pre-treatment before intracoronary application of BM-derived progenitor cells is already under clinical evaluation in patients with CMI. In addition, homing of cells via the intracoronary route can be improved by disrupting cell-carrying microbubbles via ultrasound. Without evoking undesired damage, this technology appears to facilitate vascular transmigration by creating capillary pores.[136,137]

With all limitations and shortcomings in mind, the idea to realize effective cardiac tissue repair by using only one cell type is likely challenging. Depending on further mechanistic and clinical insights, we envision combined, precisely timed, multi-step approaches that incorporate various stem and stromal cells, paracrine factors, and specifically bioengineered tools for the future cardiac regenerative medicine in order to advance this exciting field to the next level, the level of true ischaemic tissue repair.

Summary

We are currently in the phase of translation of cardiac cell-based therapy, and good translation without any surprise takes time and efforts. After the initial hype revolving cell-based cardiac tissue repair led to a rather rapid translation into early-phase clinical trials, we have now reached the stage where conclusive answers and thoughtful fine-tuning, which includes a step back to the bench to find answers, are needed. There is some consensus that cell-based therapy has potential beneficial effects on top of standard medial therapy as it has been expressed by the task force of European Society of Cardiology.[138] Apart from uncertainties and limitations discussed above, several pressing tasks need to be addressed: sustained efficacy needs to be proven for each cell type depending on the treated entity. Comparative studies may allow discriminating superior cell populations, sources, timing, dosing, and delivery mode considering with regard to certain applications. Safety profiles dependent on cell type, delivery route, and underlying disease need to be characterized by long-term follow-up of larger collectives. To substantiate these aspects, large-scale, multicentre trials are clearly needed. In order to predict benefit, influencing factors need to be identified with the aim to focus resources and efforts. Local retention and fate of cells in the therapeutic target zone must be improved. Further understanding of regenerative mechanisms will enable optimization at all levels. Cell priming, bionanotechnology, and tissue engineering are emerging tools and may merge into a combined biological approach of ischaemic tissue repair.

After a hectic decade, it is time to catch breath and focus efforts on concise pre-clinical work, and thoughtfully designed, sufficiently powered clinical trials to find answers to important remaining questions, to clarify on uncertainties, and to overcome limitations with the aim to optimize cell-based therapy. Despite all promise, eventually, we are challenged to show robust effects on disease progression, morbidity, and mortality associated with an acceptable safety profile to advance the promising concept of cell-based therapy to clinical routine.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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