

Cell Therapy Applications for Retinal Vascular Diseases: Diabetic Retinopathy and Retinal Vein Occlusion

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Retinal vascular conditions, such as diabetic retinopathy and retinal vein occlusion, remain leading causes of vision loss. No therapy exists to restore vision loss resulting from retinal ischemia and associated retinal degeneration. Tissue regeneration is possible with cell therapy. The goal would be to restore or replace the damaged retinal vasculature and the retinal neurons that are damaged and/or degenerating from the hypoxic insult. Currently, various adult cell therapies have been explored as potential treatment. They include mesenchymal stem cells, vascular precursor cells (i.e., CD34+ cells, hematopoietic cells or endothelial progenitor cells), and adipose stromal cells. Preclinical studies show that all these cells have a paracrine trophic effect on damaged ischemic tissue, leading to tissue preservation. Endothelial progenitor cells and adipose stromal cells integrate into the damaged retinal vascular wall in preclinical models of diabetic retinopathy and ischemia-reperfusion injury. Mesenchymal stem cells do not integrate as readily but appear to have a primary paracrine trophic effect. Early phase clinical trials have been initiated and ongoing using mesenchymal stem cells or autologous bone marrow CD34+ cells injected intravitreally as potential therapy for diabetic retinopathy or retinal vein occlusion. Adipose stromal cells or pluripotent stem cells differentiated into endothelial colony-forming cells have been explored in preclinical studies and show promise as possible therapies for retinal vascular disorders. The relative safety or efficacy of these various cell therapies for treating retinal vascular disorders have yet to be determined.

Keywords: stem cell, retinal vasculopathy, retinal ischemia, diabetic retinopathy, retinal vein occlusion

Retinal vascular disorders, such as diabetic retinopathy and retinal vein occlusion, are common causes of vision loss in the older adult population in the United States and worldwide.¹ Diabetes mellitus affects over 120 million people worldwide and the incidence of diabetes mellitus is anticipated to increase to 200 million in the next few decades.² The prevalence of diabetic retinopathy correlates with the duration of diabetes mellitus and is approximately 75% after 10 years and 95% after 20 years.³ Diabetic retinopathy is a leading cause of blindness among the working age adults worldwide and one of the leading causes of vision loss in the elderly.⁴ Retinal vein occlusion is the second retinal vascular cause of vision loss in the elderly population in the United States and worldwide with an incidence of 1% to 2% in 10 to 15 years.⁵ Both conditions affect all ethnic groups. Although intravitreal drug therapy and laser photocoagulation are used to treat retinal neovascularization or macular edema that can occur as visually significant complications of these common retinal vascular disorders, no treatment is available for vision loss resulting from retinal ischemia and/or retinal degeneration associated with these retinal vascular disorders.^{6,7}

Various studies have shown that the quality of life of patients with eye disease correlated with visual acuity of the better eye regardless of cause of vision loss.⁸ Vision loss from diabetic retinopathy or retinal vein occlusion can severely affect the quality of life.⁹ Thus, there is an unmet need to develop a treatment to reverse or protect the retinal

vasculature and retinal neuron in patients with retinal vascular disease.

RETINAL VASCULOPATHY AND ISCHEMIA

The inner retina is perfused by the retinal circulation provided by branches of the central retinal artery. As such, retinal vascular damage associated with diabetic retinopathy or retinal vascular occlusion can be appreciated on clinical fundus examination by the changes in retinal vascular caliber, presence of retinal hemorrhages, exudates and cotton wool spots, and development of retinal complications such as neovascularization or macular edema (Fig. 1A). The extent of retinal hypoperfusion or ischemia may be appreciated with fundus fluorescein angiography (Fig. 1B). In severe cases, the hypoperfusion can be visible on fundus examination as sclerosed, hypoperfused retinal vessels (Fig. 1C). The net result is vision loss and retinal neuronal death or degeneration manifesting clinically as a decrease in retinal thickness. (Fig. 1D).

Retinal ischemia results in impaired oxygen delivery and metabolic clearance. The net effect is a lowering of the pH, production of reactive oxygen species, and elevation of extracellular transmitters with resulting excitotoxicity.^{10,11} This may occur acutely, as in retinal vein occlusion, or chronically and progressively, as in diabetic retinopathy. The degree of



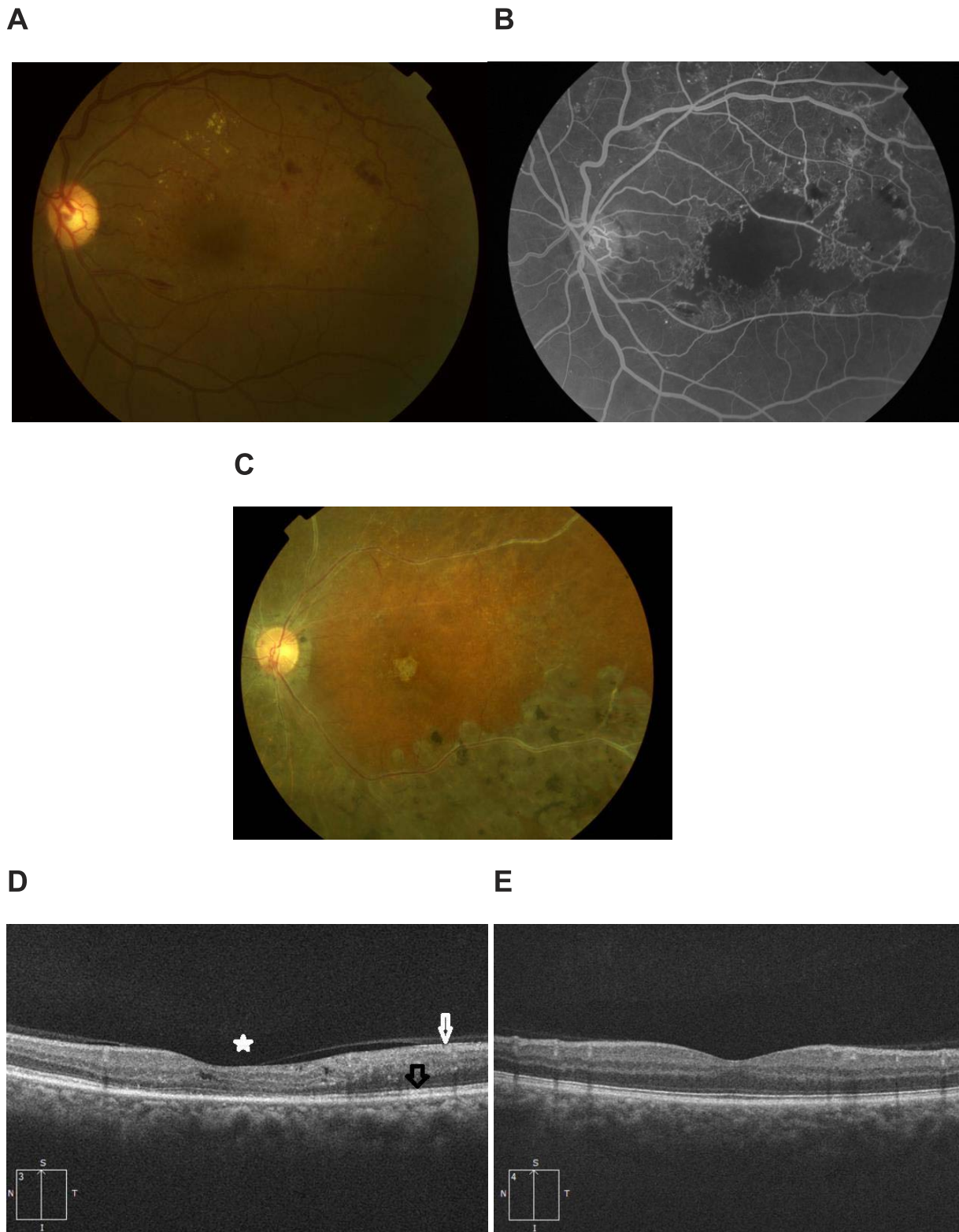


FIGURE 1. Diabetic retinopathy and associated retinal ischemia and degeneration leading to retinal atrophy. **(A)** Fundus photograph of the left eye a patient with early proliferative diabetic retinopathy showing scattered retinal hemorrhages, microaneurysms, and intraretinal exudates characteristic for diabetic retinopathy. Early fronds of retinal neovascularization is noted supratemporal to the macula from proliferative disease. **(B)** Fluorescein angiogram of the eye shown in **(A)** shows a grossly enlarged foveal avascular zone from marked macular ischemia. This explains the severe loss of vision in this eye. Note the retinal ischemia extends temporal to macula where early retinal neovascularization from proliferative disease is detected. **(C)** Fundus photograph of a second patient with more severe retinal ischemia from diabetic retinopathy. The main retinal arteries and branches appear attenuated and white from diffusely sclerosis and lack of perfusion. This eye has inactive proliferative diabetic retinopathy after panretinal laser photocoagulation. Note the macular pigment changes from chronic atrophy resulting from ischemia. **(D)** Spectral-domain optical coherence tomographic cross-sectional image of the macula of left eye shown in **(A)** showing diffuse thinning of the central macula (*star*) in the region of

ischemia. The retinal layers are poorly visualized centrally and there is partial loss of the superficial nerve fiber layer (*white arrow*) and the deeper photoreceptor inner segment-outer segment zone (ellipsoid zone, *open arrow*) in this eye with macular degeneration and atrophy from diabetes and associated macular ischemia. (E) Spectral-domain optical coherence tomographic cross-sectional image of the macula of a healthy eye for comparison showing the intact retinal layers.

vision loss associated with both diabetic retinopathy and retinal vein occlusion correlates with the degree of retinal ischemia.

In diabetic retinopathy, chronic longstanding hyperglycemia results in dysfunction and death of endothelial cells, pericytes, and vascular smooth muscle cells, resulting in breakdown of the blood-retinal barrier and retinal ischemia from capillary nonperfusion.¹² The exact pathogenesis for diabetic vasculopathy remains unclear. However, both the endothelial cells and pericytes appear to undergo premature death *in vivo*, replicating senescence.⁷ The compromised retinal vascular architecture and associated inflammation can lead to upregulation of cytokines and growth factors, especially VEGF-A.¹³ The net effect is retinal vascular leakage leading to macular edema or neovascularization leading to proliferative disease. Glial and microglial stimulation also result from associated inflammation.

Whether the retinal ischemia is acute and chronic, microvasculature remodeling, and repair occur. In retinal vein occlusion, revascularization, and/or neovascularization occur where redundant and acellular basement membrane tubes are recanalized and reconnected with existing circulation.⁷ New vessels form in close relationship with developing vessel tubes and surrounded by macrophages and hypertrophic Müller cells and astrocytes that re-establish the glial-vascular relationship. Microaneurysms and intraretinal microvascular abnormalities (IRMA) associated with diabetic retinopathy occur in association with acellular capillaries close to the arterial side of the circulation. These vascular changes are thought to represent endothelial proliferation in the absence of pericytes resulting from impaired attempts at neovascularization due to the diabetic environment.^{7,14}

RETINAL DEGENERATION ASSOCIATED WITH RETINAL VASCULOPATHY

Both diabetic retinopathy and retinal vein occlusion are retinal vascular diseases. However, electroretinography abnormalities are often noted with both conditions, especially in severe cases. In the case of diabetes, subtle changes in electroretinography, dark adaptation, and color contrast sensitivity consistent with retinal neuronal dysfunction can be detected before clinical signs of diabetic retinal vasculopathy.¹⁵ The impairment in retinal function is thought to be a direct effect of hyperglycemia on retinal neurons although subclinical hypoxic damage on retinal neuron is also a possible cause because functional abnormalities may be improved by breathing pure oxygen. Loss of retinal ganglion cells from apoptosis and microglial and glial cell reactivity have been described in diabetic eyes before signs of retinal vascular endothelial abnormalities are detected.^{16,17}

In eyes with diabetic retinopathy or retinal vein occlusion, atrophy, or disruption of the outer retina (i.e., photoreceptor layer) can be appreciated on optical coherence tomography in severe cases with irreversible vision loss (Fig. 1D) indicating that the retinal degeneration associated with retinal vasculopathy is not confined to the inner retina perfused by the retinal circulation, but may extend to the outer retina, either directly or indirectly.

Cell Therapy for Retinal Vasculopathy

This review will explore the potential use of various cell therapies as treatment for retinal vasculopathy. The goal of cell therapy in retinal vascular disease would be to restore or minimize the vision loss associated with retinal ischemia and/or retinal degeneration. As such, the cell therapy would have to restore or replace the damaged retinal vasculature and retinal neurons.

Direct cell replacement is being explored as potential therapy for macular degeneration using pluripotent stem cells differentiated into retinal pigment epithelial cells. Clinical trials have been initiated using embryonic stem cells and inducible pluripotent stem cells differentiated into RPE cells and injected into the submacular space. The treatment results of the phase I/II study using embryonic stem cell-derived RPE has been published and the treatment appears to be well tolerated with visual improvement in some cases.¹⁸ For this clinical study, systemic immunosuppressive therapy was used for 3 months to minimize rejection of transplanted cells.

In the case of cell therapy for retinal vasculopathy, direct tissue replacement might be more challenging because several different cells in the retina are affected in the pathogenesis of the vision loss. They include the vascular endothelium, vascular pericytes, vascular smooth muscle, the inner retinal neurons, the glia and microglia within the retina, and even the outer retinal photoreceptors. Nonetheless, both preclinical and clinical studies are ongoing exploring the use of cell therapy for retinal vascular disease. Most of the work has used adult mesenchymal or progenitor cells. These adult mesenchymal or progenitor cells are considered multipotent and have more limited ability to differentiate into the various different cells damaged in the retina from retinal vascular disease. However, these adult cells are known to have a regenerative effect via a local paracrine trophic effect.¹⁹⁻²¹ Such a mechanism is less tissue specific and may represent a more viable approach to regenerate or preserve the various different damaged cells in the retina resulting from retinal vascular disorders. The Table summarizes the types of adult cells that are being explored as potential therapy for retinal vascular disease. Nonetheless, the therapeutic window for treatment may be limited as paracrine trophic effect may be limited to eyes with enough viable cells in the retina. In addition, because of concerns regarding possibly enhancing ocular neovascularization or other forms of cellular proliferation using cell therapy, eyes with active ocular neovascularization may not be ideal candidates for cell therapy. Ultimately, it is possible that more than one type of cell therapy might be needed to restore and regenerate the retinal vasculature and the retinal neuron in order to improve visual function in eyes with significant ischemic retinopathy.

This review will provide an overview of the various types of cells that have been explored as potential therapy for retinal vascular disorders.

MESENCHYMAL STEM CELLS

Mesenchymal stem cells (MSCs) were originally harvested from bone marrow. These cells also have harvested from adipose tissue, placenta, umbilical cord blood, Whartons Jelly, dental pulp, heart, and liver.²² Mesenchymal stem cells readily expand

TABLE. Summary of Main Features of Various Types of Adult Cells Explored as Possible Treatment for Retinal Vasculopathy

Cell Feature	MSC	EPC/CD34	OEC	ASC
Source	BM, adipose	BM, blood	Blood	Adipose
Expandable	Yes	No	Yes	Yes
Route of transplantation*	Subretinal, intravitreal, intravenous	Intravitreal, intravenous	Intravitreal	Intravitreal
Allogenic use	Yes	No	Yes	Yes
Main cell precursor	Muscle, bone	Endothelium, blood cells	Endothelium	Pericyte
Clinical trial	Phase 1	Phase 1	None	None

BM, bone marrow.

* Routes of cell administration explored in preclinical and/or clinical studies of retinal disorders.

in culture and can be isolated by the cells' ability to adhere to the tissue culture plates. The identity of the isolated cells can be confirmed by the presence of certain cell surface markers (CD105, CD73, stromal antigen 1, CD44, CD90, CD166, CD54, and CD49) and absence of cell surface markers for hematopoietic cells (CD14, CD45, CD11a, CD34), erythrocytes (Glycophorin A), and platelets (CD31).^{22,23}

At least three different potential mechanisms of action for tissue regeneration have been proposed for MSCs. First and foremost effect of these cells in vitro and in vivo is the paracrine trophic effect. These cells have been shown to secrete neuroprotective and angiogenic factors (e.g., ciliary neurotrophic factor [CNTF], bFGF, VEGF, TGF-beta, etc.).¹⁹ In pathologic conditions such as hypoxia, this paracrine trophic effect may be enhanced. Secondly, these cells have some ability to differentiate into the damaged tissue, but the ability is somewhat limited because the cells are multipotent. Thus, they can differentiate into tissue of mesodermal origin more readily than tissue of ectodermal or endodermal origin.^{24,25} Both myogenic and endothelial differentiation of MSCs have been reported.²⁶ In an animal model of diabetic neuropathy, direct modulation of both angiogenesis and myelination in the peripheral nerves was observed by bone marrow-derived MSCs.²⁷ These stem cells were noted to have preferentially engrafted in the peripheral nerves but did not become neurons. Thus, direct tissue replacement may not be the predominant mechanism of action of these cells in tissue regeneration. In animal models of retinal degeneration, MSCs are reported to differentiate into photoreceptors and retinal pigment epithelium following local or systemic administration, but it is unclear whether these observations represent true differentiation or fusion of MSCs with pre-existing photoreceptors.²⁸ Lastly, MSCs are immune-modulating cells. In most systems, these cells are noted to be immunosuppressive by their ability to generate T regulatory cells and secretion of cytokines, such as IL-10, IL-17, TGF-beta, LIF, soluble HLA-G, and IL-1 receptor antagonist.²² Thus, they have been explored as potential therapy for autoimmune disorders, such as multiple sclerosis and Crohn's disease.²⁶ However, in certain animal models, an inflammatory reaction or fibrous proliferation has been observed after intravitreal administration (Zawadzki RJ, Pugh E, Nolta J, unpublished personal observations and communications, 2015).

MESENCHYMAL STEM CELLS FOR RETINAL VASCULOPATHY

The advantage of using MSCs clinically for treatment of retinal vasculopathy is that these adult stem cells can be easily harvested and expanded in culture although they represent less than 0.01% of the cells in bone marrow.²⁶ In addition, both autologous and allogeneic therapy have been explored in

clinical and preclinical studies because of the presumed immune privileged status of these cells. However, the immune privilege status may not be absolute because there is evidence of immune rejection of allogeneic MSC therapy in preclinical study.²⁹ Nonetheless, based on phase 1 and 2 clinical trials completed for various nonocular conditions, no long-term safety issues have been noted with intravenous, intrathecal, intramuscular, or intra-articular administration of autologous or allogeneic MSCs for various conditions ranging for joint disease, multiple sclerosis, and peripheral ischemia.²⁶ Most clinical trials have used MSCs from bone marrow. More recently adipose-derived MSCs have been explored and appear to be well-tolerated in clinical trial of stroke or ischemic cardiomyopathy following intravenous or transendocardial administration.^{30,31} They appear to be as effective as bone marrow-derived cells despite some differences in immune-phenotype, transcriptome, and proteome of these cells from different origin.³² Of note, animal studies recently have shown that intravascular injection of MSCs can lead to vascular obstruction depending on the size of the MSCs,³³ raising some potential concerns about intravascular administration of these cells.

The use of MSCs for treatment of retinal vascular disease has been explored.³⁴ Intravenous administration of adipose-derived MSCs in streptozotocin diabetic rats resulted in improved blood-retinal barrier integrity and reduction in blood glucose level after 1 week.³⁴ The donor cells were observed in the retina expressing rhodopsin and glial fibrillary acidic protein suggestive of photoreceptor and glial-like cell differentiation. However, it is unknown whether this observation resulted from true cell differentiation or fusion of donor cell with host cell.

Most preclinical studies exploring the use of subretinal injection of MSCs used murine or rat model of retinal degeneration. Although direct intraretinal incorporation of the subretinally injected MSCs is not observed typically, the cell therapy slowed down retinal degeneration.^{26,35} Mesenchymal stem cells have also been administered intravitreally in animal model of retinal degeneration or ischemia-reperfusion injury.³⁶ No intraretinal incorporation is noted in any of the studies. These injected cells typically form a cellular clump in the midvitrous and retrolentally. In animal models of retinal degeneration, some studies showed treatment effect after intravitreal MSC administration although the effect was not as pronounced as subretinal administration.³⁵ In animal model of acute ischemia-reperfusion injury, some preservation of ganglion cell layer was noted with intravitreal MSC administration, an effect that could be mimicked using the conditioned media.³⁶ These observations support the predominantly paracrine effect of MSCs on ischemic or degenerating retina. However, the immune modulatory effect of MSCs could also play a role because retinal ischemia is associated with local activation of inflammation.

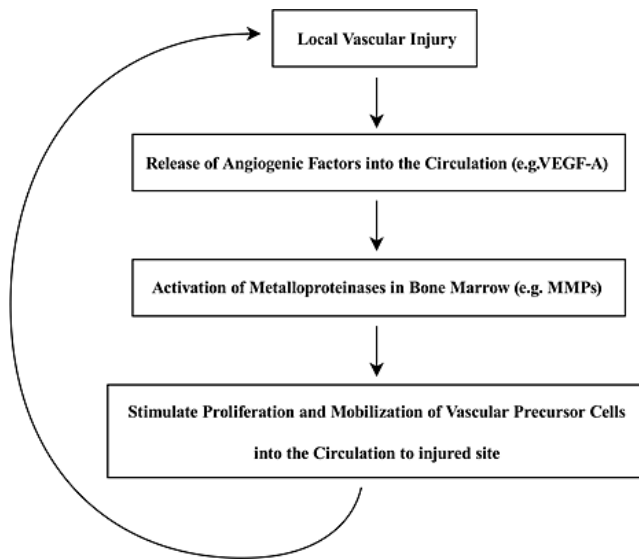


FIGURE 2. Diagram summarizing the proposed key molecular events associated with mobilization of vascular precursor cells from bone marrow.⁴² Vascular injury results in release of angiogenic factors, such as VEGF-A into the circulation, which in turn results in activation of MMP in bone marrow. This, in turn, stimulates proliferation and release of vascular precursor cells (CD34⁺ cells, endothelial progenitor cells) into the systemic circulation.

A concern regarding the use of MSC for treatment of retinal vasculopathy is that there is limited long-term safety information regarding the use of intravitreal MSCs for ischemic retinal vasculopathy. A recent study showed no long-term safety issues following subretinal implantation of MSCs from Wharton's Jelly in RCS rat.³⁷ Based on animal studies, there are some concerns regarding the effect of these cells following intravitreal injection. Bony or fibrous transformation has been observed following intravitreal administration of MSCs in some animal eyes leading to tractional retinal detachment (Nolta J, Pugh E, Zawadzki R, personal communication, 2015). This transformation of MSCs may be triggered by exposure to factors in the eye, such as aqueous humor.³⁸ In addition, some host factors, such as diabetes, might affect the efficacy of MSCs. Bone marrow-derived MSCs from diabetic mouse was noted with reduced migration and adhesion.³⁹ The diabetic environment appears to reduce the capacity of MSCs to promote neovascularization in the ischemic hind limb animal model.⁴⁰

Nonetheless, MSCs are being explored in phase 1 clinical trials for various degenerative and ischemic retinal conditions, including retinal vein occlusion and diabetic retinopathy. In order to start a clinical trial under an Investigational New Drug application approved by the Food and Drug Administration (FDA) in the United States, long-term preclinical safety studies are generally required using cells isolated and prepared under Good Manufacturing Practice conditions and using the same method that would be used to prepare the cells for the clinical trial. Most current clinical studies using MSCs for retinal vasculopathy are being conducted outside the US although there is one study listed as being conducted within the United States according to www.clinicaltrials.gov (in the public domain). Various routes of administration are being explored, ranging from intravitreal, periocular, and intravenous. It is unclear how regulated any of these studies are. The results of these clinical studies are still pending.

VASCULAR PRECURSOR CELLS: CD34⁺ CELLS, ENDOTHELIAL PROGENITOR CELLS

The potential role of circulating CD34⁺ cells as endothelial progenitor cells (EPCs) for tissue regeneration and angiogenesis following ischemia was first described by Asahara et al.⁴¹ They showed that these circulating cells differentiated into endothelial cells in vitro. In addition, in animal models of ischemia, heterologous, homologous, and autologous EPCs incorporated into sites of active angiogenesis.

CD34⁺ cells are found in highest concentration in bone marrow. In recent years, these CD34⁺ cells also have been isolated from adipose tissue, umbilical cord blood, and fetal liver. Figure 2 summarizes the molecular switches involved in mobilizing and recruiting these cells from bone marrow in response to ischemia. Vascular injury results in elevation of serum levels of angiogenic factors, such as VEGF-A and placental growth factor, which in turn activates metalloproteinases (MMPs).⁴² Activation of MMP-9 results in release of stem cell-active soluble kit ligand, resulting in proliferation and mobilization of the cells into the circulation.

The number and angiogenic capacity of circulating EPCs in peripheral blood can vary with disease states and may play a role in pathogenesis or recovery. In eyes with exudative AMD, elevated levels of CD34⁺ cells have been found in peripheral blood.⁴³ In patients with cardiovascular disease or risk factors, such as diabetes mellitus, hyperlipidemia, hypertension, or smoking, a decreased number and altered functional property EPCs in peripheral blood have been observed.⁴⁴ These alterations in EPCs are speculated to play a role in the pathogenesis of the associated systemic vascular complications. In contrast, drugs used to treat hyperlipidemia, (i.e., statins) have been shown to increase the number of EPCs and enhance the proliferative capacity of EPCs in vitro. Similarly, angiotensin 2 receptor antagonists or angiotensin-converting enzyme inhibitors have been shown to increase the number of circulating EPCs in patients with cardiovascular disease.

The adult CD34⁺ cells are multipotent and have some capacity to differentiate beyond endothelial cells.^{45,46} Bone marrow mononuclear cells have been shown to engraft and differentiate into retinal cells following intravitreal injection in a rat model of retinal injury.⁴⁷ The CD34⁺ cells are thought to represent a heterogeneous group of cells.⁴⁵ Whether the multipotent nature of this cell population results from the heterogeneity of the cell population or from true multipotency of each cell is open to debate. Nonetheless, the multipotency of these cells is evident in that they can be stimulated to differentiate into various blood cell lineages as well as endothelial cells. Thus, these cells are also often referred to as "hematopoietic cells." They have been used safely and successfully clinically for bone marrow transplantation for treatment of hematologic disorders.⁴⁸

Currently, CD34⁺ cells and EPCs are being explored in phase 1, 2, and 3 nonocular clinical trials as potential therapy for various ischemic disorders, including ischemic cardiomyopathy, peripheral ischemia, and cerebrovascular accidents.⁴⁵ Endothelial progenitor cells have been found to be more resistant to oxidative stress when compared to differentiated endothelial cells.⁴⁸ Thus, direct engraftment of these cells into the damaged vascular endothelium may lead to tissue regeneration. Differentiation of bone-derived stem cells into both myocytes and vascular cells have been shown in animal model of myocardial infarct.⁴⁶ In addition, these cells can have a paracrine trophic effect. They have been shown to secrete proangiogenic factors (VEGF, HGF, IGF-1, FGF-2) and may play a role in functional collateralization. They also secrete neurotrophic cytokines and are being explored as therapy for diabetic neuropathy.²⁰ Endothelial progenitor cells also have

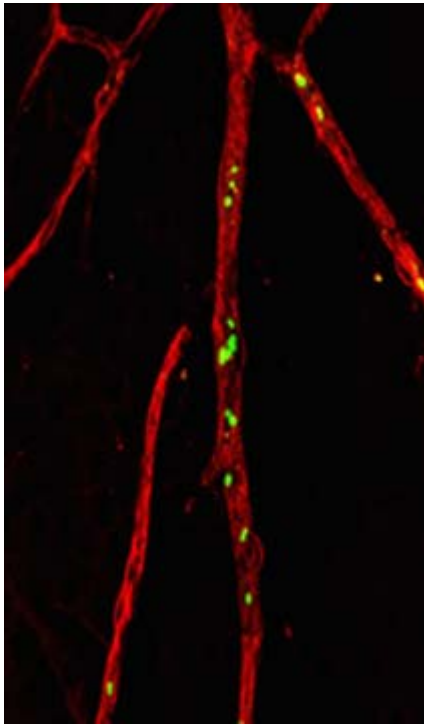


FIGURE 3. Immunohistochemical analysis of the retinal vascular of a NOD-SCID mouse 6 months after acute ischemia-reperfusion injury and treatment with intravitreal injection with human CD34+ cells from bone marrow. The human cells (green) are found incorporated into the mouse retinal vasculature which appears to have normalized (figure reproduced with permission).⁵¹

been found to prevent oxidative stress-induced apoptosis of mature endothelial cells via a paracrine effect.²¹ Direct immune suppressive and tolerance inducing ability of CD34+ cells have also been described.⁴⁹

No safety issues have been noted following intravenous infusion of EPCs. Efficacy results using EPCs for nonocular indications are somewhat variable depending on study design and how the cells are isolated.⁴⁵ Cells for clinical trial have been harvested from bone marrow or peripheral blood following mobilization. Many studies have used a crude mononuclear cellular fraction containing a low concentration of CD34+ cells, although some studies have used enriched or purified cellular fraction.

These variations in cell source and isolation are of note since CD34+ cells or EPCs consist of a heterogeneous group of cells and the relative composition may vary depending on the source of the cells and how they are harvested.⁴⁵ For example, the relative component of CD34+ cellular fraction from bone marrow may be different from CD34+ cellular fraction harvested from peripheral blood after mobilization which in turn may differ from adipose tissue-derived cells. The ideal source of these cells and the best method of isolating these cells for therapeutic trial are areas of controversy and ongoing research.

CD34 is a transmembrane cell surface protein. The exact function of CD34 is unknown.⁴⁵ However, because it is present on the surface of EPCs and mature endothelial cells, this protein is thought to play an important role in cell-cell interaction, cell migration and functions of the vascular endothelium. Although CD34 is one of the cell surface markers used to identify EPCs, it is not a universal cell surface marker used to identify or isolate EPCs.⁷ Some uniformity in nomenclature, identification, and purification of the EPCs

may eliminate some confusion regarding interpreting the results of studies using these cells.

CD34+ CELLS AND RETINAL VASCULOPATHY

The use of CD34+ cells as potential therapy for ischemic retinal disease has been explored in murine models of diabetic retinopathy and acute ischemia-reperfusion injury.⁵⁰ Intravenous or intravitreal administration of human CD34+ EPCs from peripheral blood or bone marrow resulted in rapid homing of the cells into the damaged retinal vascular. Although the engraftment rate was low, these human cells were found incorporated into the mouse retinal vasculature at long as 6 months following a single intravitreal administration (Fig. 3).⁵¹ There was an apparent normalization of the damaged retinal vasculature, suggestive of a therapeutic effect and no adverse effect was noted. In an animal model of ischemic optic neuropathy, engraftment and neuroglial differentiation of these cells from bone marrow into the inner retina were enhanced by brain-derived neurotrophic factor (BDNF) and CNTF.⁵²

Based on these promising preclinical observations, a phase 1 clinical study is ongoing exploring the safety and feasibility of intravitreal autologous administration of CD34+ cells from bone marrow as potential treatment for patients with irreversible vision loss from retinal ischemia or degeneration. The preliminary observations of the first several subjects enrolled and treated were published recently showing no safety or feasibility issues.⁵³ A high yield of CD34+ cells was isolated from a single bone marrow aspirate for intravitreal injection, regardless of the age or concurrent systemic disease such as diabetes mellitus. This observation is important because some decrease in the number of circulation EPCs have been found associated with host factors such as increasing age, concurrent cardiovascular disease or diabetes mellitus.⁴⁴ In addition, EPCs from peripheral blood of a diabetic subject was found to have defective homing capacity in animal models of retinal vasculopathy.⁵⁰ Whether these observations apply to CD34+ cells from bone marrow remains to be determined.

Nonetheless, autologous intravitreal CD34+ cell therapy shows some promise as therapy for retinal ischemia based on the findings of a study subject with retinal ischemia from a central retinal vein occlusion who was treated with this cell therapy.⁵³ Functional and anatomic improvement was noted on clinical examination during the six month follow-up course of the study. Although it is impossible to determine whether the improvement represents a cell treatment response based on the study design, no adverse intraocular neovascularization or cellular proliferation was noted in any of the study eyes. These are potential adverse effects of concern that may occur using angiogenic cell therapy. For the ongoing phase 1 clinical study, active retinal neovascularization from proliferative diabetic retinopathy or ischemic retinal vein occlusion is one of the exclusion criteria for study enrollment. This is an important exclusion because vitreous and aqueous humor from eyes with active proliferative diabetic retinopathy were noted to impair the function of bone marrow-derived CD34+ cells *in vitro*.⁵⁴ In addition, excluding eyes with active ocular neovascularization may minimize the potential proangiogenic effect of endothelial precursor cells

OUTGROWTH ENDOTHELIAL CELL/ENDOTHELIAL COLONY-FORMING CELL

Because EPCs and CD34+ cells are believed to represent a heterogeneous population of cells, one method to enhance the

therapeutic potential of EPCs is to identify the “true” endothelial precursor cells and expand them in culture. So far, investigators have identified at least two distinct subpopulation of EPCs in human blood by molecular analysis, early EPC (eEPC, also referred to as circulating angiogenic cells or myeloid angiogenic cells) and outgrowth endothelial cells (OECs).^{55–59} Comparative analysis at the transcription level revealed that eEPCs expressed hematopoietic specific transcripts (RUNX1, WAS, LYN) with links to inflammation and immunity (TLRs, CD14, HLAs). Early EPCs have minimal proliferative capacity and do not directly incorporate into the vascular endothelium but play an important role in promoting vascular repair via secreting IL-8.⁵⁷ In contrast, OECs highly expressed transcripts involved in vascular development and angiogenesis (Tie2, eNOS, Ephrin). Outgrowth endothelial cells (OECs) are committed to endothelial lineage and have significant proliferative potential.

Outgrowth endothelial cells are analogous to endothelial colony-forming cells described by other authors. This cellular subgroup of EPCs is thought to represent the “true” population of endothelial progenitors since they have been shown to form *denovo* blood vessels *in vivo*.⁵⁶ These cells interact with endothelial cells to form adherens and tight junctions. Outgrowth endothelial cells have been found to release soluble factors that reduce organ fibrosis, perhaps via attenuation of both TGF- β and angiotensin 2.^{59,60} Outgrowth endothelial cells from both healthy and diabetic mice attenuated diabetic renal injury and dysfunction via an antioxidant effect, supporting the potential use of autologous OECs as therapy for diabetic organ complications.⁶¹

The use of OECs as therapy for ischemic retinopathy also has been explored in an animal model of retinal ischemia. These cells incorporated into the damaged retinal vasculature and reduced the avascular zone of the retina, minimizing risk of pathologic preretinal neovascularization.⁶²

These OECs make up only a small fraction of isolated EPCs but can be expanded in culture.⁶³ In addition, these cells express similar levels of HLA antigen on the cell surface as MSCs, making them potential candidates for allogeneic cell therapy.⁶⁴ These features may make these cells a better choice for clinical trial than EPCs or CD34+ cells; however, it remains to be determined whether a purer cell fraction translates to more efficacy in treating retinal vascular disease. Simply reconstituting the retinal vascular endothelium may not be ideal for visual recovery or preservation in the presence of concurrent retinal degeneration; the paracrine trophic effect may be just as important in regenerating the neuronal elements of the retina damaged by retinal ischemia. The potential interplay between these endothelial colony-forming cells and other adult stem cells need to be explored further. For example, endothelial colony-forming cells have been shown to enhance engraftment and regenerative capacity of MSCs via paracrine signaling.⁶⁵

ADIPOSE STROMAL CELL: PERICYTE PRECURSOR

The presence of multipotent, undifferentiated cells in adipose tissue was first characterized by Zuk et al.^{66,67} Given the relative abundance and ease of access of adipose tissue, further research characterized these undifferentiated cells into two cell fractions: mesenchymal cell fraction or stromal vascular fraction.⁶⁸ The stromal vascular fraction consists of a heterogeneous composition of cells, including MSCs, mast cells, white blood cells, and CD34+ cells (50%–80%). Among the CD34+ cells, a distinct population of cells is identified without endothelial cell surface marker (i.e., CD31–/CD144–). These CD34+/CD31–/CD144– cells attach to uncoated plastic and

express CD10, CD13, CD90, and pericytic and smooth muscle markers. They are thought to represent resident pericytes or their precursors and display a stromal and perivascular position in blood vessel wall with engraftment.⁶⁹ However, they lack some specific pericyte markers (e.g., NG2, CD140b, or α -smooth muscle actin [α -SMA]).

Trakluev et al.⁶⁹ demonstrated bidirectional paracrine interaction of these adipose stromal cells with endothelial cells affecting their function and structure. The conditioned media resulting from coculture of adipose stromal cells and endothelial cells contained angiogenic factors (VEGF, HFG, bFGF), inflammatory cytokines (IL-6, IL-8, monocyte chemoattractant protein-1 and -2), mobilization factors (macrophage colony-stimulating factor and granulocyte/macrophage colony-stimulating factor). Adipose stromal cells also secrete anti-apoptotic factors.⁷⁰ In addition, functional vascular networks can develop *in vivo* by combining adipose stromal cells and cord blood endothelial cells.⁷¹ Lastly, endothelial cell survival was increased with adipose stromal cells coculture.⁷²

The use of adipose stromal cells has been explored as potential therapy for retinal vasculopathy, including diabetic retinopathy. The rationale for using adult pericyte or pericyte precursor cell is based on the theory that diabetic retinopathy results from the loss of pericytes that provide an anti-inflammatory and antiangiogenic environment for endothelial cells under normal conditions.^{73,74} Adipose stromal cells have been administered intravitreally and intravenously into animal models of oxygen induced retinopathy and diabetic retinopathy. Perivascular integration of these adipose stromal cells has been observed with rescue of retinal capillary damage.^{34,69,75} Improvement in electroretinography and decrease in vascular leakage and apoptosis around retinal vessels was noted within 1 to 3 weeks following intravitreal adipose stromal cell injection.⁷¹ These cells show promise as potential therapy for retinal vascular disease. Long-term safety and efficacy studies will be desired before starting clinical trial.

PLURIPOTENT STEM CELLS

To date, there is no clinical trial that has been started using pluripotent stem cells as therapy for retinal vascular disease. Undifferentiated induced pluripotent cells (iPS) have been explored in an animal model of stroke and found to be teratogenic.⁷⁶ However, more recently, Fang et al.⁷⁷ generated iPS cells without c-Myc to minimize teratogenicity. Subretinal transplantation of the non c-Myc iPS cells into the subretinal space of rat eyes with acute ischemia reperfusion injury resulted in rescue of the ischemic retinal damage via secretion of paracrine trophic factors (BDNF and CNTF) and regulation of oxidative parameters. No tumors were noted after 6 months, but the transplanted cells remained in the subretinal space without engraftment.

Other researchers have differentiated embryonic stem cells or iPS cells into endothelial precursor cells (endothelial colony unit forming cells) and have used these cells to show some efficacy in treating an animal model of retinopathy of prematurity.⁷⁸ Retinal perfusion was partially restored, but it remains to be determined whether endothelial colony unit forming cells differentiated from pluripotent stem cells are better sources of cells for retinal vascular regeneration than cells from adult sources or cord blood. When vascular progenitor cells derived from pluripotent cells derived from various sources were compared in a murine model of ischemia-reperfusion injury, vascular progenitor cells derived from embryonic cells and iPS cells from cord blood had engraftment, homing, and repair capability while those derived from iPS cells from fibroblasts did not.⁷⁹ This observation suggests the iPS cells derived from mature cells may not be as ideal.

Similarly, researchers have successfully differentiated pluripotent embryonic stem cells into MSCs. These MSCs from pluripotent cells are similar to MSCs from adult sources, but it remains to be determined whether these cells are more pluripotent and therapeutic than MSCs from adult origin.⁸⁰ In addition, preclinical studies are still pending to determine the long-term safety and efficacy of using these cells as therapy for retinal vasculopathy.

FUTURE DIRECTIONS

Cell therapy may be a feasible treatment for retinal vascular diseases, including diabetic retinopathy or retinal vein occlusion.⁵³ However, there are very limited published data on clinical applications of cell therapy for retinal vascular disease. Several pilot phase I studies have been initiated and are ongoing; the results are pending. Long-term preclinical studies will be important safety and efficacy studies that need to be conducted before initiating any clinical trial.⁵¹ Ultimately, clinical studies will need to be conducted in order to determine the full therapeutic potential and any associated limitations of any cell therapy for retinal vascular disease. Because early phase clinical studies are conducted typically in eyes with advanced disease, it is important to remember that efficacy may not be observed if the condition is beyond the therapeutic window. In addition, clinical trials should be designed to detect and minimize potential adverse effects of cell therapy, such as stimulation of ocular neovascularization or cellular proliferations.

The key needs and opportunities for future research in this area are as follows:

1. Cell therapy that can potentially regenerate both the damaged retinal vasculature and retinal neurons is desired. As such, adult stem cells with paracrine trophic effects on multiple cell types in the retina show promise. Whether endothelial precursor cells or MSCs derived from cord blood or pluripotent sources are more pluripotent and therapeutic than adult cells remains to be determined but should be investigated;
2. Adult stem cell therapies are in early clinical trial but efficacy and safety results are still pending. If these studies move forward to larger clinical studies, a more standardized approach to evaluating safety and efficacy of cell therapy for treatment of retinal vascular disorders will be developed;
3. Understanding the interplay between various precursor cells is important to developing the ideal cell therapy for vascular regeneration. The optimal cell therapy may involve a combination of stem cells or precursor cells;
4. Host factors might affect the regenerative potential of stem cells for autologous cell therapy. Pharmacologic methods to overcome these potential host factors are being developed and these methods may enhance the regenerative potential of these stem cells⁸¹; and
5. Understanding the molecular basis for the regenerative effect of stem cells in retinal vascular conditions might shed light on new pharmacologic or genetic approaches to treating retinal vascular disorders and new approaches to enhancing the therapeutics effects of currently available stem cell therapies.⁸²

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References

1. Prevent Blindness America website. Available at: <http://www.visionproblemsus.org>. Accessed June 1, 2015.
2. Zheng Y, He M, Congdon N. The worldwide epidemic of diabetic retinopathy. *Indian J Ophthalmol*. 2012;60:428-431.
3. Kempen JH, O'Colmain BJ, Leske MC, et al. The prevalence of diabetic retinopathy among adults in the United States. *Arch Ophthalmol*. 2004;122:552-563.
4. Kocur I, Resnikoff S. Visual impairment and blindness in Europe and their prevention. *Br J Ophthalmol*. 2002;86:716-722.
5. Laouri M, Chen E, Looman M, Gallagher M. The burden of disease of retinal vein occlusion: review of the literature. *Eye*. 2011;25:981-988.
6. Lois N, McCarter RV, O'Neill C, Medin RJ, Stitt AW. Endothelial progenitor cells in diabetic retinopathy. *Front Endocrinol (Lausanne)*. 2014;5:1-11.
7. Stitt AW, O'Neill CL, O'Doherty MT, Archer DB, Gardiner TA, Medina RJ. Vascular stem cells and ischaemic retinopathies. *Prog Retin Eye Res*. 2011;30:149-166.
8. Brown GC. Vision and quality of life. *Trans Am Ophthalmol Soc*. 1999;97:473-511.
9. Brown MM, Brown GC, Sharma S, Landy J, Bakal J. Quality of life with visual acuity loss from diabetic retinopathy and age-related macular degeneration. *Arch Ophthalmol*. 2002;120:481-484.
10. Bonne C, Muller A, Villain M. Free radicals in retinal ischemia. *Gen Pharmacol*. 1998;30:275-280.
11. Osborne NN, Casson RJ, Wood JP, Chidlow G, Graham M, Melena J. Retinal ischemia: mechanisms of damage and potential therapeutic strategies. *Prog Retin Eye Res*. 2004;23:91-147.
12. Kern TS, Tang J, Berkowitz BA. Validation of structural and functional lesions of diabetic retinopathy in mice. *Mol Vis*. 2010;16:2121-2131.
13. Kern TS. Contributions of inflammatory processes to the development of the early stages of diabetic retinopathy. *Exp Diabetes Res*. 2007;95103:1-14.
14. Luty GA. Effects of diabetes on the eye. *Invest Ophthalmol Vis Sci*. 2013;54: ORSF81-ORSF87.
15. Tzekov R, Arden GB. The electroretinogram in diabetic retinopathy. *Surv Ophthalmol*. 1999;44:53-60.
16. Barber A. A new view of diabetic retinopathy: a neurodegenerative disease of the eye. *Prog Neuro-Psychopharmacol Biol Psychiatry*. 2003;27:283-290.
17. Gardner T, Antonetti D, Barber A, LaNoue K, Levison S. Diabetic retinopathy: more than meets the eye. *Surv Ophthalmol*. 2002(47 suppl 2):S253-S262.
18. Schwartz SC, Rgillo CD, Lam BL, et al. Human embryonic stem cell-derived retinal pigment epithelium in patients with age-related macular degeneration and Stargardt's macular dystrophy: follow-up of two open-label phase 1/2 studies. *Lancet*. 2015;385:509-516.
19. Caplan A, Deniis J. Mesenchymal stem cells as trophic mediators. *J Cell Biochem*. 2006;98:1076-1084.
20. Kim H, Kim JJ, Yoon YS. Emerging therapy for diabetic neuropathy: cell therapy targeting vessels and nerves. *Endocr Metab Immune Disord Drug Targets*. 2012;12:168-178.
21. Yang Z, von Ballmoos MW, Faessler D, et al. Paracrine factors secreted by endothelial progenitor cells prevent oxidative

- stress-induced apoptosis of mature endothelial cells. *Atherosclerosis*. 2010;211:103-109.
22. Chamberlian G, Fox J, Ashton B, Middleton J. Concise review: mesenchymal stem cells: their phenotype, differentiation capacity, immunological features, and potential for homing. *Stem Cells*. 2007;25:2739-2749.
 23. Prockop D. Marrow stromal cells as stem cells for non-hematopoietic tissues. *Science*. 1997;276:71-74.
 24. Jiang Y, Jahagirdar BN, Reinhardt L, et al. Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature*. 2002;418:41-49.
 25. Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. *Science*. 1999;284:143-147.
 26. Liew A, O'Brien T. Therapeutic potential for mesenchymal stem cell transplantation in critical limb ischemia. *Stem Cell Res Ther*. 2012;3:28.
 27. Han JW, Choi D, Lee MY, Huh YH, Yoon YS. Bone marrow-derived mesenchymal stem cells improve diabetic neuropathy by direct modulation of both angiogenesis and myelination in peripheral nerves [published online ahead of print May 13, 2015]. *Cell Transplant*. doi:http://dx.doi.org/10.3727/096368915X688209.
 28. Megaw R, Dhillon B. Stem cell therapies in the management of diabetic retinopathy. *Curr Diab Rep*. 2014;14:498.
 29. Zangi L, Margalit R, Reich-Zeliger S, et al. Direct imaging of immune rejection and memory induction by allogeneic mesenchymal stromal cells. *Stem Cells*. 2009;27:2865-2874.
 30. Diez-Tejedor E, Gutierrez-Ferandez M, Martinez-Sanchez P, et al. Reparative therapy for acute ischemic stroke with allogeneic mesenchymal stem cells from adipose tissue: a safety assessment: a phase II randomized, double-blinded, placebo-controlled, single-center, pilot clinical trial. *J Stroke Cerebrovasc Dis*. 2014;23:2694-2700.
 31. Perin EC, Sanz-Ruiz R, Sanchez PL, et al. Adipose-derived regenerative cells in patients with ischemic cardiomyopathy: The PRECISE Trial. *Am Heart J*. 2014;168:88-95.
 32. Strioga M, Viswanathan S, Darinskas A, Slaby O, Michalek J. Same or not the same? Comparison of adipose tissue-derived versus bone marrow-derived mesenchymal stem and stromal cells. *Stem Cells Dev*. 2012;21:2724-2752.
 33. Ge J, Guo L, Wang S, et al. The size of mesenchymal stem cells is a significant cause of vascular obstructions and stroke. *Stem Cell Rev*. 2014;10:295-303.
 34. Yang Z, Li K, Yan X, Dong F, Zhao C. Amelioration of diabetic retinopathy by engrafted human adipose-derived mesenchymal stem cells in streptozotocin diabetic rats. *Graefes Arch Clin Exp Ophthalmol*. 2010;248:1415-1422.
 35. Tzameret A, Sher I, Belkin M, et al. Transplantation of human bone marrow mesenchymal stem cells as a thin subretinal layer ameliorates retinal degeneration in a rat model of retinal dystrophy. *Exp Eye Res*. 2014;118:135-144.
 36. Li N, Li X, Yuan J. Effects of bone-marrow mesenchymal stem cells transplanted into vitreous cavity of rat injured by ischemia/reperfusion. *Graefes Arch Clin Exp Ophthalmol*. 2009;247:503-514.
 37. Leow Sn, Luu CD, Nizam NH, et al. Safety and efficacy of human Wharton's Jelly-derived mesenchymal stem cells therapy for retinal degeneration. *PLoS One*. 2015;10:e0128973.
 38. Morgan JT, Kwon HS, Wood JA, et al. Thermally labile components of aqueous humor potently induce osteogenic potential in adipose-derived mesenchymal stem cells. *Exp Eye Res*. 2015;135:127-133.
 39. Li L, Xia Y, Wang Z, et al. Suppression of the P13K-Akt pathway is involved in the decreased adhesion and migration of bone marrow-derived mesenchymal stem cells from non-obese diabetic mice. *Cell Biol Int*. 2011;35:961-966.
 40. Yan J, Tie G, Xu TY, Cecchini K, Messina LM. Mesenchymal stem cells as a treatment for peripheral arterial disease: current status and potential impact of type II diabetes on their therapeutic efficacy. *Stem Cell Rev*. 2013;9:360-372.
 41. Asahara T, Murohara T, Sullivan A, et al. Isolation of putative progenitor endothelial cells for angiogenesis. *Science*. 1997;275:964-966.
 42. Rafii S, Lyden D. Therapeutic stem and progenitor cell transplantation for organ vascularization and regeneration. *Nat Med*. 2003;9:702-712.
 43. Yodoi Y, Sasahara M, Kameda T, Yoshimura N, Otani A. Circulating hematopoietic stem cells in patients with neovascular age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2007;48:5464-5472.
 44. Shantsila E, Watson T, Lip GYH. Endothelial progenitor cells in cardiovascular disorders. *J Am Coll Cardiol*. 2007;49:741-752.
 45. Mackie AR, Losordo DW. CD34 positive stem cells in the treatment of heart and vascular disease in human beings. *Tex Heart Inst J*. 2011;38:474-485.
 46. Duran JM, Makarewich CA, Sharp TE, et al. Bone-derived stem cells repair the heart after myocardial infarction through transdifferentiation and paracrine signaling mechanisms. *Circ Res*. 2013;113:539-5552.
 47. Tomita M, Adachi Y, Yamada H, et al. Bone marrow-derived stem cells can differentiate into retinal cells in injured rat retina. *Stem Cells*. 2002;20:279-283.
 48. Peters C, Cornish JM, Parikh, SH, Kurtzberg J. Stem cell source and outcome after hematopoietic stem cell transplantation (HSCT) in children and adolescents with acute leukemia. *Pediatr Clin North Am*. 2010;57:27-46.
 49. Gur H, Krauthgamer R, Bachar-Lustig E, et al. Immune regulatory activity of CD34+ progenitor cells: evidence for a deletion-based mechanism mediated by TNF-alpha. *Blood*. 2005;105:2585-2593.
 50. Caballero S, Sengupta N, Afzal A, et al. Ischemic vascular damage can be repaired by healthy, but not diabetic, endothelial progenitor cells. *Diabetes*. 2007;56:960-967.
 51. Park SS, Caballero S, Bauer G, et al. Long-term effects of intravitreal injection of GMP-grade bone-marrow-derived CD34+ cells in NOD-SCID mice with acute ischemia-reperfusion injury. *Invest Ophthalmol Vis Sci*. 2012;53:986-994.
 52. Goldenberg-Cohen N, Avraham-Lubin BC, Sadikov T, Askenasy N. Effect of coadministration of neuronal growth factors on neuroglial differentiation of bone marrow-derived stem cells in the ischemic retina. *Invest Ophthalmol Vis Sci*. 2014;55:502-512.
 53. Park SS, Bauer G, Abed M, et al. Intravitreal autologous bone marrow CD34+ cell therapy for ischemic and degenerative retinal disorders: preliminary phase 1 clinical trial findings. *Invest Ophthalmol Vis Sci*. 2014;56:81-89.
 54. Balaiya S, Grant MA, Priluck J, Chalam KV. Growth factors/chemokines in diabetic vitreous and aqueous alter the function of bone marrow-derived progenitor (CD34+) cells in humans. *Am J Physiol Endocrinol Metab*. 2014;307:E695-E702.
 55. Medina RJ, O'Neil CI, Sweeney M, et al. Molecular analysis of endothelial progenitor cell (EPC) subtypes reveals two distinct cell populations with different identities. *BMC Med Genomics*. 2010;3:18.
 56. Vaughan EE, O'Brien T. Isolation of circulating angiogenic cells. *Methods Mol Biol*. 2012;916:351-356.
 57. Medina RJ, O'Neill CL, O'Doherty TM, et al. Myeloid angiogenic cells act as alternative M2 macrophages and modulate angiogenesis through interleukin-8. *Mol Med*. 2011;17:1045-1055.
 58. Vaughan EE, O'Brien T. Isolation of circulating angiogenic cells. *Methods Mol Biol*. 2012;916:351-356.

59. Critser PJ, Yoder MC. Endothelial colony-forming cell role in neoangiogenesis and tissue repair. *Curr Opin Organ Transplant.* 2010;15:68-72.
60. Yuen DA, Connelly KA, Zhang Y, et al. Early outgrowth cells release soluble endocrine antifibrotic factors that reduce progressive organ fibrosis. *Stem Cells.* 2013;31:2408-2419.
61. Zhang Y, Yuen DA, Advani A, et al. Early-outgrowth bone marrow cells attenuate renal injury and dysfunction via an antioxidant effect in a mouse model of type 2 diabetes. *Diabetes.* 2012;61:2114-21125.
62. Medina RJ, O'Neill CL, Humphreys MW, Gardiner TA, Stitt AW. Outgrowth endothelial cells: characterization and their potential for reversing ischemic retinopathy. *Invest Ophthalmol Vis Sci.* 2010;51:5906-5913.
63. Hirshi KK, Ingram DA, Yoder MC. Assessing identity, phenotype, and fate of endothelial progenitor cells. *Arterioscler Thromb Vasc Biol.* 2008;28:1584-1595.
64. Nuzzolo ER, Capodimonti S, Marini M, et al. Adult and cord blood endothelial progenitor cells have different gene expression profiles and immunogenic potential. *Blood Transfus.* 2014;(suppl 1):S367-S374.
65. Lin RZ, Moreno-Luna R, Li D, Jaminet SC, Greene AK, Melero-Martin JM. Human endothelial colony-forming cells serve as trophic mediators for mesenchymal stem cell engraftment via paracrine signaling. *Proc Natl Acad Sci U S A.* 2014;111:10137-10142.
66. Zuk P, Zhu M, Mizuno H, et al. Multilineage cells from human adipose tissue: implications for cell-based therapies. *J Tiss Eng.* 2001;7:211-228.
67. Zuk P, Zhu M, Ashjian P, et al. Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell.* 2002;13:4279-4295.
68. Alexander RW. Understanding adipose-derived stromal vascular fraction cell biology and use on the basis of cellular, chemical, structural and paracrine components: a concise review. *J Prolotherapy.* 2012;4:e855-e869.
69. Traktuev DO, Merfeld-Clauss S, Li J, et al. A population of multipotent CD34+ adipose stroma cells share pericyte and mesenchymal surface markers, reside in a periendothelial location, and stabilize endothelial networks. *Circ Res.* 2008;102:77-85.
70. Rehman J, Traktuev D, Li J, et al. Secretion of angiogenic and antiapoptotic factors by human adipose stromal cells. *Circulation.* 2004;109:1292-1298.
71. Traktuev DO, Prater DN, Merfeld-Clauss S, et al. Robust functional vascular network formation in vivo by cooperation of adipose progenitor and endothelial cells. *Circ Res.* 2009;104:1410-1420.
72. Rajashekhar G, Ramadan A, Abburi C, et al. Regenerative therapeutic potential of adipose stromal cells in early stage diabetic retinopathy. *PLoS One.* 2014;9:e84671.
73. Von Tell D, Armulik A, Betsholtz C. Pericytes and vascular stability. *Exp Cell Res.* 2006;312:623-629.
74. Cai J, Kehoe O, Smith GM, Hykin P, Boulton ME. The angiopoietin/Tie-2 system regulates pericyte survival and recruitment in diabetic retinopathy. *Invest Ophthalmol Vis Sci.* 2008;49:2163-2171.
75. Mendel TA, Clabough EB, Kao DS, et al. Pericytes derived from adipose-derived stem cells protect against retinal vasculopathy. *PLoS One.* 2013;8:e65691.
76. Kawai H, Yamashita T, Ohta Y, et al. Tridermal tumorigenesis of induced pluripotent stem cells transplanted in ischemic brain. *J Cereb Blood Flow Metab.* 2010;30:1487-1493.
77. Fang IM, Yang CM, Yang CH, Chiou SH, Chen MS. Transplantation of induced pluripotent stem cells without c-Myc attenuates retinal ischemia and reperfusion injury in rats. *Exp Eye Res.* 2013;113:49-59.
78. Prasain N, Lii MR, Vemula S, et al. Differentiation of human pluripotent stem cells to cells similar to cord-blood endothelial colony-forming cells. *Nat Biotechnol.* 2014;32:1151-1157.
79. Park TS, Bhutto I, Zimmerlin L, et al. Vascular progenitors from cord blood-derived induced pluripotent stem cells possess augmented capacity for regenerating ischemic retinal vasculature. *Circulation.* 2014;129:359-372.
80. Kimbrel EA, Kouris NA, Yavarian GJ, et al. Mesenchymal stem cell population derived from human pluripotent stem cells displays potent immunomodulatory and therapeutic properties. *Stem Cells Dev.* 2014;23:1611-1624.
81. Hazra S, Stepps V, Bhatwadekar AD, et al. Enhancing the function of CD34(+) cells by targeting plasminogen activator inhibitor-1. *PLoS One.* 2013;8:e79067.
82. Ueki Y, Wilken MS, Cox KE, et al. Transgenic expression of the proneural transcription factor *Ascl1* in Müller glia stimulates retinal regeneration in young mice. *Proc Natl Acad Sci U S A.* 2015;112:13717-13722.