

BRIEF REPORT

Autologous Induced Stem-Cell–Derived Retinal Cells for Macular Degeneration

M. Mandai, A. Watanabe, Y. Kurimoto, Y. Hirami, C. Morinaga, T. Daimon, M. Fujihara, H. Akimaru, N. Sakai, Y. Shibata, M. Terada, Y. Nomiya, S. Tanishima, M. Nakamura, H. Kamao, S. Sugita, A. Onishi, T. Ito, K. Fujita, S. Kawamata, M.J. Go, C. Shinohara, K. Hata, M. Sawada, M. Yamamoto, S. Ohta, Y. Ohara, K. Yoshida, J. Kuwahara, Y. Kitano, N. Amano, M. Umekage, F. Kitaoka, A. Tanaka, C. Okada, N. Takasu, S. Ogawa, S. Yamanaka, and M. Takahashi

SUMMARY

The authors' full names, academic degrees, and affiliations are listed in the Appendix. Address reprint requests to Dr. Takahashi at the Laboratory for Retinal Regeneration, RIKEN Center for Developmental Biology, 2-2-3 Minatojima-minamimachi, Chuo-ku, Kobe, Hyogo 650-0047, Japan, or at retinalab@cdb.riken.jp; or to Dr. Yamanaka at the Center for iPS Cell Research and Application, Kyoto University, 53 Shogoin Kawahara-cho, Sakyo-ku, Kyoto 606-8507, Japan, or at yamanaka@cira.kyoto-u.ac.jp.

Drs. Mandai, Watanabe, and Kurimoto contributed equally to this article.

N Engl J Med 2017;376:1038-46.

DOI: 10.1056/NEJMoa1608368

Copyright © 2017 Massachusetts Medical Society.

We assessed the feasibility of transplanting a sheet of retinal pigment epithelial (RPE) cells differentiated from induced pluripotent stem cells (iPSCs) in a patient with neovascular age-related macular degeneration. The iPSCs were generated from skin fibroblasts obtained from two patients with advanced neovascular age-related macular degeneration and were differentiated into RPE cells. The RPE cells and the iPSCs from which they were derived were subject to extensive testing. A surgery that included the removal of the neovascular membrane and transplantation of the autologous iPSC-derived RPE cell sheet under the retina was performed in one of the patients. At 1 year after surgery, the transplanted sheet remained intact, best corrected visual acuity had not improved or worsened, and cystoid macular edema was present. (Funded by Highway Program for Realization of Regenerative Medicine and others; University Hospital Medical Information Network Clinical Trials Registry [UMIN-CTR] number, UMIN000011929.)

AGE-RELATED MACULAR DEGENERATION (AMD) IS ONE OF THE MOST prevalent retinal diseases that threaten vision in older populations in developed countries.¹⁻⁴ Neovascular (also called “wet”) AMD is more prevalent than atrophic (or “dry”) AMD in Japan⁵ and is associated with the ectopic development of a choroidal neovascular membrane in the subretinal space of the center of the retina (the macula). Physical disruption and functional impairment of the retinal pigment epithelium (RPE), a monolayer sheet of cells that supports the overlying photoreceptors and underlying choroidal vasculature, occur in the course of wet AMD.⁶

Current treatments of AMD that involve the intravitreal injection of anti-vascular endothelial growth factor (VEGF) drugs^{7,8} do not target the underlying degeneration inherent in the disease, and a high rate of recurrence is seen when such treatments are discontinued.⁹ Earlier treatments of wet AMD included laser photocoagulation to ablate the neovascular membrane, surgical removal of the neovascular membrane with or without retinal translocation, and photodynamic therapy.^{10,11} Although surgical removal of the neovascular membrane is usually followed by immediate resolution of exudative changes, improvement in vision is often limited because of previous damage to the RPE and because the surgical procedure can involve the inadvertent removal of the remaining RPE, along with some photo-

receptors, when the neovascular membrane is removed. Atrophy of the retina and choroid can result.¹² To overcome these problems, allogeneic transplantation of sheets of RPE cells derived from human fetuses was performed in the 1990s, but graft rejection usually occurred.^{13,14} Transplantation of a sheet made up of autologous RPE cells was performed in the early 2000s, and long-term vision was preserved in some patients^{15,16}; however, harvesting an RPE cell sheet with or without choroidal vasculature from the peripheral part of the eye and then reinserting it into the submacular space is an invasive and complex surgical procedure that is associated with a high risk of massive hemorrhage and retinal detachment.¹⁷⁻²⁰

Several clinical trials of the use of RPE cell suspensions derived from embryonic stem cells for the treatment of advanced dry AMD have been conducted.²¹⁻²³ The most common adverse events in these clinical trials have been associated with the use of immunosuppressants; therefore, we decided to test the use of an *ex vivo* preparation of autologous RPE cell sheets^{24,25} for transplantation. Here we report the results of induced pluripotent stem-cell (iPSC)-based transplantation in a patient with wet AMD.

CASE REPORTS

PATIENT 1

A 77-year-old Japanese woman received a diagnosis of polypoidal choroidal vasculopathy (a subtype of neovascular AMD) in both eyes in 2010. She had undergone cataract surgery in both eyes 2 months before her initial visit to our hospital, and her best corrected visual acuity was 0.15 (visual acuity is expressed as a reciprocal of minute of arc as visual angle, which ranges from 0.01 to 2.0, with higher numbers indicating better visual function [≥ 1.0 is considered normal visual function in a healthy eye]; 0.15 is equivalent to 20/130 on a Snellen chart) in the right eye and 0.2 (equivalent to 20/100 on a Snellen chart) in the left eye. Since her initial visit, she received a total of 13 intraocular injections of an anti-VEGF drug in the right eye (9 injections of ranibizumab and 4 injections of aflibercept), as clinically indicated, over a 29-month period, but the best corrected visual acuity in her right eye gradually declined to 0.09 (lower than 20/200 on a Snellen chart). The patient was enrolled in our study,

and the right eye was selected for transplantation. On September 12, 2014, we removed the neovascular membrane and transplanted an autologous iPSC-derived RPE cell sheet (hereafter referred to as an iPSC-RPE sheet) that measured 1.3×3.0 mm under the fovea (Fig. 1B, 1C, and 1D; and Fig. S8B and S8C in the Supplementary Appendix, available with the full text of this article at NEJM.org).

PATIENT 2

A 68-year-old Japanese man received a diagnosis of polypoidal choroidal vasculopathy in the right eye in 2009. He had received 2 photodynamic treatments, 10 injections of ranibizumab, and 6 injections of aflibercept, as clinically indicated, over 5 years, and he was enrolled in our study. At the time of enrollment, his best corrected visual acuity was 0.15 (20/130 on a Snellen chart) in the right eye. Patient 2 did not undergo transplantation because of concerns about the genetic changes that occurred in the iPSCs and iPSC-derived RPE cells and because the neovascular membrane had a moderate response to anti-VEGF therapy.

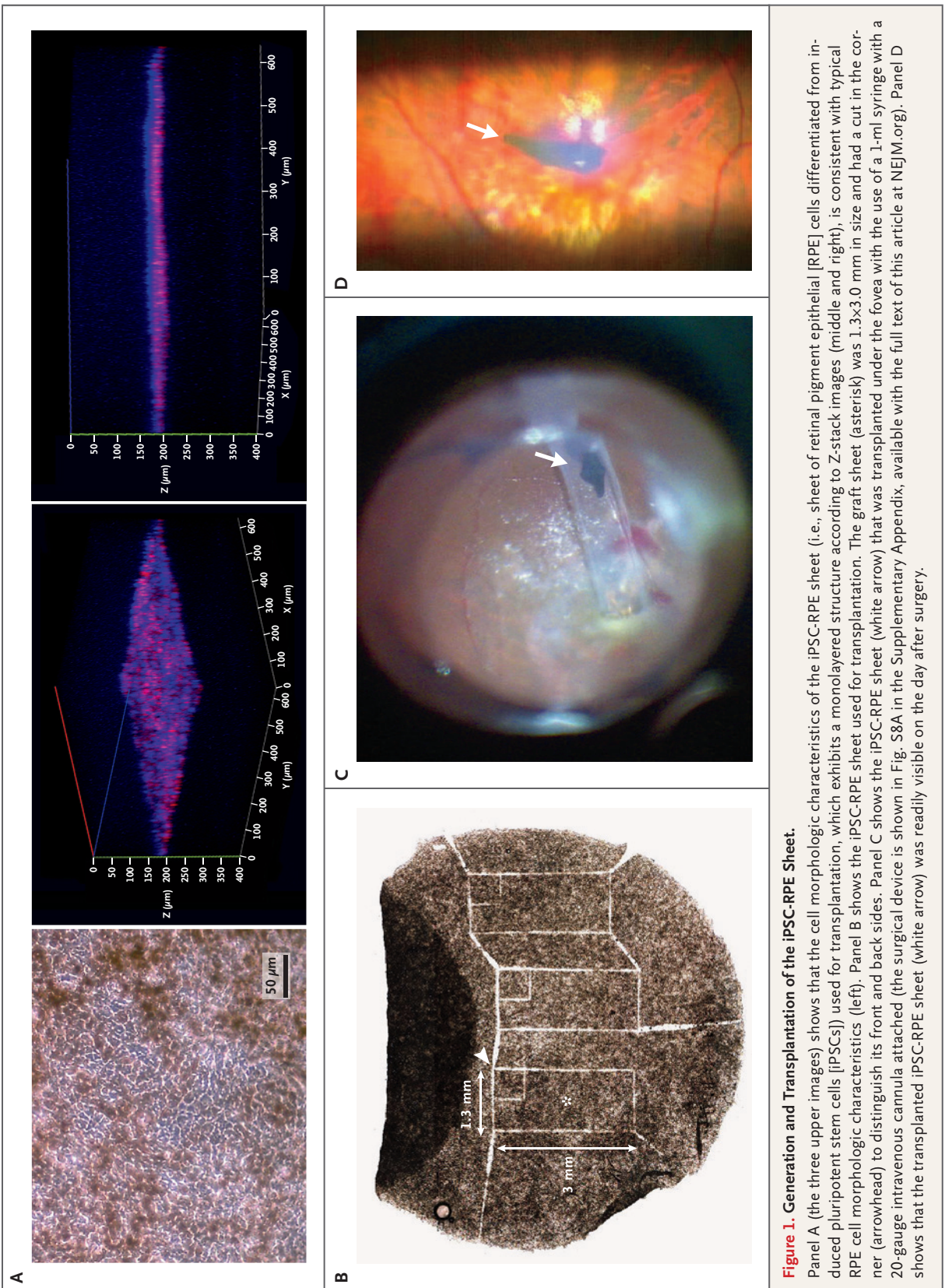
METHODS

STUDY OVERSIGHT

The protocol was approved by the institutional review boards and ethics committees at the collaborating sites and by the Minister of Health, Labor, and Welfare in Japan after a review by the Committee on Science and Technology of Health Sciences Council. Written informed consent was obtained from the patients, and the study was conducted in accordance with the tenets of the Declaration of Helsinki. All experiments that involved the use of samples obtained from humans and all studies in animals were reviewed and approved by the institutional review board at the Foundation for Biomedical Research and Innovation and RIKEN Center for Developmental Biology. The authors vouch for the completeness and accuracy of the data and analyses and for the fidelity of the study to the protocol.

OUTCOME MEASURES

The primary outcomes of this case study were the safety and adverse-event profile of iPSC-derived RPE cells as a graft source and assessment of the transplantation procedure of the iPSC-RPE sheet in persons with neovascular AMD. Secondary outcomes included the effectiveness of iPSC-based



autologous transplantation as a treatment option, with retinal morphologic characteristics and visual function after surgery taken into account (additional details are provided in the Supplementary Appendix and the protocol, available at NEJM.org).

STUDY DESIGN

We generated iPSCs using nonintegrating episomal vectors and differentiated them into RPE cells as described previously.²⁵ The autologous iPSC-derived RPE cells were assessed for quality and safety before transplantation according to our protocol, and whole-genome sequencing, whole-genome methylation profiling, and expression analyses were also performed. Additional details about the episomal vectors, procedures and plans for the cell preparation, patient recruitment, transplantation, quality and safety assessments, and postoperative examination are provided in the Supplementary Appendix and the protocol. The statistical analysis that was initially planned in the protocol was not performed, because only one patient received an iPSC-RPE sheet.

RESULTS

PATIENTS AND IPSC GENERATION

The clinical study was initiated in August 2013. The first patient was enrolled on November 13, 2013, and underwent transplantation with an iPSC-RPE sheet in September 2014. The second patient was enrolled on March 12, 2014. The enrollment of further patients for this study was halted in 2015 owing to the enactment of Japan's Regenerative Medicine Law in November 2014, which requires that the clinical study plans of regenerative medicine are submitted by medical institutions but not research institutions.

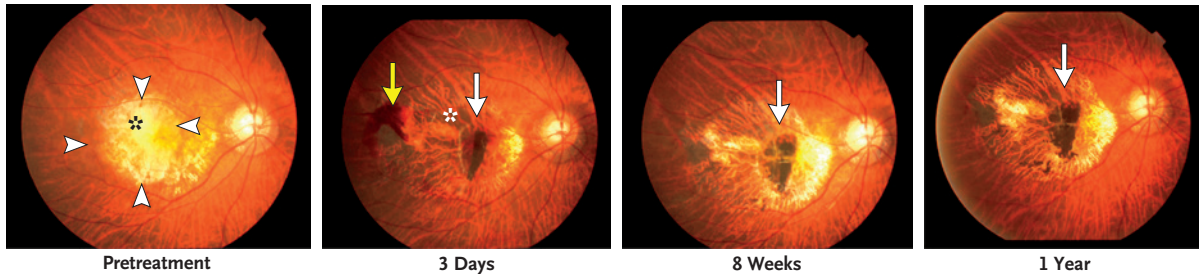
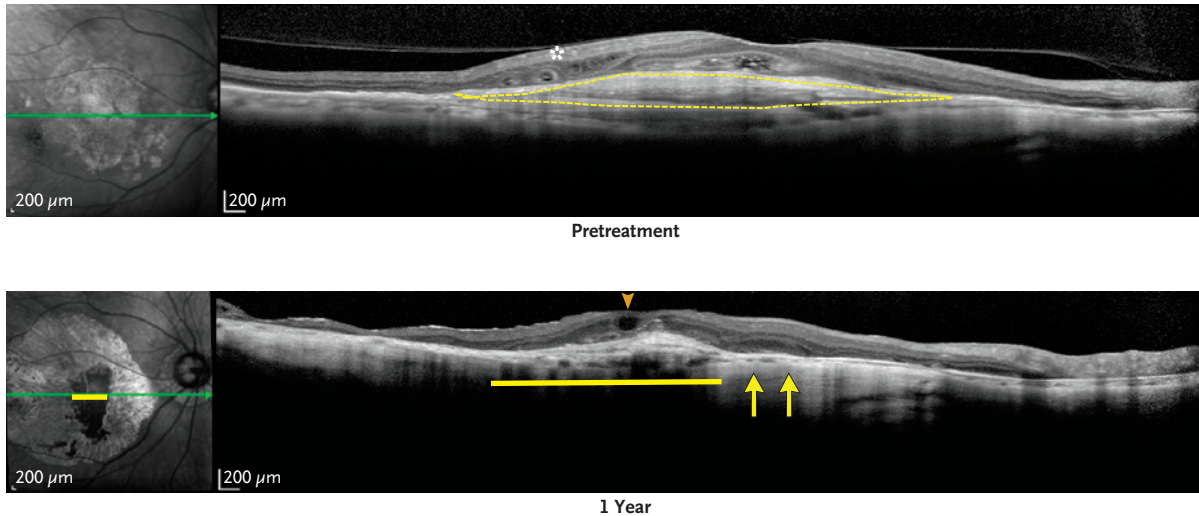
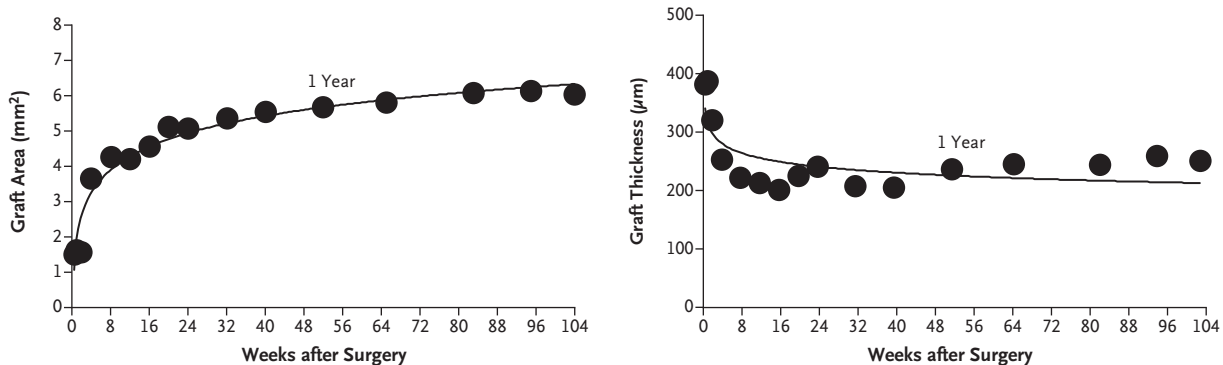
We obtained two iPSC-derived RPE cell lines from Patient 1 (Patient1-28-RPE and Patient1-6-RPE; a third cell line from Patient 1 [Patient1-13-RPE] failed a test for adhesion) and one iPSC-derived RPE cell line from Patient 2 (Patient2-1-RPE) that met our criteria (Fig. 1A, and Figs. S2, S3, and S4 and Tables S2 through S6 in the Supplementary Appendix). These iPSC-derived RPE cells exhibited DNA methylation and gene expression profiles consistent with those of RPE tissue; in addition, using single-cell quantitative polymerase chain reaction, we detected levels of RPE-specific gene expression that were consistent

with those of RPE tissue. (See Figs. S5 and S6 in the Supplementary Appendix.)

We then used immunodeficient mice (non-obese diabetic/Shi-scid/IL2 γ ^{null} [NOG] mice) to test the tumorigenic potential of Patient1-28-RPE cells; no tumor was observed in these mice. Single-nucleotide variations that were detected by means of whole-genome sequencing, including those detected in the original fibroblasts at low frequencies, had not been reported as cancer driver mutations. No large de novo insertions or deletions or DNA copy number alterations were detected in protein-coding regions. Furthermore, we confirmed that the plasmid DNA was not integrated into the genomic DNA. (See Fig. S7A and Tables S7 through S12 in the Supplementary Appendix.)

PATIENT 1

After surgery on the right eye of Patient 1, the choroidal vessels that had been masked by the presence of the fibrotic neovascular membrane were readily visible and appeared to have a nearly normal pattern. The graft sheet was initially curled on the margin but flattened gradually by 8 weeks (Fig. 2A). A large, fibrotic neovascular membrane and polyps were visualized preoperatively by means of fluorescein angiography and indocyanine green angiography. At 1 year after surgery, a well-demarcated hyperfluorescent area was observed at a location corresponding to the atrophic area that resulted from the removal of the neovascular membrane–RPE complex; no sign of graft rejection or recurrence of the neovascular membrane was present. Preoperative optical coherence tomographic images showed a large hyperreflective mass under the macula and retinal edema, which reappeared after anti-VEGF treatment was stopped before surgery. The RPE cell line could not be traced on the surface of the fibrotic neovascular membrane in the foveal lesion, where outer retinal tubulations were forming.²⁶ After surgery, the large hyperreflective mass that was observed preoperatively had disappeared completely. A highly reflective line indicating the presence of RPE cells in the optical coherence tomographic image — which corresponded to the presence of the pigmented graft sheet in the color fundus photographs — lengthened gradually over time. At 3 months and beyond, the highly reflective RPE-like cell line was observed to extend farther from the graft sheet

A Color Fundus Photographs Taken before and after Surgery**B Vertical Sectional Views by OCT before and 1 Year after Surgery****C Changes in Graft Area and Thickness after Transplantation**

to the nasal side, and an external limiting membrane and choroid space were retained over and under the line, respectively, which indicated the presence of functional RPE cells. On the graft sheet, the outer nuclear layer, with high-density reflectivity in the space corresponding to the inner and outer segments, was retained at 1 year. (Fig. 2B, and Figs. S9, S10, and S11 in the Supplementary Appendix.)

Macular edema, as evidenced by the intraretinal cystic change that was detected on optical coherence tomography, disappeared immediately after surgery but then reappeared at 4 weeks. When the dose of glucocorticoid eyedrops was increased, the extent of cystoid macular edema was reduced, but the edema persisted without substantial worsening or adverse changes in or around the graft. We observed no remarkable

Figure 2 (facing page). Ocular Findings before and after Surgery to Remove the Neovascular Membrane and Transplant the iPSC-RPE Sheet and Postoperative Changes in Graft Area and Thickness.

Panel A shows color fundus photographs taken before and after surgery. The left-most image shows a large fibrotic neovascular membrane (black asterisk, with white arrowheads indicating the margin). After the removal of neovascular membrane, the underlying choroid vessels became readily visible and were almost intact (white asterisk in the left-middle image). Hemorrhages were observed at the graft insertion site 3 days after surgery but were absorbed in 2 weeks (yellow arrow, the second image from left). The graft sheet was initially curled at its edge but flattened by 8 weeks (white arrows in the two middle images and the right image). Panel B shows vertical sectional views by optical coherence tomography (OCT) before and 1 year after surgery. Before surgery, the neovascular membrane was observed as a dense hyperreflective mass under the macula (marked by the yellow dotted line, upper image). The tubules of the photoreceptor cell layer were observed to form as rosette-like structures in the fovea (asterisk, upper image). At 1 year, a highly reflective RPE-like cell line was observed extending nasally from the graft sheet, and the structured photoreceptor cell layer and choroid space were retained above and below the line, respectively (yellow arrows). Cystoid macular edema persisted (orange arrowhead; see also Figure S11 in the Supplementary Appendix). Panel C shows changes in the graft area (left) and thickness (right) after transplantation. The graft area was determined by the presence of a pigmented sheet or fragments on the color fundus photographs, and the graft thickness was measured at the thickest point of the graft in the OCT images (see also Fig. S9A in the Supplementary Appendix).

changes in fluorescein angiographic findings at 6 months and 1 year and no evidence of leakage at the site of cystoid macular edema until the late phase (7 to 8 minutes after intravenous injection of fluorescein), which led us to conclude that the degenerative cystic change was part of the ongoing disease process and not due to graft rejection or recurrence of the neovascular membrane (Fig. S10 in the Supplementary Appendix).

The size of the graft sheet increased and the thickness decreased in the first 2 months after surgery, a finding that suggests that the sheet, which was curled initially, flattened over time (Fig. 2C). Histologic analysis of the neovascular membrane that had been removed revealed multiple vessels, a finding that suggests that the lesion was active. (See Figs. S9A and S12 in the Supplementary Appendix.)

At 1 year after transplantation, there were no serious complications (Table S13 in the Supple-

mentary Appendix) and no unexpected proliferation or sign of local or systemic malignant disease. The transplanted iPSC-RPE sheet showed no sign of rejection during the 1-year study period and at the most recent evaluation on December 9, 2016. The patient had a transient elevation in intraocular pressure to 28 mm Hg in the early postoperative period (5 to 8 weeks), but the pressure returned to normal with temporary administration of antiglaucoma eyedrops. We conclude that the iPSC-based autologous transplantation was safe and feasible in the treatment of this patient.

We assessed efficacy at 1 year after transplantation by measuring foveal thickness and evaluating visual function. The foveal thickness was reduced successfully through the removal of the neovascular membrane, and there was no recurrence. Postoperative best corrected visual acuity did not improve but was maintained at approximately 0.1 (equivalent to 20/200 on a Snellen chart) at 1 year and throughout the follow-up period. (See Fig. S9B and S9C in the Supplementary Appendix.) The patient received no anti-VEGF injections after surgery. Her score on the National Eye Institute Visual Functioning Questionnaire (VFQ)-25 (on which scores range from 0 to 100, with higher scores indicating better visual function and general health) increased from 48.8 (before surgery) to 58.3 (1 year after surgery). Retinal sensitivity, as assessed by microperimetry, remained at 0 dB within the fovea and juxta-fovea before and after surgery, but the fixation points shifted closer to the fovea on the grafted area, which indicated that the graft was functional, although the fixation was unstable. (See Fig. S13 in the Supplementary Appendix.) Reliable results could not be obtained with multifocal electroretinography because of the unstable fixation.

PATIENT 2

An iPSC line and iPSC-derived RPE cells obtained from Patient 2 met all our requirements in the protocol. We did not detect single-nucleotide variations previously reported to be cancer driver mutations. However, we detected three aberrations in DNA copy number (deletions) that would probably affect expression of genes encoded by both the deleted DNA and by DNA flanking the deletions. The fact that one of the three deletions was on the X chromosome (and not in a pseudoautosomal region) was of particular con-

cern, given the male sex of the patient. The iPSC-derived RPE cells passed the *in vivo* tumorigenicity test and an additional tumorigenicity test involving subretinal injection of iPSC-derived RPE cells into five nude rats. However, we decided not to perform the transplantation because of concerns about the possible effects of the deletions and the moderate response of the neovascular membrane to anti-VEGF therapy in this patient. (See Figs. S7B and S14 and Tables S2 through S7 and S14, S15, and S16 in the Supplementary Appendix.)

DISCUSSION

We performed iPSC-based autologous transplantation in the treatment of neovascular AMD in one patient, and no serious adverse event was noted at 25 months of follow-up. Although it has been reported that autologous iPSC-derived cells can trigger immune-mediated rejection in mice,²⁷ Patient 1 did not receive immunosuppressants, and we observed no signs of rejection. We advise caution in extrapolating our findings to other types of iPSC-based transplantation, especially because iPSC-derived RPE cells have been shown to inhibit T-cell activation,²⁸ and we emphasize that our findings from treating a single patient are uninformative with respect to the risks associated with the procedure.

At 1 year after transplantation in Patient 1, the best corrected visual acuity of the treated eye had not improved or worsened, and her VFQ-25 score had improved, although it is possible that this outcome could have been obtained without surgery. The patient expressed satisfaction with “brighter” vision, which was probably due to removal of the neovascular membrane. The presence of a vascular-rich neovascular membrane, despite the repeated injections of anti-VEGF drugs, and the reappearance of fluid immediately after the anti-VEGF treatment was stopped before the surgery suggested that removal of the neovascular membrane was a reasonable therapeutic choice.

The expansion of the graft area, due partly to the cell attachment and flattening, may indicate survival of the grafted cells. A similar enlargement of the pigmented area was observed in a previous study that used human embryonic stem cell–derived RPE cells.²¹ Optical coherence tomographic images indicated good retinal integrity over the graft over the 1 year after the transplan-

tation, with a high-density area, which may indicate an area of recovering inner and outer segments of photoreceptor cells. Similar observations in the area of inner and outer segment formation after retinal transplantation in animals have been reported.^{29,30} However, we have yet to evaluate the extent of photoreceptor function of the RPE graft in Patient 1.

A major concern about cell-based transplantation therapies is tumorigenicity. Highly proliferative graft cells, such as progenitor cells, require assessments of genome integrity; for cells with a low rate of proliferation, such as iPSC-derived RPE cells, *in vivo* tumorigenicity tests are considered to be adequate. We performed *in vivo* tumorigenicity tests that have sensitivities that are approximately 10,000 times as high as those of conventional methods.³¹ Although it was not prespecified in the protocol, we also performed a series of genomic analyses. We did not observe genomic aberrations suggestive of tumorigenicity (such as those affecting tumor suppressor genes) in the iPSC-derived RPE cells obtained from Patient 1; however, we detected copy-number alterations in iPSCs obtained from Patient 2, although we were unable to gauge the likely effect of these alterations on tumorigenicity on the basis of information in the published literature. Perhaps postponing surgery in Patient 2 was unnecessarily strict, given the small number of cells (approximately 100,000 cells in a sheet) transplanted per protocol, the fact that no metastatic tumors originating from RPE cells have been reported, and the ease of detecting aberrant proliferation after transplantation. Our decision not to perform the procedure was influenced by the stable best corrected visual acuity with anti-VEGF therapy in this patient and because genomic analyses in the evaluation of iPSC-derived cells have yet to be sufficiently adapted for clinical use.

Supported by grants from the Highway Program for Realization of Regenerative Medicine (to Dr. Takahashi), the Research Project for Practical Applications of Regenerative Medicine (to Drs. Takahashi, Kurimoto, and Watanabe), the Research Center Network for Realization of Regenerative Medicine from the Japan Agency for Medical Research and Development (a grant from the Core Center for iPS Cell Research to Dr. Yamanaka, a grant from the Centers for Clinical Application Research on Specific Disease/Organ [Type A] to Dr. Takahashi, and a grant from the CREST program to Dr. Watanabe), and the Japan Society for the Promotion of Science (Grant-in-Aid for Scientific Research [KAKENHI] 15K06921) (to Dr. Watanabe).

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

We thank Dr. Hidenobu Tanihara (Kumamoto University), Dr. Motohiro Kamei (Aichi Medical University), and Dr. Hiroshi Goto (Tokyo Medical University) for the independent data monitoring; Drs. Noriko Miyamoto, Akihiro Nishida, Takanori Kameda, Matsutaka Shimozone, and Seiji Takagi (Kobe City Medical Center General Hospital) for patient recruitment and follow-up; Hoshimi Kanemura, Masayuki Shikamura, and Naoki Nishishita (Foundation for Biomedical Research and Innovation) for tumorigenicity tests; Dr. Hiroyuki Kitajima, Tomoyo Hashiguchi, and Chikako Yamada (RIKEN Center for Developmental Biology) for immunohistochemical analyses; Dr. Satoshi Okamoto (Yokohama City University) for differentiation of RPE cells from iPSCs; Drs. Takahiro Yamashita and Yoshinori Shichida (Kyoto University) for the phagocytosis assay of RPE cells; Tokiko Okame, Mila Nishimura, Rika Hirai-Doi, Chikako Okubo, Kenta Sutou, Koichi

Kaneko, Yoshiko Sato, Yasuko Matsumura, and Kazuhiko Kitajima, and Drs. Shiori Furukawa, Masaki Nomura, Tomoko Takahashi, and Yasutaka Mizoro (Center for iPS Cell Research and Application [CiRA], Kyoto University) and Reow Mohri (Mitsubishi Space Software) for technical assistance; Hiromi Dohi, Mayumi Ikeda, and Hiroko Endo (CiRA) for administrative assistance; Dr. Peter Karagiannis (CiRA) for proofreading an earlier version of this article; and Drs. Fumihito Miura and Takashi Ito (Kyushu University), Drs. Hideyuki Takeshima and Toshikazu Ushijima (National Cancer Center), Drs. Kazutoshi Takahashi, Keisuke Okita, Yoshinori Yoshida, and Masato Nakagawa (CiRA), Dr. Shin-ichi Nishikawa (All About Science Japan), Dr. Yoshihito Honda (Japan Red Cross Osaka Hospital), and Dr. Yoshiki Sasai (who passed away during the preparation of the manuscript), for helpful comments.

APPENDIX

The authors' full names and academic degrees are as follows: Michiko Mandai, M.D., Ph.D., Akira Watanabe, Ph.D., Yasuo Kurimoto, M.D., Ph.D., Yasuhiko Hiram, M.D., Ph.D., Chikako Morinaga, Ph.D., Takashi Daimon, Ph.D., Masashi Fujihara, M.D., Ph.D., Hiroshi Akimaru, Ph.D., Noriko Sakai, B.S., Yumiko Shibata, M.S., Motoki Terada, Yui Nomiya, M.S., Shigeki Tanishima, B.S., Masahiro Nakamura, M.D., Ph.D., Hiroyuki Kamao, M.D., Ph.D., Sunao Sugita, M.D., Ph.D., Akishi Onishi, Ph.D., Tomoko Ito, Kanako Fujita, Shin Kawamata, M.D., Ph.D., Masahiro J. Go, Ph.D., Chikara Shinohara, Ph.D., Ken-ichiro Hata, D.D.S., Ph.D., Masanori Sawada, M.D., Ph.D., Midori Yamamoto, Sachiko Ohta, Yasuo Ohara, B.S., Kenichi Yoshida, M.D., Ph.D., Junko Kuwahara, Yuko Kitano, M.S., Naoki Amano, M.S., Masafumi Umekage, M.S., Fumiyo Kitaoka, Ph.D., Azusa Tanaka, Ph.D., Chihiro Okada, M.S., Naoko Takasu, M.S., Seishi Ogawa, M.D., Ph.D., Shinya Yamanaka, M.D., Ph.D., and Masayo Takahashi, M.D., Ph.D.

The authors' affiliations are as follows: the Division of Ophthalmology, Institute of Biomedical Research and Innovation Hospital, Foundation for Biomedical Research and Innovation (M.M., Y. Kurimoto, Y.H., M.F., M.Y., S. Ohta, M. Takahashi), Laboratory for Retinal Regeneration, RIKEN Center for Developmental Biology (M.M., C.M., H.A., N.S., Y.S., M. Terada, H.K., S.S., A.O., M.Y., M. Takahashi), the Department of Ophthalmology, Kobe City Medical Center General Hospital (Y. Kurimoto, Y.H., M.F.), the Department of Clinical Trial Management, Institute of Biomedical Research and Innovation Hospital, Foundation for Biomedical Research and Innovation (T.I., K.F.), and Research and Development Center for Cell Therapy, Foundation for Biomedical Research and Innovation (S.K., M.J.G.), Kobe, Center for iPS Cell Research and Application (CiRA) (A.W., Y.N., M.N., J.K., Y. Kitano, N.A., M.U., F.K., A.T., N.T., S.Y.), Institute for Integrated Cell-Material Sciences (iCeMS) (A.W.), and Department of Pathology and Tumor Biology, Graduate School of Medicine (K.Y., S. Ogawa), Kyoto University, Kyoto, the Department of Biostatistics, Hyogo College of Medicine, Nishinomiya (T.D.), Platform of Therapeutics for Rare Disease, National Institutes of Biomedical Innovation, Health, and Nutrition, Ibaraki (H.A.), Mitsubishi Space Software, Tokyo (S.T., Y.O., C.O.), the Department of Ophthalmology, Kawasaki Medical School, Kurashiki (H.K.), Japan Tissue Engineering, Gamagori (C.S., K.H.), and HEALIOS K.K., Tokyo (M.S.) — all in Japan; and Gladstone Institute of Cardiovascular Disease, San Francisco (S.Y.).

REFERENCES

1. Fine SL, Berger JW, Maguire MG, Ho AC. Age-related macular degeneration. *N Engl J Med* 2000;342:483-92.
2. Klein R, Klein BE, Linton KL. Prevalence of age-related maculopathy: the Beaver Dam Eye Study. *Ophthalmology* 1992;99:933-43.
3. Vingerling JR, Dielemans I, Hofman A, et al. The prevalence of age-related maculopathy in the Rotterdam Study. *Ophthalmology* 1995;102:205-10.
4. Mitchell P, Smith W, Attebo K, Wang JJ. Prevalence of age-related maculopathy in Australia: the Blue Mountains Eye Study. *Ophthalmology* 1995;102:1450-60.
5. Oshima Y, Ishibashi T, Murata T, Tahara Y, Kiyohara Y, Kubota T. Prevalence of age related maculopathy in a representative Japanese population: the Hisayama study. *Br J Ophthalmol* 2001;85:1153-7.
6. Holz FG, Pauleikhoff D, Klein R, Bird AC. Pathogenesis of lesions in late age-related macular disease. *Am J Ophthalmol* 2004;137:504-10.
7. Rosenfeld PJ, Brown DM, Heier JS, et al. Ranibizumab for neovascular age-related macular degeneration. *N Engl J Med* 2006;355:1419-31.
8. Holash J, Davis S, Papadopoulos N, et al. VEGF-Trap: a VEGF blocker with potent antitumor effects. *Proc Natl Acad Sci U S A* 2002;99:11393-8.
9. Rofagha S, Bhisitkul RB, Boyer DS, Sadda SR, Zhang K, SEVEN-UP Study Group. Seven-year outcomes in ranibizumab-treated patients in ANCHOR, MARINA, and HORIZON: a multicenter cohort study (SEVEN-UP). *Ophthalmology* 2013;120:2292-9.
10. Macular Photocoagulation Study Group. Laser photocoagulation for juxtafoveal choroidal neovascularization: five-year results from randomized clinical trials. *Arch Ophthalmol* 1994;112:500-9.
11. Hawkins BS, Bressler NM, Miskala PH, et al. Surgery for subfoveal choroidal neovascularization in age-related macular degeneration: ophthalmic findings: SST report no. 11. *Ophthalmology* 2004;111:1967-80.
12. Falkner CI, Leitich H, Frommlet F, Bauer P, Binder S. The end of submacular surgery for age-related macular degeneration? A meta-analysis. *Graefes Arch Clin Exp Ophthalmol* 2007;245:490-501.
13. Peyman GA, Blinder KJ, Paris CL, Alturki W, Nelson NC Jr, Desai U. A technique for retinal pigment epithelium transplantation for age-related macular degeneration secondary to extensive subfoveal scarring. *Ophthalmic Surg* 1991;22:102-8.
14. Algvere PV, Gouss P, Dalfarg Kopp E. Long-term outcome of RPE allografts in non-immunosuppressed patients with AMD. *Eur J Ophthalmol* 1999;9:217-30.
15. Binder S, Krebs I, Hilgers R-D, et al. Outcome of transplantation of autologous retinal pigment epithelium in age-related macular degeneration: a prospective trial. *Invest Ophthalmol Vis Sci* 2004;45:4151-60.
16. van Meurs JC, ter Averst E, Hofland LJ, et al. Autologous peripheral retinal pigment epithelium translocation in patients with subfoveal neovascular membranes. *Br J Ophthalmol* 2004;88:110-3.
17. van Meurs JC, Van Den Biesen PR. Autologous retinal pigment epithelium and choroid translocation in patients with

- exudative age-related macular degeneration: short-term follow-up. *Am J Ophthalmol* 2003;136:688-95.
18. Heussen FM, Fawzy NF, Joeres S, et al. Autologous translocation of the choroid and RPE in age-related macular degeneration: 1-year follow-up in 30 patients and recommendations for patient selection. *Eye (Lond)* 2008;22:799-807.
 19. van Zeeburg EJT, Maaijwee KJM, Missotten TOAR, Heimann H, van Meurs JC. A free retinal pigment epithelium-choroid graft in patients with exudative age-related macular degeneration: results up to 7 years. *Am J Ophthalmol* 2012;153(1):120-7.e2.
 20. Falkner-Radler CI, Krebs I, Glittenberg C, et al. Human retinal pigment epithelium (RPE) transplantation: outcome after autologous RPE-choroid sheet and RPE cell-suspension in a randomised clinical study. *Br J Ophthalmol* 2011;95:370-5.
 21. Schwartz SD, Regillo CD, Lam BL, et al. Human embryonic stem cell-derived retinal pigment epithelium in patients with age-related macular degeneration and Stargardt's macular dystrophy: follow-up of two open-label phase 1/2 studies. *Lancet* 2015;385:509-16.
 22. Schwartz SD, Hubschman J-P, Heilwell G, et al. Embryonic stem cell trials for macular degeneration: a preliminary report. *Lancet* 2012;379:713-20.
 23. Song WK, Park K-M, Kim H-J, et al. Treatment of macular degeneration using embryonic stem cell-derived retinal pigment epithelium: preliminary results in Asian patients. *Stem Cell Reports* 2015;4:860-72.
 24. Hiram Y, Osakada F, Takahashi K, et al. Generation of retinal cells from mouse and human induced pluripotent stem cells. *Neurosci Lett* 2009;458:126-31.
 25. Kamao H, Mandai M, Okamoto S, et al. Characterization of human induced pluripotent stem cell-derived retinal pigment epithelium cell sheets aiming for clinical application. *Stem Cell Reports* 2014;2: 205-18.
 26. Zweifel SA, Engelbert M, Laud K, Margolis R, Spaide RF, Freund KB. Outer retinal tubulation: a novel optical coherence tomography finding. *Arch Ophthalmol* 2009;127:1596-602.
 27. Zhao T, Zhang Z-N, Rong Z, Xu Y. Immunogenicity of induced pluripotent stem cells. *Nature* 2011;474:212-5.
 28. Sugita S, Kamao H, Iwasaki Y, et al. Inhibition of T-cell activation by retinal pigment epithelial cells derived from induced pluripotent stem cells. *Invest Ophthalmol Vis Sci* 2015;56:1051-62.
 29. Assawachananont J, Mandai M, Okamoto S, et al. Transplantation of embryonic and induced pluripotent stem cell-derived 3D retinal sheets into retinal degenerative mice. *Stem Cell Reports* 2014; 2:662-74.
 30. Shirai H, Mandai M, Matsushita K, et al. Transplantation of human embryonic stem cell-derived retinal tissue in two primate models of retinal degeneration. *Proc Natl Acad Sci U S A* 2016;113:E81-E90.
 31. Kuroda T, Yasuda S, Kusakawa S, et al. Highly sensitive in vitro methods for detection of residual undifferentiated cells in retinal pigment epithelial cells derived from human iPS cells. *PLoS One* 2012; 7(5):e37342.

Copyright © 2017 Massachusetts Medical Society.