Stem Cell Therapies for Ischemic Cardiovascular Diseases

Colin E. Murdoch[1,*], Sophie A. Broadway-Stringer[1], Milda Bartkeviciute[1], Camilla Siciliano[2,3], Roberta Altobelli[2] and Elena De Falco[2,*]

[1]*Reproductive and Vascular Biology Division, Aston Medical School, Aston University, Birmingham, B4 7ET, UK;* [2]*Faculty of Pharmacy and Medicine, Department of Medical-Surgical Science and Biotechnologies, University of Rome "Sapienza" C.so della Repubblica 79, 04100 Latina, Italy;* [3]*Center for Life Nano Science@Sapienza, Istituto Italiano di Tecnologia, Rome, Italy*

*Address correspondence to these authors at the Reproductive and Vascular Biology Division, Aston Medical School, Aston University, Birmingham, B4 7ET, UK; Tel: +44 121 204 3920; Email: c.murdoch@aston.ac.uk

Faculty of Pharmacy and Medicine, Department of Medical-Surgical Science and Biotechnologies, University of Rome "Sapienza" C.so della Repubblica, 79, 04100, Latina, Italy; Tel: +39-0773-1757234; Fax: +39-0773-1757254; E-mail: elena.defalco@uniroma1.it

Abstract: Myocardial infarction results in loss of cardiac muscle and deficiency in cardiac performance. Likewise, peripheral artery disease can result in critical limb ischemia leading to reduced mobility, non-healing ulcers, gangrene and amputation. Both of these common conditions diminish quality of life and enhance risk of mortality. Successful advances in treatment have led to more people surviving incidences of myocardial infarction or living with peripheral artery disease. However, the current treatments are inadequate in repairing ischemic tissue. Over the last 5 years, a vast number of patents have been submitted concerning the use of stem cells, which correlates with the exponential growth in stem cell publications. Exploiting stem cell therapy offers a real potential in replacing ischemic tissue with functional cells. In this paper, we review recent patents concerning stem cell therapy that have the potential to provide or potentiate novel treatment for ischemic cardiovascular disease. In addition, we evaluate the promise of the inventions by describing some clinical trials that are currently taking place, as well as considering how current research on ischemic cardiovascular disease may change the patent landscape in the future.

Keywords: Cardiovascular, cell therapy, heart, ischemia, regenerative medicine, stem cells.

INTRODUCTION

The use of cultured cells in tissue repair dates back to the beginning of the 20th century [1]. The huge potential for stem cells to deliver cell-driven repair arises from their distinct properties that include immunological tolerance as an allogenic transplant, potential to develop into multiple lineages and relative ease of harvesting and expansion. These properties initiated a great determination to understand how stem cells can be fully utilized in a wide range of diseases. The 90's saw a great burst in publications on stem cells which has been growing exponentially ever since Fig. (**1a**) [2]. Research in stem cells has driven the unmistakable confidence, ability and ambition in regenerative medicine, which is reflected in the number of patent submissions claiming unique intellectual property within this field. Since 1990 there has been approximately 5500 patents regarding stem cells, remarkably half of these have been submitted in the last 5 years Fig. (**1b**) [2]. We have focused this review on how stem cell technology would benefit ischemia cardiovascular disease of the heart (myocardial infarction (MI)) and lower limb (peripheral artery disease (PAD)). We have highlighted how stem cell technologycan be utilized and document recent submitted patents and current clinical trials associated with these diseases.

MYOCARDIAL INFARCTION

The European Society of Cardiology estimated that cardiovascular disease is responsible for 1.9 million deaths each year in the EU, which equates to 40% of all deaths within the EU. In the UK, it is projected that there are 103,000 MIs each year [3], with similar numbers found in most other European countries. The survival rate of people who suffer a heart attack is increasing as a result of better knowledge and treatment of the disease. Subsequently, more patients are now living with their heart in a compromised state [3]. In the post-infarcted heart, a large area of the myocardium remains non-functional as a result of ischemia, causing remodeling of the non-infarcted region and fibrosis, triggering abnormal filling, pumping and electrical signaling of the heart. Current treatment with therapy only treats the symptoms but does not tackle the primary issue of loss of cardiac tissue. Repair of the ischemic heart using a pool of easily sourced human cells is the holy grail of cardiovascular medicine and would increase the long term survival rate and quality of life of people who have suffered from a myocardial infarction [1].

PERIPHERAL ARTERY DISEASE

PAD affects an estimated 27 million people across Northern America and Europe [4, 5]. PAD is caused by

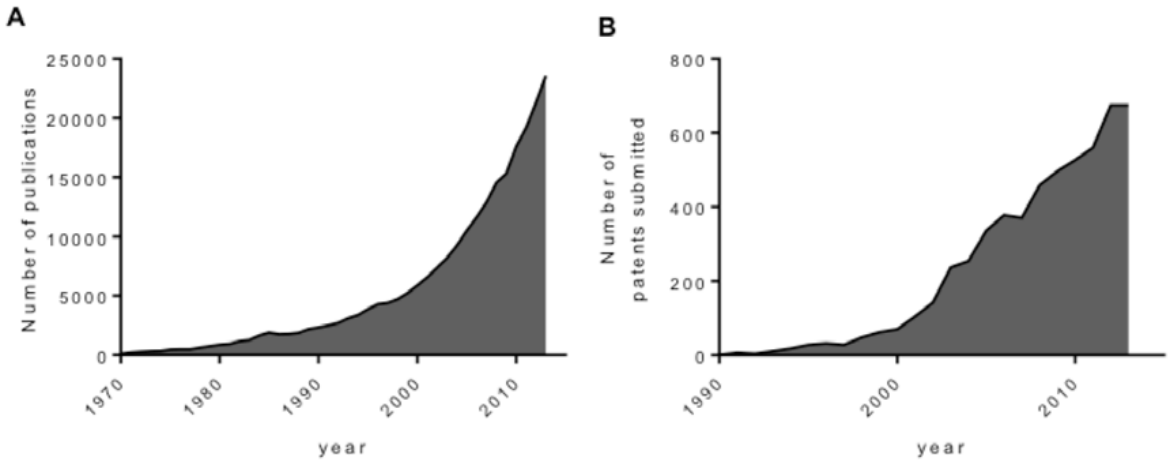

Fig. (1). Number of publications and patents submitted relating to stem cell corresponding to their publication year. Fig. (**1a**), number of publications regarding " stem cell" returned corresponding to the publication year using NCBI biomedical search site (pubmed.gov [77]). Fig. (**1b**); number of patents submitted worldwide over the corresponding years searched using Espacenet worldwide patent search (www.epo.org) [2].

atherosclerosis in the lower extremities or abdomen, usually presented clinically as intermittent claudication [5]. Patients are initially faced with worsening burden from chronic pain whilst walking, to non-healing ulcers, gangrene and finally potential limb loss [6]. Increased risk of other cardiovascular diseases (CVD) events are high for instance PAD is the third leading cause of atherosclerotic cardiovascular morbidity. As
7-8% of the population in Europe [4] is predicted to suffer from PAD, this has a significant consequence on the econ- omy and health care systems [6]. Restoring perfusion in PAD patients to improve blood flow to the ischemic tissue would alleviate the pain and also offer the best possible chance of preventing amputation. Stem cells have the ability to promote collateral vessel growth, which would achieve the aspired therapeutic angiogenesis [7].

SOURCES OF STEM CELL

A number of stem cell and progenitor cell populations have the potential to be employed to aid cardiac repair or relieve peripheral ischemia. There are disadvantages and advantages for each cell type and some such as embryonic stem cells have further ethical limitations to overcome. The health status of the donor patient has a great impact on the number and effectiveness of stem cells [8]. We briefly de- scribe some of the sources of stem cells.

Endothelial Progenitor Cells (EPCs) have been defined to express CD133+, CD34+ and vascular endothelial growth factor receptor-2 [9]. EPCs have been suggested to potentially bolster neovascularization by differentiating into endothelial cells at the site of ischemia. Isolation of EPC cells were found
to mostly contain a mononuclear cell ($CD14^{+}/CD34^{+}$) sub-
population, interestingly these cell have the potential to aid angiogenesis through release of paracrine factors [10]. There is controversy in use of EPCs in stem cell research as EPC characterization, isolation, and mechanism of action are far from being fully understood and standardized. In addition, elderly, diabetics and patients with cardiovascular disease all

have low EPC numbers [11]; moreover the angiogenic capac- ity of their EPCs is significantly reduced thus limiting the therapeutic usefulness.

CD133+ Cells. Early hematopoietic stem cells (HSCs) and EPCs both express the cell surface antigen CD133 and both synergistically enhance vascularization of ischemic tissues by differentiating into endothelial cells at the site of ischemia. CD133+ cells numbers are severely limited for therapeutic value, very small proportion (approximately 1%) of bone marrow cells (BMCs) are CD133+. Furthermore CD133+ cells can't be expanded *ex vivo* [12].

Mesenchymal Stem Cells (MSCs) are routinely isolated from bone marrow, selected and expanded in culture. MSCs have the potential in theory to differentiate into any of the mesodermal linage which includes smooth, skeletal and car- diac muscle. However, the ability of MSCs to differentiate into cardiomyocytes is rare *in vivo*. MSCs may have a posi- tive paracrine effect by secreting anti-inflammatory, anti- apoptotic and pro-angiogenic factors [13].

Resident Stem Cells A pool of resident cardiac progenitor cells (CPCs) have been identified in the heart that can differ- entiate into new cardiomyocytes [14], endothelial cells or smooth muscle cells [12], thus changing the textbook-concept of the terminally differentiated heart. CPC population com- prises of cardiosphere-dervived cells (CDCs), cardiomyocyte progenitor cells (CMPCs) and c-kit+ cardiac stem cell (CSCs) [15]. CPCs have the potential advantage of fully replacing damaged myocardium. However, the disadvantages of using CPCs include low yield, specialist requirement to obtain car- diac biopsy and slow amplification [16].

Vascular Resident Stem Cells. Similar to the heart, all three layers of the vasculature (intima, media and adventitia) contain a pool of resident progenitor cell populations which include EPCs, MSCs, CD34+ and Sca-1+ [17, 18] Likewise, these cells have the potential to enhance neovascularization.

Bone Marrow Mononuclear Cells (BMMSCs) are popular candidates for cell based therapy owing to their rela-

tively ease and amplitude of harvesting under GMP condi- tions. Moreover, BMMNCs comprise of HSCs, MSCs and EPCs, therefore ensure the synergetic advantages of various stem cell populations. BMMNCs have the potential ability to differentiate into cardiac or endothelial cell types, providing paracrine signals [16].

Embryonic Stem Cells (ESCs) are totipotent stem cells harvested from the inner cell mass of blastocysts (4-5 days post fertilization). ESCs have the immense potential to dif- ferentiate into derivatives of all the three cell types ectoderm, endoderm and mesoderm, provide a possible source for car- diomyocytes or endothelial cells. Importantly, human ES cell-derived cardiomyocytes are able to couple electrically with the host myocardium as well as displaying similar struc- tural and functional properties. The major obstacles are the ethical issues related to the use and destruction of human embryos. Nevertheless, clinical trials in the US on hESC have been approved for treating spinal cord injury and macu- lar degeneration [19].

Induced Pluripotent Stem Cells (iPSCs) may hold the key to bypassing ethical issues in ESCs [10]. This technol- ogy reprograms adult differentiated cells to pluripotency, matching the potential and capability of ESCs. Some of the major limitations of iPSC technology include efficacy of generation rates and potential for mutation [20].

PRE-CLINICAL STEM CELLS THERAPY TO TREAT MYOCARDIAL INFARCTION

In an early study, by Orlic *et al*. HSCs were injected into the infarct boarder zone in mice subjected to coronary artery ligation [21]. Newly formed HSC-derived myocytes were observed that had replaced the infarcted area and importantly vascular structures were evident. There is controversy in the field regarding these experiments, debating whether the HSC injection actually replaced cardiomyocytes directly or via an indirect method such as paracrine signaling or stimulating an endogenous cardiomyocyte progenitor cell pool [12]. Never- theless, it is clear that cardiac function is enhanced through HSC injection. Indeed some studies have shown that condi- tioned media from human MSC can improve cardiac func- tion post-MI [22].

More recent studies have shown that human CPCs are able to survive and differentiate into cardiac cell types after injection into the border-zone minutes after infarction in murine hearts. Most promising, CPCs had a positive impact left ventricle (LV) function, infarct size, vascularization and fibrosis [23].

A more realistic timeline for clinical application whereby administration of CPCs occurred several months post-MI, still resulted in positive effects on LV function [24]. Trans- plantation of clinical scale human ES-derived cardiomyo- cytes that had been successfully cryopreserved was recently investigated. Administration of human ESC-derived cardio- myocytes were effectively engrafted to the myocardium in a non-human primate model of myocardial infarction [19]. Crucially, these were able to provide substantial new cardiac

muscle, that had regular calcium transients and synchronized electrocardiograms, and vascularization of the graft from host vessels [19]. Scaling up the cryopreservation of hESC-derived cardiomyocytes promises a fast and effective methodology that could be widely utilized in medical centers that lack stem cell expertise. Such is the promise of this technology clinical trials have been raced through without complete understanding of the exact mechanism [12]. Small trials with a vast range of different conditions have taken place, not all of which have been positive. However, taken together with the growing ex- perimental evidence from *in vivo* models stem cell therapy is still a viable method to treat MI [25]. Studies are now investi- gating ways in which the survival of the transplanted stem cell population can be enhanced in the myocardium. Currently, early pre-clinical *in vivo* models provide information on po- tential future patents. Inhibition of the Renin pathway [26] protection by enhancement of the pro-angiogenic receptor Notch 1 [27], and overexpression of myocardin, a transcrip- tion co-activator [28] are all approaches adapting the host en- vironment to potentiate the effect of the stem cells. Other tac- tics consider modifying the progenitor cells themselves. Inhi- bition of HDAC4, involved in transcription regulation [29] or inositol hexakisphosphate kinases (IP6Ks) to enhance AKT [30] are two current trends published this year.

PRE-CLINICAL STEM CELL THERAPY TO TREAT PAD

Over 15 years ago, isolation of endothelial progenitor cells (CD34+) from human peripheral blood was described to differentiate into endothelial cells and incorporate into the vasculature of the experimental ischemic limb [31]. Shortly afterwards, Hamano *et al.* demonstrated that bone marrow injected into the ischemic muscle could induce angiogenesis promoting blood flow recovery in rodent ischemic model via elevated bFGF, IL-1β levels and possible stem cell incorpo- ration [32].

Over the preceding years studies have taken place to at- tempt to improve the effectiveness of stem cell therapy in the ischemic limb, these include ways to initiate resident stem cell populations, modify exogenous stem cells, or change the host environment. MSCs can release cytokines such as VEGF and bFGF to promote recovery [33]. Mobilization of resident hematopoietic stem cell niche by combined treat- ment of G-CSF and parathyroid hormone (PTH) improved blood flow recovery in hindlimb ischemia [34]. A similar beneficial effect of PTH was observed in MI model [35]. Combination of mural and endothelial cells derived from ES cells provided an alternative method of enhancing the thera- peutic benefit. Endothelial and mural cell differentiated from embryonic cells-positive for VEGFR2 and were incorporated into the host vasculature as endothelial cells and mural cells improving vessel integrity thus benefiting recovery of the ischemic hindlimb [36]. Alternatively, priming hMSCs to induce VEGF and HGF secretion potentiated stem cell therapeutic effectiveness [37].

An encouraging development for PAD treatment is the use of CTX0E03 a clinical grade human neural stem cell line, which has been previously shown to be beneficial in pre-clinical stroke models by promoting neurogenesis and angiogenesis [38]. Dose dependent improvements in blood flow recovery was observed in mouse model of PAD (hind limb ischemia), suggesting that CTX0E03 may have wider benefits than treatment of stroke [39]. Patents for use of CTX0E03 in treating PAD have been submitted and a clini- cal trial is underway both of which will be discussed later.

Another example of how the host microenvironment can be altered to enhance the effectiveness of BMC therapy is through co-treatment with antioxidants to reduce oxidative stress, inflammatory cell infiltration in the ischemic limb as well as enhancing plasma NO bioavailability [40].

OXIDATIVE STRESS

Oxidative stress is a hallmark of cardiovascular diseases, strong correlations have been found in MI, PAD, stroke, atherosclerosis, LV hypertrophy, intermittent claudication, critical limb ischemia, and insulin resistance [41, 42].

Oxidative stress involves the generation of reactive oxy- gen or nitrogen species (ROS/RNS), for example superoxide, peroxides, hydrogen peroxide and peroxynitrite to name a few. High levels of ROS/RNS have long-term detrimental effects on cellular function under pathological conditions, but are also recognized as pivotal to cellular signaling in physiological pathways [43]. ROS/RNS can regulate cellular signaling through oxidative post-translation modification of cysteine residues on key proteins. The distinct properties of cysteine residues allow a range of reversible or irreversible oxPTMs, which are largely dependent upon the level of ROS/RNS or antioxidants. Thus, cysteine residues act as sensors detecting ROS/RNS levels providing a mechanistic switch to control protein function [44]. There are a number of antioxidant pathways within the cell to balance the cellu- lar and microenvironments (redox state) such as superoxide dismutase, catalase, glutathione peroxidase, peroxiredoxins, and sulfiredoxin [44].

Of interest to this review, oxidative stress can perturb tissue homeostasis by damaging stem and progenitor cells, leading to aberrant cell proliferation and anomalous differen- tiation patterns in the affected tissue [45].

ROS levels correlate with stem cells (SCs) differentiation capability. High ROS levels are associated with greater differ- entiation of SC, whereas low ROS levels are thought to be protective towards SC by maintaining them in a quiescent state [46]. Comparison of mature endothelial Cells (ECs) with EPCs suggests that EPCs have lower ROS levels as a result of higher antioxidant (MnSOD, catalase and glutathione peroxi- dase) expression. Low ROS levels are thought to preserve EPCs undifferentiated and self-renewing properties essential for EPCs ability to aid in treatment of disease. Whereas, cyto- kine stimulation (e.g. G-CSF) of HSC mobilization into the circulation is mediated via ROS signaling [47]. Redox signal- ing plays an important role in modulating SC function, upset- ting of the redox homeostatsis outside a narrow window may be detrimental to SC function [48]. Exploiting the fine tuning through regulation of antioxidant and ROS generating en- zymes may provide a therapeutic advantage to aid stem cell therapy.

ENHANCING RETENTION OF STEM CELLS

Crucial to successful stem cell therapy is the strategy for cell delivery. Adequate numbers are needed to be supplied to the organ and these numbers need to be retained. Delivery approaches to the heart are more complicated than peripheral muscle. Intravenous MSC therapy is the easiest and most practical, however a major drawback are stem cells becom- ing confined in the pulmonary circulation [49]. Transendo- cardial injection provides a low invasive method of stem cell delivery. Stem Cells are directly delivered to the infarcted region using a catheter guided by fluoroscopic guidance or electroanatomic mapping [50]. Cardiac perforation or ar- rhythmias are risk factors that require managing. In contrast, direct intramyocardial injection allow direct visualization of the infarcted myocardium, and perforation can be controlled [51]. However, this method is highly invasive requiring a thoracotomy or sternotomy. Intracoronary infusion of stem cells using a standard over-the-wire balloon angioplasty al- lows a brief period facilitating stem cell retention in the myocardium while the balloon is inflated to stop blood flow [50]. Although, reduced blood flow in the ischemic muscle may prevent effective stem cell delivery; in addition inflation of catheter-balloon may cause further ischemia. However, angioplasty techniques are common procedures for cardiolo- gists, in addition these techniques are now used as front line methods in rapid treatment for acute MI upon presentation in emergency rooms. Therefore, this method of delivery could be dovetailed into current treatment strategies.

The local microenvironment is pivotal to the cell reten- tion as this can impact on cell adhesion, migration and stem cell survival [12]. Co-administration or priming the muscle prior to stem cell delivery could modify the microenviron- ment to a more favorable status for stem cell function. Alter- natively, genetic manipulation or pharmacological treatment of the stem cells may also have beneficial effects. BM-MSCs were genetically modified to express the anti-apoptotic and anti-inflammatory enzyme, heme oxygenase-1 (HO-1). Im- proved cardiac function was observed in a swine MI model 3 months after treatment with HO-1 transfected-MSCs [52].

PATENTS AND PUBLICATIONS

Some of the recent patents that have submitted to utilize stem cells for the treatment or that can benefit patients with MI or PAD are reviewed below and further summarized in Table **1**. The expected therapeutic aims of the patents are to improve retention of stem cells to the site of ischemia, stabi- lization, efficacy by synergistic mechanism such as paracrine secretion.

STEM CELL- BASED THERAPY

The patent WO2013126590 [53] describes the use of a cell population comprising of CD34+ stem cells isolated

Table 1. Patents Submitted for Treatment of Myocardial Ischemia or Peripheral Artery Disease by Stem Cell Therapy

Patent Number [Reference]	Title	Inventors/Assignees	Published Date	Description
Stem cell				
WO2013126590 [53]	Pharmaceutical composition comprising CD34+ cells	Palmer, L., Motlagh, D., Cohen, A., Amrani, D.L.	2013	Treatment of ischemic conditions and diseases using a cell population comprising CD34+ cells isolated from peripheral blood of a subject
US20040258670 [54]	Introducing enriched human endothelial generating cells and mesenchymal stem cells; enhancing vasculogenesis and collateralization around blocked and/or narrowed vessels	Mary, L., Stephen, H., Vincent, P.	2004	Administration of endothelial precursor or Mesenchymal stem cells enriched for CD133+/CD34+ cells to improve vascularization in the preferred setting of ischemic myocardium.
EP2428563 [55]	Vascular/lymphatic endothelial cells	Prosper, F., Verfaillie, C.M., Lopez-Aranguren, X., Claver, C.C., Luttun, A.	2012	Method to differentiate cells into more than one embryonic lineage
WO2014022373 [56]	Treatment of pulmonary arterial hypertension with mesenchymal stem cells	Jeffs, R., Petersen, T., Ilagan, R.M., Wade, M.	2014	Method for treating or preventing vasculopathy administrating pharmaceutical composition comprising mesenchymal precursor cells
US20110250182 [57]	Angiogenesis using placental stem cells	Abbott, S., Edinger, J.W., Francki, A., Hariri, R.J., Jankovic, V., Kaplunovsky, A., Labazzo, K., Law, E., Padliya, N.D., Paredes, J., Wang, J.L./ Anthrogenesis Corporation	2011	Methods of treating individuals having diseases or disorders of the circulatory system, using placental cells

US20130156726 [58]	Endometrial stem cells and methods of making and using same	Ichim, T.E., Meng, X., Riordan, N.H./ Medistem Laboratories, Inc.	2013	Pluripotent stem cells and methods for making and using pluripotent stem cells
US20130315875 [59]	Amnion derived adherent cells	Abbott, S., Edinger, J.W., Francki, A., Hariri, R.J., Jankovic, V., Kaplunovsky, A., Labazzo, K., Law, E., Padliya, N.D., Paredes, J., Wang, J.L./ Anthrogenesis Corporation	2013	Isolation of novel angiogenic cells from amnion (AMDAC) for the treatment of disrupted blood flow in ischemic disease such as ischemic limb or myocardium Cells are adherent to tissue culture plastic, OCT-4-., CD49f+, CD90+ and HLA-G-.
US8617538 [60]	Mesodermal-like cell population for treating ischemia in mammals	Zoldhelyi, P., Willerson, J.T., Liu, Q., Chen, Z.Q./ Board Of Regents Of The University Of Texas System	2013	Compositions containing mesodermal-like multipotent mammalian mononuclear cells used for treating ischemia. CD34 and M-cadherin
WO2010089605 [61]	Treatment of limd ischemia	John, S., Erik, M., Paolo, M R. Ltd.	2010	The use of neural stem cells in the manufacture of a medicament for the treatment of a patient suffering from peripheral arterial disease. The invention is particularly suited for treating limb ischemia or Buerger's disease
Modification of host environment or stem cells				
US8455435 [62]	Combination of granulocyte-colony stimulating factor (G-CSF) and DPP-IV inhibitors like Vildagliptin or Sitagliptin	Franz, W.M., Theiss, H., Zaruba, M.M., Brunner, S./ Ludwig-Maximilians-Universitat Munchen	2013	Uses and methods of parathyroid hormone (PTH, and/or parathyroid hormone-related peptide (PTHrP), for recruiting stem cells into tissue suffering from ischemia

Table (1) contd.…

Patent Number [reference]	Title	Inventors/Assignees	Published Date	Description
US20130236433 [64]	Methods, compositions, cells, and kits for treating ischemic injury	Webster, K.A.	2013	Preconditioning of the ischemic tissue with hypoxia-regulated human VEGF and human IGF-1, prior to stem cell transplantation
WO2011011092 [66]	Methods and compositions to reduce oxidative stress	Messina, L.M.	2011	Therapeutic applications for compositions that reduce the level of oxidative stress on cells using stem cells
WO2011053896 [68]	Hypoxia regulated conditionally silenced aav expressing angiogenic inducers	Webster, K.A.	2011	A composition comprising of a conditionally silenced associated viral vector (AAV) encoding at least one of a list of pro-angiogenic factors, growth factors, cytoprotective/cell survival, cellular migration factors and anti-inflammatory factors, in the presence of a hypoxia response element (HREs)
US8343485 [69]	Compositions and methods of vascular injury repair	Andrew, L.P., Robert, A.P.	2013	A sterile pharmaceutical compositions comprising of CD34+ enriched HSC containing a CXCR-4+ subpopulation stabilized with addition of serum.

from peripheral blood. The invention provides a pharmaceu- tical composition comprising of CD34+ cells, a plasma pro- tein and an isotonic solution. Methods of obtaining CD34+ cells from a subject are also provided, illustrating all the steps from promoting mobilization of CD34+ cells from bone marrow and collection of the mobilized CD34+ cells from peripheral blood (which optionally involves apheresis). In some embodiments, the method further includes an en- riching step in which CD34+ cells are separated from CD34- by employing specific antibodies or antigen-binding frag- ments. Pharmaceutical compositions described in the patent are to be administered in an amount effective to increase development of blood vessels in the damaged tissue or to repair the tissue in the subject. The pharmaceutical composi- tion comprising the cells is formulated for different types of administration, such as parenteral, subcutaneous, intrave- nous, intramuscular, intra-arterial, intrathecal, or intraperito- neal, via nasal, spray, oral, aerosol, rectal, or vaginal admini- stration. Cells obtained through these methods can also

be administered via a cell delivery matrix. This patent also cov- ers association of the stem cell population with a second moiety, such as a therapeutic agent or a diagnostic agent.

The invention US20040258670 [54] described in this patent relates to delivering a therapeutic quantity of CD133+/CD34+ enriched human MSCs and/or endothelial generating cells. The patent covers isolation and enrichment of CD133+/CD34+ endothelial precursor cells or MSC pref- erably from umbilical cord blood but also covers isolation from peripheral blood and bone marrow. The preferred use of this invention is to enrich endothelial generating cells prior to administration and expansion in culture. This inven-

tion is designed to treat ischemic myocardium by increasing blood flow to the ischemic region but also covers treatment of other ischemic tissue for example ischemic limb. Route of administration includes intravenous injection or infusion in close proximity to the ischemic tissue to facilitate migration of the cells to the ischemic tissue such as an intracardiac infusion. The patent also covers genetic manipulation of the enriched endothelial generating cells to additionally express a recombinant polypeptide such as VEGF.

The invention described in the patent EP2428563 [55] relates to methods and compositions for differentiation of Multipotent Adult Progenitor Cells (MAPCs) towards the endothelial lineage with arterial, venous and lymphatic endo- thelial characteristics, this will be beneficial *in vivo* with the differentiation to vascular cells such as arterial or venous cells. MAPCs are non-embryonic, non-germ and non- embryonic germ cells that can differentiate into ectodermal, endodermal and mesodermal cells types. They are positive for telomerase and Oct-3A (Oct-3/4), and isolated from bone marrow, brain, muscle, placenta, umbilical cord and cord blood, liver, spinal cord, blood or skin. MAPCs can differen- tiate *in vivo* where they can form vascular cells, such as arte- rial or venous cells. MAPCs are capable of extensive culture without loss of differentiation potential and show efficient, long term, engraftment and differentiation along multiple developmental lineages *in vivo* without evidence of teratoma formation. MAPCs cultured in the presence of VEGF165 were found to acquire endothelial cell markers, including VEGF-R1 and 2, Tie-1, Tie-2, KDR, Flt-1, CD26, CD105, avp3, CD34, VE-cadherin and von Willebrand Factor. They also had increased expression of markers for arterial (Hey-2,

Dll-4, EphrinB2 and EphrinB1) and venous (EphB4) endo- thelium, demonstrating the potential for arterial and venous endothelial differentiation of these cells. A subset of the population of differentiated cells expressed smooth muscle actin, a marker of smooth muscle, showing that MAPCs can differentiate into both endothelial cells and smooth muscle cells. Either autologous, allogeneic or xenogeneic cells can be administered to a patient, moreover in undifferentiated, terminally differentiated or in a partially differentiated form, genetically altered or unaltered, by direct introduction to a site of interest, on or around the surface of an acceptable matrix, systemically or in combination with a pharmaceuti- cally acceptable carrier in order to repair, replace or to pro- mote the growth of existing and new blood vessels.

The patent WO2014022373 [56] describes a pharmaceu- tical formulation of MSCs, which can be isolated from autologous and/or heterologous bone marrow and its admini- stration with/without prostacyclin for treating and preventing peripheral arterial disease. This method also includes the use of MPC-derived conditioned culture medium or the MSCs- conditioned culture medium pre-treated with prostacyclin. In some embodiments the formulation also contains endothe- lial precursors cells (EPCs) that are transformed with a nu- cleic acid that increases the expression or biological activity of a protein selected from the following group: endothelial nitric oxide synthase (eNOS), heme oxygenase (HMOX1) and prostacyclin synthase (PTGIS).

The patent US20110250182 [57] provides methods of using PDACs (placenta derived adherent cells), to promote angiogenesis, and to treat diseases or disorders of the circula- tory system (for example Ischemic Diseases) by improving angiogenesis. Disruption of placental tissue using enzymatic digestion or perfusion allows for the isolation of the desired PDACs. Administration of PDACs can be implanted alone or in combination with a matrix by injection, infusion and by delivery via catheter. PDACs could be incubated or cultured in the presence of factors that stimulate stem or progenitor cell differentiation according to a cardiogenic, angiogenic, hemangiogenic, or vasculogenic pathway, such as growth factors, chemokines, cytokines, cellular products, demethy- lating agents, and other factors which are known to stimulate cell trans-differentiation. Inventors report that the control of the trans-differentiation can be assessed by evaluating the expression of at least one of the following markers such as cardiomyosin, skeletal myosin, or GATA4, or by functional parameters as the acquisition of a beating rhythm which can be spontaneous or otherwise induced, or by the ability of cell engraftment into the cardiac muscle of the patient without inducing arrhythmias. The number and type of cells collected from a mammalian placenta can be moni- tored, for example, by measuring changes in morphology and cell surface markers using standard cell detection tech- niques such as flow cytometry, cell sorting, immunocyto- chemistry, fluorescence activated cell sorting (FACS), magnetic activated cell sorting (MACS), by examination of the morphology of cells using light or confocal microscopy, and by measuring changes in gene expression by PCR and gene expression profiling. Different preparation of placen-

tal cells, obtained from different subjects, can be stored in a dedicated cell bank for long-term storage. PDACs could be genetically engineered to produce recombinant or exogenous cytokines associated with and they can be conditionally im- mortalized by transfection with any suitable vector contain- ing a growth-promoting gene. Kits ready to use for the treatment of MI, provide a therapeutic cell composition comprising of PDACs, which can be prepared in a pharma- ceutically acceptable form, for example by mixing with a carrier, and an applicator. The kits are suitable for the treat- ment of an individual who has a disease or disorder of the circulatory system which would allow this therapy to be used in wider medical centers.

The invention US20130156726 [58] describes the use and isolation of pluripotent stem cells to induce *in vitro, ex vivo* and *in vivo* cell trans-differentiation into various cell lineages and to produce conditioned medium. The use of adult stem cells in therapy is limited by their availability, invasiveness of extraction, and in some cases limited prolif- erative capacity, it is also necessary to avoid karyotypic ab- normalities and potential oncogenic transformation during *in vitro* culture, this patent addresses these critical issues. The invention describes isolated and purification of undifferenti- ated mammalian pluripotent stem cells obtained from endo- metrium, endometrial stroma, endometrial membrane or menstrual blood. These cells retain the ability to differentiate into one or more different cell types and thus offers the op- portunity to treat a range of conditions. The conditioned me- dium can potentially stimulate cell survival and viability, growth, proliferation and differentiation of totipotent, pluri- potent, multipotent or differentiated stem cell. It has also the ability to stimulate and to enhance hematopoiesis and/or to inhibit, reduce and limit inflammation. The patent also de- scribes a kit that can be used to readily access pluripotent stem cells, which would benefit medical centers without spe- cialist stem cell isolation expertise and equipment.

The patent US20130315875 [59] provides novel angio- genic cells isolated from amnion, called “amnion derived adherent cells” (AMDACs). Amnion derived adherent cells are extracted from amnion tissue by enzymatic digestion using one or more tissue-digesting enzymes. The number and type of cells collected from amnion can be monitored, for example, by measuring changes in morphology and cell sur- face markers using standard cell detection techniques such as immunolocalization, flow cytometry, cell sorting, immuno- cytochemistry, fluorescence activated cell sorting (FACS), magnetic activated cell sorting (MACS), by examination of the morphology of cells using light or confocal microscopy, and by measuring changes in gene expression using PCR and gene expression profiling. These techniques can also be used to identify cells that are positive for one or more particular markers. The patent covers: i) differentiation of AMDACs, to exhibit at least one characteristic of an endothelial cell, a myogenic cell, or a pericytic cell; ii) genetic modification of AMDACs, to additionally produce a nucleic acid or polypep- tide of interest directly or to produce a differentiated cell (osteogenic cell, myocytic cell, pericytic cell, or angiogenic cell) that produces a nucleic acid or polypeptide of interest;iii) a range of compositions comprising AMDACs, which can be used in the clinical practice, for instance in pharma- ceutical compositions matrices and scaffolds, and media conditioned by amnion derived adherent cells; iv) Condi- tional immortalization of AMDACs by transfection with any suitable vector containing a growth-promoting gene. The patent suggests that the benefits from these cells can be used in a number of conditions including ischemic disease to in- duce angiogenesis and differentiation into cardiac or endo- thelial cells.

The patent US20110104124 [60] reports various compo- sitions containing an effective amount of mesodermal-like multipotent mammalian mononuclear cells that express both CD34 and M-cadherin cell surface markers. These composi- tions can be used in different embodiments such as prevent- ing, treating or reducing the severity of tissue ischemia or an ischemia associated disorder. The method comprises of iso- lation of cells from autologous/heterologous bone marrow displaying a positive expression for CD34 and M-cadherin (both 95% of positive surface expression). A minor fraction (10%) of this population can also express Pax3 or Pax7. Subsequent administering directly to an ischemic tissue siteor an adjacent site, wherein the dose comprises of 10^2-10^{10}
cells bearing both CD34 and M-cadherin cell surface mark- ers. The employment of this cell population may include the *in vivo* repopulation with new myocytes and vascularization of the ischemic site. By administrating the said cell popula- tion, functional new blood vessels formation can be im- proved, and consequently one or more ischemia symptoms. In addition, the method can comprise the additional admini- stration of angiogenic cytokines to the ischemic or adjacent ischemic tissue, specifically the myocardium or ischemic limb, to provide synergetic benefit.

This patent additionally utilizes the invention as a diag- nostic marker detecting the level and/or distribution of CD34+/M-cadherin+ mesodermal-like precursor cells in a mammalian tissue sample. This enables indication of self- repairing ability. Alternatively, it can be used to measure success of stem cell transplant.

The patent application WO2010089605 [61] describes the use of neural stem cells for the treatment of patients suf- fering from peripheral arterial disease. Neural stem cells offer an alternative to bone marrow derived stem cells pro- viding a scalable, safe and potent allogenic treatment. Neural stem cells are derived from ventricular and hippocampal regions of fetal and adult brain or derived from ESCs which have undergo differentiation to neural stem cells.

The invention US8455435 [62] described in this patent relates to uses and methods of parathyroid hormone (PTH), and parathyroid hormone-related peptide (PTHrP), for re- cruiting stem cells into tissue suffering from ischemia. The patent also covers the use of a combination of G-CSF and a dipeptidyl peptidase IV (DPP IV) inhibitor/antagonist. The DPP IV antagonist/inhibitor can be used in combination with G-CSF or a G-CSF fragment. Additionally the patent relates to a pharmaceutical composition comprising of PTH, and PTHrP and G-CSF, with and without DPP IV inhibitor/antagonist.

As discussed previously the influence of parathyroid hormone on the HSC niche in the bone marrow strengthens survival and self-renewal of hematopoietic stem cells. It is known that PTH has cardiovascular functions such as vaso- dilatation, increased

myocardial blood flow, hypotensive effects, myocardial hypertrophy, positive chronotropic and contractility effects. PTH has the ability to work with G-CSF in mobilizing circulating progenitor cell numbers and tissue perfusion [34]. Whereas, inhibition of DPP-IV significantly improved cardiac function after MI in a mouse model [63], in correlation with increased mobilization and a higher rate of endothelial cell proliferation. This treatment is useful for the recruiting of stem cells from the bone marrow into the periphery and, further, is useful for the prevention and treat- ment of ischemia.

The invention US20130236433 [64] described in this patent is based on the discovery that stem cells, when in- jected into ischemic tissue of mammals, can be protected by preconditioning of the ischemic tissue with hypoxia- regulated human Vascular endothelial growth factor (VEGF) and human Insulin Growth Factor-1 (IGF-1) [65]. In the pat- ent compositions, cells, kits and methods are reported. They include the use of hypoxia-regulated, inflammation-responsive conditionally-silenced nucleic acids to promote stem cell survival and vascularization in ischemic disease. It was hy- pothesized that tissue engineering with hypoxia-regulated growth and survival factors may reduce toxicity, before stem cell injection, thus promoting cell survival and the efficacy of the therapy. By using such combination of gene and stem cell therapy, it has been proven to improve both cell survival and tissue reperfusion. The invention also describes a typical method of treating tissue already injured or at risk of ischemic injury in a subject. For instance, the administration to the patient of a therapeutically effective amount of a com- position which includes at least one nucleic acid encoding at least one cell survival factor for protecting stem and progeni- tor cells from ischemia, and/or alternatively at least one nu- cleic acid operably linked to a hypoxia-regulated promoter and subsequently administration to the subject prior to injection stem and/or progenitor cells.

The invention WO2011011092 [66] is based on the un- derstanding that oxidative stress is a critical factor regulating stem cell function. A physiological balance of the redox state in a cell or a tissue can be achieved by administering a com- position or a combination of agents resulting in reversal or reduction of oxidative cell or tissue injury. These include the administration of one or more activators of an anti-oxidative pathway, co-factors, anti-oxidants or free radical scavengers; for example L-Arginine, N-acetyl-cysteine, and/or L- Cysteine, BH4 (tetrahydrobiopterin) prior to stem cell ther- apy.

The patent, US20130131152 [67], discusses a method relating to the treatment of hypoxia and associated conditions, especially directional angiogenesis for therapeutic ad- vantage. The method uses a conditionally silenced adeno-

associated vector (AAV) expression system which expresses the desired factor. The method uses a combination of silenc- ers such as NRSE and TOAD/FROG to regulate growth fac- tor expression in hypoxia and ischemic affected tissues to give a more rapid and efficient revascularization and tissue salvage before, during or post injury. This invention can be used to replace the preclinical and gene therapy models fo- cused on angiogenesis which are primitive in comparison due to the inadequate delivery vehicles and constitutively active gene expression that provides non-directional vessel growth.

The AAV gene expression is regulated by the promoter, for example phosphoglycerate kinase (PGK) promoter, in conjunction with a combined cassette of hypoxia response element (HRE) along with the combination of silencer ele- ments, which are activated by ischemic conditions. The ad- ministration of pro-angiogenic growth factor genes such as; endothelial growth factor, fibroblast growth factor (FGF), platelet derived growth factor (PDGF), insulin-like growth factor (IGF), epidermal growth factor (EGF), transforming growth factor (TGF), hepatocyte growth factor (HGF), pro- liferin, angitropin, angiopoietin, vascular endothelial growth factor (VEGF), transforming growth factor beta (TGF-beta) or erythropoietin (EPO) is a possibility. Further examples of factors are c-kit ligand/ stem cell factor, insulin, insulin like growth factor-l (IGF-l), nerve growth factor (NGF), bone morphogenetic protein (BMP), leukemia inhibitory factor (LIF), brain derived neurotrophic factor (BDNF), interleu kins such as but not limited to interleukin 3 (IL-3), interleu- kin 6 (IL-6), interleukin 7 (IL-7), and interleukin l3 (IL-l3), stromal derived factor (SDF), stem cell factor (SCF), granu- locyte colony stimulating factor (G-CSF), and matrix metal- loproteinase (MMP) inhibitors.

The conditionally silenced vectors may be used to treat hypoxia associated condition such as MI in order to reduce the severity of cell/tissue damage during the post ischemic period.

The methods outline ways to isolate subpopulations in- cluding but not exclusively adherence to plastic culture dishes followed by culture in a selective medium, separation via specific cell markers whether it be due to their expression or lack of expression, markers such as: CD133, CD45, CD34, CD31, Sca-1, c-kit, Thy1, and CD105. The stem cells used in this invention can be autologous, allogeneic or xeno- genic. The choice of which depends on the urgency of the need for treatment.

The patent, WO2011053896 [68], outlines methods for treating hypoxia and the conditions associated with it by directional angiogenesis/arteriogenesis using conditionally silenced vectors such as adeno-associated virus (AAV) or lentiviral vector, which express the required factor for direc- tional angiogenic manipulation. The system will transport the desired genes to target cells such as skeletal and cardiac myocytes, endothelial cells, smooth muscle cells, pericytes and stem cells. Different stem cells can be used such as mus- cle, cardiac, mesenchymal, hematopoietic or endothelial pro- genitor stem cells. The patent describes the purification,*ex vivo* culture, and transfection/infection with conditionally silenced vectors.

This invention is potentially important for the treatment of ischemic diseases and conditions, where new vessel growth is vital for the repair and recovery of affected cells, tissues and microenvironments. The treatment relies upon hypoxic conditions to regulate the AAV vector through a silencer element, which includes but is not limited to NRSE and TOAD/FROG in a heterogeneous combination, which are activated in ischemic conditions while inhibited during aerobic conditions. The vector also depends upon promoters such as phosphoglycerate kinase (PGK) to regulate pro- angiogenic gene expression alongside hypoxia response ele- ments (HREs), inflammatory response elements (IREs) or shear-stress activated elements (SSAEs). The treatment can be used in concert with a specific drug regime such as vaso- dilators (adenosine, nitric oxide donors such as prostagland- ins or antioxidants), to have additional benefits.

Current therapeutic methods to induce angiogenesis have flaws in their methods; for example, inadequate delivery vehicles that extinguished gene expression too early and the delivery is unregulated so genes do not provide the direc- tional cues needed for the correct new vessel growth. This invention could be the answer to deliver a more efficient and robust therapeutic cue to regulate angiogenesis.

METHODS AND DIAGNOSTIC

The patent US8343485 [69, 70] provides to a sterile pharmaceutical formulation composed of an enriched CD34+ population that contains a subpopulation of CD34+/CXCR-4 cells holding a CXCR-4-mediated chemotactic activity, the methods of preparation and its use for the treatment of vas- cular-injury repair, including MI. Moreover, the pharmaceu- tical composition contains a stabilizing amount of serum that is characterized as having the said properties for at least 24 hours after that acquisition of the chemotactic hematopoietic stem cell product, when tested *in vitro* after passage through a catheter.

The chemotactic hematopoietic stem cell factor is prepared by isolating and purifying CD34+ hematopoietic stem cells from a population of mononuclear cells isolated from autolo- gous bone marrow and peripheral blood after treatment with a hematopoietic stem cell mobilizing agent (such as G-CSF, GM-CSF or a pharmaceutical acceptable analog or derivate).

The chemotactic hematopoietic stem cell factor contains varying proportions of pure CD34+ cells. The sterile composi- tion patented is formulated for parentheral administration in coronary blood vessel, in myocardium, artery, vein or muscle and it can contain one of the compatible active agent (hema- topoietic stem cell mobilizing agent), such as angiotensin- converting enzyme inhibitor, beta-blocker, a diuretic, anti- arrhythmic agent, anti-anginal agent, anticoagulant, vasoactive agent, fibrinolytic agent, or hypercholesteromic agent.

The sterility of the chemotactic hematopoietic cell prod- uct is confirmed by a multi-step procedure patented in this protocol.

Table (2) contd….

CURRENT & FUTURE DEVELOPMENTS

Reviewing clinical trials offers an informative method to establish the potential, validity, progress and success of claims put forward in patents involving medical innovations. Unlike documentation available for patents it is relatively difficult to accurately establish the exact number of worldwide clinical trials currently taking place in any particular field. There are different publically available open databases on clinical trials where information can be sourced; however the data does not always match between databases. Nevertheless, these data- bases provide a good reflection of the current trends in the usage of this technology. The United States National Institute of Health Clinical Trial database (ClinicalTrials.gov [71]) cur- rently provide >1765 open records of worldwide trials involv- ing stem cells. Within that group, 53 trials are currently re- cruiting patients for stem cells trials involving MI, whereas 27 stated PAD or critical limb ischemia as the target condition

Table **2**. Within the UK there are currently 41 clinical trials ongoing involving stem cells, cardiovascular is the second most common target disease with oncology leading the way (Sourced from Cell Therapy Catapult UK Clinical Trial Data- base [72]). Five of the current trials involving stem cells in- clude MI while one is investigating PAD.

Clinical trial SRCTN65630838, is a prospective, double- blind randomized trial that will enrich bone marrow derived cells using CD133+ selection and test this in patients under- going coronary surgery. This clinical trial appears to be us- ing the invention described in patent US20040258670. Autologous CD133+ stem cells will be transplanted into scarred areas to induce angiogenesis and neomyogenesis. The clinical endpoint will assess left ventricular thickening by MRI, 6 months after injection. Secondary outcome meas- urements include left ventricular function, scarring, troponin I levels and quality of life scores.

Table 2. Ongoing Clinical Trials for Treatment of Myocardial Infarction and Peripheral Artery Disease.

NCT Number	Title	Sponsor/Collaborators Phase	Primary/ Secondary Outcome Measures
NCT00350766	Cell therapy in myocardial infarction	Ministry of health, Brazil Phase 3	Global left ventricular ejection fraction change. death. Acute myocardial infarction, stroke and hospital admission due to cardiovascular cause
NCT00725738	Intracoronary autologous stem cell trans- plantation in ST elevation myocardial infarction: Tracia study	National Heart Institute, Mexico|National Center of Blood Transfusion Mexico. Phase 2/3	Evaluate the mean LVEF increase by magnetic resonance imaging (MRI) at 6 months of follow up between the stem cell group and the control group
NCT01625949	Stem cell therapy in patients with myocar- dial infarction and persistent total occlusion of infarct related artery	All India institute of medical sciences, New Delhi	Left ventricular function
NCT00275977	Treatment of myocardial infarction with bone marrow derived stem cells	Odense University Hospital Phase 1	Safety Change in left ventricular funtion at 4 months followup using contrast enhanced echocardiography
NCT01652209	Relief(A randomized, open labeled, multi- center trial for safety and efficacy of intra- coronary adult human mesenchymal stem cells acute myocardial in Farction)	Pharmicell Co., Ltd. Phase 3	Left ventrical function by MRI
NCT01536106	Rapid delivery of autologous bone marrow derived stem cells in acute myocardial infarction patients.	Totipotent RX cell therapy Pvt. Ltd.|TotipotentRX Corpo- ration Phase 1/Phase 2	Number of adverse events as a measure of safety. Changes in the global left ventricular ejection fraction(LVEF), LV volumes-end systolic volume (ESV) and end diastolic vol- ume (EDV), infarct size, myocardial mass, myocardial viability and regional wall motion abnormalities. Major adverse cardiac events (MACE)/Quality of life
NCT00501917	MAGIC cell-5-combicytokine trial	Seoul National University Hospital Phase 2/Phase 3	Change of left ventricular ejection fraction measured by cardiac MRI Wall motion score index exercise capacity BNP
NCT00437710	Safety and efficacy of bone marrow cell transplantation in humans myocardial infarction	Azienda UnitÌÊ Sanitaria Locale di Piacenza Phase 1/Phase 2	Mortality and morbidity. left ventricular function and re- modeling, baroreflex sensitivity, stress induced myocardial ischemia

Table (2) contd….

NCT Number	Title	Sponsor/Collaborators Phase	Primary/ Secondary Outcome Measures
NCT00650143	Sitagliptin plus granulocyte-colony stimulating factor in acute myocardial infarction	Ludwig-Maximilians - University of Munich|Heinz nixdorf-foundation Phase 2/Phase 3	Change of global myocardial function from baseline to 6 months of follow-up Segmental myocardial thickness and volumes in MR/ extent of non-viable myocardium will be monitored from baseline up to 6 months measured by MRI delayed enhancement
NCT00529932	A trial using CD133 enriched bone marrow cells following primary angioplasty for acute myocardial infarction	Onze Lieve Vrouw Hospital|King's College London	Comparison of changes in myocardial thickening in non-viable akinetic / hypokinetic LV wall segments as determined by cardiac magnetic resonance imaging (cMRI) in treated and control groups
NCT01781390	Safety study of allogeneic mesenchymal precursor cell infusion in myocardial infarction	Angioblast Systems|Mesoblast, Inc.|Mesoblast, Ltd.|Teva Pharmaceuticals USA Phase 2	Frequency of the total major adverse cardiac and cerebrovascular events (MACCE)
NCT01974128	Study to assess the safety and cardiovascular effects of autologous adipose-derived stromal cells implantation in patients during the acute recovery phase of st-elevation myocardial infarction	Ageless Regenerative Institute/Instituto de Medicina Regenerativa, S.A. de C.V. Phase 1/Phase 2	Cardiac improvement primary safety objective
NCT01394432	Estimation study for endocardial mesenchymal stem cells implantation in patients after acute myocardial infarction	Meshalkin research institute of pathology of circulation Phase 3	Reduction in left ventricle systolic volume on 15% mesured by MRI/All-cause death|number of patients with thromboembolic events|number of heart failure hospitalizations/Distance during 6-minute walking test
NCT01969890	Stem cells mobilization in acute myocardial infarction outcome trial	Heart care foundation/A. manzoni hospital/centro cardiologico monzino Phase 3	The composite endpoint of: - All cause death or, - recurrence of myocardial infarction (MI) or, - hospitalization due to heart failure./All cause death and cardiovascular events
NCT00936819	The enhanced angiogenic cell therapy - acute myocardial infarction trial	Ottawa hospital research institute/Canadian institutes of health research (CIHR) Phase 2	Assessment of global LVEF, Assessment of: cardiac wall motion and volumes, time to clinical worsening (TTCW)/Safety measurements
NCT01454323	Intracoronary infusion of bone marrow mononuclear cells in patients with previous myocardial infarction	Fundaciì n PÌYblica Andaluza Progresoy Salud Phase 2	Change from baseline in left ventricular ejection fraction (LVEF), major adverse cardiac events (MACE), functional grade of the new york heart association (NYHA)
NCT00711542	Effects of intracoronary progenitor cell therapy on coronary flow reserve after acute MI	Johann wolfgang goethe University Hospitals/University of Leipzig Phase 1/Phase 2	Improvement of coronary flow reserve in the infarct vessel, Improvement of relative coronary flow reserve, Improvement of global and regional left ventricular ejection fraction Major adverse cardiac events (death, MI, rehospitalization for heart failure, revascularization).
NCT01753440	Allogeneic stem cells implantation combined with coronary bypass grafting in patients with ischemic cardiomyopathy	AHEPA University Hospital Phase 2/Phase 3	Left ventricular ejection fraction. Myocardial segmental perfusion All-cause mortality and all-cause morbidity. Major adverse cardiac and cerebrovascular events
NCT01758406	Transplantation of autologous cardiac stem cells in ischemic heart failure	Royan Institute Phase 2	Death, arrhythmia, hospitalization, ejection fraction. Pro BNP changes. NYHA functional class

Table (2) contd….

NCT Number	Title	Sponsor/Collaborators Phase	Primary/ Secondary Outcome Measures
NCT01615250	Implantation of peripheral stem cells in patient with ischemic cardiomyopathy	Odessa national medical University Phase 1	Change in global left ventricular ejection fraction and regional wall motion score index. Incidence of the major adverse cardiac events
NCT01337011	Intra-coronary versus intramyocardial application of enriched CD133pos autologous bone marrow derived stem cells	Asklepios proresearch/Miltenyi Biotec GmbH Phase 1/Phase 2	Change in left ventricular global ejection fraction measured via echocardiography improvement of 6min walk. Improvement of peak oxygen consumption. Improvement of LV function as measured by cardiac MRI
NCT01693042	Compare the effects of single versus repeated intracoronary application of autologous bone marrow-derived mononuclear cells on mortality in patients with chronic post-infarction heart failure	Johann wolfgang goethe University Hospitals Phase 2/Phase 3	Mortality at 2 years after inclusion into the study. Morbidity at 2 and 5 years after inclusion into the study
NCT01467232	Impact-CABG Trial: Implantation of Autologous CD133+ stem cells in patients undergoing coronary artery bypass grafting	University Health Network, Toronto|Miltenyi Biotec, Inc. Phase 2	Freedom from major adverse cardiac event. freedom from major arrhythmia. Regional myocardial perfusion and function assessed by magnetic resonance scans. Global ventricular function assessed by echocardiographic measures of ejection fraction.
NCT00418418	Combined CABG and stem-cell transplantation for heart failure	Helsinki University Phase 2	Ejection fraction and cardiac function of the heart measured with MRI or PET ischemia area
NCT01946048	Umbilical cord derived mesenchymal stem cells therapy in ischemic cardiomyopathy	Hebei Medical University Phase 1	The examination of heart function. all-cause mortality and morbidity
NCT01913886	Mesenchymal stem cells to treat ischemic cardiomyopathy	PontifÌcia Universidade CatÌlica do ParanÌÁ/Danielle Malheiros.Santa Casa de MisericÌ_rdia de Curitiba, Brazil/FundaÌ¤Ì£o AraucÌÁria, Brazil Phase 1/Phase 2	Change from baseline in left ventricular ejection fraction (LVEF) measured by echocardiogram. Change in quality of life. Changes in exercise capacity Changes in plasma inflammatory markers
NCT01720888	Intracoronary autologous mesenchymal stem cells implantation in patients with ischemic dilated cardiomyopathy	National University of Malaysia/Cytopeutics Pte. Ltd. Phase 2	Change in LV volumn, functional status LV ejection fraction as measured by echocardiogram and MRI after implantation
NCT01670981	An efficacy, safety and tolerability study of ixmyelocel-t administered via transendocardial catheter-based injections to subjects with heart failure due to ischemic dilated cardiomyopathy (IDCM)	Aastrom biosciences Phase 2	Average number of clinical events over 12 months post-treatment Change from baseline to 12 months post-treatment in 6-minute walk test, left ventricular function as evaluated by echocardiography, in quality of life
NCT02057900	Transplantation of human embryonic stem cell-derived progenitors in severe heart failure	Assistance Publique - HÌ«pitaux de Paris Phase 1	number and nature of adverse events Feasibility of patch's generation and its efficacy on cardiac functions
NCT01098591	Meta-analysis of cell-based cardiac studies: Accrue	Medical University of Vienna/ and across Europe	Freedom from occurrence of major adverse cardiac and cerebrovascular events (MACCE), including all-cause death, re-infarction, revascularization and stroke Hard clinical end point Changes in end-diastolic volume, end-systolic volume and ejection fraction

Table (2) contd….

NCT Number	Title	Sponsor/Collaborators Phase	Primary/ Secondary Outcome Measures
NCT02059512	Autologous bone marrow mononuclear cells in the combined treatment of coronary heart disease	St. Petersburg State Pavlov Medical University Phase 3	All-cause mortality associated with the progression of basic disease. Quality of life
NCT01905475	CXCR4 Antagonism for cell mobilisation and healing in acute myocardial infarction (CATCH-AMI)	Polyphor Ltd. Phase 2	Change in LVEF and additional measures of cardiovascular function as determined by MRI Mobilization of stem and progenitor cells Pharmacokinetic outcome Safety of POL6326 by intravenous infusion
NCT01033617	Impact-CABG Trial: Implantation of Autologous CD133+ stem cells in patients Undergoing CABG	Centre hospitalier de l'Universitì© de Montrì©al (CHUM)/Miltenyi Biotec, Inc./ Centre de Recherche du Centre Hospitalier de l'Universitì© de Montrì©al/Maisonneuve-Rosemont Hospital Phase 2	Freedom from major adverse cardiac event: cardiac death, myocardial infarct, repeat coronary bypass grafting or percutaneous intervention of bypassed artery or major arrhythmia Regional myocardial perfusion and function assessed by magnetic resonance scans. Device performance end point: Feasibility to produce from 100ml of bone marrow aspiration a final cell product that contains a target CD133+ cells higher than 0.5 million with a purity superior to 30% and a recovery superior to 10%.
NCT01458405	Allogeneic heart stem cells to achieve myocardial regeneration	Capricor Inc./National Institutes of Health (NIH)/National Heart, Lung, and Blood Institute (NHLBI) Phase 1/Phase 2	Infarct size assessed by MRI
NCT00394498	Stem cell mobilization by G-CSF post myocardial infarction to promote myocyte repair	University of Ottawa/Canadian Institutes of Health Research (CIHR) Phase 2/Phase 3	6 month Left ventricular ejection fraction, myocardial FDG-PET uptake, myocardial ammonia-PET perfusion, 6 week/month left ventricular diastolic and systolic volume
NCT01234181	Clinical study of hypoxia-stressed bone marrow mononuclear cell transplantation to treat heart diseases	Second affiliated hospital, School of Medicine, Zhejiang University	Heart function
NCT01569178	BAMI. The effect of intracoronary reinfusion of bone marrow-derived mononuclear cells(BM-MNC) on all-cause mortality in acute myocardial infarction	Barts & The London NHS Trust Phase 3	Time from randomization to all-cause death/Time from randomization to cardiac death time from randomization to cardiovascular re-hospitalisation incidence and severity of adverse events bleeding by BARC definition
NCT01813045	Angiogenesis and fibrosis in myocardial infarction	University of Edinburgh	The primary outcome is heart function determined by ejection fraction (in %) 6 months following a heart attack. Extent of fibrosis (% late gadolinium enhancement) & blood flow 6 months post-MI, and the correlation with integrin expression at 9 weeks (fluciclatide distribution through the myocardium viewed on CTPET images)
NCT01127113	Inflammatory cell trafficking after myocardial infarction	University of Edinburgh/British Heart Foundation	Change in cardiac MRI signal intensity from baseline after administration of labelled vs. unlabelled mononuclear cells Correlation of myocardial MRI signal intensity change from baseline with markers of systemic inflammation.

Table (2) contd.…

NCT Number	Title	Sponsor/Collaborators Phase	Primary/ Secondary Outcome Measures
NCT02052427	Safety & efficacy of adipose-derived regenerative cells in the treatment of chronic myocardial ischemia (ATHENA II)	Cytori therapeutics Phase 2	Primary efficacy - Change in minnesota living with heart failure questionnaire Secondary efficacy - change in mVO_2. Change in LVESV/LVEDV, Ejection Fraction, perfusion defect, heart failure symptoms, angina, and quality of life.
NCT00950274	Intramyocardial transplantation of bone marrow stem cells in addition to coronary artery bypass graft (CABG) surgery	Miltenyi Biotec GmbH/German Federal Ministry of Education and Research Phase 3	Left ventricular ejection fraction at rest, measured by MRI Change in LVEF as assessed by MRI and echocardiography Regional contractility in the AOI / Change in LV dimensions Physical exercise capacity determined by 6 minute walk test
NCT01267331	Cell therapy in patients with chronic ischemic heart disease undergoing cardiac surgery	Chinese PLA general hospital Phase 1/Phase 2	Major adverse cardiac events Left ventricular function
NCT00984178	Trial of hematopoietic stem cells in acute myocardial infarction	Tecam Group|Hospital General Universitario Gregorio Maraìon Phase 2	The change in left ventricular ejection fraction and left ventricular end-systolic volume relative to baseline measured by magnetic resonance The change in left ventricle end-diastolic volume, segment contractility, wall thickness and intravascular ultrasound reendothelization relative to baseline measured by magnetic resonance and other imaging techniques To determine the safety of the study procedures
NCT01770613	A study of allogeneic mesenchymal bone marrow cells in subjects with st segment elevation myocardial infarction (STEMI)	Stemedica cell technologies, Inc./Mercy gilbert medical center at AZ/Chandler regional medical center at chandler AZ/University of California, San Diego Phase 2	The safety and tolerability of a MBMC intravenous administration during the twelve month study period as determined by major adverse events MACE endpoint.| LV end diastolic and systolic volume Infarct size measured by MRI, with and without contrast (only for patients eligible for MRI Global left ventricular ejection fraction (measured by echocardiography
Peripheral artery disease			
NCT01456819	Intramuscular mononuclear cells and mesenchymal stem cells transplantation to treat chronic critical limb ischemia	National University of Malaysia/Cytopeutics Pte. Ltd. Phase 2	Change in angiogenesis Change in blood supply Change in ulcer size Visual Analog Score Exercise Treadmill Test
NCT02099500	Autologous adipose-derived stromal cell delivered via intramuscular injections for the treatment of critical limb ischemia	Ageless regenerative institute Phase 1/Phase 2	Improvement from baseline in perfusion as measured by ankle-brachial index and collateral artery number/ Number of adverse events reported Improvement from baseline in improvement or resolution of ulcer or gangrene Limb Salvage
NCT01867190	Study to assess efficacy and safety of bone marrow derived stem cells in patients with critical limb ischemia	Lifecells, LLC. Phase 1/Phase 2	To assess the efficacy and safety of intra-arterial infusion and intramuscular injection of ASCT01 on the combined primary endpoint of major amputation (above the ankle) or persisting critical limb ischemia (no clinical or perfusion improvement)

Table (2) contd….

NCT Number	Title	Sponsor/Collaborators Phase	Primary/ Secondary Outcome Measures
NCT02145897	To evaluate the safety and efficacy of IM and IV administration of autologous ADMSCs for treatment of CLI	Kasiak Research Pvt. Ltd. Phase 1/Phase 2	To assess the safety To assess the efficacy
NCT01408381	Intra-arterial infusion of autologous bone marrow mononuclear cells in non-diabetic patients with critical limb ischemia	Fundaciìn PÌ\1blica Andaluza Progresoy Salud/Iniciativa Andaluza en terapias Avanza-das Phase 2	Adverse events Ankle-brachial index Transcutaneous oxygen pressure (TcO_2)/Greater ulcer size Degree of rutherford-becker Perimeter calf muscle faster opacity in infra-popliteal vessels at 6 months com-pared with the basal situation of the patient
NCT01484574	A clinical trial to study the efficacy and safety of different doses of bone marrow derived mesenchymal stem cells in patients with critical limb ischemia due to buergers disease	Stempeutics Research Pvt Ltd Phase 2	Relief of the rest pain Healing of ulcerations or reduction of ulcer area in the target limb|Relief of the rest pain Healing of ulcerations or reduction of ulcer area in the target limb|Pain free walking distance Major amputation free survival Ankle brachial pressure index (ABPI) - measured by Dop-pler Increase in transcutaneous partial oxygen pressure (TcPO2) Quality of life by King's College VascuQOL questionnaire Angiogenesis - collateral blood vessels by Magnetic reso-nance angiogram (MRA) The type of adverse events AE(s), number of AE(s) and proportion of patients with AE(s)
NCT01257776	Human adipose derived mesenchymal stem cells for critical limb ischemia in diabetic patients	Fundaciìn PÌ\1blica Andaluza Progreso y Salud Phase 1/Phase 2	Angiographic assessment of neovasculogenesis (angiogene-sis plus arteriogenesis) Major adverse event (death, target limb amputation) Ankle brachial index/University of Texas Classification at target limb
NCT00488020	Stem cells for treating critical ischemia	Instituto de Molestias Cardio-vasculares Phase 1	Suppress pain and heal ischemic ulcers/improve quality of life
NCT01049919	Safety and efficacy study of autologous concentrated bone marrow aspirate (cBMA) for Critical Limb Ischemia (CLI)	Biomet, Inc./Biomet Biol-ogics, LLC	Time to treatment failure/Perfusion and quality of life meas-urements
NCT01351610	Tolerability and Efficacy of Intravenous Infusion of Autologous MSC_Apceth for the Treatment of Critical Limb Ischemia	Apceth GmbH & Co. KG Phase 1/Phase	Collection of adverse events Safety laboratory values ECG findings Analysis of inflammation markers Comparison of course of haemodynamic and vascular processes
NCT00922389	A clinical trial on diabetic foot using pe-ripheral blood derived stem cells for Treat-ing Critical Limb Ischemia	Beike Biotech India Pvt.ltd Phase 1/Phase 2	Adverse events and laboratory parameters Trans cutaneous partial pressure of Oxygen: $TCpO_2$

Table (2) contd….

NCT Number	Title	Sponsor/Collaborators Phase	Primary/ Secondary Outcome Measures
NCT01216865	Umbilical cord mesenchymal stem cells injection for diabetic foot	Qingdao University Phase 1/Phase 2	Angiographic evaluation of angiogenesis at ischemic limb and pain Ankle-brachial pressure index Wound healing (wound size, wound stage) Walking distance Rate and extent of amputations
NCT01686139	Safety study of stem cells treatment in diabetic foot ulcers	Sheba Medical Center Phase 1/Phase 2	Frequency of adverse events/Healing of all wounds in the target limb
NCT01824069	Treatment CLI nonrevascularizable lower limb with cell therapy	Instituto de Investigaciìn Hospital Universitario La Paz/Hospital Universitario La Paz Phase 1/Phase 2	Safety of inject mesenchymal stem cells in MMII Quality of life of patients after treatment
NCT01916369	Safety trial of CTX cells in patients with lower limb ischaemia	Reneuron Limited Phase 1	Incidence of adverse events
NCT00919516	Autologous bone marrow mononuclear cell implantation for moderate to severe peripheral arterial disease	The vascular and vein center, columbus, OH	Major limb amputation Improved ABI measurements Relief of rest pain Ulceration healing
NCT00411840	Novel therapy of PAD by combined transplantation of BMCs	Heinrich-Heine University, Duesseldorf Phase 1	ABI, walking distance, capillary-venous oxygen saturation, venous occlusion plethysmography
NCT00113243	Safety study of using stem cells to stimulate development of new blood vessels in peripheral vascular disease	Murphy, Michael P., MD/Indiana University School of Medicine Phase 1	Adverse events recorded in the 12 week study period Serious Adverse events recorded for one year Changes in limb perfusion after treatment with stem cells will be assessed with arteriography, blood pressure recordings, oxygen measurements, and wound healing
NCT01558908	Phase I/II trial of endometrial regenerative cells (ERC) in patients with critical limb ischemia	Medistem Inc. Phase 1/Phase 2	Safety Efficacy
NCT01903044	Safety and efficacy of autologous bone marrow stem cells for lower extremity ischemia treating	Universidade Catìlica do Paranìá/Instituto de Mo-lÌ©stias Cardiovasculares Rio Preto, Brazil Phase 1/Phase 2	Wound healing (wound size, wound stage) - monitoring the healing of trophic lesions Pain and analgesics use Quality of life outcome Improvement of the coronary and collateral circulation. Survival without amputation
NCT00442143	Treatment of severe limb ischemia with autologous bonemarrow derived mononuclear cells	Odense University Hospital Phase 1	Transcutaneous oxygen pressure. 1st toe blood pressure (strain gauge)/Ankle blood pressure. Wound healing. Pain amputation. Infection

Clinical trial number NCT00747708, is a randomized trial investigating the co-administration of bone marrow pro- genitor cells with Granulocyte colony-stimulating factor (G- CSF) in patients with left ventricular dysfunction secondary to ischemic heart disease. The study has three arms; Arm 1, patients will receive G-CSF or placebo peripheral injection. Arm 2, percutaneous intracoronary injection of G-CSF fol- lowed by BM progenitor cells or placebo. Arm 3, percutaneous intracoronary injection of G-CSF followed by intramyo- cardial injection of BM progenitor cells or placebo. G-CSF is widely used in bone marrow transplantation to induce hema- topoietic stem cell mobilization, more recently G-CSF was observed to influence stromal cell-derived factor 1 (SDF-1) and its receptor CXCR4 levels, increasing homing of trans- planted cells and reducing apoptosis of

cardiomyocytes [73-76].

An interesting clinical trial is currently investigating the effectiveness of rapid stem cell therapy in MI. NCT00765453 is a Phase 2 trial aiming to assess if myocardium can be recovered by combining stem cell therapy with primary angioplasty. BMMNC will be administered within 5 hours of a MI at the same time as primary angioplasty. Primary outcome will be assessed by longitudinal change in LV ejection fraction over 1 year.

Clinical trial NCT01569178 and 2006-000280-28 are both assessing safety of stem cells administration.

Clinical trial 2006-000280-28, will deliver bone marrow mononuclear cells via intracoronary injection in patients with dilated cardiomyopathy secondary to myocardial ischemia. Its aim is to demonstrate that a single intracoronary infusion of autologous bone marrow-derived mononuclear cells is safe and reduces all-cause mortality in patients.

Clinical trial 2006-000280-28, is a Phase I/II safety toler- ability test administering a dose escalation of adult haema- topoietic stem cells to patients with established myocardial ischemia.

Clinical trial NCT01916369 sponsored by ReNeuron is currently recruiting in the UK focusing on PAD. It is a Phase I dose safety study of intramuscular administration of CTX0E03 which is a neural stem cell line. The primary objective is to assess the safety of increasing doses of CTX0E03 in addition to tolerability of intramuscular injec- tion. This trial to be conducted in patients with PAD is re- lated to the patent WO2010089605, which describes the in- vention of CTX0E03 for PAD treatment.

Clinical trial NCT01558908 by Medistem Inc. are utiliz- ing endometrial regenerative cells in patients with critical limb ischemia. This promising use of endometrial-derived cells is being fully utilized in heart failure. Currently endo- metrial regenerative cells are in Phase II clinical trials for congestive heart failure [74]. Regenerative cells from the endometrium are reported to be superior to other sources of regenerative cells as they have greater expansion capability [74]. Bearing in mind that endogenous growth factors reside in the endometrium and this is an organ with great angio- genic potential. These clinical trials are probably utilizing patent US20130315875 [59] describing the use of novel angiogenic cells isolated from amnion, called "amnion derived adherent cells" (AMDACs)

CONCLUSION

Similar to industrial revolution which saw a steep rise in patent applications the advent of potential far-reaching technologies brings forward today's prospectors and pioneers driving substantial increase in patent submission laying claim to their invention. So, it is no surprise that the explosion in stem cell publications has led to a rapid and sustained growth in patent submissions in regenerative medicine. Applications range from sourcing new stem cell populations from different tissue or providing selection criteria. These patents are currently being vigorously tested in clinical trials for safety and efficacy, in the hope that real breakthroughs can be made. Investigations are now focusing on how to im- prove patency of stem cells and include methods modifying the microenvironment, timing of stem cell treatment and genetic makeup of the stem cells themselves. With the sheer number of patents and scientists focusing on this field that even if a small proportion of these patents can be realized it is great hope this would lead to unlimited benefit for ischemic cardiovascular disease.

CONFLICT OF INTEREST

The authors confirm that this article content has no con- flict of interest.

ACKNOWLEDGEMENTS

Colin E. Murdoch and Elena De Falco designed the concept, wrote and revised the manuscript. Milda Bartkeviciute, Sophie A.B.-S, Camilla Siciliano and Roberta Altobelli gathered and analysed the patents. CEM is funded by Framework 7 Marie Sklodowska-Curie International Incoming Fellowship [PIIF-GA-2013-626633]. The Authors thank Fondazione Roma.

LIST OF ABBREVIATIONS

MI = Myocardial infarct

PAD = Peripheral artery disease HSCs = Hematopoietic stem cells PTH = Parathyroid Hormone

PTHrP = Parathyroid Hormone-Related Peptide G-CSF = Granulocyte Colony Stimulating Factor DPP IV = Dipeptidyl Peptidase IV

HSCs = Hematopoietic Stem Cells

IGF-1 = Insulin Growth Factor-1

VEGF = Vascular Endothelial Growth Factor

EPCs = Endothelial Progenitor Cells

MSCs = Mesenchymal Stem Cells

MAPCs = Multipotent Adult Progenitor Cells MPCs = Mesenchymal Precursors Cells PTGIS = Prostacyclin Synthase

HMOX1 = Heme Oxygenase

eNOS = Endothelial Nitric Oxide Synthase

PAH = Pulmonary Arterial Hypertension

PVD = Peripheral Vascular Disease

CLI = Critical Limb Ischemia

PDACs = Placenta Derived Adherent Cells MACS = Magnetic Activated Cell Sorting FACS = Fluorescence Activated Cell Sorting CHD = Congenital Heart Disease

AMDACs = Amnion Derived Adherent Cells

BH4 = Tetrahydrobiopterin

G-CSF = Granulocyte Colony-Stimulating Factor

PDACs = Placenta Derived Adherent Cells

REFERENCES

[1] Nerem RM. Regenerative medicine: The emergence of an industry. JR Soc Interface 2010; (7 Suppl 6): S771-5.

[2] Espacenet worldwide patent search available at www.epo.org n.d. (Accessed on: August 27, 2014).

[3] Smolina K, Wright FL, Rayner M. Determinants of the decline in mortality from acute myocardial infarction in England between 2002 and 2010: Linked national database study 2012; 8059: 1-9.

[4] Fowkes FGR, Rudan D, Rudan I, Aboyans V, Denenberg JO, McDermott MM, *et al.* Comparison of global estimates of prevalence and risk factors for peripheral artery disease in 2000 and 2010: A systematic review and analysis. Lancet 2013; 382: 1329-40.

[5] Tendera M, Aboyans V, Bartelink M-L, Baumgartner I, Clément D, Collet J-P, *et al.* ESC Guidelines on the diagnosis and treatment of peripheral artery diseases: Document covering atherosclerotic disease of extracranial carotid and vertebral, mesenteric, renal, upper and lower extremity arteries: The task force on the diagnosis and treatment. Eur Heart J 2011; 32: 2851-906.

[6] Marrett E, DiBonaventura M daCosta, Zhang Q. Burden of peripheral arterial disease in Europe and the United States: A patient survey. Health Qual Life Outcomes 2013; 11:175.

[7] Botham CM, Bennett WL, Cooke JP. Clinical trials of adult stem cell therapy for peripheral artery disease. Methodist Debakey Cardiovasc J 2013; 9: 201-5.

[8] Itzhaki-Alfia A, Leor J, Raanani E, Sternik L, Spiegelstein D, Netser S, *et al.* Patient characteristics and cell source determine the number of isolated human cardiac progenitor cells. Circulation 2009; 120: 2559-66.

[9] Hristov M, Erl W, Weber PC. Endothelial progenitor cells: Mobilization, differentiation, and homing. Arterioscler Thromb Vasc Biol 2003; 23: 1185-9.

[10] Zhang L, Xu Q. Stem/Progenitor cells in vascular regeneration. Arterioscler Thromb Vasc Biol 2014; 34: 1114-9.

[11] Shantsila E, Watson T, Lip GYH. Endothelial progenitor cells in cardiovascular disorders. J Am Coll Cardiol 2007; 49: 741-52.

[12] Wollert KC, Drexler H. Clinical applications of stem cells for the heart. Circ Res 2005; 96: 151-63.

[13] Goumans M-J, Maring JA, Smits AM. A straightforward guide to the basic science behind cardiovascular cell-based therapies. Heart 2014; 100: 1153-7.

[14] Bergmann O, Bhardwaj RD, Bernard S, Zdunek S, Barnabé-Heider F, Walsh S, *et al.* Evidence for cardiomyocyte renewal in humans. Science 2009; 324: 98-102.

[15] Beltrami AP, Barlucchi L, Torella D, Baker M, Limana F, Chimenti S, *et al.* Adult cardiac stem cells are multipotent and support myocardial regeneration. Cell 2003; 114: 763-76.

[16] Mirotsou M, Jayawardena TM, Schmeckpeper J, Gnecchi M, Dzau VJ. Paracrine mechanisms of stem cell reparative and regenerative actions in the heart. J Mol Cell Cardiol 2011; 50: 280-9.

[17] Torsney E, Xu Q. Resident vascular progenitor cells. J Mol Cell Cardiol 2011; 50: 304-11.

[18] Tang Z, Wang A, Yuan F, Yan Z, Liu B, Chu JS, *et al.* Differentiation of multipotent vascular stem cells contributes to vascular diseases. Nat Commun 2012; 3: 875.

[19] Chong JJH, Yang X, Don CW, Minami E, Liu Y-W, Weyers JJ, *et al.* Human embryonic-stem-cell-derived cardiomyocytes regenerate non-human primate hearts. Nature 2014; 510: 273-7.

[20] Yoshida Y, Yamanaka S. iPS cells: A source of cardiac regeneration. J Mol Cell Cardiol 2011; 50: 327-32.

[21] Orlic D, Kajstura J, Chimenti S, Bodine DM, Leri A, Anversa P. Bone marrow stem cells regenerate infarcted myocardium. Pediatr Transplant 2003; 7 (Suppl 3): 86-8.

[22] Timmers L, Lim SK, Hoefer IE, Arslan F, Lai RC, van Oorschot AM, *et al.* Human mesenchymal stem cell-conditioned medium improves cardiac function following myocardial infarction. Stem Cell Res 2011;6: 206-14.

[23] Smits AM, van Laake LW, den Ouden K, Schreurs C, Szuhai K, van Echteld CJ, *et al.* Human cardiomyocyte progenitor cell transplantation preserves long-term function of the infarcted mouse myocardium. Cardiovasc Res 2009; 83: 527-35.

[24] Bolli R, Tang X-L, Sanganalmath SK, Rimoldi O, Mosna F, Abdel-Latif A, *et al*. Intracoronary delivery of autologous cardiac stem cells improves cardiac function in a porcine model of chronic ischemic cardiomyopathy. Circulation 2013; 128: 122-31.

[25] Ratcliffe E, Glen KE, Naing MW, Williams DJ. Current status and perspectives on stem cell-based therapies undergoing clinical trials for regenerative medicine: Case studies. Br Med Bull 2013; 108: 73-94.

[26] Franchi F, Ezenekwe A, Wellkamp L, Peterson KM, Lerman A, Rodriguez-Porcel M. Renin inhibition improves the survival of mesenchymal stromal cells in a mouse model of myocardial infarction. J Cardiovasc Transl Res 2014: 7(6): 560-9.

[27] Androutsellis-Theotokis A, Leker RR, Soldner F, Hoeppner DJ, Ravin R, Poser SW, *et al.* Notch signalling regulates stem cell numbers *in vitro* and *in vivo*. Nature 2006; 442: 823-6.

[28] Wang N, Xu Y, Qin T, Wang F-P, Ma L-L, Luo X-G, *et al.* Myocardin-related transcription factor-A is a key regulator in retinoic acid-induced neural-like differentiation of adult bone marrow-derived mesenchymal stem cells. Gene 2013; 523: 178-86.

[29] Zhang LX, DeNicola M, Qin X, Du J, Ma J, Zhao TY, *et al.* Specific Inhibition of HDAC4 in cardiac progenitor cells enhances myocardial repairs. Am J Physiol Cell Physiol 2014; 307(4): C358-72.

[30] Zhang Z, Liang D, Gao X, Zhao C, Qin X, Xu Y, *et al.* Selective inhibition of inositol hexakisphosphate kinases (IP6Ks) enhances mesenchymal stem cell engraftment and improves therapeutic efficacy for myocardial infarction. Basic Res Cardiol 2014; 109(4): 417.

[31] Asahara T. Isolation of putative progenitor endothelial cells for angiogenesis. Science 1997; 275: 964-6.

[32] Hamano K, Li TS, Kobayashi T, Tanaka N, Kobayashi S, Matsuzaki M, *et al.* The induction of angiogenesis by the implantation of autologous bone marrow cells: A novel and simple

therapeutic method. Surgery 2001; 130: 44-54.

[33] Kinnaird T, Stabile E, Burnett MS, Lee CW, Barr S, Fuchs S, *et al.* Marrow-derived stromal cells express genes encoding a broad spectrum of arteriogenic cytokines and promote *in vitro* and *in vivo* arteriogenesis through paracrine mechanisms. Circ Res 2004; 94: 678-85.

[34] Napoli C, William-Ignarro S, Byrns R, Balestrieri ML, Crimi E, Farzati B, *et al*. Therapeutic targeting of the stem cell niche in experimental hindlimb ischemia. Nat Clin Pract Cardiovasc Med 2008; 5: 571-9.

[35] Zaruba M-M, Huber BC, Brunner S, Deindl E, David R, Fischer R, *et al*. Parathyroid hormone treatment after myocardial infarction promotes cardiac repair by enhanced neovascularization and cell survival. Cardiovasc Res 2008; 77: 722-31.

[36] Yamahara K, Sone M, Itoh H, Yamashita JK, Yurugi-Kobayashi T, Homma K, *et al*. Augmentation of neovascularization [corrected] in hindlimb ischemia by combined transplantation of human embryonic stem cells-derived endothelial and mural cells. PLoS One 2008; 3: e1666.

[37] Lee EJ, Park H-W, Jeon H-J, Kim H-S, Chang M-S. Potentiated therapeutic angiogenesis by primed human mesenchymal stem cells in a mouse model of hindlimb ischemia. Regen Med 2013; 8: 283-93.

[38] Stroemer P, Patel S, Hope A, Oliveira C, Pollock K, Sinden J. The neural stem cell line CTX0E03 promotes behavioral recovery and endogenous neurogenesis after experimental stroke in a dose-dependent fashion. Neurorehabil Neural Repair 2009; 23: 895-909.

[39] Katare R, Stroemer P, Hicks C, Stevanato L, Patel S, Corteling R, *et al.* Clinical-grade human neural stem cells promote reparative neovascularization in mouse models of hindlimb ischemia. Arterioscler Thromb Vasc Biol 2014; 34: 408-18.

[40] Napoli C, Williams-Ignarro S, de Nigris F, de Rosa G, Lerman LO, Farzati B, *et al*. Beneficial effects of concurrent autologous bone marrow cell therapy and metabolic intervention in ischemia-induced angiogenesis in the mouse hindlimb. Proc Natl Acad Sci USA 2005; 102: 17202-6.

[41] Murdoch CE, Chaubey S, Zeng L, Yu B, Ivetic A, Walker SJ, *et al.* Endothelial NADPH oxidase-2 promotes interstitial cardiac fibrosis and diastolic dysfunction through pro-inflammatory effects and endothelial-mesenchymal transition. J Am Coll Cardiol 2014; 63(24): 2734-41.

[42] Murdoch CE, Grieve DJ, Cave AC, Looi YH, Shah AM. NADPH oxidase and heart failure. Curr Opin Pharmacol 2006; 6: 148-53.

[43] Ray R, Murdoch CE, Wang M, Santos CX, Zhang M, Alom-Ruiz S, *et al*. Endothelial Nox4 NADPH oxidase enhances vasodilatation and reduces blood pressure *in vivo*. Arterioscler Thromb Vasc Biol 2011; 31: 1368-76.

[44] Pimentel D, Haeussler DJ, Matsui R, Burgoyne JR, Cohen RA, Bachschmid MM. Regulation of cell physiology and pathology by protein S-glutathionylation: Lessons learned from the cardiovascular system. Antioxid Redox Signal 2012; 16: 524-42.

[45] Liang R, Ghaffari S. Stem cells, redox signaling, and stem cell aging. Antioxid Redox Signal 2014; 20: 1902-16.

[46] Jang Y, Sharkis SJ. Stem cells in hematology. A low level of reactive oxygen species selects for primitive hematopoietic stem cells that may reside in the low-oxygenic niche 2007; 110: 3056-63.

[47] Tesio M, Golan K, Corso S, Giordano S, Schajnovitz A, Vagima Y, *et al.* Enhanced c-Met activity promotes G-CSF-induced mobilization of hematopoietic progenitor cells via ROS signaling. Blood 2011; 117: 419-28.

[48] Urao N, Ushio-Fukai M. Redox regulation of stem/progenitor cells and bone marrow niche. Free Radic Biol Med 2013: 54: 26-39.

[49] Barbash IM, Chouraqui P, Baron J, Feinberg MS, Etzion S, Tessone A, *et al.* Systemic delivery of bone marrow-derived mesenchymal stem cells to the infarcted myocardium: Feasibility, cell migration, and body distribution. Circulation 2003; 108: 863-8.

[50] Sherman W, Martens TP, Viles-Gonzalez JF, Siminiak T. Catheter-based delivery of cells to the heart. Nat Clin Pract Cardiovasc Med 2006; 3 (Suppl 1): S57-64.

[51] Dixon JA, Gorman RC, Stroud RE, Bouges S, Hirotsugu H, Gorman JH, *et al.* Mesenchymal cell transplantation and myocardial remodeling after myocardial infarction. Circulation 2009; 120: S220-9.

[52] Tang YL, Tang Y, Zhang YC, Qian K, Shen L, Phillips MI. Improved graft mesenchymal stem cell survival in ischemic heart with a hypoxia-regulated heme oxygenase-1 vector. J Am Coll Cardiol 2005; 46: 1339-50.

[53] Palmer, L., Motlagh, D., Cohen, A., Amrani, D.L. Pharmaceutical composition comprising CD34+ cells. WO2013126590 (**2013**).

[54] Laughlin, M., Haynesworth, S., Pompili, V. Introducing enriched human endothelial generating cells and mesenchymal stem cells; enhancing vasculogenesis and collateralization around blocked and/or narrowed vessels. US20040258670 (**2004**).

[55] Prosper, F., Verfaillie, C.M., Lopez-Aranguren, X., Claver, C.C., Luttun, A. Vascular/lymphatic endothelial cells. EP2428563 (**2012**).

[56] Jeffs, R., Petersen, T., Ilagan, R.M., Wade, M. Treatment of pulmonary arterial hypertension with mesenchymal stem cells. WO2014022373 (**2014**).

[57] Abbot, S., Edinger, J.W., Francki, A., Hariri, R.J., Jankovic, V., Kaplunovsky, A., Kristen, L., Eric, L., Neerav, D., Padliya, J.P., Jia-Lun, Wang. Angiogenesis using placental stem cells. US20110250182 (**2011**).

[58] Ichim, T.E., Meng, X., Riordan, N.H. Endometrial stem cells and methods of making and using same. US20130156726 (**2013**).

[59] Edinger, W., Kaplunovsky, A., Labazzo, K., Padliya, N., Hill. C. Amnion derived adherent cells. US20130315875 (**2013**).

[60] Zoldhelyi, P.H., Willerson, J.T., Liu, Q., Chen, Z.-Q. Mesodermal-like cell population for treating ischemia in mammals. US8617538 (**2013**).

[61] Sinden, J., Miljan, E., Madeddu, P. Treatment of limb ischemia. WO2010089605 (**2010**).

[62] Franz, W.M., Theiss, H., Zaruba, M.M., Brunner, S. Combination of granulocyte-colony stimulating factor (G-CSF) and DPP-IV inhibitors like Vildagliptin or Sitagliptin. US8455435 (**2013**).

[63] Zaruba M-M, Theiss HD, Vallaster M, Mehl U, Brunner S, David R, *et al.* Synergy between CD26/DPP-IV inhibition and G-CSF improves cardiac function after acute myocardial infarction. Cell Stem Cell 2009; 4: 313-23.

[64] Webster, K.A. Methods, compositions, cells, and kits for treating ischemic injury. US20130236433 (**2013**).

[65] Herrmann J, Abarbanell A. Optimizing stem cell function for the treatment of ischemic heart disease. J Surg 2011; 166: 138-45.

[66] Messina, L.M. Methods and compositions to reduce oxidative stress. WO2011011092 (**2011**).

[67] Webster, K.A., Biscayne, K. Hypoxia regulated conditionally silenced AAV expressing angiogenic inducers. US20130131152 (**2013**).

[68] Webster, K.A. Hypoxia regulated conditionally silenced aav expressing angiogenic inducers. WO2011053896 (**2011**).

[69] Andrew, L.P., Robert, A.P. Compositions and methods of vascular injury repair. US8343485 (**2005**).

[70] Webster, K.A. Methods, compositions, cells, and kits for treating ischemic injury. WO2012064920 (**2012**).

[71] Clinical trial database avaliable at http://clinicaltrials.gov/ct2/search **(**Accessed on: August 27, 2014).

[72] Catapult cell therapy clinical trial database at https://ct.catapult.org.uk/clinical-trials-database n.d. (Accessed on: August 27, 2014).

[73] Yu J, Hu K, Smuga-Otto K, Tian S, Stewart R, Slukvin II, *et al.* Human induced pluripotent stem cells free of vector and transgene sequences. Science 2009; 324: 797-801.

[74] Bockeria L, Bogin V, Bockeria O, Le T, Alekyan T, Woods E, Brown AA, Ichim T and Patel A. Endometrial regenerative cells for treatment of heart failure: A new stem cell enters the clinic. J of Trans Med 2013. 11: 56.

[75] Shim W, Mehta A, Lim SY, Zhang G, Lim CH, Chua T, *et al.* G-CSF for stem cell therapy in acute myocardial infarction: Friend or foe? Cardiovasc Res 2011; 89: 20-30.

[76] Petit I, Szyper-Kravitz M, Nagler A, Lahav M, Peled A, Habler L, *et al.* G-CSF inG-CSF induces stem cell mobilization by decreasing bone marrow SDF-1 and up-regulating CXCR4. Nat Immunol 2002; 3: 687-94.

[77] Pubmed search engine at www.ncbi.nlm.nih.gov/pubmed/ n.d. (Accessed on: August 27, 2014).