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Concise Review: Mending a Broken Heart: The Evolution of Biological Therapeutics

Caressa Chen, Vitavat Termglunchan, Ioannis Karakikes

Key Words. Cardiac regeneration • Cell therapy • Gene therapy • Regenerative medicine • Heart failure

ABSTRACT

Heart failure (HF), a common sequela of cardiovascular diseases, remains a staggering clinical problem, associated with high rates of morbidity and mortality worldwide. Advances in pharmacological, interventional, and operative management have improved patient care, but these interventions are insufficient to halt the progression of HF, particularly the end-stage irreversible loss of functional cardiomyocytes. Innovative therapies that could prevent HF progression and improve the function of the failing heart are urgently needed. Following successful preclinical studies, two main strategies have emerged as potential solutions: cardiac gene therapy and cardiac regeneration through stem and precursor cell transplantation. Many potential gene- and cell-based therapies have entered into clinical studies, intending to ameliorate cardiac dysfunction in patients with advanced HF. In this review, we focus on the recent advances in cell- and gene-based therapies in the context of cardiovascular disease, emphasizing the most advanced therapies. The principles and mechanisms of action of gene and cell therapies for HF are discussed along with the limitations of current approaches. Finally, we highlight the emerging technologies that hold promise to revolutionize the biological therapies for cardiovascular diseases.

INTRODUCTION

Cardiovascular diseases are debilitating and often deadly, responsible for more deaths worldwide than any other disease [1]. Specifically, heart failure (HF)—the common sequela of many cardiovascular diseases—affects over 38 million people, 50% of which are estimated to die within 5 years of diagnosis [2]. Given the limited intrinsic regenerative potential of the adult heart [3], the endogenous sources of regeneration and repair are insufficient to halt the progression of HF. In end-stage cases, the last available option for treatment is cardiac transplantation, which is limited by the shortage of organs available. Pharmacological and device-based treatments that are currently implemented into HF treatment guidelines have improved patient survival [4, 5]. Although these therapies are beneficial, they are limited in relieving symptoms and do not address the molecular mechanisms underlying the pathogenesis of HF and hence cannot reverse or slow the adverse remodeling of the heart. This limitation in available remedies has raised the need for therapies that could repair or even regenerate the injured myocardium. Biological therapies, such as gene- and cell-based approaches, have emerged as alternative therapies to treat both acute cardiac events such as myocardial infarction (MI) and chronic cardiovascular diseases, representing a new generation in biological therapeutics for HF.

In this review, we discuss the progress in the gene therapy field and the use of stem and precursor cells for stimulating endogenous regeneration and/or as a source for cardiomyocyte renewal, emphasizing the recent human clinical trials. In addition, we highlight
the biological processes that underpin the reported therapeu-
tic benefits, and discuss the shortcomings, challenges, and
future perspectives of gene- and cell-based therapies for HF.
Finally, we examine the most exciting advances in the field,
which hold promise as alternative approaches to cardiac
regeneration and repair.

PROGRESS AND CHALLENGES IN STEM CELL CARDIAC
REGENERATIVE THERAPY

Over the past two decades, substantial progress has been
made in the field of stem cell therapy for cardiac repair.
Despite the absence of an understanding of the mechanism
through which donor cells improve cardiac function in
patients, research has proceeded rapidly from preclinical mod-
els to clinical studies. To date, a plethora of relatively small
clinical trials have tested the potential benefit of various cell
types in patients with HF (Table 1).

BONE MARROW-DERIVED CELLS

Bone marrow consists of hematopoietic stem cells (HSCs) and
nonhematopoietic multipotent cells, such as mesenchymal
stem cells (MSCs), which can be induced to differentiate into
the adipocytic, chondrocytic, or osteocytic lineages [28]. Bone
marrow mononuclear cells (BMMNCs) can be readily obtained
from patients by bone marrow aspiration and density gradient
centrifugation without the need for culture in vitro before
administration.

Since its inception over 15 years ago [29], the use of autologous
BMMNCs as cell therapy for HF has been investi-
gated in multiple randomized and nonrandomized trials, yield-
ing conflicting and controversial clinical outcomes. Early
clinical trials such as TOPCARE-CHD [15], REPAIR-AMI [9], and
FINCELL [10] reported improved systolic function in treated
acute MI (AMI) patients, while others reported either no sig-
nificant improvements (ASTAMI, Leuven-AMI) [7, 8] or
absence of any long-term benefits (BOOST) [6]. More recent
trials with larger cohorts that were adequately controlled
(FOCUS-CCTRN, TIME, Late TIME, REGENERATE-AMI) [11–14]
found modest or no effect of BMC therapy on ventricular
function and prespecified endpoints. Overall, all trials failed to
show any improvements in clinical outcomes in the treated
patients.

In an attempt to improve the therapeutic potential of
autologous bone marrow cells, multiple trials have assessed
the safety and efficacy of selected and ex vivo expanded sub-
populations, such as MSCs. The C-CURE [18] trial was one of
the first studies that implemented the concept of delivering
cardiac lineage primed bone marrow-derived MSCs (termed
“cardiopoietic stem cells”) before myocardial implantation
in patients with ischemic cardiomyopathy. This cardiopoietic cell
population was derived by exposure of MSC (CD105, CD166,
CD29, and CD44 and negative for CD14, CD34, and CD45) to a
growth factor cocktail, including transforming growth factor-β,
bone morphogenetic protein, activin A, fibroblast growth fac-
tor 2, cedartophilin, and α-thrombin, which triggers hallmark
traits of cardiac development [30]. Despite inconsistencies in
the reported data [31], the study suggested that patients who
received cells showed evidence of improved function versus
the control arm 6 months after treatment, suggesting that
therapy with cytokine-primed MSCs is safe and feasible
with signs of benefit in chronic ischemic HF. Another autolog-
ous bone marrow-derived subpopulation, termed ixmyelocel-
T, has been tested in clinical trials in HF patients. Ixmyelocel-T
is an expanded population of mesenchymal stromal cells and
M2-like macrophages, as well as many of the CD45+ cells
found in the bone marrow. Although the precise mechanism
of action is unknown, it is hypothesized that this expanded
multicellular product induces tissue remodeling, immunomo-
dulation, angiogenesis, and endothelial protection [32, 33].
The early phase, open label clinical trials (IMPACT-DCM and
CATHERET-DCM) suggested that intramyocardial delivery of
ixmyelocel-T might improve clinical, functional, symptomatic,
and quality-of-life outcomes in patients with HF due to ische-
mic dilated cardiomyopathy (DCM) [16]. More recently,
the phase IIB randomized, double-blind ixCELL-DCM [17] study
showed that this multicellular therapy resulted in a significant
reduction in adjudicated clinical cardiac events compared with
placebo leading to improved patient outcomes, corroborating
the findings of early trials.

The widespread use of BMMNCs can be attributed to
immediate availability from the patient. Nonetheless, allogene-
ic cells could provide an even more readily available “off-
the-shelf” therapeutic agent, avoiding the need for bone mar-
row aspiration and tissue culture delays before treatment. As
such, allogeneic BM-derived MSCs have recently emerged as
the leading candidate for an “off-the-shelf” product for HF
cell-based therapy [34]. MSCs are considered immune-
privileged and can be expanded in quantities unattainable
from an autologous source, undergo cryopreservation, and be
available for delivery. The early-stage study (POSEIDON) [21]
was the first to demonstrate that alloimmune reactions in patients receiving allogeneic MSCs for ischemic left ventricle/
ventricular (LV) dysfunction were low, suggesting that alloge-
nic MSC transplantation might be accomplished without the
need for significant host immunosuppression. The trial
reported similar safety profiles between the autologous and
allogeneic MSCs. Although it was not powered to show effica-
cy, the MSC treatment favorably affected ventricular remodel-
ing of patients with ischemic cardiomyopathy. Similarly,
immunoselected bone marrow-derived mesenchymal precur-
sor cells (MPCs), an enriched Stro-1/Stro-3+ population, were
evaluated in a phase II, multicenter, dose escalation study to
determine feasibility and safety of three doses in patients
with chronic HF. This study concluded that the high-dose allo-
genic MPC treatment may reduce adverse cardiovascular
events and provide beneficial effects on adverse left ventricu-
lar remodeling [20]. Taken together these studies suggest that
allogeneic immune-selected MSCs are safe and potentially
beneficial in treating patients with ischemic cardiomyopathy,
offering an off-the-shelf readily available cell product. This
beneficial effects attributed to multiple mechanisms have
been proposed, including transdifferentiation, paracrine factor
secretion with antiapoptotic, proangiogenic, and possibly
immunomodulatory effects. However, to date, the precise
mechanisms involved in the positive impact of MSCs remain
to be identified.

Despite rapid clinical translation and widespread enthusi-
asm, the therapeutic benefits of bone marrow-derived cell
(BMC) therapy in patients with heart disease remains
controversial. Differences in cell types, cell preparation standards, delivery techniques, imaging methods, and patient profiles can lead to incorrect inferences, and the effects of the therapies are difficult to interpret. Systematic review and meta-analysis of data from eligible randomized controlled trials could be informative, but have also yielded conflicting results, highlighting the lack of consistent efficacy in cell-based cardiac regeneration therapies [35–37].

Table 1. Cell therapy clinical trials

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Phase</th>
<th>Name</th>
<th>Patient number</th>
<th>Condition</th>
<th>Treatment outcomes</th>
<th>Clinical trial identifier</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMMNC</td>
<td>I</td>
<td>BOOST</td>
<td>60</td>
<td>5–7 days post-MI</td>
<td>Improvement of LV systolic function after 6 months; no significant benefit after 18 months</td>
<td>NCT00224536</td>
<td>[6]</td>
</tr>
<tr>
<td>II</td>
<td>Leuven-AMI</td>
<td>67</td>
<td>1 day post-MI</td>
<td>No significant effect on recovery of global LV function</td>
<td>NCT00264316</td>
<td>[7]</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>ASTAMI</td>
<td>50</td>
<td>5–7 days post-MI</td>
<td>No improvement in global LVEF after 6 months</td>
<td>NCT00199823</td>
<td>[8]</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>REPAIR-AMI</td>
<td>204</td>
<td>3–6 days post-MI</td>
<td>Significant increase in LVEF, reduced adverse events after 1 year</td>
<td>NCT00279175</td>
<td>[9]</td>
<td></td>
</tr>
<tr>
<td>II/III</td>
<td>FINCELL</td>
<td>80</td>
<td>2–6 days post-MI</td>
<td>Improvement of global LVEF after 6 months</td>
<td>NCT00363324</td>
<td>[10]</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>REGENERATE-AMI</td>
<td>100</td>
<td>1 day post-MI</td>
<td>Not significant improvement in LVEF after 1 year</td>
<td>NCT00765453</td>
<td>[11]</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>TIME</td>
<td>120</td>
<td>3 or 7 days post-MI</td>
<td>No significant effect on recovery of LV function</td>
<td>NCT00684021</td>
<td>[12]</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>Late-TIME</td>
<td>87</td>
<td>14–21 days post-MI</td>
<td>No significant improvement in LV function after 6 months</td>
<td>NCT00684060</td>
<td>[13]</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>FOCUS-CCTRN</td>
<td>153</td>
<td>CAD</td>
<td>No significant improvement in LV volume, oxygen consumption or defect</td>
<td>NCT00824005</td>
<td>[14]</td>
<td></td>
</tr>
<tr>
<td>CD90+ MSC and CD45+ CD14+ Macrophages (Ixmylelocel-T)</td>
<td>III</td>
<td>BAMI*</td>
<td>3,000</td>
<td>AMI</td>
<td>Significant increase in LVEF</td>
<td>NCT01569178</td>
<td>[15]</td>
</tr>
<tr>
<td>IIa</td>
<td>TOPCARE-CHD</td>
<td>75</td>
<td>MI</td>
<td>Improves symptoms in patients with ischemic DCM</td>
<td>NCT00289822</td>
<td>[16]</td>
<td></td>
</tr>
<tr>
<td>IIa</td>
<td>IMPACT-DCM</td>
<td>39</td>
<td>DCM</td>
<td>Improved symptoms in patients with ischemic DCM</td>
<td>NCT00765518</td>
<td>[16]</td>
<td></td>
</tr>
<tr>
<td>Bone marrow-derived mesenchymal cardiopoietic cells</td>
<td>II/III</td>
<td>CURE</td>
<td>47</td>
<td>HF</td>
<td>Reduced cardiac events with treatment</td>
<td>NCT01670981</td>
<td>[17]</td>
</tr>
<tr>
<td>III</td>
<td>CHART-1*</td>
<td>240</td>
<td>HF</td>
<td>Improved LVEF and quality of life</td>
<td>NCT00810238</td>
<td>[18]</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>CHART-2*</td>
<td>240</td>
<td>HF</td>
<td></td>
<td>NCT01768702</td>
<td>[19]</td>
<td></td>
</tr>
<tr>
<td>Allogeneic MPC Stro-1/Stro-3+</td>
<td>II</td>
<td>ixCELL-DCM*</td>
<td>109</td>
<td>DCM</td>
<td>High-dose significantly reduced adverse cardiac events</td>
<td>NCT00721045</td>
<td>[20]</td>
</tr>
<tr>
<td>Autologous and allogeneic MSC</td>
<td>III</td>
<td>DREAM-HF*</td>
<td>600</td>
<td>HF</td>
<td>Improved functional capacity, quality of life, ventricular remodeling</td>
<td>NCT02032004</td>
<td>[21]</td>
</tr>
<tr>
<td>II/II</td>
<td>POSEIDON</td>
<td>30</td>
<td>HF</td>
<td>Increased LVEF and decreased infarct size</td>
<td>NCT01087996</td>
<td>[22, 23]</td>
<td></td>
</tr>
<tr>
<td>CSC</td>
<td>I</td>
<td>SCPIO</td>
<td>33</td>
<td>HF</td>
<td></td>
<td>NCT00474461</td>
<td>[22, 23]</td>
</tr>
<tr>
<td>CDC</td>
<td>I</td>
<td>CADUCEUS</td>
<td>31</td>
<td>HF</td>
<td>No significant improvement on LVEF or scar reduction</td>
<td>NCT00893360</td>
<td>[24, 25]</td>
</tr>
<tr>
<td>I/II</td>
<td>ALLSTAR*</td>
<td>134</td>
<td>MI</td>
<td></td>
<td>NCT01458405</td>
<td>[21]</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>DYNAMIC*</td>
<td>42</td>
<td>DCM</td>
<td></td>
<td>NCT02293603</td>
<td>[21]</td>
<td></td>
</tr>
<tr>
<td>CDCs with bFGF</td>
<td>I</td>
<td>ALCADIA</td>
<td>7</td>
<td>HF</td>
<td>Safe and effective in patients with ischemic cardiomyopathy</td>
<td>NCT00981006</td>
<td>[26]</td>
</tr>
<tr>
<td>CSC + MSC</td>
<td>II</td>
<td>CONCERT-HF*</td>
<td>144</td>
<td>HF</td>
<td>No major complications after 3 months</td>
<td>NCT02501811</td>
<td>[27]</td>
</tr>
<tr>
<td>ESCs CD15+ Isl-1+</td>
<td>I</td>
<td>ESCORT*</td>
<td>6</td>
<td>HF</td>
<td></td>
<td>NCT02057900</td>
<td>[27]</td>
</tr>
</tbody>
</table>

Abbreviations: AMI, acute myocardial infarction; bFGF, basic fibroblast growth factor; BMMNC, bone marrow mononuclear cell; CAD, coronary artery disease; CDC, cardiosphere-derived cell; CHD, coronary heart disease; CSC, cardiac stem cell; DCM, dilated cardiomyopathy; EF, ejection fraction; ESC, embryonic stem cell; HF, heart failure; LV, left ventricle/ventricular; MI, myocardial infarction; MPC, mesenchymal precursor cell; MSC, mesenchymal stem cell.

*Ongoing.
discrepancies in design, methods, or results in many of the early phase clinical trials have also raised concerns over the validity of the reported benefits of bone marrow stem cell therapy [38]. It is apparent that only well-designed and adequately powered trials will establish whether BMC therapy offers a new hope to patients with HF. A series of studies have been designed as phase III confirmative randomized controlled clinical trials, including the BAMi trial (NCT01569178; http://www.bami-fp7.eu) a mortality trial enrolling 3,000 patients post-AMI throughout the European Union; the CHART-1 trial [19], which successfully enrolled 240 high-risk patients with advanced congestive HF; and the DREAM-HF study (NCT02032004), with a target enrollment of more than 600 high-risk patients with congestive HF have been designed as phase III confirmative trials. These studies are the most scientifically rigorous human experiments to date in the field of cardiac cell therapy. It is anticipated that the results of these clinical trials will be crucial in establishing whether BMC therapy represents an effective strategy for HF treatment.

ENDOGENOUS CARDIAC STEM CELLS

Recent findings have refuted the long-held belief that the adult mammalian heart is a terminally differentiated organ. There is, in fact, a constant cardiomyocyte turnover within human hearts throughout life, although at a very low rate [3]. Although the mechanisms of endogenous heart regeneration remain highly debatable, the discovery of putative resident cardiac stem cells (CSCs), such as c-kit+ cells [39], provided the rationale that these cells could be isolated and harnessed to regenerate the failing heart [40]. Despite discrepant results, a plethora of preclinical studies demonstrate beneficial effects of c-kit+ cell administration to ischemically damaged hearts despite the observed paucity of cardiomyogenic differentiation of these cells. The phenotype of postnatal c-kit+ cardiac cells resembles traditional MSCs, suggesting their major mechanism of action involves paracrine actions [41].

The SCiPIO trial was the first human, randomized, open-label trial of autologous c-kit+ CSCs in patients with ischemic HF undergoing coronary artery bypass grafting [22]. The initial results of the study showed a striking improvement of LV function and decreased infarct size at 4 months and 1 year after intracoronary infusion [22, 23]. However, concerns regarding the integrity of the published data have been raised, casting doubts over the validity of the study [42]. Another potential source for cardiac-derived stem cell therapy is cardiosphere derived cells (CDCs), a heterogeneous mixture of many different cell types derived by ex vivo culture of right ventricular endomyocardial biopsies [43]. The enhanced potency of cardiospheres for myocardial repair has been attributed to their growth properties that mimic stem cell niche properties with enhanced “stemness” and expression of ECM and adhesion molecules [44]. The CADUCEUS trial was a proof-of-concept study that evaluated the safety and efficacy of autologous CDCs in patients with a recent MI. The results showed no significant difference in heart function, end-systolic, or end-diastolic volumes with the treatment, but analysis of exploratory efficacy endpoints revealed an increase in viable myocardium after 6 and 12 months, suggestive of therapeutic cardiac regeneration [24, 25]. In addition, the ALCADIA [26] study tested a novel approach of combining CDCs with a hydrogel-based delivery method of basic fibroblast growth factor (bFGF) [45] in patients with advanced HF. The interim results of the study demonstrated that the combination of CDCs and bFGF is safe, but given the small size of the study and the absence of a control group, no conclusion can be drawn regarding the safety and the efficacy of this approach.

The aforementioned proof-of-concept studies have the potential to revolutionize the treatment of HF. However, the small number of enrolled patients, the short period of follow up, and the preliminary nature of the findings preclude any safe conclusions. In addition, although the c-kit+ CSCs and CDCs are cardiac-derived cells that have been named “cardiac progenitor cells,” there is no compelling evidence that they can differentiate into myocardial cells, and therefore the mechanisms involved in the beneficial actions observed remain unknown. Despite these uncertainties, prospective, randomized, placebo-controlled clinical trials are currently investigating the efficacy of cardiac-derived cell therapies in HF. For example, the DYNAMIC (NCT02293603) and ALLSTAR (NCT01458405) trial are currently evaluating the safety and efficacy of allogeneic CDCs in patients with DCM and MI, respectively. Similarly, a hybrid cell therapy composed of autologous c-kit+ CSCs and bone marrow-derived MSCs is currently being tested in the CONCERT-HF, a phase II trial (NCT02501811) in patients with ischemic cardiomyopathy. The results of these promising studies are eagerly awaited.

EMBRYONIC STEM CELLS

Pluripotent stem cells (PSCs)—either embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs)—have emerged as a renewable cell source for heart regenerative applications [46]. Human PSCs are attractive because they can be differentiated with great efficiency into cardiomyocytes (CMs) [47], providing an unlimited supply of cardiomyocyte-like cells in vitro, before transplantation. After a long period of preclinical and translational work, the first human trial, ESCORT [27], was initiated in 2013 with the first patient receiving purified ESC-derived cardiac progenitors (CD15 Isl-1+) in an epicardial fibrin gel patch. Although the preliminary results suggested an improvement in the kinetics of the non-bypassed cell-patched area at 3 months and 6 months follow up, it would be meaningless to draw any conclusions regarding the efficacy of this treatment based on a single patient. This landmark study demonstrated the feasibility of producing clinical-grade ESC-derived cardiac progenitor cells and represents the first clinical application of this approach in the setting of HF, but additional studies are necessary to evaluate its safety and efficacy. Although the capacity of ESCs to differentiate toward the cardiac lineages is well established, numerous challenges remain for the clinical implementation of ESC-based therapies [48, 49]. For example, allogeneic ESCs face immunological challenges that might require life-long immunosuppression; theoretically autologous iPSC-derived cardiomyocytes circumvent this issue. PSC derivatives pose the inherent risk of forming teratomas. In addition, cell survival, retention, and engraftment are major obstacles. Even when cells successfully engraft and survive in the injured heart,
PSC-derived CMs could potentially trigger malignant arrhythmias, due to the presence of phenotypically immature cells with spontaneous beating activity [50]. Larger preclinical studies investigating cell dose, timing, and delivery modalities using consistent and efficient methods are necessary to address the aforementioned concerns and to conclusively demonstrate that the PSC-derived CMs and/or cardiomyocyte progenitor cells can improve cardiac function.

In summary, stem cell/progenitor cell therapies have been rapidly translated from bench to bedside, and numerous clinical trials have been spurring over the last 15 years. The initial enthusiasm generated by early-stage studies has now been met with skepticism, as the clinical outcomes of most BMC-based trials have yielded inconclusive results. Similarly, putative heart-derived stem cells, such as c-kit+ cells and CDCs, have been proposed as attractive candidates for heart regeneration, but their therapeutic value remains questionable. Notably, it is not clear whether the modest beneficial effects are cell-type specific and the mechanisms of cardioprotection have not been completely unraveled yet. With the exception of PSC-derived CM progenitor cells, BMCs, MSCs, CDCs, and CSCs do not represent bona fide stem cell populations and are unlikely to regenerate the myocardium. Originally hypothesized to differentiate into new CMs, the aforementioned cell types are now known to engraft poorly, with the majority persisting less than a week after transplantation. Regardless of the cell source, the current consensus is that the transplanted cells do not generate new tissue, and it has been postulated that their beneficial effect is exerted via paracrine mechanisms that stimulate the endogenous repair pathways through the release of various factors. Nevertheless, these paracrine mechanisms have yet to be elucidated, and studies to determine exact mechanisms of action in the diseased human heart are needed to develop more targeted and robust cell therapies. Despite these mechanistic uncertainties, it is important to acknowledge that the safety and feasibility of BMCs, MSCs, CDCs, and CSCs has been consistently established. Recent advances with PSCs hold promise for successful clinical translation, but it will take time to develop effective and safe protocols for the use of PSCs in heart failure. The completion of the ongoing Phase II/III studies will certainly contribute knowledge and most likely provide valuable information to the cardiac regenerative medicine conundrum.

### Table 2. Gene therapy clinical trials

<table>
<thead>
<tr>
<th>Molecular target</th>
<th>Delivery mode</th>
<th>Phase</th>
<th>Name</th>
<th>Patient number</th>
<th>Treatment outcomes</th>
<th>Clinical trial identifier</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF Adenovirus</td>
<td>II</td>
<td>KAT</td>
<td>103</td>
<td>Significant increase in myocardial perfusion</td>
<td>NCT01002430</td>
<td>[52]</td>
<td></td>
</tr>
<tr>
<td>Plasmid</td>
<td>I</td>
<td>VIVA</td>
<td>178</td>
<td>Enhanced myocardial perfusion</td>
<td>NCT00454818</td>
<td>[53]</td>
<td></td>
</tr>
<tr>
<td>Adenovirus</td>
<td>I</td>
<td>KAT301</td>
<td>30</td>
<td>No difference in myocardial perfusion</td>
<td>NCT00143585</td>
<td>[54]</td>
<td></td>
</tr>
<tr>
<td>Plasmid</td>
<td>II/III</td>
<td>EUROINJECT-ONE</td>
<td>80</td>
<td>No difference in myocardial perfusion</td>
<td>NCT01002430</td>
<td>[55]</td>
<td></td>
</tr>
<tr>
<td>Plasmid</td>
<td>II/III</td>
<td>NORTHERN</td>
<td>93</td>
<td>No difference in myocardial perfusion</td>
<td>NCT01002430</td>
<td>[56]</td>
<td></td>
</tr>
<tr>
<td>Adenovirus</td>
<td>III</td>
<td>REVASC</td>
<td>17</td>
<td>No beneficial effect</td>
<td>NCT01002430</td>
<td>[57]</td>
<td></td>
</tr>
<tr>
<td>FGF4 Adenovirus</td>
<td>II/III</td>
<td>AGENT-3</td>
<td>416</td>
<td>No beneficial effect</td>
<td>NCT00354437</td>
<td>[58]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>II/III</td>
<td>AGENT-4</td>
<td>116</td>
<td>No beneficial effect</td>
<td>NCT00185263</td>
<td>[59]</td>
<td></td>
</tr>
<tr>
<td>AC6 Adenovirus</td>
<td>I/I</td>
<td>AC6 Gene Transfer</td>
<td>56</td>
<td>Dose-related improvement of cardiac function</td>
<td>NCT00787059</td>
<td>[60]</td>
<td></td>
</tr>
<tr>
<td>SERCA2a AAV1</td>
<td>I/II</td>
<td>CUPID</td>
<td>51</td>
<td>Decreased HF symptoms remodeling</td>
<td>NCT01643330</td>
<td>[61]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>II/III</td>
<td>CUPID-2b</td>
<td>250</td>
<td>No improvement in the clinical course of HF</td>
<td>NCT01643330</td>
<td>[62–65]</td>
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<td>SERCA2a I</td>
<td>II</td>
<td>AGENT-2</td>
<td>10</td>
<td>Improvements 6-minute walk</td>
<td>NCT01966887</td>
<td>[63]</td>
<td></td>
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<tr>
<td>SERCA2a II</td>
<td>II</td>
<td>AGENT-HF*</td>
<td>5</td>
<td>Improvements 6-minute walk</td>
<td>NCT00534703</td>
<td>[64]</td>
<td></td>
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<tr>
<td>SDF1 Plasmid</td>
<td>I</td>
<td>ACRX-100</td>
<td>17</td>
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<td>NCT01082094</td>
<td>[65]</td>
<td></td>
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<tr>
<td></td>
<td>II</td>
<td>STOP-HF*</td>
<td>90</td>
<td>Improved clinical outcomes</td>
<td>NCT01643590</td>
<td>[66]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IIb</td>
<td>STOP-HF2*</td>
<td>180</td>
<td>Improved clinical outcomes</td>
<td>NCT01643590</td>
<td>[67]</td>
<td></td>
</tr>
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</table>

Abbreviations: AAV, adeno-associated virus; AC6, adenyl cyclase 6; FGF, fibroblast growth factor; HF, heart failure; LV, left ventricle/ventricular; SDF1, stromal-derived factor 1; SERCA2a, sarcoplasmic reticulum Ca2+ ATPase; VEGF, vascular endothelial growth factor.

aTerminated.
bOngoing.

PROGRESS AND CHALLENGES IN GENE THERAPY FOR CARDIOVASCULAR DISEASE

Over the past decade, our understanding of the complex disease mechanisms underlying the pathogenesis of HF has significantly improved [51], and advances in molecular cardiology have identified key targets within the progression of HF. Gene therapy has emerged as a viable therapeutic strategy for specifically modulating underlying disease mechanisms, potentially replacing the symptomatic approach of existing treatments. Rectifying the disease at the gene level could mean a more permanent therapeutic benefit that could slow down or even reverse the detrimental course of HF. Extensive investigation into new treatment modalities has led to the development of gene-based therapeutic interventions, and in recent years there have been rapid advancements in gene therapy for HF (Table [2–68, 72, 75, 76]).

Gene therapy was proposed to be particularly valuable in the context of coronary artery disease (CAD), the most common type of cardiovascular disease. Preclinical studies have shown that a number of growth factors including vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), hepatocyte growth factor (HGF), platelet-derived growth factor (PDGF), and hypoxia-inducible factor (HIF) could...
promote angiogenesis and induce vascular permeability and cytoprotective effects. Most gene therapy clinical trials for CAD were focused on the administration of genes encoding angiogenic growth factors, such as VEGF and FGF4, aiming to promote the development of collateral blood vessels in ischemia-related conditions. Early trials, such as VIVA [53] and KAT [52], suggested a functional improvement in myocardial perfusion and cardiac function in patients with CAD after the administration of either an expression plasmid or an adenoviral vector expressing VEGF-A

**Ad. VEGF-A**

respectively. However, subsequent, double-blind, randomized, placebo-controlled trials (EUROINJECT-ONE, NORTHERN) [55, 56] failed to demonstrate any improvement in myocardial perfusion. Similarly, the phase II randomized, controlled REVASC [57] trial that evaluated the efficacy of an adenoviral vector-mediated VEGF delivery (Ad. VEGF-A

**Ad. VEGF-A**

1) did not show any significant improvement in the primary endpoint of myocardial perfusion and alleviation of symptoms. Recently, another member of the VEGF family, VEGF-D, has also been clinically evaluated in patients with severe CAD. The preliminary data of the KAT301 trial [54], a randomized, placebo-controlled, single-blinded phase I/II study, suggest that adenoviral-mediated VEGF-D gene therapy is safe and could enhance myocardial perfusion. In an alternative approach, early phase I/II trials demonstrated that FGF4 treatment improved exercise capacity and reduced ischemic defect size in CAD patients (AGENT and AGENT-2) [59, 60]. However, the larger phase III trials (AGENT-3 and AGENT-4) [58] failed to corroborate these benefits. Consequently, both studies were terminated after an interim analysis of the AGENT-3 trial indicated that there were no significant differences regarding the primary endpoint in the between the treatment and placebo groups.

In advanced HF, cardiac calcium (Ca

**Ca**

2+) cycling—the release and reuptake of intracellular Ca

**Ca**

2+ that drives muscle contraction and relaxation—is profoundly altered, resulting in impaired contractility and fatal cardiac arrhythmias [69]. Key components of the machinery that regulates Ca

**Ca**

2+ cycling in the heart have emerged as prominent targets for human HF therapy [70, 71]. Notably, heart failure is associated with depressed sarcoplasmic reticulum (SR) calcium cycling, reflecting impaired SR Ca

**Ca**

2+ transport and Ca

**Ca**

2+ release, which has been attributed to reductions in gene and protein expression, or activity of the SR Ca

**Ca**

2+ uptake pump (SERCA2a). SERCA2a plays a key role in transporting Ca

**Ca**

2+ from the cytosol into the lumen of the SR following cardiac contraction, thus regulating cardiac contractility and relaxation. Targeting SERCA2a showed beneficial results in preclinical testing, leading to the first-in-human trial to enhance SR Ca

**Ca**

2+ uptake in 2007 [62, 63]. In this phase I/II trial (CUPID), a small number of patients with advanced HF received an intracoronary administration of an adeno-associated viral (AAV1) vector expressing SERCA2a (AAV1.SERCA2a) and later showed improvements in key clinical outcomes [64]. Although individual patients did not show improvements across all parameters, improvements in prespecified primary endpoint criteria were observed in the highest dose cohort [65]. The promising outcome of the initial trial led to a larger Phase IIIB, double-blind, placebo-controlled study, CUPID2, and two smaller auxiliary studies, AGENT-HF and SERCA-LVAD trials. The recently completed CUPID2 study did not meet its primary or secondary endpoints, and overall failed to demonstrate any improvement of clinical outcomes in patients with advanced HF [66] Following the outcome of the CUPID2 trial, patient enrollment in both AGENT-HF and SERCA-LVAD studies was suspended. Importantly, no safety issues emerged—from the procedure of delivering the virus or long-term effects—in the participant patient population at the tested AAV1 therapeutic dose.

Adenylyl-cyclase type 6 (AC6) is an enzyme that serves as the effector molecule for β-adrenergic signaling, playing a key role in contractile responsiveness, cardiac relaxation, and LV diastolic function [72]. In preclinical studies, adenoviral-mediated delivery of an AC6 transgene improved LVEF and increased survival rates in animal models of cardiomyopathy in part due to increased SERCA2a activity and improved Ca

**Ca**

2+ handling in CMs [73, 74]. A recent randomized, double-blinded, placebo-controlled clinical trial evaluated the safety, tolerability, and clinical effectiveness of ascending doses of adenovirus-5 encoding human AC6 (Ad5.hAC6) in patients with stable but severe HF [61]. Although the rates of serious adverse events were similar in both groups, the findings of this small clinical study suggest that intra coronary delivery of Ad5.hAC6 in patients with HF appears to be safe with a dose-related improvement in cardiac function at 4 and 12 weeks after randomization. The size of the study, however, was too small to draw any definitive conclusions regarding the efficacy and long-term benefit of this promising new gene therapy target in patients with advanced HF.

The stromal cell-derived factor-1 (SDF-1), and its receptor, chemokine receptor type 4 (CXCR4) has emerged as a key regulator in endogenous tissue repair. Preclinical studies indicate that a SDF-1 promotes tissue repair through the SDF-1:CXCR4 axis by promoting cell survival, endogenous stem cell recruitment, and vasculogenesis [75, 76]. The safety and potential efficacy of SDF-1 gene therapy was initially demonstrated in an open-label Phase I study in patients with ischemic cardiomyopathy [67]. According to the results of the more recent STOP-HF trial [68], the transient overexpression of SDF-1 has the potential to improve cardiac function in patients with ischemic cardiomyopathy. Although the trial failed to meet its primary efficacy endpoint, a prespecified subanalysis demonstrated that the potential benefits were more pronounced in patients with advanced cardiac dysfunction for at least 1 year post-treatment. These promising findings have led to the design of a larger, prospectively designed clinical study (STOP-HF2) that is expected to enroll up to 180 HF patients.

In short, despite extensive preclinical evaluation and encouraging results from early clinical studies, to date none of the gene therapy approaches have provided compelling evidence of a significant clinical benefit in HF patients. Early studies that focused on neovascularization have shown limited efficacy and consequently the angiogenic gene-therapy approaches with the goal to improve cardiac vascularization have largely been abandoned. More recently, the first human clinical trial of viral vector-based gene transfer for advanced HF was initiated after pilot clinical trials targeting the Ca

**Ca**

2+ cycling pathway in HF patients showed favorable clinical outcomes without safety issues. However, the milestone CUPID2 trial failed to meet its prespecified endpoints, demonstrating that establishing clinical efficacy of novel therapeutic principles is a long and arduous path.
CONCLUSION AND FUTURE DIRECTIONS

HF, a complex clinical syndrome, represents a major global health problem. Significant progress has been made over the past two decades in cell- and gene-based therapies for HF, promising the development of innovative therapeutic strategies for both treatment and prevention (Fig. 1). There are, of course, substantial gaps in knowledge that pose obstacles to the realization of the full potential of such novel biological therapies for clinical benefit. There is still a tremendous amount of work to be done, especially in addressing the need for deeper insights into the underlying disease mechanisms (i.e., which cell types, which genes, and at what levels, which pathways are relevant to any given pathogenic process, and which patients to treat).

Perhaps one of the most promising developments in the field of the regenerative cardiology is the emerging notion of using pre-existing cardiomyocytes as the source for cardiomyocyte replacement to maintain normal myocardial homeostasis as well as after myocardial injury [77–80]. The stimulation of proliferation of pre-existing cardiomyocytes could provide new avenues for future therapeutic strategies to regenerate the heart. However, further evidence and characterization for this putative pool of cycling cardiomyocytes as well as development of the means of therapeutic manipulation is a prerequisite to harness the endogenous regenerative properties of the adult heart.

Finally, genome-editing tools such as programmable engineered nucleases [81] are becoming more accessible [82] and are being used to increase our understanding of disease mechanisms as well as to develop novel therapeutic approaches. Gene correction by genome editing has shown great promise for clinical translation, as highlighted by recent studies for the treatment of Duchenne Muscular Dystrophy in vivo [83–85] and DCM in vitro [86]. Nevertheless, these novel approaches will likely have to address the problem of delivery that has been a key issue in gene therapeutic strategies targeting the heart. Although the genome editing field is in its infancy, these studies represent an important step towards the treatment of hereditary forms of cardiovascular diseases.

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AUTHOR CONTRIBUTIONS

C.C. and V.T.: conception and design, manuscript writing, collection and/or assembly of data and final approval of manuscript; I.K.: conception and design, manuscript writing, collection and/or assembly of data, financial support and final approval of manuscript.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors indicate no potential conflicts of interest.
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