

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

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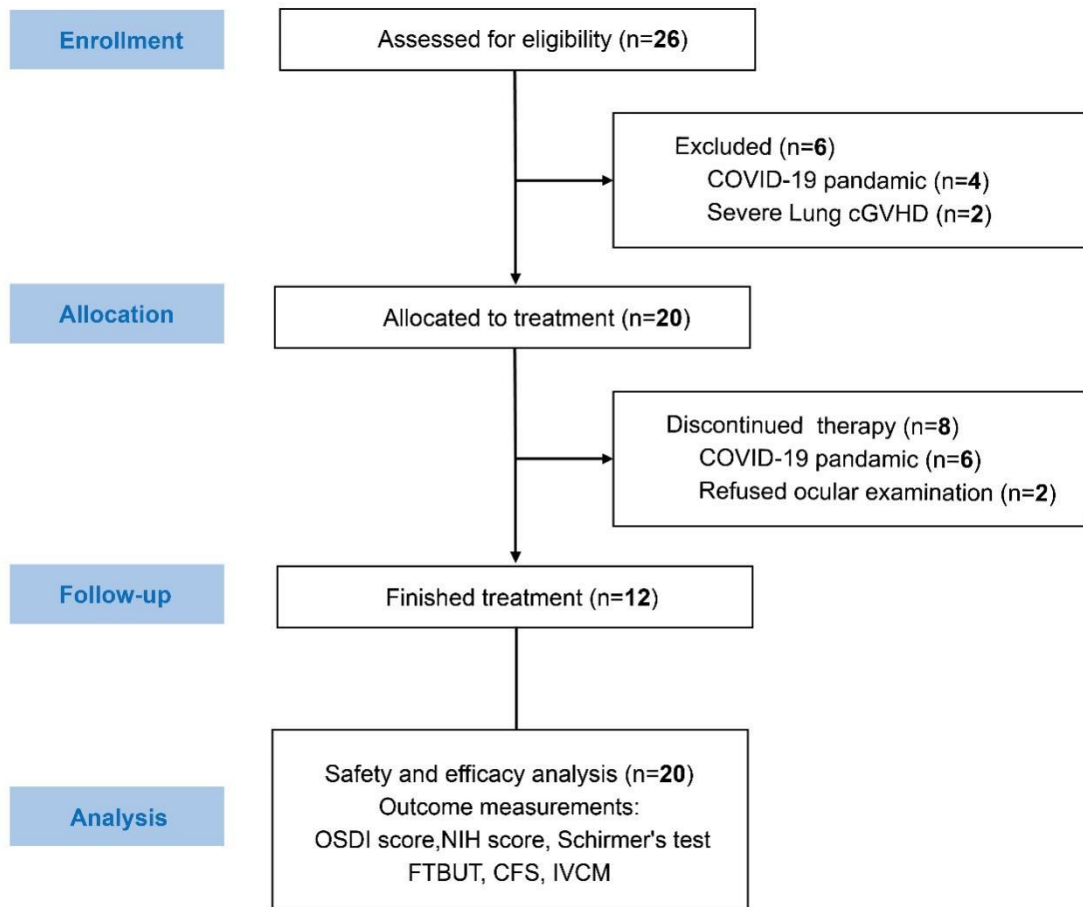
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### Online Supplementary Figure 1



Consolidated Standards of Reporting Trials (CONSORT) diagram of the hAESC eye drops trial. OSDI=ocular surface disease index; FTBUT=fluorescein tear break-up time; CFS=corneal fluorescein staining; IVCM=in vivo confocal microscopy.

**Online Supplementary table 1** Manufacturing details of the hAESC eye drop products

The amniotic membrane was peeled from the placental chorion and washed in Hank's balanced salt solution (HBSS) to remove blood cells. The amniotic membrane was digested with 0.25% trypsin (EDTA) for 30 min at 37 °C in a water bath. Culture medium (F12/DMEM, 10% KSR (Knock Out Serum Replacement), 2 mmol/L L-glutamine, 1% nonessential amino acid, 55 µmol/L 2-mer-captoethanol, 1 mmol/L sodium pyruvate, 1% antibiotic-antimycotic (all from Gibco) and 10 ng/mL EGF (Peprotech) was added to the trypsin digestion medium, and the sample was centrifuged for 10 min at 300×g. The cell pellet was suspended in complete culture medium for subsequent cell culture. The cultured hAESC were frozen in CELLBANKER (ZENOAQ, Fukushima, Japan) at a concentration of 5×10<sup>6</sup>/mL and stored at -80 °C overnight. Then, frozen vials were transferred into liquid nitrogen for long-term preservation. The hAESC were thawed by gently agitating the vial in a 37 °C water bath. Then, the hAESC eye drops were made at a concentration of 1×10<sup>6</sup>/mL. In addition, 12 growth factors were identified in the suspension of hAESC using enzyme-linked immunosorbent assay research.

Items	Results
Appearance	Colorless translucent cell suspension, no foreign matter or granular material
Cell Viability	≥90%
Cell Phenotypic CD324	≥90%
Cell Phenotypic CD146	≤5%
HLA-DR	≤2%
Bacteria	Negative
Fungi	Negative
Mycoplasma	Negative
Endotoxin	≤2.5EU/ml
TGF-β2	Negative
TGF-β3	Negative
bFGF	Negative
EGF	Negative
HGF	Negative
TGF-β1	7.4 ng/ml

AGN	35.5 pg/ml
IGF	5.1 ng/ml
KGF	210.3 pg/ml
NGF	3.0 ng/ml
NT-3	7.5 pg/ml
VEGF	438.0 pg/ml

TGF- $\beta$ 2=Transforming growth factor- $\beta$ 2; basic fibroblast growth factor=bFGF; epidermal growth factor=EGF; Hepatocyte growth factor=HGF; AGN=angiogenin; IGF=insulin-like growth factor; KGF=keratinocyte growth factors; NGF=nerve growth factor; NT-3=neurotrophin-3; VEGF=vascular endothelial growth factor

**Online Supplementary table 2.** Characteristics of patients with ocular chronic graft-versus-host disease

Pts	Sex	Age	Diagnosis	Type of Transplant	Baseline Time after HSCT (months)	Systemic cGVHD	Overall NIH Score	Topical treatment before hAESC treatment	Baseline ophthalmic characteristics				
									OSDI score	Schirmer (mm) (OD,OS)	FTBUT (s) (OD,OS)	CFS score (OD,OS)	Eye NIH score
1	M	56	AML	UD-HSCT	66	Oral, Eye	2	AT, PSL	12.5	5, 6	5, 3	1, 0	1
2	M	49	AML	Sib-HSCT	90	Eye	3	AT, PSL, CSA, ASD, Punctal plug	81.25	0, 0	1, 1	15, 15	3
3	M	38	AML	Sib-HSCT	60	Oral, Eye	4	AT, PSL	79.17	2, 3	3, 4	9, 9	3
4	F	29	MDS	Sib-HSCT	43	Skin, Oral, Joint, Eye	6	AT, PSL, CSA, FK506	41.67	1, 1	1, 1	8, 8	3
5	M	33	TCL	UD-HSCT	5	Skin, Liver, Eye	4	AT, PSL	35.42	2, 15	3, 5	15, 1	2
6	M	30	MDS	Sib-HSCT	40	Oral, Liver, Eye	5	AT, PSL, CSA, FK506	97.92	1, 0	0, 0	0, 0	3
7	M	51	MDS	haplo-HSCT	16	Liver, Eye	4	AT, CSA, FK506	70.83	0, 0	3, 3	15, 15	3
8	M	24	AML	UD-HSCT	32	Lung, Eye	3	AT, PSL, CSA, FK506	52.08	1, 0	3, 4	13, 12	2
9	F	51	AML	UD-HSCT	24	Lung, Eye	3	AT, PSL, CSA, FK506, Punctal plug	45.83	0, 2	0, 0	15, 15	2
10	M	21	ALL	Sib-HSCT	10	Skin, Oral, Eye	5	AT, PSL, CSA	45.83	0, 1	6, 7	9, 15	3
11	M	35	AMOL	haplo-HSCT	19	Skin, Liver, Lung, Joint, Eye	7	AT, PSL, CSA, FK506	81.25	0, 0	0, 0	15, 15	3
12	M	33	AML	haplo-HSCT	21.5	Eye	1	AT, PSL, FK506	47.92	5, 5	4, 2	1, 4	1
13	F	37	ALL	Sib-HSCT	50	Eye	2	AT, CSA	62.5	5, 2	8, 5	3, 4	2
14	F	52	AML	Sib-HSCT	30	Liver, Eye	3	AT, PSL	41.67	2, 0	5, 3	3, 15	2
15	F	30	MDS	Sib-HSCT	4	Liver, Lung, Joint, Eye	5	AT, PSL, CSA, FK506	33.33	2, 0	4, 2	7, 15	2

16	M	48	ALL	Sib-HSCT	40.5	Oral, Liver, Eye	4	AT, PSL	35.42	7, 3	3, 1	2, 2	2
17	M	43	AML	Sib-HSCT	58	Oral, Eye	3	AT, PSL, CSA, FK506	16.67	4, 3	8, 3	1, 6	2
18	M	40	ALL	UD-HSCT	14	Liver, Eye	4	AT, PSL, CSA, FK506	14.58	8, 11	10, 1	0, 3	2
19	M	26	ALL	UD-HSCT	34.5	Skin, Eye	4	AT, CSA, FK506	37.5	1, 1	3, 3	15, 15	3
20	M	53	AML	Sib-HSCT	50	Oral, Liver, Eye	4	AT, CSA	14.58	0, 0	1, 2	15, 15,	2

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ALL=acute lymphoblastic leukemia; AML=acute myelogenous leukemia; CML=chronic myelogenous leukemia; AMOL =acute monocytic leukemia; BMs =bone marrow stem cells; PBSCs =peripheral blood stem cells; TCL= T-cell lymphoma; UD-HSCT= unrelated donor hematopoietic stem cell transplantation; Sib-HSCT= sibling donor hematopoietic stem cell transplantation; haplo-HSCT=haploidentical hematopoietic stem cell transplantation; AT=artificial tear; PSL=prednisolone; CSA=cyclosporin; FK506=tacrolimus; ASD=autologous serum eye drops; OS=oculus sinister; OD=oculus dexter