Safety and efficacy of human amniotic epithelial stem cell eye drops in ocular chronic graft-*versus*-host disease

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the only curative treatment for certain hematologic diseases. Chronic graft-*versus*-host disease (cGVHD) is the most common complication after allo-HSCT, occurring in 30% to 70% of patients.[1] The eye is one of the major target organs affected by cGVHD, with ocular GVHD (ocGVHD) present in more than 50% of patients with cGVHD.[2] The common symptoms of ocGVHD are dryness, ocular pain, and even visual impairment, which severely decrease the patient's quality of life.[3] Nevertheless, the current clinical therapies for ocGVHD, including artificial tears, glucocorticoids, cyclosporine and tacrolimus, usually provide limited benefits.[4] Thus, novel therapeutic strategies are urgently needed.

Previously, we discovered that amniotic membrane transplantation can significantly alleviate ocular symptoms in ocGVHD patients.[5] Human amniotic epithelial stem cells (hAESC), a stem cell population isolated from the epithelium of the human amnion membrane, present unique characteristics, including pluripotency, low immunogenicity, and non-tumorigenicity, which suggests that they can be used as a safe cell therapy.[6] Moreover, hAESC also could inhibit the activation and proliferation of T lymphocytes by initiating their programmed death and produce a variety of immunoregulatory factors that suppress the functions of inflammatory cells and promote tissue repair and downregulate the expression of extracellular matrix deposition-related cytokines to decrease fibrosis.[7] ocGVHD is predominantly characterized by T-cell-mediated inflammatory damage, which leads to ocular tissue damage and progressive fibrosis.[8,9] Hence, we postulate that the application of hAESC could improve ocular symptoms in ocGVHD patients and investigate the safety and efficacy of hAESC for treating ocGVHD in the clinical study.

This was designed as a single-center, prospective study for patients following HSCT. The study was reviewed and approved by the Army Medical University Xinqiao Hospital Medical Science Research Ethics Committee and performed in accordance with the tenets of the Declaration of Helsinki. The study was registered at the Chinese Clinical Trials Registry (ChiCTR2200057857). ocGVHD patients were diagnosed in accordance with the National Institutes of Health (NIH) consensus, and the inclusion criteria were set as ocGVHD patients between the age of 18 and 60 who were willing to participate in the study. Eligible patients also had to present with controlled and stable systematic GVHD signs in other organs but show poor responses to the available ocular treatments, including artificial tears, glucocorticoids, immunosuppressors such as topical cyclosporine and tacrolimus, or punctual occlusion, thus resulting in a diagnosis of refractory ocGVHD. The exclusion criteria included ocular surgery within the preceding 6 months, ocular injury, other ocular diseases (such as infection, allergy, glaucoma, retinopathy, and autoimmune disease), pregnancy, long-term use of contact lenses, and uncooperative patients. At the time of recruitment, clinical data, including demographic information, diagnosis, source of transplant, type of transplant, occurrence of systemic acute/chronic GVHD, and manifestations of cGVHD, were collected (*Online Supplementary Figure S1*). Then, the patients were advised to apply two drops four times daily per eye for 6 weeks. The hAESC eye drops were obtained from iCell Biological Technology Co., Ltd. (*Online Supplementary Table S1*) and are stored at -4°C for 12 hours. The patients were further advised to report any adverse event (AE) immediately either by phone or through scheduling an immediate appointment. A team of hematologists and ophthalmologists diagnosed and graded the severity of ocGVHD in the patients based on the consensus of the NIH cGVHD score criteria. Visual acuity, ocular pressure, slit-lamp biomicroscopy, ocular surface disease index (OSDI), Schirmer's test without anesthesia, corneal fluorescein staining (CFS), fluorescein tear break-up time (FTBUT) and *in vivo* confocal microscopy (IVCM) results were all evaluated for all participants by the same ophthalmologist. Patients were followed at 2 weeks, 4 weeks, 6 weeks, 3 months and 6 months after using hAESC eye drops and whenever clinically indicated. A complete ophthalmologic examination was conducted at each visit to the extent tolerated by the patient. The differences in ocular examination results between baseline and after treatment were analyzed using the paired Student's *t* test. GraphPad Prism 10.0 for Windows was used to conduct statistical analysis and image graphing (GraphPad Software Inc.; San Diego, CA, USA). Adobe Illustrator 2020 was used for drawing. $P<0.05$ was considered to indicate statistical significance.

In total, 26 patients were included in the study. However, six patients did not complete the 2-week treatment and second ophthalmic examination and were excluded. Consequently, the population considered for analysis consisted of 20 patients, whose characteristics are summarized in the *Online Supplementary Table S2*. More male than female patients were recruited (5 females, 15 males; range, 21-56 years). The average time of ocGVHD onset following allo-HSCT was 9 months. The ocGVHD patients had cGVHD involvement in other organ systems, including the skin, liver, gastrointestinal system, oral mucosa, and lungs.

No serious AE were reported (Table 1). A total of four AE, in-

cluding hypersensitivity, application site pain, ocular itching, and increased intraocular pressure (IOP), were evaluated during this study. During treatment with the hAESC eye drops, one patient developed ocular itching rated 1 (mild) on a 1 to 5 scale, which did not stop immediately after discontinuation of the study medication for 1 day. Hence, it was considered unlikely to be related to the study medication, and the patient resumed hAESC eye drop therapy. After 2 weeks of treatment (N=20), patients demonstrated a significant reduction in OSDI from 47.40±24.73 at baseline to 35.64±24.89 (P=0.0003). After 4 weeks (N=15) and 6 weeks (N=12) of treatment, the mean OSDI decreased from 51.81±24.52 to 30.56±24.30 at baseline (P=0.0002) and from 48.49±25.38 to 23.67±22.60 at baseline (P=0.0001) (Figure 1A). After treatment with hAESC eye drops, most patients also exhibited increased tear secretions (day [d]0 *vs*. 2 weeks: 2.48±3.30 *vs*. 4.25±5.60, P=0.0237; d0 *vs*. 4 weeks: 2.67±3.57 *vs*. 5.89 ±8.09, P=0.0216) (Figure 1B). Most importantly, we also observed a marked reduction in corneal epithelial damage and improvement in epithelial recovery as detected by fluorescein staining after use of hAESC eye drops (d0 *vs*. 2 weeks: 9.23±5.83 *vs*. 7.00±6.06, P<0.0001; d0 *vs*. 4 weeks: 8.93 ±5.70 *vs*. 6.27±5.44, P=0.0003; d0 *vs*. 6 weeks: 7.96±6.04 *vs*. 6.87±5.72, P=0.0393) (Figure 2A, B). Interestingly, we found that corneal subbasal nerve density was significantly increased after hAESC treatment (d0 *vs*. 2 weeks: 9.84±5.23 *vs*. 12.53±5.83, P=0.006; d0 *vs*. 4 weeks: 10.44±5.47 *vs*. 12.85±5.18, P=0.0221) (Figure 2C, D). This result indicated that hAESC eye drops might enhance corneal sensitivity in ocGVHD patients.

ocGVHD is devastating because of limited therapeutic options, as patients usually fail to respond to current drugs, causing them to suffer from unbearable pain and apparent visual impairment, severely affecting their quality of life.[10] In the current trial, all enrolled patients had used long-term artificial tears without relief of dry eye symptoms. Artificial tears are usually administered to complement the insufficiency of tears but are unable to prevent the progression of dry eye disease.[11] Moreover, steroids and immunosuppressants are non-specific and do not restore immune homeostasis on the ocular surface well. Meanwhile, long-term steroids are always avoided in clinical practice because of an increased risk of side effects, including infection, glaucoma, and cataracts.[12] Stem cell therapy is considered an ideal option because of its ability to induce long-term recovery for injuries. In a prospective clinical trial, 14 ocGVHD patients exhibited substantial relief after mesenchymal stem cell-derived exosome (MSC-exo) treatment, showing reduced fluorescein scores and lower OSDI scores.[13] However, the flexible immune response and controversial tumorigenicity under pathological environments restrict their use for autoimmune disease patients. Moreover, autologous MSC therapy requires invasive extraction for harvesting in most cases. In contrast, hAESC, deriving from the placenta, can be non-invasively harvested without ethical concerns and have no tumorigenicity due to the lack of telomerase.[14] Importantly, hAESC have demonstrated immunomodulatory properties in inhibiting the activity of

Table 1. Overall summary of adverse events in the human amniotic epithelial stem cell eye drop trial.

Adverse events	Exist/severity N (%)	Relationship	Action taken
Hypersensitivity	0 (0)	N/A	N/A
Application site pain	0 (0)	N/A	N/A
Ocular itching	1 (5)	N/A	Discontinuation hAESC for 1 day and continue
IOP	0(0)	N/A	N/A

hAESC: human amniotic epithelial stem cell; IOP: increased intraocular pressure; N/A: not applicable.

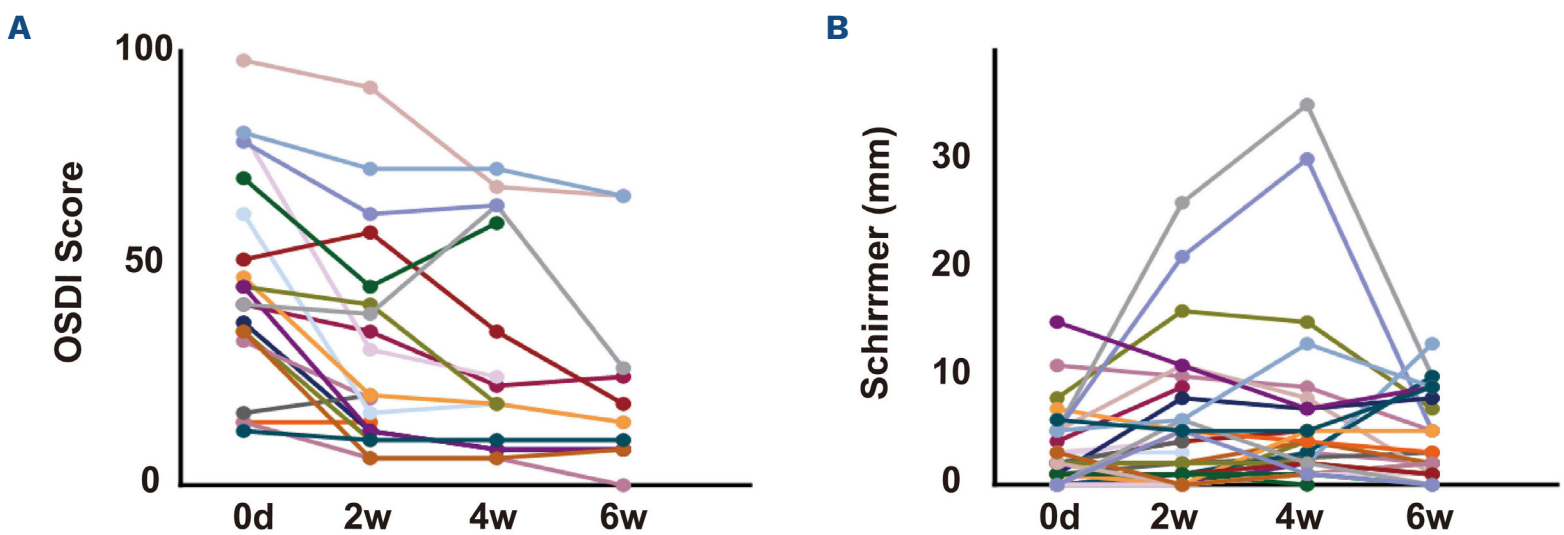

Figure 1. Human amniotic epithelial stem cell eye drops ameliorated ocular symptoms in ocular graft-*versus*-host disease patients. (A) After 2 weeks of treatment with human amniotic epithelial stem cell (hAESC) eye drops, most of patients ameliorated ocular symptoms and improved their quality of life, as reflected by decreased Ocular Surface Disease Index (OSDI) scores. Each line represents 1 patient (N=20). (B) After 2 weeks (w) of treatment with hAESC eye drops, patients presented with increased tear volume by Schirmer's test. Each line represents 1 eye (N=40). d: day.

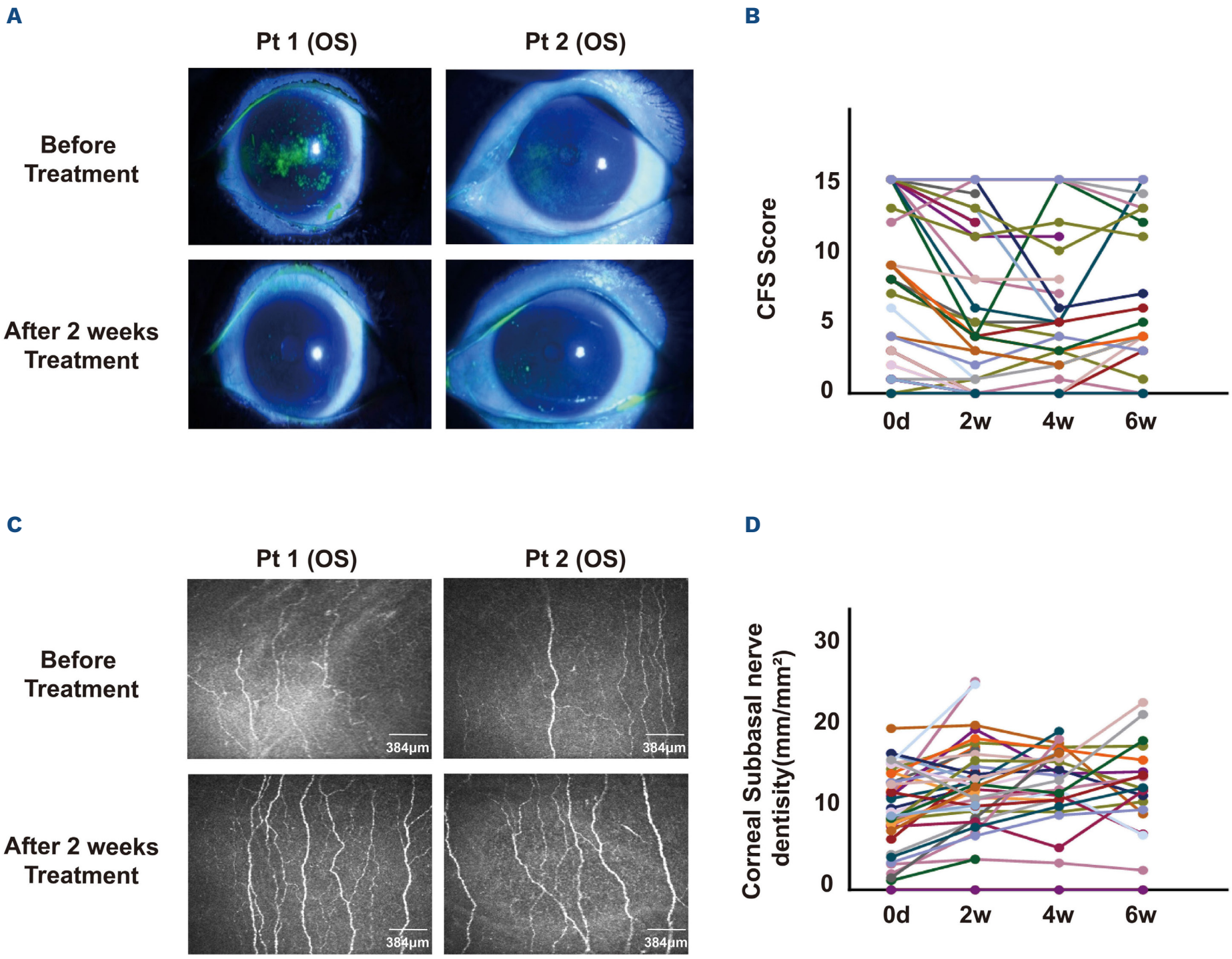

Figure 2. Changes in corneal fluorescein staining and corneal subbasal nerve density. (A, B) Representative images of the ocular anterior segment under slit-lamp microscopy and fluorescein examination before and 2 weeks (w) after human amniotic epithelial stem cell (hAESC) treatment. After hAESC eye drops usage, the cornea was more transparent, with fewer punctate areas of erosion. Fluorescein scores of individual eyes before and 6 weeks after treatment with hAESC eye drop showed that the fluorescein score tended to decrease in most eyes. (C, D) *In vivo* confocal microscopy images of corneal subbasal nerve density in patients before and after treatment, showing an increased corneal nerve density after using hAESC eye drops. (A-D) Each line represents 1 eye (N=40). OS: oculus sinister; Pt: patient; CFS: orneal fluorescein staining.

immune cells and efficaciousness and have regenerative repair and antifibrotic properties.[15] The relative stability of hAESC when administered non-invasively has led to the application of cell eye drops rather than injections as a therapeutic approach for ocGVHD.

Our clinical trial showed that the application of hAESC eye drops four times daily improved the clinical signs and symptoms of ocGVHD within 2 weeks. The hAESC caused no irritation of the eyes, resulting in excellent compliance. According to our results, the mean reductions in the total OSDI was more apparent as the duration of treatment increased. All changes were greater than 10 points, which is generally considered to indicate a significant change in this group of severely affected patients. The eye symptoms in ocGVHD patients, including stinging, burning sensations, crust, or redness, were relieved after hAESC eye drop treatment. In our study, the treatment also showed an increase in tear production in Schirmer's test. We showed that in addition to prominent symptomatic improvement, the CFS was significantly improved, indicating healing of the epitheliopathy. Corneal innervation is one of the most important aspects indicating the state of the ocular surface. As evidenced by previous studies using *in vivo* confocal microscopy, the density of subbasal nerves in ocGVHD

was lower than that in normal eyes. A review revealed that the density of the subbasal corneal nerve is thought to be positively correlated with corneal sensitivity. Our study showed that the density of subbasal nerves improved after using hAESC eye drops. The *Online Supplementary Table S1* provides a description of the growth factors found in hAESC eye drops, such as transforming growth factor-β1, angiogenin, insulin-like growth factor and so on. These growth factors play a crucial role in the recovery of the corneal epithelium and nerves for ocGVHD patients. Further research is necessary to ascertain the exact mechanisms by which growth factors repair corneal epithelial cells and nerves.

In conclusion, hAESC eye drops are a safe, non-invasive, and efficient therapy for ocGVHD patients. Consistent with a safety study, sample sizes were relatively small in the study population. Further trials using hAESC eye drops in larger groups are planned to validate these findings. Furthermore, it would be appropriate for us to conduct relevant animal research in the future to explain the mechanism by which hAESC treat ocGVHD.

Authors

Xianjing Cheng,[1,2,3*] Wei Fan,[4*] Ruihao Huang,[1,2,3*] Yuancheng Zhao,[4] Yonghong Tang,[4] Shiqin Huang,[1,2,3] Guanghui Zhang,[5] Xi Zhang,[1,2,3,6] Rongdi Yuan[4] and Xiaoqi Wang[1,2,3]

[1]Medical Center of Hematology, Xinqiao Hospital of Army Medical University, Chongqing, [2]Chongqing Key Laboratory of Hematology and Microenvironment, Chongqing; [3]State Key Laboratory of Trauma and Chemical Poisoning, Army Medical University, Chongqing; [4]Department of Ophthalmology, Xinqiao Hospital, Army Medical University, Chongqing; [5]Shanghai iCELL Biotechnology Co., Ltd, Shanghai and [6]Jinfeng Laboratory, Chongqing, China

**XC, WF and RH contributed equally as first authors.*

Correspondence:
X. ZHANG - zhangxxi@sina.com
R. YUAN - yuanrongdi@126.com
X. WANG - xiaoqiwang27@gmail.com

https://doi.org/10.3324/haematol.2023.284571

Received: May 16, 2024.
Accepted: September 19, 2024.
Early view: September 26, 2024.

Disclosures

No conflicts of interest to disclose.

Contributions

XC, WF and RH performed the research and prepared the manuscript. YZ and HT performed the ophthalmic index analysis. SH collected and analyzed the data. GZ prepared the hAESC eye drops. XZ and RY provided advice on the study design and critical review and editing of the manuscript. XW is the PhD advisor for XC and WF and helped with selecting the research project, designing and supervising the study and editing the manuscript. All authors had access to the data and were involved in the interpretation of data, contributed to the manuscript review and revisions, and approved the final version for submission.

Acknowledgments

The authors wish to thank the study patients, their support and the clinical research staff who assisted in collecting samples and clinical information.

Funding

This work was supported by the National Natural Science Foundation of China (82020108004, 82100235), the National Key R&D Program of China (2022YFA1103300, 2022YFA1103304), the Natural Science Foundation of Chongqing Innovation Group Science Program (cstc2021jcyj-cxttX0001), the Natural Science Foundation of Chongqing (CSTB2022NSCQ-MSX1060), and the Special Project for Talent Construction in Xinqiao Hospital (2022YQB004, 2022XKRC001).

Data-sharing statement

The data underlying this article will be shared on reasonable request addressed to the corresponding author XW.

References

1. Jagasia MH, Greinix HT, Arora M, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. The 2014 Diagnosis and Staging Working Group Report. Biol Blood Marrow Transplant. 2015;21(3):389-401.
2. Cheng X, Huang R, Huang S, et al. Recent advances in ocular graft-versus-host disease. Front Immunol. 2023;14:1092108.
3. Stem Cell Application Group, Chinese Society of Hematology, Chinese Medical Association. [Chinese expert consensus on ocular chronic graft-versus-host disease (2023)]. Zhonghua Xue Ye Xue Za Zhi. 2023;44(6):449-457.
4. Cheng XJ, Yuan RD, Zhang X. [Treatment of chronic ocular graft versus host disease]. Zhonghua Xue Ye Xue Za Zhi. 2022;43(10):877-880.
5. McDonald MB, Sheha H, Tighe S, et al. Treatment outcomes in the DRy Eye Amniotic Membrane (DREAM) study. Clin Ophthalmol. 2018;12:677-681.
6. Yang P-J, Yuan W-X, Liu J, et al. Biological characterization of

human amniotic epithelial cells in a serum-free system and their safety evaluation. Acta Pharmacol Sin. 2018;39(8):1305-1316.
7. Li J, Qiu C, Wei Y, et al. Human amniotic epithelial stem cell-derived retinal pigment epithelium cells repair retinal degeneration. Front Cell Dev Biol. 2021;9:737242.
8. Cheng X, Huang R, Fan W, et al. The tear cytokine panel is a useful biomarker for early diagnosis and severity-evaluating of ocular chronic graft-versus-host disease. Bone Marrow Transplant. 2023;58(6):732-734.
9. Ogawa Y, Kawakami Y, Tsubota K. Cascade of inflammatory, fibrotic processes, and stress-induced senescence in chronic GVHD-related dry eye disease. Int J Mol Sci. 2021;22(11):6114.
10. Carreno-Galeano JT, Dohlman TH, Kim S, Yin J, Dana R. A review of ocular graft-versus-host disease: pathophysiology, clinical presentation and management. Ocul Immunol Inflamm. 2021;29(6):1190-1199.
11. Doughty MJ, Glavin S. Efficacy of different dry eye treatments with artificial tears or ocular lubricants: a systematic review. Ophthalmic Physiol Opt. 2009;29(6):573-583.
12. Inamoto Y, Valdés-Sanz N, Ogawa Y, et al. Ocular graft-versus-host disease after hematopoietic cell transplantation: expert review from the Late Effects and Quality of Life Working Committee of the Center for International Blood and Marrow Transplant Research and Transplant Complications Working Party of the European Society of Blood and Marrow Transplantation. Biol Blood Marrow Transplant. 2019;25(2):e46-e54.
13. Zhou T, He C, Lai P, et al. miR-204-containing exosomes ameliorate GVHD-associated dry eye disease. Sci Adv. 2022;8(2):eabj9617.
14. Strom SC, Gramignoli R. Human amnion epithelial cells expressing HLA-G as novel cell-based treatment for liver disease. Hum Immunol. 2016;77(9):734-739.
15. Ouyang X, You S, Zhang Y, et al. Transplantation of human amnion epithelial cells improves endometrial regeneration in rat model of intrauterine adhesions. Stem Cells Dev. 2020;29(20):1346-1362.