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The Therapeutic Potential of Stimulating Endogenous Stem Cell Mobilization

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1. Introduction

The past decade has seen a fast and extensive development of various therapies and treatment protocols based on Adult Stem Cells (ASC) and their application to various diseases. While some of these treatment protocols have been well documented in the scientific literature and used in well controlled clinical set ups, others have been developed and are being used by a growing numbers of clinics throughout the world, without thorough documentation though nevertheless with good clinical care and with the reports of very compelling results.

Despite the wide variety of methods, the general procedure guiding these various protocols follows a series of common steps. The first step is the isolation of stem cells from a source. For the purpose of banking or clinical application, stem cells can be isolated from a variety of sources including umbilical cord (Can and Balci, 2011; Zhang et al., 2011), adipose tissue-derived stem cells (Insausti et al., 2011; Zachar et al., 2011), peripheral blood stem cells (Kolbe et al., 2010; Hofmann et al., 2009), amniotic and placental stem cells (Klein and Fauza, 2011; Tsagias et al., 2011), dental pulp stem cells (Gronthos et al., 2011; Tirino et al., 2011), olfactory stem cells (Chen et al., 2006; Viktorov et al., 2008), and even human limbal epithelial stem cells (Vasania et al., 2011).

The second step is proliferation. This is not a necessary step with regard to stem cell function, however the small number of stem cells present in one umbilical cord, one placenta, one blood sample, one liposuction or one dental pulp makes clinical application difficult without the ability to expand the harvested stem cells. Methods to expand embryonic stem cells have been developed more than a decade ago, however it is only a few years ago that methods to significantly expand ASC have been developed, leading to an expansion of the stem cell banking market and greater clinical application (Ivanovic et al., 2011; Dos Santos et al., 2011; Pineault et al., 2011).

The third step is pre-conditioning or treatment to trigger commitment of the stem cells into a specific cellular lineage. For example, stem cells can be led to differentiate into dopamine-producing neuron by an exposure to a cocktail containing sonic hedgehog (SHH), fibroblast growth factor 8 (FGF8), and basic fibroblast growth factor (bFGF) (Trzaska and Rameshwar, 2011; Wang et al., 2011) or into neurons responding to multiple neurotransmitters by a simple exposure to retinoic acid and other growth factors (Greco et al., 2008). Likewise, stem cells can be guided to differentiate in cardiomyocytes by exposure to a cocktail containing transforming growth factor-beta(1), bone morphogenetic protein-4, activin A, vascular endothelial growth factor (VEGF), insulin-like growth factor-1, fibroblast growth factor-2, Epidermal growth factor (EGF), and interleukin-6 (Behfar et al., 2010; Behfar et al., 2008). Pre-conditioning with these cytokines can also enhance the formation of gap junction and improve therapeutic efficacy (Hahn et al., 2008). Exposure of stem cells to a cocktail containing insulin, transferrin, selenium and the GLP-1 (glucagon-like peptide-1) analogue exendin-4 leads to the formation of insulin-producing pancreatic cells (Docherty, 2009; Chandra et al., 2009). Various cocktails have been shown to trigger the differentiation of mesenchymal stem cells in into a wide variety of cell types (Snykers et al., 2011; Arufe et al., 2009; Keilhoff et al., 2006). Nevertheless, this pre-conditioning step is not essential since ASC will naturally differentiate into the cell type with which they find themselves, upon contact with cellular debris or cell marker specific to that cell type. For example, as they migrate into the heart, stem cells can be triggered to differentiate into cardiomyocytes (Orlic et al., 2001), or into keratinocytes and skin appendages, insulin-producing pancreatic cells or hepatocytes as they respectively migrate in a skin wound (Zhang and Fu, 2008), the pancreas (Hasegawa et al., 2007) or the liver (Theise et al., 2000).

The third and final step is the injection of stem cells into the target organ, in the main artery leading to the target organ or in the bloodstream from where a number of them will migrate on their own to the affected organ. In the case of a heart attack for example, stem cells can be injected in coronary artery (Wollert et al., 2004) or directly in the border zone of the infarct (Stamm et al., 2003; Tse et al., 2003). Treatment efficacy can vary significantly with the various methods of injection. For the treatment of acute myocardial infarction, injection in the border zone of the infarct seems to yield the best results, followed by intracoronary and intravenous injection, respectively (Karra and Wu, 2008). For the treatment of spinal cord injury, injection directly in the lesion or in the cerebrospinal fluid seems far superior to intravenous injection (Lima et al., 2010), yet very compelling cases have been documented following intravenous stem cell injection or simple bone marrow stem cell mobilization (see Section 4). For the treatment of diabetes however, intravenous injection seems to yield better results than stem cells transplantation directly into the pancreas (Hasegawa et al., 2007).

In this multistep procedure in which each step can be accomplished according to a wide variety of protocols and methods, it remains that peripheral blood stem cells (PBSC) can, without expansion, pre-conditioning or injection reach various target organs and participate to the process of tissue repair. This observation has led a number of researchers to look at the therapeutic potential of simply stimulating Endogenous Bone Marrow Stem Cell Mobilization (ESCM). This chapter will look in detail into the clinical and therapeutic potential of ESCM by describing its physiological basis, by reviewing the existing literature on the clinical application of ESCM and by presenting a few clinical cases.

2. The repair system of the body

For ESCM to have any clinical relevance, the demonstration must be made that one of the natural roles of stem cells in the body is to participate to tissue repair in cases of injury or degenerative diseases. Therefore, the clinical relevance of mobilizing endogenous bone marrow-derived stem cells (BMSC) would be to increase the number of circulating stem cells available to migrate into affected tissues and contribute to tissue repair. For this phenomenon to be natural: 1) the body must have a mechanism that triggers BMSC mobilization after an injury; 2) BMSC must traffic in the blood and be recruited by the injured tissue; 3) in the injured tissue BMSC must proliferate and 4) a mechanism must exist to trigger the differentiation of BMSC into cells of that tissue.

2.1 Signaling for mobilization

The most common compound known to naturally stimulate BMSC mobilization is Granulocyte-Colony Stimulating Factor (G-CSF). Discovered in 1985 by Welte et al., G-CSF is a cytokine secreted by various tissues that stimulates the proliferation, differentiation and function of neutrophil precursors and mature neutrophils. But G-CSF was also shown to stimulate BMSC mobilization (Petit et al., 2003; Cottler-Fox et al., 2003), making it a common tool in protocols of stem cell apheresis for the purpose of cryopreservation and stem cell transplant (Gordon et al., 2008; Croop et al., 2001).

Given the vital importance of the heart and the fact that cardiovascular diseases are a leading cause of death in the world, much of the clinical stem cell research has focused its efforts on the role of stem cells in cardiac repair taking place after acute myocardial infarction (AMI). A number of studies have revealed the sequence of events taking place after AMI. A few hours after AMI, the cardiac tissue releases or causes to release G-CSF (Leone et al., 2006). As its concentration slowly increases in the bloodstream, G-CSF triggers the release of stem cells from the bone marrow, increasing the number of PBSC which peaks at around 4-7 days after AMI (Shintani et al., 2001; Leone et al., 2006). It is worth mentioning that the serum level of G-CSF and the number of PBSC are also increased in cases of chronic angina (Leone et al., 2006). Similar stem cell mobilization and increase in PBSC have been documented following skeletal muscle injury (Stout et al., 2007).

Other chemokines such as interleukine-8 (IL-8), Stromal-Derived Factor-1 (SDF-1), Stem Cell Factor (SCF), Groß, and vascular endothelial factor (VEGF) have been shown to trigger BMSC mobilization (King et al., 2001; Lapidot & Petit, 2002; Fukuda et al., 2007; Lapid et al., 2009). Contrary to G-CSF and SCF, which lead to a slow increase in the number of PBSC over a period of a few days, other cytokines such as IL-8 lead to a significant increase in the number of PBSC within hours (Fibbe et al., 1999).

As has been described with the heart following AMI, a stroke also triggers the release of cytokines that induce the mobilization of BMSC and their migration into the brain. For example, the number of PBSC in stroke patients nearly tripled within 7 days after the stroke (Hennemann et al., 2008; Paczkowska et al., 2005). In one study, the magnitude of stem cell release was actually correlated with the functional recovery of the patients (Dunac et al, 2007). Interestingly, the number of circulating stem cells did not increase in patients who received thrombolysis therapy immediately after their stroke. Therefore, it appears that it is

the lingering injury that slowly leads to the mobilization of stem cells from the bone marrow.

Finally, injuries to the skin and bones were also shown to trigger mobilization of BMSC and their migration into the injured tissue. For example, within 24 hours of a severe burn, a rapid increase of up to 9-fold in the number of PBSC has been observed in the blood of burn patients (Fox et al., 2008). Furthermore, the size of the area of the body affected by the burns strongly correlated with the magnitude of the mobilization. The affected skin also released cytokines such as SDF-1 and VEGF, which are involved in the migration of PBSC to the skin and their differentiation into blood vessels, respectively (Mansilla et al., 2006). In one study (Lee et al., 2008), the number of PBSC peaked around 3 days after a bone fracture and rapidly returned to basal level within a few days. These results were confirmed in another study in which stem cells were shown to migrate to the fracture site and to promote neovascularization. The formation of new vessels was shown to peak at the fracture site 7 days after the fracture, which corresponds to the early phase of ossification of the fracture line (Matsumoto et al., 2008). Therefore BMSC mobilization was documented to naturally follow an injury or even be associated with chronic conditions.

The natural process by which stem cells are mobilized from the bone marrow is still not fully understood. Contrary to most tissues in which SDF-1 is secreted consequent to an injury or a degenerative condition, in the bone marrow SDF-1 is constitutively produced and released, and binding of SDF-1 to its exclusive receptor CXCR4 leads to the externalization of adhesion molecules, namely integrins, which allow for the adherence of stem cells to the bone marrow matrix. The binding of SDF-1 to CXCR4 is referred to as the SDF-1/CXCR4 axis. The general understanding is that disruption of the SDF-1/CXCR4 axis reduces the expression of adhesion molecules, leading to a reduction in the adherence of stem cells to the bone marrow matrix and the consequent mobilization of stem cells. Various compounds known to trigger stem cell mobilization all affect the SDF-1/CXCR4 axis in various ways.

For example, G-CSF disrupts the SDF-1/CXCR4 axis by activating a series of proteolytic enzymes including elastase, cathepsin G, and various matrix metalloproteinases (MMP2 and MMP9) that inactivate SDF-1 (Mannello et al., 2006; Jin et al., 2006; Carion et al., 2003). AMD3100 is a newly developed BMSC mobilizer and it acts by blocking CXCR4, disrupting the SDF-1/CXCR4 axis (Broxmeyer et al., 2005). A blocker of L-selectin was recently isolated from the cyanophyta *Aphanizomenon flos-aquae* and shown to trigger BMSC mobilization (Jensen et al., 2007). Inhibition of L-selectin leads to a down-regulation of CXCR4 expression, partially disrupting the SDF-1/CXCR4 axis. The mobilization mechanism of IL-8, SCF, VEGF and Groß are not well understood.

Therefore, the human body has a mechanism to naturally mobilize BMSC which can then traffic to various areas of the body and contribute to tissue regeneration and repair.

2.2 Extravasation & recruitment

Recruitment is the process by which PBSC are recruited by a specific tissue signaling for repair. The process of PBSC recruitment in a tissue takes place predominantly at the level the postcapillary venule, in a manner similar to neutrophils (Henschler et al., 2008).

In brief, the sudden drop in blood pressure taking place at the postcapillary venule triggers turbulence whose shear force mechanically activates L-selectin which in turn triggers the externalization of CXCR4, making PBSC more responsive to signals coming from tissues. If the tissue is in need of repair, it is secreting SDF-1 as well as other compounds such as stem cell factor (SCF) and hepatocyte growth factor (HGH) (Kucia et al., 2004; , Neuss et al., 2004) that diffuse locally to the capillaries. Binding of SDF-1 and SCF to their specific receptors (e.g. CXCR4 and c-Kit) leads to the expression of adhesion molecules at the surface of stem cells (Voermans et al., 2000; Peled et al., 1999; Peled et al., 2000). Through a complex interaction of microvilli at the surface of both the capillary and PBSC, the stem cells initiate the process of tethering and then arrest on the capillary endothelium (Middleton et al., 1997; 2002).

Following firm attachment, SDF-1 and HGH continue to bind to their respective receptors CXCR4 and c-met at the surface of SC, which triggers the release and activation of matrix metalloproteinases (MMPs) which digest the endothelial lining, allowing for the extravasation of PBSC (Mannello et al., 2006; Janowska-Wieczorek et al., 2000; Neuss et al., 2004; Ries et al., 2007).

When studying the migration behavior of SCs to a wide array of chemokines, SCs were found to migrate only toward SDF-1 (Wright et al., 2002; Jo et al., 2000). The migration to SDF-1 is a polarized phenomenon toward the chemokine source (chemotactic) and not a simple random motion (chemokinetic), as SCs only migrated in a gradient of SDF-1 and not when SDF-1 is uniformly distributed in the media.

SDF-1 is normally secreted, to some extent, by cardiomyocytes (Askari et al., 2003), skeletal muscles (Ratajczak et al., 2003), liver (Hatch et al., 2002; Kollet et al., 2003), brain (Bagri et al., 2002; Lazarini et al., 2003; Zou et al., 1998), and kidney (Schrader et al., 2002). However, its secretion increases during tissue damage such as AMI (Wojakowski et al., 2004; Abbott et al., 2004), ischemia (Takahashi et al., 1999, Iwaguro et al., 2002), toxic liver damage (Kollet et al., 2003; Swenson et al., 2008; Hatch et al., 2002), and excessive bleeding (Ratajczak et al., 2004).

In the tissue, the process of migration toward the site of injury relies on the interaction between CD44 and its ligand hyaluronic acid (HYA). HYA is one of a family of polysaccharides known as glycosaminoglycans (GAGS), typically found in the connective tissues of vertebrates. Alongside other proteins such as collagen, elastin, fibrillin, fibronectin and laminin, GAGS and HYA constitute the extracellular matrix (ECM) of most tissues. Studies in rats have shown that half of the HYA found in the body is in the skin, while 25% is found in joints and bones together (Reed et al., 1988). The rest is distributed somewhat equally in muscles and viscera (Clarris and Fraser,1968; Comper and Laurent, 1978) where the highest concentrations are found in connective tissue that form the ECM of most tissues.

After extravasation, CXCR4 on the surface of SC and SDF-1 secreted by the effected tissue continue their interaction which, in the tissue, leads to the formation of pseudopodia (see Figure 1) toward the source of SDF-1 secretion. As SDF-1 binds to CXCR4 in the tissue, CD44 adhesion molecules are externalized at the tip of the pseudopodia, leading to adhesion to HYA pathways within the tissue. In the tissue, the binding of CD44 to HYA is transient, as CD44 molecules shed soon after binding to HYA (Friedl et al., 1995), thus enabling pseudopodia detachment from the ECM. CD44 can also be cleaved by specific enzymes whose secretion is enhanced by SDF-1 (Heissig et al., 2002; Okamoto et al., 1999;

Janowska-Wieczorek et al., 1999). Through this process, stem cells can migrate within the tissue toward the site of injury.

The fact that PBSC primarily migrate to organs affected by an injury or a degenerative process has been documented in several studies. For example, in the cases of male recipients of liver transplants from female donors, biopsies performed 4-13 months after transplantation contained a significant number of Y-chromosome positive hepatocytes (16% to 43%). In one patient who suffered from hepatitis C after liver transplant and died 4.5 months after transplantation, up to 43% of the transplanted liver was made of Y-chromosome positive hepatocytes in comparison with 5% after 4.5 months in a woman with a sound liver who received a bone marrow transplant from a man (Theise et al., 2000). Investigations carried out on archival autopsy and biopsy liver specimens obtained from two women who received a bone marrow transplant from male donors for the treatment of leukemia revealed that 5 to 10% of the liver had been replaced by donor derived BMSCs after 4.5 and 13 months, respectively (Theise et al., 2000).

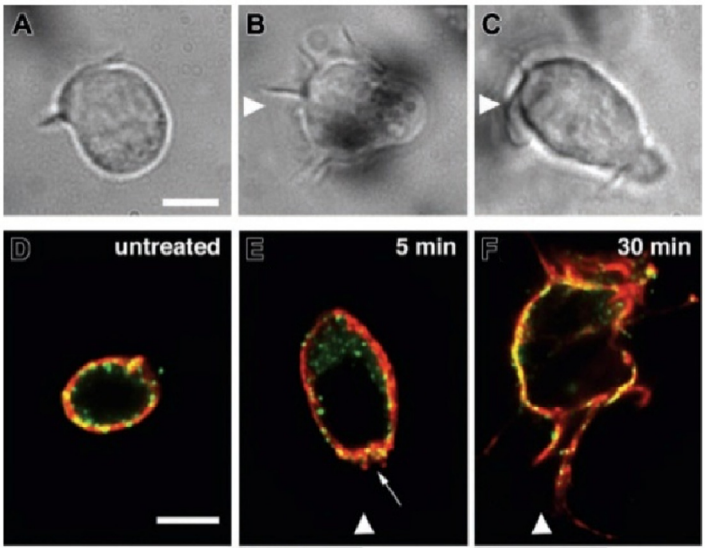

Fig. 1. **CD44 is localized to the leading edge of polarized human stem cells migrating toward SDF-1.** Cord blood-derived CD34+ cells were plated on HA coverslips and allowed to adhere for 30 minutes before recording cell movement. The position of SDF-1 source is indicated by arrowheads. (A-C) Phase contrast microscopy of untreated cells (A), cells stimulated with polarized source of SDF-1 (B), and cells treated with anti-CD44 mAb F10-44-2 and stimulated with polarized source of SDF-1 (C). (D-F) Cells treated as above were fixed 5 and 30 minutes after exposure to polarized source of SDF-1 and indirectly immunolabeled with antihuman CD44 mAb (red) and anti-CXCR4 mAb (green). An arrow is pointing to the fine CD44-positive protrusions at the direction of SDF-1. Bars = 5μm.
(Taken from Avigdor et al., 2004)

Similar observations were made on men who received cardiac transplants from female donors. Analyses of tissue samples from biopsies revealed that an average of 0.1% (Muller et

al., 2002) to 15% of cardiomyocytes were Y-chromosome positive (Quaini et al., 2002; Laflamme et al., 2002). In one patient who died of cardiac rejection, 29% of the cardiomyocytes contained the Y chromosome in ''hot spots'' of high cardiac repair. In male patients who received lung transplants from female donors, recipient-derived BMSCs cells were detected as bronchial epithelial cells, type II pneumocytes, and seromucous glands in the transplanted lungs of all tested patients. In patients suffering from chronic damage, up to 24% of bronchial epithelial cells carried the Y-chromosome, indicating ongoing repair of damaged tissue by the recipient's own stem cells (Kleeberger et al., 2003).

In one study investigating this process of tissue repair by BMSC, irradiated mice were transplanted with GFP-positive SC before being injected in the right tibialis muscle with a large dose of cardiotoxin, which led to a loss of mobility within a few days. Yet, after eight weeks the injured muscle showed massive regeneration, with the right tibialis muscle significantly reconstructed with GFP-positive myocytes. By contrast, the contralateral non-injured leg showed very small incorporation of GFP-positive myocytes (Drapeau et al., 2010). Similar observations were made by Abedi et al. (2004), using smaller injections of cardiotoxin. Four weeks after the injection of cardiotoxin in the right leg, the area of the injection contained 1-2% of GFP-positive muscle cells while the left leg showed no fluorescence at all.

Similar observations were made in models of skin injury (Abedi et al., 2004). In a similar protocol, irradiated mice were transplanted with GFP-positive stem cells. BMSCs were then mobilized during five days using G-CSF. On the fourth day, mice were subjected to punch biopsies on their flank. The area of the injury was rebiopsied and sutured 48 hours and 1 month after the injury, in order to assess the incorporation of GFP-positive cells in the healing skin. While analysis at 48 hours showed significant infiltration by GFP-positive undifferentiated stem cells in the deep layer of the skin (hypodermis), after 4 weeks there was a large number of GFP-positive tissue cells in the dermis composing the structure of the healed skin, such as keratinocytes, sebaceous glands, blood vessels, and some rare muscle fibers and hair follicles. In the control animals that received G-CSF but no punch biopsy, none of these skin structures were positive for GFP, indicating that the few SC that had migrated in the skin had done so randomly.

In other studies looking at the incorporation of BMSCs in injured tissues, directed migration was demonstrated in the gut after section of an intestinal segment, (Hayakawa et al., 2003) in the heart after AMI (Orlic et al., 2001a; Fukuhara et al., 2004) or induced cardiomyopathy, (Hisashi et al., 2004) in the brain after stroke, (Sanchez-Ramos et al., 2002; Hoehn et al., 2002) and in the liver after drug-induced liver damage (Abedi et al., 2004). Taken altogether these studies clearly establish that BMSCs primarily migrate to areas subjected to injury, damage or simple degeneration.

2.3 Proliferation

When stem cells reach the site of an injury they must proliferate and expand, as there are not enough PBSC to accomplish full repair of any significant injury or degenerative process.

Several chemokines such as SDF-1 have been reported to enhance stem cell proliferation (Bonavia et al., 2003). The direct effect of SDF-1 on cell proliferation and survival is not well understood, but SDF-1 has been found to stimulate the proliferation and survival of stem

cells under certain experimental conditions (Broxmeyer et al., 2003). In tissues, SDF-1 appears to act as a "cellular survival factor" (Hwang et al., 2006). Insulin-like growth factor (IGF-1), when coupled with epidermal growth factor (EGF) or fibroblast growth factor-2 (FGF-2), was shown to support the proliferation and survival of neural (Arsenijevic et al., 2001) and muscle stem cells (Deasy et al., 2002). Extracellular nucleotides were also shown to support the proliferation of brain stem cell (Mishra et al., 2006).

2.4 Differentiation

The ability of adult stem cells to differentiate into various cell types has been well documented, though the mechanism behind such transformation is still not well understood.

As reported by Krause et al. (2001), 11 months after injection of male stem cells in female mice, Y-chromosome bearing cells were found in various tissues including the liver, muscle, skin, lung, and intestine. It has been well demonstrated that BMSC can differentiate into a wide variety of cell types including myocytes (Ferrari et al, 1998), hepatocytes (Lee et al., 2004), epithelial cells (Krause et al., 2001), neurons (Mezey et al., 2003; Sanchez-Rarnos et al., 2000; Woodbury et al., 2000), retinal cells (Tomita et al., 2002), endothelial cells and cardiomyocytes (Jackson et al., 2001; Orlic et al, 2001), gastrointestinal epithelium (Krause et al., 2001; Okamoto et al., 2002), pancreatic endocrine cells (Ianus et al., 2003), bone and cartilage (Pereira et al., 1995; 1998).

Although little work has been done in this field and many questions remain to be answered, two possible mechanisms have been proposed for SC differentiation.

One proposed mechanism is cellular fusion, which takes place when two cells fuse together to become one cell. A few studies have suggested that SC have the ability to fuse with somatic cells, rescuing the target cell (Tarada et al., 2002; Vassilopoulos et al., 2003; Spees et al., 2003). Although there is clear evidence that this phenomenon did take place in a few experiments and that it may take place naturally in certain tissue such as the heart (Nygren et al., 2004), it is unlikely to be a significant physiological phenomenon (Wurmser and Gage, 2002). For example, while the process of fusion involves the interaction of one single SC with one somatic cell, therefore a ratio of 1:1, the extent of SC-mediated tissue repair that has been documented in numerous studies, involving various tissues, far exceeds the actual number of SCs migrating in the tissue. Furthermore, the process of cellular fusion results in a cell that is tetraploid. Although this has been observed in a few in vitro studies, it has been a very rare observation in vivo. In fact, even in vitro, relatively harsh conditions had to be used in order to obtain cellular fusion. Finally, cellular fusion would imply the merging of two different cellular membranes, a process that in itself is rigged with challenges, as cells are designed not to fuse.

So, although cellular fusion could possibly naturally take place in the body, it is unlikely to contribute significantly to the process of repair that has been documented with ASCs. The other most likely mechanism is differentiation through contact with cellular components when the affected tissue is locally subjected to the action of various matrix metalloproteinases (MMPs) or differentiation induced by cytokines released by neighboring cells.

This process was beautifully put in evidence by the study of Jang et al. (2004) where hematopoietic stem cells (HSC) were co-cultured with either normal or damaged liver tissue separated by a semi-permeable membrane with 0.4 μm pores. Using immunofluorescence to detect markers specific to either HSC (CD45) or hepatocytes (albumin), the authors could follow the transformation of the HSC population. When HSCs were cultured alone for 8 hours, they only expressed CD45 and no albumin. However, when HSCs were exposed to injured liver tissue, they rapidly became positive for albumin. Over time, the population of cells positive for CD45 began to decrease as the population positive for albumin began to increase. Albumin-positive cells were seen as early as 8 hours and constituted 3.0% of the cell population at 48 hours. The conversion was minimal and delayed when HSC were exposed to undamaged liver (control for injury). Therefore the presence of an injury appears to be an important factor in the process of SC differentiation into a specific type of somatic cells.

The authors further investigated the phenomenon of differentiation by tracking the presence of various markers found in developing fetal liver cells, such as αFP, and in mature hepatocytes, such as CK18, albumin, fibrinogen, and transferrin. They found that αFP was expressed only at 8 hours and was lost thereafter. On the other hand, the expression of CK18, albumin, fibrinogen and transferrin each increased over time. While at time 0 the HSCs expressed only CD45, after as little as 8 hours all liver-specific markers were positive. So during the differentiation process, the SC seemed to take a route similar to the development of hepatocytes in the developing fetus, leading to mature hepatocytes within less than 48 hours. This retracing of fetal development has also being documented in cardiopoiesis (Behfar et al., 2008).

In this study, differentiation did not involve cellular fusion, as the new liver cells only contained one set of chromosomes. The differentiation was necessarily triggered by signaling molecules produced by the damaged tissue. It has been suggested that MMPs produced by damaged tissue could be playing an important role in SC differentiation by digesting specific ECM components that would then diffuse and get into contact with SCs. Binding of such compounds to specific receptors would activate internal messengers that would trigger the process of differentiation by activating specific genes, as suggested by the high level of mRNA found in differentiating cells. (Mannello et al., 2006; Chavey et al., 2003)

Gap junction intercellular communication (GJIC) and tunneling nanotubules (TNTs) could constitute other mechanisms playing a significant role in SC differentiation, by direct cell-to-cell contact (Behfar et al., 2010). The diameter of one gap junction is around 2 nm and the molecular size cut-off level is around 1-2 kDa, which is sufficient for the intercellular exchange of ions, nucleotides and even small proteins (Neijssen et al., 2005). GJIC is known to play a crucial role in modulating several cellular functions, to the point that impaired or lack of GJIC has been associated with severe diseases (Dasgupta et al., 1999). It has been suggested that GJIC could play an important role in SC differentiation (Loewenstein and Rose, 1992). Likewise, recent studies indicated that two cells can also exchange information via TNTs (Rustom et al., 2004). TNTs would form a cytoplasma bridge between two cells that could be large enough to allow the transport of large molecules or even whole cell organelles. Such information could play a role in the finalizing process of differentiation, though much work needs to be done in this field before we obtain a better understanding of the role of GJIC and TNTs in stem cell differentiation.

2.5 Paracrine effect

Finally, a number of studies investigating the effect of BMSC on various diagnostic entities have revealed that oftentimes the extent of the benefits observed cannot be accounted for by the sheer number of BMSC that have differentiated into somatic cells, even when there is clear evidence of tissue regeneration mediated by newly formed cells. For example, irradiated female mice transplanted with transgenic GFP+ BMSC showed much better recovery from experimental stroke after G-CSF-induced BMSC mobilization when compared to non-mobilized control mice (Kawada et al., 2006). Both motor and cognitive functions were improved by BMSC mobilization, and the treatment also reduced infarct size. However, using bromodeoxyuridine (BrdU), it was observed that a significant number of the newly formed brain cells did not derive from BMSC but rather from local neural stem cells. Similar results were seen with spinal cord lesion and muscle injury (Kinnaird et al., 2004; Osada et al., 2010). The regenerative effect is believed to be triggered by the secretion of growth factors and paracrine signaling by BMSC (Uccelli et al., 2011).

The paracrine effect has been best put in evidence in the heart after AMI. Delivery of BMSC to ischemic cardiac tissue has led to a significant increase in the concentration of IL-10, interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α) and other cytokines in the cardiac tissue, which contributed to neovascularization and reduction of infarct size (Kamihata et al., 2001; Burchfield et al., 2008; Gnecchi et al., 2005; Mirotsou et al., 2011). Condition media in which BMSC were exposed to hypoxia proved to be cytoprotective to cardiomyocytes and was able to reduce infarct size (Gnecchi et al., 2006).

In all, a growing body of evidence supports the hypothesis that as BMSC migrate into tissues, aside from differentiating into cells of the target tissue, they also exert their regenerative effect at least in part -and maybe even to a large extent- through the secretion of paracrine signaling compounds.

3. ESCM as a treatment approach

Given the fact that SDF-1 is secreted by various organs and tissues upon injury or degeneration, it would follow that the number of PBSCs should be an important parameter in the overall effectiveness of SC-mediated tissue repair and regeneration. A higher number of PBSC would mean more SC available to respond to SDF-1 signaling and migrate into tissues. In this regard, Tomoda et al. (2003) reported that after a heart attack individuals with a higher number of PBSC showed greater recovery of cardiac functions after 6 months when compared to people having fewer PBSC at the time of the cardiac event.

In a prospective study using more than 500 individuals, Werner et al. (2005, 2007) put in evidence that the number of PBSC is a critical parameter in the role of SCs in tissue repair. The authors quantified the baseline number of PBSC in 519 individuals (average 66.6±10.8 years old) at risk for cardiovascular problems, and monitored their condition for one year. A first major cardiovascular event occurred in 214 patients. After adjustment for age, sex, vascular risk factors, and other relevant variables, increased levels of PBSC were associated with reduced risk of death from cardiovascular causes, lesser risk of a first major cardiovascular event, greater revascularization and lesser frequency of hospitalization.

Similar data has been obtained by Vasa et al. (2001) who documented that a higher number of endothelial progenitor cells (EPC) was associated with greater cardiovascular health. In fact, hypoxia has been associated with the secretion of SDF-1 and VEGF by the ischemic tissue (Schioppa et al., 2003; Bachelder et al., 2002). Circulating EPCs therefore are attracted to the ischemic heart by the action of SDF-1 and the migrating EPCs contribute to the formation of new blood vessels upon the action of VEGF (Lee et al., 2007). Therefore, a higher number of EPCs or PBSC helps maintain optimal blood flow and a strong cardiac tissue.

The link between disease formation and the number of circulating stem cells is not limited to the heart. Similar observations have been made with muscular dystrophy where the rate of progression of the disease has been linked to the number of circulating stem cells and for which the number of PBSC is now considered one of the most important predictors of the disease progression (Marchesi et al., 2008). Likewise, the progression of pulmonary arterial hypertension (Diller et al., 2008; Junhui et al., 2008), arthritis (Herbrig et al., 2006; Grisar et al., 2005), atherosclerosis (Zhu et al., 2006), lupus erythematosus (Westerweel et al., 2007; Moonen et al., 2007), kidney failure (Choi et al., 2004; Herbrig et al., 2004; Eizawa et al., 2003), migraine (Lee et al., 2008), erectile dysfunction (Baumhakel et al., 2007) and other diseases have all been linked to a reduction in the number of PBSC. Recently, a direct relationship has been established between the number of PBSC and the development of diabetes, linking impaired fasting glucose, impaired glucose tolerance, and insulin-dependent diabetes mellitus to progressively lower levels of PBSCs (Fadini et al., 2010).

If the number of PBSC constitutes such a key parameter in the process of SC-mediated tissue repair, therefore increasing the number of PBSC could constitute a therapeutic approach. Following is the description of a number of clinical trials investigating the clinical potential of ESCM in various diseases or injuries.

3.1 Acute myocardial infarction

The heart has been traditionally seen as having little regenerative capabilities after birth, although many recent studies have challenged this view. Evidence clearly suggests that there is a low level of constant regeneration of cardiac cells (Soonpaa and Field, 1998; Bergmann et al., 2009; Quaini et al., 2002), and the number of dividing cells can increase by up to 10-fold in chronic heart disease or after AMI (Kajstura et al., 1998; Beltrami et al., 2001). Yet, this level of proliferation seems insufficient to rescue the cardiac muscle after AMI (Schwartz and Kornowski, 2003) and survivors of heart attacks are left with reduced quality of life and little prospect for improvement.

A number of studies in animals using G-CSF and SCF have shown that ESCM can lead to significant cardiac repair after AMI. Injection of SCF and G-CSF for 8 days after inducing AMI significantly increased the number of PBSCs, which led to the migration of PBSCs into the myocardium (Orlic et al., 2001b). Twenty-seven days after AMI, a band of newly formed cardiac tissue occupied more than 75% of the infarcted region of the ventricule and newly formed blood vessels were supplying the infarcted tissue. The blood vessels were surrounded by smooth muscles and microscopic observations revealed the presence of red blood cells, indicating that the newly formed arterioles integrated structurally with the remaining functional vasculature. By comparison, in control animals the ventricular wall

was filled with scar tissue covering the entire area of the infarct and no new blood vessels could be seen.

In summary, while only 17% (9 of 52) of the untreated animals survived AMI, showing severe signs of cardiomyopathy and compromised blood circulation, up to 73% (11 of 15) of the animals treated with G-CSF survived with significantly improved cardiac function and restored blood circulation. After 27 days, ejection fraction was 114% greater in the treated group and other parameters such as end-diastolic pressure, systolic pressure, and other parameters of cardiovascular function were all improved in treated versus non-treated mice.

Injection of G-CSF, however, can have significant negative effect in humans if done at large dose for more than 5-6 days (Bensinger et al., 1996; Shimoda et al., 1993). At lower dose and with shorter treatment duration, human trials have so far delivered mitigated results, though the approach remains promising. While some groups did report very promising results (Ince et al., 2005; Sesti et al., 2005), others reported no effect at all (Ellis et al., 2006; Zohlnhofer et al., 2007; Ripa and Kastrup, 2008) . A comprehensive review of the various studies however reveals that each study used slightly different protocols with regard to the time of treatment after AMI (from hours to 3 months), as well as the intensity and duration of the treatment, suggesting that ESCM could indeed hold great promise once the most effective treatment protocol has been developed (Abdel-Latif et al., 2008).

For example, Wojakowski et al. (2006) reported in 43 cardiac patients that if the patients were treated early after AMI (<12 hours) with G-CSF, the number of PBSCs following BMSC mobilization correlated with the extent of cardiac repair. In another study, G-CSF was injected within 5 days post-AMI in 41 patients at high risk for unfavorable left ventricular remodeling. Five months after G-CSF treatment, ejection fraction had improved 12.5% compared to no improvement in the control group (Leone et al., 2007). The improvements in cardiac function appeared to be linked to the prevention of left ventricular remodeling.

A meta-analysis reviewing the effectiveness of BMSC mobilization for the treatment of AMI included 7 studies and a total of 364 patients. The analysis concluded that treatment with G-CSF can improve LV ejection fraction if the treatment is administered early after the heart attack (Kang et al., 2007). However, in spite of the improvements in ejection fraction, other general parameters of cardiovascular health such as ventricular arrhythmia, rehospitalization for heart failure, and the composite of other cardiovascular events (i.e., death from heart attack, recurrent heart attack, and stroke), were not significantly different in the G-CSF treatment groups compared with the control groups. Similar results were reported by another meta-analysis that included eight studies and 385 patients (Abdel-Latif et al., 2008).

So, it remains unclear whether the simple mobilization of BMSCs can constitute an effective treatment for AMI. While some studies have yielded promising results, others suggest no benefits at all. However, positive results obtained in some studies should not be denied on the basis of the negative results obtained in others. Reconciliation of all this data and the development of an effective treatment protocol will most probably come through the determination of optimal treatment parameters: 1) intensity of ESCM, 2) duration of the treatment, 3) time after AMI, 4) number of treatments received over time, and 5) other yet unidentified parameters. Compounds other than G-CSF might also be discovered that could provide more consistent results (Broxmeyer et al., 2005; DeClercq, 2005).

3.2 Stroke

Many studies have shown that extensive neuronal death in the brain after a stroke triggers the migration of neural stem cells to the site of injury, followed by their proliferation and differentiation into neurons and glial cells (Peterson, 2002; Fallon et al., 2000; Arvidsson et al., 2002; Nakatomi et al., 2002; Schmidt and Reymann 2002). However, this natural process does not appear to be sufficient to produce significant functional recovery (Yamamoto et al., 2001; Magavi and Macklis, 2002).

As with the heart after AMI, stroke has been associated with BMSC mobilization. Studies have shown that the number of PBSC in stroke patients can increase up to 3-fold within 7 days after the stroke (Hennemann et al., 2008; Paczkowska et al., 2005). In one study, the magnitude of BMSC mobilization was correlated with the patients' functional recovery (Dunac et al, 2007).

When rats were injected with rat (Chen et al., 2001; Pavlichenko et al., 2008; Willing et al., 2003) or human SC (Li et al. 2002) after an induced stroke, significant motor and cognitive improvements were observed. Although a significant number of BM-derived cells could be identified as newly formed neurons and glial cells in the stroke foci, they accounted for only a small percentage of the total number of newly formed brain cells. Most of the newly formed brain cells are believed to be derived from neural SC upon the action of paracrines secreted by the migrating SC. Similar results were obtained using human umbilical cord stem cells (HUCSCs) where intravenous injection of HUCSCs 24 hours after a stroke greatly improved functional recovery (Chen et al., 2001). Injection of HUCSCs 7 days after the stroke still led to significant functional recovery, though the extent of the recovery was less than with treatment at 24 hours.

Mobilization of BMSCs induced by G-CSF was shown in a number of studies to improve the outcome of a stroke. For example, when tested 14 and 28 days after a stroke, animals treated with G-CSF showed much greater body coordination than control animals (Shyu et al., 2004). When the brains were analyzed using imaging, the infarcted area was much smaller in the treated group (61 mm^3) when compared to the control group (176 mm^3). All these benefits were greatly reduced when the animals were pre-treated with a blocker of CXCR4, indicating that the observed effects were dependent upon the migration of BMSCs into the brain.

Similar results have been reported by other scientific teams (Six et al., 2003; Kawada et al., 2006). For example, after repopulating the bone marrow with GFP-positive stem cells, G-CSF-induced BMSCs mobilization immediately and roughly 2 weeks after inducing a stroke dramatically improved motor and cognitive performances four weeks after the stroke, as measured by using the Morris water maze (Kawada et al., 2006). In this study, while all the mice in the treated group reached the platform within 40 seconds, none of the control mice reached the submerged platform within the allotted 120 seconds. As in the study by Shyu et al. (2004), the infarct size was much smaller in the treated animals when compared to control. Using BrdU it was observed that the number of new brain cells found in the infarcted area was much higher in the treated group than in the control group. Yet very few of the new brain cells were GFP-positive, supporting the view that as they migrate in the brain BMSCs secrete growth factors that support the proliferation and differentiation of neural stem cells (Yoo et al., 2008). BMSCs also support neovascularization, which further

contributes to the regeneration of the brain tissue (Lee et al., 2005; Hess et al., 2002; Kan et al., 2005).

Although much of this work needs to be reproduced in humans, ESCM for the treatment of stroke appears promising and would constitute a safe approach to the treatment of stroke.

3.3 Diabetes

The ability of BMSCs to leave the bone marrow, migrate to the pancreas and become insulin-producing cells was beautifully shown by Ianus et al. (2003). In brief, female mice were lethally irradiated and then transplanted with male BMSCs that express, using a CRE-LoxP system, GFP if the insulin gene is actively transcribed. When analyzed 4-6 weeks after the transplantation, GFP-positive cells were found in the pancreas (Figure 2). The GFP-positive

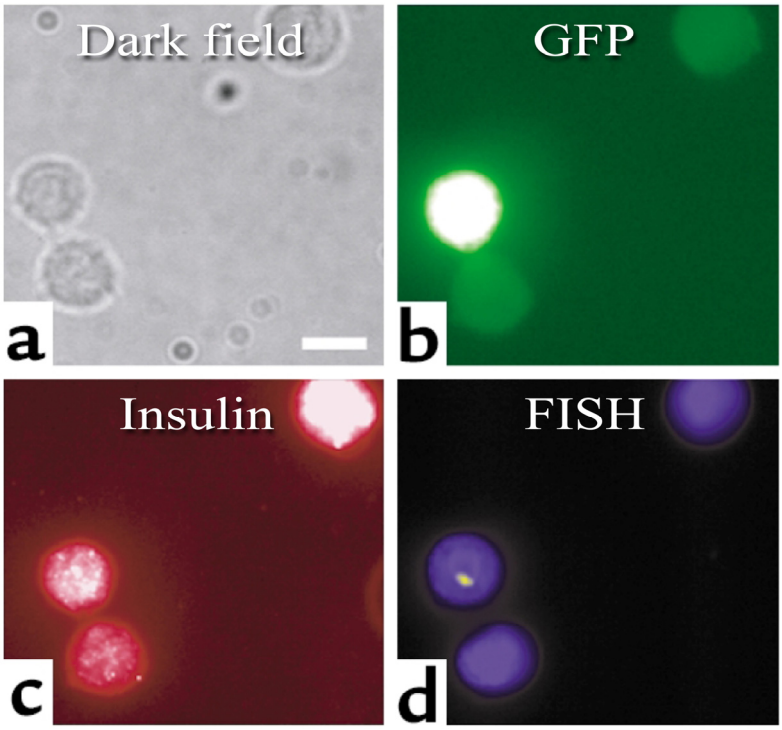

Fig. 2. **FISH and immunofluorescence marking of BM-derived insulin-producing cells.** Immunofluorescence and FISH of isolated, dispersed pancreatic islet cells after transplantation of lethally irradiated female mice with male BMSCs that express, using a CRE-LoxP system, GFP if the insulin gene is actively transcribed. a) Bright-field phase, b) GFP imaging note slight autofluorescence of control isolated islet cells; c) Immunostaining with rhodamine X–labeled secondary antibody for insulin; d) FISH for Y chromosome (in yellow) and nucleus stain with DAPI (blue). Y chromosome is present only in GFP-positive cells. Scale bar, 5 μm; X630. (Taken from Ianus et al., 2003)

cells were also positive for insulin, for insulin RNA, and for Y-chromosome, demonstrating that they originated from the transplanted BMSCs. These cells showed functional characteristics typical of normal pancreatic ß-cells, such as fluctuations of intracellular calcium upon exposure to various concentrations of glucose. Within the time frame of that study (4–6 weeks), 1.7–3% of BM–derived GFP-positive cells were detected in the pancreatic islets. In a similar study, BMSCs were also shown to participate into the development of new blood vessels, further supporting the regeneration of the pancreatic tissue (Mathews et al., 2004; Gao et al., 2008).

Then, using a protocol similar to that used by Ianus et al., Hasegawa et al. (2007) further demonstrated that mobilization of BMSCs was not only effective but essential for pancreatic regeneration. Hasegawa et al. induced diabetes by injection of streptozotocin (STZ) in lethally irradiated female mice followed by infusion of BMSC from GFP transgenic mice. Infusion of BMSCs led to the incorporation of GFP-positive BMSCs into islets of Langerhans in the pancreatic tissue, partially restoring pancreatic islet number and size, and improving STZ-induced hyperglycemia. However, when the same experiment was done while simply infusing the pancreas with BMSCs, without preirradiation, no improvement was obtained. Furthermore, when the experiment was repeated with full BMSC transplant in a model of mice with impaired ability to mobilize stem cells, no benefits were obtained. Therefore, natural mobilization of BMSCs from the bone marrow appears essential for the regeneration of pancreatic function after inducing diabetes with STZ.

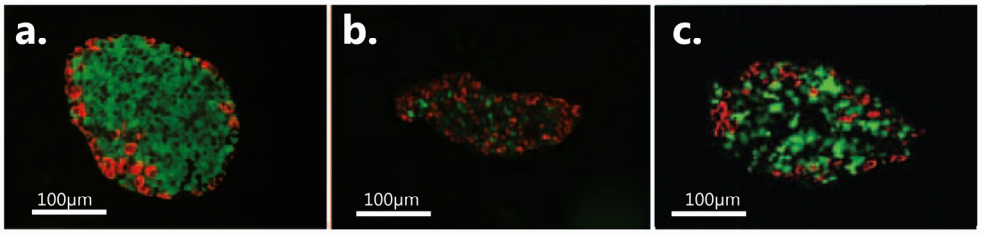

Fig. 3. **Pancreatic islets of STZ-treated mice receiving subsequent bone marrow transplant (BMT)**. Double immunostaining of pancreases with anti-insulin and anti-glucagon antibodies. *Green* indicates insulin-positive and *red* glucagon-positive cells. Pancreases from normoglycemic control mouse (a), hyperglycemic control mouse (b), and STZ-treated mouse receiving BMT (c). BMT improved STZ-induced hyperglycemia. (Taken from Hasegawa et al., 2007)

In one recent study in humans, ESCM showed great promise in the treatment of diabetes. The study selected individuals recently diagnosed for diabetes and the treatment consisted of both stem cell mobilization and autologous stem cell transplant. The patients first received injections of G-CSF in order to harvest PBSC, followed later by autologous stem cell thansplant and, 5 days post-transplant, a second round of G-CSF treatment. The endpoints monitored in the study were overall morbidity along with temporal changes in exogenous insulin requirements. Before the treatment, all patient required daily insulin injection. By the end of the study, 14 of the 15 patients had experienced insulin-free episodes ranging between 1 and 35 months (mean 16.2 months) (Voltarelli et al., 2007).

In this study the patients benefited from two instances of ESCM and one instance of autologous SC transplant. It is not possible to determine what were the respective contributions of the ESCM and SC injection, however it is likely that the mobilizations by themselves significantly contributed to the benefits experienced. While the first mobilization lasted several days and the second mobilization lasted about one week, there was only one instance of stem cell injection.

Diabetes is an interesting disease to study the link between disease progression and the number of circulating PBSCs, as it follows a series of relatively well defined stages with regard to carbohydrate metabolism status, namely normal glucose tolerance (NGT), impaired fasting glucose (IFG), impaired glucose tolerance (IGT), and newly diagnosed diabetes mellitus (DM). Fadini et al. (2010) quantified the number of circulating CD34+ cells by flow cytometry in 425 individuals divided among these four stages of disease progression. The data showed a clear trend of decreased number of PBSCs with disease progression through IFG, IGT and DM (Figure 4). The number of circulating PBSCs was significantly lower in the IGT and DM groups when compared to the NGT group. The reduction in the number of PBSCs can either be a consequence of higher blood glucose levels that might affect the ability of stem cells to mobilize from the bone marrow or a causal factor in the development of DM whereby a reduced number of circulating PBSCs reduces the ability of the pancreas to renew itself over the years, or both. This supports the view previously suggested that diabetes could be a stem cell disease (Fadini et al., 2009).

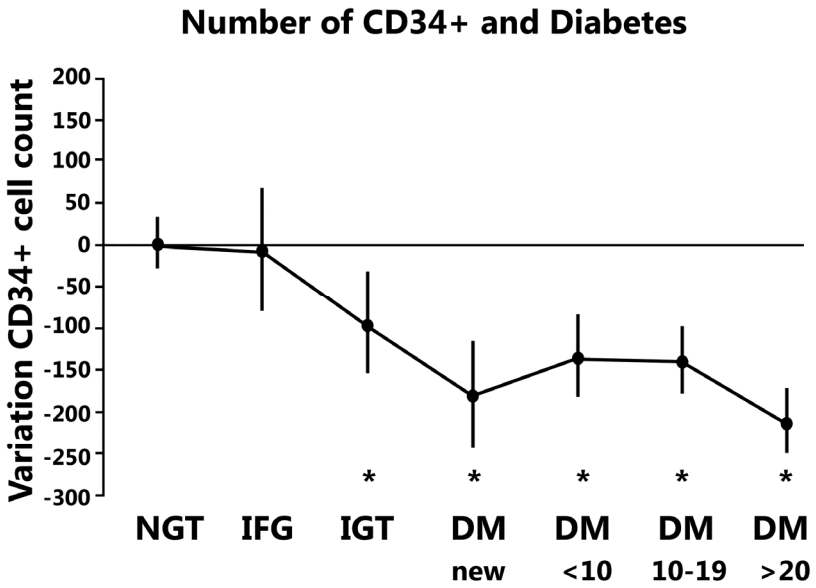

Fig. 4. Variation of circulating CD34+ cells and diabetes. **V**ariation of circulating CD34+ cells in patients grouped according to carbohydrate metabolism, namely normal glucose tolerance (NGT), impaired fasting glucose (IFG), impaired glucose tolerance (IGT) or diabetes (DM) duration, as appropriate. The mean value of patients with NGT was taken to represent the zero point. Bars indicate 95% CIs of means. * Values significantly different when compared to NGT. (Taken from Fadini et al., 2009)

4. Clinical application (SE)

Investigation of the clinical potential of ESCM has been limited, largely due to the significant risk associated with the use of G-CSF, the main stem cell mobilizer used in clinical trial, for extended periods of time (Bensinger et al., 1996; Shimoda et al., 1993). Recently, a new stem cell mobilizer (StemEnhance®; SE) has been developed that triggers a much milder increase in the number of PBSC, but its safety allows for a sustained oral daily consumption over long periods of time, allowing for safe daily ESCM (Jensen et al., 2007).

In brief, SE is an extract from the cyanophyta *Aphanizomenon flos-aquae* that concentrates a protein with an estimated molecular weight of 160–180 kDa, which was shown to be a selective L-selectin blocker. Oral consumption of 1 gram of SE was been shown to trigger an average 25% increase in the number of PBSC within 60 minutes after consumption. The magnitude of the mobilization induced by SE is much smaller than that triggered by G-CSF, however its safety allows for continuous use and therefore offers a novel approach in the study of ESCM. To test its therapeutic potential, SE was used in a number of preliminary clinical trials involving a number of diagnostic entities.

4.1 Parkinson

MC is a 62 year old male with a 17 year history of Parkinson's Disease. MC was initially diagnosed with Parkinson's type resting pill-rolling tremor affecting his left hand and then progressing to the limbs on left side of his body. Over the course of five years the tremors increased gradually affecting the right side as well. At ten years into the disease process, MC was no longer able to practice as an attorney, mobility was affected and limitations increased compromising his personal and professional abilities.

In 2009, before the treatment of SE, the left side tremors had significantly worsened, symptoms of stiffness and bradykinesia which introduced a shuffling gait and poor balance made it unsafe for HM to ambulate without the use of a cane. At the time MC began consuming SE he was unable to dress himself, put on his watch, write, feed himself or drive his car. After 45 days on the product, consuming 1 gram of SE, three times per day, MC showed significant improvement with decreased tremors, less stiffening and bradykinesia; at this time MC was able to get around without the use of his cane. After 60 days on the product, judging that the benefits had plateaued, MC discontinued the use of SE. Forty - five days later, signs of tremor and bradykinesia returned and MC was seeking medical attention, once again. MC resumed SE with a dose of 2 grams, three times per day and within six weeks showed much improvement with less tremors, stiffness and bradykinesia. MC was able to participate in activities of daily living, such as dressing and feeding himself, he was also able to tie his own tie, put on his watch, and once again, he could ambulate without using a cane. To date MC has been on SE for two years, he has returned to driving his car without limitations, he is independent with all activities of daily living and he also participates in some level of professional activity.

Another patient, MT, is a 52 year old woman with an early onset of Parkinson at age 36, with tremor as the primary symptom. Early treatment consisted of Pergolid, then Budipin up to 30 mg three times a day. However due to QTc increase, Budipin was later reduced to 10 mg three times a day. The patient was also treated with Methydopamin 62.5 mg four times a day. With this treatment, MT's main problem was the experience of fluctuations and

the beginning of ON-OFF syndrome. One year ago MT began consuming StemEnhance 1 gram three times a day. Today MT's experiences only minor tremor accompanied with some dyskinetic syndrome and sometimes propulsion. Her quality of life has increased and she is much more socially active.

4.2 Traumatic spinal cord injury

A preliminary trial with 8 individuals with spinal cord injuries was performed in a community center in Hawaii. The cases all involved paraplegia and various degrees of quadriplegia. Of the 8 cases, 4 dropped due to various circumstances unrelated to the consumption of SE. Of the 4 remaining participants, 2 had repetitive periods of hospitalization that made their consumption of SE irregular. Of the two remaining participants, one experienced mild though significant improvement in mobility while the other participant experienced significant improvements in mobility.

The latter participant, VS, had a serious car accident 17 years prior to SE consumption and was left with traumatic brain injury that affected her speech and a significant spinal cord lesion. At the beginning of the trial, VS was able to lift her right leg approximately 15 centimeter from her chair with no lateral movement, and showed a total absence of movement in her left leg. She could move her arms, hands and fingers though the movements were very slow with little dexterity and precision. She had some ability to move in her bed but was unable to turn herself without assistance. No data was available to document peripheral sensory perception of the lower limbs or nerve conductivity. After 6 months of daily consumption of 3 grams of SE three times a day, VS could lift her right leg more than 30 centimeters from her chair, with lateral movement outside of her chair. She could also lift her left leg off the chair and laterally outside of the chair. VS had less control over the movement of her left leg, though the magnitude of the movements was comparable to the movements seen with the right leg. After 10 months, VS was able to rotate herself in her bed unassisted and from a supine position she could lift both legs to a 90 degree angle and sit in her bed unassisted. Over the period of the trial her upper limbs also improved in dexterity and her speech showed mild though significant improvement. VS comes from a disadvantage socio-economic environment and did not have access to physical therapy beyond the first few years of her injury, she therefore developed leg and feet deformities that prevented her from bearing weight and possibly resuming physical therapy.

4.3 Coronary Artery Disease

JP is a 60 year old South African male who experienced a heart attack at 51 years of age. After diagnosis of Coronary Artery Disease was made a stent placement was performed. Dietary and lifestyle changes were implemented immediately by JP following the hospitalization. Unfortunately, 3 years later, JP suffered four additional heart attacks. During the hospitalization, the angiogram revealed the right and left coronary arteries were obstructed 100% and 40%, respectively, thus determining that JP was not a good candidate for bypass surgery. At the time of the last hospitalization, JP had decreased energy and experienced "stable" angina with any exertion, and his overall quality of life was greatly compromised. JP was put on a medication regimen which consisted of Atenolol 50mg daily,

Perindopril 4 mg daily, Elantan 20 mg twice per day, Simvostatin 20 mg daily, Adelat xl 30 mg daily, and Aspirin 1 per day. JP was then put on a list for possible heart transplant.

Three weeks after discharge JP began consuming SE, 1 gram three times per day. After 3 months of taking the product, he received a call for a possible heart transplant and returned to see his cardiologist for evaluation and comprehensive testing. The cardiologist reported that JP was making a remarkable recovery, the heart transplant surgery was postponed. Four months later JP was re-evaluated and found to have made a complete recovery. JP has since been returning at 6 month intervals for follow up evaluation, and to date he has been stable with no further coronary incidents. The evaluations and most recent ECG conclude that JP has normal heart function. Presently, JP shares that he is not on any medications, blood pressure is 126/65 mm Hg, he continues to take SE and experiences good quality of life.

4.4 Diabetes and rheumatoid arthritis

NA is a 47 year old Colombian woman who was diagnosed at 18 year of age with deforming rheumatoid arthritis. Over the years she managed her condition with the use of Prednisone, Methotrexate, Sulfasalazina, and Diclofenac. Four years ago NA was diagnosed with diabetes mellitus with glycemia reaching 308 mg/dL and assumed treatment with Euglucon 5 mg twice a day along with NPH insulin. About one year ago, due to her arthritic condition she became wheelchair bound and required assistance for bathing and dressing. At that time her medical records show levels of C-Reactive Protein (CRP) of 96.2 mg/L, Erythrocytes sedimentation rate (ESR) of 81 mm/H, Platelet count of 535 K/ul, fasting glucose of 147 mg/dL and HgbA1c (or Glycosylated Hemoglobin) of 8.07. A few months after becoming wheelchair bound NA began consuming 1 gram of SE once a day. After one month she subjectively reported a reduction in pain and inflammation, while 3 months later her mobility had improved to the point that she could bathe and dress alone. After 6 months she began using a walker, then switched to a cane and one year later she was walking unaided. Her last medical records indicate a level of CRP of 2.1 mg/L, ESR of 34 mm/H, Platelet count of 485 K/ul. NA has also discontinued the use of any diabetes medication; her records indicate a fasting glucose of 106 mg/dL with a glycemia not exceeding 120 mg/dL and HgbA1c of 5.62.

At the time of writing this report, NA does not take any anti-inflammatory drug, undergoes a remarkable improvement in her quality of life, keeping daily consumption for 15 consecutive months of 1 gram of SE, bone support supplementation of 500 mg Calcitriol (1,25-dihydroxycholecalciferol) every month, milk of magnesia and an annual dose of Zoledronic Acid.

4.5 Cerebrovascular accident (Stroke)

In September 2008, GE, a 78 year old male surgical oncologist who was otherwise in good health, had a stroke. The MRI/MRA revealed an acute infarct involving the right lentiform nucleus, moderately extensive chronic small vessel ischemic changes, chronic lacunar infarct involving the right ventromedial thalamus, and intracranial atherosclerotic vascular disease. The stroke left GE with aphasia and a reduced ability to perform any physical activity. October 2009, 13 months after the stroke, GE began taking SE; GE consumed 1 gram twice

per day and after 8 weeks on the product GE noticed improvement with his speech and experienced more energy with improved balance. In April 2010 a repeat MRI/MRA showed no evidence of an acute infarct. GE's aphasia was completely resolved at this point and his overall mobility was improved allowing him to perform all activities of daily living. In January of 2011 a repeat MRI/MRA of the anterior and posterior cerebral arteries demonstrated no evidence of hemodynamically significant stenosis, and revealed normal vertebrobasilar arteries with no evidence of intracranial aneurysm or vascular malformation.

To date, GE has resumed playing tennis at age 81, walks around the mall in the neighborhood with other senior citizens and has returned to his professional activity as a surgeon.

4.6 Kidney failure

The whole paragraph should read: "GW is 36 months old; he was born with a malfunctioning valve in the urethra, compromising the flow of urine at birth. At ten days old he had surgery to repair the urethra, however, the damage to the kidneys and the bladder were already evident, caused from the retention of urine at birth. Following the surgical procedure, scar tissue was observed to the wall of the bladder and one of the kidneys did not respond, eventually that kidney quit working and began to atrophy. A scar was also left on his hand caused from an IV being ripped out after surgery. Before SE was introduced, GW had stopped growing at 15 months of age and multiple health issues were leading him toward a kidney transplant. Furthermore, GW had been on antibiotics for one year due to multiple infections. His body was not eliminating fluid as it should, as evidenced by the worsening of edema resulting in the use of diuretics. GW had purple feet caused from compromised circulation, dark spots were observed on his back, his eyes were blood shot and his overall presentation was pale in appearance. GW's activity was nothing normal for a 1 ½ year old child; his energy level was very depleted and he was unable to play like a normal toddler.

GW was 21 months old when SE was introduced at 250 mg per day. After 3 days on SE his mother reported that his eyes were crystal clear and he was observed running through the house full of energy. The dark spots on his back began to fade after one week of SE consumption. Red color was seen on GW cheeks for the first time since he was born. After 75 days on SE, GW had grown 6cm taller and the shrinking kidney at birth had measured 0.8cm larger. GW is off all pharmaceutical medications to date and his bladder and kidney are back to normal functioning to their fullest capacity, the scar to his hand is completely gone.

5. Conclusion

The benefits of ESCM on various degenerative conditions have been documented in several animal models and in humans. In some cases, BMSC clearly migrate into tissues and directly contribute to the formation of new functional somatic cells of the target tissue. However in other cases, especially diseases affecting the heart and central nervous system, a primary mechanism of action appears to be the secretion of paracrines that stimulate the

proliferation and differentiation of tissue stem cells. Much work remains to be done to clearly elucidate the mechanisms of action behind the benefits of ECSM in various diagnostic entities, however from a clinical standpoint it remains that regardless of the mechanism of action, ESCM appears to be a valuable approach to increase the quality of life of patients affected by various degenerative diseases.

Various stem cell mobilizers have been documented in the scientific literature and many of them have been associated with side effects that prevent the application in humans of what has been documented as effective in various animal models. Such mobilizers include G-CSF, Stem Cell Factor, interleukin-8 and plerixafor (Lemery et al., 2011), which have all been associated with side effects going from diarrhea, nausea, pain and numbness to pericarditis and thrombosis. In spite of the potential benefits, such side effects have largely prevented the use of such compounds for ESCM in humans, and the lack of safe stem cell mobilizers largely explains the limited interest so far in this therapeutic approach. The main challenge in further investigating the therapeutic potential of ESCM remains therefore the development of safe stem cell mobilizers. In the meanwhile, SE appears to be a valuable tool to study the clinical benefits of ESCM.

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