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**Review**

# Application of Stem Cell Technologies to Regenerate Injured Myocardium and Improve Cardiac Function

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Stem Cell Technologies • Spermatogonial Stem Cells • Injured Myocardium

**Abstract**
In the recent decades, cardiovascular diseases emerged as the major leading cause of human mortality. However, current clinical approaches still do not encompass a thorough therapeutic solution for improving heart function of the patients who suffered an extensive myocardial injury. Based on this status quo, stem cells could become a novel option, as a natural source of the new myocardium lineage cells, being capable of paracrine factors secretion, protection or even regeneration of the damaged heart muscle. Efficient stem cell-based therapy of the heart should lead to repair or/and replacement of the degenerated tissue with functional myocardial and endothelial cells. Hereon, various types of pluripotent and multipotent stem cells have been already studied in the pre-clinical and clinical settings, demonstrating their cardiomyogenic and regenerative potential. In this context, as a type of male adult stem/ progenitors, spermatogonial stem cells feature a remarkable ability for a formation of cardiovascular lineages, based on our own observations. Presented data supports the presumption, that spermatogonial stem cells not only have a suitable capacity to generate functional heart cells but can also potentially improve the function of an injured myocardium. In this review article, we first describe the essential molecular and pathophysiological mechanisms involved in the heart tissue injury. Afterwards, based on our ongoing study, we review the impact of the stem cell technologies on the regeneration therapy in cardiovascular and myocardial diseases. Particular emphasis is being put on the usability of spermatogonial stem cells in cardiac therapy.



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## Introduction

According to previously published reports, cardiovascular diseases (CVDs), such as coronary artery diseases (CAD) and/or ischemic heart disease (IHD), are known as one of the leading cause of human mortality worldwide [1, 2]. Globally, a number of 422.7 million CVD cases and 17.92 million CVD related deaths have been registered only in 2015 [3].

Extensive variety of the risk factors and pathophysiological variables are involved in development of the CVDs and subsequent heart failure (HF) [4]. Through the HF development, a reduction in ventricular wall thickness and dilatation, as well as heart dysfunction are accrued into the injured heart areas through degeneration of cardiomyocytes (CMCs), vascular smooth muscle cells (VSMCs), and vascular endothelial cells (VECs), as the main heart lineage cells, in response to the pro-inflammatory cytokines secretion [5, 6]. While several pharmaceutical and interventional therapeutic strategies have been developed to improve CVDs patient's heart function to some extent, clinically effective CVD treatment still remains one of the most important challenges in the foreground of the public health [7].

During the past decades, stem cells (SCs) technology has opened a new promising perspective towards treatment of the CVDs patients, particularly IHDs, with ultimate goal to regenerate the damaged myocardium. The SCs generally play a vital role in preserving individual's hemostasis and development during their entire lifetime [8, 9]. Technically, based on the SCs differentiation potential and origin, they are classified into the two main pluripotent stem cell populations (PSCs, iPSCs) and adult stem/progenitor cell (ASPCs) types. For the purpose of heart regeneration, different kinds of the PSCs such as embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) [10, 11], as well as the ASPCs such as mesenchymal stem cells (MSCs) [12, 13], $CD34^+$ bone marrow mononuclear stem cells (BMNSCs) [14, 15], cardiac stem/progenitor cells (CSPCs) [16, 17], and spermatogonial stem cells (SSCs) [18, 19] have been already administrated in numerous of experimental and clinical studies. Although all of those administered PSCs and ASPCs have shown a capacity to generate the heart lineage cells, possible teratogenic potential of the PSCs made the direct clinical implementation difficult [20].

From the translational aspect, the concept of utilizing the stem cell technology in CVDs regeneration, consists of two main stages comprising experimental/pre-clinical and clinical phases. It can successfully help us to measure the SCs safety and recovering potential in a standard, a reliable, and a scientific framework (Fig. 1). The safety and efficacy of heart SCs-based therapeutic approaches have been proved through several experimental and clinical trials [21]. Theoretically, the utilized SCs, act first by secreting different types of paracrine/autocrine factors into the injured heart microenvironment, then by stimulating the activation and proliferation of the endogenous (native) CSPCs around the infarcted zone, and finally by potentially substituting the new cardiac lineage cells – altogether inducing protective and regenerative effects in an infarcted myocardium [22-25]. In accordance with the above-mentioned mechanisms, a significant improvement in the animal and patient's heart functions have been already observed [23, 24]. Given the importance of this novel and challenging therapeutic approach, the present study reviews the potential and strengths of different types of the SCs, particularly SSCs, for treating and regenerating the heart, damaged in the course of different CVDs.

## Stem cells

Generally, SCs have referred to the parts of individual's pluripotent, multipotent, and unipotent cellular population with the ability for proliferation, self-renewal, and differentiation into mature cells. The SCs play an essential role in organ formation and development during mammalian embryogenesis and also constitute the major platform of tissue regeneration in the adults [26]. The characteristics, biological behavior and differentiation potential of these cells have been reported in several studies [10-19].

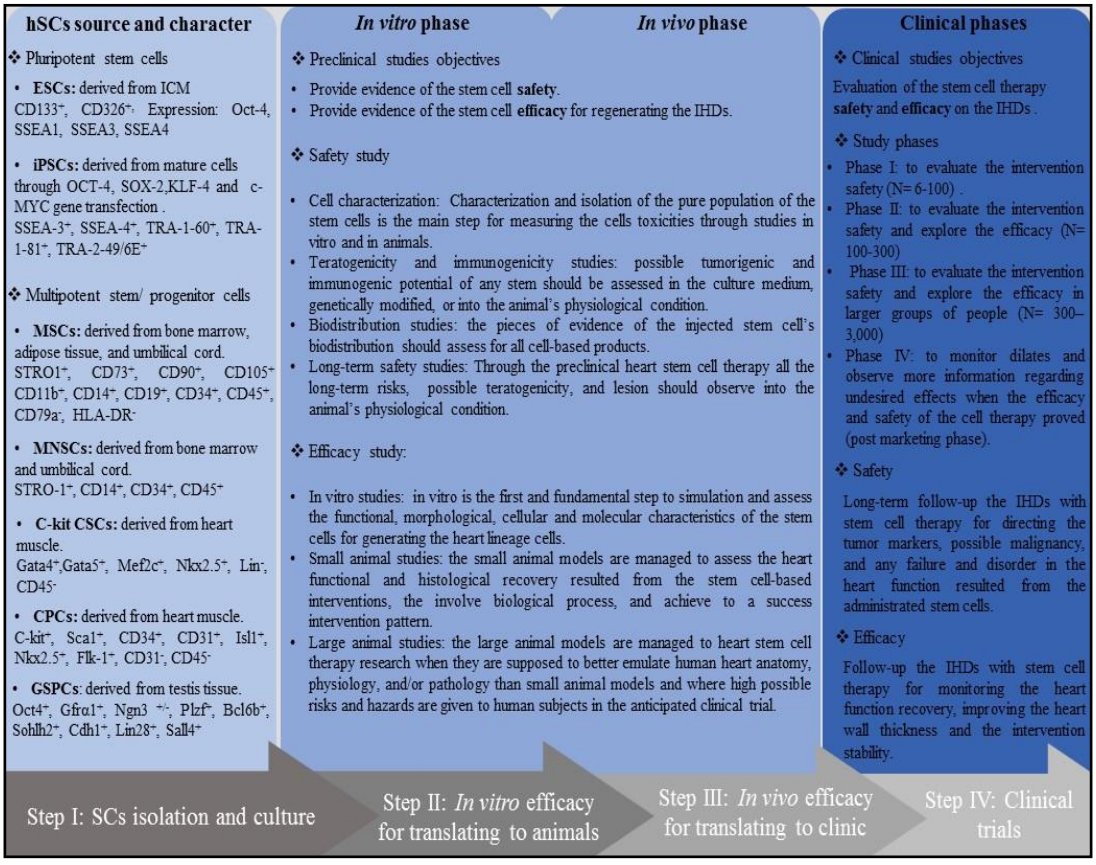


**Fig. 1.** Translational cascade of CVDs stem cell therapy.

Germline stem cells (GSCs) represent a type of testis-derived PSCs, with a self-renewal and differentiation ability [27, 28]. In males, embryonic germline stem primordial germ cells (PGCs) differentiate into spermatogonial stem cells, from which directly originate the sperm production and male fertility [27, 29]. Previous study has demonstrated that PGCs could be converted into embryonic germ cells (EGCs) under an *in vitro* condition [30]. A unique characteristic of EGCs germline stem cells is their pluripotent differentiation ability comparable to the ESCs, referring to the fact that germline lineage may retain this potential throughout their differentiation into SSCs. Seandel, et al. (2007) could establish a type of multipotent adult germline stem cells (maGSCs) *via* using a mouse neonatal testis-derived SSCs [31]. It should be noted that high differentiation capacity of the mammalian maGSCs has been demonstrated by several studies [32-34]. In fact, these stem cells, derived from adult mouse testis (GSCs), could show the characteristics ESC-pluripotency including the expression of the PSCs specific transcription factors and differentiation into three embryonic germ layers including heart lineages and functional CMCs [18, 35, 36]. Similarly, having an equal ESC property in the human GSCs has been reported by Meyer, et al. in 2010 [37]. Adult mouse testis derived SSCs, acquire the ESCs properties and can directly differentiate into derivatives of three embryonic germ layers [8]. These cells have been designated as the maGSCs. This evidence has clearly suggested the maintenance of pluripotency of the GSCs in all stages of development (Fig. 2).

Within the framework of a major discovery, PSCs has been generated in an *in vitro* condition by transfection of four different genes including octamer-binding protein 3/4 (OCT3/4), SRY (sex determining region Y) -box 2 (SOX2), Krüppel-like factor 4 (KLF4) and MYC (collectively referred to as OSKM) into the adult mature cells by reprogramming them to an ESC-like state, designating them as iPSCs [38, 39]. This methodology enabled the researchers to take a little biopsy and generate different types of the mature cells, ASPCs

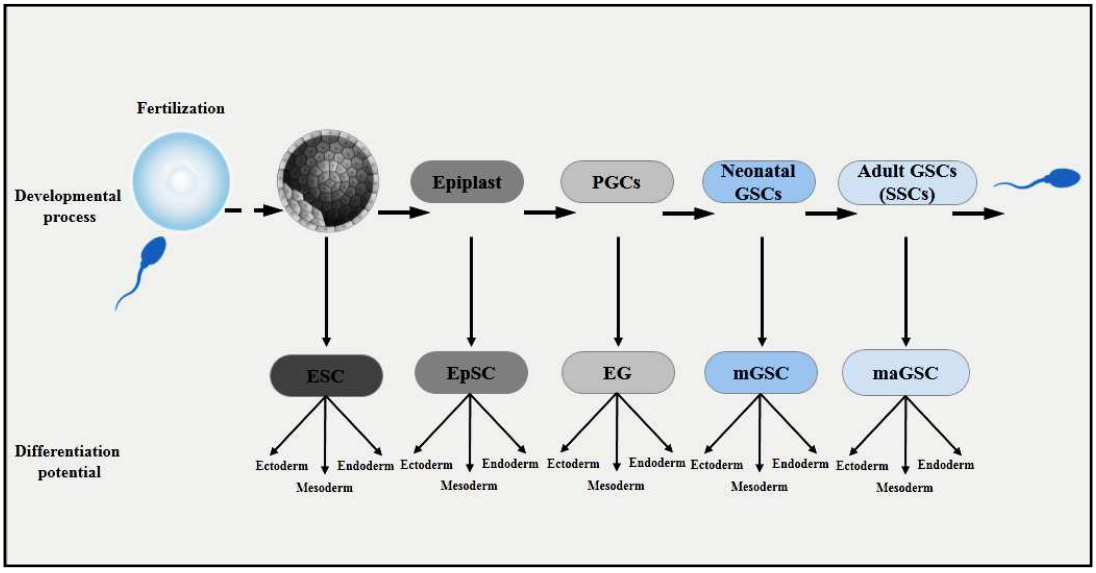


**Fig. 2.** Germline stem cells developmental process and its ability to reprogram to pluripotent stem cells in all stages of development. Abbreviations: PGCs, primordial germ cells; GSCs, germline stem cells; SSCs, spermatogonial stem cells; ESC, emberionic stem cell; EG, embryonic germ cells; mGSC, multipotent germline stem cells; maGSC, multipotent adult germline stem cells.

including the SSCS [40] and heart lineage cells for the regenerative medicine subjects [41]. As an imperative discovery, Guan, Nayernia, and their team (2006) could show that the human SSCs have a similar pluripotent character as the iPSCs [28]. Their transcriptional analysis has shown the SSCs could express the main pluripotency specific transcription factors including the Oct 3/4, Nanog, undifferentiated embryonic cell transcription factor 1 (Utf1), embryonic stem cell-specific gene 1(Esg1), and zinc finger protein 42 (zfi-42, or Rex1). Additionally, they proved the SSCs to encompass cardiomyogenic, myogenic, vasculogenic and also neurogenic differentiation under *in vitro* condition [28]. Like the iPSCs, this discovery could provide a new source of the pluripotent cell population in adults.

Multipotent cells are able to divide or self-renew successfully even in frequent passages and retain their capacity to generate a range of cell types from originating organ. It is generally thought that the adult SCs have a limited proliferation and differentiation capacity, compared to ESCs. In this manner, HSPCs are multipotent cells residing in the mammalian myocardium that are capable of self-renewing and generating vessels and heart muscle cells [17, 42, 43]. During the heart development, the creation of two different initial cardiac plates including the first heart field (FHF) and the second heart field (SHF) are managed by two primary and separate CSPCs [44, 45]. Expression of LIM-homeodomain transcription factor Islet-1 (Isl1) protein was introduced as the main marker for identification of the SHF [46]. $Isl1^+$ cardiac progenitors constitute a significant contribution to the heart morphogenesis as they are incorporated into the right ventricle, part of the left ventricle, and also the atria development. Important role of the $Isl1^+$ cardiac SCs in the heart development has been demonstrated by the lack of formation of the above mentioned tissue portions in Isl1 homozygous knock-out rodent heart [47]. Tracking and isolating the $Isl1^+$ CSPCs from adult myocardium let scientific community realize the fact that SCs play an impactful character in the heart healing during entire individual's lifetime.

In contrast to the SHF, the FHF is involved exclusively in the left ventricle and portions of the atrial chambers formation. Within a retrospective clonal analysis, it has been obviously conferred that both FHF and SHF cellular lineages are directly derived from a common primary stem cell before crescent heart formation [48]. The process of cardiac CMs, smooth muscles and VECs accruement underlies a control of several main protected and non-overlapping mechanisms. Furthermore, it should be remarked that evolution and development of the

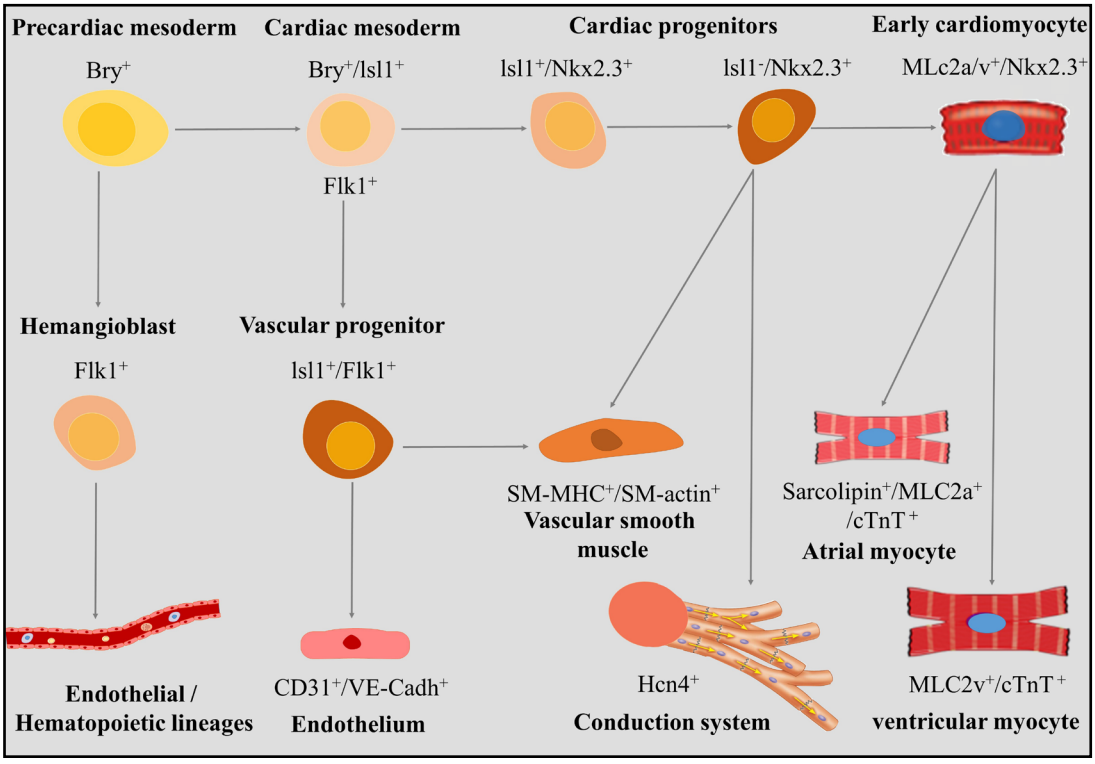


**Fig. 3.** A glance into the process of heart progenitor cell lineages development (From Laugwitz et al., 2008) [54] . The expression of lineage markers shown is based on the following studies: Kattman et al., 2006, Moretti et al., 2006 and Wu et al., 2006. The expression of Nkx2.5 in the precardiac mesoderm controls both endothelial and hematopoietic lineages development from the myocardial lineage [46, 54]. Abbreviations: Bry, brachyury T; MLC2a, atrial myosin light chain 2; MLC2v, ventricular myosin light chain 2; cTNT, cardiac troponin T; HCN4, hyperpolarization-activated cation channel 4; SM-MHC, smooth muscle myosin heavy chain; VE-Cadh, VE-Cadherin.

two heart fields is processed through activation of separate signaling [46, 49]. The process of the myocardium development has been clarified to start from a common primary stem cell, which further differentiate into a hierarchy of downstream cellular populations which actively facilitate the cardiogenesis in an embryo [50, 51]. An array of proteins and factors is involved in the process of heart morphogenesis, which can be used as markers at different stages of development (Fig. 3).

## IHD pathophysiological pathways and its impact on the implanted stem cells

Ischemic heart disease, in particular the acute myocardial infarction (MI), emerges as the most prevalent diagnose among the CVD patients [52]. It should be noted that the myocardial injuries resulting from heart ischemia are to great extent developed by a similar pathological mechanism like MI [6, 53]. Thus, the restoration of the previously interrupted blood flow into the heart muscle and preventing further consecutive harmful cascades is known as the central common dogma of MI therapeutic strategies [54].

In the context of MI, several pathophysiological mechanisms and pathways are involved in development of an ischemic myocardial injury. Early after ischemia, changes in the cellular metabolisms, induction of the cellular oxidative stress as well as vascular cell dysfunction and eventually CMCs death are actively initiated during the infarction process. This progressive cascade, through the production of free radicals and flowing them activation of the free radical scavenging enzyme [55, 56], storage of intracellular calcium [6], and also decreasing

the ischemic cell's level of adenosine triphosphate (ATP) [57] can induce tissue and heart lineage cells degeneration. In this regard, generation of high level of reactive oxygen species (ROS) and reactive nitrogen species (RNS) from the stressed cell's mitochondria, well known as free radicals, directly promotes some of the harmful cascades in CMCs [57]. It has been proven that several apoptosis-related cascades are promoted through the accumulation of ROS and RNS in the heart cells. Functionally, these reactive species directly lead to switch-on of the caspase-3 related apoptosis, increased activation of pro-apoptotic Bcl-2-associated X (Bax) protein, and repression of the anti-apoptotic B-cell lymphoma 2 (Bcl-2) protein activity as well [58].

From an immunological point of view, through promoting the expression of an array of pro-inflammatory factors and cytokines such as tumor necrotic factor-α (TNF- α), interleukin-1 (IL-1) family, and interleukin-6 (IL-6) free radicals also indirectly contribute to the cellular death in the infarcted zone [59]. Moreover, the expression and activation of main death ligand receptors including toll-like receptors (TLR), apoptosis antigen-1 (APO-1 or FAS), and also TNF-related apoptosis-inducing ligand-receptor (TRAIL-R) are the other targets of the free radicals in this pathological cascade [6, 60] (Fig. 4). Following this process, by inducing the expression of different integral membrane proteins such as cysteine-X-cysteine (CXC) and cysteine-cysteine (CC) chemokine, free radicals production promotes

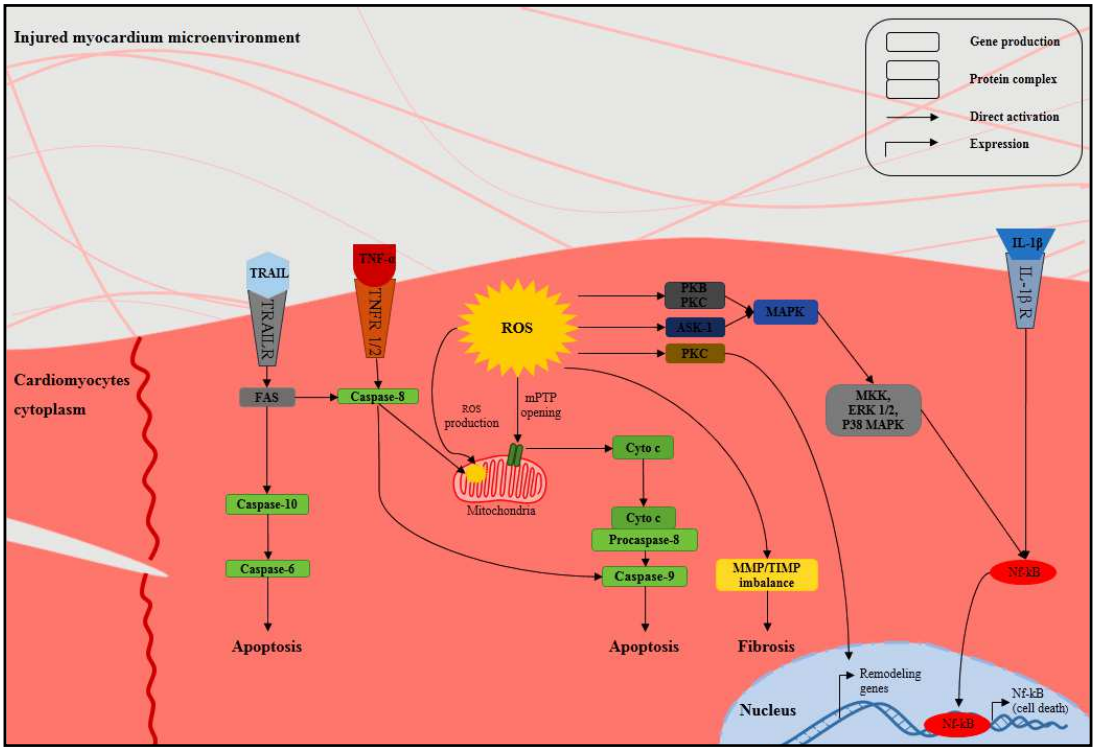


**Fig. 4.** A schematic representation of the cellular and molecular mechanisms of the cardiomyocytes death through the heart injury. Endogenic ROS production acquires early after injuries. Cytoplasmic ROS via changes mPTPs opening on the surface of mitochondria, releases of the cyto c into the cytoplasm, and activation and intra nucleus accumulation of Nf-kB through MAPK signaling stimulation leading to the cardiomyocyte apoptosis and necrosis. Beside, stimulation of the TNF-R1/2, IL-1R, and TRAIL-R as the death ligands by activation of the caspases cascades have a central role in developing the myocardium injuries. Abbreviations: Cyt-C: cytochrome -C, ERK: extracellular signal–regulated kinases, Fas: apoptosis antigen 1 (APO-1 or APT), IL-1R: interleukin-1 receptor, IL-1β: interleukin-1 β , MAPK: mitogen-activated protein kinase, NFκB: nuclear factor kappa-light-chain-enhancer of activated B cells , ROS: reactive oxygen spices, SCs: stem cells, TNF-R: tumor necrosis factor receptor, TNF-α: tumor necrosis factor- α, TRAIL: TNF-related apoptosis-inducing ligand, and TRAIL-R: TNF-related apoptosis-inducing receptor.

the infiltration of inflammatory leukocytes and thus contributes to the establishment of a harmful inflammatory myocardium microenvironment within the infarcted heart segment [61, 62].

In a post-infarcted myocardium, circulating blood monocytes which are known to be the primary responder to the chemotactic factors, extensively infiltrate the infarcted zone and immediately differentiate into the mature macrophages under the stimulation of the inflammatory-microenvironment-associated factors [63]. The macrophages have a pleiotropic role within the progress of myocardial pathophysiologic and also regenerative response after the infarction. These cells release various pro-inflammatory and anti-inflammatory cytokines, inducing their complex pleiotropic effects on the healing heart tissue [64-67]. Macrophages, classified into M1 and M2 class, possess a meaningful role in removing the dead cells and additionally interact with cardiac fibrosis [63, 64, 68]. In the course of MI, the macrophage activation takes place first through the M1 pathway and then shifts to the M2 pathway within the differentiation of the infiltrating monocytes [69]. Briefly, under the stimulation and by expression of some of the main chemokine family such as C-C motif chemokine ligand 2 (CCL2) and C-X3-C motif chemokine ligand 1 (CX3CL1) on the infarcted heart cell's surface, Ly-6C$^{high}$ monocytes infiltration, polarization, and differentiation takes place in the injured myocardial microenvironment. It seems that M1 macrophages are generated by the Ly-6C$^{high}$ monocytes differentiation [70]. It has been shown that the M1 macrophages, unlike the M2 type, play a harmful pro-inflammatory role through secreting high levels of TNF-α, IL-1β, IL-6, and also interferon gamma (INF-γ) into the injured myocardial environment [63, 64, 71, 72]. Switching the expression of CCL2 to CX3CL1, in the heart healing phases, causes the recruitment and the increase in the number of M2 macrophages through differentiation of the Ly-6C$^{Low}$ monocytes [63, 64, 71, 72]. M2 macrophages can play a vital role in promoting revascularization, regeneration, and remodeling mechanisms in the infarcted healing heart *via* secretion of several anti-inflammatory cytokines and growth factors such as transforming growth factor- β (TGF-β), IL-10, vascular endothelial growth factor (VEGF), and basic fibroblast growth factor (b-FGF) [63, 64, 71-73]. Previously published experimental studies demonstrate that the macrophages infiltrating the injured myocardium, besides the expression of pro-inflammatory cytokines, can actively release wingless/integrated (Wnt) protein, a family of 19 secreted glycoproteins, into the infarcted zones. In course of this process, activation of Wnt/b-catenin cascade intensifies cardiac damage and disrupts the myocardium hilling [74-76]. Additionally, Palevski et al. (2017) observed that "loss of macrophage Wnt secretion improves remodeling and function after myocardial infarction in mice" [63]. In addition to macrophages, infiltrating CD4$^{+}$ T cells and myeloid cells can promote cardiac cell apoptosis through the secretion of different pro-apoptotic cytokines [77, 78].

In the progress of the secretion of pro-inflammatory cytokines by infiltrating leukocytes, the myocardial cell death is extensively induced through stimulation of the death ligands and activation of cellular-death specific signaling pathways (Fig. 4). It has been proven that, nuclear factor- kappa B (NF-κB) is a main target of TNF-R (TNF-α receptor), in addition to IL-1βR (IL-1β receptor) and other death ligands such as FAS and TRAIL-R. Activation and intra-nuclear accumulation of the NF-κB plays a critical role in expression of pro-apoptotic genes and has a positive feedback on pro-inflammatory cytokines secretion [79, 80].

Whilst the cytotoxic effects of the TNF- α and IL-1 family, as well as their related mechanisms on the CMCs are carefully proven, the IL-6 affected mechanisms on the CMCs survival are not fully clarified, yet. The IL-6 and IL-6 family members play both pro- and anti-inflammatory roles in a post-infarcted heart [2, 13, 81, 82]. In some studies, it has been observed that the IL-6, as a pro-inflammatory cytokine, can induce CMCs apoptosis and cause the thinning of myocardial wall through stimulation of the NF-κB signaling pathway [5, 8, 10, 11, 17, 19, 30, 33-35, 37, 39, 41, 63, 64, 68, 73-75, 83-87].

It has been recognized that the regeneration potential of implanted SCs is directly affected by the noted inflammatory microenvironment creating early after MI. Based on some observations, into the post-infarcted heart regions, about 90% of all types of injected cells

degenerate just during 4 days following transplantation [81, 88]. Through activation of NF-κB signaling pathway, it seems that promotion of the TNF-R and the IL-1βR related cascades play a vital role in the implanted SCs survival [89, 90]. It has been also shown that, into a post-infarcted heart, TNF-α and IFN-γ networks can synergistically enhance the autophagy and apoptosis cell death process through stimulating ROS/ mitogen-activated protein kinase 1/3 (ERK) pathway, inducing Bcl-2-homology (BH)-3 domain only protein (Beclin-1) gene expression, and inhibiting anti-apoptotic B-cell lymphoma 2 (Bcl-2) expression on the SCs [84]. Moreover, other observations have obviously demonstrated that the TNF-α and its mediated signaling pathways inhibit the heart lineage differentiation of the both PSCs and ASPCs. Flowing this mentioned mechanism, generation of neuroaderenergic-like fate can significantly increase through differentiation of the injected stem cells [91, 92]. In agreement with this hypothesis, it is cleared activation of both TNF-R1 and TNF-R2 into the infarcted heart tissue can promote myocardium innate regeneration responses through decreasing the CSPCs differentiation potentials and proliferation through stimulation of the NF-κB and mitogen-activated protein kinase (MAPK) signaling pathways around the infarcted zone [92].

## Cellular therapy approaches for cardiovascular regeneration

For a clinically effective regeneration in patients with CVDs, a suitable and a safe source SCs with a high cardiomyogenic potential is greatly needed. Till now, beneficial effects and manifold positive feedback on different types of PSCs and ASPCs implemented in CVDs patients and animal models have been reported in a line-up of preclinical and clinical studies [93-95]. Over the past few decades, main portion of our efforts has been focused on discovery of the SCs physiological and biological behavior, as well as their capacity to generate the functional heart cells. Moreover, we could successfully report stem cell's outstanding potential for regenerating and recovering the function of an injured heart in the experimental animal models.

In the field of PSCs, our previous studies have carefully outlined the cardiomyogenic and angiogenic potential of ESCs and iPSCs in both *in vitro* and *in vivo* conditions [96-103]. Regrettably, the immunogenicity issues and especially the teratogenic potential of undifferentiated PSCs hindered the use of undifferentiated ESCs and iPSCs in the clinical phases [20, 104-106]. However, the application of the *ex vivo* generated CMCs from the PSCs not only could be a safe strategy but also seemed to be an effective method for replacing lost myocytes in the context of heart SCs therapy.

Within a translational view, pre-clinical studies on the IHD animal models have shown a significant efficacy during intramyocardial implantation of undifferentiated hESCs and hiPSCs [87, 107-109]. The current researches are trying to display the iPSCs as the main PSCs candidate in order to translational and clinical goals. However, there still some challenges to achieve this purpose. Ethically, direct application of the PSCs because of their unpleasant behaviors into the patient's myocardium is too controversial. Nevertheless, it has been clime that application of the PSCs would be safer in the case of using ex vivo generated mature CMCs.

According to several observations, the iPSCs have been introduced as a cost-effective, reliable, and efficient source of the PSCs in compared with the ESCs although both of them are similar in morphology, phenotype, and specific cell marker. Having a complete histocompatibility of the iPSCs with the patients and lifetime availability to the production of this PSCs, besides their great cardiogenic differentiation potential make them as a powerful SCs for the future heart regeneration goals [110, 111]. In contrast, some of the investigations have already realized that the iPSCs normal behavior and differentiation potential can be affected by an array of genetic and epigenetic alterations during the reprogramming [112]. Method for inducing pluripotency, type of the utilized somatic cell for iPSC preparation, and the preformed material to the cell isolation and culture are the other well-known effectors of the iPSCs normal behavior [113].

Despite the above stated challenges, the potential of the PSCs to the regenerate heart injuries has been proven so far in experimental set-up [114]. Hodgson et al. (2004) observed a significant therapeutic efficacy and the improvement of the rat's heart function after application of undifferentiated human ESCs into the infarcted areas of laboratory rodents. They also remarked that this notable efficacy was related to the differentiation of implanted SCs into heart lineage cells [10]. Furthermore, a similar result has been obtained in another study, after administration of undifferentiated human ESCs for improving the heart function in the experimental animal model of myocardial injury [115]. Similarly to ESCs, several observations have proven that using undifferentiated iPSCs would alike be a powerful tool for regenerating the injured myocardium, as well [10, 116-118].

In order to understand the therapeutic benefits of the *ex vivo* generated CMCs, within the concept of an orthotopic intramyocardial implantation of the murine iPSC-derived CMCs (iPSCs- CMCs) in a syngeneic mice model of cryoinfarcted heart injury, we have tracked implanted long-term surviving cells and importantly observed a significant improvement in the animals heart function after the cell implantation [119]. Our results were supported through another observation in context of intramyocardial injection of human iPSCs-CMCs in non-human primate model of MI [114]. Furthermore, we have been able to improve the iPSCs-CMCs regenerative function based on implementation of a new kind of gelatin microspheres. Notably, we concluded, "intramyocardial transfer of iPSCs-CMCs bound to gelatin microspheres enhances cell retention in the early stage after transplantation significantly" [120].

Because of the main fundamental and developmental difference between the ESCs and iPSCs, it seems there will be some functional discrepancy between CMCs generated out of these two respective cell types. To evaluate and compare the action potentials of the *ex vivo* generated CMCs from the ESCs and the iPSCs with a mature CMCs in an experimental study, we have first functionally examined and then compared the activity of cardiac-specific voltage-gated $Na^+$, $Ca^{2+}$, and $K^+$ channels in the ESCs-CMCs and iPSCs-CMCs with the mature CMCs counterparts at the early and late differentiation stages. Our observation has demonstrated that the CMCs generated from the iPSCs had a delayed action against the physiological stimulators compared with ESCs-CMCs and mature harvested CMCs. We also concluded that this notable difference might be related to the incomplete reprogramming of the iPSCs [111, 121, 122].

During the past decades, considerable efforts were undertaken to launch regeneration strategies based on the implementation of autologous and/or identical adult SPCs, in particular MSCs and BMNSCs. Easier access and increased safety, compared to PSCs, in addition to their high proliferative, differentiative and paracrine/ autocrine secretory potential make them the most favorite cells type for the CVDs regeneration concepts in the clinical phases [83, 123-128]. Chen et al. (2004) performed an intracoronary injection of the 8-10 × $10^9$ (cell/ ml) autologous bone marrow-derived MSCs in the acute MI and observed a significant improvement in the patient left ventricular (LV) functions in a time span of several months after the cell therapy [124]. Among all of these interesting reports, including our own observations, some unreliable differentiation processes of the implanted adult SPCs have been observed [121]. Based on our evidence, it should be noted that the bone marrow-derived MSCs implanted into the murine infarcted myocardium may acquire incorrect fates of differentiation such as into the osteocytes, in addition to the heart lineage cells [121]. Our finding clearly presented the fact that the utilized MSCs for the heart regeneration were not as safe as initially considered.

Apart from MSCs, intracoronary implantation of the autologous BMNSCs had a safe suitable,and remarkable therapeutic response in the patients with heart disorder and infarction [126-128]. It seems that this noted regeneration has largely resulted from an angiogenic response and revascularization of the damaged tissues *via* implanted SCs stimulation and differentiation [86, 129]. Unfortunately, many of the clinical MSCs and BMNSCs-based therapies have not had a long-term efficacy to improve the patient's heart function, as demonstrated by the lately published meta-analysis [130].

CSPCs were introduced as a convenient adult SCs option for regenerating the injured myocardium [16, 131-133]. In both human and rodent heart, the CSPCs were classified by expression of the cardiac-specific transcription factors such as GATA Binding Protein-4 (GATA-4), NK2 Homeobox 5 (Nkx-2.5), and myocyte enhancer factor-2 (MEF-2). C-kit$^+$ cardiac stem cells (CSCs), Sca-1$^+$ CSCs, cardiosphere-derived SPCs; side population CSCs are some of the well-known populations of the mammalians heart SCs [43, 53]. Differentiation potential into all heart lineage cells, promoting the cardioprotective signaling *via* secretion of various kinds of paracrine/ autocrine factors besides their high ability to survive in the damaged myocardial microenvironment made them another useful stem cell type for managing the heart regeneration [134, 135]. Practically, regenerative response of the CSPCs is being controlled by regulation of specific mechanisms and signaling pathways. Inside of the infarcted heart microenvironment, increasing expression of stem cell factor (SCF) from the injured CMCs stimulates heart's endogenous c-kit$^+$ CSPCs activity through activation of Wnt/ β-catenin cascade [136]. Furthermore, in response to the activation of phosphoinositide 3-kinase/ protein kinase B (PI3K/ Akt) and Notch pathways, CSPCs can raise their regenerative capacity [137-139]. It seems that the activation of the endogenous innate CSPCs can be an attractive target for developing new CVDs therapeutic agents. Study on the experimental mice model of MI has shown that systemic administration of extracellular high-mobility group box 1 protein (HMGB1),could dramatically induce a regenerative response and a significant improvement of the mice heart function through a stimulation of the C-kit$^+$ CSCs located around the infarction zone [140].

Among various types of the heart SPCs, cardiosphere-derived SPCs are the most trialed cell type in different clinical studies [16, 82, 122, 133]. According to a clinical trial performed by Malliaras, et al. (2014), in response to intracoronary implantation of the autologous cardiosphere-derived SPCs in the patients with acute MI, a significant improvements in the patient's infarcted-segment regional function, the mass of scar size, and also the viable myocardium were observed [133]. Similarly, an improvement in the viable myocardium after the intracoronary implantation of cardiosphere-derived SPCs was proven in other clinical studies [16, 82, 122].

Number of studies have also described a possible potential of testis-derived SPCs to regenerate the injured heart [8, 125-129]. Through one of our studies in 2006, we have successfully identified and isolated a new sort of SCs from the adult mammalian testis with the pluripotent features and high potential to generate different mature cellular lineages. Results of our study suggested the testis as a new source of the PSCs for organ-regenerating strategies [4]. In this regard, our previous research has introduced a fact that the SSCs have a natural potential for creating the functional CMCs and may be able to regenerate lost CMCs [14]. The results of our study have been supported by additional *in vitro* experiences.

### Spermatogonial stem cells possible impacts for heart regeneration

Testis-derived SCs inclosing the maGSCs are a unique and extremely resistant cellular population against numerous types of stresses. A primary study by Meistrich, et al. (1974) has shown an incredible regenerative behavior of the mice SSCs after the animals were exposed to an ionizing radiation [142]. Furthermore, later published studies have also reported that the rate of SSCs survival, proliferation, and migration significantly increased in response to such pathophysiological and physical stress like inflammation and gamma irradiation [85, 142-144]. Interestingly, it has been proven that the testis-derived SCs are resistant to the harmful cellular factors and cascades such as free radicals mediated pathways. In this context, according to an experimental study, Morimoto, et al. (2013) have explained that the creation of ROS and the activation of its related signaling pathways in mice SSCs not only had no apoptosis-inducing effects, but in contrast, proliferative and a self-renewing response in the SSCs were observed to be mediated *via* activation of NADPH oxidase 1 (Nox1) enzym. The results of these observations determined testis-derived SCs to be potentially highly

suitable for regeneration of the damaged tissues with an extensive harmful inflammatory environment.

To verify the hypothesis about maGSCs being beneficial in heart regeneration strategies, we launched one of our current ongoing *in vivo* trials and carefully evaluated the potential of murine maGSCs in the regeneration of rodent heart. In this study, we have clearly observed that intramyocardial implantation of the mice maGSCs into the healthy and also infarcted myocardium elicited a safe and efficient therapeutic response. Here, administrated maGSCs into a healthy contracting myocardium have shown an acceptable survival besides a specific differentiation into heart lineage cells (Fig. 5).

In case of testicular SCs, we believe that the post-infarcted myocardial inflammatory microenvironment not only lacks a disruptive effect on this utilized maGSCs population but also seems to be a stimulating factor for the testicular SCs survival, proliferation, and differentiation. Accordingly, in a C57BL/6 mice model of MI, murine maGSCs orthotopic implantation into infarcted heart areas showed a significant potential for regeneration of the lost heart tissue through a successful differentiation. Besides a significant survival, our immunohistochemical assessments indicated that the implanted maGSCs could create a large number of CMCs and VECs within 4 weeks after application into the post-infarcted heart (Fig. 6). Moreover, in none of maGSCs-treated mice, any signs of malignancy resulting from aberrant differentiation of implanted SCs could be observed. The results from our pre-clinical study, in line with our other previous experiences, outline the SSCs to be potentially highly suitable for application and translation into future clinical studies for CVD-regeneration in a safe and efficient way.

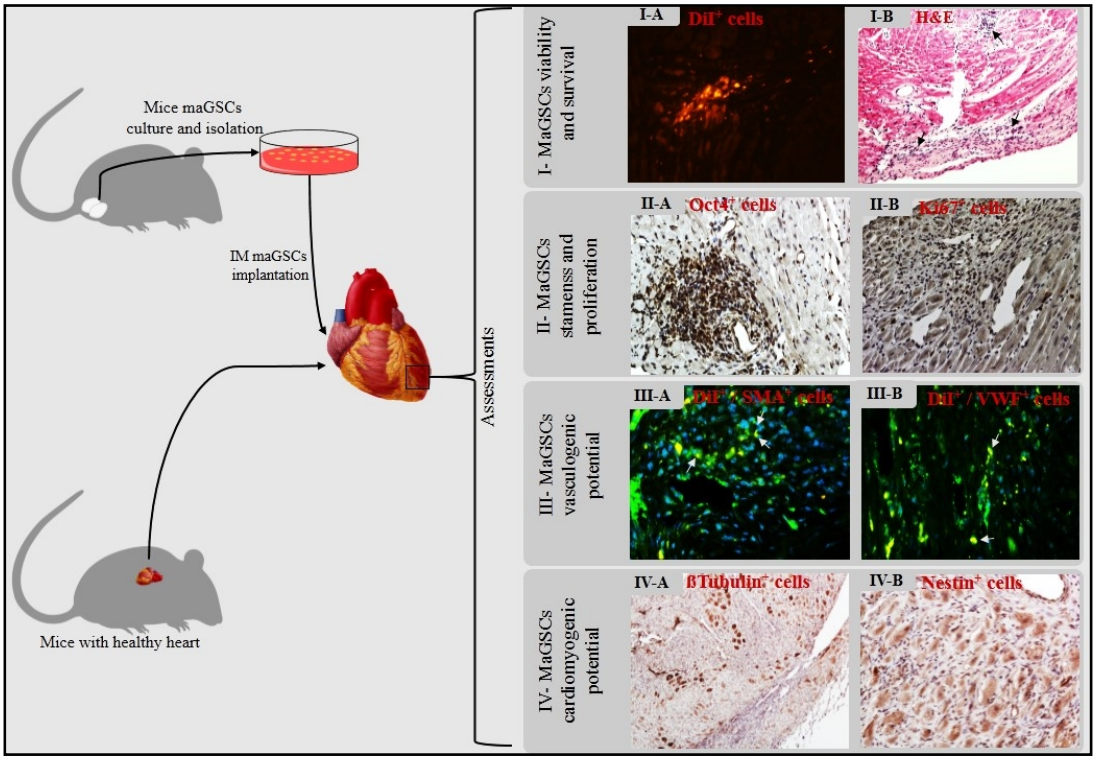


**Fig. 5.** Assessing behavior of murine maGSC into the healthy myocardium. Tracing of the I) implanted pre-differentiated maGSC (red labeled cells) and implanted cell nucleus (H & E), II) Stemness-related transcriptional factor (Oct4) and proliferation specific marker (II-B: Ki67), III) Vasculogenic specific markers SMA (III-A) and VWF (III-B), and IV) Cardiomyogenic specific markers βTubulin (IV-A) and Nestin (IV-B) into the heart muscle. Abbreviations: maGSC, multipotent adult germline stem cells; H&E, hematoxylin and eosinOct4, octamer-binding transcription factor 4; Ki67, MKI67; VWF, von Willebrand Factor; and SMA, smooth muscle alpha actin.

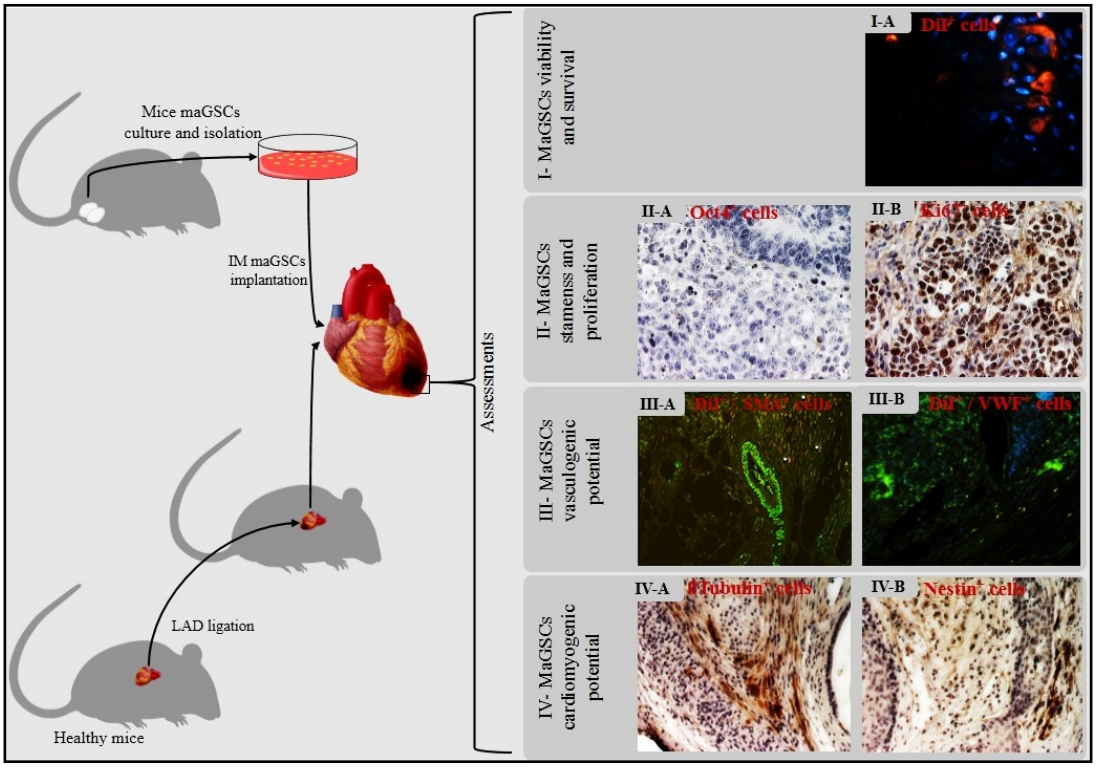


**Fig. 6.** Assessing behavior of murine maGSC into the infarcted myocardium. Tracing of the I) implanted pre-differentiated maGSC (red labeled cells), II) Stemness-related transcriptional factor (Oct4) and proliferation specific marker (II-B: Ki67), III) Vasculogenic specific markers SMA (III-A) and VWF (III-B), and IV) Cardiomyogenic specific markers βTubulin (IV-A) and Nestin (IV-B) into the infarcted zoon. Abbreviations: maGSC, multipotent adult germline stem cells; Oct4, octamer-binding transcription factor 4; Ki67, MKI67; VWF, von Willebrand Factor; and SMA, smooth muscle alpha actin.

## Conclusion

More than two decades have passed since the first scientific efforts for stem cell based regeneration of injured heart were launched. Although, a complete and effective repair in cell-treated patients with myocardial damage could not be unequivocally demonstrated, it is believed that future studies will be able to introduce more efficient technologies for the rehabilitation of the damaged myocardium by means of stem cell therapy. To achieve more effective cardiac regeneration policies, based on application of the stem cell technology, broadening our horizons on pathophysiologic mechanisms of the myocardial damage as well as stem cell behavior and its associated molecular pathways, plays an inevitable role. Accurate and reliable implementation of alternative stem cell sources including the GSCs and SSCs, shown to possess a suitable potential for the generation of the CMCs and endothelial cells (see our own above cited observations), can offer an opportunity with a higher potential to replace the previously lost heart cells.

In addition to undisputable cardiogenic differentiation potential of SSCs, these stem cells may hence encompass a translational capability in heart regeneration strategies, as supported by following propositions: I. SSCs, as a type of PSCs-like cells, have not shown as high tumorigenicity and immunogenicity as their ESCs/iPSCs counterparts in the previous experimental set-ups, II. SSCs feature a low sensitivity and a high resistance to the adverse effects of detrimental local microenvironment of the infarcted heart, III. SSCs present a readily accessible resource, as their isolation and large-scale culturing is possible during

the male's whole lifetime. The above stated paradigms suggest that implementation of SSCs might become a part of solution to overcoming barriers of the heart stem cell therapy. Further studies will be essential for optimizing and translating experimental accomplishments into stem cell based regeneration therapy of the heart diseases in clinical routine. Despite previous achievements, further progressive questions have to be addressed on the way towards effective translation of heart stem cell therapy. Can a personalized medicine play an influential role in management of effective stem cell therapy? To which extent does patient's specific geno/phenotype determine their characteristics of myocardial ischemia and consecutive host's response to the SC therapeutics? Is there a way to implement personalized medicine in order to optimize the modalities of stem cell therapy, based on patient's unique myocardial specifications? Considering previous experiences from personalized cancer therapy, the implementation of personalized methodologies might be a smart leap towards solving some of the obstacles along the way of stem cell based heart regeneration.

## Disclosure Statement

The authors declare to have no competing interests.

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