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Potential Strategies to Address the Major Clinical Hurdles Facing Stem Cell Regenerative Therapy for Cardiovascular Disease: A Review

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Abstract

Importance—While progress continues to be made in the field of stem cell regenerative medicine for the treatment of cardiovascular disease, significant barriers to clinical translation still exist that have thwarted the delivery of cell therapy to the bedside.

Objective—The purpose of this review is to summarize the major current hurdles for the clinical implementation of stem cell therapy and discuss potential strategies to overcome them.

Evidence Review—Information for this review was obtained through a search of PubMed and the Cochrane database for English language studies published between January 1, 2000 and June 15, 2016. Ten randomized clinical trials and eight systematic reviews were included in this review.

Findings—One of the major clinical hurdles facing the routine implementation of stem cell therapy is the limited and inconsistent benefit observed thus far. Reasons for this are unclear but may be due to poor cell retention and survival, as suggested by numerous preclinical studies and a handful of human studies incorporating cell fate imaging. Additional cell fate imaging studies in humans are needed to determine how these factors contribute to limited efficacy. Treatment strategies to address poor cell retention and survival are under investigation and include the following: 1) co-administering of immunosuppressive and pro-survival agents, 2) delivering cardioprotective factors packaged in exosomes rather than the cells themselves, and 3) using tissue engineering strategies to provide structural support for cells. If larger grafts are achieved using the aforementioned strategies, it will be imperative to carefully monitor the potential risks of tumorigenicity, immunogenicity, and arrhythmogenicity.

Conclusions and Relevance—Despite important achievements to date, stem cell therapy is not yet ready for routine clinical implementation. Significant research is still needed to address the clinical hurdles outlined herein before the next wave of large clinical trials is underway.

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Introduction

Stem cell therapy still holds promise despite conflicting reports of efficacy from recent adult stem cell clinical trials.[1–7] Like any high-risk, high-reward scientific endeavor, initial efforts are fraught with challenges, but the scientific community and general public remain optimistic that continuing effort will realize the full potential of stem cells. In this review, we outline the major clinical hurdles facing stem cell regenerative therapy and potential strategies to overcome these obstacles.

Major Clinical Hurdles for Routine Clinical Implementation

Recent clinical trials have found that transplantation of adult bone marrow mononuclear cells (BMMNCs) produces only modest benefit, ranging from an improvement of 2–5% in left ventricular ejection fraction (LVEF),[4,7] a degree of change with uncertain clinical significance given the inherent variation of traditional imaging modalities. Although efficacy questions remain, these studies have confirmed that the administration of these cells appears to be safe; however, the risks of tumorigenicity, immunogenicity, and arrhythmogenicity may increase if larger grafts are achieved. In the following section, we will highlight the major clinical hurdles facing stem cell regenerative therapy, including our incomplete knowledge of cell fate post-delivery, poor cell survival and engraftment, and major safety concerns. Additional economic, regulatory, and ethical hurdles have been described in other comprehensive reviews.[8]

Lack of knowledge regarding the fate of cells post-delivery—One of the primary challenges of bringing stem cell therapy into the clinic is our limited knowledge of cell fate after delivery in humans. Unlike drugs whose presence in the blood can be used to correlate with response, for stem cell therapy, we need to be able to locate the cells, quantify their number, evaluate their viability, and determine whether they could integrate into the host tissue to correlate dose with benefit.

Without sufficient knowledge about cell fate after delivery, it has been difficult to interpret previous dose response studies. Of the five clinical studies evaluating the relationship between cell dose and efficacy,[9–13] two studies have shown an inverse relationship,[10,11] whereas the other three have shown a positive dose relationship.[9,12,13] In a study of 167 patients with refractory angina who received transendocardial injection of autologous $CD34^+$ cells, Losordo et al. observed a significant improvement in angina frequency and exercise tolerance in the low dose group compared to the high dose group (e.g., 1×10^5 vs. 5×10^5 cells per kg).[10] Similarly, Hare et al. found a significantly greater increase in LVEF and reduction in infarct size in patients with ischemic cardiomyopathy (ICM) receiving transendocardial injection of only 20 million mesenchymal stem cells (MSCs) compared to those receiving higher doses of 100 and 200 million.[11] By contrast, after delivering escalating doses of 5, 10, or 15 million autologous $CD34^+$ BMMCs into the myocardium of patients with ST elevation MI via intracoronary injection, Quyyumi et al. found that patients with ≥≥10 million cells had the greatest improvement in myocardial perfusion.[9] Although the reasons for these discrepant findings remain unclear, one possible explanation is that cell influx and retention at the target site might vary depending on the operators, the target

patients, and even the delivery methods. However, these studies, like many others published to date, contain little information on whether these cells arrived and were retained at the site of injury, leaving many questions unanswered.

To address this limitation, Vrtovec et al. performed two sequential studies in patients with nonischemic cardiomyopathy (NICM) by imaging cell fate shortly after delivery.[12,13] Patients with evidence of greater myocardial homing via intracoronary injection or higher retention rates via transendocardial injection had better improvement in LVEF. These studies demonstrated that the ability to track cells post-delivery plays a critical role in understanding the dose-response relationship and ultimately predicting overall clinical efficacy. Future studies should examine cell fate to better define the relationship between cell survival/retention and clinical outcomes.

Tracking cell fate may also help determine the optimal timing of cell delivery following MI, a period during which the tissue microenvironment might be hostile to cells and could lead to cell death.[14] The two randomized controlled trials sponsored by the Cardiovascular Cell Therapy Research Network (e.g., Timing In Myocardial infarction Evaluation [TIME],[15,16] and Late-TIME trials[17]) that were designed to evaluate whether timing of delivery affects outcome failed to show a significant benefit from cell therapy when cells were transplanted early (e.g., day 3 and 7) or late (e.g., a mean of 17 days). Neither TIME[15,16] nor Late-TIME[17] tracked cell fate *after* delivery, so it remains unclear whether the observed lack of benefit was due to poor cell retention/survival or whether timing truly had no significant effect on efficacy.

Finally, understanding the fate of cells post-delivery can help identify the optimal mode of delivery. In clinical trials to date, cells have been delivered via intravenous injection, catheter-based intracoronary injection into a culprit vessel, catheter-directed transendocardial injection, or transepicardial injection at the time of cardiac surgery. The choice of delivery mode is often based on the target patient cohort and their specific disease. While an intracoronary approach may be best for patients with acute myocardial infarction (AMI), a transendocardial or intramyocardial approach is better suited for patients with chronic ICM who may have completely occluded arteries. In a preliminary study of 40 patients, Vrtovec et al. found that patients receiving transendocardial injections of $CD34^+$ cells had higher myocardial retention rates than those receiving intracoronary injections,[13] echoing findings from several preclinical studies.[18,19]

Few studies, however, have directly correlated cellular retention rates after intravenous, intracoronary, or intramyocardial delivery with efficacy. Replicating findings from two stem cell tracking studies performed in large animal studies,[18,20] Vrtovec et al. demonstrated that patients with NICM, after treatment with transendocardial injection of BM-derived $CD34^+$ cells, had higher myocardial cell retention rates with better ventricular function and exercise capacity compared to patients who underwent intracoronary injections.[13] The study, however, was small in size (n=40) and conducted as an open-label in a single center. Larger multi-center clinical studies that incorporate cell fate imaging are needed to investigate the comparative effectiveness of these delivery methods.

Poor engraftment and survival of cells, limiting their potential efficacy— Arguably the most significant barrier for clinical translation is poor cell engraftment and survival, which has been reported in multiple small animal studies and a handful of large animal studies (including humans) incorporating stem cell fate imaging.[21–23] As shown in Figure 1, once cells are delivered to the patient, a percentage of cells may arrive at the target area and engraft while others may migrate to distant organs. Those that remain in the heart, however, may or may not survive long-term to provide cardio-protection. Indeed, the handful of human studies evaluating stem cell fate show that only 5% of stem cells engraft within 24 hours, but longer-term data in humans are limited.[24] Long-term data from small animal studies suggest that cells are present near the site of injury for only a short time, with the majority migrating to distant organs and then dying *in situ*.[19,24] Taken together, these results suggest that even if a patient receives 100 million cells at the time of delivery, perhaps only ~50,000 will survive in the heart at 4–6 weeks to contribute to its repair.

Not surprisingly, the lack of viable cells in the infarct area after delivery has been associated with reduced efficacy in a rodent model,[25] which was later confirmed in human studies.[12] Although an intuitive approach, large clinical trials have yet to incorporate *in vivo* imaging to better correlate factors such as cell retention, engraftment, and survival in individual patients with their responses afterwards.

Risk of Tumorigenicity, Immunogenicity, and Arrhythmogenicity—Before cells reach patients, they need to undergo rigorous testing to ensure their purity, quality, and sterility. Compliance with good manufacturing practice (GMP) is mandatory before human transplantation can be performed. Once cells are safely isolated and then delivered, patients are monitored for the development of potential tumors, activation of the immune system, and/or the development of arrhythmias.

Stem cell transplantation carries a risk of tumorigenicity, which varies depending on the cell source. Adult stem cells, for example, have a low risk of tumorigenicity because they have limited growth and differentiation potential. As expected, not a single case of cancer has been reported to date from any clinical trial using adult stem cells for heart disease. It should be noted, however, that a tumor developed in a one patient with ataxia telangiectasia who received fetal neural stem cells[26] and in a second patient who received autologous bone-marrow derived stem cells for the treatment of lupus nephritis.[27] The risk for tumor formation is higher when transplanting cardiomyocytes derived from embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs). Fortunately, the cardiac differentiation process has improved significantly over the past decade,[28] and the minimal threshold needed to form a tumor also appears to be quite high, on the order of 10^5 undifferentiated cells,[29] in part due to poor survival of transplanted cells.

Another potential safety risk for stem cell transplantation is increased immunogenicity. Like other organ transplants, stem cell transplantation may activate the immune system, especially if cells are allogenic (i.e., donor cells are not obtained from the recipient), potentially leading to graft rejection and progressive donor cell death.[30] As expected, immune activation has not been reported in large-scale clinical trials, because most patients receive stem cells harvested from their own organs (e.g., autologous cells).

Additional evidence comes from preclinical studies that imaged cell fate after transplantation into immunodeficient mice after transplantation of allogeneic cells.[22,32] Some cells survived longer than others because they were less immunogenic (e.g., MSCs and iPSC derivatives),[19] although majority of transplanted cells died within 4–6 weeks of transplantation. Administration of adjuvant agents including immunosuppressants and pro-survival agents mitigated graft rejection, reduced cell death, and improved left ventricular function,[33,34] providing further evidence that regulating the immune response may be one of the keys to improving efficacy.

Perhaps the most feared complication of transplantation is the development of life-threatening arrhythmias due to possible enhanced automaticity or development of re-entry circuit induced by stem cells. Evidence for a pro-arrhythmia effect in trials using BMMNCs has been scant, but evaluation has not been rigorous. In one of the earliest trials, Wollert et al. found no evidence of arrhythmia on 24-hour Holter monitoring or on electrophysiological testing.[35] Limited survival of implanted stem cells and the fact that favorable effects of adult stem cells are mediated by mechanisms other than direct cardiomyocyte differentiation, however, might account for this favorable safety profile.

Future research using cardiomyocytes derived from ESCs or iPSCs will need to incorporate more extensive surveillance with event monitors, insertable long-term recorders, microvolt T-wave alternans, and invasive electrophysiological studies, especially because these cells have been shown to exhibit heterogeneous electrophysiological phenotypes, immature action potentials, immature gap junctions, and spontaneous automaticity in cell culture.[36] Similarly, more careful monitoring will also be required if sizeable grafts are achieved using other cell sources.

Potential Strategies for Addressing Clinical Hurdles

Overcoming the clinical hurdles outlined above will require a multi-faceted approach that centers on the incorporation of advanced imaging techniques to track cell fate. Additional strategies to address these limitations include the use of pluripotent stem cell (PSCs)--which includes ESCs and iPSCs--that can potentially contribute to cardiomyogenesis and novel tissue engineering approaches to improve cell survival (Figure 2). While improving stem cell survival will be crucial for addressing efficacy concerns for all cell types, tackling safety as well as ethical, regulatory, and economic hurdles may be more challenging for the clinical implementation of PSCs.[8]

The Use of Pluripotent Stem Cells—As discussed above, one major hurdle for the routine clinical implementation of stem cell therapy is that only modest, if any, efficacy was achieved in adult stem cell trials. Because this lack of significant efficacy may be related more to poor cell survival and retention post-delivery than the inability of these cells to repair myocardial injury, jettisoning this approach in favor of using PSCs may be premature.[37] Nevertheless, PSCs are an attractive alternative because, unlike adult stem cells, they can be differentiated into cardiomyocytes, endothelial cells, and other reparative cells with the potential to restore functional myocardium.[38,39] Although a recent study has shown that ESC-derived cardiomyocytes can develop extensive grafts in non-human

primates,[40] results from this study were criticized because: 1) only variable benefit in LVEF was achieved; 2) only a small number of animals that had small infarcts were included; 3) an incomplete evaluation of pump function and electrical properties was performed; and 4) a high incidence of ventricular arrhythmias was observed,[41] which contradicted earlier observations from the same group.[42] The underlying cause for the high arrhythmia rate is unclear, but may be related to the high number of cells injected (i.e., 1 billion), the large size of the grafts that developed, as well as the presence of a prominent non-cardiac cell population. Although findings from this study raises concerns about the use of ESC- and iPSC-derivatives in humans, a Phase I/II study led by Menasche et al. has reported no adverse arrhythmias associated with the first transplantation of ESC-derived cardiac progenitor cells embedded in a fibrin patch that is sutured directly onto the epicardium, a strategy which may reduce myocardial irritation and, thus, arrhythmia risk.[43]

PSCs and their derivatives may also have a greater risk of tumorigenicity because of possible contamination from residual undifferentiated cells, culture-acquired mutations (e.g., karyotypic abnormalities, copy number variants, as well as the loss of heterozygosity and phenotypic features),[44,45] and the risk of de-differentiation after transplantation.[46–48] Strategies to minimize the risk of oncogenic transformation include the development of safer methods for reprogramming and use of efficient and reproducible differentiation protocols.[39,49–53] In our experience with rodent and porcine models (unpublished data), we have not seen tumor formation following the injection of cardiomyocytes derived from ESCs or iPSCs. Long-term monitoring of potential tumor development is needed in the future. This may involve a combination of magnetic resonance imaging (MRI) and serum biomarkers, which was recently shown to have greater sensitivity in the detection of stem cell derived tumors than MRI or echocardiography alone.[54] Additional imaging with positron emission tomography (PET) can potentially differentiate between ESC-derived tumors, which express higher levels of $\alpha_v\beta_3$ integrin than other tumors.[55]

Incorporating Molecular Imaging in Cardiac Stem Cell Trials—In addition to the lack of significant clinical improvement, large randomized controlled clinical trials to date have failed to show a *consistent* benefit across different trials and across different patients within the same trial, raising questions about the reasons behind these perplexing findings.[2,16,56–59] Perhaps in patients who showed no detectable improvement, transplanted cells never arrived to the area of injury or died shortly after delivery. Although this may be a plausible explanation, whether or how variations in cell retention or survival affect efficacy remains unknown because the majority of randomized controlled clinical trials did not image cell fate.

While the details of how to perform stem cell fate imaging is beyond the scope of this review, we will briefly review the fundamentals of this approach and refer the reader to other more comprehensive reviews.[19,24] To follow the spatiotemporal distribution of cells following delivery, stem cells must be labeled with a molecular probe emitting signals that can be detected by an appropriate imaging modality. In small animals, *in vivo* serial imaging of cell fate can be performed using small animal imaging systems (e.g., bioluminescence imaging [BLI] and microPET) over weeks or months, because cells can be labeled with reporter genes that integrate into their genome and generate signals in the presence of

reporter probes as long as the cells are viable. Emitted signals are then detected by cameras with high spatial resolution (BLI: ~3–5 mm; microPET: ~1–2 mm) and imaging sensitivity (BLI: ~10^{-15} to 10^{-17} mol/L probe; microPET: ~10^{-11} to 10^{-12} mol/L probe), enabling a lower detection limit of ≥≥10^3 and ≥≥10^4 for BLI and microPET, respectively.[60] The resolution and sensitivity of BLI, however, is depth-dependent, and thus cannot be used in large animals and humans.

In humans, however, stem cell imaging is currently limited by the sensitivity of clinical imaging systems. Even clinical PET, the most sensitive modality available for human imaging, has a relatively inferior spatial resolution (e.g., 6–10 mm) and imaging sensitivity (e.g., 10^{-11} to 10^{-12} mol/L) compared to small animal imaging systems, resulting in a lower detection limit of ≥≥10^4 cells.[60] Clinical stem cell fate imaging is also limited by the short half-lives of radiolabelled probes (≤3 days) that are used for direct cell labeling. As technology advances, longer-term imaging of stem cell fate will likely be feasible in humans using reporter genes, as reported by Yaghoubi et al., who demonstrated that successful tracking of cytolytic T cells labeled with a viral PET reporter gene in a patient with gliobastoma.[61] Concerns over issues of immune activation of non-human reporter genes and the risk of oncogenic transformation secondary to random insertions into the genome, however, have impeded further application of this approach in humans. The development of humanized reporter genes and techniques for site-specific integration of these reporter genes may circumvent these safety issues.[62]

Immunosuppressive Therapy, Pro-survival Agents and Exosomes—Several hypotheses have emerged to explain the potential causes of poor cell survival and engraftment that have contributed to the limited efficacy found in previous trials. One notable theory is that the hostile tissue microenvironment, characterized by ischemia and inflammation, promotes cell death. Inflammation and ischemia, however, can be modulated by the delivery of local immunosuppressive therapy, pro-survival agents, and exosomes that reduce inflammatory cytokines, promote angiogenesis, and decrease apoptosis. As shown in two independent small animal studies,[33,34] immunosuppressive therapy can decrease cell death in immunocompetent mice. Other studies have shown that co-administration of pro-survival factors[63] or pro-survival miRNA cocktail[64] can significantly enhance cell survival. Alternatively, cells can be genetically engineered to express genes to potentially enhance survival and biological function.[65,66] Finally, recent studies have suggested that cardioprotective factors, including miRNAs released by stem cells, packaged into exosomes can be potentially delivered as stand alone therapy.[67,68] Importantly, the local delivery of these agents enables the generation of a microenvironment that is less hostile to cells while avoiding unintended systemic effects seen in systemic immunosuppression regimens.

Finally, using autologous cell sources, such as patient-specific BMMNCs, MSCs or iPSC derivatives as opposed to allogenic cell sources, may potentially reduce immune-mediated cell death and potentially avoid the use of immunosuppressive agents. Human iPSCs, for example, can be obtained by reprogramming adult somatic cells from a patient, differentiating them into cardiomyocytes, and transplanting them into the same patient.[8] Before iPSCs from patients with inherited cardiomyopathies could be used, however, the genetic defect within the iPSC must be first corrected by genome editing using techniques

such as zinc finger nuclease (ZFN), transcription activator-like effector nuclease (TALEN), and clustered regularly-interspaced short palindromic repeats (CRISPR)/Cas9.[69] Because using autologous derivatives from iPSCs may be economically infeasible, it is not surprising that only one trial, which uses autologous iPSC-derived retinal pigment epithelial cells to treat patients with macular degeneration, has been initiated to date.[70]

Tissue Engineering Approaches: Injectable Scaffolds, Myocardial Patches, and Engineered Heart Muscle—Cell death may also result from the delivery of isolated cells that are not anchored to extracellular matrix, which may increase programmed cell death, commonly referred to as “anoikis”. Embedding cells in biomaterials, however, can potentially reduce anoikis by providing structural and mechanical support for cells to survive and mature in the host myocardium.[71] For example, cells can be delivered in an injectable scaffold made of fibrin,[43] hydrogel,[72] or other biomaterials whose topology (e.g., pore/channel and structures) can facilitate vascularization within the implant and minimize fibrosis.[73] To improve cell affinity, these synthetic and natural biomaterials have been modified with cell adhesive molecules (e.g. collagen, fibronectin, and laminin.[74] These scaffolds have also been loaded with small molecules and trophic factors to provide a multifaceted approach to improve cell survival. Scaffolds have been built to continually release oxygen,[75] pro-angiogenic cytokines,[76] anti-apoptotic agents,[77] and extracellular matrix proteins.[78]

More recently, efforts have been put into the creation of functional heart tissue constructs using ESC- or iPSC-derived cardiomyocytes in the form of a myocardial patch, cardiomyocyte sheet, or engineered heart muscle (EHM), which can be implanted directly onto the myocardium.[79,80] In one study, tissue constructs prepared from neonatal cardiomyocytes were preconditioned using a cyclic stress system and then sutured onto the infarcted rat myocardium, resulting in improvement in LVEF.[81] More recently, EHM and cell sheet technologies have moved into large animal models,[82] showing great promise for re-muscularization of the infarcted heart.

Importantly, we have already seen early phase clinical trials testing the safety and efficacy of biomaterials or extracellular matrix products in the setting of acute MI or HF. As discussed previously, Menasche et al. initiated a Phase I/IIa trial to evaluate the efficacy and safety of delivering ESC-derived cardiac progenitor cells embedded in a fibrin scaffold, with a planned enrollment of 6 patients (https://clinicaltrials.gov/ct2/show/NCT02057900).[43] LoneStar Heart Inc. (Laguna Hills, CA) recently reported successful one-year results of their randomized clinical trial on the efficacy of Algisyl, a biocompatible, inert hydrogel that can potentially be used to embed cells for more effective transplantation.[83] Similarly, in a randomized, double blind, placebo-controlled study, Bellerophon Therapeutics, Inc. (Warren, NJ) reported that its bio-absorbable alginate hydrogel product designed to mitigate the adverse remodeling could be safely administered in patients after MI.[84] Finally, Anker et al. reported significant improvement in exercise capacity estimated by measurements of maximal oxygen consumption in HF patients treated with alginate hydrogel directly injected into the heart compared with control patients.[85] Studies such as these four early-stage clinical trials are paving the way for incorporating tissue-engineering strategies to improve the clinical translation of stem cell regenerative therapy.

Conclusion

Although the last two decades have seen great strides in stem cell therapy, significant clinical hurdles remain. Arguably the most difficult challenge in translating this therapy into routine clinical use is the inconsistent and relatively limited efficacy observed in adult stem cell clinical trials to date. The inability to determine cell fate and survival in humans has been a significant obstacle to understanding the mechanisms of the variable efficacy. The incorporation of cell fate imaging in clinical trials may help address these significant hurdles. Furthermore, concerns over tumorigenicity, immunogenicity, and arrhythmogenicity exist with the use of different stem cell sources. A multifaceted approach will be vital in addressing these issues to enable successful clinical translation of stem cell therapies.

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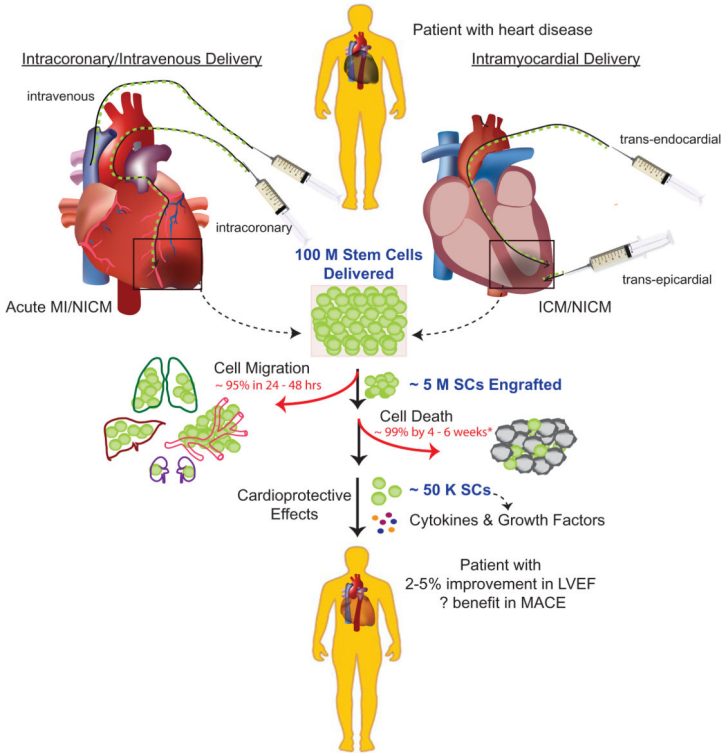

Figure 1. A major clinical hurdle is poor stem cell survival and retention post-delivery

Most trials published to date have employed intravenous, intracoronary, or intramyocardial delivery of autologous bone marrow mononuclear cells to treat both ischemic and non-ischemic heart diseases. Although a dose of 100 million cells are delivered, typically only a small fraction of cells (<5%) are retained at the site of transplantation after 24–48 hours in humans. Of those that are retained at the site of transplantation, many (~99%) do not survive beyond 4–6 weeks, as demonstrated in preclinical studies. Poor cell retention and survival likely limit improvement in LVEF and the incidence of MACE, although these issues have not been well studied (*denotes data that are only available in humans). SCs: stem cells; LVEF: left ventricular function; MACE: major adverse cardiac events.

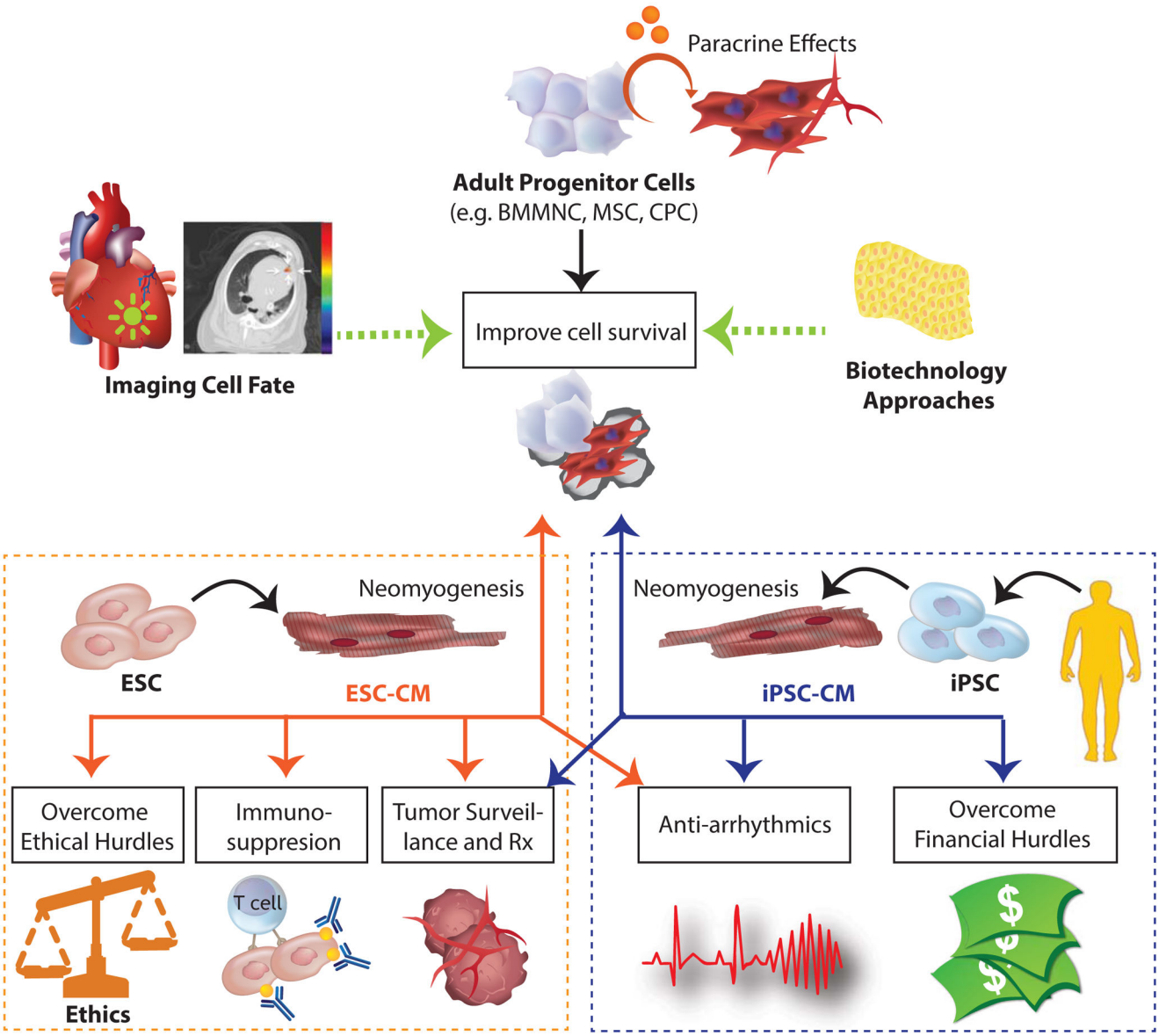

Figure 2. Potential solutions to clinical hurdles faced by the implementation of adult progenitor cells and pluripotent stem cells

Unlike adult stem cells, pluripotent stem cells such as ESCs and iPSCs can be differentiated into cardiomyocytes, which can generate new myocardial tissues. However, when cell death is pervasive, the extent of "neomyogenesis" and their subsequent functional output is debatable. Nevertheless, imaging technologies can be used to better identify strategies that can improve cell survive, engraftment, and efficacy. Pluripotent stem cells also face additional hurdles. ESCs require immunosuppression and face difficult regulatory and ethical challenges, whereas iPSCs may not be economically feasible (yet). Both cell types will likely require antiarrhythmic therapy if large grafts are achieved. ESCs: embryonic stem cells, iPSCs: induced pluripotent stem cells, Rx: treatment.

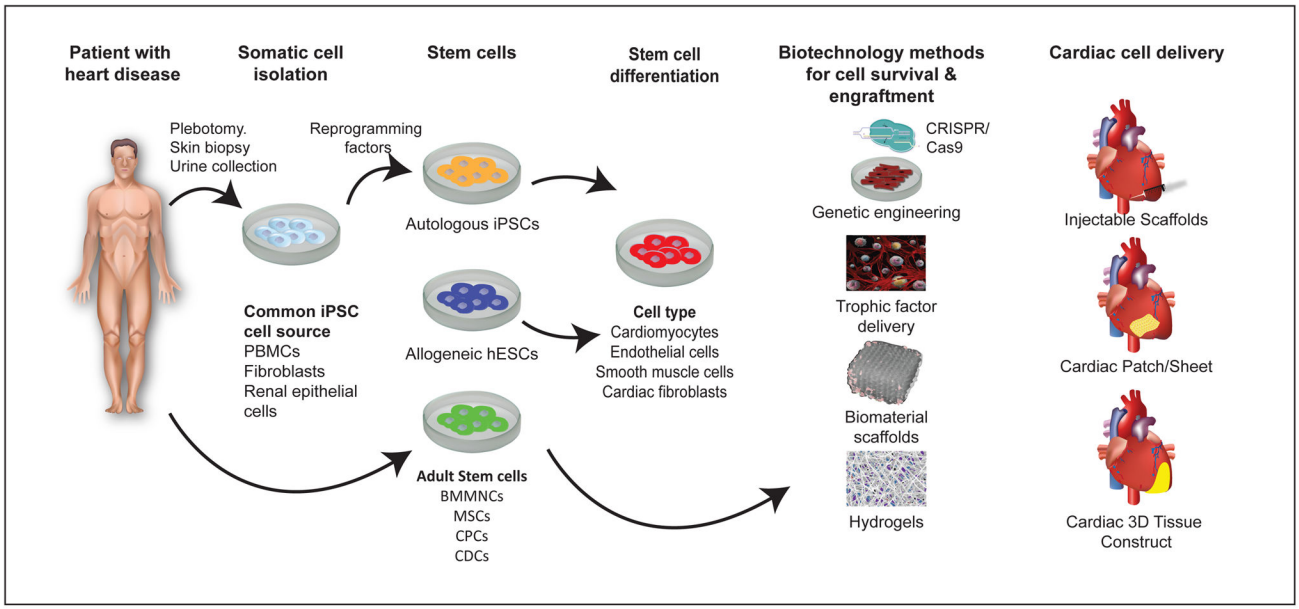

Figure 3. Schematic of how tissue engineering can be incorporated into stem cell therapy

Patients with heart disease can be treated with different cell sources, including autologous iPSCs, allogenic ESCs, and adult stem cells. Human iPSCs are generated by reprogramming adult somatic cells that are isolated from blood, skin or renal epithelial cells into the pluripotent state, and then differentiating them into cells that can repair the heart (e.g., cardiomyocytes, endothelial cells, smooth muscle cells, or fibroblasts). Similarly, ESCs can be differentiated into cell derivatives (e.g., cardiomyocytes, endothelial cells, smooth muscle cells, cardiac fibroblasts, etc.). By contrast, adult stem cells such as bone marrow mononuclear cells, mesenchymal stem cells, cardioprogenitor cells, and cardiosphere-derived cells do not require reprogramming or differentiation. To improve their survival post-transplantation, these cells can be genetically altered to express pro-survival genes using CRISPER/Cas9, transplanted together with trophic factors, or embedded in scaffolds or hydrogels. These cells together with their adjuvant agents can be delivered as an injectable scaffold, patch, or 3D tissue construct to further improve cellular retention and efficacy.