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Cell and gene therapy for severe heart failure patients: The time and place for Pim-1 Kinase

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Abstract

Regenerative therapy in severe heart failure patients presents a challenging set of circumstances including a damaged myocardial environment that accelerates senescence in myocytes and cardiac progenitor cells. Failing myocardium suffers from deterioration of contractile function coupled with impaired regenerative potential that drives the heart toward decompensation. Efficacious regenerative cell therapy for severe heart failure requires disruption of this vicious circle that can be accomplished by alteration of the compromised myocyte phenotype and rejuvenation of progenitor cells. This review focuses upon potential for Pim-1 kinase to mitigate chronic heart failure by improving myocyte quality through preservation of mitochondrial integrity, prevention of hypertrophy and inhibition of apoptosis. In addition, cardiac progenitors engineered with Pim-1 possess enhanced regenerative potential, making Pim-1 an important player in future treatment of severe heart failure.

Keywords

cell therapy; cardiac progenitor cell gene therapy; heart failure; Pim-1 senescenc

Optimal care for a cardiac patient requires a dual approach: on one hand limiting damage and salvaging viable myocardium and on the other hand replacing dead myocardium with newly formed force-generating myocytes. To date, the majority of therapeutic options seem to prefer one side of this dichotomy: salvaging jeopardized cells. Clinical trials using Bone Marrow-Derived Stem Cells (BMSCs) were first initiated more than a decade ago. In

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retrospect, after hundreds of preclinical studies and over a dozen BMSC-clinical trials, the meta-analysis of collective findings shows the approach to be safe with beneficial reduction of infarct size without consistent marked improvement in Left Ventricular Ejection Fraction (LVEF [1–6]). Outcomes with use of BMSC prompt the prevailing interpretation that BMSCs may be able to limit myocardial damage and potentially recruit endogenous stem cells in a paracrine fashion [7] Lack of improvement in LVEF remains a point of concern as an important clinically relevant endpoint for efficacy, making more enduring cell-based therapeutic interventional strategies mandatory. Sustainable improvement in LVEF will inevitably require regeneration, prompting incorporation of newer stem cell types cardiac-lineage differentiation potential into the clinic as exemplified by the SCIPIO (cardiac **S**tem **C**ell Infusion in Patients with Ischemic Cardiomyopathy) trial [8]. SCIPIO Phase 1 provided initial proof of concept, safety and optimistic sustainable improvement with regards to LVEF and cardiac scar reduction [8] In comparison, conclusions from an alternative approach as represented by the CADUCEUS (Cardiosphere-Derived Autologous Stem Cells to Reverse Ventricular Dysfunction) trial appear to recapitulate findings from the aforementioned BMSC trials insofar as the major reported benefit was myocardial scar-reduction without evidence of increased LVEF [9,10]. Although these phase I trials are indeed primarily designed to assess safety rather than efficacy, the good news is that Cardiac progenitor cell (CPC)-mediated therapy has ripened from a proof of concept at laboratory benches to therapeutic reality at a patient's bedside. Currently, while we await further expansion into phase II trials for both SCIPIO and CADUCEUS, enthusiasm and optimism is tempered by circumspection of how broadly applicable such cell-based interventions will be to a broader patient population. Patients enrolled in the SCIPIO-trial were selected within criteria for being less than 75 years of age together with baseline LVEF < 40%. Similarly, patients with New York Heart Association Class IV were excluded from the CADUCEUS trial. These rational and legitimate criteria for the initial clinical assessment of stem cell treatment have, by design, excluded a substantial patient population that desperately needs regenerative therapy: severe heart failure patients currently on a Left Ventricular Assist Device (LVAD) who cling to life as cardiac transplant candidates.

Terminal heart failure is a chronic disease involving progressive organ damage that clearly demonstrates myocardial regenerative capacity as insufficient to mediate functionally meaningful repair [10]. Aging, oxidative stress, DNA damage, and inflammation contribute to development of replicative and premature cellular senescence with subsequent secretome-mediated tissue impairment that drags neighboring cells into senescence [11–18] Severe heart failure, cardiac patients predominantly correlate with a plethora of cardiovascular disease-associated risk factors such as smoking, excess caloric intake, or alcohol abuse that all participate in acceleration of telomere erosion and descent into cellular senescence [19–22]. Thus, cardiac stem cells residing within the embattled environment of severe heart failure may not necessarily reflect the reparative potential of cells employed in clinical trials to date. Collectively, the very target population of aged and infirmed patients destined to be at the forefront of interventional therapy are also likely to possess the most compromised stem cell population in terms of functional capacity and regenerative potential. Therefore, a reasonable question to ask is whether "youthful vigor" could be restored in these aged and pathologically embittered stem cells without altering programming for context-dependent

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recognition of the environment and appropriate integration into the local environment in a delicate fashion (as is problematic for current inducible pluripotent stem cell-based approaches). In this special report, we will elaborate upon the role of the Serine/threonine kinase Pim-1 as a “rejuvenating” approach in cardiac stem cell therapy and myocardial regeneration. In addition, the potential role of Pim-1 as a gene therapy target in salvaging damaged myocardium will be discussed.

Pim-1

The proto-oncogene *Pim-1* gene displays characteristics of *primary response genes* that are induced by activation of transcription factors downstream of growth factor signaling such as Akt, JAK-STAT and NF-B [23–25]. Pim-1 mRNA has a very short half-life due to presence of the destabilizing AUUU (A) sequence in the 3′ UTR. The 5′ UTR sequences of Pim-1 mRNA contains a GC-rich region representing a ‘weak transcript’, thereby imposing a cap-dependent translation [26,27] Due to presence of alternative translation initiation sites, two isoforms of this calcium/calmodulin-regulated kinase family member are produced (44kDa and 33kDa). Pim-1 protein is known to autophosphorylate, thereby being constitutively active [27,28]. Once activated, the kinase has a half-life time of 10 minutes, indicative of a tightly regulated production/degradation process. Indeed, Pim-1 has been shown to physically interact with the prolylisomerase Pin-1, which allows interaction with protein phosphatase 2A leading to dephosphorylation, ubiquitinylation and subsequent proteosomal degradation [29,30]. In addition, Hsp90[31] and Hsp70[32] have been correlated with regulation of Pim-1 stability and degradation respectively. Expression and activity of Pim-1 is induced by multiple growth factors, mitotic stimuli and cytokines in various cell types. In most cells, Pim-1 activity is associated with cell survival and proliferation.

Although belonging to different kinase families, Pim-1 is a downstream target of Akt kinase and shows similar substrate specificities. In fact, Akt–dependent survival signaling is attributed in part to physical interaction of Pim-1 with Bad, a major apoptotic initiator [32,34]. Phosphorylation and inactivation of Bad leads to increased levels of pro-survival proteins Bcl-2 and Bcl-xl in various cell types. In addition, Pim-1 has been reported as anti-apoptotic, independent of Akt, via phosphorylation of p38 MAPK in hematological cells [35].

The well-accepted role of Pim-1 in fostering cell cycle progression occurs in conjunction with phosphorylation of the phosphatase Cdc25A, a positive regulator of G1-phase of the cell cycle [36]. Increased phosphatase activity leads to amplification of Cdc25A and increased G1-S-phase transition rate. In addition, the main inhibitory protein of G1-S-phase transition, p21, is phosphorylated and inactivated by Pim-1 resulting in an increased cellular proliferation [37]. In addition, the pro-proliferative function of Pim-1 is not restricted to cell cycle progression. NuMa protein, responsible for organization of the spindle apparatus in the M-phase, is regulated by Pim-1 phosphorylation [38]. Similarly, Pim-1 mediates C-TAK1 and Cdc25C phosphorylation [39,41]. Pim-1-dependent regulation of c-Myc transcription and protein levels have been demonstrated in multiple tumor types. Both c-Myc transcription and protein stabilization by Pim-1 have been reported, contributing to the perception of Pim-1 as a protein with proto-oncogenic activity [41,42].

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Pim-1: The “P” as a Proto-oncogene, “M” as a Mediator

Aforementioned molecular mechanisms of Pim-1 expression and activity have prompted indictment of Pim-1 as an instigator of cellular transformation in the field of oncology, based largely upon detection of increased Pim-1 levels in various hematological and solid tumors. In a group of malignancies, high Pim-1 protein level is associated with poor prognosis (e.g gastric cancer, head and neck tumors [43–46]). Intriguingly, a major study on 2000 tumor samples reported an inverse correlation of Pim-1 levels and tumor recurrence rate [47]. Consistent with this counter-culture viewpoint, Pim-1 overexpression in prostate cancer, pancreatic ductal carcinoma and non-small cell lung carcinoma has been reported to be correlative with favorable prognosis [48–50] emphasizing the *cell-dependent/context dependent* role of Pim-1. Appreciation of the dogmatic perspective that Pim-1 contributes to cancer requires consideration of the cellular context for Pim-1associated Oncogenic behavior. In most tumors, Pim-1 is overexpressed in conjunction with increases in c-Myc levels. High level c-myc activity has been correlated with induction of apoptosis, necessitating a molecular compensatory response by cells to preserve survival and potentially promote oncogenesis. Indeed, Pim-1 counteracts apoptosis in c-Myc–transformed cells [51,52]. Similarly, high Pim-1 activity has been reported in K-Ras-mutation-based-transformed pancreatic malignancies [53]. Unlike many types of cellular transformation in tumor specimens characterized by gene rearrangement or dysregulated amplification for typical oncogenes, Pim-1 overexpression is thought to rest with altered transcriptional regulation. To our knowledge, the only cases of Pim-1 hypermutation occur in a select few lymphoma subtypes (Hodgkin, DLBCL and MALT). In this lymphoma, Pim-1 mutational hyperactivity is accompanied by simultaneous mutation in other genes such as c-myc [51,54]. Taken as a whole, oncology literature would seem to implicate Pim-1 as a ‘proto-oncogenic-mediator’ rather than an ‘oncogenic-initiator’.

Another facet of consideration in the context of this myocardial-centric exposition is the decades of incontrovertible experience with the heart as an organ virtually refractory to oncogenic transformation. Cardiac-specific over expression of canonical oncogenes such as c-myc and c-fos during mouse development increase cardiac myocyte number in early stages after birth without persistent myocyte proliferation [55]. In fact, c-myc-driven increases in myocyte proliferation during development do not lead to abnormal myocyte formation in adulthood [55]. Even forced expression of telomerase by cardiac-specific transgenesis in mice causes hyperplastic heart development followed by overriding mitotic arrest and typical hypertrophic growth in adolescence, underscoring the legendary resistance of myocardium to transformation. The mechanism of mature myocardial resistance to proliferation is yet to be elucidated, but it is worth contemplating that cardiac myocytes possess shortened telomeres, particularly after pathologic injury. The concept of Oncogene Induced Senescence (OIS) or a ‘hypermitogenic arrest’ is based upon failure of Oncogene-mediated transformation due to eroded and shortened telomeres. Thus, proto-proliferative proteins display distinct and unique phenotypic consequences in the heart relative to other cells or organs that retain mitotic activity as part of their normal homeostasis in adult life.

Taking our interpretation of Pim-1 mechanism in oncology together with an established legacy of non-transformation using cardiac-specific overexpression for otherwise canonical

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oncogenes in the heart, we now propose that Pim-1 can be reasonably and safely considered as an important target in rejuvenating aged stem cells for cardiac cell therapy of severe heart failure patients.

Pim-1 as a Rejuvenating-tool for CPCs

Pim-1 overexpression in CPCs leads to elongation of telomeres that is tightly regulated as evidenced by normalization of telomere length with prolonged cell passage, although during the transient phase of telomere elongation the CPCs exhibited a youthful phenotype characterized by higher proliferation rate and metabolic activity. In addition, Pim-1 overexpression in CPCs does not inhibit the capacity for cardiac lineage commitment [56], as has been observed for stem cells modified by activated Akt [57]. Chromatid segregation in CPCs is non-random (also referred to as asymmetric) ensuring that one daughter cell receives an enriched number of “immortal” chromosomes associated with preservation of stemness, whereas the other daughter cell will possess a disproportionate number of newly synthesized chromosomes that are thought to promote lineage commitment and cellular differentiation. Asymmetric chromosome distribution is important to create daughter cells participating in tissue regeneration, a phenomenon linked to expression of Pim-1 in CSC that increases asymmetric chromosome segregation by nearly twofold [58]. Similarly, transgenic mice with cardiac-specific overexpression of Pim-1 exhibit significantly higher number of asymmetric dividing CPCs as compared to their normal control brethren [59]. CPCs overexpressing Pim-1 and cocultured with neonatal rat cardiac myocytes (NRCMs) show normal acquisition of I_{ca} current and Ca^{2+} signaling consistent with cardiac lineage [60], electrical connections through Cx43 gap junctions, and an authentic response to paracrine signals from NRCMs [60]. Collectively, these observations highlight expansion of *progeny-targeted* cardiogenesis mediated by Pim-1 activity as opposed to dysregulated and unproductive CPC-pool expansion.

In the setting of myocardial infarction injury, intramyocardial adoptive transfer of c-kit(+) BMSC modified to overexpress Pim-1 (BMSC-Pim) at time of infarction supported enhanced anterior wall dimension thickening and blunting of left ventricle dilation compared with hearts treated with vehicle alone [61]. Early recovery of cardiac function conferred by BMSC-Pim facilitated modest improvements in hemodynamic function up to 12 weeks after infarction between cell-treated groups and persistence of BMSC-Pim was improved relative to BMSC-GFP. The number of recruited endogenous c-kit (+) cells mobilized to the site of infarction injury was increased with BMSC-Pim compared to BMSC-GFP61. Interestingly, the paracrine effects of BMSC in these mouse studies promoted cellular hypertrophy in the border and infarcted regions coupled with an upregulation of hypertrophic genes61. Although the conclusion using BMSC-Pim supported a net improvement in structural remodeling relative to BMSC-GFP, the lack of functional commitment of BMSC-Pim echoed the limited efficacy of BMSC clinical trials. The need to use a more specialized and better adapted CPC cell population was reinforced by these results.

Fischer *et al.* conducted the first *in vivo* study using CPCs engineered to express Pim-1 (CPCeP) in the context of myocardial infarction, demonstrating significantly higher hemodynamic performance and LVEF as compared to CPC expressing green fluorescent

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protein (CPCeG) as controls that persisted for up to 32 weeks post-injection [62]. The persistent functional improvement was attributed to increased *do novo* myogenesis, neovascularization, and engraftment of CPCeP relative to CPCeG [62]. The stage was now set to move toward translational studies by incorporating human-derived CPCs into the next set of studies.

Rejuvenating CPCs by overexpression of Pim-1 was, for the first time, extrapolated to Human CPCs (hCPCs) in 2012 [63]. The experimental study was designed specifically for the clinically-relevant patient population with severe heart failure in mind. Mohsin *et al.* isolated CPCs from tissue samples harvested from patients undergoing an LVAD-placement procedure. Stem cells were isolated and selected on the expression of stem cell surface marker c-kit. hCPCs were then lentivirally engineerd with Pim-1 or a GFP-control. Similar to prior findings by Fischer *et al*. using the mouse CPCs, these human CPCs expressing Pim-1 (hCPC-eP) showed enhanced proliferation, metabolic activity and telomerase activity relative to human CPCs expressing GFP control (hCPC-eG[63]). The aged CPCs in this possessed a normal karyotype even while concomitantly exhibiting their boosted proliferation rate, negating a potential safety issue that has plagued the embryonic stem cell field for years. Long-term *in vivo* assessment of heart function upon myocardial infarction and hCPC-eP delivery to immunocompromised SCID-mice showed significant improvement in cardiac function within six weeks after cell injection relative to the hCPC-eG control group. Interestingly, differences between hCPC-eP versus hCPC-eG groups became increasingly evident with the ensuing months, where salutary effects of hCPC-eP remained at a superior level for 20 weeks relative to hCPCeG [63]. Enhanced hemodynamic performance in hCPC-eP-treated hearts correlated with prolonged persistence and evidence of cardiogenic lineage commitment, increased *de novo* myocyte formation and neovascularization [63,64].

Sustained improvement of LVEF along with findings of *de novo* myocyte formation and neovascularization seem a desirable cocktail for the Holy Grail of myocardial regeneration. Realization of enduring improved cardiac function using HCPCeP isolated from an aged and severely diseased population is highly encouraging. However, confounding factors of patient clinical variability, inter-individual inherent stem cell differences and myocardial response to cell therapy remain as important and as yet unresolved uncertainties. At this point, ensuring the future success of myocardial cell therapy may very well require patient-specific assessment of inherent regenerative potential and endogenous stem cell exhaustion. Recent results combining Hcp

Ceg and bone marrow mesenchymal stem cells (MSCs) advocate that a blend of cell types is more beneficial than CPCs only or MSCs only [65]. Future combinatorial avenues are mind-boggling when one considers the plethora of potential stem cells candidate types for adoptive transfer therapy. The major relevant aspect in salvaging jeopardized cells and myocardial regeneration is *time*. Rescue-time for myocytes in the wake of acute injury is severely limited and requires a ready to go ‘off-the-shelf’ product. On the other hand, autologous CPC-growth and associated regeneration will inevitably require a more protracted interventional time course to isolate, expand, and eventually reintroduce the donated cell population. Together with cell therapy, the field of gene therapy has witnessed

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major progress in development of broadly applicable minicircle plasmids, site-specific gene insertions using lentiviral construct and a great level of experience and expertise in the field of Adeno-Associated Viruses (AAV). In fact, current clinical trials are ongoing using AAV6 as a vector for cardiac gene therapy. Indeed, gene therapy appears a pragmatic and tractable 'off-the-shelf' approach to rescue myocytes at time of an interventional procedure such as Percutaneous transluminal coronary angioplasty (PTCA). However, a heart failure patient presents distinct challenges not amenable to an acute interventional strategy. The complexity of underlying etiology, pervasiveness of degenerative changes, and deterioration of structural and functional characteristics in the failing myocardium will likely require a variety of combinatorial strategies to reverse cellular losses and combat accumulation of senescent underperforming cells. Conceptual fusion of genetic engineering to potentiate myocardial repair with *ex vivo* manipulation of stem cells offers distinct advantages: controlled manipulation of donated cells without concerns related to *in vivo* gene therapy issues of delivery and cell targeting.

Pim-1 as a target for gene therapy

Pim-1 is abundantly expressed in neonatal hearts and decreases upon aging. Postnatal expression levels of Pim-1 declines but remains significantly elevated until 8 weeks of age when protein becomes comparable to seven months of age [66]. Subcellular localization of Pim-1 switches from predominantly nuclear in neonates to cytosolic in early adulthood when protein levels start decreasing. In failing mouse and human hearts, Pim-1 expression re-emerges and is localized in the nucleus of myocytes. After acute pathologic injury, Pim-1 is reactivated to play a cardioprotective role in the cytosol of borderzone myocytes. Cardiac-specific overexpression of Pim-1 results in higher levels of anti-apoptotic Bcl-XL and Bcl-2 compared to samples from normal control hearts. Genetic ablation of Pim-1 does not provoke an overt cardiac phenotype under physiologic conditions presumably due to compensatory upregulation of Pim-2 and Pim-3, but an impaired compensatory cardiac phenotype becomes evident upon pathologic challenge[66]. Cardiac-specific overexpression of Pim-1 (Pim-WT) in transgenic mice exhibit a 33% higher number of myocytes reflected in decreased average myocyte size relative to wild-type controls [66]. The preponderance of smaller, more numerous myocytes in Pim-WT hearts results in a hyperdynamic myocardium with an enhanced cellular reserve to cope with pathologic challenge, without abnormal myocyte formation, transformation or tumorogenesis. In fact, Pim-1 overexpression actually inhibits hypertrophy induced by endothelin-1 in neonatal rat cardiac myocytes. Pim-1 overexpression in cultured neonatal rat cardiac myocytes is characterized by enhanced calcium reuptake and decreased relaxation period with increasing sarcomeric shortening and SERCA expression. These cardioprotective actions extend to preservation of cardiac structure and function *in vivo* following hypertrophic challenge in Pim-WT hearts, which exhibit blunted hypertrophic remodeling under pressure overload challenge and preservation of functional output as evidenced by increased anti-hypertrophic signaling, decreased pro-hypertrophic proteins and increased hemodynamic function [67]. The cellular basis of response to hypertrophic stimulation in the Pim-WT heart consists of increased cellular proliferation and decreased apoptosis. Moreover, coping capacity of the Pim-WT heart in response to myocardial infarction (MI) is also superior to identically challenged

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nontransgenic controls with a 40% decrease in infarct size. Although, baseline hemodynamic performance of NTG and Pim-WT mice was the same, Pim-WT mice show higher contractile function after MI than NTG-controls [66]. Biochemical, molecular, and microscopic analyses have demonstrated beneficial effects of Pim-1 upon mitochondrial integrity [68]. Pim-1 levels increase in the mitochondrial fraction with a corresponding decrease in the cytosolic fraction of myocardial lysates from hearts subjected to 30 minutes of ischemia followed by 30 minutes of reperfusion. In response to oxidative stress, Pim-1 preserves inner mitochondrial membrane potential ($\Delta\Psi m$ [68]). Mitochondrial ultrastructure is maintained by Pim-1 activity, preventing swelling-induced calcium overload. Finally, mitochondria isolated from Pim-WT mice show inhibition of cytochrome *c* release triggered by a truncated form of pro-apoptotic Bad. In addition to preservation of mitochondrial integrity, Pim-1 also serves to blunt mitochondrial fission through inhibition of Drp-1 [69]. High glucose treatment of adult rat cardiomyocytes leads to cell death. Increased level of Pim-1 mitigates high glucose induced cell death by increased survival signaling [70]. The common, non-ischemic/hypertensive, diabetic cardiomyopathy progresses from diastolic dysfunction to cardiac failure. Pim-1 expression is decreased in diabetic transgenic mice along with an increase in protein phosphatase 2A. In addition, diabetic hearts show low levels of anti-apoptotic proteins and increased caspase-3 activity. Adeno-Associated-Virus9 mediated delivery of Pim-1 in diabetic mouse hearts, has been shown to improve cardiac function and prevent cardiac failure, symbolizing Pim-1 cardioprotection on a mitochondrial level [70]. Collectively, this constellation of cardioprotective and anti-hypertrophic properties exhibited by cardiac-specific Pim-1 activity stands in stark contrast to forced expression of typical oncogenic mediators such as Ras or myc that induce and contribute to progression of hypertrophy, making Pim-1 an effective genetic modification to promote stem cell-mediated regeneration and preserve myocardial structure function in the pathologically injured heart. Combinatorial therapy using Pim-1 as a myocardial genetic approach and CPCs-engineered with Pim-1 captures advantages of both sides of the *stem cell myocyte environment* dichotomy Figure 1.

Expert commentary

Over a century ago the American writer and philosopher Elbert Hubbard wrote: “Optimism is a kind of heart stimulant- the digitalis of failure.” Unfortunately, in the intervening 100 years neither optimism nor digitalis has provided any substantive progress for the prognosis or outcome of severe heart failure patients. Instead, alternative approaches for treatment of severe cardiac failure patients are required now more than ever before. Severe cardiac failure patients are challenged by a seemingly intractable combination of chronic stress, debilitating conditions and/or premature and replicative cellular senescence of the myocardium. All this, together with the high probability of compromised regenerative potential incapacitates the relatively modest ability of the heart to ameliorate or prevent further progression of cardiac failure. In retrospect, experience and expertise from studies using BMSCs and CPCs suggest that priming myocardial environment and myocyte regeneration could serve as a complementary approach. However, combinatorial-based cell therapy will require substantial investigation involving “trial and error” to sort through the cornucopia of various cell types, their respective combinations, relative percentages, and

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preparation delivery protocols through rigorous testing and mechanistic studies prior to reaching a tractable clinical platform. Uncertainties of the innate stem cell biology for therapeutic use are further complicated by inter-patient variability, individual differences in stem cell behavior, and inherent deficiencies in myocardial responsiveness. Patient-specific assessment and choice of interventional time-line remains a critical unresolved area of investigation and requires expert assessment of the physician depending on patient-etiology and medical history. While one group of heart failure patients may require advanced environment remodeling to improve outcome prior to cell-based therapy, others might require a one-time procedure receiving a stem cell cocktail in combination with the gene therapy vector. In addition, manipulation of the pathologically damaged heart by preemptive gene therapy would allow much needed time and opportunity for longitudinal assessment of the myocardial environment to improve survival and persistence prior to cell delivery for regenerative treatment.

In order to figuratively “level the playing field” in this daunting task of regenerative therapy for heart failure we as researchers and implementers of technology must begin to reduce the number of possible approaches and optimize characteristics of the cells involved in order to standardize treatment protocols. “Rejuvenating” the patient’s own stem cells with a validated and safe *ex vivo* genetic modification for autologous therapy avoids many pitfalls currently plaguing regimens implementing embryonic stem cells, inducible pluripotent stem cells, or allogeneic stem cells. Furthermore, this genetic engineering strategy should ideally confer improved survival and functional characteristics not only to the stem cell population, but also to their daughter progeny responsible for the arduous task of rebuilding the damaged myocardium. At present, Pim-1 is the only genetic modification to our knowledge that has been comprehensively studied and proven effective in both stem cell engineering as well as preserving/upgrading of myocyte quality. Ultimate success for myocardial regenerative treatment involving severe cardiac failure will likely require teamwork from multiple areas of investigation involving cell biology, gene therapy, and the clinical practitioners responsible for turning optimism into reality.

Five-year view

The outcome of ongoing and planned clinical trials for adoptive cell transfer therapy and gene therapy for heart failure will provide a foundation for development and implementation of clinical cardiac cell and gene therapy protocols worldwide. Thereby, further improvement of issues such as efficiency, broadening of the patient population and modification of compromised stem cells and myocytes will be essential for ongoing success in the clinical arena given the inherent variability of the target patient population. Overall, the evolution of cell therapy in the field of cardiology is likely to follow the fundamental clinical influences as the first cardiac transplant procedures, LVADs, beta-blocker application and other critical milestones. However, impatience of a desperate public, pressure from political concerns, and the often over-hyped allure of translational medicine tempers the reality that ongoing CPC and gene therapy trials result from many years of incremental scientific discovery and testing that culminate in a clinical “breakthrough.” Pressures for interventional “deliverables” from scientists and physicians has prompted an atmosphere of “translation-centric” and patient-oriented focus that sometimes can be dismissive of the importance for

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basic mechanistic understanding and relatively incremental, but necessary, slow progress. In the scientific "Maslow's hierarchy of needs", clinical trials require a foundation in basic scientific and mechanistic knowledge. The natural shift in focus towards translational medicine and subsequent mechanistic arrears could conceivable lead to a new dichotomy: either the clinics will be confronted with a protracted waiting time for major future therapeutic discoveries or patients will be confronted with premature clinical application of certain drugs or interventions with the subsequent consequences for the patients themselves and the scientific fields. Cell signaling studies *in vitro*, studies on epigenetics, molecular mechanistic cell cycle studies in all stem cell types (adult, ES/iPS, etc…), calcium-handling studies, and much more might not be directly clinically applicable in the short term, but nevertheless remain crucial in delineation of knowledge and expertise essential for development of major discoveries such as cell or gene therapy.

References

1. Willerson JT, Perin EC, Ellis SG, et al. Cardiovascular Cell Therapy Research Network (CCTRN). Intramyocardial injection of autologous bone marrow mononuclear cells for patients with chronic ischemic heart disease and left ventricular dysfunction (First Mononuclear Cells injected in the US [FOCUS]): Rationale and design. Am Heart J. 2010; 160(2):215–223. [PubMed: 20691824]
2. Strauer BE, Brehm M, Zeus T, et al. Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. Circulation. 2002; 106(15):1913–1918. [PubMed: 12370212]
3. Perin EC, Willerson JT, Pepine CJ, et al. Cardiovascular Cell Therapy Research Network (CCTRN). Effect of transendocardial delivery of autologous bone marrow mononuclear cells on functional capacity, left ventricular function, and perfusion in chronic heart failure: the FOCUS-CCTRN trial. JAMA. 2012; 307(16):1717–1726. [PubMed: 22447880]
4. Meyer GP, Wollert KC, Lotz J, et al. Intracoronary bone marrow cell transfer after myocardial infarction: 5-year follow-up from the randomized-controlled BOOST trial. Eur Heart J. 2009; 30(24):2978–2984. [PubMed: 19773226]
5. Leistner DM, Fischer-Rasokat U, Honold J, et al. Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction (TOPCARE-AMI): final 5-year results suggest long-term safety and efficacy. Clin Res Cardiol. 2011; 100(10):925–934. [PubMed: 21633921]
6. Assmus B, Schächinger V, Teupe C, et al. Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-AMI). Circulation. 2002; 106(24):3009–3017. [PubMed: 12473544]
7. Zeng L, Hu Q, Wang X, et al. Bioenergetic and functional consequences of bone marrow-derived multipotent progenitor cell transplantation in hearts with postinfarction left ventricular remodeling. Circulation. 2007; 115(14):1866–1875. [PubMed: 17389266]
8. Bolli R, Chugh AR, D'Amario D, et al. Cardiac stem cells in patients with ischaemic cardiomyopathy (SCIPIO): initial results of a randomised phase 1 trial. Lancet. 2011; 378(9806): 1847–1857. [PubMed: 22088800]
9. Makkar RR, Smith RR, Cheng K, et al. Intracoronary cardiosphere-derived cells for heart regeneration after myocardial infarction (CADUCEUS): a prospective, randomised phase 1 trial. Lancet. 2012; 379(9819):895–904. [PubMed: 22336189]
10. Kajstura J, Rota M, Cappetta D, et al. Cardiomyogenesis in the aging and failing human heart. Circulation. 2012; 126(15):1869–1881. [PubMed: 22955965]
11. Tchkonia T, Zhu Y, van Deursen J, Campisi J, Kirkland JL. Cellular senescence and the senescent secretory phenotype: therapeutic opportunities. J Clin Invest. 2013; 123(3):966–972. [PubMed: 23454759]

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12. Mallat Z, Fornes P, Costagliola R, et al. Age and gender effects on cardiomyocyte apoptosis in the normal human heart. J Gerontol A Biol Sci Med Sci. 2001; 56(11):M719–M723. [PubMed: 11682581]
13. Duque G. Apoptosis in Cardiovascular Aging Research: Future Directions. Am J Geriatr Cardiol. 2000; 9(5):263–264. [PubMed: 11416577]
14. Grodzicki T, Michalewicz L, Messerli FH. Aging and essential hypertension: effect of left ventricular hypertrophy on cardiac function. Am J Hypertens. 1998; 11(4 Pt 1):425–429. [PubMed: 9607380]
15. Lakatta EG. Introduction: chronic heart failure in older persons. Heart Fail Rev. 2002; 7(1):5–8. [PubMed: 11790918]
16. Patel MB, Sonnenblick EH. Age Associated Alterations in Structure and Function of the Cardiovascular System. Am J Geriatr Cardiol. 1998; 7(2):15–22. [PubMed: 11416448]
17. Sussman MA, Anversa P. Myocardial aging and senescence: where have the stem cells gone? Annu Rev Physiol. 2004; 66:29–48. [PubMed: 14977395]
18. Chimenti C, Kajstura J, Torella D, et al. Senescence and death of primitive cells and myocytes lead to premature cardiac aging and heart failure. Circ Res. 2003; 93(7):604–613. [PubMed: 12958145]
19. Beltrami AP, Cesselli D, Beltrami CA. Stem cell senescence and regenerative paradigms. Clin Pharmacol Ther. 2012; 91(1):21–29. [PubMed: 22089268]
20. Cesselli D, Beltrami AP, D'Aurizio F, et al. Effects of age and heart failure on human cardiac stem cell function. Am J Pathol. 2011; 179(1):349–366. [PubMed: 21703415]
21. Kajstura J, Rota M, Urbanek K, et al. The telomere-telomerase axis and the heart. Antioxid Redox Signal. 2006; 8(11–12):2125–2141. [PubMed: 17034355]
22. Torella D, Rota M, Nurzynska D, et al. Cardiac stem cell and myocyte aging, heart failure, and insulin-like growth factor-1 overexpression. Circ Res. 2004; 94(4):514–524. [PubMed: 14726476]
23. Miura O, Miura Y, Nakamura N, et al. Induction of tyrosine phosphorylation of Vav and expression of Pim-1 correlates with Jak2-mediated growth signaling from the erythropoietin receptor. Blood. 1994; 84(12):4135–4141. [PubMed: 7527668]
24. Matikainen S, Sareneva T, Ronni T, Lehtonen A, Koskinen PJ, Julkunen I. Interferon-alpha activates multiple STAT proteins and upregulates proliferation-associated IL-2Ralpha, c-myc, and pim-1 genes in human T cells. Blood. 1999; 93(6):1980–1991. [PubMed: 10068671]
25. Wierenga AT, Vellenga E, Schuringa JJ. Maximal STAT5-induced proliferation and self-renewal at intermediate STAT5 activity levels. Mol Cell Biol. 2008; 28(21):6668–6680. [PubMed: 18779318]
26. Domen J, Von Lindern M, Hermans A, Breuer M, Grosveld G, Berns A. Comparison of the human and mouse PIM-1 cDNAs: nucleotide sequence and immunological identification of the *in vitro* synthesized PIM-1 protein. Oncogene Res. 1987; 1(1):103–112. [PubMed: 3329709]
27. Selten G, Cuypers HT, Berns A. Proviral activation of the putative oncogene Pim-1 in MuLV induced T-cell lymphomas. EMBO J. 1985; 4(7):1793–1798. [PubMed: 2992942]
28. Hoover DS, Wingett DG, Zhang J, Reeves R, Magnuson NS. Pim-1 protein expression is regulated by its 5′-untranslated region and translation initiation factor elF-4E. Cell Growth Differ. 1997; 8(12):1371–1380. [PubMed: 9419425]
29. Losman JA, Chen XP, Vuong BQ, Fay S, Rothman PB. Protein phosphatase 2A regulates the stability of Pim protein kinases. J Biol Chem. 2003; 278(7):4800–4805. [PubMed: 12473674]
30. Ma J, Arnold HK, Lilly MB, Sears RC, Kraft AS. Negative regulation of Pim-1 protein kinase levels by the B56beta subunit of PP2A. Oncogene. 2007; 26(35):5145–5153. [PubMed: 17297438]
31. Mizuno K, Shirogane T, Shinohara A, Iwamatsu A, Hibi M, Hirano T. Regulation of Pim-1 by Hsp90. Biochem Biophys Res Commun. 2001; 281(3):663–669. [PubMed: 11237709]
32. Shay KP, Wang Z, Xing PX, McKenzie IF, Magnuson NS. Pim-1 kinase stability is regulated by heat shock proteins and the ubiquitin-proteasome pathway. Mol Cancer Res. 2005; 3(3):170–181. [PubMed: 15798097]
33. Fox CJ, Hammerman PS, Cinalli RM, Master SR, Chodosh LA, Thompson CB. The serine/threonine kinase Pim-2 is a transcriptionally regulated apoptotic inhibitor. Genes Dev. 2003; 17(15):1841–1854. [PubMed: 12869584]

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34. Yan B, Zemskova M, Holder S, et al. The PIM-2 kinase phosphorylates BAD on serine 112 and reverses BAD-induced cell death. J Biol Chem. 2003; 278(46):45358–45367. [PubMed: 12954615]
35. Didichenko SA, Spiegl N, Brunner T, Dahinden CA. IL-3 induces a Pim1-dependent antiapoptotic pathway in primary human basophils. Blood. 2008; 112(10):3949–3958. [PubMed: 18768389]
36. Mochizuki T, Kitanaka C, Noguchi K, Muramatsu T, Asai A, Kuchino Y. Physical and functional interactions between Pim-1 kinase and Cdc25A phosphatase. Implications for the Pim-1-mediated activation of the c-Myc signaling pathway J Biol Chem. 1999; 274(26):18659–18666.
37. Wang Z, Bhattacharya N, Mixter PF, Wei W, Sedivy J, Magnuson NS. Phosphorylation of the cell cycle inhibitor p21Cip1/WAF1 by Pim-1 kinase. Biochim Biophys Acta. 2002; 1593(1):45–55. [PubMed: 12431783]
38. Bhattacharya N, Wang Z, Davitt C, McKenzie IF, Xing PX, Magnuson NS. Pim-1 associates with protein complexes necessary for mitosis. Chromosoma. 2002; 111(2):80–95. [PubMed: 12111331]
39. Bachmann M, Kosan C, Xing PX, Montenarh M, Hoffmann I, Möröy T. The oncogenic serine/threonine kinase Pim-1 directly phosphorylates and activates the G2/M specific phosphatase Cdc25C. Int J Biochem Cell Biol. 2006; 38(3):430–443. [PubMed: 16356754]
40. Bachmann M, Hennemann H, Xing PX, Hoffmann I, Möröy T. The oncogenic serine/threonine kinase Pim-1 phosphorylates and inhibits the activity of Cdc25C-associated kinase 1 (C-TAK1): a novel role for Pim-1 at the G2/M cell cycle checkpoint. J Biol Chem. 2004; 279(46):48319–48328. [PubMed: 15319445]
41. Zhang Y, Wang Z, Li X, Magnuson NS. Pim kinase-dependent inhibition of c-Myc degradation. Oncogene. 2008; 27(35):4809–4819. [PubMed: 18438430]
42. Kim J, Roh M, Abdulkadir SA. Pim1 promotes human prostate cancer cell tumorigenicity and c-MYC transcriptional activity. BMC Cancer. 2010; 10:248. [PubMed: 20515470]
43. Alizadeh AA, Eisen MB, Davis RE, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature. 2000; 403(6769):503–511. [PubMed: 10676951]
44. Wright G, Tan B, Rosenwald A, Hurt EH, Wiestner A, Staudt LM. A gene expression-based method to diagnose clinically distinct subgroups of diffuse large B cell lymphoma. Proc Natl Acad Sci USA. 2003; 100(17):9991–9996. [PubMed: 12900505]
45. Poulsen CB, Borup R, Nielsen FC, et al. Microarray-based classification of diffuse large B-cell lymphoma. Eur J Haematol. 2005; 74(6):453–465. [PubMed: 15876249]
46. Hsi ED, Jung SH, Lai R, et al. Ki67 and PIM1 expression predict outcome in mantle cell lymphoma treated with high dose therapy, stem cell transplantation and rituximab: a Cancer and Leukemia Group B 59909 correlative science study. Leuk Lymphoma. 2008; 49(11):2081–2090. [PubMed: 19021050]
47. Rhodes DR, Sanda MG, Otte AP, Chinnaiyan AM, Rubin MA. Multiplex biomarker approach for determining risk of prostate-specific antigen-defined recurrence of prostate cancer. J Natl Cancer Inst. 2003; 95(9):661–668. [PubMed: 12734317]
48. Dhanasekaran SM, Barrette TR, Ghosh D, et al. Delineation of prognostic biomarkers in prostate cancer. Nature. 2001; 412(6849):822–826. [PubMed: 11518967]
49. Reiser-Erkan C, Erkan M, Pan Z, et al. Hypoxia-inducible proto-oncogene Pim-1 is a prognostic marker in pancreatic ductal adenocarcinoma. Cancer Biol Ther. 2008; 7(9):1352–1359. [PubMed: 18708761]
50. Warnecke-Eberz U, Bollschweiler E, Drebber U, et al. Frequent down-regulation of pim-1 mRNA expression in non-small cell lung cancer is associated with lymph node metastases. Oncol Rep. 2008; 20(3):619–624. [PubMed: 18695914]
51. Dang CV, O'donnell KA, Juopperi T. The great MYC escape in tumorigenesis. Cancer Cell. 2005; 8(3):177–178. [PubMed: 16169462]
52. Shirogane T, Fukada T, Muller JM, Shima DT, Hibi M, Hirano T. Synergistic roles for Pim-1 and c-Myc in STAT3-mediated cell cycle progression and antiapoptosis. Immunity. 1999; 11(6):709–719. [PubMed: 10626893]
53. Xu D, Allsop SA, Witherspoon SM, et al. The oncogenic kinase Pim-1 is modulated by K-Ras signaling and mediates transformed growth and radioresistance in human pancreatic ductal adenocarcinoma cells. Carcinogenesis. 2011; 32(4):488–495. [PubMed: 21262926]

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54. Hemann MT, Bric A, Teruya-Feldstein J, et al. Evasion of the p53 tumour surveillance network by tumour-derived MYC mutants. Nature. 2005; 436(7052):807–811. [PubMed: 16094360]
55. Jackson T, Allard MF, Sreenan CM, Doss LK, Bishop SP, Swain JL. The c-myc proto-oncogene regulates cardiac development in transgenic mice. Mol Cell Biol. 1990; 10(7):3709–3716. [PubMed: 1694017]
56. Cottage CT, Neidig L, Sundararaman B, et al. Increased mitotic rate coincident with transient telomere lengthening resulting from pim-1 overexpression in cardiac progenitor cells. Stem Cells. 2012; 30(11):2512–2522. [PubMed: 22915504]
57. Fischer KM, Din S, Gude N, et al. Cardiac progenitor cell commitment is inhibited by nuclear Akt expression. Circ Res. 2011; 108(8):960–970. [PubMed: 21350213]
58. Sundararaman B, Avitabile D, Konstandin MH, Cottage CT, Gude N, Sussman MA. Asymmetric chromatid segregation in cardiac progenitor cells is enhanced by Pim-1 kinase. Circ Res. 2012; 110(9):1169–1173. [PubMed: 22441844]
59. Cottage CT, Bailey B, Fischer KM, et al. Cardiac progenitor cell cycling stimulated by pim-1 kinase. Circ Res. 2010; 106(5):891–901. [PubMed: 20075333]
60. Tufan H, Zhang XH, Haghshenas N, Sussman MA, Cleemann L, Morad M. Cardiac progenitor cells engineered with Pim-1 (CPCeP) develop cardiac phenotypic electrophysiological properties as they are co-cultured with neonatal myocytes. J Mol Cell Cardiol. 2012; 53(5):695–706. [PubMed: 23010478]
61. Quijada P, Toko H, Fischer KM, et al. Preservation of myocardial structure is enhanced by pim-1 engineering of bone marrow cells. Circ Res. 2012; 111(1):77–86. [PubMed: 22619278]
62. Fischer KM, Cottage CT, Wu W, et al. Enhancement of myocardial regeneration through genetic engineering of cardiac progenitor cells expressing Pim-1 kinase. Circulation. 2009; 120(21):2077–2087. [PubMed: 19901187]
63. Mohsin S, Khan M, Toko H, et al. Human cardiac progenitor cells engineered with Pim-I kinase enhance myocardial repair. J Am Coll Cardiol. 2012; 60(14):1278–1287. [PubMed: 22841153]
64. Karantalis V, Schulman IH, Hare JM. Nitroso-redox imbalance affects cardiac structure and function. J Am Coll Cardiol. 2013; 61(9):933–935. [PubMed: 23449427]
65. Williams AR, Hatzistergos KE, Addicott B, et al. Enhanced effect of combining human cardiac stem cells and bone marrow mesenchymal stem cells to reduce infarct size and to restore cardiac function after myocardial infarction. Circulation. 2013; 127(2):213–223. [PubMed: 23224061]
66. Muraski JA, Rota M, Misao Y, et al. Pim-1 regulates cardiomyocyte survival downstream of Akt. Nat Med. 2007; 13(12):1467–1475. [PubMed: 18037896]
67. Muraski JA, Fischer KM, Wu W, et al. Pim-1 kinase antagonizes aspects of myocardial hypertrophy and compensation to pathological pressure overload. Proc Natl Acad Sci USA. 2008; 105(37):13889–13894. [PubMed: 18784362]
68. Borillo GA, Mason M, Quijada P, et al. Pim-1 kinase protects mitochondrial integrity in cardiomyocytes. Circ Res. 2010; 106(7):1265–1274. [PubMed: 20203306]
69. Din S, Mason M, Völkers M, et al. Pim-1 preserves mitochondrial morphology by inhibiting dynamin-related protein 1 translocation. Proc Natl Acad Sci USA. 2013; 110(15):5969–5974. [PubMed: 23530233]
70. Katare R, Caporali A, Zentilin L, et al. Intravenous gene therapy with PIM-1 via a cardiotropic viral vector halts the progression of diabetic cardiomyopathy through promotion of prosurvival signaling. Circ Res. 2011; 108(10):1238–1251. [PubMed: 21474815]

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Key issues

- CPCs have entered clinical practice for patients with moderate heart failure.
- Severe heart failure patients may not be good candidates for early stage clinical trials due to impairment of their endogenous regenerative responses.
- Heart failure patients suffer from aged-senescent myocytes and CPCs.
- Therapy for heart failure requires a dual approach; modification of the environment through reversal of senescence in myocytes and reversal of senescence in CPCs.
- Pim-1 has been proven to upgrade myocyte quality through prevention of hypertrophy, increased mitochondrial integrity and quality, less apoptosis and subsequent improved contractile function.
- Pim-1 rejuvenates CPCs through telomere elongation, increased proliferation & metabolic activity and higher regenerative capability without alteration of cardiac-lineage commitment.
- Optimal Pim-1 mediated therapy for severe heart failure patients will require Pim-1 gene therapy for priming the environment and application of “rejuvenated” CPCs for new myocyte formation.

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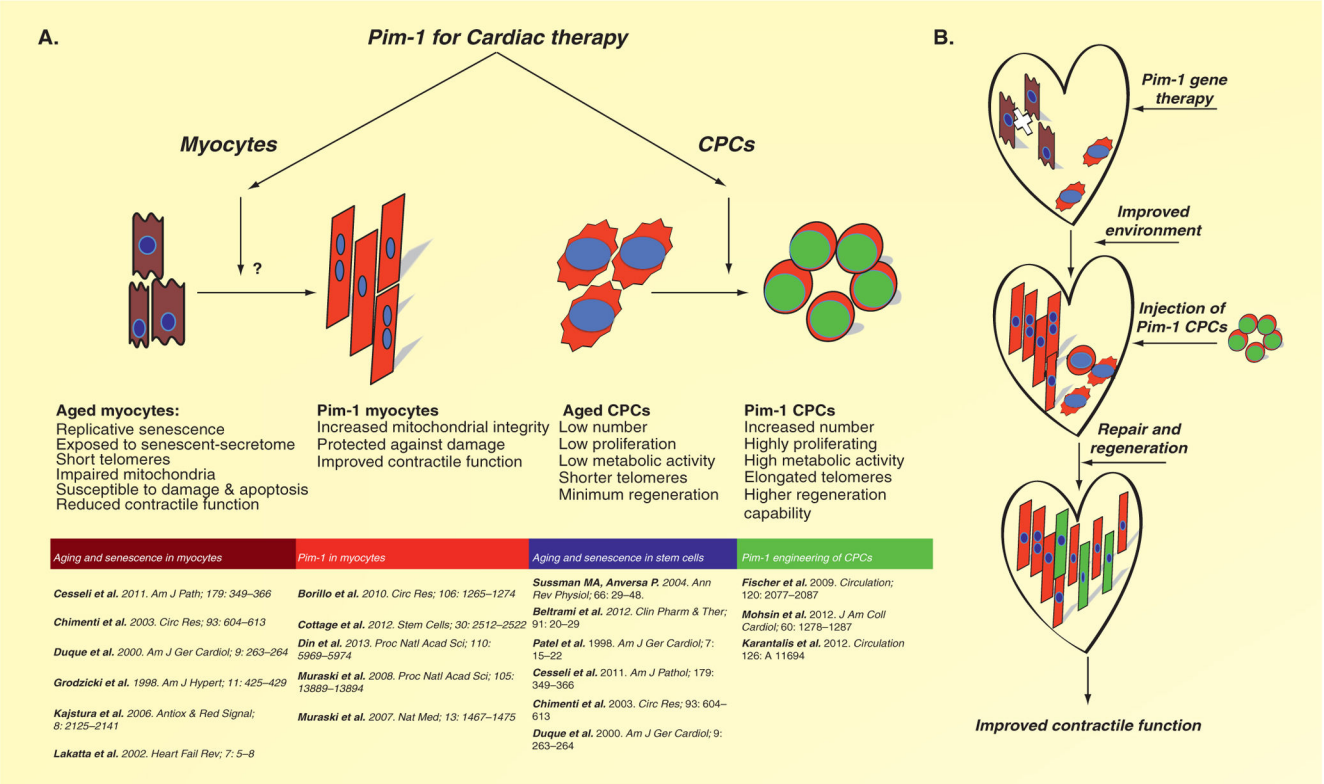

Figure 1. Application of Pim-1 genetic engineering for cardiac progenitor cells, cardiomyocytes, and the pathologically damaged myocardium of severe heart failure

(**A) 1**. Myocytes suffering from replicative senescence, exposure to the senescent "secretome", with shortened telomeres and impaired mitochondrial function exhibit reduced contractile function. **2.** Myocytes "rejuvenated" by Pim-1 overexpression possess heightened survival and metabolic activity together with increased contractile function. **3.** CPCs with compromised proliferative capability, short telomeres as a result of replicative and premature senescence, exhibiting compromised regenerative potential. **4.** CPCs engineered with Pim-1 recover proliferative potential, higher metabolic activity and elongated telomeres, thereby having an increased regenerative potential. (**B)** Conceptual representation of Pim-1 mediated molecular interventional strategy to treat severe heart failure. Decompensated heart suffering from replicative and premature senescence with aged/ damaged myocytes and senescent stem cells (top). Time and place for Pim-1 gene therapy intervention is indicated by the arrow (upper panel). Pim-1 mediated priming of the environment results in improved myocyte quality with subsequent beneficial paracrine effect on the endogenous CPCs (middle of panel). Delivery of Pim-1 "rejuvenated" CPCs into a modified environment is indicated by the arrow (middle panel). Injection of Pim-1 engineered CPCs results in myocardial regeneration and improved contractile function (lower panel) in mouse CPCs, human CPCs in mouse and human CPCs in swine (*Fischer et al., Mohsin et al., Karantalis et al.).* CPCs: Cardiac progenitor cell