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Pursuing Candidate Stem Cells for Optimal Cardiac Regeneration in Patients Suffered from Acute Coronary Syndrome

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1. Introduction

It has been estimated that cardiovascular diseases will increase to 23.3 million in 2030 [Mathers & Loncar, 2006]. Acute myocardial infarction (AMI) typically occurs as a result of death of millions of myocytes, replaced by non-contractile scar tissue, which imposes a great load burden on surviving myocytes [Segers & Lee, 2010]. Despite of the fundamental progress in the treatment of cardiovascular diseases, a substantial limitation is still present. Current reperfusion strategies afford a great myocardial salvage but limited regenerative capacity of the human heart is a barrier for complete myocardial recovery after necrotic events. Thus, the heart responds to injury by scar formation and persistence muscle damage. Massive cell death and replacement of fibrotic tissue lead to cavitary dilatation and negative left ventricular (LV) remodeling [Ren et al., 2005]. The aftermath event is contractile dysfunction and terminal failure. Thus, regenerating the infracted heart should be adjunct to the therapeutic strategies capable to restore the blood supply to the territory of the infarct related artery. These needs can be met using a cluster of cells with self-renewal capacity, clonal expansion and ability to differentiate into multiple cell lineages. Pluripotent embryonic stem cells (ESCs) or multipotent adult stem cells (ASCs) showed remarkable capacity in heart regeneration. But it needs to be emphasized that heart is not just a pump and orchestrated temporospatial activities consisting sequential electrical stimulation and mechanical contraction are highly demanded. By now, our knowledge about the genetic bases and natural underlying events of cardiovascular disease precede the advancement of therapeutic strategies. A candidate therapeutic strategy should improve cardiac remodeling and function through formation of new blood vessels and inducing reconstitution of functional myocardium. Thus, the aim of this chapter is to focus on the different aspects of stem cell therapy as a growing field for cell-based strategies.

2. Stem cells

Human ESCs (hESCs) isolated from the inner cell mass of the blastocyst stage of human embryo [Ding et al., 2011]. These cells have a unique ability to differentiate into various derivatives of three germ layers and construct ~ 220 diverse cell types of adult human body [Mingxia et al., 2011, Ding et al., 2011]. Application of clinically unsuitable or

developmentally arrested embryos can overcome the ethical problems related to embryo manipulation. Obtaining cells from single blastomeres of human embryo hampers the need for embryo destruction. Induced pluripotent stem cells (iPSCs) using cellular reprogramming via forced expression of certain stimulating factors essential for maintenance of stemness, brought the ultimate solution without the need to human embryo [David et al., 2011]. By transferring the nuclear materials of somatic cells into the oocytes conferred pluripotency or totipotency of somatic cells became possible [Gurdon & Wilmut, 2011]. Despite of some epigenetic variations between iPSCs (mainly factor-free) and ESCs, these cells are similar in terms of proliferation, morphology, differentiation potential, imprinting, chromatin profiles and global gene/protein expression signature [Nordin et al., 2011]. This technology bypasses the need for the embryo or the desired tissue as heart. Thus, it is ethically accepted for both therapeutic applications and diagnostic measures like patient's disease modeling in vitro in order to find a treatment. Disease-specific iPS cells are also of paramount importance for achievement of this goal. In this way, tissue matching for organ transplantation is not a matter more. These goals are achievable using exogenous expression of two pluripotency transcription factors (e.g. Nanong, Oct4 and Sox2) and two proto-oncogenes (e.g. c-Myc and Klf4) [Nordin et al., 2011]. Induction of these programming factors is possible through application of retroviral and lentiviral vectors. Both of these vectors act for a period of time and then get silenced state once the endogenous genes had taken over the management of pluripotency [Stadtfeld & Hochedlinger, 2010]. The larger insert capacity of defective lentiviral vectors let them to deliver all of the programming factors without the need for separate individual vectors [Sinn et al., 2005]. In contrast with retroviruses, lentiviral vectors potently infect both dividing and non-dividing cells [Škalamera et al., 2011]. Viral vectors without integration into the genomic material of the target cells, like Adenovirus, have been used but their extremely low efficiency has faded their wide application [Okita & Yamanaka, 2011, Stadtfeld et al., 2008]. In addition to genetic manipulation, cell preconditioning and reprogramming could be performed thorough chemical and pharmacological cell manipulation. The advent of virus-free induction methods seems a revolutionary step in stem cell biotechnology. Despite of their low efficiency, Transposons, protein and mRNA-based induction methods seem advantageous due to their transgene-free nature [Si-Tayeb, 2010]. Small molecules as chromatin-modifying agents like Valporic acid (VPA) are also promising options for transgene-free cell reprogramming with replacement of potentially oncogenic-reprogramming factors [Medvedev et al., 2011]. In addition to the induction methods, knowledge about the through interplay between reprogramming factors will help in identification of more powerful reprogramming strategies. In this context, the effect of c-Myc in augmentation of the Oct4, Sox2 and Klf4 has been consequently associated with enhanced proliferation and differentiation arrest [Takahashi. et al., 2007]. Fully programmed cells raised safety concerns due to induced tumorigenicity by applied preconditioning factors as c-Myc [Kooreman & Wu, 2010]. Recently it has been suggested that creation of iPSCs using L-Myc instead of c-Myc brings less tumorigenicity [Nakagawa et al., 2010]. iPSCs are characterized by the expression of the above mentioned transcription factors and cell surface molecules (e.g. SSEA-3/4, Tra-1-60 and Tra-1-81) [Swelstad & Kerr, 2009]. High alkaline phosphatase and telomerase activity, rapid proliferation, lack of contact inhibition and high nucleus to cytoplasmic ratio with prominent nuclear growing in flat colonies are also further confirmatory indices of successful iPSC achievement [Kooreman & Wu, 2010]. The major

criterion for pluripotency is demonstration of the cell lineage's ability to reconstitute tissue composed of three layers by creating chimeras, tetraploid complementation or teratoma formation tests [Swelstad & Kerr, 2009]. Teratoma assay in immunodeficient SCID mice is currently used to test pluripotency in vivo for human iPSCs [Tan et al., 2008]. Teratoma formation (mature or immature) with differentiated ESC- or –iPSC-derived cells is attributed to the insufficient purity and remnant undifferentiated cell population within the transplanted cells [Kooreman & Wu, 2010]. Teratoma formation with injected mouse ESC (mESC)-derived beating embryoid bodies and undifferentiated mESCs is seen in experimental studies [Lin et al., 2010]. Transplantation of pure hESC-derived cardiomyocytes (82.6±6.6%) into immunodeficient rats was not associated with teratoma formation [Laflamme et al., 2007]. For ultimate translation of pluripotent stem cells into clinical benefits, highly purified cells and early detection of teratoma using novel non-invasive tracking strategies and advanced molecular imaging are warranted.

Despite of the substantial progresses made in the field of reprogramming, low reprogramming efficiencies (0.01-0.1% of input cells), slow kinetics of process, partial reprogramming and genetic instability of the manipulated cells hamper clinical application of iPSCs [Utikal et al., 2009, Kanawaty & Henderson, 2009, Stadtfeld et al., 2008]. The type of original somatic cell used for iPSCs, its cycle status and genetic/epigenetic background affect the functional/molecular characteristic of the derived cells [Polo et al., 2010]. These factors, in addition to the "epigenetic memory" of hiPSCs affect the total reprogramming efficiency [Polo et al., 2010]. Thus, alternative promising stem cell source with remarkable plasticity as easily extracted adult stem cells (ASCs), hematopoietic stem cells (HSCs), adipose-derived stem cells and derived MSCs seem useful surrogates [Lodi et al., 2011]. ASCs showed wide range of paracrine effects as cytoprotection, enhanced angiogenesis, recruitment of hematopoietic stem cells and activation of resident cardiac stem cells for endogenous repair [Gnecchi et al., 2008]. Umbilical cord blood (UCB) containing hematopoietic (UC-HS) and mesenchymal stem cells (UC-MS) with higher immunological tolerance are another cell source [Mihu et al., 2008]. Generally, UC-MS grafts are more beneficial than BM-MSCs [In 't Anker et al., 2010].

The activated vs. silenced pluripotency gene cluster is needed for proper programming. Mitotic errors, mutation occurrence and karyotypic changes have been observed in hESCs cultured over long passages [Kooreman & Wu, 2010]. Alterations in imprinted region on chromosome 12, location of pluripotency marker Nanog gene, have been proposed in the tumorigenicity of pluripotent cells [Draper et al., 2004]. Suppressed p53 signaling necessary for reprogramming brings tumorigenicity to the derived stem cells [Hong et al., 2009]. Tumorigenicity is an inherent property of pluripotent cells which is reduced upon differentiation. Thus, decreased tumorigenicity of the pluripotent cells means parallel decrease in their pluripotency and self-renewal potentials. Despite of the presence of intact spindle assembly checkpoints (SAC), mitotic failure-induced polyploidy has been observed in ESCs without occurrence of apoptosis [Kooreman & Wu, 2010]. In contrast with phenotypically resistant ESCs to DNA-damaging agents, embryoid bodies (EB) undergo caspase-3-induced apoptosis by these agents [Kooreman & Wu, 2010]. Human EB aggregates could be propagated from embryonic germ (EG) cells with multi-lineage differentiation potential and limited proliferation [Wobus & Löser, 2011]. It has been speculated that hEG cells might be an alternative to hESCs in future for therapeutic

applications [Wobus & Löser, 2011]. Embryonic-like stem cells as Spermatogonial stem cells (SGSCs), parthenogenetic stem cells (PSCs) and male germline stem cells in pre-menopausal women can also give rise into fully active cardiomyocytes [Guan et al., 2007, Zimmermann, 2011].

3. Image platform

Molecular imaging for in vivo tracking the proliferating and viable stem cells made a substantial help in the field of bench to bedside application of stem cells. Pre-transplantation labeling through cell inoculation with nanoparticles or reporter gene is helpful [Kooreman & Wu, 2010]. Semiconductor quantum dots capable to emit different light wavelengths show photostable bright image signals but their aggregation inside the cytosol made the process of cell delivery difficult [Kooreman & Wu, 2010]. Non-specific binding is another issue. Mesenchymal stem cells have been tracked by MRI after labeling with Ferumoxides [Kraitchman et al., 2003, Amado et al., 2005, Arai et al., 2006]. MRI signals elicited by changes in T2 relaxation are induced due to the endocytosis of the iron oxide particles (SPIOs) or ultrasmall superparamagnetic iron oxide particles (USPIOs) [Kooreman & Wu, 2010]. MRI signals are detectable for a period between three weeks up to two months but these signals can already exist in the presence of dead stem cells due to engulfed iron particles in scavenging macrophages [Lee et al., 2009]. Alternatively, direct stem cell labeling applying radionucleotides has been used for circulating-progenitor cells successfully [Hofmann et al., 2005]. The radionucleotide-bound cells could be detected using SPECT, PET, gamma camera and cardiac magnetic resonance tracing [Kooreman & Wu. 2010]. Ultimately, the tracking duration of the radionucleotide-bound cells depends greatly on the individual half-lives of the applied radionucleotide [Kooreman & Wu, 2010]. The enhanced false-positive rate, attributed to the radionucleotide leakage into the non-target cells is still a remained limitation for this highly valuable labeling technique [Kooreman & Wu, 2010].

Reporter gene imagining using intracellular enzyme, cell surface receptor, transmembrane protein and intracellular storage protein probes can provoke detectable signals after interaction with the used exogenous reporters [Cao et al., 2006, MacLaren et al., 1999, Miyagawa et al., 2005, Liu et al., 2009]. Facilitated evaluation of survival and proliferation of the mother stem cells is possible through transferring stably integrated reporter genes [Kooreman & Wu, 2010]. However, concerns persist with regards to the altered cellular behaviors due to the inserted gene [Kooreman & Wu. 2010]. Assurance can be achieved using safer site-specific integration approaches [Keravala et al., 2009]. Double fusion construct containing firefly luciferase (Fluc) which interact with the reporter probe D-luciferin and enhanced green florescence protein (eGFP) can be used for cell tracking in small animals [van der Bogt et al., 2006]. Low-energy photons (2-3 ev) made by Fluc suitable for high-throughput bioluminescence imaging (BLI) and signals of eGFP can be detected using ultrasensitive CCD camera and postmortem histology experiments, respectively [Kooreman & Wu, 2010]. In vivo monitoring of survival, proliferation and migration of the injected intramyocardial mESCs were performed using triple-fusion construct composed of Fluc, monomeric red fluorescent protein (mRFP) and Herpes simplex virus truncated thymidine kinase (HSVttk) [Cao et al., 2006]. PET is preferred to BLI due to the greater anatomical details and applicability in humans [Yaghoubi et al., 2009]. Indeed, easily performed and sensitive imaging modalities like BLI are able to detect early stages of

teratoma formation [van der Bogt et al., 2006, Lee et al., 2009]. Early detected teratomas can be ablated by its targeting using reporter-suicide gene construct [Cao et al., 2007]. But limited signal penetration in larger animals and lack of provided spatial three-dimensional data hurdles clinical application of BLI using Fluc reporter gene [Kooreman & Wu, 2010]. Higher spatial resolution of PET and MRI made them good candidate for clinical application but their substantial low detection threshold remained an obstacle [Kooreman & Wu, 2010]. Thus, combining image modalities is of crucial importance especially for clinical insights about identification of the safe limit of stem cell numbers without teratoma formation. Using BLI, Lee et al found safe limit of undifferentiated hESCs for cardiac transplantation into SCID mice to be 1×10^4 [Lee et al., 2009]. Progress in tracking strategies should be in parallel with identification of the appropriate markers for tracking of both stem cell homing and cardiac differentiation. Markers of undifferentiated cells as Oct4, hTert (human telomerase reverse transcriptase) and Dusp6 (dual specifity phosphatase 6) have been shown to be decreased during cardiac trans-differentiation [Wobus & Löser, 2011]. Thus, markers of mesoderm and early cardiogenesis as GATA-4 and Brachyury were found to be suitable for tracking cardiac differentiation [Wobus & Löser, 2011]. Precise imaging technologies should solve uncertainties real cardiomyocyte trans-differentiation vs. cell fusion. Cell fusion as an overlooked phenomenon occurs due to the autoflorescence problems regarding label transfer to neighboring cells or fusion of donor and recipient cells [Reinecke et al., 2008]. The former is avoidable using cell lineage markers as genetic materials [Reinecke et al., 2008]. This process gives rise to bi-nucleated, mono-nucleated cells with tetraploid synkaryon or cells with normal karyotype during division [Wang et al., 2003]. This phenomenon might be occurred after trans-differentiation into myocardium.

4. Culture conditions

The first ESC was cultured on mouse embryonic fibroblast- feeder layer cells (MEF-FL) [Wobus, 2010]. Growing of stem cells in suspension as aggregates or removing of feeder fibroblasts promotes differentiation of ESCs [Dambrot et al., 2011]. Derivatives of three germ layers like mesoderm (cardiomyocytes, blood and vascular endothelial cells) were derived by this method [Dambrot et al., 2011]. By advent of novel culture media stem cells could be kept in undifferentiated state even in the absence of feeder cells. Commercial culture media as mTeSR®1 and TeSR™2 (STEMCELL technologies) in combination with matrix containing a mixture of human collagen IV, fibronectin, vitronectin and laminin are beneficial in this context [Dambrot et al., 2011]. The goal of these culture conditions is to promote stem cell scale up while keeping karyotypic stability through successive enzymatic passages or suspension cultures. This field of stem cell technology needs to be promoted further.

5. Special considerations

Surveys to find the candidate stem cell should be parallel with search to find the candidate animal model. By now, mice are the most common used animal models due to the feasibility of the mutation induction and targeted deletion in them. Despite of these advantages, there are some fundamental differences between mouse and human heart. The predominantly expressed isoform of Myosin heavy chain (MHC) in fetal and adult mice are βMHC and αMHC, respectively [Dambrot et al., 2011]. The inverse pattern is seen in humans. The higher beating rate of mice (500 bpm) is surprisingly different from human heart (70 bpm)

[Dambrot et al., 2011]. Some inherent properties of mESCs and hESCs are noteworthy but their clinical significance is still unknown. mESCs and hESCs differ in expression of surface markers and culture requirements, mainly attributed to the more naïve state of mESCs [Dambrot et al., 2011]. Regarding culture requirements for maintenance of undifferentiated state, mESCs are leukemia inhibitory factor (LIF)-dependent but humans are dependent to basic fibroblast growth factor (bFGF) and Activin/Nodal-controlled signaling pathways [Tesar et al., 2007, Xu et al., 2008]. Indeed, mESCs express SSEA-1 surface molecule rather than SSEA-3/4 in hESCs [Wobus & Löser, 2011]. These basic differences make scientist to seek for a more comprehensively matched research model for regenerative purposes and diagnostic applications. In vitro drug screening, drug geneotoxicity/mutagenecity, chemical safety assessment, predictive toxicology and cardiac safety pharmacology are other avenues for beneficial application of stem cell technology. Due to species-specific pharmaco-toxicological effects, animal models are not representative for human beings. Indeed, high number of animals needed for in vitro compound screening and toxicology tests [Wobus & Löser, 2011]. In vitro human cellular tests overcame the limitations of inadequate standardized animal-based tests [Wobus & Löser, 2011]. These species specific toxicology tests using immortalized human cell cultures were not real representative of normal cell types and mortal primary human cells loose their tissue-specific functions in cultures [Wobus & Löser, 2011]. Thus, stem cells can provide a good source of cells without the need for immortalization measures; facilitate human-specific cardiac pharmaco-toxicology test systems. These stem-cell based compound screening, is of paramount importance for drugs synthesized for treatment of acute ischemic events. Stem cells can be potentially used for preimplantation genetic diagnosis (PGD) and –screening (PGS) of cases with genetic predisposition to cardiac ischemic events, but this aspect of stem cell technology needs to be wrought further: extended EST (Embryonic Stem Cell Test).

Creating predictive in vitro human models of acute coronary events may be possible using cardiac stem cells. Stem cell-based models might be helpful both diagnostically and therapeutically. In terms of diagnosis and treatment, induction of gain-of function (selective turn-on) and loss-of-function (selective turn-off) mutations allows selective genetic manipulation of stem cells serving as vehicles. In addition, these assays are complementary to understand the effects of constitutively expressed genes in cell function and during the differentiation process. Loss-of-function mutations will potentially serve in identifying the cardiac lethality and survival genes. Generally, non-homologous joint recombination, homologous recombination, site-specific double-strand breaks and transpositional recombination are used strategies for genomic manipulation of stem cells [Dambrot et al., 2011]. Direct stem cell reprogramming using three cardiac transcription factors, mouse fibroblasts can be differentiated into the cardiomyocytes [Ieda et al., 2010]. Examination of a cocktail of genes, introduced Gata4, Mef2c and Tbx5 as "master regulator genes" for rapid and stable direct reprogramming of fibroblasts into cardiomyocytes [Dambrot et al., 2011]. This method showed superior efficiency to iPSC technology by eliminating concerns regarding the presence of residual undifferentiated cells [Ieda et al., 2010]. Accelerated delivery of cells to the patients with lower costs is other benefit of this method [Dambrot et al., 2011]. But the inability of these emerged cardiomyocytes to expand in vitro is its major limitation [Dambrot et al., 2011]. Adipose-derived stem cells are also an attractive easily accessible source of stem cells for clinical application. These cells safely improve both

angiogenesis and myogenesis in injured heart. Finally, it should be beer in mind that the goal of stem cell differentiation methods should be achievement of functional myocardium.

6. Cardiac differentiation

By depletion of differentiation-repressing factors or growing cells as EBs, hESCs are easily committed to the target lineage [Dambrot et al., 2011]. EB-based directed differentiation occurs on specific matrixes in the presence of multiple inducers as growth factors, differentiation repressors or small molecules [Mohr et al., 2010, Boheler et al., 2002, Zwi et al., 2009, Mummery et al., 2007, Passier et al., 2006]. This method showed more success with mESCs rather than hESCs [Huangfu et al., 2008]. Spin EBs created from exactly defined cell numbers and centrifugated in V-shaped wells enhanced directed differentiation down the cardiac lineage [Ng et al., 2005, Ng et al., 2008]. Its yield is comparable with cardiomyocyte achievement of less than 5% of all cells using "hanging drop" EBs [Yoon et al., 2006]. Spontaneously beating cardiac clusters in the EB-outgrowths, varying in number from 8 to 70%, will be stable for up to three months [He et al., 2003, Kehat et al., 2001, Xu et al., 2002]. Contracting EB depends on the applied growth factors, cell line used and size of EB [Burridge et al., 2007, Pal & Khanna, 2007, Mikkola et al., 2006, Niebruegge et al., 2009, Mohr et al., 2010]. Moreover, crucial additions of major regulators of cardiac development as fibroblast growth factor (FGF), transforming growth factor-β (TGF-β), bone morphogenic protein (BMP), activin, vascular endothelial growth factor (VEGF), stem cell factor (SCF), ascorbic acid and members of Wnt family added the yield of this technique [Dambrot et al., 2011]. Some inhibitors like Wnt-inhibitor DKK1 (added to culture media at late stages), mitogen-activated protein kinase inhibitor (p38 MAPK) and glycogen synthase kinase 3 (GSK3) inhibitor added more to this yield [Dambrot et al., 2011]. Co-cultures with visceral endoderm-like cell lines (END) in serum-free media supplanted with Insulin or their conditioned medium is an alternative approach [Passier et al., 2005, Freund et al., 2008]. This method, applies mechanical rather than enzymatic passage of undifferentiated cells [Dambrot et al., 2011]. Co-culture with END-2 cell line led a more homogenous ventricular cardiomyocyte population [Mummery et al., 2003]. Laflamme et al achieved cardiomyocytes more efficiently than EB-derived cells using high-density monolayer model in serum-free medium in the presence of BMP4 and activin A [Laflamme et al., 2007]. Achievement of homogenous mature cardiac cells as homogenous atrial, ventricular, conduction fibers or a mixture of them is the main goal of directed cardiomyocyte differentiation [Dambrot et al., 2011]. Currently applied technique yield a heterogeneous cell population with cardiomyocytes ranging from 1% to ~50% of the total cell mass [Dambrot et al., 2011]. Indeed, the premature phenotype of induced cells is an important issue which needs maturation induction using cell re-plating or END-2 co-culture methods followed by limited three-dimensional culturing [Otsuji et al., 2010]. Cyclic stretches or forcing alignments might enhance tissue maturity further. Gradient centrifugation method isolates largely-sized cardiomyocytes, physically [Xu et al., 2006]. Cardiomyocyte harvesting based on the cell surface receptors like protein fetal liver kinase 1 [Flk1; also known as VEGF-receptor or kinase insert domain-containing receptor (KDR)] has been demonstrated [Yang et al., 2008]. This method will provide a mixed population of cells like endothelial progenitor cells (EPCs), endothelial cells, smooth muscle cells and some other undifferentiated cells [Dambrot et al., 2011]. An easy and reversible method of isolation has been introduced using reversible mitochondria labeling by tetramethylrhodamine methyl ester perchlorate

(TMRM) in the mixed cell population [Hattori et al., 2010]. This labeling yields three cell fractions as follow: cardiomyocyte with high fluorescent fraction, intermediate fraction of non-cardiomyocyte viable cells and dead low fraction or blood cells [Hattori et al., 2010]. In this way, cardiomyocytes are maintained more than 50 days in cultures [Hattori et al., 2010]. Alternation in culturing protocols might insight to valuable information about alternative approaches for obtaining cardiomyocytes. A yield up to 50% cardiomyocytes was achieved using feeder-dependent enzymatic passage of hESCs in knockout serum replacement (KOSR) followed by spin EBs and addition of BMP4 and activin A with subsequent re-plating [Ng et al., 2008]. As a complication of acute coronary syndrome, conduction defects are notable which calls attention for the importance of nodal cell achievement besides cardiomyocytes. For this purpose, inhibition of neuregulin (NRG)-1β/ErbB pathway has been shown to enhance nodal-like cell achievement, in vitro [Zhu et al., 2010].

7. Resident cardiac stem cells

Mammalian cardiomyocytes are not totally terminally differentiated post-mitotic cells and cardiomyocyte turn-over has been observed in adult hearts [Walsh et al., 2010]. In aggregate, CSCs seem more efficient and natural for cardiogenesis than other non-heart origin stem cells. Resident CSCs restore the dead myocardium by proliferation and differentiation into newly mechanically effective myocardium [Dergilev et al., 2011]. These cells are tissue-specific, mostly pre-committed to cardiac lineage fate [Limana et al., 2011]. Thus, activation of the few resident cardiac stem cells (rCSCs) available in the heart via exogenous factors might exert beneficial effects [Leri et al., 2005]. But their insufficient numbers limit the benefits derived from their activation. Some resident cardiac progenitor cells are explained here.

Side-population (SP) cells are some resident which trans-differentiate into mature cardiomyocytes by co-culture with mature ventricular myocytes. SP cells express Abcg2 transporter and exporters of Hoechst dye [Balbuena et al., 2011]. The immediate replacement of SP cell by bone marrow cells after AMI suggests the presence of homing mechanism and phenotypic conversion [Guan & Hasenfuss, 2007]. This inspires the possibility of SP cell hunting from peripheral blood and avoiding their extraction by surgery and cardiac biopsy. Among SP cells the maximum potency for cardiac differentiation belongs to the Sca-1$^+$, CD31$^-$ cells [Pfister et al., 2005]. Cardiac SP-derived Cardiospheres, self-adherent clusters derived from mild enzymatic digestion of cardiomyocytes, express both endothelial and stem cell markers [Chamuleau et al., 2009, Reinecke et al., 2008]. These cells belong to CSCs and contain firm cardiac stemness phenotypes [Guan & Hasenfuss, 2007]. Cardiosphere-derived stem cells as well as C-Kit$^+$ cells are able to differentiate into the major cardiac vascular and muscular specialized cells [Guan & Hasenfuss, 2007].

Skeletal myoblasts were the first relevant cells used clinically [Guan & Hasenfuss, 2007]. Upon transplantation into the infracted myocardium, these cells were clonally expanded, propagated and differentiated into myotubes clustering in specific foci and improved cardiac contractility [Guan & Hasenfuss, 2007, Taylor et al., 1998, Scorsin et al., 2000]. However, there is some controversies regarding the arrhythmogenicity of these cells [Moreno et al., 2010]. Another cardiac stem cell capable to contribute to approximately all cellular elements of the cardiac interstitium and coronary vasculature is referred as epicardium-derived cell (EPDC) [Lie-Venema et al., 2007]. EPDCs transplanted into mouse

heart, improved LV function and attenuated pathologic remodeling mostly through an indirect paracrine pathway [Winter et al., 2007]. The clinical efficacy of these cells for human application is not yet well clarified.

8. Stem cell-based therapies for ACS

Inadequate cardiac regeneration and cell death with subsequent progressive remodeling following acute ischemic insults make a vicious cycle toward further degeneration: degeneration begets degeneration. Measures should be performed to break this vicious cycle at earlier reversible stages. Despite of the potential role of stem cells in the regeneration of advanced stages of disease spectrum, it will bring battery of influential effects if used at the acute phase of ischemic event. Final goal should be replacement of the damaged and necrotic regions with alive and regenerative cells. Stem cell nomenclatures based on their function, phenotype, special characteristics and practical applications for heart regeneration seem helpful. Stem cell-based cardiac repair put forth new therapeutic paradigms for treatment of relentless progression of heart diseases after acute myocardial insults. But it is still in its infancy.

9. Pre-administration perquisites

Prior planning for stem cell-based therapies, issues regarding their safety and feasibility should be determined. Ethical considerations for stem cell-based therapies should be identified based on the district rules. The risks and benefits of the proposed procedure, the superiority of this procedure over other approaches and its probable durability and reproducibility should be reviewed with patient. Signed informed consents are perquisite for stem cell-based therapies. Pre-hospital issues to potentiate outcomes of the cases intended for this treatment modality are unknown. In clinically indicated cases, electrolyte abnormalities should be corrected before stem cell implantation. Patients should be evaluated for arrhythmogenic clinical grounds. It is not yet determined if patients at high risk for development of life-threatening arrhythmia are eligible for stem cell-based therapies. By virtue of the predisposition to arrhythmia in ischemic myocardium, the amplified arrhythmia risk might not be clinically favored. The benefits should be weighted in patients with currently under treatment of fatal arrhythmia or cases with remarkable past history respective to these arrhythmias.

After all, it is noteworthy that each stem cell-based therapy should be administrated in equipped hospitals. Isolated stem cells should be transferred to intervention room easily. Perhaps, the best condition is performance of stem cell-based therapies in hospitals equipped with stem cell laboratories. This minimizes troubles and cautions related to cell transferring. Moreover, time wasting would be minimized. For this purpose, standardized cell isolation protocols and scale-up procedures should be emerged before wide clinical applications. Expanded stem cells should be characterized prior to clinical application. Of note, pre-administration evaluation of cell sterility, quality and functionality both in vitro (migratory and colony forming abilities) and in vivo (ability to reperfuse blood flow to ischemic district) should be performed. Technical challenges must be met thoroughly before clinical stem cell application. The impact of quality of cell processing and purity of the final cell on the final outcomes has been previously clarified. In an equipped hospital, a trained assembled team composed of basic stem-cell researcher, cardiologist and nurses is

necessary. Caring nurses must be able to recognize and deal with the challenges specified to stem cells recipients. Cardiac surgeon should also be attendant if surgical cell delivery is intended. Thus, those qualified centers should have on-site surgical back-up. Both team and hospital should have certificates for these operations by maintenance of good tissue practice (GTP) and GMP (good manufacturing practice). Periodic quality control should not be missed. Minimal stem-cell based procedures per year for the team and hospital need to be defined by experts. High-volume operation centers might offer less risk to the patient compared with low-volume ones. Poor clinical attainments might be reflection of technical failure. But, to date there is no absolute definition for primary and late procedural success and failure. Of course, clinical failure attributed to time delay to reperfusion and major adverse cardiac events due complications of angioplasty should be discriminated from pure cell-related outcomes.

10. Administration of regenerative agents

By now the maximized cell migration and adhesion through percutaneous delivery of stem cells is done with stop-flow balloon catheter to achieve total flow occlusion within three minutes followed by stem cell infusion and reflow through deflation [Nuri & Hafeez, 2011]. In the case of extensive MI and multi-vessel disease, the eligible vessel should be identified. The superiority of antegrade vs. retrograde and proximal vs. distal stem cell delivery is not yet elucidated. Inflamed necrotic myocardium makes a hot microenvironment for delivered stem cells. The impact of this even slightly higher temperature on the efficacy of stem cells should undergo exploration. If any, cooling devices assembled with mechanical cell delivery instruments seem attractive. Perhaps stem cell eluting stents containing stem cell seeding on stem cell-friendly biomaterials without the problem of much scaffolding become available in future. If so, combination of drug-release plus stem cell-eluting stents and other combination might become revolutionary. Cells delivered directly through intracoronary route, need migration out of the vessel walls into the adjacent myocardium. This method brings the risk of coronary artery obstruction due to the plagued stem cells and consequently leads to further myocardial damage [Grieve et al., 2010]. The diameter of the target vessel and number of delivered cells seem detrimental with respect to cell stasis and vessel obstruction. The inherent risk of embolic events "cell embolism" is another limitation for intracoronary administration of stem cells [Zhang et al., 2007]. The underperfused myocardium potentially makes an unfavored environment for stable graft survival.

Intravenous stem cell injection has been shown to be safe for allogenic MSCs [Vassalli & Moccetti, 2011]. By intravenous cell administration, the majority of infused cells were shown to be harbored in kings and this cell trapping consequently limit the efficacy of this approach [Wang. et al., 2011]. Poor cell survival and drastic safety outcomes due to the extensive cell redistribution throughout the body limits this delivery approach. Alternatively, direct intramyocardial cell delivery is possible through transendocardial (percutaneous) or transepicardial (surgical) cell injection into the LV walls [Nuri & Hafeez, 2011]. Percutaneous route or "interventional cardiomyoplasty" requires retrograde passage of specially designed injection catheters into the left ventricle via femoral or arterial access [Psaltis et al., 2010]. Direct cell injection into the scar tissue or hibernated myocardium can be performed during open heart surgery or minimally invasive thoracostomy. Traumatic myocardial perforation especially at the site of freshly infracted tissue is a major side effect of this method.

The goal of catheter based needle intramyocardial cell injection should be promoted cell dispersion with limited immediate cell washout. Since formation of cell clusters rather than cell dispersion is proposed as a mechanism of arrhythmia-induction following stem cell application, dispersed-delivery techniques should be sought. Strategies which enhance homogenous and aligned cell integration with host tissue are more desired. But the most important item is delivery of healthy cells, not crushed or squeezed cells.

Myocyte-specific strategies prevent tumor formation and growth in other tissues and allow safe systemic delivery bypassing complicated local delivery approaches. Factors specific for cardiomyocytes in the contracting walls bordering the infract zone should be identified. This strategy might be potentially with pronounced efficacy without imposing side effects like hypertrophy on remote resident cardiomyocytes. Implantation of tissue-engineered autologous myoblast sheets showed promising results in rat, canine and porcine ischemic models [Sawa et al., 2010]. Sheets would cover larger area with fewer arrhythmogenic potential. Since stem cell therapy is not just based on the administration of crude stem cells, approaches for delivery of other regenerative agents should also be discussed here.

Stem cell application might be possible through seeding of stem cells on appropriate scaffolds and cell delivery at the site of damage. A step further back, might be approaches based on the enhanced homing of stem cells via promoted endogenous or exogenous stimuli with high specifity for stem cells involved in cardiac regeneration. Providing an accommodation for stem cells released after acute ischemic insult into the circulation, will be another alternative. These approaches will minimize the untoward effects of the exogenously delivered stem cells. A combination of exogenous stem cell administration and activation of endogenous stem cells using endogenous or exogenous stimulating factors might be attractive. Last, integrated and multi-disciplinary stem cell therapy for ACS needs fusing basic and clinical researches to narrow the gaps. Moreover, identification of key proteins involved in cardiac regeneration and cell differentiation opened the field of "Protein therapeutics". Proteins should be modified in the way to limit immunogenicity and rapid degradation in plasma and tissues. Delivered proteins exert paracrine effects on neighboring myocardium. Regardless of the type of regenerative agent, each candidate method should elicit a durable effect in a significant number of myocytes.

Irrespective of the applied method for myocardial cell delivery, cells should be engrafted in suitable place. Viable myocardial segments are most desired sites for cell delivery. Patients who suffered from ACS might have chronic scar tissues rather than freshly made scar due to the acute event. Obviously, cell grafting at the sites of chronic scars would be of no benefit. In addition, most of the directly injected cells die off soon due to the lack of nutrient and blood supply from necrotic tissue without live myocyte syncytium. Cell loss during and after transplantation lowers the efficacy of stem cell therapy.

Targeting the ideal site of cell deployment is of paramount value. This site reflects the mentioned regenerative focus which can send out constructive signals. Thus, numerous 3-D intracardiac navigation systems as electromechanical mapping techniques have been developed for correct cell seeding [Banovic et al., 2011]. Interrogation using intracardiac echocardiography might yield more. Mapping catheters integrated with injection ports conjunct with manipulated guiding catheters might help in direct endomyocardial injection mapping for targeted cell delivery. Optimal imaging techniques should be applied to both guide characterization of the cell-delivery site and monitoring the functionality and efficacy

of the transplanted cells. Stem cell scintigraphy, lineage tracing and intravital imaging protocols might be helpful. Since the heterogeneity of the grafted stem cells with native myocytes is the principal cause of arrhythmia induction, one potential application of imaging modalities might be detection of patients with greatest uncoupling between grafted and native cells. In this way, patents at high risk for development of arrhythmia presumably could be identified earlier. Patients at high risk of arrhythmogenicity can receive prophylactic measures before development of life-threatening arrhythmias. Whether if routine stem cell tracking after application of stem cell-based therapies is valuable or not should be determined clinically. Labeled grafts facilitate following of applied cells, but need advent of non-toxic and or timely degrades labels. Non-invasive objectifying of myogenic cell grafts and assessment of the fate and bio-distribution of applied cells might be valuable in certain patient populations at risk of early stem cell failure. If so, such at risk patients should be characterized by risk assessment algorithms. Indeed, the importance of early detection of residual ischemia in patients seems valuable. Residual ischemia might limit the potential benefits of applied stem cell-based therapies as soon as the time of application. All of the deteriorating underlying conditions like anemia and poor glycemic control, in recipients of stem-cell based therapies should be approached similar to the patients receiving standard medical cares.

11. Post-administration perquisites

Induction of malignant arrhythmia by transplanted stem cells has been demonstrated in several studies. This increased incidence has been attributed to the non-synchronized contraction and electromechanical non-incorporation of novel myocytes with background cells [Song et al., 2011]. Other mechanisms as Anisotropy, scar-implanted cell interaction and the presence of immature cardiomyocytes with intrinsic pacemaker activities are also speculated [Dambrot et al., 2011]. Recipients should be monitored in-hospital with more intense attention to electrocardiographic evidences of arrhythmia. Electrolyte abnormalities in favor of arrhythmogenicity should be identified and eliminated earlier. Routine electrolyte evaluation might be remarkable after stem cell implantation for reduction of the risk of arrhythmia. In the case of life-threatening arrhythmia, prophylactic and therapeutic anti-arrhythmic approaches should be provided for the patient. At least in-hospital monitoring interval before discharge should be determined. Indeed, the optimal hospitalization place for the recipient should also be evaluated. When the recipient should be sent to the step-down wards out of CCU? Certainly, the observation unit for the recipient should be equipped with central monitoring systems for the occurrence of fatal and non-fatal arrhythmias. Really, the interval with the greatest risk of arrhythmia induction should be determined. The impact of traditional risk factors and multiple comorbidities on either exogenous and mobilized endogenous stem cell outcomes or organ toxicities should be determined clearly. Irrelative to the type of applied therapies, ongoing risk factor modification and life-style modification should be started at early hospitalization in patients suffered from acute coronary syndrome. These strategies impact the general efficiency of applied therapies as stem cell-based treatments in the recipient patients. After stem cell transplantation, recipient should be followed-up for functional performance, clinical complications and mortality. Follow-up modalities and intervals should be determined. In this context, stem cell-specific comorbidity index (CI) charts derived from long-term patient follow-ups are helpful.

12. Clinical trials

The first clinical attempt of cell therapy was performed using BMC (bone-marrow derived cells). Rapid transfer of BMCs from bench to bedside without the need for ex-vivo expansion facilitates their clinical applications. BMCs showed modest and reproducible improvement in cardiac function by enhanced angiogenesis, augmented myocardial perfusion [Segers & Lee, 2008, Sun et al., 2009]. Reduced end-systolic volume by these cells reflects reduced negative remodeling [Orlic et al., 2001]. Un-fractionated BMCs encompass heterogeneous stem cell population including stromal cells, circulating progenitor cells (CPCs), EPC, MSC and HSC [Soejitno et al., 2010]. These cells release biologically active factors in favor of healing of the infracted myocardium [Henning, 2011]. Meta-analysis demonstrated a mean absolute increase 3-4% in ejection fraction by intracoronary infusion of patient's own reconstituted BMC aspirate [Rangappa et al., 2010]. Despite of the patient's pain relief and improved systolic and diastolic cardiac performance, the ultimate long-term effects were limited [Passier et al., 2008, Mummery et al., 2010, van Ramshorst et al., 2009]. Practically, there is no proved superiority between BMCs and CPCs in terms of clinical utilities and both are readily aspirated and administrated in contrary with difficult expansion of competent cardiac stem cells (CSCs) [Soejitno et al., 2010]. Ex-vivo expanded BMCs and unfractionated CPCs were infused post AMI via intracoronary rout in Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction (TOPCARE-AMI) trial [Schächinger et al., 2004]. It showed improved regional and global wall motion up to 9 and 1.2 percentage points from baseline during 4-month follow-up [Soejitno et al., 2010]. Significant enhancement was found in coronary blood flow reserve, cardiac geometry and myocardial viability [Schächinger et al., 2004]. EPCs augment tissue perfusion through pro-neovasculogenic functions and show very low efficient cardiac trans-differentiation in co-culture with mature cardiomyocytes [Kawamoto & Asahara, 2007, Badorff et al., 2003]. These cells increase perfusion of the ischemic tissue [Murohara et al., 2000, Kawamoto et al., 2001, Kalka et al., 2000]. In bone marrow transfer to enhance ST-elevation infarct regeneration (BOOST) trial, Mononucleated BMCs applied days after post-MI coronary intervention, increased regional and global cardiac performance during 6-month follow-up [Wollert et al., 2004]. Similar to TOPCARE-AMI trial, most improvement was seen in infarct border zone. But unexplained statistically insignificant decline in LVEF after 18 and 61 months later occurred in treatment arm [Meyer et al., 2006, Meyer et al., 2009]. Sustained improvements in LV function after 12-month of follow-up has been underlined through post-PCI BMC application in Reinfusion of Enriched Progenitor cells And Infarct Remodeling in Acute Myocardial Infarction (REPAIR-AMI) trial [Schächinger et al., 2006]. There are some discouraging data from Autologous Stem Cell Transplantation in Acute Myocardial Infarction trial **(**ASTAMI) and Multicenter, randomized trial of intracoronary infusion of autologous mononuclear bone marrow cells or peripheral mononuclear blood cells after primary PCI (HEBE) trial which failed to demonstrate clinical benefits of intracoronary transfusion of BMC and BMC or CPC, respectively [van der Laan et al., 2008, Lunde et al., 2006]. Similar negative results with BMC were achieved by Leuven AMI trial with intracoronary application of BMCs 24 hrs after reperfusion in AMI cases [Janssens et al., 2006]. These patients showed improvement in regional function of only infracted segment [Janssens et al., 2006]. The same, Regeneration by Intracoronary Infusion of Selected Population of Stem Cells in Acute Myocardial Infarction (REGENT) trail failed to show promising outcomes of intracoronary infusion of bone marrow-derived selected $CD34^{+}CXCR4^{+}$ cells and non-selected mononuclear cells on LVEF or volumes [Tendera et

al., 2009]. However, a trend in favor of cell therapy was found in patients with severely depressed LVEF [Schaefer et al., 2010]. In TOPCARE-AMI study, the potential role of BMCs in the pathogenesis of In-Stent restenosis and thrombosis has been advocated [Assmus et al., 2002, Schächinger et al., 2004]. These concerns are based on the advocates respective to enhanced restenosis due to progenitor cell-mediated plaque angiogenesis or inflammation [Assmus et al., 2006]. Increased restenosis rate seen with $CD133^{+}$cells arose these questions, but evidences support the risk related to the local inflammation due to applied mouse antibody for cell isolation [Assmus et al., 2006]. Contrary, mobilized BM-derived putative EPCs designated to decrease complications of iatrogenic vascular injury [Kawamoto & Asahara, 2007]. In this regard, REPAIR-AMI trial showed a substantial decreased necessity for revascularization procedures in patients with engrafted BMCs [Schächinger et al., 2006]. FINCELL study has declared that there is no globally increased risk of restenosis attributable to BMCs [Huikuri et al., 2008]. The increased risk of intramyocardial calcification in rats received BMCs have been found with no similar finding in related human cases [Kang. et al., 2004, Yoon. et al., 2004]. The clinical safety of BMCs regarding to adverse events and induction of malignant arrhythmia has been shown in BOOST and FINnish study of autologous bone marrow-derived stem CELLs in acute myocardial infarction (FINCELL) trials [Wollert et al., 2004, Huikuri et al., 2008]. SWiss multicenter Intracoronary Stem cells Study in Acute Myocardial Infarction. SWISS-AMI trial using autologous bone marrow mononucleated cells (BM-MNCs) is currently underway [Sürder et al., 2010].

The limited efficacy of BMCs led scientists for alternative stem cell source. MSCs are potentially differentiated into functional beating cardiomyocytes, especially by exposure to certain DNA-methylation inhibitors [Lu et al., 2010]. MSCs improved contractility by prevention of remodeling in the vicinity of non-infracted myocardium [Soejitno et al., 2010]. MSCs, easily isolated and expanded in vitro, are effective in restriction of ischemic wave propagation in the area adjacent to infarction by accelerating inflammation, angiogenesis activation, prevention of apoptosis and reducing the scar size and volume [Sokolova & Pavlichenko, 2010]. Unsurprisingly, efficacy of these cells proved clinically without adequate clues of remuscularization [Soejitno et al., 2010].Very low degree of cardiogenesis and donor cell incorporation (just seven MSC-derived cardiomyocytes per heart) was also seen [Soejitno et al., 2010]. These data inspire the presence of an indirect paracrine mechanism of actions [Mangi et al., 2003, Noiseux et al., 2006, Gnecchi et al, 2005, Gnecchi et al., 2006]. These paracrine effects also impact on the extent of scar and fibrotic tissue by exertion of anti-inflammatory, anti-apoptosis and anti-remodeling properties [Gnecchi et al., 2008, Burdon. et al., 2011]. Immunomodulatory actions and low immunogenicity of MSCs allow their safe application for allogenic transplantation [Pittenger & Martin, 20004, Aggarwal & Pittenger, 2005]. Another examined stem cell source for cardiac regeneration is hESCs. hESCs, cultured reliably and differentiated robustly into cardiomyocytes regenerate myocardium in infracted hearts, attenuate heart remodeling and contribute to LV systolic force development [Henning. 2011]. Despite their unambiguous cardiogenesis, limited data are available regarding their advanced maturation and seamless myocardial integration in vivo. To overcome obstacles regarding the risk of teratocarcinoma formation by hESCs, application of differentiated hESCs toward cardiac progenitor cells prior to transplantation into the hearts seems beneficial. The large pool of generated hESCs cardiac progenitor cells needed for this purpose is a limitation for this proposition. Ultimately, prevention of immune rejection and enhanced graft survival over long term are necessary to improve

myocardial performance. There are some few studies with CD34 or CD133 positive cells, CD34 and CXCR4 double positive cells, hESC-CMs (cardiomyocytes) and hESC-CPCs (cardiac progenitor cells). A quiescent progeny of epiblast-derived progenitor stem cells, BM-derived very small embryonic-like cells (VSELs), enhanced LV contractility more efficient than that of HSCs in experimental MI models [Wojakowski et al., 2011]. But studies are limited for proper conclusions regarding clinical readiness and utilities of these cells.

13. Experiments on stem cell homing after ACS

Granulocyte-Colony stimulating factor (G-CSF) is the only safe exogenous factor has been investigated widely for the treatment of acute ischemic phase. Despite of the low myogenic differentiation potential of the homed hematopoietic stem cells by G-CSF, its angiogenic and anti-apoptotic effects on cardiomyocytes assumed to be beneficial [Segers & Lee, 2008, Harada et al., 2005]. It also accelerates infarct healing through facilitated infiltration of macrophages into the necrotic tissue and activation of matrix metalloproteinases (MMPs) [Minatoguchi et al., 2004]. G-CSF used within the early 37 hours after MI, showed modest but tangible effects on cardiac performance without any significant impact on mortality rate or vessel stenosis [Abdel-Latif et al., 2008]. G-CSF enriches and mobilizes a specific $CD34^{+}CD133^{+}$ sub-fraction of hematopoietic cells from whole blood [Powell et al., 2005]. Some contradictory results seen with application of G-CSF are attributed to its inhibitory effects on CXCR4 activity with consequent decrease in migration and homing of progenitor cells into the infarct tissue [Dimmeler, 2010]. Application of G-CSG mobilized cells in AMI showed more benefits rather than application of G-CSF alone [Kang et al., 2007]. Regarding stem cells homing, Hepatocyte-growth factor (HGF) has been demonstrated to decrease infarct size by improving angiogenesis and exerting anti-apoptosis properties on the damaged myocardium [Urbanek et al., 2005, Wang et al., 2004, Nakamura et al., 2000]. HGF has chemotactic effects on putative cardiac stem cells [Segers & Lee.2010]. Alternatively, Delivered protease resistant Stroma-derived factor (SDF-1 S4V) using self-assembled peptide nanofibers enhanced both vascular density and cardiac function in rats with AMI [Segers et al., 2007].

Stimulating Factor in Acute Myocardial Infarction (SITAGR-AMI) trial with administration of CD26/dipeptidyl peptidase 4 inhibitor in AMI, which augments SDF concentration, is currently underway [Theiss et al., 2010]. SDF potentially recruits endothelial progenitor cells (EPCs) [Segers & Lee.2010]. EPCs and BM-derived putative progenitor cells are potentially homed into infarcted heart tissue after by Erythropoietin (EPO) [Westenbrink et al., 2007]. Due to le discrepancies between preclinical and clinical results, large randomized placebo-controlled clinical trials are underway to scrutinize therapeutic benefits of EPO treatment for acute coronary syndromes. After all, the efficacy of each promising homing factor depends on the presence of relatively intact stem cell pool.

AMI is not just a fault with cardiac myocyte and defects in large vessels and microvasculature compose one tail of the spectrum. Although angiogenic factors as VEGF and FGF seem hypothetically beneficial, they failed to meet the primary endpoints in clinical trials [Segers & Lee, 2008]. This unsuccessful application has been attributed to the formation of tortuous, aberrant and leaky vessel walls [Carmeliet, 2005, Lee et al., 2000]. Formation of non-leaky vessels need orchestrated action of various proteins at different time intervals beyond mere VEGF and FGF. Thus, a comprehensive approach would be

application of functional proteins at various time points but it is yet impossible. The tumorgenecity of angiogenic factors is also a great concern [Segers & Lee, 2008].

There are some limited experiments regarding the beneficial role of proteins capable to induce cell cycle reentry of cardiomyocytes for regeneration purposes. Some are explained here. Extracellular matrix play a fundamental role in remodeling is prevention of ventricular rupture after AMI [Matsumur et al., 2005]. Periostin delivered into the infracted rat myocardium through a patch of Gelfoam, induced cell cycle reentry of adult myocytes in the border zone of MI and stimulated mitosis of surviving myocytes [Kühn et al., 2007]. Although controversial, it improved cardiac function with decreased scar formation and remodeling of non-infarcted myocardium [Segers & Lee, 2010]. Another protein able to establish myocyte reentry is neuregulin, a member of epidermal growth factor family involved in proliferation and differentiation of cardiomyocytes [Lemmens et al., 2007]. Increased cardiac function and decreased infarct size were seen with daily intraperitoneal administration of neuregulin [Bersell et al., 2009]. This improvement was attributed to the proliferation of existing myocytes rather than prohibition of apoptosis or differentiation of progenitor cells [Bersell et al., 2009].

14. Limitations and clinical pearls

Ladder diagrams, evidence-based guidelines and evidence-based therapies for the use of stem cells in the cases afflicted with ACS should be prepared. The witnessed "Go with Guidelines" in the treatment of acute coronary syndrome should be extended to the field of cardiac stem cell-based therapies. It will be possible only after pooled analysis of large-scale proof-of-concept studies, clinical trials and sufficient data analysis for validity/reliability measurements and success/failure rate of the applied methods. Identification of ideal stem cell type and dose-regimen, optimal timing for initiation of stem cell therapy, safe cell hunting method, proper patient selection and administration method, justified cell tracking strategies and directed follow-up sessions, makes this a very difficult task. Lack of trials with long follow-up period is potentially problematic. Thus far, trials with longest follow-up period were BOOST (5 year), ASTAMI and REPAIRE-AMI trials (both 12 months). Indeed, some cell-related endpoints as engraftment rate, cell retention and dose-response relations besides remodeling, regional and global LVEF, death, MI quality of life, symptom relief and hospitalization for heart failure should be considered in trials. This will mirror both technical and clinical success.

Candidate stem cell must have remarkable capacity for expansion and unquestioned potential for cardiogenesis. And only cells with true cardiac differentiation could likely effect scar regeneration. Candidate stem cell should posses the potential to form long-term stable grafts with no or less inflammation. Absolutely, it should be kept in mind that there can't be such a stem cell ranking. Appropriate stem cell selection is partly a function of patient's general conditions. Advance age and chronic illness are among factors with significant effect on the proper propagation of isolated stem cells due to their premature death. Stem-cell's age, a reflection of patient's age, is a determinant factor of stem-cell's plasticity. Although it can't be simply stated that younger is better, advanced age, old stem-cells or in the other terms octa- and nonagenarian stem cells might be unable to construct a virtually unexhausted cellular reserve pool. Apparently, stem cell therapy should be tried in patients with anticipated life expectancy. Underlying disease sates as cardiovascular

diseases per se, could directly or indirectly affect functional activity of the endogenous progenitor cell reservoir. Diminished production and mobilization of EPCs from bone marrow demonstrated in the milieu conflicted by endothelial cell dysfunction [Hill et al., 2003, Tepper et al., 2002, Vasa et al., 2001, Schmidt-Lucke et al., 2005, Werner et al., 2005]. Short telomere length of CPCs has been observed in these circumstances [Dimmeler & Leri, 2008]. The positive or negative effects of ischemic preconditioning on stem cells of cases with cardiovascular diseases are unknown. Hence, the impact of extracellular tissues and signals derived from non-myocardial component on patterned cardiac differentiation should not be overlooked. In addition, some stem cells work better in the presence of underlying comorbidities. It has been demonstrated that BMCs are more effective in ageing and DM individuals but less effective in males [Bai et al., 2010]. So, selection of best cell or "cardiogenic cell preference" should be tailored to the patient's primary clinical ground. Certainly, the infarct size is also of paramount vale in determination of the best stem cell source and mode of delivery. Large amounts of infarct territory might need cell type with more restoring capacity. The hypothetical "patient-specific stem cells" might be adjoined into "patient's cardiac specific stem cell" in foreseeable future. The optimum dose should also be determined per case; larger infarcts might need more cells. Inadequate cell number leads to delayed recovery and decreased patient survival, but stem cell oversizing and heart-cell mismatch would not be free of risk. Sufficient number of individual stem cells for clinical benefits seems to be a function of its natural properties related to survival potential and capacity of mass production. In the other word, enough new myocyte mass with appropriate vascular density should restore sufficiently mechanical function of heart. Routinely, dose regimens contain notes regarding administration intervals. Thus, heart as a challenging organ for repair and integration of reparative stem cells might need more than one simple stem cell transplantation. Do booster cells engrafted at time intervals add more clinical benefits? Hypothetically, is it beneficial to primarily transplant one type of stem cell with superior cardiac benefits following by application of another cell type of stimulating agent with hours or days later? In addition, is there any difference between required doses for males and females? Generally, longer telomere length was observed in females' hematopoietic stem cells which might lead to lower senesce of these endogenous reparative stem cells [Sidorov et al., 2009].

Regarding optimal timing, some investigators demonstrated that BMCs applied up to four days after AMI had no benefits whereas later cell engraftment (4-8 days) showed beneficial effects [Dimmeler et al., 2008]. In the other hand, some investigators recommend early timing of cell administration due to microenvironment alteration at the first week after AMI which modulates functionality of the homed cells. It has been attributed to the initial edema formation as a consequence of inflammation which disrupts optimal stem cell homing. In contrast, some evidences support the clinical benefits of stem cells with early stem cell application in AMI. As a matter of fact, stem cell harvesting is not an immediate process. Since some culture-dependent cell hunting methods require days prior to application, it might not be possible to isolate cells on the patient arrival to the hospital during acute events. But at not too distant future, progress in cell banks may provide some facilities for having available stem cells at the hospital stem-cell rooms. And physicians can order one cell type based on the patients' clinical scenario. Perhaps, some day stem cells become as available as chewing aspirin in emergency rooms. Cells derived from sources like bone-marrow, peripheral or umbilical cord blood are not sufficient in number. Ex-vivo cell

expansion provides enough number of required cells from clinical point of view, but a bit concerns remains regarding induced stem cell differentiation than self-renewal in cultures rather. Anyway, harvested autologous stem cells can be expanded in cultures in contamination-free ambient soon in life and stored for possible use in future. But the large number of cell banks needed for individual patients and the costly procedure for scaling-up of these cells are serious drawbacks should be solved. Reasonably, stem cells can be pre-preserved for patients recognized to be high risk for acute cardiac events. This risk would be assessed using perfect risk predictors in males and females more than 45 and 55 years old, respectively. The overhanging concept of stored allogenic stem cells is interesting from clinical point-of-view. But the elicited immune-mediated inflammatory reactions at the site of cell delivery might accelerate tissue damage and stem cell removal. Obviously, the cardiopoietic paracrine effects of allogenic stem cells might exceed the disadvantages of immune-mediated reactions but needs to be determined. In the case of allogenic stem cell harvesting, the eligible donor should be defined. Perhaps a donor with fresh stem cells without any underlying disease is better. Females with longer stem cells compared with age-matched males seem more suitable for this application but it is not as simple as a coin flip. However, the necessity of immune-matched cells at the time of application is a great obstacle for clinical utilities of allogenic stem cells. Cardiomyocytes derived from iPSC lines are promising in this context due to obviation of this necessity, but iPS technology can't merit the cost-benefit criteria for clinical usefulness. However, there might be some tricks bypassing these problems as isolation of stem cells from umbilical cord blood (UCB) as an accessible and less immunogenic source of stem cells. By the progress made in perseveration of human umbilical vein blood banks every person can have unlimited access to homologues stem cells for application in acute coronary syndrome. But the outcomes of the freshly used vs. stored or cultured cells needs to be clearly determined. LVEF enhancement with BMCs was inversely correlated with their storage duration [Jiang et al., 2010]. Anyway, since exerted beneficial effects of cells might be related to their basic characteristics, the optimal timing factor for each stem cell should be identified individually.

Patient subsets that stem cell application could be possibly beneficial need to be determined carefully. Ample evidences support the significant clinical benefits of applied stem cells in ACS case with viable hibernated myocardium, large regional wall motion abnormalities or depressed LVEF below median. Patients with these eligibility indices could be identified clinically using imaging modalities as echocardiography and myocardial perfusion imaging. Other factors as severity of symptoms, extent of jeopardized myocardium, percentage of scar tissue, number and extent of diseased vessels, left main diseases, involvement of proximal part of LAD, coronary flow characteristic, presence and grade of collateral arteries to the ischemic region, the percentage of downstream viable myocardium, clinical signs of heart failure, prior MI, history of angina, high-risk sub maximal exercise test, rhythm stability, age, gender and comorbidities might help physicians in decision making.

There is still lack of risk-stratification strategies based on low-, intermediate- or high-risk patient categories relied on the positive cardiac enzymes and diabetes. Current cell-based therapies for all patients are expensive and with a high risk of failure. Therefore, patients hospitalized for AMI predicted clinically that current therapies are modestly effective and or nature of their acute event seems to be vexing, benefit more from stem cell therapies. Indeed, subgroups of patients with limited options and "no-option" or incurable cases are

also ideal candidate for cell-based therapies. Elderly patients, cases with contraindication for thrombolytic therapy, poor vascular anatomy for intervention and high risk patients for CABG will put in this group. However, knowledge about the decreased In-stent thromboses and restenosis by homed EPCs in the site of stent deployment tempt routine post-angioplasty stem cell application [Silber et al., 2011]. This would be also limited to the cases with reliable clinical predictors of high rate of In-stent restenosis and thrombosis.

Furthermore, it should be answered if hemodynamically unstable persons due to complicated ACS can undergo stem cell therapy or not? If the most clinical benefits of stem cells are within the first hours of ACS, are cooling-off periods spent for patient stabilization are still beneficial or not? Since heart is intrinsically an integrated organ with both vascular and muscular components, re-visualization and re-muscularization should be simultaneous and in-parallel. In the other word, enhanced neovascularization and neomyogenesis are both needed and any mismatch between these two arms will be associated with lack of sufficient success. Benefits of improved neomyogenesis in the territory of infarct related artery without optimal revascularization should always be scrutinized. Certainly, the restoring cardiac stem cells have their own nutritional requirements and the improvement process will down-hill in the case of deficient supply. Stem cell therapy as a risk-mitigating treatment and ad-on therapy or primary therapeutic choice should be well evaluated in the management of patients in acute setting. Trials should asses the benefits of initial stem cell therapies vs. initial conventional or invasive strategies. Peliotropic effects of Angiotensin-converting enzymes or Satins might be beneficial on stem cell functionality but the stem cell protective dose of these agents might differ from routinely used in emergency departments, which seeks to be determined. The optimal timing of beneficial medications on stem cells should be identified which could be even as early as ex-vivo stem cell processing. Do dual and triple anti-platelets exert effects on the ultimate benefits of applied regenerative cells? If invasive strategies are certainly needed, which of the upstream stem cell therapy (at present and before PCI) or deferred (at the time of PCI) are superior? Are all patients with ACS good candidate for stem cell therapy? Acute coronary *syndromes* encompass a wide spectrum from unstable angina pectoris to non-ST elevation MI and ST-elevation MI (transmural and non-transmural). Are stem cell-based therapies beneficial in medically or spontaneously relieved unstable angina? Like indications for thrombolytic therapy, should stem cell-based therapies restricted to transmural STEMIs? Do patients suffered from isolated right ventricular or posterior AMI benefit from stem cell therapies?

These questions need tight answers before widespread use of stem cells in clinical practice. Some considerations for complicated acute ischemic events by cardiogenic shock, with the need for cardiogenesis in its pure sense, should also be determined. These cases should be stabilized using bridge-to-treatment or bridge-to-transplant interventions. Simultaneous application of regenerative stem cells might be helpful in this case prospectively. Whether stem cell therapy would impact other complications of AMI as ventricular septal defect, aneurysms and free wall rupture is not yet demined. Stem cell therapy may move to be applied simultaneous with surgical ventricular repair.

Items in which clinicians can determine the success rate of treatment based on the pre-and post-test probabilities should be characterized. The advent of algorithms for stem cell therapies is necessary for future perspectives to determine selective stem cell application of routinely for “all comers” in the subset of ACS. Even if these caveats are solved and chart

lines become available, physicians should not be boxed in chart cages. Short of that, recipients should be reviewed in cardiac stem cell clinics conversant with issues particular for patients undergoing such therapeutic measures. In aggregate, progression of these stem and progenitor cell-based cardiac repair, require close interaction between scientists, clinicians and patients. Stem cell encyclopedia needs question-directed state-of-science trails.

Challenges for finding appropriate cell source offers clues toward creation of more conductive myocardium through environmental modifiers with enhanced cell survival, persistence, differentiation and proliferation. Cardiac regeneration is not merely relied on stem cells and the underlying scaffold for applied stem cells, both mobilized and injected, should not be overlooked. Engagement and disengagement of stem cells to heart niche is a finely tuned dynamic process. This delicate balance is remarkably altered under stress conditions as ischemia. Physical contact of delivered stem cells with cardiac nascent niche and neighboring cells enhance cardiomyocyte differentiation. Stem cells decide to become incorporated physical elements of newly formed vessels or myocytes partly based on the niche they have exposed. Knowledge about stem cell microenvironment and trafficking mechanisms would lead to profound understanding of enhanced engagement and depressed stem cell regression to the desired organ. In acutely infracted myocardium, the dominant harsh ischemic and cytokine-rich microenvironment, infiltrated by inflammatory cells imposes a Strong challenge to the transplanted donor stem cells. Will stem cells in delivered in this niche fate with cardiac ischemic memory? With niche therapy, niche environment can be modified in such a way to boost stem cell homing, migration, engraftment, retention and commitment. Niche-based fate determination is possible by providing stromal matrix, physical contact and chemical dialogue for invited and activated resident stem cells. A prepared niche regulates mobilization of cells into the circulation and enhances stem cell recruitment, homing and cell retention. In the other word, a favored niche helps in maintenance of stem cell compartment. In contrast, a disturbed niche might lead to cell dysfunction probably by exhaustion. The clinical significance of niche-based strategies or niche-targeting is not yet clarified in the field of coronary artery disease and needs to be addressed. Cardiac niche therapy should be adjoined to cardiac stem cell-based therapies, in a mutually complementary manner.

15. Concluding remarks future recommendations

Despite of the remarkable advances in the care of ACS, missed opportunities are still troublesome. These mismanagements include failure to deliver any form of reperfusion in about 30% of patients or delayed application of reperfusion strategies related to perpetuation of inefficient system of care [Moon et al., 2002]. Pathologic remodeling, which evolves immediately after AMI, alters the contractile properties of non-infarct zone and consequently impairs systolic and diastolic performance of the LV. Thus, cases with missed reperfusion are at risk of suffering from complications of adverse remodeling. To modify the disease process, recapitulation of stem cells has been proposed to ameliorate adverse remodeling and consequently improve LV function and angiogenesis. Tremendous effort is being put to functionally revitalize scar, non-contractile myocardium. Stem cells added one new dimension for the treatment of AMI with multi-faceted nature. It can be applied singly or combined to boost the efficacy of other therapies. The final aim of SCT for myocardial

regeneration has been directed to compensating myocardial loss and restoring myocardial deficits. Encourages due to the positive preliminary results and solved safety and tolerability issues paced cardiovascular stem cell therapies, but so far it has faced with contradictory results. This field is plagued by paucity of trials and trials with skepticism should definitely reinforce whether stem cell therapy should be augmented or not. Although emerging field stem cell based cardiac regenerative therapies are still limited, continued development in this field will impact this area in the very near future. Advent of magic stem cell bullet(s) or cocktails of stem cell with or without growth factors and homing agents might be revolutionary events achievable in future. As Feynman stated at 1959 "There is plenty of room at the bottom".

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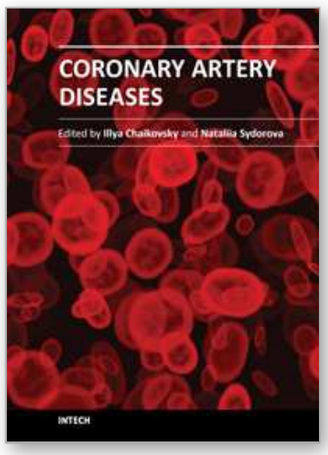

Coronary Artery Diseases
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This book has "wide geography" both literally and figuratively. First of all, this book brings together contributions from around the world, both from post-industrial countries and developing world. This is natural, because coronary artery disease is becoming pandemic worldwide. CAD is the single most frequent cause of death in developed countries, causes about 1 in every 5 deaths. Mortality from cardiovascular disease is predicted to reach 23.4 million in 2030. Moreover, in the developing world, cardiovascular disease tends to affect people at a younger age and thus could negatively affect the workforce and economic productivity. The morbidity, mortality, and socioeconomic importance of CAD make its diagnosis and management fundamental for all practicing physicians. On another hand, the book widely represents "geography" of CAD itself, i.e. many various aspects of its pathophysiology, epidemiology, diagnosis, treatment are touched in this book. This book does not pretend on complete and integral description of the Coronary artery disease. Rather, it contains selected issues on this complex multifactorial disease. Nevertheless, we hope that readers will find Coronary Artery Disease useful for clinical practice and further research.

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