INTRAVITREAL INJECTION OF AUTOLOGOUS BONE MARROW–DERIVED MONONUCLEAR CELLS FOR HEREDITARY RETINAL DYSTROPHY

A Phase I Trial

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Purpose: To evaluate the short-term (10 months) safety of a single intravitreal injection of autologous bone marrow–derived mononuclear cells in patients with retinitis pigmentosa or cone–rod dystrophy.

Methods: A prospective, Phase I, nonrandomized, open-label study including 3 patients with retinitis pigmentosa and 2 patients with cone–rod dystrophy and an Early Treatment Diabetic Retinopathy Study best-corrected visual acuity of 20/200 or worse. Evaluations including 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 1, 7, 13, 18, 22, and 40 weeks after intravitreal injection of 10×10^6 autologous bone marrow–derived mononuclear cells (0.1 mL) into 1 study eye of each patient.

Results: No adverse event associated with the injection was observed. A 1-line improvement in best-corrected visual acuity was measured in 4 patients 1 week after injection and was maintained throughout follow-up. Three patients showed undetectable electroretinography responses at all study visits, while 1 patient demonstrated residual responses for dark-adapted standard flash stimulus (*a* wave amplitude approximately 35 μV), which remained recordable throughout follow-up, and 1 patient showed a small response (*a* wave amplitude approximately 20 μV) recordable only at Weeks 7, 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.

Conclusion: Intravitreal injection of autologous bone marrow–derived mononuclear cells in eyes with advanced retinitis pigmentosa or cone–rod dystrophy was associated with no detectable structural or functional toxicity over a period of 10 months. Further studies are required to investigate the role, if any, of autologous bone marrow–derived mononuclear cell therapy in the management of retinal dystrophies.

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Hereditary retinal dystrophies, such as retinitis pigmentosa (RP) or cone–rod dystrophy, affect as many as 1 in 3,500 individuals.[1] They are characterized by progressive visual field and visual acuity loss, night blindness, optic nerve atrophy, arteriolar attenuation, and altered vascular permeability, often progressing to complete blindness.[1]

Molecular genetic analysis of these diseases has identified mutations in more than 110 different genes accounting for only a relatively small percentage of the known affected individuals[2,3]; many of these mutations are associated with enzymatic and structural components of the phototransduction machinery, including rhodopsin,[4] cyclic guanosine monophosphate phosphodiesterase,[5] peripherin,[6,7] and RPE65.[8] Despite these observations, there are still no effective treatments to slow or reverse the progression of these dystrophies.

Preliminary results from clinical trials indicate that the treatment of a form of RP, Leber congenital amaurosis, with gene therapy can be safe and

effective.[9] Moreover, encouraging advances in gene therapy have led to partial reversal of the phenotypic changes observed in some animal models of RP, such as the rd mouse,[10] the rhodopsin gene–mutated mouse,[11] and the Royal College of Surgeons rat,[12,13] and in models with mutations of RPE65.[8,14,15] However, specific genetic defects have been found in a relatively few retinal degenerative diseases, which thereby limits the potential application of gene therapy for those few patients with a known mutation.

Trophic factors,[16–18] calcium channel blockers,[19] and electrical stimulation[20] have demonstrated positive effects in slowing disease progression in some retinal degeneration models. Furthermore, it has been shown that intraocular adenovirus-mediated gene transfer of ciliary neurotrophic factor may reduce photoreceptor loss in an RP model.[21–23]

There are reports of successful transplantation of neural stem cells, but this approach is limited by cell rejection and by political and ethical controversies related to the use of embryonic stem cells.[24] Recent experimental studies in animal models have reported that bone marrow–derived stem cells administered intravitreally have the potential to treat retinal diseases, improving perfusion through angiogenesis and replacing damaged cells.[25–27]

Recently, Jonas et al[28] demonstrated the clinical feasibility of intravitreal administration of autologous bone marrow–derived mononuclear cells (ABMC) in patients with advanced degenerative retinopathies. We report a prospective Phase I trial to investigate the safety of intravitreal ABMC in patients with RP or cone–rod dystrophy.

Methods

The study protocol adhered to the tenets of the Declaration of Helsinki and was approved by the local and national institutional review boards. All participants gave written informed consent. Patients were evaluated at the Retina and Vitreous Section of the Department of Ophthalmology, Otorhinolaryngology and Head and Neck Surgery, School of Medicine of Ribeirão Preto, between May 2009 and February 2010.

Throughout the study, a single certified examiner performed Early Treatment Diabetic Retinopathy Study best-corrected visual acuity (BCVA) measurement before any other study procedure. Ophthalmic evaluation was performed by a single retinal specialist (A.M.), and stereoscopic fundus photography, fluorescein and indocyanine green (ICG) angiography, and optical coherence tomography (OCT) were performed by a single certified ophthalmic technician.

Posterior iliac crest puncture and aspiration of bone marrow–derived cells were performed by J. C. Voltarelli. Intravitreal injections were performed by R. C. Siqueira.

Patients' Eligibility

Patients were included if they had a 1) diagnosis of hereditary retinal dystrophy classified clinically as RP or cone–rod dystrophy and 2) Early Treatment Diabetic Retinopathy Study BCVA of 20/200 or worse. Exclusion criteria included 1) previous ocular surgery other than cataract extraction; 2) presence of cataract or other media opacity that would prohibit high-quality ocular imaging or that would affect electroretinography (ERG) or visual field evaluation; 3) presence of other ophthalmic disease such as glaucoma or uveitis; 4) history of blood disorders such as leukemia; 5) known allergy to fluorescein or ICG angiography; or 6) known coagulation abnormalities or current use of anticoagulative medication other than aspirin. If both eyes were eligible for treatment, the eye with worse visual acuity was included in the study.

Ophthalmologic Evaluation

Patients underwent a comprehensive ophthalmologic examination, including undilated and dilated slit-lamp biomicroscopic examinations, applanation tonometry, and dilated funduscopic indirect ophthalmoscopy. Presence of cells in the anterior chamber was graded from 0 to 4, where 0 = none (no cells), 1 = mild (1–5 cells), 2 = moderate (6–15 cells), 3 = severe (16–30 cells), and 4 = very severe (>30 cells). Anterior chamber flare was also scored from 0 to 4, where 0 = none (no Tyndall effect), 1 = mild (barely discernible Tyndall effect), 2 = moderate (moderately intense Tyndall beam in anterior chamber), 3 = severe (severely intense Tyndall beam), and 4 = very severe.

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Fluorescein Angiography

Digital color fundus photography and fluorescein and ICG angiography were performed using a Topcon fundus camera system (TRC-50IA/IMAGEnet; Topcon, Tokyo, Japan).

Optical Coherence Tomography

Third-generation OCT evaluation (Stratus Tomographer, Model 3000; Carl Zeiss Ophthalmic Systems, Inc, Humphrey Division, Dublin, CA) was performed in all patients and consisted of 6 linear 6.00-mm scans obtained at intervals of 30° and centered on the foveal region. To optimize accuracy of OCT data, automatic delineation of the inner and outer boundaries of the neurosensory retina generated by OCT built-in software was verified for each of the six scans using the ''retinal thickness (single eye)'' analysis protocol. Central macular thickness values were calculated automatically as the average thickness of a central macular region 1,000 μm in diameter centered on the patient's foveola by built-in OCT3 software using ''retinal thickness/volume'' analysis protocol.

Visual Acuity

Best-corrected visual acuity measurement was performed according to a standardized refraction protocol using a retroilluminated Lighthouse for the Blind distance visual acuity test chart (using modified Early Treatment Diabetic Retinopathy Study Charts 1, 2, and R).

Visual Fields

Goldman kinetic perimeter using a standard protocol was used. Visual fields were scanned (CanoScan LiDE) at 600 dpi, and the area of the residual field surrounded by Goldman mark V4 was drawn and measured using Image J software.

Electroretinography

Electroretinography was performed according to the International Society for Clinical Electrophysiology of Vision standard[29] using the Espion E2 (Diagnosys LLC) recording unit and light coupled to the ColorDome (Diagnosys LLC, Impington, Cambridge, United Kingdom) as Ganzfeld LED stimulator. The protocol included 4 stimuli under dark-adapted (30 minutes) conditions: ROD response (0.01 cd·second/m^2), followed by dark-adapted maximum response (3 cd·second/m^2) and a high-intensity flash (10 cd·second/m^2). Oscillatory potentials were filtered from the second stimulus using a band-pass filter (Espion built-in) set between 75 Hz and 100 Hz.

Thereafter, eyes were light adapted for 10 minutes using a light background of 30 cd/m^2, with the same Ganzfeld. Cone single-flash response (3 cd·second/m^2; background of 30 cd/m^2) and 30-Hz flicker response (3 cd·second/m^2 at 30 Hz; background of 30 cd/m^2) were then recorded.

Preparation of Autologous Bone Marrow–Derived Mononuclear Cells

Aspiration of autologous bone marrow cells was performed under local anesthesia. Ten milliliters of bone marrow were harvested from the posterior iliac crest and mononuclear cells were separated by Ficoll–Hypaque gradient centrifugation and suspended in buffered saline containing 5% human albumin at a concentration of 10^7 cells per milliliter.[30] Final product demonstrated absence of microbial contamination. The final 0.1 mL of cell suspension used for the intravitreal injection contained from 0.92×10^4 to 2.91×10^4 (mean, 1.68×10^4) bone marrow–derived hematopoietic stem cells (CD34$^+$).

Intravitreal Injection Technique

All patients received one intravitreal injection using topical proparacaine drops under sterile conditions (eyelid speculum and povidone–iodine). Autologous (freshly isolated) bone marrow–derived mononuclear cells were injected into the vitreous cavity using a 27-gauge needle inserted through the inferotemporal pars plana 3.0 mm to 3.5 mm posterior into the limbus. After the injection, central retinal artery perfusion was confirmed with indirect ophthalmoscopy. Patients were instructed to instill 1 drop of 0.3% ciprofloxacin into the injected eye 4 times daily for 1 week after the procedure.

Safety Outcome Measures

Primary safety outcomes included 1) severe visual acuity loss, defined as a loss of $\geq$15 letters on the Early Treatment Diabetic Retinopathy Study visual acuity scale; 2) decrease in ERG *a* wave amplitude of 100 μV; 3) decrease in visual field seeing area of 1 square radians; and 4) genesis of abnormal tissues (teratomas) or tumors. Secondary safety outcomes included 1) signs of intraocular inflammation, defined as anterior chamber cells or flare with a score $>$3; 2) decrease in central macular thickness of $>$50 μm; 3) qualitative changes in retinal or choroidal perfusion on fluorescein or ICG angiography; and 4) intraocular pressure changes of $\geq$5 mmHg.

Results

Patients' age and gender were as follows: Patient 1: 23 years (F), Patient 2: 31 years (F), Patient 3: 33 years (M), Patient 4: 35 years (M), and Patient 5: 35 years (M). All patients completed the 10-month follow-up evaluation. Patient 1, Patient 3, and Patient 5 showed classic RP, while Patient 2 and Patient 4 had cone–rod dystrophy (Figure 1).

The procedure was well tolerated, and no clinical evidence of uveitis, endophthalmitis, or ocular toxicity was observed. The scores for cells and flare in the anterior chamber were 0 for 4 patients and 1 for 1 patient. No changes in lens status were observed in any of the 5 eyes during the 10-month follow-up period.

Only one patient reported minor local adverse events related to the treatment procedure (subconjunctival hemorrhage and foreign body sensation). These events were transient and resolved by 1 week after injection.

Visual Acuity

Best-corrected visual acuity at baseline was hand movements for Patient 1 and Patient 4, 20/400 for Patient 2 and Patient 5, and 20/200 for Patient 3. Figure 2A shows the BCVA distribution during follow-up for all subjects. Hand motions and count fingers values have been converted to logarithm of the minimum angle of resolution according to the scale published by Holladay.[31] No patient demonstrated a reduction in BCVA at any study visit. In 4 patients, a 1-line improvement in BCVA was measured 1 week after injection and was maintained throughout follow-up. After the 40-week follow-up, Patient 1 showed count fingers at 10 cm, Patient 4 showed count fingers at 1.5 m, Patient 2 and Patient 5: 20/300, and Patient 3: 20/100 (Figures 1 and 2A).

Visual Field

No reduction in residual visual field area (Goldman V4) was observed in any eye throughout follow-up. Figure 1 shows the visual field at baseline and at 40 weeks after injection for all study eyes, and Figure 2B demonstrates the distribution of seeing area during follow-up.

Electroretinography

The response elicited by ROD *b* wave stimulus was undetectable in all patients throughout follow-up. In addition, Patient 1, Patient 3, and Patient 5 demonstrated undetectable ERG electric potentials for all dark- and light-adapted stimuli throughout follow-up.

Patient 2 showed no detectable ERG responses at baseline, but residual responses for dark-adapted standard flash were detected at 7, 13, 22, and 40 weeks after injection. Table 1 shows dark-adapted standard flash a and *b* wave amplitude and implicit times for all visits. This patient showed no detectable response for light-adapted stimuli at baseline and throughout follow-up (Table 1, Figure 3). Importantly, the same ERG responses were observed in the nontreated eye of this patient, suggesting that this might be explained by variability in the signal-to-noise ratio at different visits.

Patient 4 showed residual ERG responses for dark-adapted standard flash and light-adapted 30-Hz flicker throughout follow-up (Table 1, Figure 3). Dark-adapted high-intensity responses and cone single-flash responses showed similar results to dark-adapted standard flash and cone 30-Hz flicker responses, respectively, for all patients in all visits.

Macular Thickness and Volume and Retinal Angiography

Macular thickness ranged from 51 μm to 169 μm, and macular volume ranged from 5,718 μm^3 to 110,007 μm^3 at baseline. No significant changes were observed in macular thickness or volume measurements during follow-up (Figure 4). No change in retinal perfusion status compared with baseline was observed on fluorescein and ICG angiography at any study follow-up visit in any patient.

Discussion

In the present study, 5 patients with RP or cone–rod dystrophy received intravitreal injection of ABMC, and no important adverse events were observed throughout the 10-month follow-up. The procedure was not associated with clinically important intraocular inflammation, and there was no detectable impairment in visual function. Patients who showed recordable ERG responses before injection demonstrated no changes in these electrical responses, and no reduction in visual field sensitivity was observed. No important changes in macular thickness were observed on OCT, and no important changes in retinal perfusion were observed on fluorescein and ICG angiography.

Consistent with the findings of the current study, Jonas et al[28] reported no significant adverse events after intravitreal ABMC transplantation. In the latter study, polymorphic cells in the anterior chamber were observed only during the first 4 weeks after the procedure.[28] The absence of important adverse events may be explained by the lack of immunogenicity of

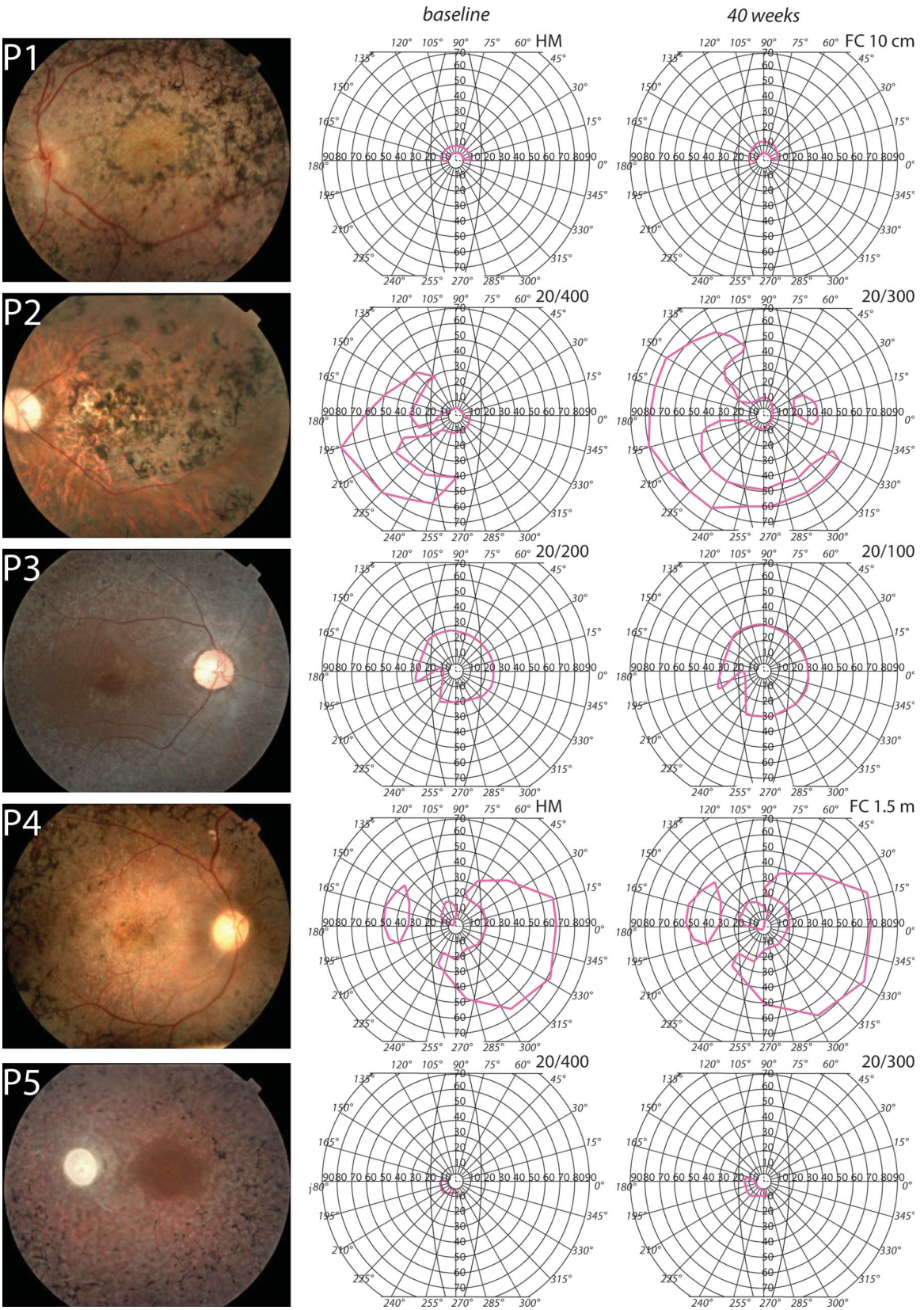

Fig. 1. Fundus photographs at baseline, visual fields (Goldman V4), and Early Treatment Diabetic Retinopathy Study best-corrected visual acuity for all 5 patients at baseline and 40 weeks after intravitreal administration of ABMC. P1 to P5, Patient 1 to Patient 5.

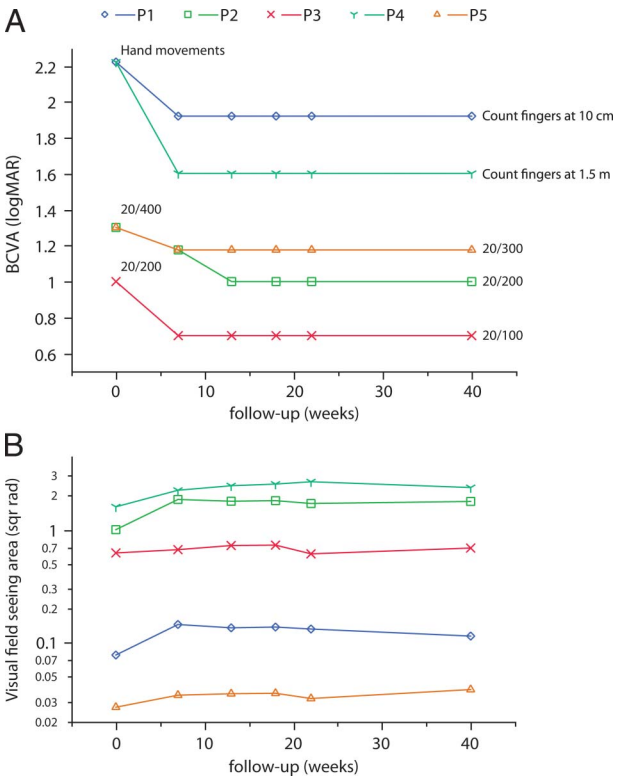

Fig. 2. Distribution at baseline and during follow-up of BCVA (**A**) and visual field seeing area (**B**) for all five patients. logMAR, logarithm of the minimum angle of resolution. P1 to P5, Patient 1 to Patient 5.

ABMC and use of biologically compatible suspension material for cell processing and injection.

Although increased central macular thickness has been reported after intravitreal administration of ciliary neurotrophic factor–secreting implant in patients with geographic atrophy,[32] no increase in central macular thickness was observed in the present study. Central macular thickness may not be a sufficiently sensitive measurement to evaluate treatment efficacy in the short term because a longer time may be needed for a significant increase in the number of retinal neurons. Besides the hypothesis of a small effect below spectral-domain OCT sensitivity detection, the presence of endothelial progenitors derived from bone marrow hematopoietic stem cell populations might have made the vasculature more resistant to degeneration and at the same time facilitates retinal neuronal survival.[27] By this mechanism, ABMC would contribute to the maintenance of healthy neuronal cells or survival of apoptotic ones, without replacing the dead ones and by this way contributing to the maintenance of central macular thickness but not to its increase, as verified in the present study.

Hematopoietic cells have been reported to play an active role in the formation of blood vessels,[33] and it has been shown that ABMC injection in mice may promote endothelial rescue.[34] In this context, angiographic tests were performed to investigate macular perfusion status and a possible effect of ABMC injection on retinal capillaries. However, in our study, no change in macular perfusion status was noticed on fluorescein and ICG angiography during 10 months after injection. In addition, and further supporting the safety of the procedure, no macular cystoid changes that may reflect an inflammatory reaction were observed on angiography or OCT.

There is evidence that ABMC may have the ability to differentiate into retinal neuronal cells.[35] In addition, interleukins and other important mediators may be produced by stem cells and have a paracrine effect, as well as upregulation of antiapoptotic genes, contributing to the rescue of retinal neurons.[27]

Table 1. Electroretinography *a* and *b* Wave Amplitude and Implicit Time Elicited Under Dark-Adapted Conditions Using a Standard Flash (4 milliseconds; 3.0 cd·second/m^2) Stimulus*

		a Wave		*b* Wave	
ID	Visit	Amplitude (μV)	Implicit Time (ms)	Amplitude (μV)	Implicit Time (ms)
Patient 2	Baseline	—	—	—	—
	7 weeks	24.3	18	26.5	74
	13 weeks	16.0	18	10.9	86
	18 weeks	—	—	—	—
	22 weeks	18.5	25	10.5	76
	40 weeks	17.4	25	13.8	72
Patient 4	Baseline	30.8	15	44.6	98
	7 weeks	58.8	20	60.6	93
	13 weeks	39.9	28	50.1	99
	18 weeks	34.5	12	45.4	79
	22 weeks	46.1	20	46.5	90
	40 weeks	41.0	18	46.8	70

*Electroretinography responses for Patient 1, Patient 3, and Patient 5 were not detectable throughout follow-up.

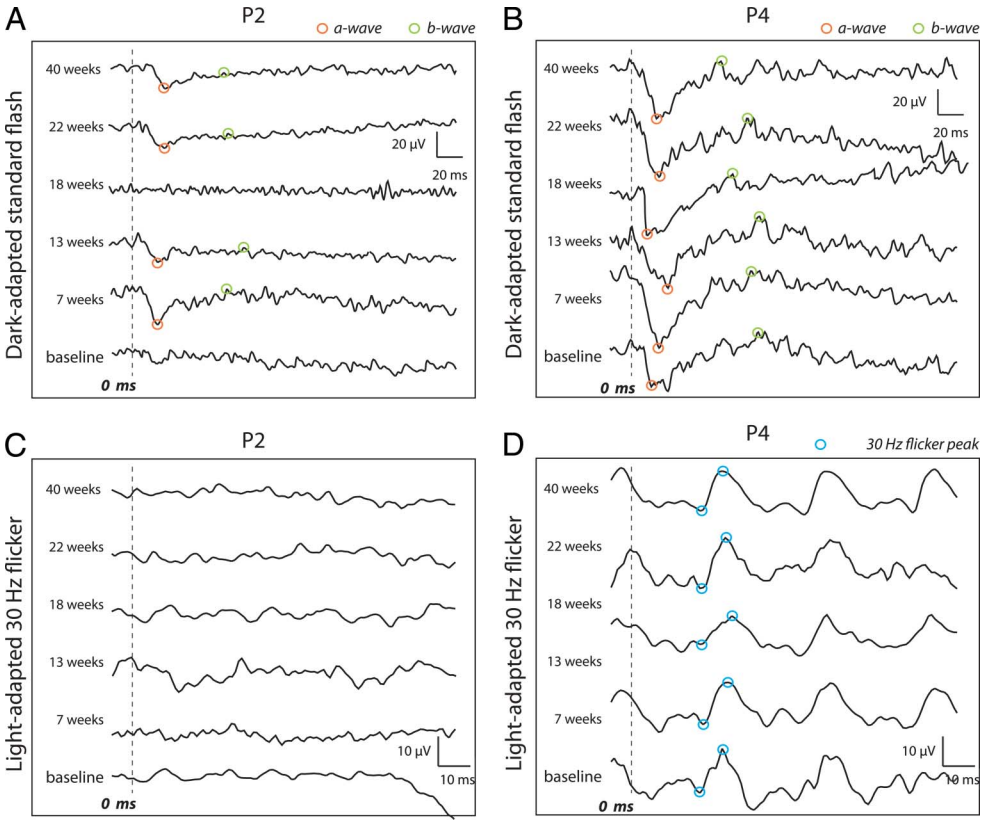

Fig. 3. Electroretinograms from Patient 2 and Patient 4 at baseline and during follow-up. **A** and **B.** Right eye and left eye responses elicited by dark-adapted standard flash (3 cd·second/m^2) from Patient 2 and Patient 4, respectively. **C** and **D.** Responses elicited by the 30-Hz stimulus after light adaptation.

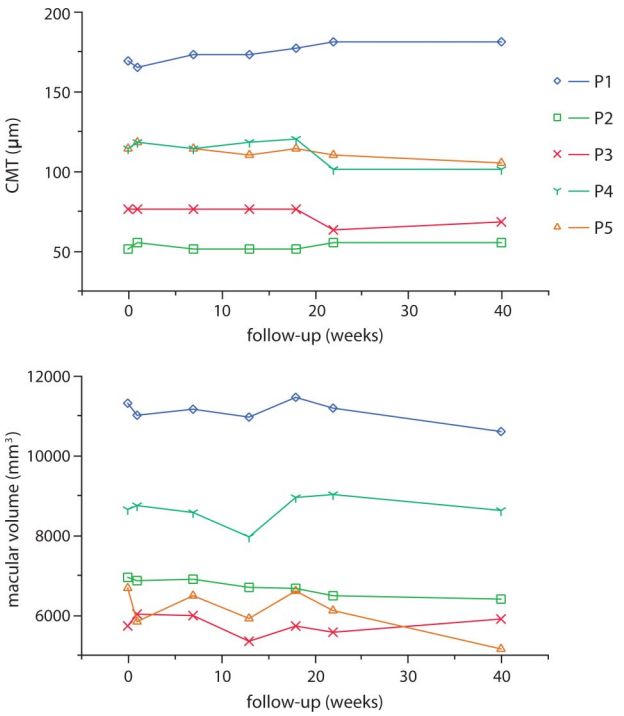

Fig. 4. Distribution at baseline and during follow-up of CMT and macular volume measured with OCT for all five patients. CMT, central macular thickness.

It is reasonable to speculate that the use of ABMC may not yield positive long-lasting effects because the transplanted cells may have the same genetic defects as the retinal cells. However, studies in mice models for retinal dystrophy have shown rescue of retinal neurons after the use of ABMC from genetically defective mice and from wild-type mice.[27] This observation suggests that patients' own bone marrow cells may provide retinal rescue, which allows for avoiding unwanted potential side effects associated with the use of viral vectors in long-term gene therapy and rejection that may follow administration of embryonic stem cells.

The present study has important limitations such as a few patients and short follow-up. However, to the best of our knowledge, this is the first report of the safety of intravitreal ABMC injection in patients with hereditary retinal dystrophies.

Key words: retinitis pigmentosa, electroretinography, stem cells.

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