Stem cell therapy in retinal diseases?

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The term evidence based medicine (EBM) was first published in 1992, with the term defined in 1996 using a definition that is still widely used today: "The conscientious, explicit and judicious use of current best evidence about individual patient care." The practice of EBM allows the integration of available evidence from systematic research, specifically clinical research, and the best clinical judgment of the clinician[1,2].

EBM is defined as the link between scientific research and good clinical practice[3,4]. In other words, EBM trials use existing scientific data with good internal and external validity, to enforce the results in clinical practice. Evidence in this context is related to the effectiveness, efficiency, efficacy and safety of treatment. Effectiveness refers to how treatment functions in real-world conditions, efficiency to cheap and affordable treatment for patients, efficacy is how the treatment works in conditions of the ideal world, and safety means that an intervention is consistent and unlikely to cause any adverse effects[5]. A study with high internal validity must comply with these characteristics[6].

The concept of stem cell therapy has been advocated for more than 10 years and there has been a growing number of clinical reports documenting the use of stem cell therapies in humans in particular in respect to myocardial infarction[7-9].

In respect to ophthalmology, several important cell types in the eye have little, if any, capacity for endogenous regeneration. As a result the only viable treatment option for patients with hereditary disorders that involve the loss of such cells is some type of cell replacement therapy. Although the replacement of highly differentiated cells, such as photoreceptors, poses challenges, a number of recent experiments suggest that the use of stem cells to achieve this goal is now feasible[10].

Distinct stem cell types have been established from embryos and identified in fetal tissues and umbilical cord blood as well as in specific niches in many adult mammalian tissues and organs such as in the bone marrow, brain, skin, eyes, heart, kidneys, lungs, gastrointestinal tract, pancreas, liver, breast, ovaries, and prostate gland[11].

Stem cell-based therapy has been tested in animal models for several diseases including neurodegenerative disorders, such as Parkinson disease, spinal cord injury, and multiple sclerosis. Replacing lost neurons which have not been physiologically replaced is pivotal to therapeutic success.

Stem-cell therapy has the potential to treat a wide range of retinal diseases. The neuroretina is a complex structure whose health depends on blood vessels and retinal pigment epithelium (RPE), each of which is affected differently in the spectrum of retinal disease. Therefore, three distinct cell types are conceivable targets for future cell therapy in the retina: the neuroretina (photoreceptors, bipolar cells, ganglion cells and glial cells), RPE and vascular endothelial cells. Depending on the type of retina disease, different cell replacement strategies will need to be developed[12].

Degeneration of neural cells in the retina is a hallmark of such widespread ocular diseases as age-related macular degeneration (AMD) and retinitis pigmentosa (RP). In these cases the loss of photoreceptors that occurs as a primary event (as in RP) or secondary to loss of retinal-pigment epithelium (in AMD) leads to blindness[11,13,14].

Preclinical studies

Several experimental studies in animals have shown the potential of stem cell use to treat retinal disease (Table 1). Routes of administration of the cells frequently used in these studies were intravitreal, subretinal (the cells are injected under the retina with vitrectomy surgery) and systemic (intravenous injection).

Different types of stem cells such as hematopoietic, mesenchymal, retinal progenitor cells, embryonic stem cells and induced pluripotent stem cells were tested[28-32].

Different models of injuries including retinal ischemia induced by increased intraocular pressure, the induction of inflammatory lesions in the retina using laser photocoagulation

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Table 1 - Stem cell use to treat retinal disease in animal models

Study	Experimental model	Route used	Type and source of cells	Results
Otani et al.[15]	Mice with retinal degenerative disease	Intravitreal transplantation	Adult bone marrow derived lineage-negative hematopoietic stem cells	Stem cells (Lin- HSCs) containing endothelial precursors stabilizes and rescues retinal blood vessels that would ordinarily degenerate completely, a dramatic neurotrophic rescue effect is also observed. Electroretinogram recordings were observed in rescued mice at times when they are never observed in control-treated or untreated eyes.
Wang et al.[16]	Retinitis pigmentosa	Systemic	Pluripotent bone marrow-derived mesenchymal stem cells	Both rod and cone photoreceptors were preserved (5-6 cells thick) at the time when control animal had just a single layer of photoreceptors remaining; Visual function was significantly preserved compared with controls
Tomita et al.[17]	Retinas mechanically injured using a hooked needle	Intravitreal transplantation	Stem cell-enriched bone marrow cells	The stem cell-enriched bone marrow cells had been incorporated and had differentiated into retinal neural cells in the injured retina. They had accumulated mainly in the outer nuclear layer around the injury sites
Zhang & Wang[18]	Light-damaged retinal structure	Subretinal space	Bone marrow mesenchymal stem cells	Apoptotic outer nuclear layer cells were significantly reduced in the bone marrow mesenchymal stem cell transplantation group than in the injected phosphate-buffer solution group and expressed brain-derived neurotrophic factor
Tomita et al.[19]	Rhodopsin knockout mice	Intravitreal transplantation	Bone marrow-derived stem cells	This study shows that retinal progenitor cells are likely to be a preferred cell type for retinal transplantation studies compared to marrow stromal cells (MSCs). However, MSCs may remain an attractive candidate for autologous transplantation.
Meyer et al.[20]	Retinal degeneration	Intravitreal transplantation	Embryonic stem cells	Donor cells had been incorporated into most layers of the retina, where they resembled retinal neurons in terms of morphology, location in the retina, and expression of cell type-specific marker proteins. The presence of transplanted donor cells was also accompanied by enhanced survival of host retinal neurons, particularly photoreceptors
Siqueira et al.[21]	Chorioretinal injuries caused by laser red diode 670N-M	Intravitreal transplantation	Bone marrow-derived stem cells	The sites of retinal laser injury showed greater preservation of the histological structure of the stem cell group compared with the control group
Wang et al.[22]	Mice with laser-induced retinal injury	Intravitreal transplantation	Bone marrow-derived stem cells	Bone marrow-derived stem cells (BMSCs) participate in the repair of retinal lesions by differentiating into retinal cells. Intravitreal transplantation of BMSCs is a potential treatment for laser-induced retinal trauma.
Johnson et al.[23]	Glaucoma	Intravitreal transplantation	Bone marrow-derived mesenchymal stem cell	Bone marrow-derived mesenchymal stem cell transplantation resulted in a statistically significant increase in overall retinal ganglion cell (RGC) axon survival and a significant decrease in the rate of RGC axon loss normalized to cumulative intraocular pressure exposure
Castanheira et al.[24]	Laser damage	Intravitreal transplantation	Bone marrow-derived mesenchymal stem cell	Grafted cells survived in the retina for at least eight weeks and almost all bone marrow-derived mesenchymal stem cell migrated and were incorporated into the neural retina, specifically in the outer nuclear layer, inner nuclear layer and ganglion cell layer while a subset of grafted cells were found in the subretinal space after transplantation.
Lee et al.[25]	Developing mouse retina	Intravitreal transplantation	Bone marrow-derived mesenchymal stem cells	The transplanted bone marrow-derived mesenchymal stem cell survived and showed morphological differentiation into neural cells and some processes within the host retina
Chung et al.[26]	Retinotomies were made by applying an Nd:YAG laser to rat retina	Systemically administered	Bone marrow-derived mesenchymal stem cells.	Systemically administered GFP-marked MSCs may be incorporated into neuroretinal tissues and play an important role in the wound modulation of physically damaged retinal tissues.
Stanke & Fischer[27]	Ganglion cells were selectively damaged	Intravitreal transplantation	Embryonic stem cells	Embryonic retinal cells promoted the survival of ganglion cells

and also models of retinal degeneration using transgenic animals were studied[33-36].

These studies demonstrated that stem cells are capable of differentiation into some retinal cell types with this capability being more limited in adult stem cells.

Thus, when the primary purpose of the study was cell replacement, there was a tendency to use cells with the greatest differential potential, such as embryonic stem cells, induced pluripotent stem cells and retinal progenitor cells. These cells have higher differentiation potential but also have higher risk of complications, especially embryonic and induced pluripotent stem cells including the formation of teratomas, differentiation into tissues other than the target organ and rejection (embryonic stem cells).

Mesenchymal stem cells have a reduced ability of cell differentiation when compared to embryonic stem cells although they may differ in some cells such as retinal pigmented epithelium cells and retinal glial cells. However, these cells secrete large amounts of trophic factors that could theoretically increase the longevity of retinal cells in distress and also to produce a recovery of function[29,37,38].

Some studies have shown that, on using transgenic animals with retinal degeneration of both eyes, the eye that was treated with stem cells preserved much of its morphological structure (assessed histologically) and function (assessed by electroretinography) compared to the eye that received no stem cells and followed the course of degeneration with cellular loss and hence with loss of

retinal function[26,30]. Otani et al. demonstrated that, whenever a fraction of mouse or human adult bone marrow-derived stem cells (Lin-HSCs) containing endothelial precursors stabilizes and rescues retinal blood vessels that would ordinarily degenerate completely, a dramatic neurotrophic rescue effect is also observed[15,29].

Retinal nuclear layers are preserved in two mouse models of retinal degeneration, rd1 and rd10, and detectable, albeit severely abnormal, electroretinogram recordings are observed in rescued mice at times when they are never observed in control-treated or untreated eyes. The normal mouse retina consists predominantly of rods, but the rescued cells after treatment with Lin-HSCs are nearly all cones. Microarray analysis of rescued retinas demonstrates significant upregulation of many anti-apoptotic genes, including small heat shock proteins and transcription factors[15,29].

Another important aspect is that these studies demonstrated that the intravitreal route for infusion of these cells is a good option for rescue therapy with adult stem cells, because there is a migration of cells into the retina and also because of the growth factors they produce. In the case of cell replacement, the technique used was the application of cells in the subretinal space (under the central area of the retina called the macula) in order to replenish the cells of the retinal pigment epithelium[15,29].

To decrease the possibility of complications arising from embryonic stem cells and enable their use in humans, a North American company called Advanced Cell Technology has developed a technique to differentiate embryonic stem cells into cells of the retinal pigment epithelium to be used as cell replacement with the injection of these cells in the macular region under the retina.

In studies in the Royal College of Surgeons (RCS), subretinal transplantation of embryonic stem cell-derived retinal pigment epithelium in a rat model of deterioration of vision due to retinal pigment epithelium dysfunction resulted in extensive photoreceptor rescue and improvement in vision without evidence of untoward pathological effects. These and other safety studies suggest that embryonic stem cells could serve as a potentially safe and inexhaustible source of retinal pigment epithelium for the efficacious treatment of many retinal degenerative diseases[27-30].

Clinical trials

Currently there are two lines of research for the treatment of retinal disease.

The first is the use of adult stem cells (bone marrow-derived) that are administered intravitreally and the other technique is the use of embryonic stem cell-derived retinal pigment epithelium that are injected into the subretinal space.

Two case reports have demonstrated the clinical feasibility of the intravitreal administration of autologous bone marrow-derived mononuclear cells (ABMCs) in patients with advanced degenerative retinopathies and retinal capillary occlusion[39,40].

Three clinical trials are being conducted in Brazil with the use of autologous bone marrow-derived stem cell transplantation for the treatment of retinal dystrophy (RP), dry age-related macular degeneration and ischemic retinopathy (including diabetic retinopathy with macular ischemia). These studies are registered with ClinicalTrials.gov, numbers NCT01068561, NCT01518127 and NCT01518842[41-43].

The study of the treatment of retinal dystrophy (RP) completed the first phase and the data have already been published. This was a prospective, phase I, non-randomized, open-label study that studied the intravitreal administration of ABMCs in three patients with RP and two patients with cone-rod dystrophy and an early treatment diabetic retinopathy best-corrected visual acuity of 20/200 or worse.

Evaluations such as best-corrected visual acuity, full field electroretinography, kinetic visual field (Goldman), fluorescein and indocyanine green angiography and optical coherence tomography were performed at baseline and at one, seven, 13, 18, 22, and 40 weeks after intravitreal injection of approximately 1 million cells (0.1 mL) into one eye of each patient. No adverse event was observed associated with the injection. A one-line improvement in best-corrected visual acuity was measured in four patients one week after the injection and was maintained throughout the follow-up. Three patients had undetectable electroretinography responses at all study visits, whereas one patient demonstrated residual responses for dark adapted standard flash stimulus (a wave amplitude of approximately 35 mV), which remained recordable throughout follow-up, and one patient showed a small response (a wave amplitude of approximately 20 mV) recordable only at weeks seven, 13, 22, and 40. Visual fields showed no reduction (with a Goldman Standard V5e stimulus) for any patient at any visit. No other changes were observed on optical coherence tomography or fluorescein and indocyanine green angiograms. It was concluded that the intravitreal injection of ABMCs in eyes with advanced RP or cone-rod dystrophy was associated with no detectable structural or functional toxicity over a period of 10 months[35,41,44].

The second phase of this study (NCT01560715)[44] and phase I/II of the protocols of ischemic retinopathy and macular degeneration already started and results are expected later this year (2012).

Another line of research is currently under clinical trial consists of using embryonic stem cells to treat diseases of the retina.

Schwartz et al.[45] started two prospective clinical studies to establish the safety and tolerability of subretinal transplantation of embryonic stem cell-derived retinal pigment epithelium in patients with Stargardt's macular dystrophy and dry age-related macular degeneration the leading cause of blindness in the developed world. Preoperative and postoperative ophthalmic examinations included visual acuity, fluorescein angiography, optical coherence tomography, and visual field testing. These studies are registered with ClinicalTrials.gov, numbers NCT01345006 and NCT01344993[46].

After surgery, structural evidence confirmed cells had attached and continued to persist during the study. The authors did not identify signs of hyperproliferation, abnormal growth or immune-mediated rejection in either patient during the first four months. Although there is little agreement between investigators on visual endpoints in patients with low vision, it is encouraging that during the observation period neither patient lost vision. Best-corrected visual acuity improved from hand motions to 20/800 [and improved from 0 to 5 letters on the Early Treatment Diabetic Retinopathy Study (ETDRS) visual acuity chart] in the study eye of the patient with Stargardt's macular dystrophy, and vision also seemed to improve in the patient with dry age-related macular degeneration (from 21 ETDRS letters to 28).

Table 2 - clinical trials currently being conducted with the use of stem cells to treat retinal diseases

Clinical trial	Disease	Administration route	Type and source of cells	Status	Conclusions
Siqueira et al.[41] NCT01068561	Hereditary retinal dystrophy (retinitis pigmentosa) Phase I	Intravitreal transplantation	Autologous bone marrow-derived stem cells	Completed	Intravitreal injection of autologous bone marrow-derived mononuclear cells in eyes with advanced retinitis pigmentosa or cone-rod dystrophy was associated with non-detectable structural or functional toxicity over a period of 10 months.
Siqueira et al.[41,44] NCT01068561 NCT01560715	Hereditary retinal dystrophy (retinitis pigmentosa) Phase II	Intravitreal transplantation	Autologous bone marrow-derived stem cells	20 of 50 patients were treated	Intravitreal injection of autologous bone marrow-derived stem cells in advanced retinitis pigmentosa was associated with slight improvement on macular sensitivity measured by microperimetry
Siqueira[43] NCT01518842	Ischemic Retinopathy Phase I/II	Intravitreal transplantation	Autologous bone marrow-derived stem cells	study has already started	Results not yet available
Siqueira et al.[42] NCT01518127	Advanced Age-related Macular Degeneration	Intravitreal transplantation	Autologous bone marrow-derived stem cells	study has already started	Results not yet available
Schwartz et al.[45] NCT01345006	Stargardt's Macular Dystrophy	subretinal	Human embryonic stem cell-derived retinal pigmented epithelial (MA09-hRPE) Cells	study has already started	The human embryonic stem cell-derived retinal pigmented epithelial cells showed no signs of hyperproliferation, tumorigenicity, ectopic tissue formation, or apparent rejection after four months (initial report of a patient)
Schwartz et al.[45] NCT01344993	Advanced Dry Age-related Macular Degeneration	subretinal	Human embryonic stem cell-derived retinal pigmented epithelial (MA09-hRPE) Cells	study has already started	The human embryonic stem cell-derived retinal pigmented epithelial cells showed no signs of hyperproliferation, tumorigenicity, ectopic tissue formation, or apparent rejection after four months (initial report of a patient)

They concluded that embryonic stem cell-derived retinal pigment epithelium cells showed no signs of hyperproliferation, tumorigenicity, ectopic tissue formation, or apparent rejection after four months[46].

There are different systems to evaluate the level of scientific evidence for the clinical use of a therapeutic procedure.

In this article, I found the system used at the Oxford Centre for Evidence-based Medicine the best[44-48].

Conclusion

Stem cell therapy for the treatment of retinal diseases still has a low level of evidence for clinical use. Pre-clinical studies were consistent and evaluated the behavior of different types of stem cells injected via three routes (intravitreal, subretinal and systemic).

Different models of retinal injuries were tested and the results were evaluated according to anatomic and functional characteristics. With these data we would consider a level of evidence 5 according to Oxford Centre for EBM. With respect to the clinical trials that are currently underway (Phase I/II), they are non-randomized prospective studies with small sample sizes and therefore on completion, they will provide a level of evidence of 1B according to the Oxford Centre for EBM.

References

1. Sackett DL, Rosenberg WM, Gray JA, Haynes RB, Richardson WS. Evidence based medicine: what it is and what it isn't. BMJ. 1996;312(7023):71-2. Comment in: BMJ. 1996;313(7050):170; author reply 170-1, BMJ. 1996;313(7050):169; author reply 170-1, Aust Health Rev. 2008;32(2):204-7, J Psychiatr Ment Health Nurs. 2005;12(6):739-44.
2. Clegg PD, Pinchbeck GL. Evidence-based medicine and stem cell therapy: how do we know such technologies are safe and efficacious? Vet Clin North Am Equine Pract. 2011;27(2):373-82.
3. Nobre MR, Bernardo WM, Jatene FB. [Evidence based clinical practice. Part 1. well structured clinical questions]. Rev Assoc Med Bras. 2003;49(4):445-9. Portuguese.
4. Bernardo WM, Nobre MR, Jatene FB. [Evidence-based clinical practice. Part II. Searching evidence databases.] Rev Assoc Med Bras. 2004;50(1):104-8. Portuguese.
5. Fletcher RH, Fletcher SW, Wagner EH. Epidemiologia clínica: elementos essenciais. 3rd. ed. Porto Alegre: Artes Médicas; 1996.
6. Sackett DL, Haynes RB, Tugwell P, Guyatt GH. Clinical epidemiology: a basic science for clinical medicine. 2nd. ed. Boston: Little Brown; 1992.
7. Davis DR, Stewart DJ. Autologous cell therapy for cardiac repair. Expert Opin Biol Ther. 2011;11(4):489–508.
8. Hare JM, Traverse JH, Henry TD, Dib N, Strumpf RK, Schulman SP, et al. A randomized, double-blind, placebo-controlled, dose-escalation study of intravenous adult human mesenchymal stem cells (prochymal) after acute myocardial infarction. J Am Coll Cardiol. 2009;54(24):2277–86. Comment in: J Am Coll Cardiol. 2009;54(24):2287-9.
9. Wollert KC, Meyer GP, Lotz J, Ringes-Lichtenberg S, Lippolt P, Breidenbach C, et al. Intracoronary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomised controlled clinical trial. Lancet 2004;364(9429):141–8. Comment in: Lancet. 2004;364(9429):121-2, Lancet. 2004;364(9449):1935-6.
10. Sheffield VC, Stone EM. Genomics and the eye. N Engl J Med. 2011;364(20):1932–42.
11. Siqueira RC, Voltarelli JC, Messias AM, Jorge R. Possible mechanisms of retinal function recovery with the use of cell therapy with bone marrow-derived stem cells. Arq Bras Oftalmol. 2010;73(5):474–9.
12. Baker PS, Brown GC. Stem-cell therapy in retinal disease. Curr Opin Ophthalmol. 2009;20(3):175–81.
13. Lanza R, Rosenthal N. The stem cell challenge. Sci Am. 2004;290(6):92-9.
14. Machaliñski A, Baumert B, Kuprjanowicz L, WiszniewskaB, Karczewicz D, Machaliñski B. Potential application of adult stem cells in retinal repair-challenge for regenerative medicine. Curr Eye Res. 2009;34(9):748–60.

15. Otani A, Dorrell MI, Kinder K, Moreno SK, Nusinowitz S, Banin E, et al. Rescue of retinal degeneration by intravitreally injected adult bone marrow-derived lineage-negative hemat opoietic stem cells. J Clin Invest. 2004;114(6):765-74. Comment in: J Clin Invest. 2004;114(6):755-7.

16. Wang S, Lu B, Girman S, Duan J, McFarland T, Zhang QS, et al. Non-invasive stem cell therapy in a rat model for retinal degeneration and vascular pathology. PLoS One. 2010;5(2):e9200.

17. Tomita M, Adachi Y, Yamada H, Takahashi K, Kiuchi K, Oyaizu H, et al. Bone marrow-derived stem cells can differentiate into retinal cells in injured rat retina. Stem Cells. 2002;20(4):279-83.

18. Zhang Y, Wang W. Effects of bone marrow mesenchymal stem cell transplantation on light-damaged retina. Invest Ophthalmol Vis Sci. 2010,51(7):3742-8.

19. Tomita M, Mori T, Maruyama K, Zahir T, Ward M, Umezawa A, etal. A comparison of neural differentiation and retinal transplantation with bone marrow-derived cells and retinal progenitor cells. Stem Cells. 2006;24(10):2270-8.

20. Meyer JS, Katz ML, Maruniak JA, Kirk MD. Embryonic stem cell-derived neural progenitors incorporate into degenerating retina and enhance survival of host photoreceptors. Stem Cells. 2006;24(2):274-83.

21. Siqueira RC, Abad L, Benson G, Sami M. Behaviour of stem cells in eyes of rabbits with chorioretinal injuries caused by laser red diode 670N-M. In: Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO), 2008, Fort Lauderdale. April 27-May 1. Invest Ophthalmol Vis Sci. 2008;49:536.

22. Wang HC, Brown J, Alayon H, Stuck BE. Transplantation of quantum dot-labelled bone marrow-derived stem cells into the vitreous of mice with laser-induced retinal injury: survival, integration and differentiation. Vision Res. 2010;50(7):665-73.

23. Johnson TV, Bull ND, Hunt DP, Marina N, Tomarev SI, Martin KR. Neuroprotective effects of intravitreal mesenchymal stem cell transplantation in experimental glaucoma. Invest Ophthalmol Vis Sci. 2010;51(4):2051-9.

24. Castanheira P, Torquetti L, Nehemy MB, Goes AM. Retinal incorporation and differentiation of mesenchymal stem cells intravitreally injected in the injured retina of rats. Arq Bras Oftalmol. 2008;71(5):644-50.

25. Lee ES, Yu SH, Jang YJ, Hwang DY, Jeon CJ. Transplantation of bone marrow-derived mesenchymal stem cells into the developing mouse eye. Acta Histochem Cytochem. 2011;44(5):213-21.

26. Chung JK, Park TK, Ohn YH, Park SK, Hong DS. Modulation of retinal wound healing by systemically administered bone marrow-derived mesenchymal stem cells. Korean J Ophthalmol. 2011;25(4):268-74.

27. Stanke JJ, Fischer AJ. Embryonic retinal cells and support to mature retinal neurons. Invest Ophthalmol Vis Sci. 2010;51(4):2208-18.

28. MacLaren RE, Pearson RA. Stem cell therapy and the retina. Eye (Lond). 2007;21(10):1352-9.

29. Siqueira RC. Stem cell therapy for retinal diseases: update. Stem Cell Res Ther. 2011;2(6):50.

30. Inoue Y, Iriyama A, Ueno S, Takahashi H, Kondo M, Tamaki Y, et al. Subretinal transplantation of bone marrow mesenchymal stem cells delays retinal degeneration in the RCS rat model of retinal degeneration. Exp Eye Res. 2007;85(2):234-41.

31. Wang S, Qu X, Zhao RC. Clinical applications of mesenchymal stem cells. J Hematol Oncol. 2012 Apr 30;5(1):19.

32. Buchholz DE, Hikita ST, Rowland TJ, Friedrich AM, Hinman CR, Johnson LV, et al. Derivation of functional retinal pigmented epithelium from induced pluripotent stem cells. Stem Cells. 2009;27(10):2427-34.

33. Otani A, Kinder K, Ewalt K, Otero FJ, Schimmel P, Friedlander M. Bone marrow-derived stem cells target retinal astrocytes and can promote or inhibit retinal angiogenesis. Nat Med. 2002;8(9):1004-10. Comment in: Nat Med. 2002;8(9):932-4.

34. Yue F, Johkura K, Shirasawa S, Yokoyama T, Inoue Y, Tomotsune D, Sasaki K. Differentiation of primate ES cells into retinal cells induced by ES cell-derived pigmented cells. Biochem Biophys Res Commun. 2010 Apr 16;394(4):877-83.

35. Siqueira RC, Messias A, Voltarelli JC, Scott IU, Jorge R. Intravitreal injection of autologous bone marrow-derived mononuclear cells for hereditary retinal dystrophy: a phase I trial. Retina. 2011 Jun;31(6):1207-14.

36. Cheng AS, Yau TM. Paracrine effects of cell transplantation: strategies to augment the efficacy of cell therapies. Semin Thorac Cardiovasc Surg. 2008;20(2):94-101.

37. Siqueira RC. [Autologous transplantation of retinal pigment epithelium in age related macular degeneration]. Arq Bras Oftalmol. 2009;72(1):123-30. Portuguese.

38. Nistor G, Seiler MJ, Yan F, Ferguson D, Keirstead HS. Three-dimensional early retinal progenitor 3D tissue constructs derived from human embryonicstem cells. J Neurosci Methods. 2010;190(1):63-70.

39. Jonas JB, Witzens-Harig M, Arseniev L, Ho AD. Intravitreal autologous bone marrow-derived mononuclear cell transplantation: a feasibility report. Acta Ophthalmol. 2008;86(2):225-6.

40. Jonas JB, Witzens-Harig M, Arseniev L, Ho AD. Intravitreal autologous bone marrow-derived mononuclear cell transplantation. Acta Ophthalmol. 2010;88(4):e131-2

41. Siqueira RC. Autologous bone marrow-derived stem cells transplantation for retinitis pigmentosa. In: ClinicalTrials.gov identifier: NCT01068561 [Internet]. Bethesda (MD): National Library of Medicine; 2012 [cited 2012 Apr 15]. Available from: http://clinicaltrials.gov/ct2/show/NCT01068561

42. Siqueira RC. Intravitreal bone marrow-derived stem cells in patients with advanced age-related macular degeneration (AMDCELL). In: ClinicalTrials.gov identifier: NCT01518127 [Internet]. Bethesda (MD): National Library of Medicine; 2012. [cited 2012 Apr 15]. Available from: http://clinicaltrials.gov/ct2/show/NCT01518127?term=siqueira&rank=3

43. Siqueira RC. Effect of intravitreal bone marrow stem cells on ischemic retinopathy (Retina Cell). In: ClinicalTrials.gov identifier: NCT01518842 [Internet]. Bethesda (MD): National Library of Medicine; 2012. [cited 2012 Apr 15]. Available from: http://clinicaltrials.gov/ct2/show/NCT01518842?term=NCT01518842.&rank=1

44. Siqueira RC. Autologous bone marrow-derived stem cells transplantation for retinitis pigmentosa (RETICELL). In: Clinicaltrial identifier: NCT01560715 [Internet]. Bethesda (MD): National Library of Medicine; 2012. [cited 2012 Apr 15]. Available from: http://clinicaltrials.gov/ct2/show/NCT01560715?term=NCT01560715&rank=1

45. Schwartz SD, Hubschman JP, Heilwell G, Franco-Cardenas V, Pan CK, Ostrick RM, et al. Embryonic stem cell trials for macular degeneration: a preliminary report. Lancet. 2012;379(9817):713-20. Comment in: Lancet. 2012;379(9817):689-90.

46. Cook DJ, Guyatt GH, Laupacis A, Sackett DL, Goldberg RJ. Clinical recommendations using levels of evidence for antithrombotic agents. Chest. 1995;108(4 Suppl):227S-30S.

47. Manser R, Walters EH. What is evidence-based medicine and the role of the systematic review: the revolution coming your way. Monaldi Arch Chest Dis. 2001;56(1):33-8.

48. Centre for Evidence Based Medicine. Oxford Centre for Evidence-based Medicine. Levels of Evidence (March 2009) [Internet]. Oxford University; 2012. [cited 2012 Apr 15]. Available from: http://www.cebm.net/?o=1025