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Advances in bone marrow stem cell therapy for retinal dysfunction

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A B S T R A C T

The most common cause of untreatable vision loss is dysfunction of the retina. Conditions, such as age-related macular degeneration, diabetic retinopathy and glaucoma remain leading causes of untreatable blindness worldwide. Various stem cell approaches are being explored for treatment of retinal regeneration. The rationale for using bone marrow stem cells to treat retinal dysfunction is based on preclinical evidence showing that bone marrow stem cells can rescue degenerating and ischemic retina. These stem cells have primarily paracrine trophic effects although some cells can directly incorporate into damaged tissue. Since the paracrine trophic effects can have regenerative effects on multiple cells in the retina, the use of this cell therapy is not limited to a particular retinal condition. Autologous bone marrow-derived stem cells are being explored in early clinical trials as therapy for various retinal conditions. These bone marrow stem cells include mesenchymal stem cells, mononuclear cells and CD34+ cells. Autologous therapy requires no systemic immunosuppression or donor matching. Intravitreal delivery of CD34+ cells and mononuclear cells appears to be tolerated and is being explored since some of these cells can home into the damaged retina after intravitreal administration. The safety of intravitreal delivery of mesenchymal stem cells has not been well established. This review provides an update of the current evidence in support of the use of bone marrow stem cells as treatment for retinal dysfunction. The potential limitations and complications of using certain forms of bone marrow stem cells as therapy are discussed. Future directions of research include methods to optimize the therapeutic potential of these stem cells, non-cellular alternatives using extracellular vesicles, and in vivo high-resolution retinal imaging to detect cellular changes in the retina following cell therapy.

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1. Introduction

1.1. Retinal dysfunction—leading cause of vision loss

There are 37 million people who are blind and 124 million people who are visually impaired excluding those with uncorrected refractive error (Foster and Resnikoff, 2005). The most common causes of untreatable blindness and vision loss are conditions associated with dysfunction or degeneration of the retina. The most common conditions include macular degeneration, diabetic retinopathy, and glaucoma (Bunce and Wormald, 2006; http://medlineplus.gov, 2008). These conditions usually affect both eyes and are progressive. The end result could be severe visual impairment that impacts the quality of life of the individual (Brown, 1999).

Age-related macular degeneration (AMD) is the leading cause of irreversible vision loss in the elderly in the developed world. As the world population increases in age, the prevalence of AMD is expected to increase significantly and 288 million people are projected to have this condition by 2040 (Wong et al., 2014). Diabetic retinopathy is the leading cause of blindness in the working-age adult population and one of the leading causes of vision loss in the elderly (Kocur and Resnikoff, 2002). Since the prevalence of diabetes mellitus is on the rise globally, the prevalence of diabetic retinopathy and associated vision loss is expected to rise as well (Shaw et al., 2010). Finally, glaucoma, a condition that is usually considered an optic neuropathy, is associated with degeneration and loss of the ganglion cells that constitute the inner retina. Glaucoma is the second leading cause of blindness globally (Kingman, 2004).

In recent years, advances in laser and surgical treatment and drug development have provided therapeutic options to minimize vision loss associated with many of these conditions. Intravitreal anti-VEGF (vascular endothelial growth factor) therapy or corticosteroid treatment can limit the vision loss associated with macular edema or neovascular complications of retinal vasculopathies, such as diabetic retinopathy and retinal vein occlusion (Diabetic Retinopathy Clinical Research Network et al., 2015; Kriechbaum et al., 2014). Intravitreal anti-VEGF therapy has limited the vision loss associated with exudative AMD (Comparison of Age-related Macular Degeneration Treatments Trials Research Group et al., 2012). In addition, advances in surgical and medical management of glaucoma aim to minimize vision loss associated with progression of glaucoma (http://ni.nih.gov, 2016).

Even with these new treatments, much of the vision loss associated with degenerative or ischemic retinal conditions remains irreversible and often progresses over time. It is sobering to note the long-term visual acuity results of individuals treated with intravitreal anti-VEGF therapy for exudative AMD as part of the Comparison of Age-related Macular Degeneration Treatment Trials study (Comparison of Age-related Macular Degeneration Treatment Trials Research Group et al., 2016). The mean visual acuity gain achieved during the first two years with intravitreal anti-VEGF therapy was lost by five years follow-up. About 20% of eyes had final visual acuity of 20/200 or worse in the treated eye despite regular intravitreal anti-VEGF treatment. Thus, the visual benefit of intravitreal anti-VEGF therapy appears to be short-term in some eyes with exudative AMD. For the more common non-exudative AMD, no treatment exists except for vitamin supplementation and lifestyle modifications (Shah et al., 2013). Thus, there is an unmet need to regenerate the retinal tissue that is lost or damaged by these pathologic processes.

Tissue regeneration is possible with stem cell therapy. Stem cell therapy is being explored clinically to treat a variety of medical disorders with variable success (Rafii and Lyden, 2003; Mackie and Losordo, 2011; Squillaro et al., 2016; Park, 2016). In this article, we will review the rationale for exploring bone marrow stem cells (BMSCs) as therapy for retinal dysfunction and discuss current and future advances in this area of research. The retinal anatomy and the retinal neuronal network that may be altered and remodeled in
response to various disorders of the retina, makes direct cell replacement challenging and perhaps less efficacious. The paracrine trophic effects of BMSCs can reach multiple damaged cells in the retina, making the use of these stem cells potentially more viable as a therapy for a broad range of conditions that result in retinal dysfunction (Gnecchi et al., 2008). It may be a preferred approach over direct cell replacement using a single cell type. In addition, the homing capability of some BMSCs can result in integration of the cells into the damaged retina, making the delivery of these cells to the target tissue easier and potentially safer than the subretinal method of cell delivery used for other types of stem cell therapy. This review will discuss: i) various stem cell therapy options currently being explored for retinal dysfunction relative to the studies using BMSCs, ii) the various types of BMSCs being explored in preclinical and clinical studies, iii) non-cellular alternatives, iv) host factors that may affect the success of BMSC therapy, and v) new retinal imaging methods to study the therapeutic effects. Finally, vi) future directions to optimize the effect the BMSC therapy for retinal dysfunction will be discussed.

2. Pathogenesis and remodeling of the retina in disease

2.1. Interplay between inner and outer retina

The retina consists of neurons, vasculature and glial cells (Park, 2004). Among the retinal neurons are photoreceptors that constitute the outer retina, the ganglion cells that constitute the inner retina and optic nerve and the connecting interneurons. The inner retina is perfused by the retinal vessels. In contrast, the outer retina and retinal pigment epithelium (RPE) are perfused by the choroidal circulation.

Retinal degeneration is usually characterized by degeneration of the photoreceptor layer with the inner retina remaining relatively intact. On the other hand, disorders of the optic nerve, such as glaucoma, are characterized by loss of ganglion cells. These disorders where only a single cell type is affected primarily may be preferred candidates for stem cell therapy where the goal is direct cell replacement. While only one cell type may need to be replaced and reconnected with the remaining neuronal network to restore vision, this is a highly complex process.

Disorders of the optic nerve, such as glaucoma, have been noted with photoreceptor cell loss using adaptive optics in vivo retinal imaging (Choi et al., 2011). This observation is suggestive of interplay between neurons in the inner and outer retina. The net result would be that degeneration of one layer of retina likely affects the health of the other. Similarly, in disorders of the retinal vasculature, such as diabetic retinopathy, where only the inner retina is expected to be affected via retinal ischemia, concurrent photoreceptor degeneration has been noted. This has been appreciated using adaptive optics in vivo retinal imaging and electroretinography (ERG, Tzekov and Arden, 1999; Soliman et al., 2016). In vivo retinal imaging studies have shown progressive loss of photoreceptors in eyes with worsening severity of diabetic retinopathy. Whether the photoreceptor degeneration associated with diabetes is from concurrent choroidal ischemia, direct photoreceptor dysfunction from hyperglycemia or secondary to inner retinal damage is unknown and open to speculation.

Such interplay of the inner and outer retinal neurons is seen in...
eyes with primary retinal photoreceptor degeneration as well. Eyes with diffuse retinal degeneration, such as retinitis pigmentosa develop optic nerve pallor, suggestive of optic nerve atrophy from ganglion cell loss, and retinal vascular attenuation suggestive of retinal ischemia in advanced stages (Thinda and Moshiri, 2015). Disorders of outer retina characterized by photoreceptor loss can be associated also with concurrent atrophy of the retinal pigment epithelium and choroidal circulation, as in eyes with AMD (Yau et al., 2015).

Fig. 1 illustrates the variable mechanisms potentially leading to vision loss in eyes with different causes of retinal dysfunction. Fundus photographs and multifocal ERG recordings of the macula of three subjects with 20/200 to 20/400 best-corrected visual acuity show interesting differences and similarities. The first subject has inactive proliferative diabetic retinopathy after panretinal laser photocoagulation. The fundus photograph shows atrophy of the central macula suggestive of macular degeneration (Fig. 1a). There is diffuse suppression of macular function by multifocal ERG demonstrating that retinal dysfunction extends beyond the area of macular atrophy seen on fundus photography. Full-field ERG is severely suppressed and not recordable in this eye (data not shown). These findings suggest a diffuse advanced retinal dysfunction in this eye with diagnosis of retinal vasculopathy, diabetic retinopathy. The second subject has the same visual acuity and severe central vision loss from Stargardt’s disease, a hereditary form of macular degeneration (Fig. 1b). The multifocal ERG shows a generalized suppression of macular function similar to the first subject with diabetic retinopathy. However, the full-field ERG is minimally decreased. These findings suggest a diffuse dysfunction of the retina primarily in the macular region. The third patient has vision loss from dry atrophic AMD with central geographic atrophy (Fig. 1c). The multifocal ERG shows only focal areas of macular dysfunction mostly corresponding to the areas of geographic atrophy seen on fundus photography. This finding suggests that the pathogenesis of dry AMD is different from hereditary macular degeneration, such as Stargardt’s disease, and is not associated with a diffuse retinal dysfunction of the macula. Focal choroidal ischemia and structural changes in Bruch’s membrane leading to focal abnormalities in the RPE and photoreceptors can be considered in the pathogenesis of dry AMD (Hanus et al., 2016).

2.2. Implications for stem cell therapy and retinal regeneration

Tissue regeneration is possible with stem cell therapy. However, the treatment efficacy may vary depending on the nature and extent of the cells affected by the underlying retinal pathology. Retinal conditions with primary dysfunction of a single cell type, such as photoreceptor or RPE, may be amenable to direct tissue replacement or rescue therapy using stem cells differentiated into the particular cell of interest. However, the concept of direct cell replacement to restore vision using a single type of retinal cell might be overly simplistic and optimistic especially in eyes with significant longstanding vision loss. First, the retinal disorder often involves dysfunction of more than one retinal cell type. Second, the neuronal circuitry within the retina and the retinal structure itself might be permanently altered by the chronic retinal dysfunction involving multiple retinal neurons. Photoreceptor degeneration has been shown to trigger extensive retinal remodeling, involving retinal neuronal migration and rewiring (Marc et al., 2003). These factors provide some explanation for the disappointing visual outcome of eyes with retinal degeneration treated with subretinal transplantation of intact sheets of fetal retina (Zhang et al., 2003; Radkte et al., 2002). Similarly, in eyes with diabetic retinopathy, progressive loss of endothelial cells and pericytes leads to formation of naked basement membrane tubes. These naked basement tubes become acellular capillaries with irreversible retinal ischemia (Douglas et al., 2012). Thus, there is likely a limited window of opportunity for stem cell treatment to reverse or limit the structural damage within the retina. In such a condition, direct cell replacement using just a single cell type to reconstitute the vascular endothelium might have a limited therapeutic effect. Harvested BMSCs with heterogeneous mixture stem cells, some with paracrine trophic effects and some capable of direct engraftment, might allow the stem cells to affect a variety of cells in the retina that might be affected in eyes with retinal dysfunction, including diabetic retinopathy and retinal degeneration.

The positive aspects of the retinal anatomy that makes stem cell therapy appealing for treatment of retinal dysfunction are that the eye is a confined small space and local delivery of cells to the retina can be achieved relatively easily using a limited number of stem cells. In addition, the optically clear media of the eye would allow in vivo retinal imaging tools to be used to study the effects of the cell therapy noninvasively and at cellular detail. Furthermore, the effects of cell therapy can be determined with both objective and subjective functional testing. Finally, the relative immunologically privileged nature of the eye allows allogeneic cell transplantation to be more feasible potentially than in other organs.

3. Stem cell options

Stem cells are defined as cells that are capable of self-renewal and at the same time can give rise to multiple cell types. The ideal stem cell for cell replacement in eyes with retinal dysfunction would be a cell that can be expanded and easily directed to differentiate into the various cells of interest in the retina that may be affected by the pathologic condition. The ideal transplanted cell should have a long-term effect by integrating and surviving in the retina and restoring the neuronal activity within the retina. The cell should be stable and display limited proliferative potential after integration in the retina in order to minimize the risk of abnormal cellular proliferation and teratoma formation in the eye.

3.1. Pluripotent stem cells

To date, there is no ideal stem cell that has all the features listed above. However, various types of stem cells have been explored as potential therapy for retinal dysfunction. Pluripotent stem cells, such as embryonic stem cells and induced pluripotent stem cells, have unlimited potential to differentiate and expand (Leeper et al., 2010). As such, they have some of the ideal features of stem cells for retinal regeneration. Thus, these pluripotent stem cells have received a lot of attention in recent years. However, these pluripotent stem cells need to be differentiated before clinical application in order to minimize the risk of abnormal cellular proliferation and teratoma formation (Kawai et al., 2010). Preclinical studies have shown that these pluripotent stem cells can differentiate into hemangioblasts (Lu et al., 2007). These hemangioblasts can integrate into damaged retinal vasculature following intravitreal or intravenous administration and play a role in vascular repair, similar to observations made with adult endothelial progenitor cells (Caballerio et al., 2007). Similarly, vascular progenitor cells derived from induced pluripotent stem cells from cord blood can incorporate into retinal vessels following intravitreal or systemic administration (Park et al., 2014b). However, these cells have not been explored in clinical trials since more long-term preclinical safety studies are needed.

Similarly, preclinical studies have shown that pluripotent stem cells can be differentiated into cells expressing features of retinal pigment epithelial (RPE) cells, retinal progenitor cells and retinal ganglion cells (Lu et al., 2009; Hirami et al., 2009; Chen et al., 2010; Parameswaran et al., 2015). However, these cells do not readily...
incorporate into the retina following intravitreal injection (Chen et al., 2010). The RPE cells derived from embryonic stem cells and induced pluripotent stem cells had been transplanted into the subretinal space and shown to slow down retinal degeneration in animal models (Lu et al., 2009; Sun et al., 2015). Thus, this is the route of delivery being explored in clinical trials.

3.2. Clinical trials using subretinal cell transplantation

Phase I/II clinical trials have been completed recently using RPE cells derived from embryonic stem cells as potential therapy for age-related and/or hereditary macular degeneration (Schwartz et al., 2015). This allogeneic therapy required systemic immunosuppressive therapy for the first three months after cell transplantation surgery in order to minimize cell rejection until the blood retinal barrier is re-established. A majority (72%) of the eyes enrolled in this clinical trial treated with this cellular therapy had visible pigmented cells in the submacular space that remained for the duration of the study follow-up (mean 22 months). However, 28% of the enrolled subjects had adverse effects from systemic immunosuppression. Since the cells were injected into the submacular space during vitrectomy surgery, some eyes developed complications of surgery such as endophthalmitis and cataract progression. Epiretinal membrane from preretinal migration of the subretinal transplanted cells developed in 16% of eyes, but none was felt to be visually significant. Most importantly, almost half the treated eyes had some improvement in visual function in this industry-sponsored clinical trial. Thus, a larger study is planned by industry to further explore the safety and efficacy of this cell therapy. Interestingly, a parallel study recently completed in the United Kingdom showed similar persistent submacular pigmented cells after cell transplantation but no visual improvement among treated eyes with Stargardt’s disease (Meht, ARVO annual meeting presentation, May 3, 2016).

The first clinical trial exploring RPE cell sheets derived from induced pluripotent stem cells started in Japan. Since this cell therapy is autologous; no systemic immunosuppression is needed. However, the current protocol requires extensive time for the pluripotent stem cell line to be established and differentiated into RPE cell sheets for transplantation. In addition, the cell transplantation requires vitrectomy surgery for submacular injection, similar to studies using RPE cells derived from embryonic stem cells. The first study subject with exudative AMD successfully completed this study and received the cell treatment without any adverse effects (Takahita et al., 30th Asia-Pacific Academy of Ophthalmology Congress, Guangzhou, China, April 2, 2015). However, there was no visual acuity gain in the study eye after cell therapy. The study is on hold temporarily due to genetic instability noted in the induced pluripotent stem cell line generated for the second subject in this study (Kurimoto et al., ARVO annual meeting presentation, May 3, 2016). Thus, significant technical hurdles remain for this technology.

An early clinical trial has been initiated by industry using subretinal allogeneic transplantation of human fetal neural progenitor cells as treatment for atrophic AMD. This study is based on promising preclinical studies in the RCS rat model showing that subretinal transplantation of fetal neural progenitor cells from the forebrain cortex preserved photoreceptors and visual function (Gamm et al., 2007; Wang et al., 2008). These cells appear to survive in the subretinal space, engraft into the degenerating retina and provide neuroprotection.

Another clinical trial has been initiated using subretinal transplantation of human retinal progenitor cells partially differentiated into photoreceptor cells. These cells have been harvested and expanded from fetal neural retina and preclinical studies show incorporation of these cells into various layers of the retina after subretinal transplantation in the RCS rat model of retinal degeneration with some preservation of the photoreceptor layer (Luo et al., 2014). A phase II/III industry-sponsored clinical trial has been initiated in the United Kingdom to treat individuals with retinitis pigmentosa. The results are pending as the study has just started.

All these approaches to stem cell therapy aim to replace a specific cell in the retina by introducing a differentiated retinal or RPE cell, usually from a pluripotent stem cell, into the sub-macular space. Pluripotent stem cells are differentiated into a target cell type before exploring therapeutic applications in order to minimize risk of teratoma (Lu et al., 2009; Hiram et al., 2009; Chen et al., 2010; Parameswaran et al., 2015; Sun et al., 2015). In reality, most retinal conditions have more than one cell type affected in the retina. As such, introducing a single differentiated cell type to the retina may limit efficacy.

3.3. Alternative approach using bone marrow stem cells

An alternative approach to stem cell therapy is to revive and regenerate the affected cells in the retina by introducing stem cells that have a paracrine trophic effect. Such a stem cell treatment would not be disease specific and can have broad clinical applications. This is potentially possible using BMSCs (Park, 2016). If these stem cells can be delivered intravitreally without adverse effects, the ease of such a route of administration would be highly desired. Unlike vitrectomy surgery for sub-retinal administration of cells that requires hospital admission and significant recovery time from surgery, intravitreal injection of cells can be performed in the clinic, with minimal recovery time. Finally, BMSCs are usually harvested from adult tissue. Thus, there are no ethical issues. For autologous use, the need for systemic immunosuppression is avoided. These adult stem cells are multi-potent and have more limited capacity to differentiate and divide when compared to embryonic and induced pluripotent stem cells. These features of adult BMSCs may make them less ideal for regenerative therapy in terms of tissue replacement (Leeper et al., 2010). However, these features of adult stem cells potentially make them safer cells to explore for tissue regeneration in clinical trials since there is minimal risk of teratoma.

4. Rationale for exploring bone marrow stem cells

Bone marrow derived stem cells (BMSCs) are being explored as a potential source of cells for retinal regeneration (Machalska et al., 2009; Enzmann et al., 2009; Siqueira, 2011; Park, 2016). This is based on the observation that hematopoietic stem cells from bone marrow appear to be plastic and can repopulate the bone marrow in transplant recipients and play an important role in vascular repair (Mackie and Losordo, 2011; Rafii and Lyden, 2003). Initially, BMSCs were believed to trans-differentiate into various cells, including retinal neurons (Tomita et al., 2002; Sun et al., 2011; Duan et al., 2013). More recently, this notion has been debated and current thinking suggests that these observations were due to cell fusion. The observed multi-potency of BMSCs may result from the heterogeneous make-up of the isolated cells (Park, 2016; Muller-Sieburg et al., 2002). The rationale for exploring the use of BMSCs as potential therapy is based on preclinical observations demonstrating a paracrine trophic effect of certain BMSCs on degenerating or ischemic retina (Otani et al., 2004; Gnecci et al., 2008; Lu et al., 2010; Park, 2016; Moisseev et al., 2016). These paracrine trophic effects on the damaged retina may represent a more viable approach to treating retinal dysfunction than direct cell replacement since most retinal disorders are associated with damage to more than one cell type in the retina and extensive remodeling.
often occurs in response to the damage. Such a cell therapy would not be disease-specific and more universally applicable.

Preclinical studies have shown that some BMSCs have many of the ideal features desired in an effective stem cell treatment. Long-term incorporation of the stem cells into the retina with survival and restoration of structure or function would be desired without ocular or systemic safety issues. This has been observed using intravitreally injected bone marrow CD34^+ cells in NOD-SCID mice with acute ischemia-reperfusion injury (Park et al., 2012). Long-term safety and retention of mesenchymal stem cells from human Wharton’s Jelly in the subretinal space also has been shown in RCS rats with some preservation of retinal degeneration on histology (Leow et al., 2015). Finally, BMSCs are easily harvested and administered. Some of the bone marrow CD34^+ cells or endothelial progenitor cells have the ability to home into the damaged retina and retinal vasculature and become incorporated after intravitreal delivery (Caballero et al., 2007; Park et al., 2012; Moisseiev et al., 2016). So, administration of the cells to the target tissue is less of an issue. Finally, since BMSCs are usually harvested from adults, autologous stem cell therapy is possible, negating the need for donor matching or systemic immunosuppressive therapy.

5. Types of bone marrow stem cells

Bone marrow contains the highest concentration of adult stem cells. These adult stem cells appear to play an important role in regenerating damaged tissue and organs after trauma, disease or aging. Thus, research has been conducted to understand the mechanism of action of these BMSCs in an attempt to maximize the regenerative effects. There are two classes of BMSCs that have been studied; mesenchymal stem cells and hematopoietic stem cells.

Fig. 2 illustrates the two types of BMSCs and the different methods of harvesting these cells (Mesentier-Louro et al., 2016). Mesenchymal stem cells are easily harvested and expanded in tissue culture. These cells are isolated from bone marrow aspirate or mononuclear cell fraction of bone marrow aspirate by their ability to adhere to the plastic tissue culture plates and grow in culture. Mesenchymal stem cells are identified by the presence of specific cell surface markers (for instance CD105, CD73, stromal antigen 1, CD44, CD90, CD166, CD146 CD54, and CD49) and absence of cell surface markers for hematopoietic stem cells (CD14, CD45, CD11a, CD34), erythrocytes (Glycophorin A) and platelets (CD31) (Bara et al., 2014). Mononuclear bone marrow cells are isolated by Ficoll Paque density gradient centrifugation of the bone marrow aspirate. Alternatively, the Percoll density gradient centrifugation or immunomagnetic depletion of polymorphonuclear cells and erythrocytes can be used (Posal et al., 2012). This cell fraction is a crude cellular mixture that contains <0.2% hematopoietic stem cells (Pang et al., 2011). It also contains endothelial progenitor cells, mesenchymal stem cells (<0.01%), monocytes and lymphocytes. Using a magnetic cell sorter, hematopoietic stem cells can be selected positively from the mononuclear cell fraction using cell surface markers. CD34 is a commonly used cell surface marker to identify human hematopoietic stem cells and endothelial progenitor cells although monocytes in this cell fraction may also express CD34 (Berenson et al., 1988). The result is a purified enriched fraction containing usually >70% concentration of CD34^+ cells.

5.1. Mesenchymal stem cells

Mesenchymal stem cells were first described in 1968 by Friedenstein et al. who isolated adherent, fibroblast-like clonogenic cells with a strong capacity to replicate and differentiate into osteoblasts, adipocytes and bone marrow stromal cells (Friedenstein et al., 1968). Mesenchymal stem cells constitute less than 0.1% of the cells in bone marrow. However, mesenchymal stem cells can be harvested and expanded easily in culture, making these cells appealing to explore in research. These cells were first harvested from bone marrow and most of the research has been conducted using bone marrow-derived cells. In recent years, similar cells also
have been harvested from adipose tissue, heart, skeletal muscle, synovial membrane, dental pulp, peritoneal ligaments, liver, c ervical tissue, Wharton’s jelly, amniotic fluid, and umbilical cord blood (Park, 2016). These cells are multi-potent, but the primary mechanism of action of these cells appears to be a paracrine trophic effect rather than direct cell replacement (Phinney and Prockop, 2007). These cells secrete neurotrophic and angiogenic trophic factors such as ciliary neurotrophic factor (CNTF), bFGF, VEGF (Caplan and Denis, 2006). The biologics secreted by these stem cells have been referred to as secretomes, some of which are packages of extracellular vesicles (Section 10; Drago et al., 2013). Secretomes from mesenchymal stem cells have been shown to have neuroprotective effects on the retina (Johnson et al., 2014). Under pathologic conditions, such as hypoxia, the mesenchymal stem cells can increase the production and secretion of these trophic factors (Anderson et al., 2016). These stem cells have the capacity to differentiate into cells of mesodermal origin, such as bone, carti lage, muscle, ligament, tendon, adipose, and stroma (Pittenger et al., 1999) These cells are multi-potent but may have limited ca pacity to differentiate into cells of ectodermal or endodermal origin (Jiang et al., 2002).

Mesenchymal stem cells have the capacity to home into injured tissue following intravenous injection via interaction with cell surface proteins in endothelial cells. However, the mechanism is not fully understood. Animal and human studies suggest that mesenchymal stem cells specifically migrate to damaged tissue with inflammation, likely in response to chemokines, adhesion molecules, and matrix metalloproteinases. Similar to hematopoietic stem cells, important signals for migration and homing of mesenchymal stem cells appear to be stromal-derived factor 1 (SDF-1/CXCL12), C-C chemokine receptor type 4 (CXCR4), and hepatocyte growth factor-MET proto-oncogen, receptor tyrosine kinase (c-MET) axes (Neuss et al., 2004; Son et al., 2006; Shi et al., 2007; De Becker et al., 2007; Cheng et al., 2015). These stem cells appear to adhere to vascular endothelium and migrate by coordi nated rolling and adhesion behavior via vascular cell adhesion molecules (Ruster et al., 2006).

5.1.1. Mesenchymal stem cell and retinal disease

Mesenchymal stem cells have been explored as potential cell therapy for retinal regeneration. In vitro studies showed that mesenchymal stem cells can develop features of RPE cells (Duan et al., 2013). In animal studies of retinal degeneration and retinal ischemia, mesenchymal stem cells were reported to differentiate into photoreceptors, RPE, and express neuronal markers following local or systemic administration but whether the study findings represent true differentiation or fusion with pre-existing photore ceptors is open to debate (Parr et al., 2002; Gong et al., 2008; Li et al., 2009). Many studies report a protective effect of subretinal injection of mesenchymal stem cells in animal models of retinal degeneration even though the incorporation of these mesenchymal stem cells into the retina itself was limited (Arnhold et al., 2007; Tzameret et al., 2014). Rescue of retinal degeneration was noted following intravitreal injection of mesenchymal stem cells, but the effect was less prominent and shorter lasting than that noted after subretinal cell injection. In addition, the intravitreally injected mesenchymal stem cells formed cellular clusters in the vitreous cavity (Tzameret et al., 2014). Protective effects on retinal degeneration have also been observed following intravenous administration of bone marrow mesenchymal stem cells in a rat model (Wang et al., 2010).

In an animal model of retinal ischemia-reperfusion injury, intravitreal injection of mesenchymal stem cells had a protective effect on ganglion cell loss, and the cells were noted to secrete neurotrophic growth factors for at least 4 weeks following injection (Li et al., 2009). Since this effect can be simulated by conditioned medium, a primary paracrine trophic effect via secretome is implicated (Dreixler et al., 2014). In animal models of diabetic retinopathy, bone marrow mesenchymal stem cells were found to integrate into the inner retina, stimulate retinal gliosis and improve ERG amplitude (Cerman et al., 2016) However, long-term studies of intravitreal and subretinal injection of human bone marrow mesenchymal stem cells noted that some of these human cells integrated into other ocular structures and bypassed the blood-retinal barrier to migrate into non-target tissue (choroid), raising some long-term safety concerns regarding the use of these cultured cells (Tzameret et al., 2014).

5.1.2. Advantages and limitations for clinical applications

For clinical application, the main appealing feature of these mesenchymal stem cells is their potential allogeneic use. These cells express low levels of HLA class I antigens and do not express HLA class II antigens (Ryan et al., 2005). Allogeneic use of mesenchymal stem cells has been conducted in both preclinical and clinical studies (Squillaro et al., 2016). These cells are considered immune privileged, but this status may not be absolute since there is some evidence of immune rejection in preclinical studies with allogeneic use. In most systems, mesenchymal stem cells have immunosuppressive effects by generating regulatory T cells and secreting cytokines (Le Blanc, 2006). The immune modulatory effect of mesenchymal stem cells may play an important role in tissue regeneration, especially in conditions such as diabetic retinopathy where inflammation has been shown to play a role in disease progression. Nonetheless, the immunomodulatory effects of mesenchymal stem cells are complex. A proinflammatory effect has been noted in some environments (Bernardo and Fibbe, 2013; Galderisi and Giodano, 2014), including following intravitreal injection of mesenchymal stem cells in some animal models. The resulting inflammatory and fibrous proliferation of cells in the vitreous raised some concerns about intravitreal clinical application of mesenchymal stem cells. As shown in Fig. 3, our group has used in vivo retinal imaging to study the effect of intravitreal injection of mesenchymal stem cells from human bone marrow in NOD-SCID mice. Within days following intravitreal injection, fundus photography shows progressive media haze. In this immune deficient murine model, the vitreous haze is not from inflammation. In vivo optical coherence tomography (OCT) imaging of the retina shows progressive formation of tractional epiretinal membrane filling the vitreous cavity. This effect varied in severity with the number of mesenchymal stem cells injected into the vitreous.

One of the main limitations of mesenchymal stem cell application is the heterogeneity in isolation and culture procedures among laboratories. In 2006, the International Society for Cellular Therapy (ISCT) established criteria for identifying these cells (Dominici et al., 2006) The mesenchymal stem cell population is defined as >95% positive for CD105, CD73, and CD90 and >95% negative for CD45, CD34, CD14 or CD11b, CD79α or CD19, and HLA-DR. Despite the incorporation of ISCT criteria, heterogeneity in mesenchymal stem cell populations persists. In preclinical studies, these cells often form a clump of cells that persist in the vitreous cavity following intravitreal injection that can lead to fibrous proliferation and tractional retinal detachment (Tzameret et al., 2014; Fig. 3). Graft induced reactive gliosis mediated by Muller cell activation has been documented leading to up-regulation of intermediate filament proteins and retinal folds (Tassoni et al., 2015). These potential adverse effects of mesenchymal stem cells may be limiting the therapeutic application of these cells for retinal dysfunction and other medical conditions. Although these cells have been studied extensively as potential treatment for various conditions in animal models, these preclinical studies have led to mostly early phase clinical trials so far.
injected cells (range of mesenchymal stem cells injected intravitreally that the same retinal location (near ONH) is visualized at each time point. Further experiments (not shown) revealed that severity of retinal detachment depends on the number of location of the OCT B-scan shown in the last raw. Each of the OCT B-scans was extracted from serial volumetric scans of the retina acquired during each imaging session, ensuring that the same retinal location (near ONH) is visualized at each time point. Further experiments (not shown) revealed that severity of retinal detachment depends on the number of injected cells (range of mesenchymal stem cells injected intravitreally~5000 to 50,000). Control mice injected intravitreally with 1 μl PBS was normal.

Fig. 3. Effect of intravitreal human bone marrow mesenchymal stem cells in NOD-SCID mice. Intravitreal injection (1 μl) of 50,000 mesenchymal stem cells from human bone marrow labeled with GFP resulting in gradual vitreous haze and tractional epiretinal membranes visualized using optical coherence tomography (OCT). Three rows show results of longitudinal retinal imaging using two different instruments: Fundus color photography (top) and fundus fluorescence images (middle) were obtained using a Micron III mouse retinal imaging fundus camera (Phoenix Research Laboratory Inc.). OCT B-scan images of the retinal and overlying vitreous were obtained using a custom build UC Davis EyePod Mouse OCT retinal imaging system. Fundus fluorescence images confirm successful injection of GFP labeled mesenchymal stem cells in the eye. OCT scans reveal that injected cells adhered to the stalk of the optic nerve head and the rear of the lens, and gradually formed a structure that produces a severe tractional retinal detachment. Yellow arrow indicates location of the OCT B-scan shown in the last raw. Each of the OCT B-scans was extracted from serial volumetric scans of the retina acquired during each imaging session, ensuring that the same retinal location (near ONH) is visualized at each time point. Further experiments (not shown) revealed that severity of retinal detachment depends on the number of injected cells (range of mesenchymal stem cells injected intravitreally~5000 to 50,000). Control mice injected intravitreally with 1 μl PBS was normal.

5.2. Hematopoietic stem cells/CD34+ cells

Hematopoietic stem cells are found in the highest concentration in bone marrow. These cells are identified by certain cell surface markers, including CD34 in humans (Berenson et al., 1988). They can differentiate into all the blood cells lineages and have been used for bone marrow transplantation (Goodell et al., 2015). These cells are capable of self-renewal and reconstitute the bone marrow compartment of the recipient (Stella et al., 1995). These hematopoietic BMSCs have been used extensively in clinical practice to treat hematologic disorders, but these stem cells also have been found in other tissues (Park, 2016). They play a role beyond renewing blood cells.

Asahara et al. made a landmark observation in 1997 that circulating human CD34+ cells included endothelial progenitor cells that are recruited to sites of tissue ischemia for tissue regeneration and angiogenesis (Asahara et al., 1997). The CD34+ cells are found in highest concentration in bone marrow and appear to be mobilized in response to tissue injury. Although the molecular signals involved in mobilization and recruitment of hematopoietic BMSCs may vary with the tissue injury, with vascular injury or tissue ischemia, release of angiogenic factors into the systemic circulation, such as VEGF-A, appears to activate metalloproteinase (MMP-9). Activation of MMP-9 increases soluble Kit ligand in bone marrow that results in proliferation and release of hematopoietic BMSCs (Rafii and Lyden, 2003; Park, 2016).

Various preclinical and clinical studies have been conducted using CD34+ cells or the mononuclear cell fraction of bone marrow to treat tissue ischemia (Mackie and Losordo, 2011). Hematopoietic stem cells mobilized into systemic circulation have become an alternative source of stem cells for treatment of hematologic disorders. Thus, these mobilized stem cells have been used for treatment of tissue ischemia in some clinical trials of myocardial ischemia (Zohnhofer et al., 2006). However, the behavior of mobilized hematopoietic stem cells may not be the same as stem cells from bone marrow.

Adult CD34+ cells represent a heterogeneous group of cells. Some CD34+ cells are endothelial progenitor cells and others are multi-potent hematopoietic stem cells capable of differentiating into various blood cells lineages (Medina et al., 2010a,b; Vaughan and O’Brien, 2012). These CD34+ stem cells have paracrine trophic effects (Park, 2016). They secrete proangiogenic factors, such as VEGF, HGF, IGF-1, FGF-2, etc. They also secrete neurotrophic cytokines.

Only a small fraction of endothelial progenitor cells is capable of differentiating into endothelial cells. These cells primarily function to repair the vasculature via paracrine mechanisms. This has been demonstrated in a murine model of chronic endothelial dysfunction and bone marrow ablation (Perry et al., 2009). Bone marrow transplantation did not result in the transplanted bone marrow cells lining the vascular wall. Rather the transplanted bone marrow cells could be detected in the outer vascular adventitial tissue, suggestive of a paracrine effect. Endothelial progenitor cells secrete insulin-like growth factor 1 (IGF-1) and VEGF.

A subclass of endothelial progenitor cells, not hematopoietic in origin but vascular wall-derived, are referred to as outgrowth endothelial cells or endothelial colony-forming cells. These endothelial colony-forming cells can be cultured and expanded in culture after isolation (Medina et al., 2010a,b). These cells are committed to endothelial lineage and are thought to represent “true” endothelial progenitor cells since they can form blood vessels in vivo (Critser and Yoder, 2010). However, the regenerative capacity of these cells on ischemic tissue may be enhanced by co-administration of hematopoietic stem cells.

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5.2.1. Hematopoietic/CD34+ cells and retinal disease

Intravitreal and intravenous administration of CD34+ cells from human bone marrow or peripheral blood resulted in rapid homing of these cells into the damaged retinal vascular in murine eyes with diabetic retinopathy or ischemia–reperfusion injury (Caballero et al., 2007). No long-term safety effects were noted following intravitreal injection of human CD34+ cells in NOD-SCID murine model of acute ischemia–reperfusion injury; apparent normalization of the retinal vessels was noted long-term (Park et al., 2012). The homing of CD34+ cells from peripheral blood of diabetic human subjects, i.e. circulating endothelial progenitor cells, was found to be defective due to a diabetes–associated reduction in phosphorylation and intracellular redistribution of vasodilator-stimulated phosphoprotein (VASP), an actin motor protein required for cell migration (Li Calzi et al., 2008). In fact, a defect in the migration of circulating endothelial progenitor cells might contribute to the development of diabetic retinopathy and other disorders (Douglas et al., 2012). Recent studies have shown that bone marrow–derived circulating pro-inflammatory monocytes are increased in diabetes while circulating endothelial progenitor cells (also called circulating angiogenic cells) are trapped in the bone marrow by the diabetic environment, harvesting these CD34+ cells directly from the bone marrow of diabetic subjects and injecting these cells into the eye may allow these angiogenic cells to reach the eye directly to maximize their potential therapeutic effect. By using bone marrow CD34+ cells, the paracrine neurotrophic can be combined with the paracrine vascular repair effects, both maximized by the ability of these cells to home into areas of retinal injury (Kim et al., 2012b). This makes CD34+ BMSCs a viable treatment to be considered for diabetic retinopathy.

Recent studies revealed a subclass of bone marrow-derived circulating angiogenic cells, CD14+ cells, that appear to retain the homing characteristics among diabetic subjects, unlike CD34+ cells (Caballero et al., 2013). These CD14+ cells can effect vascular repair under certain conditions and be considered as a potential source for cell therapy to treat diabetic retinopathy. An alternative approach would be to combine CD34+ with mesenchymal stem cells since the defective homing capability of CD34+ cells from peripheral blood of diabetic subjects could be minimized by co-treatment with mesenchymal stem cells (Caballero et al., 2013).

Otani et al. showed that bone marrow hematopoietic stem cells have a neuroprotective effect in eyes with retinal degeneration (Otani et al., 2004). Intravitreal injection of autologous bone marrow lineage negative hematopoietic stem cells resulted in preservation of retinal degeneration in murine models of hereditary retinal degeneration. Since the cells were found within the retinal vasculature rather than the degenerating photoreceptor layer, a paracrine trophic effect was implicated. More recent work suggests the neuroprotective effects of bone marrow derived endothelial precursor cells or hematopoietic stem cells might be mediated by the recruitment of neuroprotective macrophages (Fukuda et al., 2013). Moisseiev et al. showed that human CD34+ cells from bone marrow demonstrated rapid homing to the retina and may have a similar neuroprotective effect in a murine model of hereditary retinal degeneration despite concurrent systemic immunosuppression used to avoid cross species rejection of human cells (Moisseiev et al., 2016). Although no preservation of retinal function could be noted by ERG in this model of advanced retinal degeneration, a significant change in expression of over 300 murine retinal genes regulating photoreceptor transduction, maintenance and apoptosis was associated with intravitreal human bone marrow CD34+ stem cell treatment. In NOD-SCID murine model of lysosomal storage disease engrafted with human CD34+ cells, these human cells engrafted in murine bone marrow and other non-hematopoietic organs, including the degenerating RPE, and appeared to correct the tissue pathology (Hofling et al., 2003). This observation raises the question whether intravenous mode of CD34+ cell administration might be preferred in eyes with RPE and choroidal abnormalities leading to retinal degeneration, as in eyes with AMD. This remains to be determined. These bone marrow–derived stem cells appear to be mobilized into the systemic circulations in response to cytokines secreted by the damaged retina or RPE (Li et al., 2006). Stromal cell-derived factor 1 (SDF–1/CXCL12), C3, hepatocyte growth factor (HGF) and leukemia inhibitory factor (LIF) were increased in damaged RPE in preclinical studies and appear to play a role in recruiting hematopoietic stem cells from bone marrow. Whether bone marrow contains a specific subpopulation of retina-committed hematopoietic stem cells is open to debate. Some of the bone marrow hematopoietic stem cells recruited to regenerate RPE, adopted RPE morphology and expressed melanosomes (Harris et al., 2006; Sengupta et al., 2009). Similarly, bone marrow mononuclear cells have shown to engraft long-term and differentiate into retinal cells following intravitreal administration in a rat model of retinal injury (Tomita et al., 2002). Thus, these hematopoietic stem cells may have some potential for tissue incorporation and replacement although the primary mechanism of action is likely via paracrine trophic effects. An increased level of circulating hematopoietic stem cells has been observed in patients with exudative AMD (Yodoi et al., 2007). Whether these cells are recruited in response to retinal degeneration to rescue the RPE and photoreceptor or enhance choroidal vascularization is unknown. Hematopoietic stem cells have been shown to be recruited to the choroid and potentially contribute to formation of choroidal neovascularization in animal models (Sengupta et al., 2003) However, in animal models of retinal ischemia, bone marrow derived cells appear to play an important role in the formation of physiologic vessels in the retina and do not appear to contribute to pathologic retinal neovascularization. Ablation of the bone marrow with irradiation resulted in more prominent retinal nonperfusion and pathologic retinal neovascularization in eyes with oxygen induced retinopathy, demonstrating the protective effect of bone marrow cells (Zou et al., 2010).

6. Clinical trials

6.1. Regulatory clearance for investigational new drug (IND)

In order to initiate a clinical trial in the US to evaluate the safety and efficacy of a new therapeutic agent, including stem cells, investigators must obtain an Investigational New Drug (IND) clearance from the Food and Drug Administration (FDA). This is a lengthy process starting with a meeting (teleconference) with the FDA to discuss the preclinical information that would be required by the FDA to obtain this clearance. Typically, preclinical data justifying the study is required, including long-term safety studies showing that there are no local or systemic adverse effects associated with the new therapeutic agent. In addition, information regarding purity and stability of the product may be requested. For stem cell clinical trials, the cells must be isolated or manufactured in a certified GMP (Good Manufacturing Practice) laboratory. Details of the cell preparation, isolation and manufacturing are requested as part of the IND application. The IND application includes the requested preclinical information, all available published literature relevant to the therapeutic agent being studied, study
6.2. Intravitreal autologous bone marrow CD34+ cell therapy

Based on long term studies conducted in NOD-SCID mice showing no ocular or systemic safety issues associated with intravitreal injection of human bone marrow CD34+ cells in eyes with acute ischemia-reperfusion injury (Park et al., 2012), our group obtained an IND clearance from the FDA to initiate the first clinical trial in the US exploring the use of intravitreally injected autologous bone marrow CD34+ cells as a potential therapy for ischemic or degenerative retinal conditions (registered with clinicaltrials.gov, NCT01736059). The phase I study was conducted primarily as a safety and feasibility study. As such, only subjects with advanced persistent vision loss from retinal degeneration or ischemia were enrolled and treated with CD34+ cells. The study enrolled and treated a total of seven subjects, two subjects with retinal vascular-occlusion (one subject with combined central retinal vein and artery occlusion, one subject with inactive proliferative diabetic retinopathy) and five subjects with various retinal degenerative conditions (two subjects with Stargardt’s disease, two subjects with dry atrophic AMD, one subject with retinitis pigmentosa). All had best corrected visual acuity of 20/200 to 20/400 at study enrollment. The bone marrow aspiration was conducted as an out-patient procedure under local anesthesia by an experienced hematologist. The procedure was well tolerated and a high yield of bone marrow aspirate was obtained from each subject. At least 1 million CD34+ cells were isolated from the bone marrow aspirate from each subject for intravitreal injection. The CD34+ cells were isolated from the mononuclear cell fraction of the bone marrow aspirate by positive selection using a magnetic cell sorter (Miltenyi CliniMACS) under GMP conditions. The processing of CD34+ cells can vary depending on whether the mononuclear cell fraction is easily obtainable and within minutes of the release of the isolated cells from the GMP laboratory. The isolated cells were released for clinical use after ensuring that the isolated cells had required viability and no evidence of contaminants. The intravitreal injection of CD34+ cells was well tolerated in all of the study subjects during the follow-up period ranging from one to four years. No major safety or feasibility issues have been noted with this cell therapy among the study subjects. The results of the first six subjects treated and followed for over two years have been published (Park et al., 2014a). In 4 of 6 eyes treated, best-corrected visual acuity improved 2 or more lines after cell treatment. Although the study was not designed to detect efficacy, some improvement in visual function was noted on microperimetry, perimeter, and/or ERG in most of the study eyes following CD34+ cell injection. No worsening of visual function was noted in any of the eyes during study follow-up. However, some subtle progression of geographic atrophy was noted in an eye with non-exudative AMD. No other changes in clinical examination or spectral domain-OCT were noted in any of the eyes with retinal degeneration following cell treatment, but in vivo cellular imaging using adaptive optics-OCT of a subject with hereditary macular degeneration showed cellular changes in the degenerating macula by one month following cell treatment suggestive of intraretinal incorporation of intravitreally injected cells (Park et al., 2014a). Among eyes with retinal vasculopathy, the eye with persistent vision loss for nine months from a combined central retinal vein and artery occlusion had the more dramatic improvement in vision and clinical examination. The study was not designed to evaluate efficacy of therapy, but the dramatic improvement in clinical examination and fluorescein angiography, noted by three months following cell treatment with marked resolution of retinal hemorrhages and microvascular changes, was an encouraging outcome.

Based on these positive observations, a larger phase I/II study is planned to further explore the safety and potential efficacy of intravitreal autologous bone marrow CD34+ cell therapy for select retinal conditions.

6.3. Intravitreal autologous bone marrow mononuclear cell therapy

Autologous mononuclear cell therapy has been explored in clinical trials as therapy for myopic macular infarct and peripheral ischemia with no safety concerns noted (Mackie and Losordo, 2011). This crude cellular fraction has been used in place of isolated CD34+ cells since the mononuclear cell fraction is easily obtained (Fig. 2). The results among studies have shown variable efficacy using mononuclear cells for myocardial infarct. A part of the reason may be from the varied source of mononuclear cells and parameters for enrolling and gauging efficacy. The mononuclear cell fraction from bone marrow contains <0.2% hematopoietic stem cells (Pang et al., 2011). The relative composition of cells making up the mononuclear cell fraction can vary depending on whether the cells are obtained from bone marrow or the peripheral circulation after mobilization. The preferred source of CD34+ cells is bone marrow since the cells are less likely to be affected by host factors (Shantsila et al., 2007).

Two pilot clinical studies were conducted by two different centers exploring the use of intravitreal autologous bone marrow mononuclear cell therapy. Both studies showed no adverse effect of the treatment during follow-up ranging from 6- to 10-months. The first study was conducted in Germany and enrolled two subjects with advanced vision loss from diabetic retinopathy, glaucoma and macular degeneration (Jonas et al., 2010). No improvement in visual function was observed. Siqueira et al. conducted a second study in Brazil by treating five subjects with retinal dystrophy with autologous bone marrow mononuclear cells (Siqueira et al., 2011). In each study eye, 10 million mononuclear cells were injected. Visual acuity improved 1 line in four subjects in the study eye. More recently, Siqueira et al. published case reports of improvement in cystoid macular edema in a patient with retinitis pigmentosa and two patients with retinal vein occlusion following this cell therapy (Siqueira et al., 2013, 2015). A larger study is on-going and registered in clinicaltrials.gov exploring this cell therapy for non-exudative AMD, retinitis pigmentosa and ischemic retinopathies.

6.4. Bone marrow mesenchymal stem cell therapy clinical trials

Mesenchymal stem cells are easily harvested from bone marrow and expanded in culture. In addition, allogeneic therapy is possible. These features make mesenchymal stem cells appealing cells to explore in clinical trials. Numerous non-ocular clinical trials have been conducted to test the feasibility and efficacy of mesenchymal stem cell-based therapy (Squillaro et al., 2016). More than 2000 patients have been treated via over 400 clinical trials with allogeneic and autologous mesenchymal stem cells for treatment of various medical conditions, including graft-versus-host disease, diabetes, hematologic malignancies, cardiovascular disease, neurologic disease, autoimmune disease, and diseases in the bone and cartilage. Despite the large number of clinical trials, the efficacy
and long-term safety of this cell therapy have not been established. Most of the clinical trials are early phase trials (phase I and II). Based on these studies, mesenchymal stem cells administered intravenously are often trapped in capillary beds of various tissues, especially the lung (Ge et al., 2014). Thus local administration may be preferred.

With regard to use of mesenchymal stem cells to treat retinal dysfunction, various routes of administration of these cells is being explored including intravenous, pericocular, intravitreal and subretinal administration. All the studies listed in www.clinicaltrials.gov are early phase studies being conducted outside the US except for one study. Some appear to be supported by patients. Detailed reports of the results of these trials are pending, but several serious adverse effects associated with BMSC therapy from unregulated clinical studies have been reported recently. They include retinal detachment, severe macular pucker, and retinal artery occlusion following subretinal, intravitreal and periocular injections of BMSCs (Boudreault et al., 2016; Kim et al., 2016; Leung et al., 2016). Some of these adverse effects mirror reactive retinal gliosis, fibrovascular proliferation and vascular obstruction observed in preclinical studies using intravitreal and intravascular mesenchymal stem cells (Ge et al., 2014; Tassoni et al., 2015, Fig. 3). Thus tighter regulation of clinical trials employing BMSC treatment is warranted. A recent editorial voices ethical, regulatory and policy concerns regarding the prevalence of unregulated stem cell therapies in the US for various conditions including vision loss (Turner and Knoepfler, 2016).

7. Host factors on bone marrow stem cells

Factors that may limit the therapeutic potential of BMSCs in treating retinal dysfunction are host factors. It is known that circulating endothelial progenitor cells are affected by host factors (Shantsila et al., 2007). Age of the host affects the availability and function of circulating endothelial progenitor cells. Aging is associated with a reduced number of circulating endothelial progenitor cells among patients with coronary artery disease. In mice, the age-associated impairment in angiogenesis can be restored using bone marrow derived endothelial progenitor cells from young adult animals (Edelberg et al., 2002). It is unknown whether this age-related reduction in endothelial progenitor cells results in senescence, apoptosis and depletion of the progenitor pool in the bone marrow or from reduced levels of angiogenic and mobilizing cytokines, such as vascular endothelial growth factor and nitric oxide. It is known that exercise and physical training increases circulating endothelial progenitor cells in individuals with coronary artery disease (Rehman et al., 2004). So, at least some of the effects of aging might be reversible.

Similarly, other risk factors associated with cardiovascular disease, such as diabetes, hypertension, smoking and hyperlipidemia, also affect the biology and availability of circulating endothelial progenitor cells. The number of circulating endothelial progenitor cells and their function are impaired in these individuals with cardiovascular risk factors. Diabetes is associated with peripheral neuropathy which may affect the bone marrow and alter the circadian release pattern of the circulating cells (Busik et al., 2009).

The bone marrow neuropathy also results in altered hematopoiesis with a shift towards generation of increased numbers of pro-inflammatory circulating endothelial progenitor cells (Hazra et al., 2013). However, drug therapies can improve the physiology of circulating endothelial progenitor cells. Statins increase the number of circulating endothelial progenitor cells and improve their proliferative capacity, independent of their effect on lipid profile (Dimmel et al., 2001). Angiotensin II inhibitors also increase the number of circulating endothelial progenitor cells and improve their function (Bahlmann et al., 2005). A similar positive effect on these cells has been noted for women using estrogen and for individuals on erythropoietin.

It is unknown whether these host factors can affect stem cells in bone marrow that have not been mobilized into the systemic circulation. One study observed that mesenchymal stem cells from diabetic mice have some impaired mobilization similar to circulation endothelial progenitor cells but no human data are available (Yan et al., 2013). Nonetheless, to optimize the effect of BMSCs for tissue regeneration, optimizing the general health of the individual with exercise and appropriate drug therapy to manage any concurrent cardiovascular disease is likely beneficial.

8. Methods to enhance therapeutic effects of bone marrow stem cells

With improved understanding of the biology of BMSCs and the precise mechanisms involved in mobilization, migration, homing and inducing tissue regeneration, new methods can be developed to improve the therapeutic potential of these stem cells. Ex vivo transfection of endothelial progenitor cells with VEGF or telomerase reverse transcriptase before transplantation has been shown to improve the ability of the cells to revascularize. (Iwaguro et al., 2002; Murasawa et al., 2002). Similarly, mesenchymal stem cells altered to secrete high levels of neurotrophic factors have been injected intravitreally to preserve retinal ganglion cells in a rat model of optic nerve damage (Levkovitch-Verbin et al., 2010). Still others have shown that transient in vitro inhibition of TGF-beta improves the function of circulating endothelial progenitor cells from diabetic subjects and enhances the engraftment of these cells in eyes with diabetic retinopathy (Bhatwadekar et al., 2010). Such a treatment may potentially enhance the therapeutic potential of the CD34+ cells from both bone marrow and peripheral blood and normalize dysfunctions induced by the diabetic environment (Kramerov and Ljubimov, 2016).

More recently, selective inhibition of acid sphingomyelinase in bone marrow in diabetic animals reduced activation of bone marrow-derived pro-inflammatory monocytes infiltrating the retina and improved the function of circulating endothelial progenitor cells to home to sites of vascular injury and foster repair (Chakravartty et al., 2016a,b). Selective modulation of sphingolipid metabolism in normalizing the endogenous repair capability mediated by bone marrow cells in diabetes is a novel therapeutic approach that is being explored.

9. Systemic mobilization of endogenous bone marrow stem cells

Systemic administration of bone marrow stem cells appears to be an effective alternative approach to treating retinal ischemia and degeneration. Intravenous administration of mesenchymal stem cells via the tail vein in a rat model of retinal degeneration resulted in photoreceptor preservation and reduction in vascular leakage associated with the retinal degeneration (Wang et al., 2010). Similarly, intravenous administration of endothelial progenitor cells via injection of these cells into the retro-orbital sinus resulted in their homing and integration into the damaged retinal vasculature in animal models (Caballero et al., 2007). The effect was similar to that observed following intravitreal injection of the cells. These findings raise the question whether systemic administration of BMSCs and/or enhancing systemic mobilization of the BMSCs might be a preferred non-invasive approach to maximizing this cell therapy (Wang et al., 2010; Li et al., 2012). It is a mode of stem cell administration that can be considered to treat pathology in the RPE, choroid and even outer retina that is perfused by the choroidal plasma flow.
circulation. Although transretinal migration of intravitreally injected CD34+ cells have been documented in an animal model of laser-induced retinal injury (Li Calzi et al., 2009), this has not been observed in animal models of retinal degeneration (Moisseiev et al., 2016; Otani et al., 2004).

Under physiologic conditions, the level of circulating bone marrow cells is extremely low. By understanding the mechanism for mobilizing the BMSCs, the level of circulating BMSCs might be increased to enhance the repair effects of these cells. One method hypothesized would be by altering the SDF-1/CXCL12-CXCR4 axis that appears to play an important role in mobilizing mesenchymal stem cells and hematopoietic stem cells from bone marrow into the systemic circulation (Li et al., 2012). Plerixafor, CXCR4 antagonist, has been shown to normalize mobilization of hematopoietic stem cells and endothelial progenitor cells in mice and humans with diabetes via by-passing neuronal control and SDF-1/CXCL12 dysregulation (Fiorina et al., 2011; Fadini et al., 2015). It also has increased mobilization of both hematopoietic stem cells and endothelial progenitor cells in normal healthy humans (Cappellari et al., 2016).

10. Non-cellular approach using extracellular vesicles

There is increasing interest in recent years exploring extracellular vesicles as potential non-cellular therapy to achieve the trophic effects of stem cells. Although first identified over 20 years ago, the important role of extracellular vesicles in cell-cell communication was not elucidated or appreciated fully until recently. It is now recognized that extracellular vesicles constitute an important mechanism in complex cell-to-cell signaling pathways (Desrochers et al., 2016; Tkach and Théry, 2016; El Andaloussi et al., 2013).

Extracellular vesicles are composed of a lipid bi-layer that contains trans-membrane proteins, and are loaded with the cytosolic contents of their cell of origin. There are at least two distinct types of extracellular vesicles: exosomes and microvesicles (Desrochers et al., 2016; Tkach and Théry, 2016; El Andaloussi et al., 2013; Lawson et al., 2016; Cocucci et al., 2009). Exosomes are smaller and more homogenous in size, varying from about 50 to 150 nm in diameter. Exosomes are of endocytic origin and formed when the internal limiting membrane of an endosome buds inward forming an internal vesicle within a multivesicular body (MVB) (Fig. 4). Microvesicles, on the other hand, are larger and heterogeneous in size, varying from 100 to 1000 nm in diameter. They are formed by outward budding of the plasma membrane. Although microvesicles are heavily enriched in phosphatidylserine, the membrane composition of microvesicles reflects that of the parent cell more closely than does the membrane composition of exosomes.

Both exosomes and microvesicles contain cellular factors derived from their cell of origin, including various classes of proteins, RNA and metabolites. However, owing to their highly regulated biogenesis, exosomes are typically packaged with more distinct components (Chiba et al., 2012). After their secretion, exosomes can fuse into the plasma membrane or become internalized by surrounding cells. They are important for intercellular communication and horizontal gene regulation (Krämer-Albers and Hill, 2016). Although both exosomes and microvesicles are composed of a lipid bi-layer that contains trans-membrane proteins, they do not necessarily express the same marker proteins as their parent cells (Lawson et al., 2016; Jørgensen et al., 2015). In fact,
because exosomes are formed by endocytosis, they are usually devoid of many of the cell surface proteins and have a low risk of immune rejection following allogeneic use (Chaput and Théry, 2011).

Extracellular vesicles are made and secreted by most cell types and the content varies depending on the cell of origin and the condition under which they were produced (de Jong et al., 2012). They have been shown to participate in complex processes, such as angiogenesis (Kholia et al., 2016) and signaling between stem cells (Ratajczak et al., 2006; Katsman et al., 2012).

Exosomes derived from mesenchymal stem cells play a major role in the paracrine effect of these cells (Yu et al., 2014). These exosomes are an attractive target for clinical research, as they can offer comparable therapeutic effects of mesenchymal stem cells without the potential adverse effects associated with cell therapy. When cultured under hypoxic conditions, bone marrow mesenchymal stem cells preferentially produce many more exosomes than microvesicles. A comprehensive proteomic analysis of exosomes derived from mesenchymal stem cells cultured under these conditions revealed that they are loaded with a variety of factors that promote angiogenesis, such as protein associated with nuclear factor kappa B (NFκB), platelet derived growth factor (PDGF), epidermal growth factor (EGF) and fibroblast growth factor (FGF) signaling (Anderson et al., 2016). This proangiogenic response has been observed in other cell types, and likely reflects a compensatory reaction to the hypoxia (Park et al., 2010).

In animal models of myocardial ischemia, peripheral ischemia, and stroke, administration of purified exosomes derived from mesenchymal stem cells harvested under hypoxic conditions had a therapeutic effect (Lai et al., 2010; Doeppner et al., 2015; Hu et al., 2015). This effect was attributed to the content of the exosomes, which was shown to reduce oxidative stress and activate pro-survival signaling (Arslan et al., 2013).

Recently, our group is exploring intravitreal administration of exosomes derived from human bone marrow mesenchymal stem cells in eyes with retinal ischemia and degeneration (Moisseiev et al. Retina Society annual meeting paper presentation, San Diego, CA, September 15, 2016). No immunosuppression was needed despite the cross-species application of exosomes from human mesenchymal stem cells into murine eyes (Chaput and Théry, 2011). In addition, exosomes are easy to obtain, store and administer, making clinical application feasible and appealing. If the trophic regenerative effects of mesenchymal stem cells can be replicated using exosomes secreted by mesenchymal stem cells, this non-cellular approach may be a safer method to achieve retinal regeneration. This is a promising new therapeutic area of on-going research.

11. New in vivo retinal imaging tools

As we progress into clinical trials to better evaluate the safety and efficacy of BMSC therapy, newer in vivo retinal imaging tools are being developed that could allow us to evaluate changes in the retina associated with stem cell therapy that may not be evident on routine clinical examination or retinal imaging. This was exemplified in the clinical trial using intravitreal autologous bone marrow CD34+ cell therapy where the use of cellular in vivo retinal imaging using adaptive optics OCT revealed changes within the retinal layers suggestive of intraretinal incorporation of injected cells (Park et al., 2014a,b). Such changes were not appreciated using commercially available spectral-domain OCT instruments. With the advent of improved axial range using swept source OCT instruments, subtle changes in the vitreous, vitreo-retinal interface and structures deep to the retina may be appreciated (Unterhuber et al., 2005; Poddar et al., 2014). This may improve our visualization of the stem cells in the vitreous or vitreo-retinal interface following intravitreal injection as they migrate into the retinal layers or the retinal surface. By tracking the migration and intraretinal incorporation of injected BMSCs and analyzing structural changes within the retinal layers, the efficacy of the cells on the retina can be studied in vivo non-invasively. In individuals with retinal vasculopathy treated with BMSCs, subtle changes in the retinal vascular flow may occur that may not be appreciated with traditional fluorescein angiography or clinical examination. With the advent of motion contrast retinal imaging using OCT angiography, retinal and choroidal vascular flow information can be acquired in three-dimensions and in unprecedented detail. Our group has shown using phase-variance OCT angiography, that retinal ischemia associated with diabetic retinopathy can be detected and correlated with similar findings noted on fluorescein angiography (Kim et al., 2012a; Schwartz et al., 2014). The newer higher resolution motion contrast imaging allows segmentation of the various layers of the retina to evaluate changes in retinal perfusion at various depths (Fig. 5). Recent studies have shown that such segmentation analysis can detect subtle vascular remodeling and perfusion changes associated with diabetic retinopathy that would not be appreciated using traditional fluorescein angiography (Salz et al., 2016). By incorporating adaptive optics, cellular level imaging of the retinal vascular wall and retinal neurons is possible in vivo (Choi et al., 2011; Chui et al., 2013).

These newer methods of in vivo retinal imaging can potentially improve our ability to evaluate the effects of cell therapy in eyes with retinal dysfunction with improved accuracy. Since histologic analysis of human eyes treated with cell therapy is not possible, these newer in vivo imaging techniques allow us to evaluate the retina of treated individuals at a near cellular level non-invasively.

12. Conclusions and future directions

Retinal dysfunction from retinal degeneration and ischemia is a leading cause of vision loss that is currently untreatable. This review has provided an overview of the various options for stem cell therapy to treat retinal dysfunction and the rationale for exploring stem cells in bone marrow. Whether the treatment is hematopoietic CD34+ cells, mononuclear cell fraction or cultured mesenchymal stem cells, autologous cell therapy is possible without the need for systemic immunosuppression using adult BMSCs. Early phase clinical trials have been initiated exploring various BMSCs as potential therapy for retinal dysfunction (Jonas et al., 2010; Siqueira et al., 2011; Park et al., 2014a,b). These cells have a primary paracrine trophic effect that does not appear to be disease specific and may have broad clinical applications. These BMSCs are easily harvested. Since CD34+ cells home into the damaged tissue, intravitreal delivery is possible to treat eyes with retinal ischemia or degeneration. This route of delivery would be easier than vitrectomy surgery for subretinal transplantation. In the case of mesenchymal stem cells, both intravitreal and subretinal transplantation has been explored but some safety issues have been noted associated with reactive glialis from activation of Muller cells and fibrovascular proliferation of cells. A safer alternative to explore would be to use extracellular vesicles secreted by mesenchymal stem cells that contain the paracrine trophic factors. This is an area of on-going research. Future directions of research to enhance the therapeutic effects of BMSCs include host intervention to optimize the homing and repair capabilities of the stem cells in bone marrow and genetic and molecular alteration of the stem cells themselves to deliver more neurotrophic factors to the target tissue.

The ideal regenerative therapy may require a combination of stem cells and extracellular vesicles and modification of host factors. As we transition to clinical applications, it is important to
realize that the reparative potential of BMSC therapy may not be fully realized currently. New future genetic or molecular modifications to the BMSCs themselves may enhance further the reparative potential of this cell therapy on retinal dysfunction.

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Fig. 5. Optical coherence tomography (OCT) angiography showing retinal layers and associated perfusion. OCT angiography of a normal eye of a 30-year-old human subject imaged 8° nasal and 3° inferior from the fovea. Imaged area is 4 × 4” (~1.2 × 1.2 mm); (a) Intensity B-scan OCT image demonstrates the retinal layers (blue bar indicates position of the en face projection of the retinal vasculature shown in (d), green bar — choriocapillaris shown in (e), purple bar — Sattler’s layer vessels and inner parts of the Haller’s layer vessels of the choroid shown in (f), Blue bar — 163 μm, green bar — 16 μm, purple bar — 64 μm; (b) OCT angiography B-scan image detecting retinal vascular flow within the retina and choroid. (c) Overlay of OCT angiography B-scan (red) with intensity B-scan (gray) precisely localizing the vascular flow to the inner retinal layers and choroid. OCT angiography en face projections at various depths can be obtained (d–f): retinal vasculature (blue outline, d), choriocapillaris (green, e), and choroidal vessels (purple, f), at depth locations indicated by corresponding color bars in (a). Discontinuity visible in the middle of the images is caused by an involuntary eye movement. OCT imaging system used is a swept source (FDML) OCT at 17 MHz imaging speed, center wavelength 1060 nm, axial imaging resolution: ~5.5 μm. OCT angiography scan protocol was 392 A-scans per B-scan, 10 repeats of B-scans, 392 BM-scans (3920 B-scans total). OCT angiography method was phase variance OCT angiography and amplitude decorrelation OCT angiography (Gorczynska et al., 2016 for algorithm details). NFL — nerve fiber layer; G — ganglion cell layer; IPL — inner plexiform layer; INL — inner nuclear layer; OPL — outer plexiform layer; ELM — external limiting membrane; IS/OS — photoreceptor inner segment/outer segment junction; RPE — retinal pigment epithelium. (Image provided courtesy of Iwona Gorczynska, Justin Migacz and Raju Poddar, UC Davis Advanced Retinal Imaging Laboratory.).

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