NEURAL REGENERATION RESEARCH
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Bone marrow-derived mononuclear stem cells in the treatment of retinal degenerations

https://doi.org/10.4103/1673-5374.335692

Date of submission: April 15, 2021

Date of decision: May 11, 2021

Date of acceptance: June 21, 2021

Date of web publication: February 8, 2022

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Abstract

Retinal degenerative diseases affecting the outer retina in its many forms (inherited, acquired or induced) are characterized by photoreceptor loss, and represent currently a leading cause of irreversible vision loss in the world. At present, there are very few treatments capable of preventing, recovering or reversing photoreceptor degeneration or the secondary retinal remodeling, which follows photoreceptor loss and can also cause the death of other retinal cells. Thus, these diseases are nowadays one of the greatest challenges in the field of ophthalmological research. Bone marrow derived-mononuclear stem cell transplantation has shown promising results for the treatment of photoreceptor degenerations. These cells may have the potential to slow down photoreceptor loss, and therefore should be applied in the early stages of photoreceptor degenerations. Furthermore, because of their possible paracrine effects, they may have a wide range of clinical applications, since they can potentially impact on several retinal cell types at once and photoreceptor degenerations can involve different cells and/or begin in one cell type and then affect adjacent cells. The intraocular injection of bone marrow derived-mononuclear stem cells also enhances the outcomes of other treatments aimed to protect photoreceptors. Therefore, it is likely that future investigations may combine bone marrow derived-mononuclear stem cell therapy with other systemic or intraocular treatments to obtain greater therapeutic effects in degenerative retinal diseases.

Key Words: age-related macular degeneration; bone marrow stem cells; intravitreal injection; macroglia; microglia; photoreceptor degeneration; retinal ganglion cells; retinitis pigmentosa; subretinal injection; transplant

Introduction

It is estimated that there are at least 215 million people with moderate or severe visual impairment in the world, and 36 million of whom are blind (Flaxman et al., 2017). Retinal degenerations may compromise the cells of the outer retina (e.g., retinitis pigmentosa (RP), age-related macular degeneration (AMD), Stardgardt disease) or the inner retina (e.g., glaucoma, diabetic retinopathy). This review focused on the outer retinal degenerative diseases, which are at present one of the leading causes of irreversible blindness in the world (Wong et al., 2014; Flaxman et al., 2017), and course with photoreceptor loss, although their pathophysiological mechanisms may vary depending on the disease.

Search Strategy and Selection Criteria

We have conducted a Medline/PubMed search of articles published between 1990 and 2021 using the following search terms: bone marrow-derived mononuclear stem cells OR bone marrow-derived stem cells OR stem cells OR BM-MSC OR bone marrow AND retina OR retinal degeneration OR retinal transplantation OR photoreceptors. The results were further screened by reading titles and abstracts. In case of doubt, the methodology was also reviewed to determine the type of bone marrow stem cells used.

Inherited Retinal Degenerations

Inherited photoreceptor degenerations encompass a heterogeneous group of over 80 diseases (Tatour and Ben-Yosef, 2020) with more than 200 different genes involved (RetNet; https://sph.uth.edu/retnet/) (Tatour and Ben-Yosef, 2020), and are among the most common genetic diseases in humans. These diseases are usually distinguished according to their mode of inheritance that can be autosomal recessive (50–60% of cases), autosomal dominant (30–40% of cases) or X-linked (5–15% of cases) (Pfeiffer et al., 2020; Tatour and Ben-Yosef, 2020) and to their clinical phenotype. RP is the most common inherited photoreceptor degeneration and represents a major cause of visual disability and blindness. RP has become a worldwide health issue, as it causes irreversible blindness at working ages. Its worldwide prevalence is approximately 1 in 4000 (Daiger et al., 2013), with variations according to geographical locations. RP is the result of different genetic mutations that usually affect rods or the retinal pigment epithelium (RPE) (Di Pierdomenico et al., 2017; Dias et al., 2018), impacting on functions that are essential to the normal functioning and survival of photoreceptors (Daiger et al., 2013; Swaroop and Sieving, 2013). The onset of RP is characterized by a progressive loss of photoreceptors, first rods and then cones, making it clinically different from other forms of inherited retinal degenerations that are not progressive or in which cone degeneration

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Funding: *This work was supported by Fundación Lucha contra la Ceguera (FUNDALUCE) to MPVP; Fundación Robles Chillida to DGA; Fundación Séneca, Agencia de Ciencia y Tecnología Región de Murcia (19881/GERM/15 to MVS); and the Spanish Ministry of Science and Innovation, Instituto de Salud Carlos III, Fondo Europeo de Desarrollo Regional "Una Manera de Hacer Europa" (SAF2015-67643-P to MVS, PI19/00203 to MPVP and DGA, RD16/0008/0026 to MPVP; RED2018-102499-T to MVS).*

How to cite this article: *García-Ayuso D, Di Pierdomenico J, García-Bernal D, Vidal-Sanz M, Villegas-Pérez MP (2022) Bone marrow-derived mononuclear stem cells in the treatment of retinal degenerations. Neural Regen Res 17(9):1937-1944.*

precedes rod degeneration. This means that patients affected by RP typically experience nyctalopia and tunnel vision early in the disease due to the initial loss of rods and, in later stages, a gradual decline in visual acuity due to secondary cone degeneration. At this point, one of the big questions to be resolved in understanding this disease arises: why do cones degenerate following rod loss? Another major challenge related to inherited retinal degenerations is their heterogeneity which complicates both their clinical diagnosis and therapeutic approaches. Indeed, mutations of the same gene can cause different clinical phenotypes, whereas similar clinical phenotypes can be the result of mutations of different genes (Daiger et al., 2013; Swaroop and Sieving, 2013).

Age-Related Macular Degeneration

AMD is the most common acquired cause of photoreceptor degeneration. AMD is becoming a major public health concern due to the increase in life expectancy (Wong et al., 2014; Wong, 2020). It is estimated that by 2040, the number of people affected by AMD will increase to 288 million (Wong et al., 2014). AMD is a multifactorial disease caused by both genetic (Fritsche et al., 2016) and environmental risk factors (Jones et al., 2017). The disease is characterized by a progressive degeneration of photoreceptors and RPE cells in the central retina, causing irreversible blindness in older adults (Wong et al., 2014; Wong, 2020). There are two major subtypes of AMD: dry (non-neovascular or atrophic) and wet (exudative or neovascular). Typically, AMD starts as the dry form and in around 15% of patients progresses to the wet form (Kim and Lad, 2020). There are several effective therapeutic options for the wet form of the disease (Kim and Lad, 2020). However, no effective treatment for dry AMD, suffered by 85% of all AMD patients, has yet been found (Kim and Lad, 2020). Retinal degenerative diseases are therefore multifactorial and very complex diseases triggered by genetic and environmental factors, but that can also be exacerbated by factors such as light exposure (Garcia-Ayuso et al., 2018b, 2019) or diet (Garcia-Ayuso et al., 2018b).

Common Features of Degenerative Retinal Diseases

Photoreceptor loss is typically an early event occurring in outer degenerative retinal diseases. Indeed, it is now widely acknowledged that inherited photoreceptor degenerations trigger an irreversible sequence of events, namely retinal remodeling, which causes a progressive alteration of all retinal layers eventually leading to retinal ganglion cell (RGC) death (Villegas-Perez et al., 1998; Garcia-Ayuso et al., 2010, 2018b). RGCs are the efferent retinal neurons; their axons form the optic nerve and are therefore necessary for visual information to reach the brain. Retinal remodeling is thus considered a negative plasticity of the retina and has been divided into four different phases (Pfeiffer et al., 2020): (i) First, there is primary photoreceptor stress and death and onset of a glial reaction; (ii) later there is secondary photoreceptor degeneration, manifested in the case of RP by cone death, and involvement of microglia, Müller and RPE cells. This phase is concluded with the total loss of photoreceptors; (iii) subsequently there is tissue remodeling that encompasses neuronal rewiring, disorganization of the whole retina and neuronal death; (iv) and finally there is progressive neuroration, including RGC loss (Garcia-Ayuso et al., 2019). During the early stages of retinal degeneration, glial cells play an important role (Di Pierdomenico et al., 2020b; Pfeiffer et al., 2020). Microglial cells become activated and migrate from the inner to the outer retinal layers to phagocytose dying photoreceptors (Di Pierdomenico et al., 2017, 2018, 2020b), while astrocytes and Müller cells become hypertrophic, overexpress glial fibrillary acidic protein and fill the space left by dead photoreceptors to form a glial seal (Di Pierdomenico et al., 2017, 2018, 2020b; Pfeiffer et al., 2020). Retinal remodeling is a common feature of all photoreceptor degenerations regardless of the primary cause of degeneration (Garcia-Ayuso et al., 2018a, 2019; Pfeiffer et al., 2020). Understanding the events involved in retinal remodeling will enable scientists to find windows of opportunity to develop vision rescue therapies for the degenerative retinal diseases that currently lack of effective treatment, such as the aforementioned AMD and RP.

Strategies to Treat Retinal Degenerations

Traditionally, the main objective of research in photoreceptor degeneration has been two-fold, on the one hand to develop therapies that prevent or at least delay the death of photoreceptors and on the other hand therapies that replace them to restore vision (Bloch et al., 2019). If therapies that prevent or delay photoreceptor loss are achieved, this will in turn protect the inner retinal layers from the negative plasticity of retinal remodeling and, therefore, healthy RGCs will be preserved, enabling visual information to still reach the brain in the event that it is necessary to replace photoreceptors (Villegas-Perez et al., 1998; Garcia-Ayuso et al., 2010, 2018a, 2019). As noted above, there are many genetic defects involved in inherited photoreceptor degenerations. This makes it particularly hard to successfully apply gene therapy to many of these diseases. A genetic treatment was approved worldwide 4 years ago for inherited photoreceptor degenerations caused by mutations in the *RPE65* gene, but unfortunately this mutation affects a relatively small number of patients (Apte, 2018). Nevertheless, for gene therapy to be fully effective, the ongoing expression of the diseased gene must also be considered, since the disease will keep progressing if the defective gene is still expressed. Moreover, the aforementioned retinal remodeling needs to be avoided or reversed, otherwise this therapy would have limited effect in the later stages of the disease.

At present, research efforts in outer retinal degenerations have focused on developing pharmacological therapies to delay photoreceptor loss and/or cellular therapies to replace lost photoreceptors (Dias et al., 2018). Nevertheless, other therapies such as intravitreal injections of anti-vascular endothelial growth factor drugs or steroids have been documented to halt the progression of the neovascular form of AMD (Bakri et al., 2019) and/or ameliorate the retinal diseases that course with oedema (Barquet, 2015). Although intravitreal injections are by now widely and repeatedly used, they are not exempt from adverse effects (Di Pierdomenico et al., 2016), a fact that could limit their therapeutic potential, especially when several injections are needed. It is therefore important to explore other therapeutic options that avoid repeated intraocular injections.

Promising therapeutic results have been achieved in animal models of retinal degeneration through the use of several therapies that include: (i) neurotrophic factors such as basic fibroblast growth factor 2, ciliary neurotrophic factor (CNTF) or pigment epithelium-derived factor (Di Pierdomenico et al., 2018; Valiente-Soriano et al., 2019, 2020b); (ii) anti-apoptotic, antioxidant and anti-inflammatory drugs (Appelbaum et al., 2017; Chan et al., 2020); (iii) microglial inhibitors such as minocycline (Di Pierdomenico et al., 2018); (iv) nutritional supplements like the amino acid taurine (Trouillet et al., 2018) or the polyphenol resveratrol (Wiedemann et al., 2018); (v) melanopsin gene therapy (De Silva et al., 2017); (vi) retinal prosthesis (Prevot et al., 2020); (vii) photoreceptor transplantation (Ortin-Martinez et al., 2017; Garita-Hernandez et al., 2019; Lorach et al., 2019); and (vii) stem cell therapy (see also next section) (Otani et al., 2002; Zaverucha-do-Valle et al., 2014; Di Pierdomenico et al., 2020a; Adak et al., 2021). However, in clinical trials, these treatments have not been shown to be capable of preventing, recovering or reversing retinal degeneration or ultimately preventing or reversing the devastating effects of retinal remodeling.

Stem cell therapy

Stem cell therapy, either in the form of stem cell-derived photoreceptors or retinal pigment epithelium (Davis et al., 2016; Ribeiro et al., 2021) or in the form of undifferentiated stem cells (Grant et al., 2002), appears to be at present possibly one of the most promising treatments for retinal degenerations. One of its main advantages is that a single treatment can be used for a wide variety of diseases regardless of their genetic background. When transplanted to the retina, stem cells derived from different sources (see below) could theoretically divide and differentiate into normal retinal cells and substitute the damaged or lost retinal cells (Jones et al., 2017; Shen, 2020). In general terms, the use of stem cells shows good safety profiles, although there have been two reports of severe proliferative vitreoretinopathy, and even retinal detachments after intravitreal injection of adipose tissue-derived stem cells (Kuriyan et al., 2017) or mesenchymal stem cells (MSCs) (Satarian et al., 2017). Nowadays, to date the largest clinical trials using intravitreal injections of bone marrow derived-mononuclear stem cells (BM-MNCs), subject of this review, have not reported any adverse events (NCT01560715, NCT01518127) (Siqueira et al., 2011, 2015b; Cotrim et al., 2020; Wang et al., 2020). Also, no appreciable adverse effects have been found in animal studies using xenotransplantation of BM-MNCs to the vitreal or subretinal space (Di Pierdomenico et al., 2020a). Whether these differences in the incidence of adverse effects are due to the nature of the transplanted cells and/or the routes of

administration are questions that remain to be investigated (Dias et al., 2018). Various types of stem cells have been identified in several easily accessible tissues such as bone marrow, blood, umbilical cord and adipose tissue (Jones et al., 2017; Shen, 2020; Singh et al., 2020). Thus, stem cells have been frequently used in regenerative therapies, as they are a source of neurotrophic and pro-survival factors, including brain-derived neurotrophic factor, CNTF, glial cell line-derived neurotrophic factor and basic fibroblast growth factor 2 (Adak et al., 2021) and they also have anti-gliotic (Di Pierdomenico et al., 2020a) and other effects (Millan-Rivero et al., 2018).

The largest amount of adult stem cells is found in the bone marrow (Shen, 2020). Since these cells can be harvested from the adult bone marrow, autologous personalized treatment is possible, which could facilitate the approval of this treatment when compared to other treatments based on other types of stem cells that could pose ethical issues or donor-matching problems. In systemic diseases compromising the function of bone marrow cells such as diabetes, allogeneic rather than autologous transplantation could also be used. Then, donor-matching should be used to avoid side effects, increase survival of transplanted cells and enhance the therapeutic effect of the transplants. Donor-matched allogeneic transplants are widely used standard procedures that should not pose any ethical problems to be approved for clinical studies.

The bone marrow aspirate contains two different types of stem cells: hematopoietic stem cells (HSCs) and MSCs (Singh et al., 2020). Each cell type is obtained with a different method of isolation, and show variable cell composition and properties. The whole fraction of BM-MNCs is usually obtained from the iliac crest by needle puncture aspiration and isolated by density gradient-based separation (Di Pierdomenico et al., 2020a). This BM-MNCs suspension contains MSCs and HSCs, in proportions ranging from 0.01-0.001% for MSCs and 0.5–5% for HSCs ($CD34^+$).

The aim of this review is to assess the state of the art of the treatment of diseases that course with photoreceptor degeneration with the transplantation of BM-MNCs.

Bone marrow-derived stem cells therapies for degenerative retinal disorders

In higher vertebrates, the central cavities of axial and long bones contain the bone marrow, the main tissue of new blood cell production after birth. Among all bone marrow cell components, HSCs, which are localized close to the endosteum bone surface and around the blood vessels, have self-renewal capacity and are responsible for the production of all mature blood cells (i.e., erythrocytes, platelets, granulocytes, lymphocytes and monocytes), a process called hematopoiesis. The HSCs are contained in the total fraction of BM-MNCs, usually express the CD34 surface marker and can be classified according to their expression of certain molecules into lineage positive (Lin^+) and lineage negative (Lin^-) subpopulations that represent different differentiation potential into the different blood cell subpopulations, but they can express also other molecules such as c-kit, CD133, or Sca-1 (see below). It is well known that maintenance of HSC functional capacities needs the support of a complex and specialized bone marrow microenvironment called "HSC niche" that is mainly composed by non-hematopoietic cell types such as supporting MSCs, adipocytes, fibroblasts, perivascular cells, vascular endothelial cells, osteoclasts and osteoblasts (Wei and Frenette, 2018), which greatly influences the equilibrium between HSC self-renewal and lineage-specific differentiation (Pinho and Frenette, 2019).

In the context of regenerative medicine and tissue engineering, the use of bone marrow aspirates or concentrates, which contain the different bone marrow cell subpopulations and other soluble molecules such as cytokines and growth factors, have been extensively used in the last years to promote tissue healing (Wilkinson et al., 2020). However, there is currently no scientific consensus on which specific bone marrow cell component is most appropriate to use in each preclinical or clinical situation. Thus, in animal studies therapeutic interventions have often used the whole BM-MNCs fraction containing all its cell subtypes (Zaverucha-do-Valle et al., 2014; Di Pierdomenico et al., 2020a), or fractions containing only some specific bone marrow cell subtypes, i.e., Lin^-, $CD34^+$, $c\text{-}Kit^+$, $CD133^+$ or $Sca\text{-}1^+$ HSCs (Otani et al., 2002; Moisseiev et al., 2016; Qi et al., 2017; Shao et al., 2018), endothelial precursor cells (Otani et al., 2002), or MSCs (Lucas-Ruiz et al., 2019), either purified by different selection methods (e.g., magnetic cell separation by depletion or enrichment using monoclonal antibodies) (Otani et al., 2002), or expanded in *ex vivo* cultures with or without addition of different growth factors (Lucas-Ruiz et al., 2019). In this context, two animal studies have shown beneficial effects of bone marrow-derived stem cells: Zaverucha-do-Valle et al. (2014) have documented that, although the whole BM-MNC fraction has a limited time survival in the rat vitreous after transplantation, these cells increase RGC survival and axonal outgrowth 14 days after optic nerve crush. Otani et al. (2002) have found that the intravitreal injection of bone marrow Lin^- subpopulation contributed to injury-associated retinal angiogenesis. Some of these cell-based approaches have been also used in clinical trials and most of them have shown a good safety profile (Singh et al., 2020; Wang et al., 2020).

MSCs are a heterogeneous population of adult stem cells that can be isolated from almost every organ and connective tissue of the body. The most commonly employed source for obtaining MSCs is also the bone marrow, and indeed MSCs are contained in the total fraction of BM-MNCs, but they are also abundant in other adult tissues such as adipose tissue, dental tissues and umbilical cord blood, as well as in perinatal and foetal tissues (i.e., amniotic membrane, amniotic fluid and Wharton jelly´s within the umbilical cord stroma) (Lim, 2017). MSCs have the ability to differentiate into cells of the mesodermal lineage, such as osteoblasts, adipocytes and chondrocytes, but also into different cell types from the ectodermic lineage such as epithelial cells and neurons. Moreover, MSCs do not express human leukocyte antigen-class II molecules and possess low immunogenicity, so MSCs isolated from human leukocyte antigen-mismatched unrelated donors can be transplanted into the recipient with no systemic immunosuppression and with no immune rejection. Remarkably, numerous studies in the last years have described the important immunomodulatory and neuroprotective properties of MSCs, which are mediated by direct cell-to-cell contacts and by secretion of soluble molecules with anti-inflammatory, i.e., prostaglandin E2 (PGE2), transforming growth factor, hepatocyte growth factor, nitric oxide, and heme-oxygenase, and neurotrophic properties, i.e., nerve growth factor, brain-derived neurotrophic factor and CNTF) (Uccelli et al., 2008; Millan-Rivero et al., 2018), being therefore considered as an optimal cell type for regenerative medicine strategies. On the other hand, bone marrow HSCs have a number of unique properties including self-renewal, differentiation into cells of three germ layers, paracrine trophic and immunosuppressive effects and a prominent pro-angiogenic and neuroregenerative potential (Bakondi et al., 2009; Kamei et al., 2013; Li, 2013; Park et al., 2017). However, the use of specific bone marrow HSC subpopulations as grafts is less common in regenerative medicine strategies than the non-purified whole BM-MNCs fraction, because obtaining highly purified HSCs in sufficient quantities implies additional purification steps and increased costs. Moreover, the whole BM-MNCs fraction contains not only a mixture of HSCs at different stages of maturation, but also MSCs, lymphoid and myeloid cells, hemangioblasts and non-hematopoietic precursor cells, including those from endothelial origin, which are able of forming new blood vessels *in vitro* and *in vivo* and to differentiate into a variety of non-endothelial cell types including microglia and epithelium (Otani et al., 2002; Kucia et al., 2005). However, although there is a large number of studies demonstrating the beneficial effects of BM-MNCs transplants in ocular affectations, there are no comparative studies between the administration of this cell source, bone marrow HSCs and bone marrow MSCs and, therefore, it remains to be elucidated which of these yields the best therapeutic response.

Routes of cell delivery

Numerous animal studies have reported that bone-marrow derived cells injected into the eye, both intravitreally and subretinally, may be able to integrate into the retinas and replace dead or defective cells, or release different growth factors leading to increased cell survival, growth and function of resident retinal cells (Wang et al., 2010; Moisseiev et al., 2016), and have also showed in clinical trials some clinical improvement in visual function and absence of severe side effects (Siqueira et al., 2011). The administration route may have a significant impact in the subsequent therapeutic outcomes. Accordingly, intravitreal administration (**Figure 1A**) of bone marrow-derived stem cells into the vitreous, and thus close to the inner retina, has been shown to delay axotomy-induced RGC loss and to promote neuroregeneration by inducing axon regeneration through increased secretion of trophic factors (Zaverucha-do-Valle et al., 2014). Otherwise, the subretinal administration route (**Figure 1B**), that delivers bone marrow-derived stem cells closer to the outer retinal layers and thus to photoreceptors, has been described to possess a more direct therapeutic effects on the subretinal space,

being therefore considered as a suitable therapeutic alternative for external vitreoretinal disorders (Peng et al., 2017). However, both these routes of administration may occasionally produce some unwanted side events, such as retinal detachment, disturbance of the structures of the retina, intraocular inflammation or ocular hemorrhage (Falavarjani and Nguyen, 2013). Other reported, although less employed, cell delivery strategies include epiretinal, subretinal, retrobulbar and sub-tenon administration and have showed also beneficial therapeutic outcomes in some preclinical and clinical situations (Guan et al., 2013; Tzameret et al., 2015; Weiss and Levy, 2018, 2020, 2021).

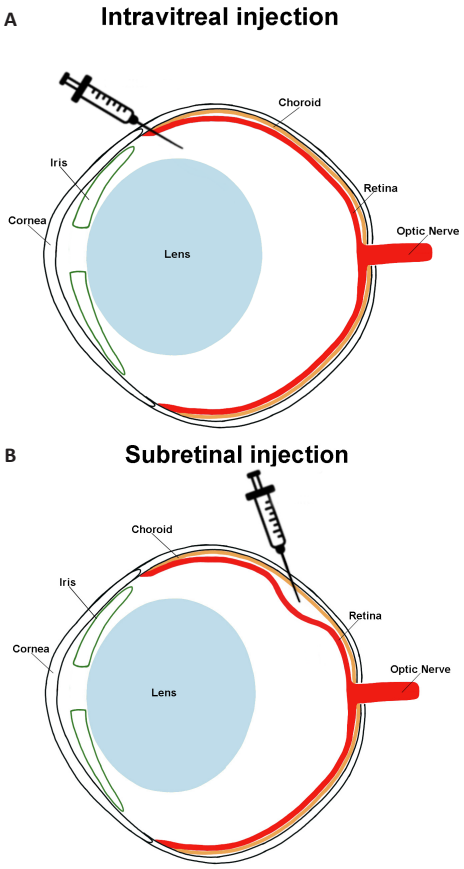

Figure 1 | **Common routes of cell delivery to the rat retina.**
Drawings depicting the procedure for performing intravitreal (A) and subretinal (B) injections in rats.

Mechanisms of action of bone marrow-derived cells transplantation

Reported mechanisms of action of allogeneic bone marrow-derived stem cell transplantation include (**Figure 2**): (i) cell replacement of degenerated retinal cells due to their trans-differentiation properties (Tomita et al., 2002; Chan-Ling et al., 2006); (ii) induction of retinal cell survival and differentiation through their paracrine effects: secretion of growth and neurotrophic factors, i.e., nerve growth factor, brain-derived neurotrophic factor, CNTF, glial cell line-derived neurotrophic factor, transforming growth factor, stem cell growth factor, platelet-derived growth factor, epidermal growth factor, fibroblast growth factor and insulin-like growth factor (Wang et al., 2010; Millan-Rivero et al., 2018); (iii) stimulation of retinal neovascularization by increasing the secretion of pro-angiogenic factors, mainly vascular endothelial growth factor, and thus favoring retinal microhemodynamics (Grant et al., 2002; Otani et al., 2002; Millan-Rivero et al., 2018); (iv) protection of photoreceptors through up-regulation of anti-apoptotic genes (i.e., *Mad1*, *Yy-1*, *Crybb2*, *Cryaa* and *Cryba1* genes) and prevention of oxidative stress damage (Otani et al., 2004); (v) promotion of neuronal rescue by establishing new synaptic connections (Otani et al., 2004); (vi) modulation of host immunological responses: secretion of anti-inflammatory molecules (i.e., transforming growth factor, prostaglandin E_2 (PGE_2), PGE_2 receptor (PGE_2R), nitric oxide, interferon and thrombospondin-1 and down-regulation of pro-inflammatory cytokines (i.e., tumor necrosis factor, interleukin-1 and interferon (Millan-Rivero et al., 2018; Hermankova et al., 2019); (vii) activation of ocular stem/progenitor cells (Crisostomo et al., 2008); and (viii) release of extracellular vesicles and exosomes, which contain proteins, mRNA, microRNA, lipids, ribosomes and mitochondria, allowing cell-to-cell communication (Mead and Tomarev, 2017; Seyedrazizadeh et al., 2020). Among all these mechanisms of action, the secretion by bone-marrow derived cells of a variety of molecules with neurotrophic properties has been the most consistent finding in numerous of the above-mentioned studies. These effects have been observed both after intravitreal transplantation of cells into the retina (Millan-Rivero et al., 2018) or even after their intravenous infusion (Wang et al., 2010). The BM-MNCs however, do not seem to integrate within the retina and replace the degenerated cells. As noted above (see section *Bone marrow-derived stem cells therapies for degenerative retinal disorders*), the whole BM-MNCs fraction contains a heterogeneous population of cells and, although it contains a small proportion of pluripotential cells that could in theory migrate into the retina, the majority tend to differentiate into mesodermal tissues (Fafian-Labora et al., 2015; Wang et al., 2020). Therefore, it is widely accepted that the adult retina is not as receptive to the integration of donor cells as previously thought and that the production of neurotrophic factors by the BM-MNCs is probably the main mechanism of action of the transplants and not cell integration and differentiation.

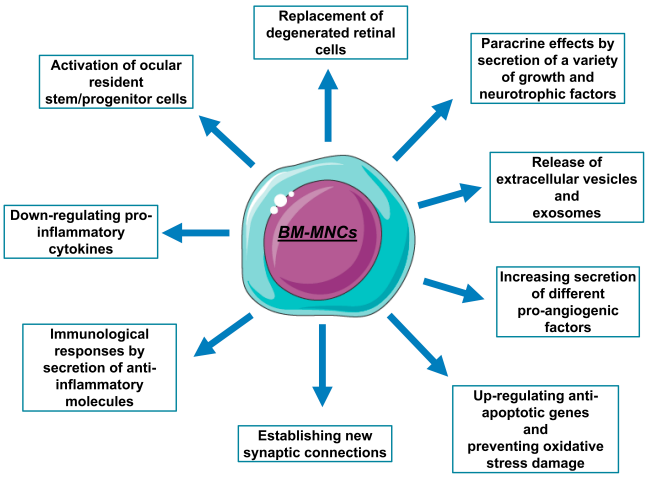

Figure 2 | **Possible mechanisms of action of bone marrow derived-mononuclear stem cells (BM-MNCs).**
Drawing showing a BM-MNC and its possible mechanisms of action. Modified using https://smart.servier.com/.

There is numerous evidence suggesting that bone marrow-derived stem cells could represent a suitable cell therapy product for ocular diseases associated with retinal gliosis and photoreceptor degeneration such as diabetic retinopathy, retinal ischemia, optic neuritis, AMD and RP (Siqueira et al., 2013; Cotrim et al., 2017; Ding et al., 2017; Satarian et al., 2017; Di Pierdomenico et al., 2020a), which have led to the implementation of different phase I and phase II clinical trials aimed at evaluating safety and efficacy of the above commented cell types.

BM-MNC therapy in retinal degenerations

To date, there is no cure or effective treatment for degenerative retinal diseases caused by different aetiologies leading to visual impairment and even irreversible blindness. Most of the currently available treatments are based on intraocular injection of neurotrophic factors (Di Pierdomenico et al., 2018; Dias et al., 2018; Valiente-Soriano et al., 2020a) or anti-angiogenic agents (Di Pierdomenico et al., 2016) in the early stages of degeneration (see above). New treatments such as gene therapy, optogenetics or cell replacement are being investigated (Dias et al., 2018; McClements et al., 2020), the latter focusing on the more advanced stages of degeneration. However, until more information is available about the outcomes of these new therapies, sustained neuroprotection seems to be, among others, one of the potential strategies applicable to the different types of degenerative diseases of the retina, mainly in their early stages. Among the different strategies, stem cells represent one of the most promising options. Its paracrine trophic effect, which includes the sustained secretion of immunomodulatory, neurotrophic, and anti-angiogenic factors (see above) (Park et al.,

2017), should lead to neuroprotective effect comparable to the achieved with neurotrophic factors. What is interesting is that, if injected cells could survive long-term in the retina, these beneficial effects could be more prolonged over time, as they could potentially be an enduring source of neuroprotection. Moreover, gene therapy could elicit long-term expression of neurotrophic factors by the transplanted cells.

Preclinical and Clinical Studies Conducted with Bone Marrow-Derived Mononuclear Stem Cells

Preclinical trials

An early preclinical study suggested that intravitreally injected BM-MNCs had the ability to incorporate and differentiate into retinal neural cells in a rat model of retinal degeneration (Tomita et al., 2002). Recent preclinical studies have shown that the allogeneic intravitreal transplantation of BM-MNCs from healthy donors increases RGC survival and axonal outgrowth in an experimental model of optic nerve crush (Zaverucha-do-Valle et al., 2014). However, the effect on RGC survival was transitory and only lasted up to 14 days after the transplantation. Attempts to improve it with a second injection were also unsuccessful (Zaverucha-do-Valle et al., 2014). A more recent work from our group studied the transplantation of adult human BM-MNCs into two rat models of inherited retinal degeneration with different aetiologies (Di Pierdomenico et al., 2020a). In this work, we suggested that although the xenotransplant of human BM-MNCs did not achieve a higher photoreceptor survival in the degenerating rat retina, it showed a promising anti-gliotic effect which encourages further study. Specifically, the proposed anti-gliotic effect could be an important key in the treatment of photoreceptor degenerations (Di Pierdomenico et al., 2020a), since it could prevent the formation of the glial seal (Garcia-Ayuso et al., 2019; Di Pierdomenico et al., 2020b). Therefore, according to preclinical studies, BM-MNCs could represent a hopeful therapy for the treatment of these and other retinal diseases and deserve further studies. It is important that the delivery routes used in the trials of BM-MNCs into the retina have a reasonably low risk so that the safety and efficacy of the cells can be adequately assessed (see above). Di Pierdomenico et al. (2016) explored the two main routes of intraocular delivery, intravitreal and subretinal injections, both having their advantages and concerns (see above), and showed that both routes are feasible and have similar outcomes (Di Pierdomenico et al., 2020a), even when the intravitreal injections release cells into the vitreous, close to the inner retina, and subretinal injections deliver them into the subretinal space, closer to the target population: the photoreceptors. Unfortunately, animal studies have not corroborated the initial idea that BM-MNCs could integrate and differentiate into retinal cells (Tomita et al., 2002; Park et al., 2017).

The effectiveness of BM-MNCs transplantation to the eye could be related to the number of $CD34^+$ HSCs present in the suspension (Moisseiev et al., 2016; Singh et al., 2020). Because their neuroprotective effect may depend on their paracrine trophic effect (Park et al., 2017), it would not depend on the integration and differentiation of the cells. However, a recently published work has proposed that these cells can integrate into the retinal surface and the retinal vasculature following their intravitreal injection (Yazdanyar et al., 2020), but failed to show an integration in the outer retinal layers, the target of the treatment in photoreceptor degenerations. Nonetheless, the fact that the neuroprotective effect of these cells relies on their paracrine trophic effect, favors that this therapy can have an impact on a wider range of diseases as they could act on several cell populations at once.

Clinical trials

Based on the early promising results on preclinical studies, two pilot clinical studies were designed to study the autologous intravitreal injection of BM-MNCs in eyes with inherited and/or acquired retinal degeneration (Jonas et al., 2010; Siqueira et al., 2011). Both studies concluded that the autologous intravitreal injection of BM-MNCs is a feasible and safe technique, but none of them were able to document a relevant improvement in visual acuity (Jonas et al., 2010; Siqueira et al., 2011). However, the results could be biased because the participants in one of the studies were at the end stage of their ocular diseases (Jonas et al., 2010) and both studies transplanted autologous cells, whose effect could be limited as they may have the same genetic defects of the retinal neurons (Siqueira et al., 2011). Two more recent studies have shown resolution of cystoid macular oedema associated with RP (Siqueira et al., 2013) and resolution of macular oedema and improvement of visual acuity in patients with diabetic retinopathy and retinal vein occlusion (Siqueira et al., 2015a) following the intravitreal injection of autologous BM-MNCs. This group has also showed an improvement in quality of life in 20 patients with RP using the same treatment, although this improvement could only be confirmed during the first 3 months following the intravitreal injection and it could have been influenced by a subjective psychological component (Siqueira et al., 2015b). More recently, the same group documented that the intravitreal injection of autologous BM-MNCs achieved a significant improvement in visual function in ten patients with atrophic AMD and no adverse events (Cotrim et al., 2017). The Stem Cell Ophthalmology Treatment Study (SCOTS) and its follow-up Stem Cell Ophthalmology Treatment Study (SCOTS 2) are registered clinical trials that have studied the effect of autologous BM-MNCs transplantation in different retinal and optic nerve diseases and have used various ocular routes of administration depending on the disease and its severity (Weiss and Levy, 2020, 2021). These studies have shown after transplantation improvements in visual acuity in diseases such as Stargardt's disease (Weiss and Levy, 2021), RP (Weiss and Levy, 2018) and dry AMD (Weiss and Levy, 2020). However, the SCOTS were uncontrolled studies, as the injections were applied bilaterally and there were no untreated eyes, and therefore it is difficult to draw conclusions from them.

To date, there are 16 registered clinical trials (http://clinicaltrials.gov) exploring the effect of bone marrow-derived stem cells in different retinal degenerations, 10 of them specifically exploring the effect of BM-MNCs (**Table 1**). However, not many results have been published so far with the mononuclear cell fraction. Interestingly, clinical trials generally use autologous transplantation in older patients, which may limit the success of the therapy as aging could impair the paracrine trophic effect of BM-MNCs (Yeganeh et al., 2021), while preclinical trials often use allogeneic transplantation from young donors.

It is important to note that the different types of cells harvested from bone marrow have been referred to generically as "bone marrow stem cells" in different studies, thereby not always clearly differentiating between the different types of cells isolated. This requires a review of the methodology of cell isolation to determine which type of bone marrow stem cell was used, and this fact was not always correctly clarified in the published studies. The main outcomes of the clinical and preclinical studies are summarized in **Table 2**.

Concluding Remarks and Future Directions

Photoreceptor degenerations are currently a leading cause of irreversible and untreatable vision loss. These incurable diseases therefore represent one of the greatest challenges of ophthalmological research. In the field of photoreceptor degenerations, investigations have traditionally focused on finding therapies to replace dead photoreceptors, such as photoreceptor prostheses or transplantation. However, it is now widely accepted that all diseases coursing with photoreceptor degeneration have a common end: the complete remodeling of the retina and the subsequent alteration of the inner retina (Garcia-Ayuso et al., 2010; Garcia-Ayuso et al., 2014; Garcia-Ayuso et al., 2019; Pfeiffer et al., 2020), which would compromise the outcomes of these treatments. Therefore, it is important to stop or slow down photoreceptor degeneration in its early stages, as this would increase the window of time in which the above-mentioned treatments aimed at replacing photoreceptors could be successful.

The BM-MNC transplantation may have, among other treatments, potential therapeutic benefits as it could be capable of slowing down photoreceptor degenerations and, therefore, improve the patients' quality of life (Siqueira et al., 2015b). The ability of these cells to substitute the lost photoreceptors is under debate, but their potential paracrine effect (Park et al., 2017) suggests for them a wide range of clinical applications since it could potentially impact on several cell types at once. This is of particular interest as retinal diseases can involve different cell types in the retina or begin in one cell and then affect adjacent cells. In addition, the use of BM-MNCs allows autologous or allogeneic transplantation without the need of immunosuppression. Furthermore, these cells are obtained from adult tissue so there would be no ethical concerns for the use of embryonic tissue, as it may be the case with the use of other stem cell types. Finally, the intraocular injection of BM-MNCs could enhance the outcomes of other treatments aimed at stopping or replacing dead photoreceptors, and therefore future lines of work

Table 1 | **Summary of the current clinical trials designed to evaluate the neuroprotective effect of BM-MNCs in retinal degenerations**

Identifier	Disease	Administration route	Status	Location
NCT01068561	Retinitis pigmentosa	Intravitreal injection	Completed phase I	University of Sao Paulo (Sao Paulo, Brazil)
NCT02280135	Retinitis pigmentosa	Intravitreal injection	Completed phase I	Clinical University Hospital Virgen de la Arrixaca (Murcia, Spain)
NCT01560715	Retinitis pigmentosa	Intravitreal injection	Completed phase II	University of Sao Paulo (Sao Paulo, Brazil)
NCT01518842	Ischemia	Intravitreal injection	Unknown	University of Sao Paulo (Sao Paulo, Brazil)
NCT01914913	Retinitis pigmentosa	Not specified	Completed phase I/II	Chaitanya Hospital, Pune (India)
NCT00550498	Behcet's syndrome	Intravitreal injection	Terminated*	Rheumatology Research Center, Behcet's Disease Unit (Shariati Hospital) Tehran University of Medical Sciences (Tehran, Iran)
NCT01518127	Age-related macular degeneration	Intravitreal injection	Completed phase I/II	University of Sao Paulo (Sao Paulo, Brazil)
NCT01834079	Ocular atrophy	Intrathecal injection	Completed phase I/II	Chaitanya Hospital, Pune (India)
NCT01920867	Age-related macular degeneration Inherited retinal dystrophy Optic nerve disease Glaucoma	Retrobulbar Subtenon Intravitreal Intravenous and/or Intraocular injections	Not applicable (Enrolling by invitation)	MD Stem Cells (Westport, Connecticut, United States)
NCT03011541	Age-related macular degeneration Retinitis pigmentosa Stargardt´s disease Optic neuropathy Nonarteritic ischemic optic Neuropathy Optic atrophy Optic nerve disease Glaucoma Leber´s hereditary optic Neuropathy	Retrobulbar Subtenon Intravitreal Intravenous and/or Intraocular injections	Not applicable (Recruiting)	MD Stem Cells (Westport, Connecticut, United States)

*No improvement obtained in three cases. Retinal detachment observed in two cases. NCT: National Clinical Trial.

Table 2 | **Summary of preclinical and clinical studies conducted with BM-MNCs**

Reference	Type of study	Disease/Animal models	Cells	Administration routes	Main findings
Di Pierdomenico et al., 2020a	Preclinic	P23H-1 rats RCS rats	BM-MNCs	Intravitreal injection Subretinal injection	Decreased retinal gliosis
Moisseiev et al., 2016	Preclinic	Retinal degeneration C3H/HeJ mice (rd1)	BM-MNCs $CD34^+$ cells	Intravitreal injection	Potential trophic regenerative effects
Park et al., 2014	Preclinic	Ischemia-reperfusion	GMP-grade BM-derived $CD34^+$ cells	Intravitreal injection	Good long-term transplant tolerance
Zaverucha-do-Valle et al., 2014	Preclinic	Optic nerve crush	BM-MNCs	Intravitreal injection	Promotion of neuroregeneration
Zaverucha-do-Valle et al., 2011	Preclinic	Optic nerve crush	BM-MNCs	Intravitreal injection	Neuroprotection and neuroregeneration mediated by FGF-2
Tomita et al., 2002	Preclinic	Mechanical retinal damage	BM-MNCs	Intravitreal injection	Differentiation into retinal neural cells Repair of damaged retinal cells
Weiss and Levy, 2021	Clinical Trial SCOTS	Stargardt Disease	BM-MNCs	Stem Cell Ophthalmology Treatment Study (SCOTS): Different combinations of retrobulbar, subtenon, intravitreal, intra-optic nerve, subretinal and intravenous injection	Improved vision (visual acuity) or stability
Weiss and Levy, 2020	Clinical Trial SCOTS	Age-Related Macular Degeneration	BM-MNCs	SCOTS	Improvement of average visual acuity or stability
Weiss and Levy, 2018	Clinical Trial SCOTS	Retinitis Pigmentosa	BM-MNCs	SCOTS	Improvement of average visual acuity or stability
Cotrim et al., 2017	Clinical Trial	Atrophic age-related macular degeneration	BM-MNCs $CD34^+$ cells	Intravitreal injection	Improved visual acuity Improved macular sensitivity threshold
Siqueira et al., 2015a	Clinical Trial	Diabetic retinopathy Central retinal vein occlusion	BM-MNCs	Intravitreal injection	Decreased macular oedema Improved retinal function Improved visual acuity
Siqueira et al., 2013	Clinical Trial	Retinitis pigmentosa	BM-MNCs	Intravitreal injection	Resolution of macular oedema Improvement in visual acuity Improvement in macular sensitivity
Siqueira et al., 2011	Clinical Trial	Retinitis pigmentosa Cone-rod dystrophy Diabetic Retinopathy	BM-MNCs	Intravitreal injection	One line improvement in best-corrected visual acuity No detectable structural or functional toxicity over a period of 10 months
Jonas et al., 2010	Clinical Trial	Age-related macular degeneration Glaucoma	BM-MNCs	Intravitreal injection	Technical feasibility of intravitreal injection

BM: Bone marrow; BM-MNCs: bone marrow derived-mononuclear stem cells; CD: cluster of differentiation; FGF-2: basic fibroblastic growth factor-2; GMP: good manufacturing practices; RCS: Royal College of Surgeons; SCOTS: Stem Cell Ophthalmology Treatment Study.

could combine this treatment with other systemic or intraocular treatments. Moreover, we have documented that intravitreal and subretinal xenotransplantation of BM-MNCs in immunosuppressed animals has beneficial therapeutic effects, particularly an anti-gliotic effect, in two models of inherited photoreceptor degenerations with different aetiologies (Di Pierdomenico et al., 2020a). We believe that this effect could be increased if autologous or allogeneic transplantation is used. We plan to continue this research work in order to improve the results of the transplants and to find out how they ultimately attain their beneficial effects. However, several questions remain unanswered: (i) could allogeneic or autologous transplant accomplish the integration and differentiation of transplants cells thus achieving higher neuroprotective effects? (ii) Could the transplant route (intravitreal or subretinal) influence these outcomes? (iii) Could BM-MNCs transplantation improve the results of other treatments aimed at replacing photoreceptors, such as retinal prostheses or photoreceptor transplants? Finally, investigators and clinicians should keep in mind that there are many types of stem cells and that for their use, it is mandatory to follow the regulations and also obtain the approval of the ethics committees of their institutions.

In summary, BM-MNC transplants to the retina may have beneficious effects that could be accomplished through different action mechanisms, although the predominant effect seems to be a paracrine neurotrophic action. This may explain why they have positive actions in different ocular diseases irrespective of their pathophysiology. Thus, we suggest that this transplantation could be used in combination with other therapies to increase their therapeutic effects. However, there remain some unanswered questions about BM-MNCs transplantation into the eye and further research will be necessary to shed light on this field and specially to elucidate the survival, mechanism of action and therapeutic potential of these cells.

Author contributions: *DGA and MPVP devised the project and the main conceptual ideas. DGA wrote the manuscript with support from MPVP, JDP, DGB and MVS. JDP and DGB helped with the literature search. All authors contributed to the interpretation of the results, discussed the results and contributed to the final version of the manuscript.*
Conflicts of interest: *The authors declare no conflicts of interest.*
Editor note: MVS is an Editorial Board member of Glioma. He was blinded from reviewing or making decisions on the manuscript. The article was subject to the journal's standard procedures, with peer review handled independently of this Editorial Board member and their research groups.
Availability of data and materials: *All data generated or analyzed during this study are included in this published article and its supplementary information files.*

Open peer reviewer: *Juan Lopez-Costa, Institute of Cell Biology and Neuroscience, Argentina.*
Additional file:
Additional file 1: *Open peer review report 1.*

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P-Reviewer: Lopez-Costa J; C-Editors: Zhao M, Zhao LJ, Qiu Y; T-Editor: Jia Y