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# Allogeneic mesenchymal stem cell therapy for dry eye disease in patients with Sjögren's syndrome: A randomized clinical trial

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ABSTRACT

*Purpose:* This double-blinded randomized clinical trial aimed to evaluate the efficacy of injecting allogeneic adipose-derived mesenchymal stem cells (ASCs) into the lacrimal gland (LG) for the treatment of dry eye disease (DED) secondary to Sjögren's syndrome (SS).

*Methods:* Fifty-four participants with severe DED secondary to SS were included and allocated to either ASCs (n = 20), vehicle (n = 20), or a non-randomized observation group (n = 14). The intervention groups received a single injection of either ASCs or an active comparator (vehicle, Cryostor® CS10) into the LG in one eye, while the observation group received lubricating eye drops only. The primary outcome measure was changes in Ocular Surface Disease Index (OSDI) score and secondary outcome measures were non-invasive tear break-up time, tear meniscus height, Schirmer's test, and Oxford score within a 12-month follow-up.

*Results:* A significant reduction in OSDI score was observed in the ASCs and vehicle groups compared to the observation group. In addition, the ASCs group demonstrated a significant increase in non-invasive tear break-up time compared to the vehicle group at the 4-week follow-up and to the observation group at the 12-month follow-up. A significant improvement in ocular surface staining, tear osmolarity, and Schirmer test score from baseline was also observed in the ASCs group; however, these changes were not significant compared to the other groups.

*Conclusion:* Improvement of subjective and objective signs and symptoms of DED was observed in both intervention groups following injection into the LG compared to the observation group. Future studies should investigate the mode-of-action of both injection treatments.

## 1. Introduction

Dry eye disease (DED) is a multifactorial condition in which tear film instability, hyperosmolarity, and ocular surface inflammation causes symptoms such as ocular discomfort and pain [1]. The clinical diagnosis of DED is difficult due to the complex etiology and the poor association between signs and symptoms. This leads to DED being underdiagnosed and undertreated [2]. DED can be categorized into two main types: primarily evaporative dry eye (EDE) and primarily aqueous-deficient dry eye (ADDE). However, patients often have a combination of both [3]. A common cause of ADDE is the autoimmune disorder Sjögren's syndrome (SS). In SS, a lymphocytic infiltration of the lacrimal and salivary glands causes glandular hypofunction leading to dryness of the eyes and mouth [4]. The diagnosis of primary SS is based

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on the "2016 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) classification criteria for primary Sjögren's syndrome": positive anti-SSA antibodies (3 points), focal lymphocytic sialadenitis (3 points), abnormal ocular surface staining (1 point), a Schirmer test score of ≤5 mm/5 min (1 point), and a reduced unstimulated salivary flow rate (1 point). A total score ≥4 for these items fulfills the diagnostic criteria for primary SS [5]. Some patients may have SS related to another autoimmune disorder such as rheumatoid arthritis (RA) or systemic lupus erythematosus (SLE), which is classified as secondary SS [6].

In research on novel treatment options for DED, the underlying inflammation is a frequent treatment target. By reducing the inflammation, the vicious circle of DED can be broken, eventually alleviating the signs and symptoms of DED [7]. Topical treatment with ciclosporin A eye drops for DED has been approved in both the US and the EU and is widely used. The subjective and objective results in many clinical trials, however, are inconsistent and may not be significantly different from vehicle or lubricating eye drops alone [8]. Surgical treatments such as salivary gland and amniotic membrane transplantation have also been investigated with varying effects [9]. Further, other lacrimal gland targeted therapies for severe ADDE are currently being investigated [10]. Mesenchymal stem (or stromal) cells (MSCs) have been shown to possess anti-inflammatory and immunomodulatory capabilities, making them suitable candidates for research on inflammatory diseases such as ADDE due to SS. Numerous pre-clinical studies in different animal models have investigated MSCs as a treatment of ADDE [11]; however, only one study investigating injection of MSCs into the lacrimal gland (LG) in humans has been published. In 2021, our research group published the results of the first clinical trial demonstrating the safety and feasibility of injecting allogeneic adipose-derived mesenchymal stem cells (ASCs) into the LG in patients with ADDE [12]. The results of this open-label safety study indicated that injection of ASCs into the LG, in a volume corresponding to maximally 50 % of the LG volume, was a safe treatment. Furthermore, within 4 months after treatment, we found a 40 % decrease in dry eye symptoms measured with the Ocular Surface Disease Index (OSDI) and a significant improvement in fluoresceine tear break up-time (FTBUT), tear osmolarity, and Schirmer test scores. We hypothesize that injection of allogeneic ASCs into the LG reduces inflammation, resulting in decreased ocular discomfort and increased tear film stability in patients with ADDE as compared to injection of vehicle or observation only.

## 2. Materials and methods

### 2.1. Patient enrolment

We performed this randomized double-blinded clinical trial to investigate the efficacy of injecting allogeneic ASCs into the LG in patients with ADDE due to SS. This trial was conducted at Copenhagen University Hospital – Rigshospitalet, Denmark, according to the Declaration of Helsinki and the ICH-GCP Guideline. The trial was approved by the Danish Medicines Agency (EudraCT no. 2020-002804-38), the Danish National Committee on Health Research Ethics, was monitored by the GCP unit in the Capital Region of Denmark, and was registered as a clinical trial at ClinicalTrials.gov (NCT04615455) prior to study initiation.

### 2.2. Inclusion criteria

Age >18 years, a diagnosis of SS according to the ACR/EULAR criteria, OSDI score ≥33, Schirmer test score 1–5 mm/5 min, and NIKBUT <10 s. If a patient signed the informed consent and met all inclusion criteria, a magnetic resonance imaging (MRI) of the orbit was performed to measure the LG volume in both eyes.

### 2.3. Exclusion criteria

LG volume on MRI <0.2 $cm^3$ in the study eye, previous treatment with ASCs or other stem cell products in the LG(s), reduced immune response, pregnancy or planned pregnancy within the next 2 years, breastfeeding, topical treatment with eye drops other than to treat DED, or any other disease/condition judged by the investigator to be grounds for exclusion.

### 2.4. Clinical and laboratory examinations

At all study visits, the examination was performed by the same investigator blinded to the allocated study treatment in the following order: Subjective symptoms of DED were quantified using the OSDI questionnaire and any adverse events (AEs) were recorded. The visual acuity was measured using a logMAR chart. A measurement of the tear osmolarity (TearLab, San Diego, CA, USA) was performed twice in each eye and the higher value was recorded. The Keratograph 5 M (Oculus, Germany) was used to obtain non-invasive keratograph tear break-up time (NIKBUT) and tear meniscus height (TMH). NIKBUT provides 2 specific values: NIKBUT *first*, which represents the time of the initial tear break-up, and NIKBUT *average*, which is the average tear break-up time during the recording. The Schirmer's I test without anesthesia was performed using sterile, standardized Tear Test Strips (Haag-Streit, Essex, UK). A single drop of sterile saline was administered onto a sterile 1 mg strip of fluorescein sodium (I-DEW FLO, Entod Research Cell UK Ltd., London, UK), which was then applied to the lower conjunctival fornix of each eye. The staining of the ocular surface was assessed using the Oxford grading scale within 3 min of applying the fluorescein.

### 2.5. Magnetic resonance imaging protocol

All qualified study participants were examined on a 3T Siemens MAGNETOM Prisma (Siemens Healthineers, Erlangen, Germany) using a single contrast-enhanced 3D T1-weighted sequence with selective water excitation and 0.9 mm isotropic resolution. The LGs were manually delineated on GE AW Server 3.2 with adjustments possible in all imaging planes and their volumes subsequently automatically calculated.

### 2.6. Allocation and randomization

If a participant fulfilled all inclusion and no exclusion criteria, they were allocated in a 1:1 ratio to injection of either allogeneic ASC product or vehicle in one eye. Treatment randomization was performed by personnel at the cell-processing unit before treatment of first patient in a double-blinded manner such that neither the participant nor the masked investigator was familiar with the allocated treatment until after the statistical analysis was performed at the end of study. If a participant fulfilled all inclusion criteria but either (1) MRI was judged to be contraindicated, (2) LG volume on MRI was <0.2 $cm^3$, or (3) the participant withdrew consent to undergo study intervention, the participant was allocated to an observation group with no intervention and a clinical follow-up > 12 months after baseline. All participants in all 3 groups were instructed to continue the existing treatment regimen with lubricating eye drops during the follow-up period. In the 2 intervention groups, the study participants were examined within 30 min following treatment and 1 week, 4 weeks, 4 months, and 12 months after study treatment. In all 3 groups, if both eyes fulfilled the inclusion criteria, the eye with the lowest Schirmer test score was determined as the study eye.

### 2.7. Outcome measures and sample size

The primary outcome measure was change in OSDI score from baseline in the ASCs group compared to the vehicle and observation groups.

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The sample size was calculated using a 2-sided *t*-test with 80 % power and $p < 0.05$ significance level, which yielded a minimum of 4–14 participants per group to detect a minimal clinical difference of 7.3–13.4 points on the OSDI score in patients with severe DED (OSDI ≥33) [13]. To compensate for the fact that DED measurements often deviate from a normal distribution and to compensate for potential drop-out in the study, 20 participants were included in each intervention group. The secondary outcome measures were change in NIKBUT, TMH, tear osmolarity, Schirmer test score, and ocular surface staining (Oxford grade). Human leukocyte antigen (HLA) antibodies were tested at baseline and 4 and 12 months after treatment. At each follow-up, any AE was recorded and classified according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 [14]. Any AE was recorded as either related or not related to the study treatment.

### 2.8. Preparation of vehicle and stem cell product

The allogeneic adipose-derived mesenchymal stromal cell products (CSCC_ASCs) were manufactured at Cardiology Stem Cell Centre (CSCC), Rigshospitalet, University Hospital Copenhagen, Denmark, under manufacturing authorization no. 38940 and tissue establishment authorization no. DK261985. Manufacturing followed EU Guidelines for Good Manufacturing Practice (GMP) of Medicinal Products for Human Use (certificate of GMP compliance no. DK IMP 130620). Liposuction from the subcutaneous adipose tissue of the abdomen of 3 healthy volunteer female donors (age 21–27 years) was performed by an experienced plastic surgeon using local anesthesia and in full in adherence to sterile cosmetic surgery protocols. The healthy donors signed an informed consent complying with the declaration of Helsinki. Within 30 days prior to liposuction and on the day of the procedure, the donors were tested for HIV, hepatitis B and C, syphilis, and human T-lymphotropic virus (HTLV) I/II serology by serum analysis. Donor tissue typing (low HLA I and II genotyping) was performed for the purpose of alloantibody screening in patients after the cell treatment. No pre-treatment tissue type matching between the donor and the recipient was performed. The stromal vascular fraction (SVF) was isolated from the lipoaspirate obtained from each donor. Subsequently, an animal-free cell expansion process was carried out using nLiven human platelet lysate (Sexton Biotechnologies) as a culture supplement. This expansion was conducted in semi-automated and functionally closed bioreactor systems (Quantum Cell Expansion System, Terumo BCT) [15]. After 2 passages in the bioreactor system, harvested ASCs were cryopreserved in CryoStor CS10 (BioLife Solutions) containing 10 % dimethyl sulfoxide (DMSO) at a dose of 22 million cells per mL. Final products were stored below −180 °C in nitrogen dry storage until clinical use. A final cell product vial contained cells from one donor only. The vehicle vials contained the active comparator CryoStor10 only.

### 2.9. Injection procedure

Each participant in the 2 intervention groups received one transcutaneous injection of either the allogeneic ASC product or vehicle into the LG in one eye. The injected volume corresponded to maximally 50 % of the LG volume as measured on MRI as previously shown to be safe [12]. The vial containing either the ASC product or vehicle came frozen as a ready-to-use product and was thawed bed side in a 37 °C water bath. All injections were performed by an experienced eye surgeon. To ensure accurate drug delivery, the injections were performed via ultrasonic guidance using a GE Logic E10 R2 (Milwaukee, Wisconsin, USA) device with a 6–24 MHz linear array transducer as previously described (Fig. 1) [16].

**Table 1**
Demographic and baseline characteristics in the ASCs, vehicle, and observation group. No significant differences between groups (besides LG volume) were present at baseline.

| | ASCs (n = 20) | Vehicle (n = 20) | Observation (n = 14) | Total (n = 54) | *p*-value |
|---|---|---|---|---|---|
| Sex, female, *n (%)* | 20 (100) | 20 (100) | 14 (100) | 54 (100) | – |
| Age (years), *median (IQR)* | 60 (51–71) | 59 (52–67) | 56 (45–71) | 59 (49–71) | 0.8201 |
| Primary SS, *n (%)* | 16 (80) | 18 (90) | 12 (86) | 46 (85) | 0.8846 |
| Study eye, right, *n (%)* | 12 (60) | 11 (55) | 7 (50) | 30 (56) | 0.9372 |
| LG volume, study eye ($cm^3$), *median (IQR)* | 0.41 (0.24–0.60) | 0.43 (0.25–0.84) | 0.07 (0.00–0.15) | 0.32 (0.20–0.60) | <.0001 |
| LG volume, fellow eye ($cm^3$), *median (IQR)* | 0.42 (0.13–0.55) | 0.44 (0.23–0.70) | 0.06 (0.00–0.13) | 0.26 (0.12–0.56) | 0.0003 |
| Injection dose ($cm^3$), *median (IQR)* | 0.18 (0.10–0.28) | 0.18 (0.10–0.40) | – | 0.18 (0.10–0.30) | 0.4811 |
| Visual acuity, study eye (logMAR), *median (IQR)* | 0.0 (0.0–0.0) | 0.1 (0.0–0.1) | 0.0 (0.0–0.1) | 0.0 (0.0–0.1) | 0.1583 |
| Visual acuity, fellow eye (logMAR), *median (IQR)* | 0.0 (0.0–0.1) | 0.1 (0.0–0.2) | 0.0 (0.0–0.1) | 0.0 (0.0–0.1) | 0.5427 |
| OSDI score (0–100), *median (IQR)* | 39.8 (35.6–52.1) | 49.0 (43.5–56.9) | 55.2 (36.1–60.4) | 46.7 (36.1–56.8) | 0.2316 |
| NIKBUT first, study eye (sec), *median (IQR)* | 3.50 (3.03–5.77) | 4.18 (2.81–5.71) | 3.76 (3.31–6.31) | 3.70 (3.06–5.74) | 0.7235 |
| NIKBUT average, study eye (sec), *median (IQR)* | 8.07 (5.14–11.82) | 10.33 (5.85–15.27) | 7.85 (5.46–14.47) | 8.59 (5.42–14.83) | 0.7555 |
| NIKBUT first, fellow eye (sec), *median (IQR)* | 5.58 (3.51–6.79) | 3.89 (2.87–6.63) | 4.62 (3.06–6.56) | 4.62 (3.06–6.69) | 0.4515 |
| NIKBUT average, fellow eye (sec), *median (IQR)* | 8.74 (6.72–14.86) | 8.66 (4.66–13.18) | 7.95 (6.20–16.21) | 8.47 (6.07–13.35) | 0.665 |
| TMH, study eye (mm), *median (IQR)* | 0.21 (0.18–0.29) | 0.24 (0.18–0.29) | 0.23 (0.17–0.30) | 0.22 (0.17–0.29) | 0.9165 |
| TMH, fellow eye (mm), *median (IQR)* | 0.24 (0.20–0.26) | 0.29 (0.20–0.40) | 0.20 (0.16–0.30) | 0.23 (0.17–0.32) | 0.3586 |
| Tear osmolarity, study eye (mosm/L), *median (IQR)* | 318 (308–328) | 308 (294–342) | 318 (305–360) | 314 (304–337) | 0.3628 |
| Tear osmolarity, fellow eye (mosm/L), *median (IQR)* | 313 (299–339) | 308 (293–330) | 309 (299–349) | 308 (298–330) | 0.7191 |
| Oxford grade, study eye, *median (IQR)* | 2 (1–3) | 3 (2–3) | 3 (2–3) | 2 (1–3) | 0.4053 |
| Oxford grade, fellow eye, *median (IQR)* | 2 (1–3) | 2 (2–3) | 2 (2–3) | 2 (1–3) | 0.4459 |
| Schirmer test score, study eye (mm), *median (IQR)* | 2.5 (1.0–5.0) | 3.5 (1.5–5.0) | 1.5 (1.0–3.0) | 3.0 (1.0–5.0) | 0.2299 |
| Schirmer test score, fellow eye (mm), *median (IQR)* | 3.0 (2.0–5.0) | 4.5 (2.0–9.5) | 2.0 (1.0–4.0) | 3.0 (2.0–5.0) | 0.0913 |

ASCs = *adipose-derived mesenchymal stem cells,* SS = *Sjögren's syndrome,* LG = *lacrimal gland,* logMAR = *logarithm of the minimum angle of resolution,* OSDI = *Ocular Surface Disease Index,* mosm/l = *milliosmole* per *liter,* NIKBUT = *non-invasive keratograph tear break-up time,* TMH = *tear meniscus height.*

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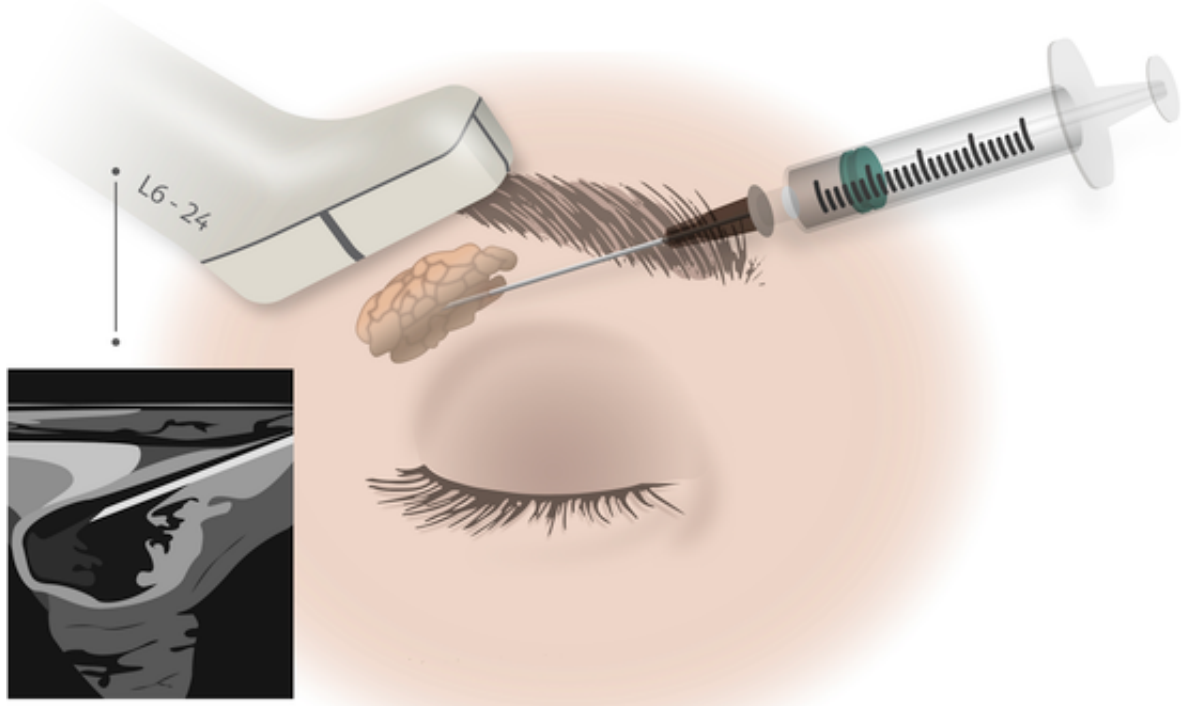


**Fig. 1.** Injection into the lacrimal gland using ultrasonic guidance.

### 2.10. Human leukocyte antigen antibodies

For HLA typing, venous blood samples were collected and contained in siliconized vacuum tubes containing protamine sulfate and ethylenediamine tetraacetic acid. Deoxyribonucleic acid was isolated and HLA typing was performed of HLA-A, B, C, DRB1, DRB3/4/5, DQA1, DQB1, DPA1, and DPB1 loci by LINKSeqTM PCR Typing kits (One Lambda, Thermo Fischer Scientific Inc) in accordance with the manufacturer's instructions. Patient serum samples were screened for anti-HLA antibodies by LABScreen™ Mixed (One Lambda, Thermo Fischer Scientific Inc). Positive samples were further analyzed by LABScreen™ Single Antigen (One Lambda, Thermo Fischer Scientific Inc) with positivity defined as mean fluorescence intensity (MFI) values ≥ 1000. Donor-specific HLA antibodies (DSA) were defined as recipient responses to single antigen beads, which each presented one or more of the donor's antigens encoded by HLA-A,-B,-C,-DRB1,-DRB3/4/5,-DQA1,-DQB1,-DPA1,-DPB1 antigens. Analyses were accredited by the European Federation for Immunogenetics and by standard DS/EN ISO 15189.

### 2.11. Data collection and statistical analysis

Study data were collected and managed using Research Electronic Data Capture (REDCap) hosted by the Capital Region of Denmark. The statistical analyses were performed in SAS 9.4 (SAS Institute, Cary, NC, USA). Prior to unblinding of the allocated study treatment, a statistical analysis plan (SAP) was prepared (Supplementary material S2) and the statistical analysis of the complete data set was performed. Baseline characteristics for the ASCs, vehicle, and observation groups were reported as frequency and percentage for categorical characteristics, and median and interquartile range (IQR) for continuous characteristics. These characteristics were compared between the 3 groups with a chi-squared test for categorical characteristics and a Kruskal–Wallis test for continuous characteristics. The mean of the outcome measures for each of the 3 groups at each of the follow-up time points was modelled in linear mixed models. To account for the excessive correlation observed between outcomes when measuring the same patient at different follow-up times, the models were adjusted by incorporating a patient random effect. For each outcome at each follow-up time point, these models produced an estimate for the mean difference from baseline and its corresponding 95 % confidence interval (CI). Whether these differences from baseline differ between the 3 groups was assessed by simple subtraction of these models. The significance level was $p < 0.05$.

## 3. Results

From November 2020 to January 2022, 65 patients recruited from the Department of Ophthalmology, Copenhagen University Hospital – Rigshospitalet, Denmark, signed the informed consent form and were screened for eligibility. Of these, 40 participants met all inclusion and no exclusion criteria and were randomly allocated to the 2 intervention groups in a 1:1 ratio. A total of 14 participants met all inclusion criteria but were excluded from intervention due to either LG vol. < 0.2 $cm^3$ (n = 11), declined intervention (n = 2), or contraindications to MRI (n = 1) and were allocated to the observation group. Thus, a total of 54 study participants were included in the study. A Consolidated Standards of Reporting Trials (CONSORT) diagram is presented in Fig. 2. All participants completed the 12-month follow-up at the end of trial at the last participant's last visit in January 2023.

All study participants were female, and the median age was 59 years (IQR 49–71). Eighty-five percent of the participants had primary SS while the remaining 15 % had secondary SS. The demographic and clinical characteristics at baseline in all 3 groups are listed in Table 1. The changes in clinical characteristics during follow-up are listed in Supplemental Table S1.

An MRI was performed in 51 participants and LG volume was measured in both eyes. The median LG volume in the study eye in the ASCs group was 0.41 $cm^3$ (IQR 0.24–0.60 $cm^3$) and in the vehicle group 0.43 $cm^3$ (IQR 0.25–0.84 $cm^3$). In the 11 participants in the observation group in which an MRI was performed, the median LG volume was 0.07 $cm^3$ (IQR 0–0.15 $cm^3$) in the qualified study eye. In both interven-

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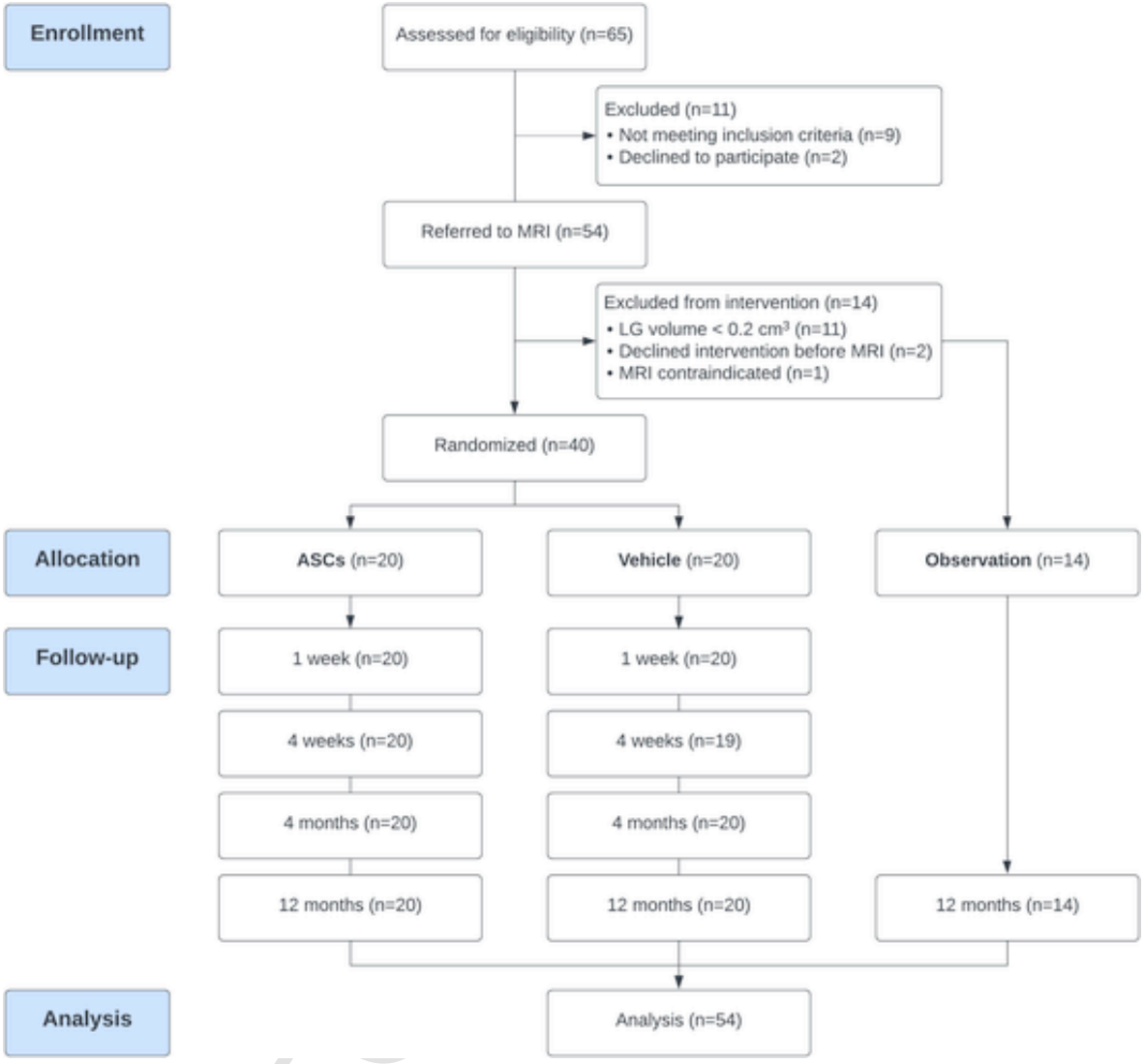


**Fig. 2.** CONSORT diagram of the enrollment, allocation, follow-up, and analysis of the study participants. CONSORT = Consolidated Standards of Reporting Trials, MRI = magnetic resonance imaging, ASCs = adipose-derived mesenchymal stem cells, LG = lacrimal gland, n = number of participants.

tion groups, the median injected volume of the allocated treatment was 0.18 ml, corresponding to 43 % of the median LG volume. In the ASCs group, this corresponded to a median dose of $3.96 \times 10^6$ ASCs per injection.

### 3.1. Ocular Surface Disease Index (OSDI)

At baseline, the 54 study participants had a median OSDI score of 46.7 (IQR 36.1–56.8) with no significant difference between the 3 groups. In the ASCs group, the OSDI score decreased significantly from median 39.8 at baseline by a mean of 16.6 points (−41.6 %, $p < 0.000$) at the 1-week follow-up, which was sustained at the 12-month follow-up (−16.1 points, −40.4 %, $p < 0.000$). In the vehicle group, the median OSDI score was 49.0 at baseline and decreased by a mean of 21.2 (−43.2 %, $p < 0.000$) 1 week after treatment, which was also sustained 12 months after treatment (−20.8, −42.4 %, $p < 0.000$). In the observation group, the OSDI score did not change significantly within 12 months follow-up. During the follow-up period, no significant difference in the decrease in OSDI score between the ASCs and vehicle group was observed, while both interventions showed a significant decrease compared to the observation group, $p = 0.03$ and $p = 0.004$ for ASCs and vehicle, respectively (Fig. 3).

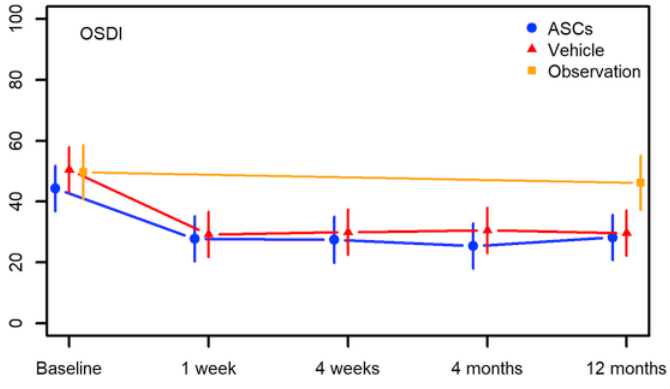


**Fig. 3.** OSDI score at baseline, 1 week, 4 weeks, 4 months, and 12 months follow-up in the ASCs, vehicle, and observation group. A significant decrease in the ASCs and vehicle group is sustained from 1 week to 12 months. OSDI = Ocular Surface Disease Index, ASCs = adipose-derived mesenchymal stem cells.

### 3.2. Non-invasive keratograph tear break-up time (NIKBUT)

At baseline, the median NIKBUT first was 3.70 s and NIKBUT average was 8.59 s with no difference between the 3 groups. In the ASCs group, a significant increase in NIKBUT first and NIKBUT average at all follow-ups was observed, which peaked at the 4 weeks follow-up with a mean increase in NIKBUT first of 6.48 s (149 %, $p < 0.001$) and in NIKBUT average of 6.54 s (73 %, $p < 0.001$). At the 12 months follow-up, a mean increase of 5.51 s (127 %, $p < 0.001$) in NIKBUT first and 4.13 s (46 %, $p < 0.004$) in NIKBUT average was observed. In the vehicle group, no significant change in NIKBUT first and NIKBUT average within the 12 months follow-up was found. In the observation group, we found a slight decrease in mean NIKBUT first of 1.57 s ($p = 0.41$) and a significant decrease of NIKBUT average of 4.03 s (−42 %, $p = 0.023$) after 12 months follow-up. The increase in NIKBUT first and NIKBUT average in the ASCs group was significantly larger than in the vehicle group, $p = 0.04$ and $p = 0.017$, respectively, at the 4 weeks follow-up. Compared to the observation group, the ASCs group showed a significant increase in NIKBUT first ($p = 0.03$) and NIKBUT average ($p = 0.007$) after 12 months follow-up (Fig. 4).

### 3.3. Schirmer test score

At baseline, the median Schirmer test score in the study eye was 3 mm (IQR 1–5 mm), with no difference between the groups ($p = 0.23$). At 4 months of follow-up, a significant mean increase in Schirmer test scores in the ASCs group of 3.55 mm (125 %, $p = 0.01$) and in the vehicle group of 3.8 mm (115 %, $p = 0.008$) was found, which was sustained at 12 months of follow-up (Fig. 5).

### 3.4. Tear meniscus height

At baseline, the median TMH in the study eye was 0.22 mm (IQR 0.17–0.29), with no difference between the groups. During the follow-up period, no significant change in mean TMH was observed in the intervention groups.

### 3.5. Tear osmolarity

The median tear osmolarity in the study eye at baseline was 314 mosm/l, with no difference between the groups. In the ASCs group, a significant mean decrease in tear osmolarity was found at the 12 months follow-up of 12.38 mosm/l (−3.9 %, $p < 0.05$) with a trend towards a significant difference compared to the vehicle group after 4 months ($p = 0.07$) and 12 months ($p = 0.098$) follow-up.

### 3.6. Ocular surface staining

Using the Oxford grading scale, the median Oxford grade at baseline was 2 (IQR 1–3) with no difference between the groups. In the ASCs group, a significant mean decrease in Oxford grade of −0.38 (−18.6 %, $p = 0.04$) was found 4 weeks after treatment compared to baseline. However, this decrease did not persist during long-term follow-up, and no differences between the groups were observed.

### 3.7. HLA antibodies

Prior to the study treatment, five participants exhibited pre-existing HLA antibodies, including DSA against HLA class II antigens, which remained present but were not boosted throughout the study. Among these participants, one initially had DSA of both HLA class I and II, which were not boosted, but later developed new DSA against other specific antigens four months after treatment. Two participants initially had no DSA, but at the 12-month follow-up, they developed new DSA targeting HLA class I antigens. No participants displayed clinical symptoms indicative of immunization during the study.

### 3.8. Adverse events

All 40 participants in the intervention groups completed the follow-up 12 months after treatment, and all 14 participants in the observation group completed a follow-up after a minimum of 12 months from baseline. During the follow-up period, no serious adverse reactions (SARs) were observed and the adverse events in both intervention groups corresponded to those found in the safety study (Supplemental Table S2) [12]. One study participant in the vehicle group was prescribed ciclosporin A eye drops between the 4 months and 12 months follow-up. No other study participants received treatment with ciclosporin A from baseline to the last follow-up.

## 4. Discussion

This study is the first double-blinded, randomized clinical trial to investigate the therapeutic application of injecting ASCs into the LG in patients with DED. The results of this study showed that injection of ASCs resulted in a reduction in subjective dry eye symptoms of about 40 % on the OSDI score sustained 12 months after treatment, a significant increase in tear film stability (NIKBUT) at every follow-up, and a significant increase in tear production (Schirmer test score) from 4 months follow-up and sustained 12 months after treatment. Furthermore, in the ASCs group we found a significant decrease in mean Oxford grade at the 4-week follow-up and a significant decrease in tear os-

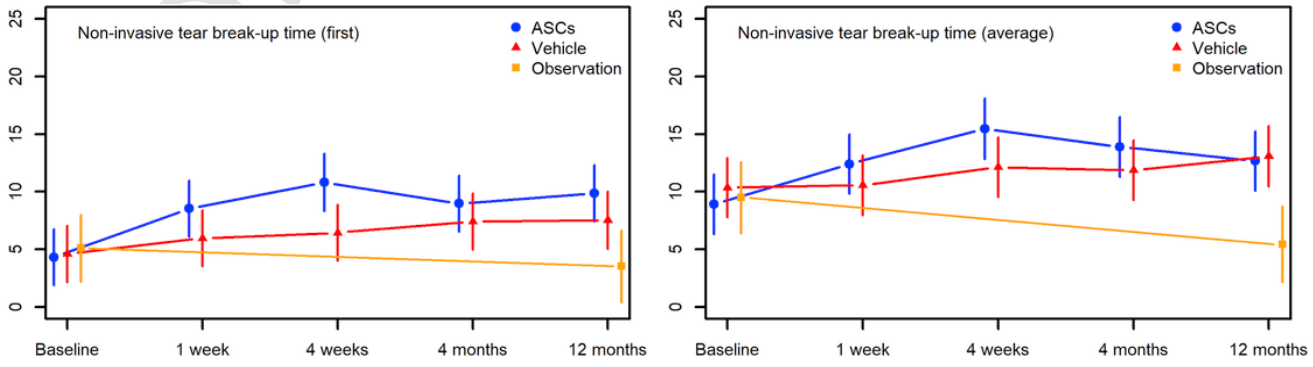


**Fig. 4.** Non-invasive keratograph tear break-up time (NIKBUT) first and NIKBUT average in the study eye at baseline, 1 week, 4 weeks, 4 months, and 12 months follow-up in the ASCs, vehicle, and observation group. In the ASCs group, a significant increase in NIKBUT first and NIKBUT average is seen compared to the vehicle group at 4 weeks and compared to the observation group at 12 months follow-up. ASCs = adipose-derived mesenchymal stem cells.

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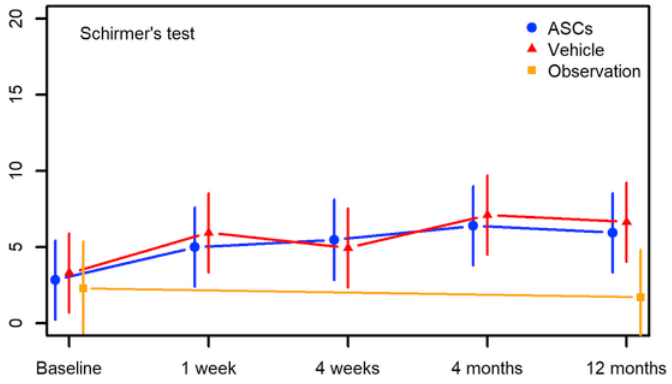


**Fig. 5.** Schirmer test scores in the study eye at baseline, 1 week, 4 weeks, 4 months, and 12 months follow-up in the ASCs, vehicle, and observation group. A significant increase in Schirmer test scores is seen in the ACSs and vehicle group from 4 to 12 months. ASCs = adipose-derived mesenchymal stem cells.

molarity at the 12-month follow-up compared to baseline. These findings are in line with the results of our open-label safety and feasibility study from 2021 [12]. Interestingly, a significant improvement was also seen in the vehicle group with regards to OSDI and Schirmer test scores, but for NIKBUT, tear osmolarity and Oxford grade, the improvement was seen in the ACSs group only. No significant improvement was seen in the observation group. When comparing the other outcome measures, the ASCs group consistently had the same or better outcome across all outcome measures compared to the vehicle group, with no examples of the vehicle group performing significantly better than ASCs at any time point. These findings align with previous pivotal trials in dry eye, where each trial has consistently found that the respective active comparator (vehicle) on its own has a positive impact on signs and symptoms of DED [17]. A potential underlying mechanism leading to improvements in DED symptoms and Schirmer test scores in both intervention groups may be attributed to the regenerative processes of the LG related to the injection-induced injury. This phenomenon has been associated with the activation and involvement of myoepithelial cells and endogenous mesenchymal stem cells [18,19].

Previous research conducted in animal models, such as SS-like mice and rabbits, have revealed that the transplantation of MSCs has anti-inflammatory and immunomodulatory properties. This effect has been attributed to an increase in the population of anti-inflammatory regulatory T cells (Tregs), resulting in the alleviation of DED in mice and an improvement in tear production in rabbits [20,21]. In mice, the underlying mechanism responsible for this therapeutic effect involves the secretion of IFN-β by MSCs, which stimulates dendritic cells to produce IL-27. Subsequently, IL-27 helps restore the balance between pro-inflammatory T helper-17 cells (Th17) and anti-inflammatory Tregs. Furthermore, this study demonstrated that in human patients with SS, lower levels of IL-27 in peripheral blood correlated with higher disease severity [20]. It is likely that this mode-of-action is the same in the human participants in our study; however, this must be investigated in future studies.

The surprising improvement observed in the vehicle group may have various causes. Firstly, a review has previously concluded that trials comparing active treatments and vehicle in DED has consistently demonstrated some beneficial effect of vehicle alone on signs and symptoms of DED [17]. Previous studies have further found DMSO, the active cryopreservative in the vehicle solution Cryostor CS10, to have anti-inflammatory and immunomodulating properties [22]. The anti-inflammatory properties of DMSO have been investigated in pre-clinical studies suggesting that DMSO represses the production of inflammatory cytokines such as TNF-α and IFN-γ while also inducing the adoptive transfer of naive $CD4^+$ T cells to Tregs [23]. Furthermore, DMSO is widely used to enhance the solubility of drugs in aqueous solutions and to enhance membrane penetration. Therefore, DMSO has previously been shown to increase the efficacy of other medical treatments. An older study in patients with RA found that intraarticular injections with corticosteroid in combination with DMSO showed better results compared to corticosteroid alone [24]. The decision to use Cryostor CS10 as an active comparator instead of a placebo such as isotonic saline in this trial served 2 purposes. Firstly, it allowed for the assessment of the specific isolated effects of the ASCs. Secondly, there is a lack of previous human studies testing the injection of isotonic saline into the LG. The potential risk of injuring the acinar structures and exacerbating the condition without any potential benefit for the patients led to the exclusion of isotonic saline as a comparator in this trial.

The decision to include OSDI score as the primary outcome measure was based on the results of our open-label safety study, in which a significant change in subjective symptoms of DED using the OSDI questionnaire was sustained after 4 months follow-up. In the present study, the effect of the assigned treatment could be confounded by several factors [25]. In certain conditions, such as pain disorders, it has been documented that placebo interventions can influence patient-reported outcomes such as the OSDI in DED, which has a considerable pain component [26]. Thus, in the future, it may be considered to choose an objective rather than a subjective primary outcome measure. When developing a clinical trial for treatments of DED, it is important to consider the mechanism of action. In the case of MSCs, which possess anti-inflammatory properties, the trial should include endpoints, such as measurements of tear film stability, that effectively reflect the role of inflammation on the ocular surface [17]. NIKBUT gives a reliable objective measurement of the tear film stability and could be used as the primary outcome measure in future trials. However, the increase in NIKBUT peaks 4 weeks after treatment, which leaves the question of whether repeated treatments with 4-week intervals would result in an additive effect. However, pre-clinical studies have indicated that repeated allogeneic MSC injections may sensitize the recipient and can lead to milder yet lasting inflammatory reactions potentially reducing the efficacy of the later treatments [27]. This risk could be minimized by using cells from different donors.

Another confounder of the results in this study is the Hawthorne effect, in which previous studies have demonstrated that simply being part of a clinical trial enhances the compliance to treatment of the study participant [28,29]. In the present trial, study participants in all 3 groups were instructed to continue the existing treatment regimen with lubricating eye drops used at baseline. At each study visit, the participant was asked about the frequency of lubricating eye drop use, which could vary considerably in the time between follow-ups. Intensified use of lubricating eye drops may increase the improvement in the individual participant, while any experienced improvement of dry eye symptoms may lead to a reduction in their use of lubricating eye drops, which would pull the effect in the opposite direction. To avoid this possible confounder in future trials, inclusion of digital devices tested to quantify the use of lubricating eye drops should be considered [30,31].

Limitations: We only included patients with severe ADDE (OSDI ≥33 and Schirmer's test 1–5 mm/5 min) due to SS. One could speculate that treatment targeting the inflammatory process in the LG may be more effective if administered closer to symptom debut in less severe cases with as many salvageable, functional acinar cells as possible. The allocation to the observation group was not randomized, which could potentially confound the comparisons to the intervention groups. In the observation group, 11/14 participants were excluded from intervention due to LG volume $<0.2$ $cm^3$ in the study eye. This could, however, be due to technical shortcomings in the MRI, which can complicate the identification and delineation of the LGs, since these are quite small. However, as no differences in the clinical characteristics were present at baseline, it seems safe to consider this group comparable to the participants in the intervention groups.

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## 5. Conclusion

Improvement of subjective and objective signs and symptoms of DED was observed in both intervention groups following injection into the LG compared to the observation group. The injection of ASCs additionally resulted in a significant increase in the tear film stability as compared to the vehicle at the 4-weeks follow-up and to the observation group at the 12-month follow-up. The reason for the improvement in both groups is speculated to be anti-inflammatory of both interventions in combination with psychological and non-specific factors including a placebo effect. Future studies should be aiming at further investigating the mode-of-action of both injection treatment and at exploring other potential ocular **indications** for the use of this treatment.

## Uncited References

[13].

## Declaration of competing interest

The research received funding from the Fight for Sight Denmark, Simon Spies Fonden, and Synoptik-Fonden. The funding sources had no role in the design or conduct of this research. All authors met the authorship criteria of the International Committee of Medical Journal Editors (ICMJE) without receiving any form of honoraria or payments. Authors M.M.-H., A.C.L., and S.H., in collaboration with Copenhagen University Hospital and the University of Copenhagen, have submitted a patent application titled “Stem cell therapy for lacrimal gland dysfunction” to the European Patent Office (EP3923960A1). M.M.-H. serves as an advisory board member for Abilion Medical Systems AB. T.P.H. is a co-founder and co-owner of The Norwegian Dry Eye Clinic and the Clinic of Eye Health in Oslo, Norway, which delivers talks for and/or receives financial support from: ABIGO, Alcon, Allergan, AMWO, Bausch & Lomb, Bayer and European School for Advanced Studies in Ophthalmology, InnZ Medical, Medilens Nordic, Medistim, Novartis, Santen, Specsavers, Shire Pharmaceuticals, and Théa Laboratories.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtos.2023.11.007.

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