Title

Vision Improvement in Retinal Degeneration Patients by Implantation of Retina Together with Retinal Pigment Epithelium

Journal

American Journal of Ophthalmology, 146(2)

ISSN

0002-9394

Authors

Radtke, Norman D
Aramant, Robert B
Petry, Heywood M
et al.

Publication Date

2008-08-01

DOI

10.1016/j.ajo.2008.04.009

Peer reviewed

Vision Improvement in Retinal Degeneration Patients by Implantation of Retina Together with Retinal Pigment Epithelium

NORMAN D. RADTKE, ROBERT B. ARAMANT, HEYWOOD M. PETRY, PARKE T. GREEN, DIANE J. PIDWELL, AND MAGDALENE J. SEILER

• PURPOSE: **To demonstrate efficacy and safety of the implantation of neural retinal progenitor cell layers (sheets) with its retinal pigment epithelium (RPE) in retinitis pigmentosa (RP) and dry age-related macular degeneration (AMD) patients with 20/200 or worse vision in the surgery eye.**

• DESIGN: **Interventional nonrandomized clinical trial.**

• METHODS: **Ten patients (six RP, four AMD) received retinal implants in one eye and were followed in a phase II trial conducted in a clinical practice setting. Early Treatment Diabetic Retinopathy Study (EDTRS) was the primary outcome measure. All implant recipients and nine of 10 tissue donors were deoxyribonucleic acids typed.**

• RESULTS: **Seven patients (three RP, four AMD) showed improved EDTRS visual acuity (VA) scores. Three of these patients (one RP, two AMD) showed improvement in both eyes to the same extent. Vision in one RP patient remained the same, while vision in two RP patients decreased. One RP patient has maintained an improvement in vision from 20/800 to 20/200 ETDRS for more than five years; at the six-year examination, it was still maintained at 20/320 while the nonsurgery eye had deteriorated to hand motion vision. This patient also showed a 22.72% increase in light sensitivity at five years compared to microperimetry results at two years; the other patients showed no improved sensitivity. Although no match was found between donors and recipients, no rejection of the implanted tissue was observed clinically.**

• CONCLUSIONS: **Seven (70%) of 10 patients showed improved VA. This outcome provides clinical evidence of the safety and beneficial effect of retinal implants and corroborates results in animal models of retinal degeneration. (Am J Ophthalmol 2008;146:172–182. © 2008 by Elsevier Inc. All rights reserved.)**

See accompanying Editorial on page 151.

Accepted for publication Apr 7, 2008.

From the Retina Vitreous Resource Center (N.D.R.), Anatomical Sciences and Neurobiology (R.B.A.), Ophthalmology andVisual Sciences (R.B.A., M.J.S.), and Psychological and Brain Sciences (H.M.P.), University of Louisville, Louisville, Kentucky; Doheny Retina Institute (R.B.A., M.J.S.), Department of Ophthalmology, University of Southern California, Los Angeles, California; Anatomy and Neurobiology (R.B.A., M.J.S.), UC Irvine, Irvine, California; Nidek Inc, Fremont, California (P.T.G.); Jewish Hospital, Louisville, Kentucky (D.J.P.); and Transplantation Center, The Cleveland Clinic, Cleveland, Ohio (D.J.P.).

Inquiries to Norman D. Radtke, Retina Vitreous Resource Center, 240 Audubon Medical Plaza, Louisville, KY 40217; e-mail: nradtke@rvrc.com

RETINITIS PIGMENTOSA (RP) DENOTES A GROUP OF inherited disorders that involve progressive loss of photoreceptors or retinal pigment epithelium (RPE)[1] and progressive visual impairments. Age-related macular degeneration (AMD) is the leading cause of blindness in the population older than 55 in the United States[2] and Western Europe. Geographic atrophy (GA) of the RPE is a form of advanced AMD that is associated with central vision loss,[2] progresses gradually, and does not regress over time.[3]

In each of these degenerative disorders, if the damaged photoreceptors can be replaced with new cells that connect with the remaining host retina,[4,5] it may be possible to restore visual function. Human fetal donor tissue transplanted to athymic nude rats can develop retinal layers in contact with a monolayer of cotransplanted RPE.[6] Transplants of fetal retinal layers have also been shown to restore visual function in the brain in several models of retinal degeneration[7,8] and to make synaptic connections with the neural circuitry of the host retina.[9] After injecting freshly harvested retinal progenitor cells in the rho$^{-/-}$ mouse, increased ganglion cell responses and pupillary reflexes have been interpreted as visual improvements,[10] similar to previous results by other groups.[11,12] However, the reliability of ganglion cell recordings and pupillometry for establishing transplant effects has been questioned.[13,14]

Limited clinical trials of retinal implantation have been underway since 1998. Between January 1, 1998 and January 1, 2001, phase I of a clinical trial was performed to demonstrate the safety of implanting freshly harvested layers of fetal retina together with its RPE. Results showed the safety of the procedure.[15]

The Food and Drug Administration (FDA) then allowed the surgery to be performed in patients with 20/200 vision or worse in the surgery eye. A phase II trial was conducted between 2002 and 2005 with a group of 10 patients (six RP and four AMD). This article presents the results.

METHODS

• STUDY DESIGN: The study was performed in a clinical practice setting. Subjects had 1) decreased central visual acuity (VA) of 20/200 or worse by Early Treatment

0002-9394/08/$34.00
doi:10.1016/j.ajo.2008.04.009

Diabetic Retinopathy Study (ETDRS) vision testing for at least one year before the procedure in the surgery eye, 2) a diagnosis of RP or AMD, and 3) better vision in the nonsurgery eye than the surgery eye. There was no control group. Success was defined as preservation or improvement of vision after one year as measured by EDTRS.

Subjects older than 21 years of age were recruited from Dr Radtke's private practice or via the practice's website. All applicants met strict inclusion/exclusion criteria. Qualified subjects were given detailed information about the study and informed consent was obtained. The Western Institutional Review Board (IRB), FDA, or Norton Healthcare Research Office did not require a data and safety monitoring committee for this study, so one was not appointed. The Investigational New Drug Application (IND) number was very stringent and subject to close FDA scrutiny.

• **DONOR TISSUE:** Donor tissues were derived from human fetal eyes, age range 10 to 15 weeks gestation, isolated from dead macerated fetuses after elective abortions. Donors were not compensated or approached to donate tissue until after the decision was made to terminate the pregnancy. All donors signed an informed consent form prior to donation.

• **IMPLANTATION METHOD AND SURGICAL PROCEDURE:** The dissection of fetal retina together with its RPE has been described previously.[6,15] Eyeballs were dissected free from surrounding tissues and incubated in dispase (Collaborative Biomedical Products, Bedford, Massachusetts, USA) for 10 to 30 minutes. The final dissection was performed in the surgery room.

A standard three-port 20-gauge vitrectomy was performed, excising the posterior hyaloid face in all cases. Immediately before implantation, 2-mm^2 to 5-mm^2 pieces of fetal retina with its RPE were cut (average 3.8 mm^2) under a dissection microscope and loaded into a custom-made implantation instrument with a flat plastic nozzle tip at a 130-degree angle.[15,16] The implantation instrument maintained the orientation of the donor tissue. After a retinotomy superior nasal to the fovea, the surgeon inserted the loaded nozzle tip into the subretinal space in the macular area and then released the nozzle, which placed prepared sheets of retina/RPE into the target area. The retinotomy was sealed by laser. Surgery success was defined as good placement of the tissue under the fovea with completely flat recipient retina and good sealing of the retinotomy superior nasal to the fovea. Immediately after implantation, the medium in which the donor tissue had been prepared was tested for sterility and endotoxins.

No immunosuppressant drugs were given. Six patients (Patients 1, 6, 7, 8, 9, and 10) received oxygen postoperatively to reduce surgical trauma.[17,18] Patient 1 was on a ventilator postoperatively and received oxygen for 10 days; the other patients received nasal cannula oxygen for one week. Since visual stimulation during development is important for the formation of synaptic connectivity (review[19]), these same patients were also asked to watch experimental visual stimulation videos on a volunteer basis. The videos consisted of moving dots and stripes with different colors and were developed by a member of this research team, who discussed their design with a low-vision specialist, a psychophysics specialist, and several patients.

• **IMMUNOLOGY TESTS:** All retinal implant recipients and nine of the 10 donors were deoxyribonucleic acids (DNA) typed for major histocompatibility complex (MHC) antigens HLA-A, B, C, DR, and DQ at Jewish Hospital (Louisville, Kentucky, USA). Recipients were typed after signing the consent form, well in advance of the transplant surgery. One donor could not be typed because of poor tissue quality. The donor DNA was extracted using a DNA tissue extraction kit (QIAGEN, Valencia, California, USA) and typed for HLA antigens by polymerase chain reaction amplification using sequence-specific primers (Pel Freeze, Brown Deer, Wisconsin, USA).

At each recipient follow-up visit, a blood sample was tested for donor-specific antibodies. Anti-HLA antibodies were detected using two techniques with sensitive flow cytometric procedures: a pool of normal T cells and HLA antigen–coated beads (One Lambda, Canoga Park, California, USA).

• **STUDY EVALUATION METHODS:** All patients were followed for one year and offered annual follow-up examinations. Four patients elected to participate in these optional annual exams (Patient 3 for two years, Patients 4 and 5 for three years, and Patient 1 for six years). All patients received a clinical examination, funduscopy, and fluorescein angiography (FA); the nonsurgery eye was also examined postoperatively to rule out sympathetic ophthalmia at each examination time point (three times preoperatively; and one week and one, three, six, nine, and 12 months postoperatively). A complete refraction was performed on each patient before VA was measured by EDTRS. All patients also had their lens removed and had an intraocular lens implant in the posterior chamber with the capsule opened preoperatively.

Early Treatment Diabetic Retinopathy Study vision was tested three times prior to surgery and at least seven times postoperatively using a previously described protocol,[20] with an ESV-3000 Illuminator cabinet (Vector Vision, Greenville, Ohio, USA; test luminance 85 cd/m^2). Testing was performed at 4 m; measurements at 1 m were performed when fewer than 15 letters were read at 4 m. The total ETDRS letters correctly identified were counted until one line (5 letters) was completely missed. The line above was then converted to Snellen acuity by measuring the distance of the patient from the chart, whether 4 m or 1 m. This value was divided into 20 and multiplied by the meter

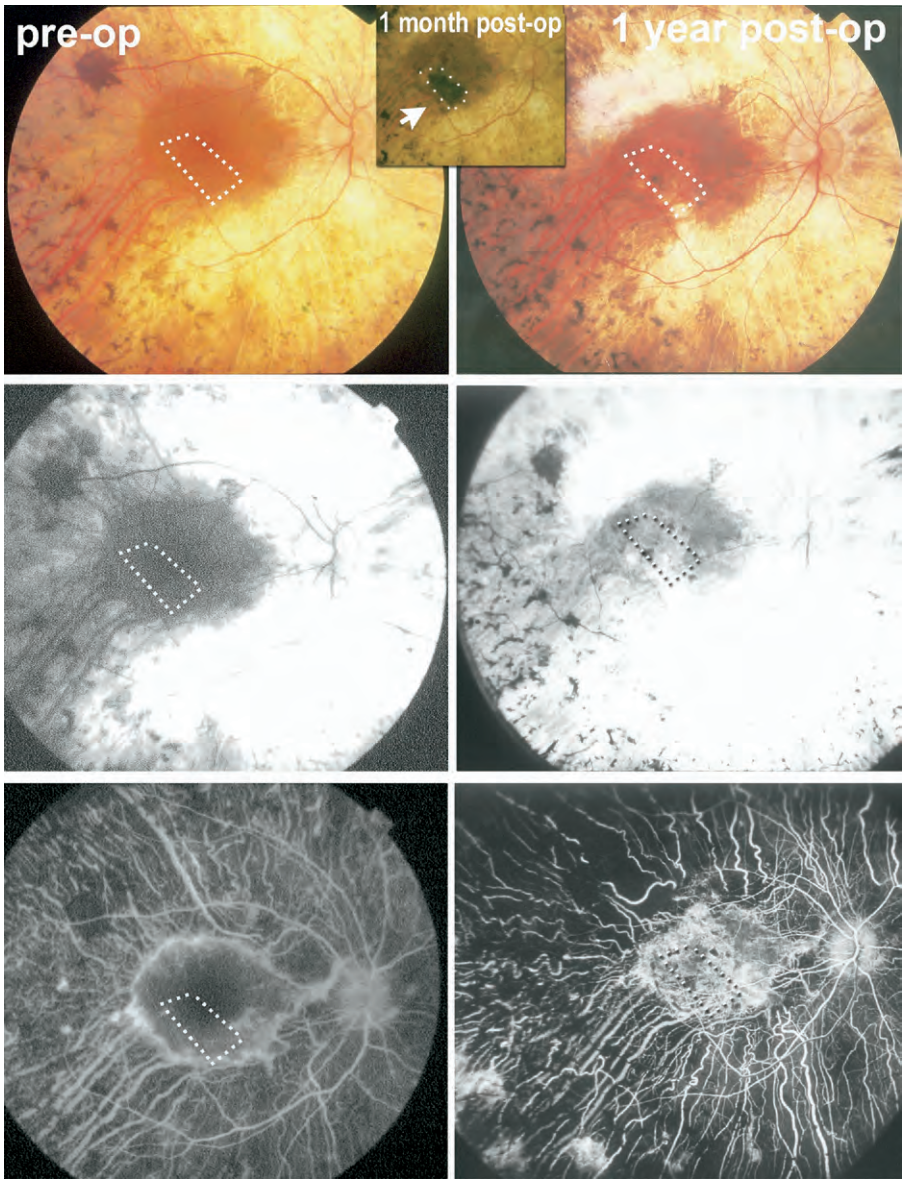

FIGURE 1. Study Patient 8 with retinitis pigmentosa (RP). The left column shows preoperative fundus images and the right column shows postoperative images. Position of the transplant is indicated by dotted rectangle. (Top left) Preoperative fundus. (Top right) Fundus at one year postoperative. (Top, small image insert) Fundus at one month postoperative with the position of the pigmented transplant indicated (arrow). (Middle left) Early fluorescein angiograph (FA), preoperative. (Middle right) Early FA, one year postoperative. (Bottom left) Late FA, preoperative. (Bottom right) Late FA, one year postoperative. The fundus images and FA document the presence of choriocapillaris underneath the transplant with no leakage. This implies evidence of no clinical rejection. Pre-op = preoperative; Post-op = postoperative.

number next to each line on the left side of the chart. For example, if the patient reads the 40-m line and is 4 m away, the vision is 20/200 by Snellen. ETDRS tests were performed in the clinical practice of Dr Radtke and at an independent test site (Patients 1, 3, 4, and 5 were tested only in Dr Radtke's practice). Testers were masked to the surgery eye. If there was a variation between test sites, the results from the independent site were reported.

Secondary evaluation methods used at the beginning of the study were scanning laser ophthalmoscopy (SLO) and multifocal electroretinography (mfERG). As the study progressed and new imaging technology became available, optical coherence tomography (OCT) and microperimetry (MP1) replaced the SLO and mfERG methods. MP1 tests were performed in the clinical practice of Dr Radtke. OCT tests were done in Louisville, Kentucky, at the clinical practices of Drs Radtke and Kumar. SLO and OCT tests were also performed at the University of Medicine and Dentistry of New Jersey (UMDNJ), Newark, New Jersey, and mfERG tests at The Smith-Kettlewell Eye Research

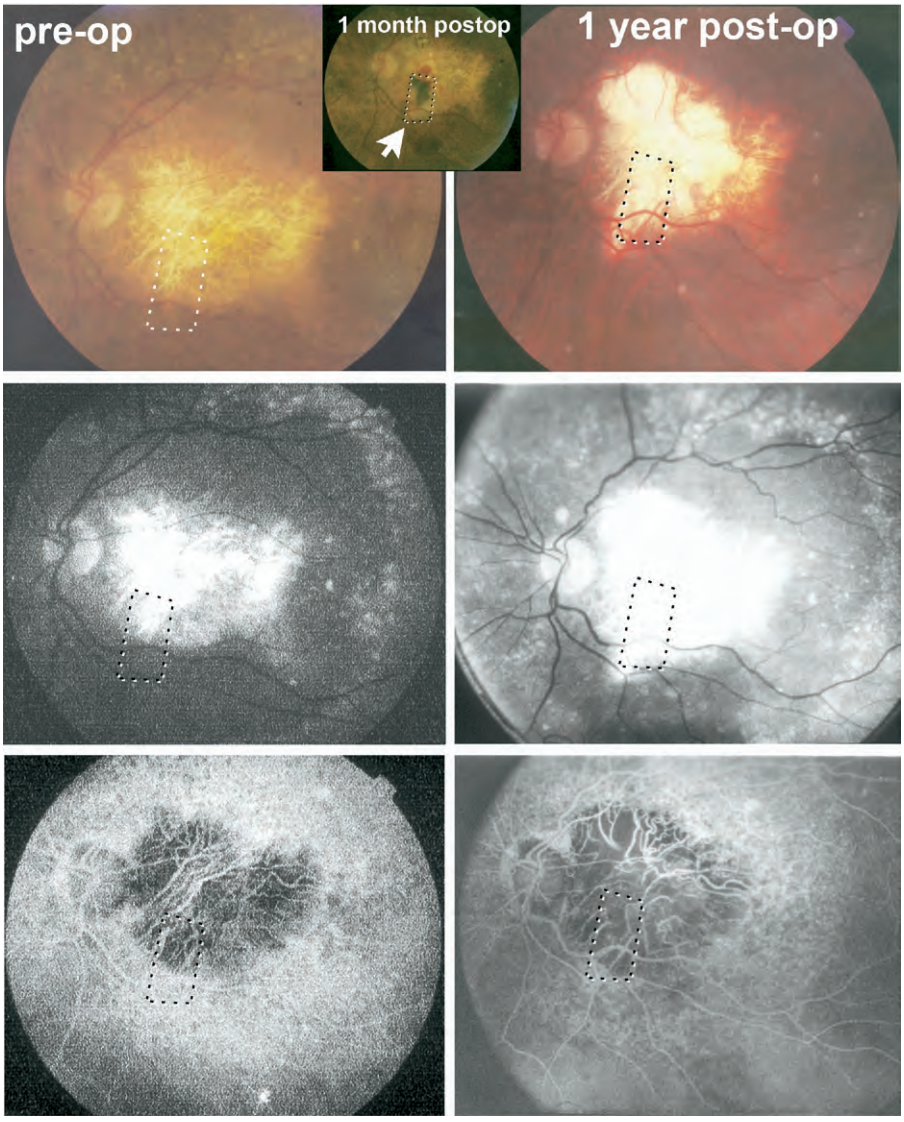

FIGURE 2. Study Patient 7 with age-related macular degeneration. The left column shows preoperative fundus images and the right column shows postoperative images. Position of the transplant is indicated by dotted rectangle. (Top left) Preoperative fundus. (Top right) Fundus at one year postoperative. (Top, small image insert) Fundus at one month postoperative with the position of the pigmented transplant indicated (arrow). (Middle left) Early FA, preoperative. (Middle right) Early FA, one year postoperative. (Bottom left) Late FA, preoperative. (Bottom right) Late FA, one year postoperative. The fundus images and FA document the presence of choriocapillaris underneath the transplant with no leakage. This implies evidence of no clinical rejection.

Institute, San Francisco, California. Again, testers were masked to the identity of the surgery eye.

Scanning laser ophthalmoscopy tests with a small, dim stimulus were performed on three RP patients preoperatively and one AMD and two RP patients postoperatively. The reference cross was fixed by manual tracking at clearly defined vascular landmarks.[21] Photopic mfERGs were recorded (VERIS Science software; EDI Inc, San Mateo, California, USA) as previously described.[22] These recordings were performed three times preoperatively on four patients (two RP and two AMD) and five times postoperatively on three patients (two RP and one AMD). Several subjects were unable to travel the distance to the independent test sites for SLO and mfERG tests. These two evaluation methods were discontinued as full retina MP1 testing proved more accurate.

As stated above, OCT and MP1 tests were introduced later in the study. OCT tests were performed preoperatively and postoperatively on two RP patients and two AMD patients, and postoperatively on one AMD patient. MP1 tests with fixation and sensitivity (MP1; Nidek Advanced Vision Information System (NAVIS); Nidek Technologies, Vigonza, Italy) were performed on three patients preoperatively (one RP and two AMD) and five patients postoperatively (two RP and three AMD). MP1 continuously registers fixation behavior by automated real-

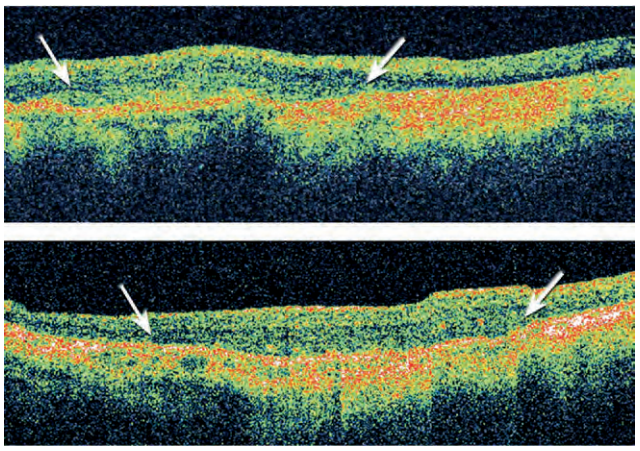

FIGURE 3. Study Patient 8 with RP. This optical coherence tomography of implant at one month (Top) and one year postoperatively (Bottom) shows the area (arrows) with thickened retina indicating implant. The retinal pigment epithelium and choroid are highly reflective (red).

time fundus tracking and alignment[21] and digitally registers the infrared photograph from the current to the previous test, allowing use of the same pattern and precise placement of the pattern's previous location.

RESULTS

• **IMPLANT SURVIVAL:** No rejection of the implanted tissue was observed clinically. No leakage was observed on FAs, suggesting that the implanted RPE cells could have reestablished a new blood-retinal barrier. Figures 1 and 2 show examples of RP Patient 8 and AMD Patient 7, respectively. The pigmentation of the transplant was lost in eight of 10 cases at three months postoperatively. Figure 3 shows OCTs of Patient 8 at one month and one year postoperatively.

• **IMMUNOLOGY TESTS:** All typed donor-recipient pairs had at least one antigen mismatch at each of the A, B, and DR loci (Table 1). These mismatches indicate that the grafts had the potential to be recognized as foreign by the recipient's immune system.

Recipients were tested for anti-HLA antibody preimplant and postimplant. Four female recipients (Patients 1 and 3, RP; Patients 10 and 6, AMD) had demonstrable anti-HLA antibody preimplant either from exposure through pregnancy or via blood transfusion. Several of these antibodies were specific for HLA antigens present on the retinal grafts but no new donor-specific antibody developed throughout the follow-up period. Patient 2 had a negative antibody screen pretransplant and developed an antibody-like reactivity at 10 months postimplant that was entirely absent in the subsequent sample at 15 months postimplant. The anti-HLA specificity of this reactivity could not be defined because of nonspecific reactivity of this patient's serum with the latex beads used in the assay. In Patients 1 and 10, the antibody titers increased at nine and 12 months postimplant, respectively. However, the HLA specificity did not change, indicating that no de novo anti–donor-specific antibody developed. In Patient 1, the antibody titer returned to preimplant levels in a sample collected three months after the apparent increase in titer. For Patient 10, the increase in titer was detected in the last sample collected and no subsequent samples were available to follow the titer. In this case, as was true for all patients with reactivity in the antibody screening assays, the specificity of the antibodies present did not change during the increase in titer. In all cases, the increase was not restricted to the antibody specific to the donor antigen but all specificities increased simultaneously. This pattern of activation may be seen in cases of infection, vaccination, or generalized immune activation and is not typical of a donor-specific response, although this cannot be entirely excluded. Patient 6 had weak antibody but the titer never increased over the two-year time period tested. With the exception of Patient 2, no de novo donor-specific antibody was detected postimplant. Anti-HLA antibody detected preimplant persisted postimplant in all cases. With the exception of Patient 2, whose anti-HLA specificity could not be defined, there were no instances where a patient developed de novo antibody that could be demonstrated to be specific for an HLA antigen present in their donor. Interestingly, all five patients with preimplant anti-HLA antibody had visual improvement (Tables 2 and 3).

• **EARLY TREATMENT DIABETIC RETINOPATHY STUDY RESULTS:** Tables 2 and 3 present the results of ETDRS testing. All subjects were evaluated three times preoperatively and at least seven times postoperatively. Four patients elected to participate in optional annual follow-up exams (Patient 1 for six years; Patients 4 and 5 for three years, Patient 3 for two years). Clinical evidence of visual improvement by ETDRS was seen in seven of 10 patients (three RP, four AMD). In Patients 1 and 3 (RP) and 5, 6, and 7 (AMD), the vision in the surgery eye improved, whereas the vision in the nonsurgery eye did not change significantly or worsened. Patients 2 (RP) and 10 (AMD) showed vision improvement in both eyes to the same extent. Patients 4 and 9 (RP) showed a decrease in vision, and Patient 8 (RP) remained the same. Patient 1 (RP) has maintained a previously reported[23] vision improvement of 20/800 to 20/200 ETDRS for five years; at the six-year examination it was still maintained at 20/320 while the nonsurgery eye had deteriorated to hand motion vision (see Table 2).

• **OTHER RESULTS:** No changes were observed in the mfERG tests. The SLO tests showed an improvement in VA in Patient 1 between nine months and 27 months postoper-

TABLE 1. Recipient and Donor Human Lymphocyte Antigen Tissue Types

Recipient/Donor	A	B	C	Bw4/6	DR	DR51/52/53	DQ
Patient 1	2, 3	***14***, 27		***4, 6***	1, 13	DR***52***	5, 6
Patient 1 donor	1, 30	13, ***14***	6, 8	***4, 6***	17, 7	DR***52***, 53	2, -
Patient 2	24, 33	13, 14	6, 8	***4***, 6	1, 7	DR***53***, -	***2***, 5
Patient 2 donor	11, -	44, 49	7, 16	***4,*** -	7, 13	DR52, ***53***	***2***, 6
Patient 3	***2***, 23	44, 57	4, 6	4, -	7, -	DR***53***, -	***2***, -
Patient 3 donor	1, ***2***	8, -	7, -	6, -	17, 7	DR52, ***53***	***2***, -
Patient 4	11, -	35, -	15, -	***6***, -	14, -	DR***52***, -	5, -
Patient 4 donor	23, 24	44, 81	4, 18	4, ***6***	7, 11	DR***52***, 53	2, 6
Patient 5	***2***, -	27, 50	6, -	4, ***6***	1, 7	DR53, -	***2***, 5
Patient 5 donor	1, ***2***	8, 45	7, 16	***6, -***	17, 15	DR52, 51	***2***, 6
Patient 6	***1***, 30	***8***, 52	***7, 16***	***4, 6***	17, 18	DR52	2, ***4***
Patient 6 donor	***1***, -	***8***, 51	***7, 16***	***4, 6***	4, 8	DR53	***4***, 8
Patient 7	***1***, 26	39, ***57***	***6, 7***	***4, 6***	7, 16	DR***53null,51***	5, ***9***
Patient 7 donor	***1***, 3	7, ***57***	***6, 7***	***4, 6***	7, 15	DR***53null,51***	6, ***9***
Patient 8	2, 32	*7, 44*	5, *7*	***4, 6***	8, 11	DR52	4, 7
Patient 8 donor	1, 3	*7, 57*	6, *7*	***4, 6***	7, 15	DR53null,51	6, 9
Patient 9	***2***, 30	51, 57	*7, 14*	4, -	11, ***15***	DR52, ***51***	***6***, 7
Patient 9 donor	***2***, 32	7, 60	*7, 10*	6, -	4, ***15***	DR53, ***51***	***6***, 8
Patient 10	1, 29	7, 44	7, 16	4, 6	7, 15	DR53, 51	2, 6
Patient 10 donor				No type available			

Bold italic indicates a recipient/donor match. As the data show, donors and recipients are mismatched in most human lymphocyte antigens (HLA). No tissue for HLA testing was available for the LC donor.

TABLE 2. Results of Study Patients with Retinitis Pigmentosa

Patient ID and Age at Implant	Vision of Surgery Eye (ETDRS)					Vision of Nonsurgery Eye (ETDRS)		
		Postoperative		Change @ 1 Year (decimal)	Last Result of Poststudy Surveillance			
	Preoperative	6 Months	1 Year			Preoperative	Vision to Date	Change @ 1 Year (decimal)
1	20/800 OS	20/400	20/160	Pos	6 years (02/08)	20/400 OD	HM	NC
62	4.0 (02/02)	2.0	0.9	+3.1	20/320 1.8	2.0 (02/02)	5.0 (02/08)	+0.2
2	LP OS	HM	HM	Pos	1 year (04/04)	LP OD	HM	Pos
51	6.0 (02/03)	5.0	5.0	+1.0	HM 5.0	6.0 (02/03)	5.0 (04/04)	+1.0
3	HM OD	20/640	20/400	Pos	2 years (06/05)	20/400 OS	20/640	Neg
41	5.0 (03/03)	3.5	2.0	+3.0	CF @ 3 feet	2.0 (03/03)	3.5 (07/04)	−1.5
4	20/640 OD	20/640	20/800	Neg	3 years (03/07)	20/800 OS	20/800	NC
43	3.5 (10/03)	3.5	4.0	−0.5	20/800 4.0	4.0 (10/03)	4.0 (03/07)	±0
8	LP OD	LP	LP	NC	1 year (10/06)	20/250 OS	20/200	Pos
69	6.0 (06/05)	6.0	6.0	±0	LP 6.0	1.6 (06/05)	1.0 (10/06)	+0.6
9	20/320 OS	LP	LP	Neg	1 year (3/07)	20/160 OD	20/160	NC
81	1.8 (12/05)	6.0	6.0	−4.2	LP 6.0	0.9 (12/05)	0.9 (03/07)	±0
	Average change in surgery eye ± SEM			0.4 ± 1.1		Average change in nonsurgery eye ± SEM		0.05 ± 0.35

CF = count fingers; ETDRS = Early Treatment Diabetic Retinopathy Study; HM = hand motion; LP = light perception; NC = no change; Neg = vision deterioration; OD = right eye; OS = left eye; Pos = vision improvement; SEM = standard error of mean.

For calculation of change in decimal vision values, HM was set as 5.0 and LP was set as 6.0. There is no significant difference in the average change between surgery eye and nonsurgery eye, but the standard deviations are significantly different.

atively.[23] MP1 tests showed no improved sensitivity with the exception of Patient 1 (see Figure 4). Her MP1 results showed a defined area of cluster points just nasal and adjacent to the implant over which the average sensitivity was calculated to have increased from 1.6 decibels [dB] (year three postoperatively) to 2.7 dB or 22.72% (year five postoperatively). This result correlates well with her improved ETDRS VA score and subjective function.

TABLE 3. Results of Study Patients with Age-Related Macular Degeneration

Patient ID and Age at Implant	Vision of Surgery Eye (ETDRS)					Vision of Nonsurgery Eye (ETDRS)		
		Postoperative						
	Preoperative	6 Months	1 Year	Change @ 1 Year (decimal)	Last Result of Poststudy Surveillance	Preoperative	Vision to Date	Change @ 1 Year (decimal)
5	20/640 OD	20/240	20/400	Pos	3 years (04/07)	20/160 OS	20/200	NC
88	3.6 (02/04)	1.4	2.0	+1.6	20/400 2.0	0.9 (01/03)	1.0 (04/07)	−0.1
6*	20/400 OS	20/240	20/200	Pos	1 year (10/06)	20/200 OD	20/200	NC
76	2.0 (08/05)	1.4	1.0	+1.0	20/200 1.0	1.0 (08/05)	1.0 (10/06)	±0
7	20/400 OS	20/340	20/160	Pos	1 year (10/06)	20/60 OD	20/200	Neg
71	2.0 (08/05)	1.8	0.9	+1.1	20/160 0.9	0.5 (08/05)	1.0 (10/06)	−0.5
10	20/400 OS	20/320	20/260	Pos	1 year (04/07)	20/240 OD	20/160	Pos
76	2.0 (03/06)	1.8	1.5	+0.5	20/260 1.5	1.4 (03/06)	0.9 (04/07)	+0.5
	Average change in surgery eye ± SEM			1.05 ± 0.22		Average change in nonsurgery eye ± SEM		−0.02 ± 0.2

ETDRS = Early Treatment Diabetic Retinopathy Study; NC = no change; Neg = vision deterioration; OD = right eye; OS = left eye; Pos = vision improvement; SEM = standard error of mean.

Difference between change in surgery eye and change in nonsurgery eye appears significant ($P < .05$); however, too few values for statistical comparisons.

*Patient 6's preoperative vision was very variable, between 20/260 and 20/640 in the surgery eye (mean, 2.27 ± 0.23) and between 20/200 and 20/640 in the nonsurgery eye (mean, 1.89 ± 0.21) (average of 11 different measurements). The other patients did not show such variability. The values shown in the table are from the last test before surgery in August 2005.

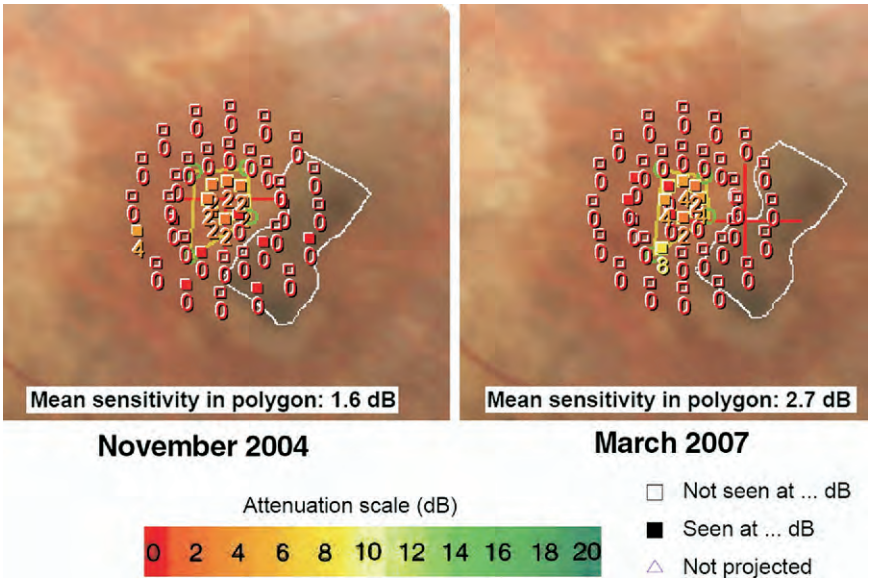

FIGURE 4. Study Patient 1 with RP. Results of microperimetry MP1 testing show retina at three years postimplant (Left) and five years postimplant (Right). A polygon in yellow and green highlights the area of interest and the average sensitivity within that area is calculated. Improvement in sensitivity is evident in the area of the polygon adjacent to the graft area. In the left image, taken in November 2004, the mean sensitivity in the polygon area is 1.6 decibels (dB); in the right image, taken in March 2007, the mean sensitivity is 2.7 dB. The 22.72% increase in sensitivity of 1.1 dB, from 1.6 dB to 2.7 dB, corresponded well with improved visual acuity by Early Treatment Diabetic Retinopathy Study and the patient's subjective assessment.

DISCUSSION

NO EFFECTIVE TREATMENT EXISTS FOR THE RECOVERY OF visual loss from RP and AMD. Oral vitamin A therapy has slowed the rate of electroretinogram loss in RP but cannot recover lost vision.[24] Gene and pharmacologic therapies are also being studied. A clinical trial of gene therapy in Leber congenital amaurosis[24] is underway, and Neurotech has started phase II clinical trials for ciliary neurotrophic factor (CNTF) for RP.[25] Multiple

centers[26–28] are actively pursuing development and use of an artificial retina.

This study demonstrates visual improvement as measured by ETDRS in seven of 10 patients. In our study, RPE absence or presence at one year in the transplant area, genetic typing, gender, or age of patients did not correlate with improvement in vision. Multiple variables such as size of implant, time from harvest to implantation, duration of surgery, fetus age, or mode of inheritance (autosomal dominant [AD], autosomal recessive [AR], gender-linked, or sporadic) were not significant in predicting visual outcomes. Without treatment, the likely outcome was that the vision of all subjects in the study would have deteriorated further.

No surgical complications occurred. The custom-made implantation instrument maintains proper orientation of the fragile donor tissue and allows the surgeon to gently place the tissue flat, without roll-up, and without any push or injection pressure and minimal additional fluid. This eliminates the requirement for a subretinal fluid bleb and thus minimizes trauma to the implant and host retina.[16]

At more than five years postoperatively, no graft encapsulation, tissue destruction, or macular edema indicating rejection was seen clinically or with FA on any subjects. However, without histologic data, the presence of more subtle graft rejection cannot be excluded. Previous reports of human retinal implantation have varied in the incidence of rejection depending on whether the implant involved patches of cultured RPE cells[29] or dissociated cells and whether the patient had exudative or nonexudative manifestations of AMD.

Human lymphocyte antigen typing of recipients and donors confirmed that none of the implants were HLA matched. In solid organ transplant, HLA mismatches at the class II DR locus are particularly conducive to immune recognition and rejection episodes.[30] In all cases here, there was at least one DR antigen mismatch, indicating that DR matching did not contribute to graft survival. Several patients that had previously been sensitized to HLA antigens demonstrated increased titers of the antibody they were previously sensitized to. However, transient increases in antibody titer without any new antibody specificity can develop after many manipulations that have pro-inflammatory effects, such as surgeries or vaccinations.[31] Since no patients developed new antibody specificity directed toward their retinal donor, there is evidence that the patients' immune systems were not recognizing the donor antigens and responding to them. Frequently, if patients are re-exposed to an antigen they have been sensitized to previously, their titer of antibody will increase and will persist.[32] We did not see that kind of response in any of our patients that demonstrated previous sensitization. With the exception of one patient (Patient 2) whose anti-HLA specificity could not be defined, there was no de novo donor-specific HLA antibody detected in any of our patients.

It is well documented that intraocular spaces are, to a large extent, immunoprivileged sites.[33] Lack of apparent cell-mediated rejection of the retinal tissue implanted to the subretinal space likely derives from this immunologic protection.[33] Additionally, the absence of detectable graft damage from donor-specific antibodies that were present in some patients before implantation would indicate that the blood-brain barrier is prohibiting antibody infiltration into the subretinal space. Placement of allogeneic tissue into the anterior chamber of the eye results in a protective response called anterior chamber–associated immune deviation (ACAID).[33] Similar immune deviation could be occurring in retinal implant recipients, which could explain graft survival despite the presence of HLA-mismatched graft antigens. If regulatory T cells are developing in response to retinal implants, they are not inhibiting memory B-cell antibody production as all antibody specificities, donor directed and non–donor specific, persisted in all sensitized patients studied here.[32]

In eight of 10 cases, the transplanted RPE cells lost their pigmentation at three to six months postoperatively, which could indicate graft rejection. However, the phenomenon of pigment loss has also been observed in co-grafts to Royal College of Surgeons (RCS) rats where the unpigmented donor RPE cells could nevertheless be identified histologically.[16] RPE cells can lose their pigment in tissue culture.[34] In the patients' eyes, with any of our imaging techniques, one cannot see individual RPE cells. If one can see pigment, one cannot tell if the pigment is extracellular, within the RPE, or within macrophages. Because patients' visual improvement started after six months postoperatively and continued, it is unlikely that there was a rejection of the donor RPE.

Several mechanisms are likely involved in the visual improvements: a trophic effect of the implant on host cones[35] and local synaptic connections between the implant and host retina.[9] Sham surgery can also have a transient trophic effect[36] by upregulation of trophic factors.[37] In adult rats, injury-related effects on the survival of neurons in the retina may be the result of the intraocular synthesis and release of molecules that are specific trophins for retinal ganglion cells. However, it would be unlikely for a sham surgery effect to persist for six years; neuroprotective effects from surgery or injury in the rat model have been reported to last only three months[37] and up to five months in pigs.[38] Various cytokines and NTFs[39,40] have been shown to protect against photoreceptor degeneration resulting from continuous light exposure or genetic defects. Most of these factors act indirectly on photoreceptors via Mueller cells.[41,42] Implanted normal rod photoreceptor cells release soluble factor(s) to enhance cone survival in primary rod photoreceptor dystrophies[35] without the need for specific synapse formation. MP1 results of patient 1, with an increased sensitivity in an area adjacent to but not inside the implant area, could indicate a trophic effect.

Visual improvements by ETDRS have been reported after subretinal microchip implantation in three of six patients,[27] two of them with a follow-up of only six months. The visual improvement was reported in areas far from the implant. Although the ETDRS improvements of two of the three

patients are comparable with the results of some of our patients, only six-month follow-up data are available.

The possibility that synaptic connections from implant to host can play an important role in the beneficial implant effect is supported by animal experiments. In different models of retinal degeneration, retinal sheet implants can restore visual responses in a small area of the superior colliculus visual center in rodents, corresponding to the placement of the graft in the retina.[7,8] Synaptic connections between subretinal implants and host retina have been demonstrated by a transsynaptic virus tracing from the host brain to the implant.[9]

Two patients (Patients 2 and 10) showed improvement in both eyes, indicating a cross-over effect that could be from several causes. It could be a placebo effect or a systemic effect like an immune response. The transplant may have trophic effects that act similar to injections of anti-VEGF drugs, possible via systemic absorption.[43] However, several examples in the literature indicate that treatment of one eye can lead to improvement in the other. In RCS rats, photoreceptor rescue in one eye can improve the ability of the contralateral eye to drive cortical cells.[44] After gene therapy of RPE $65^{-/-}$ dogs, improved cone responses were unexpectedly also found in the nontreated control eyes after long-term follow-up.[45]

Patient 6 (AMD with visual improvement) had an implant of only neural retina without the RPE attached because the RPE detached from its retina during implantation. These results support the argument that photoreceptors are more involved in the pathophysiology of AMD than the RPE. Subfoveal RPE atrophy can reoccur following macular translocation surgery in eyes with GA.[46] It is unclear why GA recurs after macular translocation in eyes with previous GA, but one possibility is that the primary defect in GA is in the photoreceptors, leading secondarily to RPE death. Results with Patient 6 may support the importance of the role of photoreceptors.

Diseases that affect the RPE and photoreceptor cells might conceivably benefit from this type of implantation. Regardless of the mode of inheritance in RP, photoreceptor degeneration is the end result, so replacement of degenerated photoreceptors by healthy cells could be useful in many types of RP.

Adverse effects related to retinal implantation have been considered to include psychological stress (knowledge of donor tissue source) and the possible risk of transmission of viral infections such as acquired immunodeficiency syndrome (AIDS) or hepatitis. These effects were not an issue in this study.

Important knowledge has been gained regarding the safety and efficacy of retinal implantation in human subjects. Conduct of a clinical trial that follows a significantly larger number of patients for five years postoperatively will be pursued to gather further evidence of efficacy. Several strategies that need further investigation for improving clinical results are implantation of several sheets of retina/RPE in the eye, postoperative visual stimulation,[19] removal of the inner limiting membrane of the donor tissue, and use of growth factors and oxygen therapy. Oxygen therapy has been shown to preserve both rods and cone photoreceptors in cats after retinal detachment,[17] to help maintain normal structure and function of Mueller cells, to reduce surgical trauma, and to mitigate the effect of detachment in Mueller cell activity.[17,47]

THIS STUDY WAS SUPPORTED BY AN ANONYMOUS DONOR; THE FOUNDATION FIGHTING BLINDNESS, OWINGS MILLS, Maryland; the Kentucky Lions Eye Foundation, Louisville, Kentucky; The Murray Foundation Inc, New York, New York; Research to Prevent Blindness, New York, New York; and Vitreoretinal Research Foundation, Towson, Maryland. Drs Radtke, Seiler, and Aramant have a proprietary interest in the implantation instrument and procedure. Involved in design and conduct of study (N.R., M.S., R.A.); collection, management, analysis, and interpretation of the data (N.R., M.S., R.A., D.P., P.G., H.P.); and preparation, review, or approval of manuscript (N.R., M.S., R.A., D.P., P.G., H.P.). The study was conducted under approval from the Western Institutional Review Board and the Norton Healthcare Research Office and FDA investigational new drug number BB IND 8354; ClinicalTrials.gov ID # NCT00345917, NCT00346060. Informed consent documents were prepared for compliance DHHS 45 CFR 46.116 and FDA 21 CFR 50.25, and patient data were handled in compliance with HIPAA privacy rules and the protocol of Helsinki. Clinical trials registration information is available to the public at http://www.clinicaltrials.gov.

The authors acknowledge the assistance of the following with independent site testing: Michael Lazar, Project Manager, Facilities Planning and Construction; Tatiana Forofonova, University of Medicine and Dentistry of New Jersey, Newark, New Jersey; Erich E. Sutter, Smith-Kettlewell Eye Research Institute, San Francisco, California; and Theodore Wandzilak and his staff, Louisville, Kentucky. The authors would like to thank Dona Wells and Ann Ahola, Ambulatory Surgical Center, Louisville, Kentucky for their invaluable support.

REFERENCES

1. 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; 43:321–334.
2. Klein R, Klein BE, Jensen SC, Meuer SM. The five-year incidence and progression of age-related maculopathy: the Beaver Dam Eye Study. Ophthalmology 1997;104: 7–21.
3. Sunness JS, Rubin GS, Applegate CA, et al. Visual function abnormalities and prognosis in eyes with age-related geographic atrophy of the macula and good visual acuity. Ophthalmology 1997;104:1677–1691.
4. Santos A, Humayun MS, de Juan E Jr, et al. Preservation of the inner retina in retinitis pigmentosa. A morphometric analysis. Arch Ophthalmol 1997;115:511–515.
5. Kim SY, Sadda S, Humayun MS, de Juan E Jr, Melia BM, Green WR. Morphometric analysis of the macula in eyes with geographic atrophy due to age-related macular degeneration. Retina 2002;22:464–470.
6. Aramant RB, Seiler MJ. Transplanted sheets of human retina and retinal pigment epithelium develop normally in nude rats. Exp Eye Res 2002;75:115–125.

7. 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;42:1669–1676.
8. Thomas BB, Seiler MJ, Sadda SR, Aramant RB. Superior colliculus responses to light—preserved by transplantation in a slow degeneration rat model. Exp Eye Res 2004;79:29–39.
9. Seiler MJ, Sagdullaev BT, Woch G, Thomas BB, Aramant RB. Transsynaptic virus tracing from host brain to subretinal transplants. Eur J Neurosci 2005;21:161–172.
10. MacLaren RE, Pearson RA, MacNeil A, et al. Retinal repair by transplantation of photoreceptor precursors. Nature 2006; 444:203–207.
11. Kwan AS, Wang S, Lund RD. Photoreceptor layer reconstruction in a rodent model of retinal degeneration. Exp Neurol 1999;159:21–33.
12. Radner W, Sadda SR, Humayun MS, et al. Light-driven retinal ganglion cell responses in blind rd mice after neural retinal transplantation. Invest Ophthalmol Vis Sci 2001;42: 1057–1065.
13. An GJ, Asayama N, Humayun MS, et al. Ganglion cell responses to retinal light stimulation in the absence of photoreceptor outer segments from retinal degenerate rodents. Curr Eye Res 2002;24:26–32.
14. Gaillard F, Sauve Y. Cell-based therapy for retina degeneration: the promise of a cure. Vision Res 2007;47:2815–2824.
15. 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;133:544–550.
16. Aramant RB, Seiler MJ. Retinal transplantation—advantages of intact fetal sheets. Prog Retin Eye Res 2002;21:57–73.
17. Lewis GP, Talaga KC, Linberg KA, Avery RL, Fisher SK. The efficacy of delayed oxygen therapy in the treatment of experimental retinal detachment. Am J Ophthalmol 2004; 137:1085–1095.
18. Birol G, Budzynski E, Wangsa-Wirawan ND, Linsenmeier RA. Hyperoxia promotes electroretinogram recovery after retinal artery occlusion in cats. Invest Ophthalmol Vis Sci 2004;45:3690–3696.
19. Yao H, Dan Y. Synaptic learning rules, cortical circuits, and visual function. Neuroscientist 2005;11:206–216.
20. Beck RW, Maguire MG, Bressler NM, Glassman AR, Lindblad AS, Ferris FL. Visual acuity as an outcome measure in clinical trials of retinal diseases. Ophthalmology 2007;114: 1804–1809.
21. Rohrschneider K, Springer C, Bultmann S, Volcker HE. Microperimetry—comparison between the micro perimeter 1 and scanning laser ophthalmoscope—fundus perimetry. Am J Ophthalmol 2005;139:125–134.
22. Hood DC. Assessing retinal function with the multifocal technique. Prog Retin Eye Res 2000;19:607–646.
23. Radtke ND, Aramant RB, Seiler MJ, Petry HM, Pidwell DJ. Vision change after sheet transplant of fetal retina with RPE to a retinitis pigmentosa patient. Arch Ophthalmol 2004; 122:1159–1165.
24. Chong NH, Bird AC. Management of inherited outer retinal dystrophies: present and future. Br J Ophthalmol 1999;83: 120–122.
25. Sieving PA, Caruso RC, Tao W, et al. Ciliary neurotrophic factor (CNTF) for human retinal degeneration: Phase I trial of CNTF delivered by encapsulated cell intraocular implants. Proc Natl Acad Sci U S A 2006;103:3896–3901.
26. Rizzo JF III, Wyatt J, Humayun M, et al. Retinal prosthesis: an encouraging first decade with major challenges ahead. Ophthalmology 2001;108:13–14.
27. Chow AY, Chow VY, Packo KH, Pollack JS, Peyman GA, Schuchard R. The artificial silicon retina microchip for the treatment of vision loss from retinitis pigmentosa. Arch Ophthalmol 2004;122:460–469.
28. Margalit E, Maia M, Weiland JD, et al. Retinal prosthesis for the blind. Surv Ophthalmol 2002;47:335–356.
29. Algvere PV, Gouras P, Dafgard Kopp E. Long-term outcome of RPE allografts in non-immunosuppressed patients with AMD. Eur J Ophthalmol 1999;9:217–230.
30. Opelz G, Wujciak T, Dohler B, Scherer S, Mytilineos J. HLA compatibility and organ transplant survival. Collaborative Transplant Study. Rev Immunogenet 1999;1:334–342.
31. Cecka JM, Zhang Q, Reed EF. Preformed cytotoxic antibodies in potential allograft recipients: recent data. Hum Immunol 2005;66:343–349.
32. Kerman RH. Understanding the sensitized patient. Heart Fail Clin 2007;3:1–9.
33. Streilein JW, Ma N, Wenkel H, Ng TF, Zamiri P. Immunobiology and privilege of neuronal retina and pigment epithelium transplants. Vision Res 2002;42:487–495.
34. Engelmann K, Valtink M. RPE cell cultivation. Graefes Arch Clin Exp Ophthalmol 2004;242:65–67.
35. 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;20:451–467.
36. Silverman MS, Hughes SE. Photoreceptor rescue in the RCS rat without pigment epithelium transplantation. Curr Eye Res 1990;9:183–191.
37. Cao W, Li F, Steinberg RH, LaVail MM. Development of normal and injury-induced gene expression of aFGF, bFGF, CNTF, BDNF, GFAP and IGF-I in the rat retina. Exp Eye Res 2001;72:591–604.
38. Mahmoud TH, McCuen BW II, Hao Y, et al. Lensectomy and vitrectomy decrease the rate of photoreceptor loss in rhodopsin P347L transgenic pigs. Graefes Arch Clin Exp Ophthalmol 2003;241:298–308.
39. Faktorovich EG, Steinberg RH, Yasumura D, Matthes MD, LaVail MM. Photoreceptor degeneration in inherited retinal dystrophy delayed by basic fibroblast growth factor. Nature 1990;347:83–86.
40. 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;89:11249–11253.
41. Liang FQ, Dejneka NS, Cohen DR, et al. AAV-mediated delivery of ciliary neurotrophic factor prolongs photoreceptor survival in the rhodopsin knockout mouse. Mol Ther 2001; 3:241–248.
42. Wahlin KJ, Adler R, Zack DJ, Campochiaro PA. Neurotrophic signaling in normal and degenerating rodent retinas. Exp Eye Res 2001;73:693–701.
43. Tezel TH, Kaplan HJ. Are intravitreal anti-VEGF antibodies safe? Ocul Immunol Inflamm 2007;15:1–2.

44. Girman SV, Lund RD. Unilateral photoreceptor rescue can improve the ability of the opposite, untreated, eye to drive cortical cells in a retinal degeneration model. Vis Neurosci 2005;22:37–43.
45. Narfstrom K, Katz ML, Ford M, Redmond TM, Rakoczy E, Bragadottir R. In vivo gene therapy in young and adult RPE65−/− dogs produces long-term visual improvement. J Hered 2003;94:31–37.
46. Cahill MT, Mruthyunjaya P, Bowes Rickman C, Toth CA. Recurrence of retinal pigment epithelial changes after macular translocation with 360 degrees peripheral retinectomy for geographic atrophy. Arch Ophthalmol 2005;123:935–938.
47. Lewis G, Mervin K, Valter K, et al. Limiting the proliferation and reactivity of retinal Muller cells during experimental retinal detachment: the value of oxygen supplementation. Am J Ophthalmol 1999;128:165–172.

Biosketch

Norman D. Radtke, FACS, received his MD at the University of Michigan in 1974. After completing a Retina Fellowship (University of Iowa) and a Vitreous Fellowship (Duke University) from 1978 to 1980, he joined the University of Louisville, Kentucky, as an Assistant Professor and Director of the VR Service until 1983. Since 1983, he has served as Clinical Professor, Department of Ophthalmology, University of Louisville and Director of the Retina-Vitreous Resource Center, Norton Audubon Hospital, Louisville, Kentucky.