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Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☒	☐ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☒	☐ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	in vitro cell images: BZ-H3CM software (Keyence Corporation, Osaka, Japan), in vivo corneal endothelial cells: built-in softwares VS-100 Ver.6V.6 from a non-contact specular microscope EM-3000 (Tomey Corporation, Nagoya, Japan) and Konan specular microscope KSSP Ver.1.40 from a KSSP slit-scanning wide-field contact specular microscope (Konan Medical, Inc., Hyogo, Japan).
Data analysis	The binarized images were analyzed with a self-written macro on Igor Pro 6.3 (WaveMetrics, Inc., Portland, OR, USA).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The authors declare that the main data supporting the results in this study are available within the paper and its Supplementary Information. The raw and analysed datasets generated during the study are available for research purposes from the corresponding authors on reasonable request.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	n = 18, among which n = 16 were subjected to the analyses for cell injection therapy, while n = 12 for penetrating keratoplasty. n = 9 lots of in vitro cell sources were analyzed. Sample sizes were determined by the number of clinical data which were available.
Data exclusions	For the analysis of patients' data undergoing cell injection therapy, n = 2 cases were excluded from the analysis due to the partial removal of Descemet's membranes at the time of surgery. As we performed the retrospective analysis of the clinical data in this study, no exclusion criteria was explicitly pre-established before the analysis.
Replication	The reproducibility and the robustness of the analyses were confirmed for in vitro cell sources and in vivo corneal endothelium by changing the region of interests, except for the damaged cornea (e.g., Figure 6c showing a sign of edema).
Randomization	All the clinical data were analyzed retrospectively. For the cell injection therapy part, all patients underwent the surgical treatment at the Department of Ophthalmology of Kyoto Prefectural University of Medicine between December 2013 and September 2015. For the penetrating keratoplasty part, all patients underwent penetrating keratoplasty at Baptist Eye Institute, Kyoto, Japan between March 2013 and December 2013.
Blinding	The blinding was not relevant to our study, because this study deals with the retrospective analyses of the clinical data from patients.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☐	☒ Human research participants
☐	☒ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	Anti-CD166 (BD Biosciences, Catalogue No.: 559263, clone name: 3A6, Lot No.: 7104824, dilution: 1:10), anti-CD24 (BD Biosciences, Catalogue No.: 561647, clone name: ML5, Lot No.: 7100601, dilution: 1:40), anti-CD105 (eBioscience, Catalogue No.: 17-1051-80, clone name: SN6, Lot No.: E07199-1633, dilution: 1:40), anti-CD44 (BD Biosciences, Material No.: 560533, clone name: G44-26, Lot No.: 6168573, dilution: 1:180).
Validation	Validation statements from manufacturers can be found in the following links: http://www.bdbiosciences.com/us/applications/research/stem-cell-research/cancer-research/human/pe-mouse-anti-human-cd166-3a6/p/559263 (CD166), https://www.bdbiosciences.com/eu/applications/research/stem-cell-research/cancer-research/human/percp-cy55-mouse-anti-human-cd24-ml5/p/561647 (CD24), https://www.thermofisher.com/antibody/product/CD105-Endoglin-Antibody-clone-MJ7-18-Monoclonal/17-1051-80 (CD105), http://www.bdbiosciences.com/ptProduct.jsp?ccn=560533 (CD44).

Human research participants

Policy information about studies involving human research participants

Population characteristics	Cell injection therapy (18 patients): female (68), male (60), male (58), male (71), male (49), female (74), female (72), female (57), male (63), female (82), female (56), female (68), female (76), male (76), female (70), male (75), male (78), male (43).

	Penetrating keratoplasty (12 patients): male (60), female (68), female (75), female (68), female (55), male (57), male (75), female (93), male (75), male (55), female (79), male (45).
Recruitment	As stated above (Life Sciences Study Design), this study deals with the retrospective analyses of the clinical data from patients. In cell injection therapy, the series of consecutive patients who underwent the injection at the Department of Ophthalmology of Kyoto Prefectural University of Medicine between December 2013 and September 2015 were included in this study. In penetrating keratoplasty, all patients who underwent penetrating keratoplasty at Baptist Eye Institute between March 2013 and December 2013, whose specular microscopy images of the corneas could be obtained at 3-, 6-, 12-, and 24-months post transplantation, were included in this study. We would like to point out that we analyzed all the cases that have complete sets of images in order to examine the potential application of our biomarker for the preemptive diagnosis. Thus we confirm that there is no self-selection bias or other biases in this study.
Ethics oversight	The protocols of this clinical trial were reviewed for compliance with the guidelines on clinical research with human stem cells in Japan and were approved by the institutional review board at Kyoto Prefectural University of Medicine and by the Special Committee of the Japanese Ministry of Health, Labor, and Welfare.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	UMIN000012534 from University Hospital Medical Information Network in Japan
Study protocol	The study protocol can be accessed https://www.nejm.org/doi/full/10.1056/NEJMoa1712770.
Data collection	The recruitment was performed from December 9, 2013 to June 30, 2016. The data were collected at Kyoto prefectural University of Medicine from December 9, 2013 to December 31, 2016.
Outcomes	The primary endpoint of this study was corneal endothelial cell density of >500 cells/mm2 at the central cornea at 24-weeks postoperative. The secondary endpoints are corneal thickness of <630μm (with decrease of corneal thickness compared with the preoperative measurement) without corneal epithelial edema at 24-weeks postoperative, over 2 levels of improvement of best-corrected visual acuity (BCVA) at 24-weeks postoperative and improvement of the National Eye Institute Visual Function Questionnaire 25 (VFQ-25) Score at 24-weeks postoperative. The corneal endothelial cell density was measured by specular microscopy. The corneal thickness was measured by ultrasonic pachymeter, anterior type Optical Coherence Tomography, or Pentacam. 5-meter vision (naked eyes, corrected, and pin hole) was measured. Questionnaire 25 (VFQ-25) was performed according to manufactured protocols.

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Human corneal endothelial cells were harvested from culture dishes by applying TrypLE Select (1 ×) and suspended at a concentration of 4 × 10^6 cells/mL in PBS buffer containing 1% BSA and 0.05% NaN3.
Instrument	BD FACS Canto II Flow Cytometry System (BD Biosciences, San Jose, CA, USA).
Software	BD FACSDiva Software (BD Biosciences, San Jose, CA, USA).
Cell population abundance	We performed flow cytometry analyses to obtain the fraction of effector cells corresponding to each lot, but did not sort the cells.
Gating strategy	As explained in Figure S1, the effector cells were selected by two gates. The first gate selects CD166+ and CD24- cells, and the second gate selects CD105- and CD44- cells.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.