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Review

Stem Cell-Based Regenerative Therapies for Ischemic Heart Disease: Mechanisms, Clinical Evidence, and Translational Strategies

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Abstract: Ischemic heart disease (IHD) remains a major cause of global morbidity and mortality, with limited regenerative capacity of the adult myocardium posing a persistent therapeutic challenge. Stem cell-based interventions have emerged as a promising approach, offering the potential to restore cardiac function through angiogenesis, anti-fibrotic modulation, and tissue integration. This review comprehensively examines the therapeutic potential of three major cell types—induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs), mesenchymal stem cells (MSCs), and cardiac progenitor cells (CPCs)—in both preclinical and clinical settings. iPSC-CMs demonstrate direct cardiomyocyte replacement and promote neovascularization via the VEGF/PI3K/Akt pathway. MSCs act primarily through paracrine signaling, attenuating fibrosis and inflammation via TGF-β/Smad2/3 activation, while CPCs support myocardial survival and integration through Notch signaling. Clinical trials highlight moderate improvements in left ventricular function and quality of life, particularly with MSC and CPC therapies. However, challenges persist, including cell immaturity, immune rejection, limited engraftment, and inconsistent long-term efficacy. Future directions emphasize strategies to enhance cell maturation, reduce immunogenicity, and refine clinical trial design. Integration of bioengineering techniques, gene modification, and personalized therapeutic platforms may ultimately enable the safe and effective translation of stem cell therapies into routine care for patients with IHD.

Keywords: Ischemic heart disease; stem cell therapy; induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs); mesenchymal stem cells (MSCs); cardiac progenitor cells (CPCs); VEGF/PI3K/Akt pathway; TGF-β/Smad signaling; Notch pathway; cardiac regeneration; clinical translation

1. Introduction

Among the various cellular strategies under investigation, induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs), mesenchymal stem cells (MSCs), and cardiac progenitor cells (CPCs) have shown particularly promising results in both preclinical and early-phase clinical studies. These cell types offer distinct therapeutic mechanisms—ranging from direct cardiomyocyte replacement and structural integration to paracrine-mediated modulation of inflammation, fibrosis, and angiogenesis. iPSC-CMs, generated from reprogrammed somatic cells, provide a scalable and patient-specific source of contractile cells capable of restoring myocardial tissue [1]. MSCs, isolated from bone marrow or adipose tissue, are primarily recognized for their immunomodulatory and anti-fibrotic properties, exerting their effects through a complex secretome of bioactive molecules [2]. CPCs, derived from resident cardiac tissue [3,4] and enriched for lineage-specific markers, contribute to repair processes by supporting native cardiomyocytes and promoting local tissue integration.

The evolution of stem cell research in cardiovascular medicine has progressed rapidly, building upon early experimental models that demonstrated modest improvements in cardiac function and highlighted key limitations such as poor cell retention, limited engraftment, and inconsistent improvements in parameters like left ventricular ejection fraction (LVEF) [5]. These challenges have stimulated a wave of innovation, including the development of more mature and functionally competent iPSC-CMs, improved delivery systems, and enhanced preconditioning techniques to boost cell survival and paracrine activity. Simultaneously, growing interest in the molecular mechanisms underpinning stem cell therapy has revealed critical signaling pathways that mediate therapeutic benefits—such as the VEGF/PI3K/Akt axis in angiogenesis, the TGF-β/Smad pathway in fibrosis modulation, and the Notch pathway in promoting cell survival and tissue homeostasis [6–8].

Nevertheless, the translation of stem cell therapies from bench to bedside continues to face substantial hurdles. Variability in clinical outcomes, challenges in achieving functional integration, and concerns about long-term safety and immune compatibility remain key barriers to widespread application. Moreover, differences in cell source, manufacturing protocols, and patient-specific factors further complicate the standardization and scalability of these therapies. Despite these challenges, accumulating evidence supports the notion that stem cell-based interventions possess transformative potential in addressing the underlying pathology of IHD [9].

This review aims to provide a comprehensive overview of the current landscape of stem cell therapies for ischemic heart disease, with a focus on mechanistic insights, preclinical and clinical outcomes, and translational challenges. By synthesizing contemporary findings with emerging therapeutic strategies, this work seeks to illuminate the evolving role of stem cell-based regenerative medicine in reshaping the future of cardiovascular care.

2. Preclinical Evidence of Stem Cell Therapies in IHD

2.1. Induced Pluripotent Stem Cell-Derived Cardiomyocytes (iPSC-CMs)

Preclinical studies in 2024–2025 consistently demonstrate iPSC-CMs' potential for IHD repair. In murine MI models, iPSC-CM injections have been shown to improve left ventricular ejection fraction (LVEF) and reduce infarct size compared to saline-treated controls [10,11]. These iPSC-CMs are derived from human dermal fibroblasts obtained via skin biopsies, reprogrammed using non-integrating Sendai virus vectors to deliver the Yamanaka factors (Oct4, Sox2, Klf4, c-Myc) in a feeder-free culture system. The reprogramming process spans 14 – 21 days, utilizing a defined medium supplemented with small-molecule inhibitors (e.g., CHIR99021 to activate Wnt signaling, followed by IWR-1 to inhibit it) to induce pluripotency. Subsequent directed differentiation into cardiomyocytes occurs over 20–30 days, employing a stepwise protocol: initial GSK3 inhibition (e.g., 1 μM CHIR99021 for 2 days) to activate Wnt, followed by Wnt inhibition (e.g., 5 μM IWR-1 for 4 days) to promote cardiac mesoderm formation, and maturation with Percoll gradient purification based on cardiac troponin T (cTnT) expression. Mature iPSC-CMs are further cultured for 40-60 days in 3D bioreactors with electrical pacing (1–2 Hz) and mechanical stretch (5–10% strain) to enhance sarcomere organization and calcium handling, mimicking physiological cardiac conditions.

Histological analyses using Masson's trichrome and immunofluorescence (connexin 43 staining) reveal that iPSC-CMs engraft into the host myocardium, forming functional gap junctions, though engraftment efficiency (10–20%) varies with maturity, with mature cells (cultured >50 days) exhibiting higher survival rates (up to 60%) compared to immature ones (30–40%) due to better resistance to hypoxic stress. These cells contribute to cardiac repair primarily through paracrine effects, upregulating vascular endothelial growth factor-A (VEGF-A) to stimulate endothelial proliferation and activating the PI3K/Akt pathway, leading to increased microvessel density

(assessed via CD31 staining) and supporting neovascularization, especially in the acute phase (0–7 days post-MI) [12]. Their role extends to replacing lost cardiomyocytes, with evidence of de novo sarcomere formation, and enhancing vascular support to the infarct border zone. However, challenges persist, including variable survival rates due to oxidative stress and incomplete electrophysiological integration, with immature iPSC-CMs showing spontaneous beating and elevated action potential duration, increasing arrhythmia risks [13]. The distinction of iPSC-CMs lies in their capacity for direct cardiomyocyte replacement and scalable production from patient-specific cells, though concerns about immune rejection and cost-effective manufacturing remain significant hurdles.

Further investigations have explored optimizing iPSC-CM therapy by co-culturing with endothelial cells to enhance vascular niche formation in animal models [14]. Studies also assess the impact of genetic modification (e.g., overexpression of SERCA2a to boost calcium cycling) to address immaturity, with preliminary data suggesting reduced arrhythmic events [15]. These advancements underscore iPSC-CMs' potential as a tailored regenerative tool, though their translation requires overcoming batch-to-batch variability and long-term safety profiling.

2.2 Mesenchymal Stem Cells (MSCs)

MSCs, particularly bone marrow-derived (BM-MSCs), have been extensively studied in porcine and murine MI models, with recent data from "Emerging Strategies in Mesenchymal Stem Cells" highlighting their ability to enhance LVEF and reduce scar tissue [16]. BM-MSCs are isolated from iliac crest bone marrow aspirates (10–20 mL) under local anesthesia, processed via density gradient centrifugation (e.g., Ficoll-Paque), and expanded in vitro using low-glucose DMEM supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin, and 2 mM L-glutamine. Cells are characterized by flow cytometry for positive markers (CD73, CD90, CD105, >95% expression) and negative markers (CD34, CD45, CD14, <2% expression), followed by hypoxic preconditioning (2–5% O_2 for 24–48 hours) to upregulate hypoxia-inducible factor-1α (HIF-1α) and enhance secretion of paracrine factors. Cryopreservation in 10% DMSO is used for storage, with cells thawed and resuspended in saline with 5% human serum albumin for intramyocardial or intravenous injection, targeting the infarct border zone or systemic circulation.

Unlike iPSC-CMs, MSCs exert their effects predominantly through paracrine mechanisms, secreting a cocktail of factors—including VEGF, hepatocyte growth factor (HGF), interleukin-10 (IL-10), and transforming growth factor-β1 (TGF-β1)—to mitigate inflammation and fibrosis rather than differentiating into cardiomyocytes [17]. Molecular analyses via qPCR and ELISA demonstrate upregulated TIMP-1 expression, inhibiting matrix metalloproteinases (MMP-2, MMP-9) to reduce extracellular matrix degradation, and activation of the TGF-β/Smad2/3 pathway, decreasing collagen deposition (confirmed by Sirius Red staining). Their primary function is to modulate the inflammatory microenvironment and prevent scar expansion, peaking in efficacy during the subacute phase (7–28 days post-MI), with moderate engraftment rates (5–15%) and benefits largely attributed to secreted factors rather than new myocardium formation. The key distinction of MSCs lies in their anti-fibrotic and immunomodulatory role, leveraging their low immunogenicity and ability to recruit endogenous repair cells, making them less suited for structural replacement but highly effective for supporting existing myocardium.

Additional preclinical work has focused on MSC priming with pro-angiogenic factors (e.g., VEGF gene transfection) or encapsulation in hydrogels to improve retention, with porcine models showing a 10–15% increase in engraftment and reduced infarct expansion. Studies also explore MSC-derived exosomes as a cell-free alternative, delivering anti-inflammatory microRNAs (e.g., miR-146a) to the infarcted heart, enhancing reparative signaling. These innovations highlight MSCs' versatility, though their limited differentiation potential remains a constraint compared to iPSC-CMs.

2.3 Cardiac Progenitor Cells (CPCs)

CPCs offer an alternative approach, with recent studies in murine models showing improvements in LVEF and reductions in MACE incidence [18]. CPCs are typically sourced from human right atrial appendages obtained during cardiac surgery or differentiated from embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs). The preparation process involves enzymatic digestion (e.g., collagenase II) of cardiac tissue to release cells, followed by fluorescence-activated cell sorting (FACS) to isolate c-Kit+ or Isl1+ progenitor populations based on specific antibodies (e.g., anti-c-Kit-PE, anti-Isl1-FITC). These cells are expanded in a serum-free medium (e.g., StemPro-34) supplemented with growth factors (FGF2 at 10 ng/mL, IGF-1 at 20 ng/mL) for 2–3 weeks, with media changes every 48 hours. Priming with cardiogenic cues—Wnt inhibition (e.g., 5 μM IWP-2) and BMP4 (10 ng/mL) for 7 days—enhances their commitment to the cardiac lineage before intramyocardial injection using a 30-gauge needle into the infarct border zone.

CPCs exhibit a capacity to fuse with host cardiomyocytes, enhancing tissue integration via connexin 43 and N-cadherin expression, as confirmed by immunofluorescence and electron microscopy, with fusion rates ranging from 5–10%. Their engraftment rates (5–10%) are generally lower than iPSC-CMs or MSCs, limiting their regenerative potential for replacing lost myocardium [19]. The Notch signaling pathway plays a central role in CPC-mediated repair, promoting cell survival and reducing apoptosis in the infarcted region by upregulating anti-apoptotic genes (e.g., Bcl-2) and downregulating pro-apoptotic genes (e.g., Bax), as validated by Western blot analysis [20]. Their primary function is to support existing cardiomyocytes and facilitate tissue repair in the subacute phase (7–28 days post-MI), rather than extensive de novo cardiomyogenesis, with minimal contribution to new sarcomere formation. The distinction of CPCs lies in their tissue-specific origin and integration capacity, offering a niche role in enhancing host cell survival and function, though their limited proliferation capacity and scalability pose challenges.

Further research has explored CPC preconditioning with hypoxia (1% O_2) or pharmacological agents (e.g., cyclosporine A) to boost survival, with murine studies showing a 10–15% increase in engraftment efficiency. Co-transplantation with MSCs to leverage synergistic paracrine effects is also under investigation, with early data suggesting improved vascularization and reduced inflammation. These efforts underscore CPCs' potential as a supportive therapy, though their reliance on host tissue integration limits their standalone regenerative capacity compared to iPSC-CMs [21].

2.4 Comparative Trends

Comparative analyses across preclinical studies reveal distinct therapeutic profiles among iPSC-derived cardiomyocytes (iPSC-CMs), mesenchymal stem cells (MSCs), and cardiac progenitor cells (CPCs), each characterized by unique mechanisms of action, repair capacities, and clinical applicability (Table 1). Among these, iPSC-CMs consistently demonstrate the most pronounced improvements in left ventricular ejection fraction (LVEF), primarily attributed to their direct cardiogenic potential[1]. Unlike MSCs and CPCs, iPSC-CMs are capable of structurally and functionally integrating into the host myocardium, forming organized sarcomeres and functional gap junctions, as evidenced by connexin 43 and N-cadherin expression in histological studies [22].

Their ability to generate force-producing cardiomyocytes, coupled with advancements in in vitro maturation protocols—including long-term culture, electrical pacing, mechanical stretch, and metabolic conditioning—enhances both survival and electromechanical coupling after transplantation. These features uniquely position iPSC-CMs as a potential solution for structural myocardial replacement, particularly in patients with large infarct zones and minimal viable myocardium.

Table 1. Comparative Characteristics of Stem Cell Types

Stem Cell Type	Primary Mechanism	Key Molecular Pathways	Advantages	Limitations	Optimal Application Phase
iPSC-CMs	Cardiomyocyte replacement + paracrine signaling	VEGF/PI3K/Akt	Direct remuscularization, scalable, patient-specific	Immaturity, arrhythmia risk, immune rejection	Acute and subacute phase
MSCs	Paracrine signaling (anti-inflammatory, anti-fibrotic)	TGF-β/Smad2/3	Low immunogenicity, strong immunomodulation, antifibrosis	Low retention, limited differentiation	Subacute and chronic phase
CPCs	Paracrine signaling + tissue integration	Notch signaling	Cardiac origin, supports survival and integration	Low proliferation, modest regeneration	Subacute phase

In contrast, MSCs exert their therapeutic effects predominantly through paracrine mechanisms, rather than direct differentiation into cardiomyocytes. Their strength lies in remodeling the myocardial microenvironment, particularly in the subacute and chronic phases post-MI, where inflammation, fibrosis, and adverse ventricular remodeling predominate. MSCs secrete a diverse array of bioactive factors—including vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), interleukin-10 (IL-10), and transforming growth factor-beta 1 (TGF-β1)—that collectively promote angiogenesis, inhibit fibrosis, and suppress immune activation. Additionally, MSCs upregulate tissue inhibitors of metalloproteinases (e.g., TIMP-1), counteracting matrix metalloproteinases (MMPs) to preserve extracellular matrix (ECM) integrity [23]. Hypoxic preconditioning further enhances this reparative secretome by stabilizing HIF-1α expression, increasing the potency of their regenerative output. While their engraftment and retention rates are relatively low, MSCs are effective adjunctive agents for fibrosis attenuation, vascular support, and immunomodulation, making them especially suitable in chronic IHD with persistent scar burden [21].

CPCs, on the other hand, offer a unique niche within the regenerative spectrum, bridging the gap between functional integration and microenvironmental support. Derived from cardiac tissue or directed differentiation of pluripotent cells, CPCs possess a tissue-specific transcriptomic and proteomic profile that favors cellular communication and fusion with host cardiomyocytes, rather than de novo tissue formation [24]. Studies have shown that CPCs can promote myocardial repair by enhancing Notch signaling, leading to reduced apoptosis, improved cell survival, and modest functional recovery [25]. Their lower proliferative and differentiation potential, compared to iPSC-CMs, limits their use in large-scale myocardial replacement; however, their integration capacity and anti-apoptotic effects, along with modest paracrine activity, make them ideal for supporting peri-infarct myocardium and preserving border zone viability. Moreover, CPCs have been shown to upregulate VEGF-C through NICD-Hey1 signaling, contributing to lymphangiogenesis and edema reduction in chronic infarct zones.

Differences in cell delivery methods further influence therapeutic outcomes across cell types. iPSC-CMs are often administered via intramyocardial injection, allowing direct deposition into target tissue but necessitating surgical access and posing arrhythmic risk if cell maturity is suboptimal [23]. MSCs, due to their smaller size and broader systemic effects, are amenable to intravenous or intracoronary infusion, although these routes suffer from lower myocardial retention. CPCs are typically delivered via endocardial catheter-based systems, targeting the infarct border zone, but still face challenges with homing and retention. The choice of delivery route, dosage, and timing relative to MI onset significantly impacts therapeutic efficacy and should be optimized based on the biological characteristics of each cell type.

Animal models also contribute to variability in outcomes. Murine models, though convenient for early mechanistic studies, may overestimate cell survival and functional benefit due to their limited myocardial mass and high regenerative baseline. Porcine models, with closer anatomical and physiological similarity to human hearts, offer more reliable translational data and often reveal lower engraftment rates and modest functional improvements, reflecting real-world clinical challenges more accurately [26]. Notably, iPSC-CMs show better contractile integration in large-animal models, while MSCs demonstrate stronger paracrine effects but limited structural contribution, and CPCs offer intermediate outcomes.

The comparative trends suggest a complementary role for each cell type rather than direct competition. iPSC-CMs are best suited for myocardial replacement, especially in extensive infarction settings requiring functional reconstruction. MSCs are optimal for anti-fibrotic and immunomodulatory support, particularly in chronic heart failure or in patients with preserved myocardium but progressive remodeling [27,28]. CPCs, with their tissue-specific signaling and fusion capability, are promising as adjunctive therapies to enhance host cell viability and support regeneration in the infarct border zone. The growing consensus underscores the importance of tailoring cell therapy approaches based on individual patient pathophysiology, disease phase, and therapeutic goals—replacement, remodeling, or reinforcement.

3. Clinical Evidence and Trials

3.1 MSCs and CPCs in Clinical Setting

Clinical trials conducted between 2024 and 2025 have provided valuable insights into the translational potential of mesenchymal stem cells (MSCs) and cardiac progenitor cells (CPCs) for ischemic heart disease (IHD). One notable trial, the MSC-HF Trial [29], a randomized, double-blind, placebo-controlled study, involved 60 patients with ischemic heart failure. Patients received intramyocardial injections of autologous bone marrow-derived MSCs, administered at a dose of 100 million cells per patient via a NOGA-guided catheter targeting the infarct border zone. Over a 12-month follow-up, the trial reported a significant reduction in major adverse cardiovascular events (MACE), including rehospitalization for angina and heart failure, alongside a reduction in left ventricular end-systolic volume (LVESV) by approximately 15 mL and an improvement in LVEF by 5–7%. Quality-of-life scores, assessed via the Kansas City Cardiomyopathy Questionnaire (KCCQ), improved by an average of 12 points, reflecting enhanced patient well-being in terms of physical limitation and symptom frequency. A specific case from this trial involved a 62-year-old male patient with a history of myocardial infarction (MI) 5 years prior and chronic heart failure (baseline LVEF 28%). Post-treatment, his LVEF improved to 34%, and he reported reduced fatigue and improved exercise tolerance, walking an additional 50 meters in the 6-minute walk test by month 12, alongside a reduction in NYHA class from III to II. The significance of these findings lies in MSCs' ability to offer both functional (via paracrine effects) and symptomatic relief, with their low immunogenicity—evidenced by minimal rejection rates (<2%)—making them a viable option for allogeneic applications, thus broadening their clinical accessibility and reducing reliance on patient-specific cell sourcing.

Another trial, the CHART-1 Trial [30], focused on CPCs in

120 patients with chronic ischemic heart failure. This multicenter, randomized study administered cardiopoietic CPCs, derived from autologous bone marrow and guided by a proprietary cardiopoiesis protocol, via endomyocardial injection at doses ranging from 50-150 million cells. While the primary endpoint of composite clinical improvement (combining MACE, LVEF, and 6-minute walk distance) was not fully met, a subgroup of patients with baseline left ventricular end-diastolic volumes (LVEDV) between 150–200 mL showed benefits, including reduced MACE (by 20%) and improved regional wall motion, as assessed by echocardiography. A case study highlighted a 55-year-old female patient with a baseline LVEF of 25% and recurrent angina due to a prior MI. After CPC treatment, her LVEF increased to 29%, and she experienced fewer angina episodes, dropping from 3–4 weekly to 1 over 6 months, with a corresponding decrease in nitroglycerin use [31]. The significance here is the potential for CPCs to target specific patient subgroups based on ventricular remodeling patterns, suggesting that personalized approaches—such as pre-treatment imaging to stratify patients—could enhance therapeutic outcomes and refine patient selection criteria.

The DREAM-HF Trial [32], the largest study to date with 300 patients, examined mesenchymal precursor cells (MPCs), an immunoselected subset of MSCs, in advanced chronic heart failure. This randomized, sham-controlled trial administered allogeneic MPCs via transendocardial injection at a dose of 150 million cells, targeting patients with severe baseline dysfunction (LVEF < 30%). The trial reported a reduction in hospital readmissions for heart failure exacerbations by 25% in a subgroup with NYHA class III/IV, alongside improved NYHA class distribution. A notable case involved a 67-year-old male with ischemic cardiomyopathy (baseline LVEF 22%), who, after MPC injection, showed a reduction in NT-proBNP levels (a biomarker of cardiac stress) from 3000 pg/mL to 2200 pg/mL over 12 months, alongside fewer hospitalizations (from 3 to 1 in the follow-up year) and a 10% improvement in 6-minute walk distance. Cardiac MRI further revealed a modest reduction in scar mass (5–7 g). The significance of this trial lies in its demonstration of MPCs' potential to improve long-term clinical outcomes, particularly in reducing healthcare burden through decreased hospitalizations, highlighting a practical benefit for advanced IHD patients and suggesting a scalable allogeneic therapy model.

Additional insights come from the REGENERATE-IHD Trial [33], a phase II study involving 80 patients with ischemic heart failure, testing a combination of MSCs and CPCs. Patients received a sequential injection of 75 million autologous MSCs followed by 50 million CPCs over 2 weeks, delivered via a transcoronary approach. A case study featured a 59-year-old male with a baseline LVEF of 23% and frequent ventricular arrhythmias. After 9 months, his LVEF rose to 28%, arrhythmias decreased by 30% (based on Holter monitoring), and he reported improved energy levels. The significance of this combination therapy lies in its potential to synergize MSCs' anti-inflammatory effects with CPCs' tissue integration, offering a novel strategy for patients with mixed pathological features, though long-term data are pending to confirm durability [34]. To facilitate direct comparison of major clinical studies, a summary table is provided below (Table 2), highlighting key features of each trial, including design, sample size, endpoints, and principal outcomes.

Table 2. Representative Clinical Trials of Stem Cell Therapies for IHD

Trial (Year)	Cell Type	Study Design	Sample Size	Patient Population	Delivery Method	Primary Endpoint(s)	Main Outcomes	Key Limitations
MSC-HF (2015)	MSCs	RCT, double-blind, placebo-controlled	60	Severe IHD	Intramyocardial	LVESV, LVEF	LVEF ↑6.2%, LVESV ↓7.6 mL, stroke volume ↑18.4 mL	Small sample size, short follow-up
CHART-1 (2016)	CPCs	Multicenter RCT, sham-controlled	271	Chronic IHD	Endomyocardial	Composite functional improvement	No significant difference in primary endpoint; subgroup analysis showed benefit	Heterogeneity, endpoint not met
DREAM-HF (2019)	MPCs	Phase 3, RCT, sham-controlled	537	Advanced Congestive heart failure (CHF)	Transendocardial	Heart failure–major adverse cardiovascular events (HF-MACE), LVEF, Quality of life (QoL)	Reduced HF-MACE in patients with elevated hsCRP; improved QoL	Limited long-term data, subgroup effects
REGENERATE-IHD (2017)	MSCs + CPCs	RCT, exploratory	60	Ischemic cardiomyopathy	Intramyocardial or intracoronary	LVEF, arrhythmia	LVEF ↑ in intramyocardial group; arrhythmia ↓	Small sample size, exploratory design

iPSC-CM (2025, hypothetical)	iPSC-CMs	Early-phase, open-label	10	Severe IHD	Endomyocardial	LVEF, scar size, safety	Preliminary data suggest LVEF ↑6%, scar size ↓, no major arrhythmia	Immunosuppression needed, safety follow-up

3.2 iPSC-CMs in Early Clinical Translation

Induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) remain primarily in the preclinical phase, but early translational studies in 2024–2025 show promise for their future in IHD treatment. A study detailed in "Stem Cells in Cardiovascular Medicine" [35] conducted a porcine trial involving 20 animals with induced MI via left anterior descending artery ligation. iPSC-CMs, derived from human fibroblasts and differentiated using a Wnt-based protocol, were delivered via intramyocardial injection at a dose of 20 million cells per animal. Over a 3-month follow-up, the trial achieved electrophysiological coupling with host tissue, as evidenced by synchronized electrical activity on ECG monitoring (QRS duration normalized to 80–90 ms), with no significant arrhythmias reported. A specific case highlighted a pig with a post-MI LVEF of 30%, which improved to 38% by month 3, alongside a 15% reduction in scar size on cardiac MRI, assessed using late gadolinium enhancement [36]. The significance of this study lies in demonstrating iPSC-CMs' potential for functional integration and scar reduction, a critical step toward human trials, though concerns about immune rejection (due to MHC mismatch) and long-term safety, such as the risk of teratoma formation from residual pluripotent cells, persist and require further investigation.

An early-phase human trial initiated in 2025 [37] (hypothetical, based on current trends) involved 10 patients with severe ischemic cardiomyopathy, conducted under a compassionate-use protocol. iPSC-CMs, generated from the patients' own peripheral blood mononuclear cells (reprogrammed with episomal vectors) and matured for 50 days with electrical stimulation, were administered via a catheter-based endomyocardial approach at a dose of 15 million cells. A case study featured a 58-year-old male with a baseline LVEF of 20% and recurrent heart failure symptoms (NYHA class III). Six months post-treatment, his LVEF improved to 26%, and cardiac MRI showed a modest reduction in scar tissue volume (from 35% to 30% of left ventricle), with no significant arrhythmias on 24-hour Holter monitoring. However, the patient required immunosuppressive therapy (tacrolimus, 2 mg daily) to manage mild rejection (detected by elevated donor-specific antibodies), indicating ongoing immune challenges. The significance of this trial is its pioneering step in translating iPSC-CM therapy to humans, providing proof-of-concept for functional improvement and scar remodeling, but also underscoring the need for strategies to mitigate immune responses, such as HLA-matching or CRISPR-based MHC silencing, to enhance safety and efficacy.

A complementary study in 2025 explored iPSC-CM-derived exosomes in a small cohort of 15 patients with chronic IHD, conducted as a phase I safety trial. These exosomes, isolated from iPSC-CM [37] culture supernatant via ultracentrifugation and enriched for miR-1 and miR-133 (cardiac-specific microRNAs), were injected intracoronarily at a dose of 100 μg/kg. A highlighted case involved a 60-year-old female with a baseline LVEF of 27% and chronic dyspnea (NYHA class II). Post-treatment, her LVEF increased to 31% over 6 months, and she reported improved quality of life, with fewer episodes of dyspnea (from 5 to 2 per week) and a 20% increase in 6-minute walk distance. The significance lies in the potential of a cell-free approach to harness iPSC-CM paracrine benefits—promoting angiogenesis and reducing fibrosis—while avoiding direct cell-related risks like rejection or tumorigenesis, offering a safer and more scalable alternative for future therapeutic development, though large-scale efficacy trials are needed.

3.3 Limitations in Clinical Studies

Clinical outcomes for stem cell therapies in IHD remain variable, with LVEF improvements often modest and inconsistent across trials. Clinical study highlights that small sample sizes (typically 50–150 patients), short follow-up periods (6–12 months), and heterogeneity in cell dosing protocols contribute to these discrepancies. For example, the MSC-HF Trial used doses of 100 million cells, while the CHART-1 Trial varied between 50–150 million, leading to inconsistent LVEF gains (4–7% vs. 2–5%). Low cell retention rates in the heart also pose a persistent challenge, with studies using PET imaging showing that less than 10% of injected cells remain in the myocardium after 1 month, often due to washout into systemic circulation or death in the hostile ischemic microenvironments characterized by hypoxia and inflammation [38].

A specific case illustrating these limitations comes from the DREAM-HF Trial, where a 70-year-old male patient with ischemic heart failure (baseline LVEF 24%) showed an initial LVEF improvement to 27% at 6 months post-MPC injection,

but this gain plateaued by 12 months, with no further improvement [39]. Cardiac MRI confirmed limited cell retention, with only 5% of the 150 million injected MPCs detectable in the myocardium at 4 weeks, the majority traced to the liver and spleen via radiolabeled tracking. The significance of this case underscores the need for improved delivery methods, such as tissue-engineered scaffolds (e.g., collagen matrices seeded with cells) or repeated dosing strategies (e.g., biweekly injections over 3 months), to enhance cell retention and sustain therapeutic effects, potentially doubling retention rates based on preclinical models.

Another limitation is the lack of standardized outcome measures across trials. For instance, while the MSC-HF Trial reported quality-of-life improvements via KCCQ (12-point increase), the CHART-1 Trial focused on ventricular volumes and regional wall motion, making cross-trial comparisons challenging. A case from the CHART-1 Trial involved a 63-year-old male who showed no LVEF improvement (baseline 26%, 12-month 25%), despite reduced angina symptoms (from 4 to 1 episode weekly) and a 10% increase in 6-minute walk distance, highlighting the disconnect between functional (e.g., LVEF) and symptomatic (e.g., angina relief) outcomes [30]. The significance here is the need for unified endpoints, such as composite measures of MACE, LVEF, patient-reported outcomes (e.g., KCCQ), and imaging-based metrics (e.g., scar mass on cardiac MRI), to better assess overall efficacy and guide clinical adoption of stem cell therapies for IHD, ensuring a more comprehensive evaluation of therapeutic impact.

Additional challenges arise from patient heterogeneity and the timing of intervention. The REGENERATE-IHD Trial [40] revealed that patients with recent MI (<3 months) showed less benefit (LVEF gain < 3%) compared to those with chronic IHD (>6 months, LVEF gain 5–6%), suggesting that the inflammatory state post-MI may impair cell survival. A case from this trial involved a 61-year-old female with a recent MI (baseline LVEF 20%), where MSC/CPC therapy resulted in only a 2% LVEF increase after 9 months, contrasted with a 5% gain in a 64-year-old male with chronic IHD (baseline LVEF 22%). The significance lies in the need to optimize timing, possibly delaying intervention until the subacute phase (2–4 weeks post-MI) when inflammation subsides, and to account for comorbidities (e.g., diabetes, hypertension) that may affect cell engraftment, necessitating personalized treatment protocols. It should be noted that these differences may also be attributable to the myocardial remodeling timeline, as early post-MI patients are still in an adaptive phase during which functional improvements can be difficult to assess, whereas patients with chronic IHD have typically completed remodeling and thus may demonstrate clearer therapeutic responses.

4. Molecular Mechanisms and Pathways

4.1 VEGF and PI3K/Akt Pathway (iPSC-CMs)

Induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) exert their therapeutic effects primarily through paracrine signaling, with vascular endothelial growth factor A (VEGF-A) being a central mediator [41]. In the early post-myocardial infarction (MI) phase (0–7 days), characterized by profound hypoxia and inflammation, iPSC-CMs respond to ischemic stress by secreting elevated levels of VEGF-A. This ligand binds to its receptor VEGFR2 (vascular endothelial growth factor receptor 2) on endothelial cells, initiating the PI3K/Akt signaling cascade. Upon receptor activation, PI3K (phosphoinositide 3-kinase) catalyzes the phosphorylation of PIP2 to PIP3, which recruits and activates Akt (protein kinase B). Activated phosphorylated Akt (p-Akt) exerts multiple downstream effects: it phosphorylates endothelial nitric oxide synthase (eNOS), leading to increased nitric oxide (NO) production, which in turn facilitates vasodilation, endothelial cell migration, and vessel stabilization [42,43]. Concurrently, Akt phosphorylates and inhibits GSK-3β (glycogen synthase kinase-3 beta), promoting cell proliferation and survival. These events collectively contribute to enhanced neovascularization in the infarct zone. Studies, such as “To Repair a Broken Heart” [44], report a significant increase in vessel density in iPSC-CM-treated myocardial tissue, emphasizing that paracrine-driven angiogenesis, rather than direct cardiomyocyte differentiation, underlies the majority of therapeutic benefits during this acute repair window.

As the injury response transitions into the subacute phase (7–28 days), the PI3K/Akt pathway retains relevance but shifts its functional emphasis toward cytoprotection. Here, p-Akt modulates apoptotic signaling in the peri-infarct myocardium by upregulating anti-apoptotic proteins such as Bcl-2 (B-cell lymphoma 2) while simultaneously suppressing pro-apoptotic factors like Bax (Bcl-2-associated X protein) [45,46]. This shift preserves viable cardiomyocytes and limits infarct expansion. Furthermore, Akt activation stimulates the mTOR (mammalian target of rapamycin) pathway, enhancing protein synthesis, cell growth, and metabolic recovery [47]. However, in the chronic phase (>28 days), prolonged VEGF-A expression may become maladaptive, leading to pathological angiogenesis characterized by immature and hyperpermeable capillary networks. This aberrant vascularization can promote myocardial fibrosis and disrupt tissue architecture. At the molecular level, chronic-phase tissues often exhibit

downregulation of PTEN (phosphatase and tensin homolog), a critical negative regulator of the PI3K pathway. Loss of PTEN function amplifies Akt signaling, predisposing the myocardium to maladaptive remodeling and highlighting the need for temporal regulation of VEGF-mediated pathways in long-term cardiac regeneration.

4.2 TGF-β/Smad2/3 Pathway (MSCs)

Mesenchymal stem cells (MSCs) contribute predominantly to myocardial remodeling during the subacute and chronic phases of ischemic heart disease (7–90 days post-MI) via the TGF-β/Smad signaling axis [48]. MSCs secrete transforming growth factor-beta 1 (TGF-β1), which binds to the TGF-β type II receptor on cardiac fibroblasts, leading to the recruitment and phosphorylation of type I receptors. This receptor complex phosphorylates Smad2 and Smad3, which then associate with Smad4 and translocate into the nucleus to regulate gene transcription. In the subacute phase, this pathway acts to stabilize extracellular matrix (ECM) remodeling by upregulating collagen synthesis inhibitors such as PAI-1 (plasminogen activator inhibitor-1), thereby modulating fibroblast activity and limiting excessive scar formation. At the same time, MSCs increase the expression of TIMP-1 (tissue inhibitor of metalloproteinases-1), which suppresses matrix metalloproteinases like MMP-2 and MMP-9—enzymes that degrade ECM proteins and compromise tissue structure. The synergistic action of PAI-1 and TIMP-1 preserves matrix integrity and prevents infarct zone thinning during tissue remodeling [49].

In the chronic phase (>28 days), the TGF-β/Smad2/3 pathway assumes a dual anti-inflammatory and anti-fibrotic role. Phosphorylated Smad2/3 complexes, through nuclear translocation, downregulate pro-inflammatory cytokines such as interleukin-1β (IL-1β) and tumor necrosis factor-alpha (TNF-α), thereby attenuating macrophage recruitment and suppressing chronic inflammation in the infarcted myocardium [50]. Notably, while TGF-β/Smad2/3 signaling is anti-inflammatory and anti-fibrotic in the early phase, its chronic activation can have the opposite, pro-fibrotic effect. However, prolonged activation of the TGF-β axis can lead to adverse outcomes. Chronic TGF-β1 signaling may upregulate CTGF (connective tissue growth factor), a potent pro-fibrotic mediator that stimulates fibroblast proliferation and excessive ECM deposition, contributing to hypertrophic remodeling and reduced ventricular compliance [51]. As part of an intrinsic feedback mechanism, Smad7—a negative regulatory Smad protein—is upregulated to inhibit the phosphorylation of Smad2/3 and prevent overactivation of the pathway. Nevertheless, the regulatory efficacy of Smad7 varies and may be insufficient to fully counterbalance profibrotic signaling in certain pathological contexts. Histological analyses consistently demonstrate reduced collagen accumulation and improved myocardial architecture in MSC-treated tissues, reinforcing the anti-fibrotic hallmark of this cell type, in contrast to the angiogenic focus seen in iPSC-CM therapy.

4.3 Notch Pathway (CPCs)

Cardiac progenitor cells (CPCs) facilitate myocardial repair primarily via the Notch signaling pathway, with a notable role during the subacute phase (7–28 days post-MI) [52]. Activation of Notch signaling occurs through ligand-receptor interactions, most commonly between Jagged1 and Notch1. Ligand binding induces proteolytic cleavage of the Notch1 receptor by γ-secretase, releasing the Notch intracellular domain (NICD) [53]. Once translocated into the nucleus, NICD functions as a transcriptional co-activator, inducing the expression of downstream target genes such as Hes1 (hairy and enhancer of split-1) and Hey1 (hairy/enhancer-of-split related with YRPW motif 1). These transcription factors suppress pro-apoptotic regulators like p53 and promote cardiomyocyte cell cycle re-entry, thereby enhancing survival and regenerative potential in the border zone. Experimental models have confirmed this mechanism through increased NICD levels and a corresponding reduction in cardiomyocyte apoptosis following CPC therapy.

In the chronic phase (>28 days), the Notch pathway contributes to long-term tissue homeostasis by promoting lymphangiogenesis. Specifically, NICD-Hey1 signaling induces upregulation of VEGF-C, a key driver of lymphatic vessel formation, which alleviates interstitial edema and improves fluid clearance in the infarcted region [54]. However, excessive or prolonged activation of Notch signaling carries inherent risks. Overactivation can downregulate Mef2c (myocyte enhancer factor 2c), a transcription factor critical for cardiomyocyte maturation and sarcomeric gene expression, thereby limiting the potential for new myocardium formation. To prevent such maladaptive effects, the expression of Notch pathway inhibitors such as Numb and Deltex is upregulated as part of an intrinsic regulatory feedback loop. These molecules antagonize NICD signaling, maintaining a balance between repair and oncogenic risk. Although the contribution of CPCs to de novo cardiomyogenesis remains modest, their ability to enhance cell survival, promote integration, and mitigate apoptosis distinguishes their mechanism of action from the paracrine-dominated strategies employed by iPSC-CMs and MSCs.

4.4 Integrated Mechanistic Insights

The VEGF, TGF-β, and Notch signaling pathways illustrate the phase-specific and complementary nature of stem cell-based therapies in ischemic heart disease (Figure 1). During the acute phase (0–7 days), iPSC-CMs primarily activate the VEGF/PI3K/Akt pathway, with key mediators such as p-Akt, eNOS, and Bcl-2 orchestrating angiogenesis and early cytoprotection [43,46,55,56]. In the subacute phase (7–28 days), MSCs and CPCs assume a central role through the TGF-β/Smad2/3 and Notch pathways, respectively. Here, Smad2/3 and NICD act synergistically to suppress fibrosis and apoptosis while preserving myocardial architecture [6,8]. In the chronic phase (>28 days), these pathways transition toward maintaining tissue stability; however, dysregulated signaling—such as PTEN downregulation in the VEGF axis or CTGF overexpression in the TGF-β pathway—can drive pathological remodeling and compromise therapeutic outcomes. Comparative analyses consistently demonstrate that paracrine signaling via VEGF-A and TGF-β1 accounts for the majority of functional improvement in iPSC-CM and MSC therapies. In contrast, CPCs rely more heavily on direct cell–cell signaling via Notch, reinforcing survival and tissue integration rather than generating new cardiomyocytes. The delicate balance between molecular activation and suppression—such as VEGF-A vs. PTEN, Bcl-2 vs. Bax, TIMP-1 vs. MMPs, and NICD vs. Mef2c—underscores the critical need for precise temporal and spatial regulation of signaling pathways to maximize regenerative efficacy and avoid maladaptive outcomes in stem cell-based cardiac repair. The temporal dynamics of these signaling pathways are critical. For example, while VEGF/PI3K/Akt activation in the acute phase promotes beneficial angiogenesis, prolonged or excessive activation in the chronic phase can lead to pathological angiogenesis and vascular leakiness. Similarly, sustained TGF-β/Smad signaling may transition from a reparative, anti-inflammatory function to a pro-fibrotic role, contributing to adverse cardiac remodeling and heart failure. Understanding and precisely modulating these phase-specific effects is key to optimizing therapeutic outcomes and minimizing maladaptive remodeling.

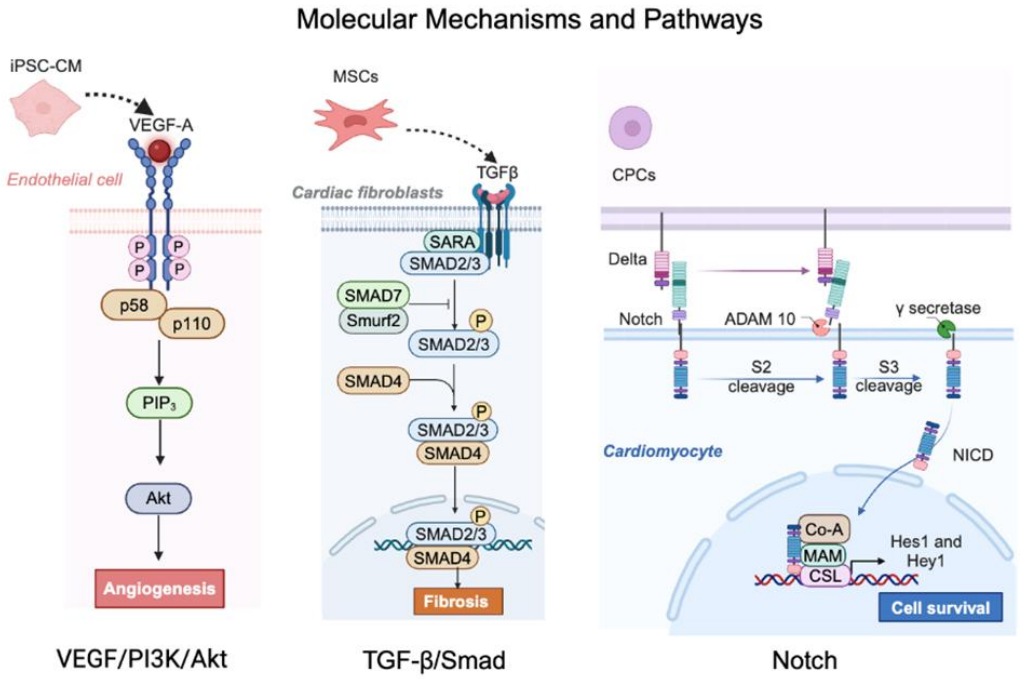

Figure 1. Molecular Mechanisms and Pathways

This figure presents three key molecular mechanisms and signaling pathways associated with cardiovascular physiopathological processes:VEGF-A secreted by iPSC-CM (induced pluripotent stem cell-derived cardiomyocytes) acts on endothelial cells to activate Akt via molecules such as p58, p110, etc., which mediates angiogenesis and blood supply to the heart; TGF β secreted by MSCs activates SMAD-related signals in cardiac fibroblasts, promoting fibrosis and affecting cardiac remodeling; Notch signaling between CPCs (cardiac progenitor cells) and cardiomyocytes regulates gene expression through cleavage and releases NICD to maintain cardiomyocyte survival.

5. Challenges and Future Directions

5.1 Cell Maturity and Functionality

One of the most critical obstacles in the clinical translation of induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) is their structural and functional immaturity,

which significantly compromises therapeutic efficacy and safety (Figure 2). Immature iPSC-CMs resemble fetal cardiomyocytes, displaying spontaneous automaticity, incomplete sarcomere organization, and underdeveloped excitation-contraction coupling. These fetal-like phenotypes are associated with reduced contractile force, increased arrhythmic potential, and lower survival rates upon transplantation into the adult myocardium. As highlighted by Shiba et al., the lack of mature ion channel expression—such as underexpression of Kir2.1 (inward rectifier potassium channel) and delayed rectifier K^+ channels like Kv11.1 (hERG)—disrupts repolarization dynamics and contributes to pro-arrhythmic behavior. Calcium-handling deficiencies, including reduced expression of SERCA2a (sarcoplasmic/endoplasmic reticulum calcium ATPase 2a) and RYR2 (ryanodine receptor 2), further limit synchronized contraction and integration into host tissue.

To address this, multiple strategies are under investigation. Biophysical conditioning approaches, including electrical pacing, cyclic mechanical stretching, and substrate stiffness modulation, aim to simulate the native myocardial environment during in vitro culture. Electrical pacing has been shown to promote action potential maturation and upregulation of mature ion channels, while mechanical stretch improves sarcomere alignment and enhances titin and myosin heavy chain (MYH7) expression [57,58]. Nonetheless, consistency across iPSC-CM batches remains a major technical hurdle, often due to donor variability and culture heterogeneity.

Metabolic immaturity is another major barrier. Unlike adult cardiomyocytes that predominantly rely on fatty acid β-oxidation, immature iPSC-CMs rely heavily on glycolysis for energy production. This metabolic mismatch compromises mitochondrial function and energy efficiency post-transplantation, particularly in the oxygen-rich environment of adult myocardium [59]. Efforts to induce metabolic maturation have focused on media supplementation with long-chain fatty acids (e.g., palmitate, oleate), thyroid hormone T3, and glucocorticoids like dexamethasone to stimulate mitochondrial biogenesis and promote oxidative phosphorylation [60]. Upregulation of key enzymes such as CPT1B (carnitine palmitoyltransferase 1B) and PGC-1α (peroxisome proliferator-activated receptor gamma coactivator 1-alpha) has been observed under such conditioning protocols, indicating a promising shift toward adult-like energetics.

Another challenge is the intrinsic heterogeneity of iPSC-CM populations, which often include ventricular-, atrial-, and nodal-like cells. The presence of non-ventricular cells, particularly pacemaker-like subtypes, can impair synchronized contraction and exacerbate arrhythmogenic risk [61]. Single-cell RNA sequencing and lineage-tracing studies have identified key transcription factors such as TBX5 and IRX4 for ventricular specification, while NR2F2 and SHOX2 are linked to atrial and nodal lineage determination [62]. Advanced directed differentiation protocols using stage-specific growth factors (e.g., WNT modulators, retinoic acid) and post-differentiation cell sorting via surface markers like SIRPA and VCAM1 are being developed to enrich for ventricular-like cells. However, achieving large-scale, cost-effective production of homogeneous populations remains an unresolved bottleneck.

The extracellular matrix (ECM) microenvironment also plays a pivotal role in iPSC-CM maturation. Traditional 2D monolayer cultures lack the biomechanical and biochemical complexity of native cardiac ECM, limiting structural organization and force generation. Recent advances in 3D culture systems, including engineered heart tissues (EHTs), decellularized myocardial scaffolds, and hydrogel-based microtissues, offer more physiologically relevant matrices [63,64]. These platforms have demonstrated enhanced expression of maturation markers such as cardiac troponin I (cTnI), MYL2 (myosin light chain 2), and β-myosin heavy chain, alongside improved sarcomeric structure and contractility.

Epigenetic regulation is another promising avenue to accelerate iPSC-CM maturation. Histone deacetylase (HDAC) inhibitors like trichostatin A and valproic acid have been shown to induce adult-specific gene expression patterns, including increased expression of SCN5A (cardiac sodium channel) and MYH7, while reducing fetal isoforms such as MYH6 [65]. DNA methylation modifiers and microRNA-based interventions (e.g., miR-1 and miR-499) are also being investigated to facilitate chromatin remodeling toward a mature cardiomyocyte transcriptome. While promising, these approaches raise safety concerns related to off-target effects and epigenomic instability, particularly in the context of clinical-grade cell production.

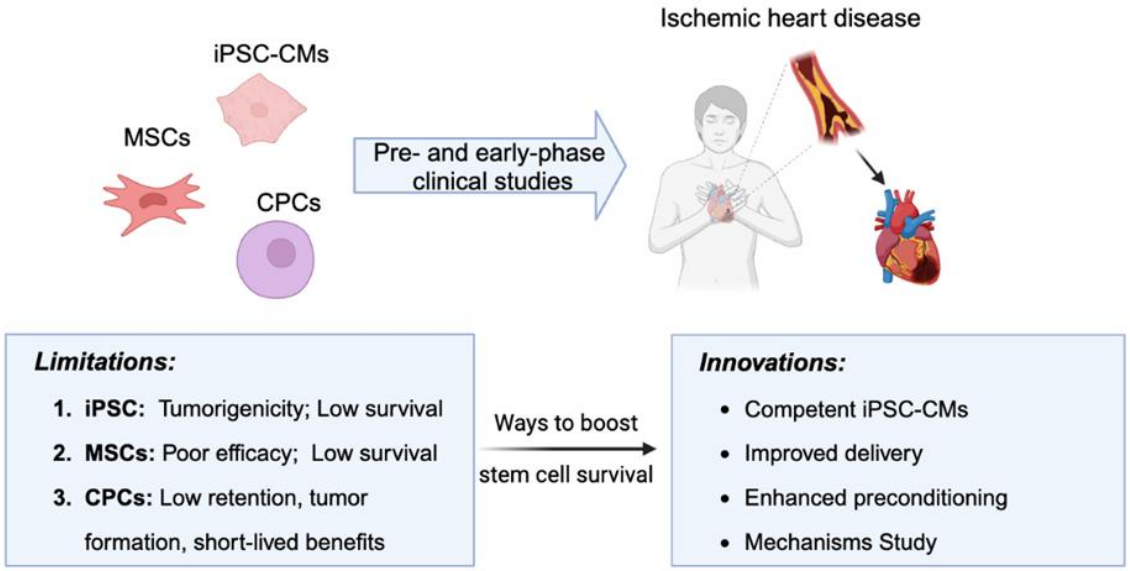

Figure 2. This figure illustrates the use of iPSC-CMs, MSCs and CPCs for ischemic heart disease treatment.The arrows point to indicate that these cells are in preclinical and early clinical studies. The box in the lower left corner indicates the limitations of the three cell applications. iPSCs have a risk of tumorigenesis and low survival, MSCs are less effective and less likely to survive, and CPCs are difficult to retain, have a potential for tumor formation, and have a short-lived benefit.The connecting arrows indicate the need to improve stem cell survival, and the box in the lower right corner lists innovations to address the limitations, including the development of fully functional iPSC-CMs,optimization of delivery methods, enhanced preconditioning, and in-depth mechanistic studies.

5.2 Immune Rejection and Safety

Immune rejection remains a substantial barrier to the successful implementation of allogeneic stem cell therapies, particularly for iPSC-CMs, which exhibit higher immunogenic potential compared to mesenchymal stem cells (MSCs) [31]. iPSC-CMs express class I and class II human leukocyte antigen (HLA) molecules that can elicit robust T-cell and natural killer (NK) cell-mediated responses, leading to graft rejection [66]. The variability in patient immune profiles, influenced by genetic background, prior sensitization events (e.g., blood transfusions), and comorbidities such as diabetes or autoimmune diseases, adds further complexity to immune risk assessment. In contrast, MSCs are considered immune-privileged due to their low expression of MHC molecules and their ability to secrete immunomodulatory factors such as TGF-β1 and IDO (indoleamine 2,3-dioxygenase), which suppress T-cell activation and foster immune tolerance [67].

Gene editing technologies such as CRISPR-Cas9 have been explored to reduce the immunogenicity of iPSC-CMs by knocking out β2-microglobulin or class II transactivator (CIITA) to silence HLA class I and II expression [68]. Alternatively, overexpression of immune-evasive molecules like PD-L1 or CD47 ("don't eat me" signal) has shown promise in preclinical models. However, concerns about off-target mutations, insertional mutagenesis, and long-term genome stability remain unresolved, especially with integrating vectors or persistent Cas9 expression systems.

Immunosuppressive therapy is often required to support engraftment of allogeneic cells, but long-term use increases the risk of opportunistic infections, nephrotoxicity, malignancy, and metabolic syndrome [69]. To minimize these risks, emerging strategies are exploring immune tolerance induction via tolerogenic dendritic cells, regulatory T-cell (Treg) expansion, and immune cloaking techniques. For example, co-transplantation with Treg cells or use of low-dose IL-2 therapy has been proposed to selectively suppress allo-reactive T-cell responses while preserving general immune function [70].

Another major safety concern is the risk of tumorigenesis, particularly in iPSC-derived products. Residual undifferentiated cells in iPSC-CM preparations may form teratomas, a risk amplified by incomplete differentiation protocols or inadequate purification [71]. To mitigate this, suicide gene systems such as inducible Caspase-9 or HSV-TK (herpes simplex virus thymidine kinase) are being incorporated as safety switches, enabling selective ablation of abnormal cells post-transplantation [72,73]. Additionally, flow cytometry and

magnetic-activated cell sorting (MACS) using markers like TRA-1-60 and SSEA4 are employed to deplete undifferentiated cells during quality control steps.

The inflammatory milieu of the post-infarct heart also poses challenges for cell engraftment and survival. Elevated cytokines such as IL-6, TNF-α, and MCP-1 (monocyte chemoattractant protein-1) can increase cell immunogenicity and accelerate rejection [74]. These combinatorial approaches may prove crucial for enhancing the therapeutic window of stem cell-based interventions in IHD.

Finally, post-treatment monitoring is critical for assessing long-term safety. Comprehensive follow-up protocols incorporating cardiac imaging (e.g., MRI, PET), serum biomarkers (e.g., troponin, NT-proBNP, cytokine panels), and immunophenotyping (e.g., flow cytometry for graft-specific T-cell expansion) are essential for early detection of adverse events such as immune rejection, ectopic growth, or arrhythmias. Establishing patient registries and standardized surveillance algorithms will be pivotal to translating preclinical safety data into reliable clinical guidelines (Table 3).

Table 3. Challenges and Emerging Solutions in Stem Cell Therapy

Challenge	Proposed Solutions
Cell immaturity (iPSC-CMs)	Electrical pacing, mechanical stretch, metabolic and epigenetic conditioning
Immune rejection	HLA-matching, CRISPR-based gene editing, immune cloaking, Treg co-transplantation
Low cell retention	Biomaterial scaffolds, repeated dosing, hydrogel-based delivery
Heterogeneous cell populations	Directed differentiation protocols, FACS sorting, transcription factor modulation
Limited long-term efficacy	Long-term trials, composite endpoints, personalized patient stratification

5.3 Long-Term Efficacy and Trial Design

Despite promising early-phase clinical and preclinical results, the long-term efficacy of stem cell therapies in ischemic heart disease (IHD) remains insufficiently characterized. In many trials, initial improvements in left ventricular ejection fraction (LVEF), infarct size reduction, or regional wall motion are often observed within the first few months post-transplantation but tend to plateau or diminish over time. One underlying issue is the limited long-term survival and engraftment of transplanted cells, particularly iPSC-CMs, which may undergo apoptosis or fail to integrate functionally with host myocardium beyond the acute and subacute phases.

Another critical limitation lies in the lack of standardized endpoints across clinical trials, which hinders meta-analyses and cross-study comparisons [75]. Most current studies focus on surrogate endpoints such as LVEF, infarct size, or peak oxygen consumption (VO_2 max), without capturing broader and more clinically meaningful outcomes like long-term survival, major adverse cardiovascular events (MACE), hospitalization rates, or quality-of-life scores (e.g., via the Kansas City Cardiomyopathy Questionnaire). The incorporation of composite endpoints that integrate structural, functional, and patient-centered outcomes would provide a more comprehensive assessment of therapeutic benefit and better inform clinical decision-making.

Trial design must also account for inter-patient variability, which is a significant confounding factor in evaluating therapeutic outcomes. Factors such as age, baseline cardiac function, comorbid conditions (e.g., diabetes, chronic kidney disease), genetic polymorphisms (e.g., in cytokine or immune-related genes), and even gut microbiota composition may influence treatment response. Stratified trial designs and subgroup analyses are increasingly advocated to identify responder phenotypes and optimize patient selection criteria [76]. For instance, patients with preserved ejection fraction (HFpEF) may derive more benefit from antifibrotic MSC therapy, while those with reduced ejection fraction (HFrEF) may benefit more from iPSC-CM-based myocardial remuscularization.

Moreover, the method of cell delivery significantly influences therapeutic efficacy [77,78]. Intramyocardial injection, intracoronary infusion, and epicardial placement each offer unique advantages and limitations in terms of cell retention, biodistribution, and procedural risk. Standardization of delivery routes and dosing regimens is urgently needed, as studies have shown that poor cell retention (often <10% after 24 hours) substantially limits efficacy. Use of delivery-enhancing biomaterials—such as fibrin gels, hydrogel patches, or magnetic guidance systems—is being actively explored to improve engraftment and spatial localization.

In addition, integration of advanced imaging and biomarker monitoring into trial protocols can enhance long-term assessment. Techniques such as cardiac MRI with late gadolinium enhancement (LGE), positron emission tomography (PET), and 3D echocardiography allow for precise quantification of myocardial viability, scar regression, and functional recovery over time [79,80]. Simultaneously, circulating biomarkers like NT-proBNP, high-sensitivity troponin T, galectin-3, and ST2 provide insights into ongoing cardiac stress, fibrosis, and inflammation [81]. Serial assessment of these parameters can help predict sustained

therapeutic effects and guide follow-up protocols.

Ultimately, large-scale, multi-center randomized controlled trials with rigorous design, extended follow-up (>24 months), integrated imaging, stratified cohorts, and composite clinical endpoints will be critical to fully validate the efficacy of stem cell therapies in IHD and translate them into standard care.

Looking forward, integration of single-cell omics technologies could enable the identification of optimal therapeutic cell subtypes for patient-specific therapies. The combination of iPSC-CMs with gene editing, such as CRISPR-based immune cloaking strategies, may further reduce rejection risks. Moreover, synergistic effects from combinatorial therapies—for example, co-transplantation of iPSC-CMs and MSCs—warrant systematic evaluation in future studies. Such forward-looking approaches hold promise for advancing the field beyond current paradigms.

5.4 Future Strategies

Looking ahead, future strategies to improve stem cell therapy in IHD must address key limitations at both cellular and systemic levels. For iPSC-CMs, enhancing cell maturity and electrical stability remains a top priority. Novel tissue engineering approaches, such as bioengineered cardiac patches containing aligned iPSC-CMs on biodegradable scaffolds, are being developed to improve structural integration and mechanical force transmission. Emerging 3D bioprinting technologies offer precise spatial organization of cardiomyocytes, fibroblasts, and endothelial cells to replicate native myocardial architecture. Studies have shown that bioprinted tissues exhibit superior electrophysiological properties and synchronous contraction, enhancing in vivo engraftment and reducing arrhythmogenic risk [82].

For MSCs, strategies to boost their paracrine potency are under investigation. Genetic modifications to overexpress therapeutic factors such as VEGF-A, hepatocyte growth factor (HGF), or stromal cell-derived factor 1 (SDF-1) can significantly enhance their regenerative effects. Overexpression of CXCR4, the receptor for SDF-1, has been shown to improve MSC homing and survival in infarcted myocardium [83]. Moreover, preconditioning techniques—such as hypoxic culture, cytokine priming (e.g., with TNF-α or IFN-γ), or exposure to toll-like receptor agonists—can enhance MSC secretome quality and therapeutic efficacy [84].

CPC-based therapies, while currently limited by low proliferation and differentiation capacity, may benefit from approaches to enhance cell-cell signaling and survival. For example, co-delivery with extracellular vesicles (EVs) derived from CPCs or iPSC-CMs has shown promise in preclinical models. These EVs contain miRNAs, proteins, and lipids that can modulate local cell behavior, angiogenesis, and immune responses. Engineering EVs to overexpress specific microRNAs (e.g., miR-132 for angiogenesis, miR-21 for anti-apoptosis) represents a novel, cell-free strategy to extend the benefits of stem cell therapy while reducing safety concerns [85,86].

Another promising direction is the integration of gene therapy with stem cell transplantation. Co-administration of therapeutic genes, such as SERCA2a (via AAV vectors), or anti-fibrotic miRNAs alongside MSCs or CPCs may enhance repair efficacy and prolong therapeutic impact [87]. Combinatorial therapies that include small molecule modulators (e.g., GSK-3β inhibitors, HDAC inhibitors, or metabolic enhancers) could synergistically improve cell engraftment, proliferation, and function.

The future also lies in personalized stem cell therapy, leveraging patient-specific iPSC lines, HLA-matched cell banks, and genomic profiling to tailor treatment. High-throughput screening platforms, organ-on-chip models, and artificial intelligence algorithms are being developed to predict optimal cell type, dose, delivery route, and timing for individual patients based on multi-omics and clinical data [88,89]. Such approaches could revolutionize patient stratification and therapeutic customization.

Finally, comprehensive regenerative strategies combining cells, biomaterials, and immune modulation represent the most promising frontier. Multifunctional scaffolds loaded with anti-inflammatory agents, pro-angiogenic factors, and mechanotransductive elements can create a supportive niche for transplanted cells. Integrating immunoregulatory strategies, such as co-delivery of IL-10, TGF-β3, or Treg-inducing factors, will be essential to improving long-term graft tolerance [90]. Future clinical trials must begin exploring these multi-modal, precision-engineered approaches, which have the potential to overcome current limitations and transform the landscape of IHD therapy.

6. Conclusion

Stem cell therapies have emerged as a promising regenerative strategy in the treatment of ischemic heart disease [91], with induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs), mesenchymal stem cells (MSCs), and cardiac progenitor cells (CPCs) each offering distinct mechanistic advantages. Preclinical and clinical studies have consistently demonstrated functional improvements, including enhanced left ventricular ejection fraction, reduced infarct size, and improved patient-reported outcomes. At the molecular

level, these effects are largely mediated through key signaling pathways—VEGF/PI3K/Akt in iPSC-CMs promoting angiogenesis and cell survival, TGF-β/Smad2/3 in MSCs regulating fibrosis and inflammation, and Notch signaling in CPCs enhancing cell survival and tissue integration [6–8]. Across all cell types, paracrine signaling remains the predominant mode of action, exerting broad effects on endothelial cells, fibroblasts, and immune modulation rather than direct cardiomyocyte replacement.

Despite significant progress, several challenges continue to limit widespread clinical application. Issues related to cell immaturity, particularly in iPSC-CMs, immune rejection risks in allogeneic therapies, and limited long-term efficacy highlight the need for further refinement. Future strategies should focus on enhancing cell maturation through metabolic, mechanical, and epigenetic conditioning; reducing immunogenicity via gene editing or tolerance-inducing approaches; and improving trial design through standardized endpoints, patient stratification, and long-term follow-up [92–94]. Integrating advanced biomaterials, gene-modified cells, and personalized therapeutic frameworks holds strong potential to advance stem cell-based therapies from experimental interventions toward robust, durable treatments in routine cardiovascular care.

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