

Improvement of Visual Sensitivity Following Transplantation of Intact Sheets of Fetal Neural Retina With its Retinal Pigment Epithelium to Retinitis Pigmentosa Patient

Short title: Visual function following transplantation

Norman D. Radtke, M.D.¹
Robert B. Aramant, Ph.D.^{2,3}
Magdalene J. Seiler, Ph.D.^{2,3}
Heywood M. Petry, Ph.D.⁴
Diane Pidwell, Ph.D.⁵

¹Retina Vitreous Resource Center, Norton Audubon Hospital, Louisville, Kentucky;

²former address: Dept. of Ophthalmology & Visual Sciences; Dept. of Anatomical Sciences and Neurobiology, University of Louisville, Louisville, KY;

³current address: Retina Vitreous Resource Center, Louisville, KY;

⁴Depts. of Ophthalmology and Visual Sciences and Psychological and Brain Sciences, University of Louisville, Louisville, Kentucky;

⁵Tissue Typing Laboratory, Jewish Hospital, Louisville, Kentucky;

CORRESPONDING AUTHOR: Norman D. Radtke, M.D.
Three Audubon Plaza Drive, Suite 240
Louisville, KY 40217
Phone – (502) 636-2823; Fax – (502)634-1646
E-mail – ret-vit.resource.ctr@prodigy.net

SUPPORT: The Murray Foundation, Inc.; Vitreoretinal Research Foundation; Kentucky Lions Eye Foundation; Research to Prevent Blindness; Anonymous Donor

N. D. Radtke, M. J. Seiler, and R. B. Aramant have a proprietary interest in the implantation instrument and procedure.

ABSTRACT

PURPOSE: To report the subjective and objective improvement in vision in a patient with autosomal dominant retinitis pigmentosa (ADRP) after transplantation of a sheet of fetal neural retina together with its retinal pigment epithelium (RPE).

DESIGN: Interventional case series.

METHODS: A sheet of fetal neural retina and RPE were transplanted together into the subretinal space under the fovea unilaterally in a patient with retinitis pigmentosa who had 20/800 vision in the treated eye. The patient was followed for one year. The main outcome measures were Early Treatment Diabetic Retinopathy Study (ETDRS) testing, scanning laser ophthalmoscope (SLO), tissue typing of both donor and recipient, fluorescein angiography, multifocal electroretinogram (mfERG), visually evoked potentials (mfVEPs), and clinical examination.

RESULTS: No evidence of rejection was observed although the patient was HLA (human leucocyte antigen) mismatched with the donor tissue. Up to one year, there was no evidence of retinal edema or scarring. The transplant sheet lost its pigmentation by 6 months. A change in vision from 20/800 to 20/400 (6 months), 20/250 (9 months) and 20/160 (1 year) was observed by ETDRS. Independently, SLO testing at a different institution at 9 months showed a vision of to 20/270 at a 40° field of view.

CONCLUSION: This study of a single patient indicates that fetal retina can be transplanted together with its RPE and survive one year without evidence of rejection and show continued improvement in both objective and subjective visual acuity.

TEXT

Previously, it has been reported that intact sheets of fetal retina together with its RPE can be safely transplanted to patients with retinitis pigmentosa (RP)¹. RP is a group of inherited diseases with mutations in photoreceptor or retinal pigment epithelium (RPE) genes (review:²). In these diseases, blindness is due to specific degeneration of photoreceptors and/or RPE cells even though the inner retina that connects to the brain may still remain functional³⁻⁵. If the diseased photoreceptors and/or RPE can be replaced and the new cells make appropriate connections to the functional part of the host retina, eyesight might be improved.

Retinal transplantation studies in animals have involved either RPE cells to rescue photoreceptors⁶⁻⁹ or cells of the neural retina to replace damaged photoreceptors¹⁰⁻¹⁵. Transplantation of healthy RPE cells in an animal model of RPE dystrophy can delay photoreceptor degeneration^{6,7}. However, RPE cell transplants have not been shown to have any effect in the RDS mouse, a model of photoreceptor degeneration¹⁶.

The success of RPE transplants in RCS rats has led to clinical trials in age related macular degeneration (ARMD) patients. The first transplants performed on patients with ARMD undergoing choroidal new vessel (CNV) excision showed signs of rejection at three months¹⁷. In ARMD patients undergoing CNV excision, Bruch's membrane is damaged and the native RPE in the dissection bed have been removed¹⁸, permitting leukocyte and humoral access to the grafts. However, in another study, patients with non-exudative ARMD, which has a largely intact blood-retinal barrier, demonstrated less clinically evident inflammation after RPE transplantation¹⁹⁻²⁰. One patient with ARMD and geographic atrophy that received adult RPE transplants showed signs of graft failure and/or rejection 5 months after surgery²¹. One AMD patient underwent CNV excision and RPE transplantation followed by immune suppression with cyclosporine, azathioprine, and prednisone. There was no clinical evidence of inflammation after surgery, and histopathology showed no signs of immune rejection, photoreceptor atrophy, and large areas devoid of RPE in the dissection bed²². RPE transplants have been done in two patients with RP. These patients experienced improved performance on visual field testing²³.

RPE transplantation can rescue existing photoreceptor cells in animal models of retinal degeneration^{6,7}. On the other hand, when photoreceptors are irreversibly lost, transplantation of RPE cells cannot be of help because the photoreceptors need to be replaced (review:²⁴). Fourteen RP patients in India²⁵, and eight patients with RP and one patient with ARMD in the U.S. received aggregate retinal transplants²⁶. Ten patients received adult photoreceptor sheet transplants²⁷⁻²⁹. These experiments have shown no clinical signs of rejection, but also no improvement in vision. However, the presence of subclinically evident graft rejection cannot be excluded.

Using an implantation instrument and procedure developed by Aramant and Seiler to transplant intact sheets of fetal retina with or without RPE to rats with retinal degeneration (review:²⁴), clinical studies have been performed in patients with RP^{1,30}. Although no objective improvement in vision was reported, these clinical studies demonstrated the safety of the procedure.

The present study is a continuation of the safety study of transplanting fetal neural retina together with RPE to RP patients with light perception or no light perception¹. The Food and Drug Administration (FDA) and the Institutional Review Board (IRB) of Norton Audubon Hospital and the University of Louisville approved to change the patients' vision criteria from light perception (LP) or no light perception (NLP) in both eyes to 20/800 or worse in one eye based on the demonstration of safety on previous patients¹. The results of the first patient with the new vision criterion of 20/800 or worse are presented because the patient showed visual improvement from 20/800 ETDRS vision before treatment to 20/160 at one year. The most effective methods to demonstrate visual function were Early Treatment Diabetic Retinopathy Study (ETDRS) and scanning laser ophthalmoscope (SLO) measurements.

METHODS: The study protocol is that of an interventional case series in which patients with RP and vision of 20/800 or worse in one eye are studied without a control group for comparison, after approval from Norton Audubon Hospital and from the University of Louisville Human

Studies Committee. The study is conducted under the Food and Drug Administration (FDA) Investigational New Drug (IND) Number BB-IND 8354.

Criteria for exclusion from the study were: any significant ocular diseases that compromised or could compromise vision in the eye to be studied and thus confound the analysis; participation in another ophthalmic clinical trial; use of any other investigational new drug within 12 weeks before the start of the study treatment; intraocular surgery within the last two months; capsulotomy within the last month in the study eye; a history of uveitis, Coat's disease, or diabetic retinopathy, glaucoma, or a cataract that prevents visualization of the posterior pole.

The patient who received the transplant had given an informed consent after extensive counseling regarding realistic expectation of the procedure. In accordance with our selection criteria, this patient had 20/800 vision measurement in the operated eye for at least one year with a diagnosis of retinitis pigmentosa, was over 21 years of age, not pregnant, and was willing to return for follow-up visits. The patient was a 64-year-old female.

The fetal tissue used was 13 weeks gestational age and was obtained by informed consent. Donors were not compensated. After the donors had decided to terminate their pregnancy, they were approached to donate tissue for research. The harvesting procedure of fetal retina with RPE has been described previously¹. A piece of 1.5 x 3.1 mm of the fetal retina with its RPE was cut out and implanted subretinally under the fovea, using a custom-made implantation instrument with a flat plastic nozzle tip at a 130° angle^{1, 24, 30}. The loaded instrument was inserted under the retina through the retinotomy site, and the donor tissue was placed into the target area with the correct orientation, the RPE towards the choroid.

Donor tissue that was dissected away was used for the tissue typing of the donor. Tissue typing of the donor was done at the histocompatibility lab at Jewish Hospital. The donor DNA was extracted using a DNA tissue extraction kit (QIAGEN, Valencia, California) and typed for the human leucocyte antigens HLA-A, B, and DR antigens by PCR amplification using sequence specific primers (Pel Freeze, Deer Brown, Wisconsin).

Anti-HLA antibodies in the recipient were detected using two techniques, both using sensitive flow cytometric procedures. Screening for antibodies was performed using a pool of normal T-cells. The specificity of antibody was determined using HLA coated beads (One Lambda, Canoga Park, California).

Complete assessments (ocular examination, fluorescein angiography, mfERG, and mfVEP) were performed pre-operatively and repeated after surgery. To assess potentially corresponding physiologic changes in the region of the transplant, photopic mfERGs and mfVEPs were recorded three times before surgery and at 2 weeks, 1, 3, 6, and 12 months post-operatively. Multifocal ERGs were recorded using a Burian-Allen electrode (Hansen Ophthalmic Development Laboratory, Iowa City, Iowa), amplified at 100K with low- and high-frequency cut-offs at 10Hz and 300Hz respectively (half amplitude, Grass preamplified P511J, Grass Instruments, Quincy, Massachusetts), and VERIS Science software (EDI, Inc., San Mateo, California). A 42° x 38° field of 103 black and white hexagons (mean luminance 200 cd/m², 75Hz frame rate) was used with the hexagons flashing on or off according to a pseudorandom binary M-sequence. A cross-correlation technique was used to extract local responses from the continuous electroretinography. For mfVEP recordings, the same testing and analysis apparatus was used with the following changes. A 60-sector dartboard stimulus was used with each sector consistent of 16 checks (VERIS stimulus Dart Board 60 with Pattern, mean luminance of 200 cd/m² 75Hz frame rate); gold cup electrodes were placed 4 cm above theinion (positive), at theinion (references) and on the forehead (ground); and signs were amplified (100K) and filtered at 3Hz and 100Hz (half-amplitude cut-offs). Multifocal VEP (mean luminance 200 cd/m², 75Hz frame rate) recorded using three Grass gold disc electrodes and the VERIS Science 4.9 system (EDI, Inc., San Mateo, California). Electrodes were placed on the forehead, at theinion and 4 cm above theinion. Dartboard pattern was used with the checks flashing on or off according to a pseudorandom binary M-sequence.

The patient was tested at nine months post-operatively at an independent testing site with the scanning laser ophthalmoscope (SLO) using a small, dim stimulus. The examiners at this

location did not know which eye had been transplanted. For SLO/Microperimetry, the following settings were used: 1) fixation cross: intensity 75 - 100 dB, size #2, square shape; background 1 Cd/m²; 2) thresholds: duration 12 flashes/sec, 255 dB intensity, size #8. A helium-neon laser was used for testing. Infrared light was used to image the fundus during testing. Manual tracking was used to compensate for the patient's eye movement after each measurement by fixing the reference cross at clearly defined vascular landmarks³¹. The patient declined the opportunity for pre-operative testing. The SLO testing was not in the experimental protocol and was additional information obtained to the other clinical tests.

RESULTS: The transplant could be observed easily after surgery by indirect ophthalmoscopy because of its heavy pigmentation (see Figure 1A,B). During the follow-up exams, the area of pigmentation eventually disappeared between 3 and 6 months (Figure 1C). The appearance of the fundus in the transplant area remained unchanged between 6 and 12 months (Figures 1 C,D). Fluorescein angiography showed no dye leakage in the area of the transplant at six to twelve months (Figure 2). Although the clinical appearance of rejection in the retina is still somewhat unclear, no evidence of tissue destruction, as evidenced by dye leakage on fluorescein angiography, subretinal fibroses, or necrosis of the retina was seen in our patient. No systemic or intraocular immunosuppression medications were used.

Tests of the medium surrounding the tissue before implantation for sterility and endotoxin levels were well within normal limits (data not shown). The HLA types for donor and recipient are listed in Table 1. As expected, the donor and recipient were not matched. The donor and recipient shared only a single HLA-B antigen (B14) and no DR antigens, indicating this was an allogeneic graft.

The patient's vision in the operated eye showed improvement both subjectively and objectively, as assessed with ETDRS acuity at 6 months to 1 year, and with SLO testing at 9 months. The patient declined the opportunity for pre-operative SLO testing but agreed to post-operative SLO testing when she noticed a marked subjective improvement at six months. She

could only see the shape of a person's head pre-operatively, but post-operatively, she could see the eyes, nose, and mouth of people's faces. Objectively, her vision improved from 20/800 pre-operatively to 20/400 at six months, 20/250 at 9 months, and 20/160 at 12 months. No vision improvement occurred prior to six months. The post-op ETDRS vision at 9 months was confirmed by SLO testing at an independent clinical center and measured 20/270 at a 40° field of view (FOV) (Figure 3). The independent clinical center did not know prior to SLO testing which eye had been given the transplant. The operated eye contained 53 seeing and 37 non-seeing areas (Figure 3). In contrast, the ETDRS vision of the non-operated eye did not change during the testing period from the 20/400 pre-operative value. This value remained the same throughout the testing period, and it was tested at every follow-up examination. Correspondingly, SLO testing of the non-operated eye at 9 months measured the vision at 20/369 (33 seeing and 37 non-seeing areas), which was consistent with the ETDRS testing of the non-operated eye of 20/400.

The overlay of the SLO fixation and the photo of the transplant shows that the patient viewed the 20/270 letter (using 40° FOV testing) with a portion of the retina nasal to but at the edge of the transplant (see Figure 4).

The mfERG and mfVEP showed no clear signal pre- or post-operatively in any region of the retina.

Immediately after surgery, the patient required ventilation for pulmonary edema following general anesthesia. This development did not appear to be related to the instrument or fetal tissue retinal implant in her eye used during the retinal transplantation. Appropriate notification was made of this adverse event to the FDA and IRB. The result of this event was that the patient had increased O₂ saturation levels for five days post-op in the range of 96.7-98.9 % as measured by blood gases and oximeter readings which had been supplemented from a baseline of 92% saturation pre-operatively. The effect of increased oxygen levels on the transplant survival is unknown.

DISCUSSION: The approach used in this patient with ADRP was to transplant an intact sheet of fetal neural retina and RPE. In RCS rats, it has been shown that freshly harvested intact sheets of fetal RPE and retina transplanted together into the subretinal space can develop approximately normal morphology³² and preserve visual responses in the superior colliculus³³. Recently, it has been shown that co-grafts of human fetal retina with RPE can develop normally in athymic nude rats³⁴. Such transplants have the potential to benefit retinal disease with dysfunctional RPE and photoreceptors such as human RP. A specialized instrument and method have been developed to transplant sheets of fetal neural retina and RPE into the subretinal space between the neurosensory retina and RPE. This approach has significant advantages over other techniques. This technique maintains the correct orientation of the retinal sheets^{24,32}. The minimal trauma associated with the procedure reduces the possibility of rosette formation or rupture of Bruch's membrane.

Fetal donor tissue has many advantages over adult tissue. Fetal cells have a high capacity to sprout processes, to produce trophic substances³⁵, and can overcome the trauma of transplantation much easier than adult cells because they do not depend so heavily on oxygen as adult cells³⁶. Fetal retinal tissue is less likely to be immunogenic than adult tissue^{37,38}. Allografted sheets of RPE, in contrast to dissociated cells, have been shown to be immunologically privileged – they are not rejected when transplanted to the kidney capsule³⁹.

To date, no effective treatment has been developed for the recovery of visual loss from RP: 1.) Oral vitamin A therapy has been demonstrated to be safe and effective in slowing the rate of ERG loss in RP but shows no effectiveness in the recovery of lost vision⁴⁰. 2.) Gene therapy and pharmacologic therapy are underway and are exciting but are still under development and not in use in clinical trials at this time, although a clinical trial of gene therapy in Leber's Congenital Amaurosis will probably be initiated in one to three years⁴¹. 3.) Development and use of a visual prosthesis is actively being pursued in many centers but it is not clear what the visual potential of the existing devices is⁴²⁻⁴⁴.

If the graft was undergoing rejection, graft encapsulation, tissue destruction, and macular edema would have been observed by fundus exams or fluorescein angiography. In this patient, by one year post-operatively, there was no rejection seen clinically or with fluorescein angiography. However, one cannot exclude the presence of more subtle graft rejection without histological data. Previous reports of human transplantation have varied in the incidence of rejection depending on whether the transplantation involved patches of RPE cells or dissociated cells and whether the patients had exudative or non-exudative manifestations of AMD^{17, 19, 20, 22}. The observed pigment loss of the transplant might have been due to death of the RPE cells (i.e., graft failure) or to the fact that the pigment production of the cells could not keep up with the growth of the cells. Pigment loss has also been observed in cografts of rat fetal retina with RPE although the donor RPE cells could still be identified by BrdU label³². Another explanation is that rejection was occurring. However, fluorescein angiography showed no dye leakage in the area of the transplant at six to twelve months.

In the patient reported here, there were no donor-specific antibodies seen at six months. Anti-MHC class I antibodies specific for HLA-A1 antigen carried on the graft was found before transplantation, possibly from past pregnancies or transfusion. The titer of this antibody did not change at the time of the transplant. However, at approximately four months after transplantation, the titer of this class I antibody increased and continued to increase through the last sample collected nine months after surgery. This result indicates that some event stimulated the patient's immune system to produce antibodies. Whether the transplant or some other stimulus (e.g., an illness) was responsible is unclear. The patient's antibodies against an antigen that the graft carried increased, but all other antibodies, including antibodies with specificities to antigens not expressed by the graft, increased concurrently. This result indicates a non-specific immunologic response as opposed to a graft-specific response. No new antibodies to graft-specific antigens developed as would be expected in the presence of graft rejection. Also, the loss of pigment in the graft occurred gradually over the 3 to 6-month period, and the beginning

of the loss of pigment in the transplant was seen well before the titer increase of the anti-class I antibody. No anti-MHC class II antibody was detected at any time before or after the transplant.

The neural retina itself is not immunogenic, but the RPE and microglial cells in the donor retina are immunogenic^{39, 45, 46}. Most microglial cells are associated with blood vessels. These cells migrate into the retina post-natally in the rat⁴⁷ and beginning at 16 weeks gestation in the human³⁸. Since the number of microglial cells in fetal rat retina is much lower than in post-natal retina³⁷, it is likely that fetal retina is less immunogenic than post-natal retina in that species because fetal retina still lacks inner retinal vessels. In an animal model using allografts of Long-Evans or ACI rat donors into Sprague-Dawley or RCS recipients, stable transplants were seen in rats six to ten months after surgery²⁴, indicating that allogeneic retinal sheets transplants can be tolerated in the subretinal space of rats with retinal degeneration.

Due to the early gestational age of the donor (13 weeks), it is unlikely that donor-derived microglial cells were present in this graft, reducing the possibility of immunologic recognition through antigen presenting cell migration to lymph nodes or spleen. The fact that the blood-brain barrier under the graft appeared to remain intact, based on clinical evaluation and fluorescein angiography, reduces the potential graft recognition by the recipient immune system.

Taken together, these immunological data indicate that changes in recipient antibody production were not initiated by graft recognition and that graft-specific sensitization and thus rejection had not occurred yet.

The subjective and objective visual acuity improvement appeared concurrently at six months after surgery. The patient's report of vision improvement was corroborated at two separate, independent clinical centers, one with ETDRS and one with SLO.

The SLO testing showed that fixation was unsteady and involved the nasal edge of the transplant as well as retinal nasal and immediately adjacent to the transplant. Viewing of the 20/270 letter was done with retina nasal to the transplant. These findings indicate that the transplanted tissue might have had a trophic effect on residual host cone cells corresponding to what has been observed in vitro and in vivo^{48, 49}. Basic fibroblastic growth factor^{3, 50-52} and

various cytokines and neurotrophic factors⁵³ have been shown to protect against photoreceptor degeneration due to continuous light exposure or genetic defects^{54,55}. These growth factors likely act indirectly on photoreceptors via Mueller cells⁵⁶. It has also been suggested that transplanted normal rod photoreceptor cells release soluble factor(s) to enhance cone survival in primary rod photoreceptor dystrophies⁵⁷ without the need for specific synapse formation.

Another mechanism by which the transplant may have improved the vision in this patient is by local synaptic connectivity between transplant and host. In RCS rats and transgenic rats with retinal degeneration mimicking human RP, transplants of fetal retinal sheets with or without RPE can restore visual responses in an area of the superior colliculus that topographically corresponds to the placement of the transplants in the retina^{33,58}. Preliminary studies suggest synaptic connections between subretinal transplants and host retina by trans-synaptic virus tracing from the host brain to the transplant⁵⁹ (Seiler et al., submitted). At the present time, however, there is no published experimental evidence that transplanted fetal neurosensory retina can reestablish appropriate synaptic connections with the residual host neural network.

The failure of the electrophysiological tests to reveal improvement was not contradictory to our conclusion of improved vision, but rather indicative of an inability to extract any clear signal from the considerable recording noise (i.e., a very low signal/noise ratio). This result was not unexpected due to fixation problems and general lack of function inherent in eyes with 20/800 vision. Because of the nature of the multifocal technique, any movement of the stimulus on the retina due to lack of good fixation will smear the stimulation and response of functional areas of retina with adjacent non-functional areas. This effect will diminish any signal that may be present. The mfERG is additionally complicated by the issue of whether the waveform recorded focally from the region of the transplant will resemble waveforms characteristic of normal retinas (e.g.,³⁰). The waveform is likely to be affected by the types of connections made between donor and host and precludes reliance on standardized templates in the analysis. Although no new tissue has been introduced to the visual cortex, waveforms composing the

normal mfVEP vary considerably across the visual field due to the convolutions of primary visual cortex and individual variability. In patients, it is possible that transplant-induced changes in input and activity of visual cortex after many years of their absence may result in neural plasticity that produces waveforms uncharacteristic of normal mfVEPs. The approval of the FDA to perform this procedure in patients with better vision (up to 20/400) and with less nystagmus will provide our future studies with greater potential for showing improved function with mfERG and mfVEP testing.

The effect of the patient's exposure to O₂ saturation levels at 97-99% for five days post-operatively on the transplant is unknown.

Diseases that affect the RPE and photoreceptor cells of the retina (e.g., RP, ARMD, rod-cone dystrophy, and Stargardt disease) might conceivably benefit from this type of transplantation. This technique has novel features, which are of considerable biological and clinical interest for patients with diseased RPE and/or neural retina. Laboratory research to improve connectivity of the donor to host should enhance the potential for functional success of this procedure.

ACKNOWLEDGMENTS

Support was provided by The Murray Foundation, Inc., Vitreoretinal Research Foundation, Kentucky Lions Eye Foundation, Research to Prevent Blindness, and an Anonymous Donor. The authors acknowledge the assistance of Michael Lazar, Tatiana Forofonova, MD, PhD, and Marco Zarbin, MD, PhD in the performance of SLO testing, creation of the overlay of the fundus photo and SLO images, and interpretation of the SLO results.

REFERENCES

1. Radtke ND, Seiler MJ, Aramant RB, Petry HM, Pidwell DJ. Transplantation of intact sheets of fetal neural retina with its retinal pigment epithelium in retinitis pigmentosa patients. *Am. J. Ophthalmol.* 2002;133:544-50.
2. van Soest S, Westerveld A, de Jong PT, Bleeker-Wagemakers EM, Bergen AA. Retinitis pigmentosa: defined from a molecular point of view. *Surv. Ophthalmol.* 1999;43:321-34.
3. Santos A, Humayun MS, de Juan E, Jr., Greenburg RJ, Marsh MJ, Klock IB, Milam AH. Preservation of the inner retina in retinitis pigmentosa. A morphometric analysis. *Arch. Ophthalmol.* 1997;115:511-5.
4. Milam AH, Li ZY, Fariss RN. Histopathology of the human retina in retinitis pigmentosa. *Prog. Retin. Eye Res.* 1998;17:175-205.
5. Humayun MS, Prince M, de Juan E, Jr., Barron Y, Moskowitz M, Klock IB, Milam AH. Morphometric analysis of the extramacular retina from postmortem eyes with retinitis pigmentosa. *Invest. Ophthalmol. Vis. Sci.* 1999;40:143-8.
6. Li L, Turner JE. Inherited retinal dystrophy in the RCS rat: prevention of photoreceptor degeneration by pigment epithelial cell transplantation. *Exp. Eye Res.* 1988;47:911-917.
7. Lopez R, Gouras P, Kjeldbye H, Sullivan B, Reppucci V, Brittis M, Wapner F, Goluboff E. Transplanted retinal pigment epithelium modifies the retinal degeneration in the RCS rat. *Invest. Ophthalmol. Vis. Sci.* 1989;30:586-588.
8. Little CW, Cox C, Wyatt J, del Cerro C, del Cerro M. Correlates of photoreceptor rescue by transplantation of human fetal RPE in the RCS rat. *Exp. Neurol.* 1998;149:151-60.
9. Lund RD, Lawrence JM, Villegas-Perez MP, Litchfield TM, Sauve Y, Whiteley SJ, Coffey PJ. Retinal degeneration and transplantation in the Royal College of Surgeons rat. *Eye* 1998;12:597-604.
10. Silverman MS, Hughes SE. Transplantation of photoreceptors to light-damaged retina. *Invest. Ophthalmol. Vis. Sci.* 1989;30:1684-1690.
11. Seiler MJ, Aramant RB. Intact sheets of fetal retina transplanted to restore damaged rat retinas. *Invest. Ophthalmol. Vis. Sci.* 1998;39:2121-31.
12. Del Cerro M, Ison JR, Bowen GP, Lazar E, Del Cerro C. Intraretinal grafting restores function in light-blinded rats. *NeuroReport* 1991;2:529-532.
13. Kwan AS, Wang S, Lund RD. Photoreceptor layer reconstruction in a rodent model of retinal degeneration. *Exp. Neurol.* 1999;159:21-33.
14. Gouras P, Du J, Kjeldbye H, Yamamoto S, Zack DJ. Long-term photoreceptor transplants in dystrophic and normal mouse retina. *Invest. Ophthalmol. Vis. Sci.* 1994;35:3145-53.
15. Ghosh F, Ehinger B. Full-Thickness Retinal Transplants: A Review. *Ophthalmologica* 2000;214:54-69.
16. Li L, Sheedlo HJ, Turner JE. Retinal pigment epithelial cell transplants in retinal degeneration slow mice do not rescue photoreceptor cells. *Invest. Ophthalmol. Vis. Sci.* 1993;34:2141-5.
17. Algreve P, Berglin L, Gouras P, Sheng Y. Transplantation of fetal retinal pigment epithelium in age-related macular degeneration with subfoveal neovascularization [see comments]. *Comment in: Graefes Arch. Clin. Exp. Ophthalmol.* 1994 Dec;232(12):706. *Graefes Arch. Clin. Exp. Ophthalmol.* 1994;232:707-16.
18. Nasir MA, Sugino I, Zarbin MA. Decreased choriocapillaris perfusion following surgical excision of choroidal neovascular membranes in age-related macular degeneration. *Br. J. Ophthalmol.* 1997;81:481-489.

19. Algere PV, Berglin L, Gouras P, Sheng Y, Kopp ED. Transplantation of RPE in age-related macular degeneration: observations in disciform lesions and dry RPE atrophy. *Graefes Arch. Clin. Exp. Ophthalmol.* 1997;235:149-58.
20. Algere PV, Gouras P, Dafgard Kopp E. Long-term outcome of RPE allografts in non-immunosuppressed patients with AMD. *Eur. J. Ophthalmol.* 1999;9:217-30.
21. Weisz JM, Humayun MS, De Juan E, Jr., Del Cerro M, Sunness JS, Dagnelie G, Soylyu M, Rizzo L, Nussenblatt RB. Allogenic fetal retinal pigment epithelial cell transplant in a patient with geographic atrophy. *Retina* 1999;19:540-5.
22. Del Priore LV, Kaplan HJ, Tezel TH, Hayashi N, Berger AS, Green WR. Retinal pigment epithelial cell transplantation after subfoveal membranectomy in age-related macular degeneration: clinicopathologic correlation. *Am. J. Ophthalmol.* 2001;131:472-80.
23. Kaplan HJ, Tezel TH, Dong F, Del Priore LV. Long-term survival of fetal human allogeneic RPE transplants in retinal degeneration. *Macula Society Annual Meeting 2003:172-173.*
24. Aramant RB, Seiler MJ. Retinal Transplantation - Advantages of Intact Fetal Sheets. *Prog. Retin. Eye Res.* 2002;20:57-73.
25. Das T, del Cerro M, Jalali S, Rao VS, Gullapalli VK, Little C, Loreto DA, Sharma S, Sreedharan A, del Cerro C, Rao GN. The transplantation of human fetal neuroretinal cells in advanced retinitis pigmentosa patients: results of a long-term safety study. *Exp. Neurol.* 1999;157:58-68.
26. Humayun MS, de Juan E, del Cerro M, Dagnelie G, Radner W, Sadda SR, del Cerro C. Human neural retinal transplantation. *Invest. Ophthalmol. Vis. Sci.* 2000;41:3100-6.
27. Kaplan HJ, Tezel TH, Berger AS, Del Priore LV. Retinal transplantation. *Chem. Immunol.* 1999;73:207-19.
28. Kaplan HJ, Tezel TH, Berger AS, Wolf ML, Del Priore LV. Human photoreceptor transplantation in retinitis pigmentosa. A safety study. *Arch. Ophthalmol.* 1997;115:1168-72.
29. Berger AS, Tezel TH, Del Priore LV, Kaplan HJ. Photoreceptor transplantation in retinitis pigmentosa: short-term follow-up. *Ophthalmology* 2003;110:383-91.
30. Radtke ND, Aramant RB, Seiler MJ, Petry HM. Preliminary report: indications of improved visual function following retina sheet transplantation to retinitis pigmentosa patients. *Am. J. Ophthalmol.* 1999;128:384-387.
31. Sunness JS, Schuchard RA, Shen N, Rubin GS, Dagnelie G, Haselwood DM. Landmark-driven fundus perimetry using the scanning laser ophthalmoscope. *Invest. Ophthalmol. Vis. Sci.* 1995;36:1863-1874.
32. Aramant RB, Seiler MJ, Ball SL. Successful cotransplantation of intact sheets of fetal retinal pigment epithelium with retina. *Invest. Ophthalmol. Vis. Sci.* 1999;40:1557-1564.
33. Woch G, Aramant RB, Seiler MJ, Sagdullaev BT, McCall MA. Retinal transplants restore visually evoked responses in rats with photoreceptor degeneration. *Invest. Ophthalmol. Vis. Sci.* 2001;42:1669-76.
34. Seiler MJ, Aramant RB. Transplanted sheets of human retina and retinal pigment epithelium develop normally in nude rats. *Exp. Eye Res.* 2002;75:115-125.
35. von Bartheld CS. Neurotrophins in the developing and regenerating visual system. *Histol. Histopathol.* 1998;13:437-59.
36. Wasselius J, Ghosh F. Adult rabbit retinal transplants. *Invest. Ophthalmol. Vis. Sci.* 2001;42:2632-8.
37. Ashwell KW, Hollander H, Streit W, Stone J. The appearance and distribution of microglia in the developing retina of the rat. *Vis. Neurosci.* 1989;2:437-48.

38. Provis JM, Leech J, Diaz CM, Penfold PL, Stone J, Keshet E. Development of the human retinal vasculature: cellular relations and VEGF expression. *Exp. Eye Res.* 1997;65:555-68.
39. Wenkel H, Streilein JW. Evidence that retinal pigment epithelium functions as an immune-privileged tissue. *Invest. Ophthalmol. Vis. Sci.* 2000;41:3467-73.
40. Sibulesky L, Hayes KC, Pronczuk A, Weigel-DiFranco C, Rosner B, Berson EL. Safety of <7500 RE (<25000 IU) vitamin A daily in adults with retinitis pigmentosa. *Am. J. Clin. Nutr.* 1999;69:656-63.
41. Chong NH, Bird AC. Management of inherited outer retinal dystrophies: present and future. *Br. J. Ophthalmol.* 1999;83:120-2.
42. Rizzo JF, 3rd, Wyatt J, Humayun M, de Juan E, Liu W, Chow A, Eckmiller R, Zrenner E, Yagi T, Abrams G. Retinal prosthesis: an encouraging first decade with major challenges ahead. *Ophthalmology* 2001;108:13-4.
43. Chow AY, Pardue MT, Perlman JI, Ball SL, Chow VY, Hetling JR, Peyman GA, Liang C, Stubbs EB, Jr., Peachey NS. Subretinal implantation of semiconductor-based photodiodes: durability of novel implant designs. *J. Rehabil. Res. Dev.* 2002;39:313-21.
44. Margalit E, Maia M, Weiland JD, Greenberg RJ, Fujii GY, Torres G, Piyathaisere DV, O'Hearn TM, Liu W, Lazzi G, Dagnelie G, Scribner DA, de Juan E, Jr., Humayun MS. Retinal prosthesis for the blind. *Surv. Ophthalmol.* 2002;47:335-56.
45. Ma N, Streilein JW. T cell immunity induced by allogeneic microglia in relation to neuronal retina transplantation. *J. Immunol.* 1999;162:4482-9.
46. Ma N, Streilein JW. Contribution of microglia as passenger leukocytes to the fate of intraocular neuronal retinal grafts. *Invest. Ophthalmol. Vis. Sci.* 1998;39:2384-93.
47. Larsson J, Juliusson B, Holmdahl R, Ehinger B. MHC expression in syngeneic and allogeneic retinal cell transplants in the rat. *Graefes Arch. Clin. Exp. Ophthalmol.* 1999;237:82-5.
48. Mohand-Said S, Deudon-Combe A, Hicks D, Simonutti M, Forster V, Fintz AC, Leveillard T, Dreyfus H, Sahel JA. Normal retina releases a diffusible factor stimulating cone survival in the retinal degeneration mouse. *Proc. Natl. Acad. Sci. U. S. A.* 1998;95:8357-62.
49. Mohand-Said S, Hicks D, Dreyfus H, Sahel JA. Selective transplantation of rods delays cone loss in a retinitis pigmentosa model. *Arch. Ophthalmol.* 2000;118:807-11.
50. Faktorovich E, Steinberg RH, Yasumura D, Matthes MT, LaVail MM. Photoreceptor rescue in retinal degenerations by basic fibroblast growth factor. In: Anderson RE, Hollyfield JG, LaVail MM, editors. *Retinal Degenerations*. Boca Raton, FL: CRC Press, 1991:101-116.
51. Faktorovich EG, Steinberg RH, Yasumura D, Matthes MT, LaVail MM. Basic fibroblast growth factor and local injury protect photoreceptors from light damage in the rat. *J. Neurosci.* 1992;12:3554-3567.
52. Uteza Y, Rouillot JS, Kobetz A, Marchant D, Pecqueur S, Arnaud E, Prats H, Honiger J, Dufier JL, Abitbol M, Neuner-Jehle M. Intravitreal transplantation of encapsulated fibroblasts secreting the human fibroblast growth factor 2 delays photoreceptor cell degeneration in Royal College of Surgeons rats. *Proc. Natl. Acad. Sci. U. S. A.* 1999;96:3126-31.
53. LaVail MM, Unoki K, Yasumura D, Matthes MT, Yancopoulos GD, Steinberg RH. Multiple growth factors, cytokines, and neurotrophins rescue photoreceptors from the damaging effects of constant light. *Proc. Natl. Acad. Sci. U. S. A.* 1992;89:11249-53.

54. Chong NH, Alexander RA, Waters L, Barnett KC, Bird AC, Luthert PJ. Repeated injections of a ciliary neurotrophic factor analogue leading to long-term photoreceptor survival in hereditary retinal degeneration. *Invest. Ophthalmol. Vis. Sci.* 1999;40:1298-305.
55. Liang FQ, Dejneka NS, Cohen DR, Krasnoperova NV, Lem J, Maguire AM, Dudas L, Fisher KJ, Bennett J. AAV-mediated delivery of ciliary neurotrophic factor prolongs photoreceptor survival in the rhodopsin knockout mouse. *Mol. Ther.* 2001;3:241-8.
56. Wahlin KJ, Campochiaro PA, Zack DJ, Adler R. Neurotrophic factors cause activation of intracellular signaling pathways in Muller cells and other cells of the inner retina, but not photoreceptors. *Invest. Ophthalmol. Vis. Sci.* 2000;41:927-36.
57. Mohand-Said S, Hicks D, Leveillard T, Picaud S, Porto F, Sahel JA. Rod-cone interactions: developmental and clinical significance. *Prog. Retin. Eye Res.* 2001;20:451-67.
58. Sagdullaev BT, Aramant RB, Seiler MJ, Woch G, McCall MA. Restoration of visual activity by retinal transplantation in a rodent model of retinitis pigmentosa. *Invest. Ophthalmol. Vis. Sci.* 2003;44:in press.
59. Aramant RB, Seiler MJ, Woch G. Retinal transplants form synaptic connections with host retinas with photoreceptor degeneration - demonstrated by transsynaptic tracing from host brain [ARVO abstract]. *Invest. Ophthalmol. Vis. Sci.* 2000;41 (Suppl.):S101; program # 528.

FIGURES AND TABLES

FIGURES:

Figure 1. Fundus images: Arrows indicate the same blood vessel landmark in all images. A) 3 weeks prior to transplantation. B) 2 weeks after transplantation, showing heavy pigmentation of transplant. The transplant area is outlined by white dots. C) 6 months after transplantation: loss of pigment. A white scar is recognizable at the retinotomy site. White dots outline the same area as in B). D) 12 months after transplantation – no change compared to 6 months.

Figure 2. Fluorescein Angiograms: Arrows indicate the same blood vessel landmark in all images (same as in Fig. 1). The transplant area (same area as in Figs. 1B-D) is outlined by white dots. A) 3 weeks before transplantation. B) 6 months after transplantation. C) 9 months after transplantation. D) 12 months after transplantation. No fluorescein leakage in transplant area.

Figure 3: Scanning Laser Ophthalmoscope (SLO): Top – Microperimetry of operated eye. Seeing areas are indicated as filled white squares, non-seeing areas as open white squares; fixation points are indicated by black crosses. The microperimetry data indicate that fixation is not stable, and sometimes involves retina over the transplant area as well as retina adjacent to the transplant. The transplant area is outlined by black-on-white dots. The operated eye contained 53 seeing and 37 non-seeing areas. In contrast, the non-operated eye contained 33 seeing and 37 non-seeing areas. Bottom – The patient fixated a large size horizontal black “E” at the nasal edge of the transplant, but outside of the area of the transplant. The patient could not consistently see the E in this location.

Figure 4: Overlay of SLO fixation image and fundus photograph of the transplant at 2 weeks postoperatively. Vascular landmarks were used to superimpose the two images accurately. The

figure shows that the patient used retina nasal to the nasal edge of the transplant to view the 20/270 letter.

TABLES:

Table 1:

DONOR HLA TYPING	RECIPIENT HLA TYPING
HLA-A1	HLA-A2
HLA-A30	HLA-A3
HLA-B13	HLA-B14
HLA-BW4	HLA-BW6
HLA-B14	HLA-B27
HLA-BW6	HLA-BW4
HLA-DR17	HLA-DR1
HLA-DR7	HLA-DR13