The retinal ganglion cells (RGCs) are not able to regenerate following optic nerve injury resulting in an irreversible vision loss in patients with optic neuropathies including glaucoma. Recent findings in ocular regeneration have opened promising avenues to apply stem cell-based modalities to restore vision in progressive optic neuropathies. Stem cell-based therapies can help to improve retinal regeneration by solving two major problems: (1) by preventing secondary degeneration of RGCs and preserving the remaining vision, and (2) by replacing degenerated RGCs and promoting RGC axon regeneration in the damaged area. The first approach, known as neuroprotective therapy, uses stem cells incorporated into the degenerating retina with an aim to offer a nourishing environment for damaged RGCs resulting in anatomic and functional improvement. The second approach, known as RGC replacement therapy, ultimately aims at replacing the damaged RGCs with healthy RGCs or RGC precursors (Gao et al., 2012; Fu et al., 2019) in order to restore the visual function. Both approaches are graphically represented in Figure 1. The implementation of cell replacement as a therapeutic approach requires successful generation of clinically safe and functional RGCs in an environment where the transplants survive, appropriately integrate and engraft, as well as establish neurites within the hosts' retina and direct consequent axons towards the relevant regions in the brain (Behtaj et al., 2020). In this work, we discuss the challenges that are required to be addressed prior to the implementation of stem cell-based therapies in clinical practice and, suggest potential solutions to overcome the current limitations.

**Stem cell-based RGC neuroprotection strategies:** Neuroprotection aims to prevent degeneration of neurons and slow down the progression of vision loss in neurodegenerative diseases such as glaucoma. The application of neurotrophic factors (NTFs) such as brain-derived-, ciliary-, glial cell-derived and nerve growth factor has been a focus of recent investigations as a prominent mean of neuroprotective strategy for it is known that NTFs prevent uncontrolled RGCs loss and aid to the cells survivability. However, the effectiveness of the neuroprotective approach is limited by a relatively short half-life, insufficient permeability and poor concentrations of NTFs in target RGCs. The use of stem cells as an intraocular slow-release delivery vehicles with the ability of sustainable and multiple NTF-secreting is release delivery vehicles with the ability of neuroprotective strategy for it is known that both anti-inflammatory cytokines and NTFs secreted by MSCs have been shown to afford neuroprotective capability to enhance RGC survival (Osborne et al., 2018; Boia et al., 2020). However, much uncertainty still exists about the relationship between the populations, sources and types of MSCs and their optimal neuroprotective impacts. Despite the positive results, the use of MSCs in animal models by intravitreal MSC injection, this treatment when applied to human subjects yielded limited success (Borkowska-Kuczkowska et al., 2019). One of the most significant drawbacks of using MSCs is that these cells are not able to penetrate to the ganglion cell layer thoroughly and, generally remain attached to the vitreous cavity and the inner limiting membrane (Guymer et al., 2019). The recently introduced cell-free stem cell therapy, which is focused on the application of microvesicles and exosomes released by MSCs, especially bone marrow-derived mesenchymal stem cells, as a novel optical neuropathic treatment modality, may be able to reduce the risks associated with the use of conventional stem cell therapies, specifically, the risk of retinal detachment (Borkowska-Kuczkowska et al., 2019). Applications in which recipient neurons are able to receive genomic material, including messenger ribonucleic acids (mRNAs) and micro ribonucleic acids (miRNAs), transported by these small extracellular vesicles may lead to the activation of target signals and facilitate the re-establishment of intercellular communications. It is important to point out that, protecting RGCs in clinical applications using exosomes derived from bone marrow-derived mesenchymal stem cells requires an efficient delivery of miRNAs, which are responsible for the exosome-mediated neuroprotection (Mead and Tomarev, 2018).

At present, the cell-free stem cell therapy is still at an early stage of development and requires further extensive pre-clinical and clinical studies. Studded with more than forty different sub-types do not display a unique morphological appearance, as well as other surface antigens such as CD184 and CD171, are expressed by RGCs and broadly used in research as potential markers for RGC or RGC precursors. However, RGCs with more than forty different sub-types do not display a unique morphological appearance or any specific electrophysiological properties. Therefore, firstly, quality-control standards that can be applied towards definitive classification of RGC sub-types need to be developed, and secondly, based on the known RGC sub-type characteristics, definitive markers, which can be applied to distinguish in-vitro generated RGCs from other neurons, have to be identified and developed as well. Another area of consideration is that the ratio of different RGC sub-types for specific RGC replacement therapies needs to be established in order to facilitate successful RGC transplantation (Miltner and La Torre, 2018; Behtaj et al., 2020).

A promising concept in generating bona fide RGCs is currently offered by CRISPR (clustered regularly interspaced short palindromic repeats), Cas9 (or ‘CRISPR-associated protein 9’) and TALEN® (transcription activator-like effector nucleases) strategies for gene editing tools, which are faster, less expensive and more accurate than conventional techniques of editing deoxyribonucleic acid (DNA) and
Conclusions: Recent research findings focused on the optic nerve neuroprotection have shown that solutions offered by stem cell-based neuroprotective therapies can be highly promising in preventing RGCs degeneration and in preserving the remaining vision. As a result, the use of MSCs owing to their unique properties has been shown to enhance neuroprotective capability and increase RGCs survival. However, a limited capacity of MSCs to effectively penetrate into the ganglion cell layer remains one of the main challenges in the successful application of these stem cells in RGC neuroprotection. The cell-free stem cell therapy has also shown a potential to become a highly efficient stem cell-based neuroprotective protocol in the near future. Before long, stem cell-based RGC transplantation therapies and other modalities could have a widespread clinical application after subsequent neuroprotection treatments, when it has been decisively determined that full RGCs replacement is justified and approved. In this work, an overview of the challenging road ahead to bring the bench results of stem cell-based therapies to the clinic has been provided, highlighting an absence of efficient differentiation protocols for producing clinically safe RGCs in large quantities, as well as the lack of a unique fingerprint that could help to distinguish RGCs from other types of neurons. The evidence shows that genome editing offered by CRISPR-Cas9 and TALEN® tools holds the potential in generating bona fide RGCs.

Although, while much of the work remains to overcome the aforementioned challenges, the limitations which we alluded to should not detract from recognising the importance of attained intermediate research outcomes, which pave the way to the future conclusive and comprehensive research solutions.

The dream of restoring the visual function in patients with progressive optic neuropathies can be fulfilled by utilising a collaborative effort in which applied pharmacological, bioengineering and/or gene therapy treatment methods have been carefully combined. This combination can offer a consolidated and precise solution of carefully targeted therapies aided by the therapeutic functions of stem cells.

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