Rigshospitalet

UNIVERSITY OF COPENHAGEN

# Stem cell-based treatment of dry eyes

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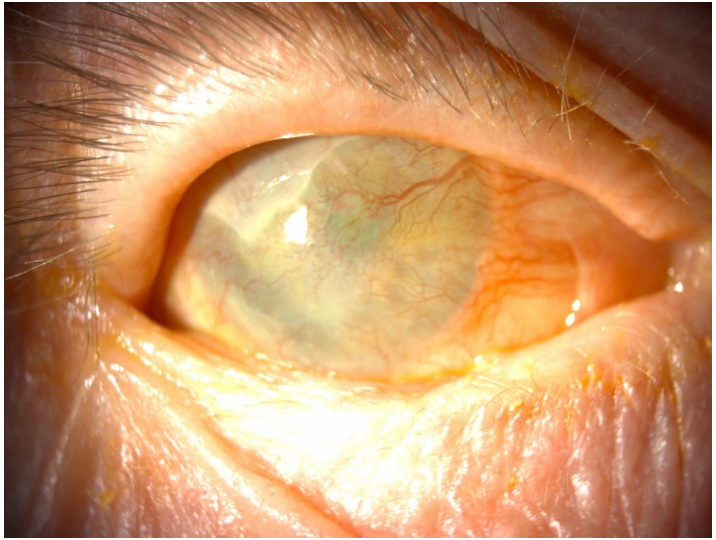
Ann-Cathrine Larsen, MD, PhD

No financial conflicts of interest to declare



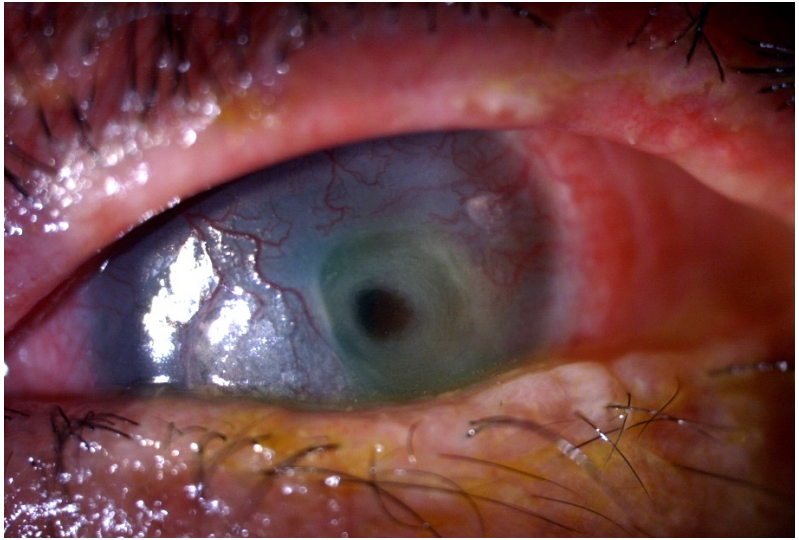


Photo: Helene Jeppesen, M.D., Rigshospitalet, Copenhagen, Denmark

Dry eye | Sjögren’s | Pre-clinical studies | Phase I | Phase II | Conclusion | Perspectives

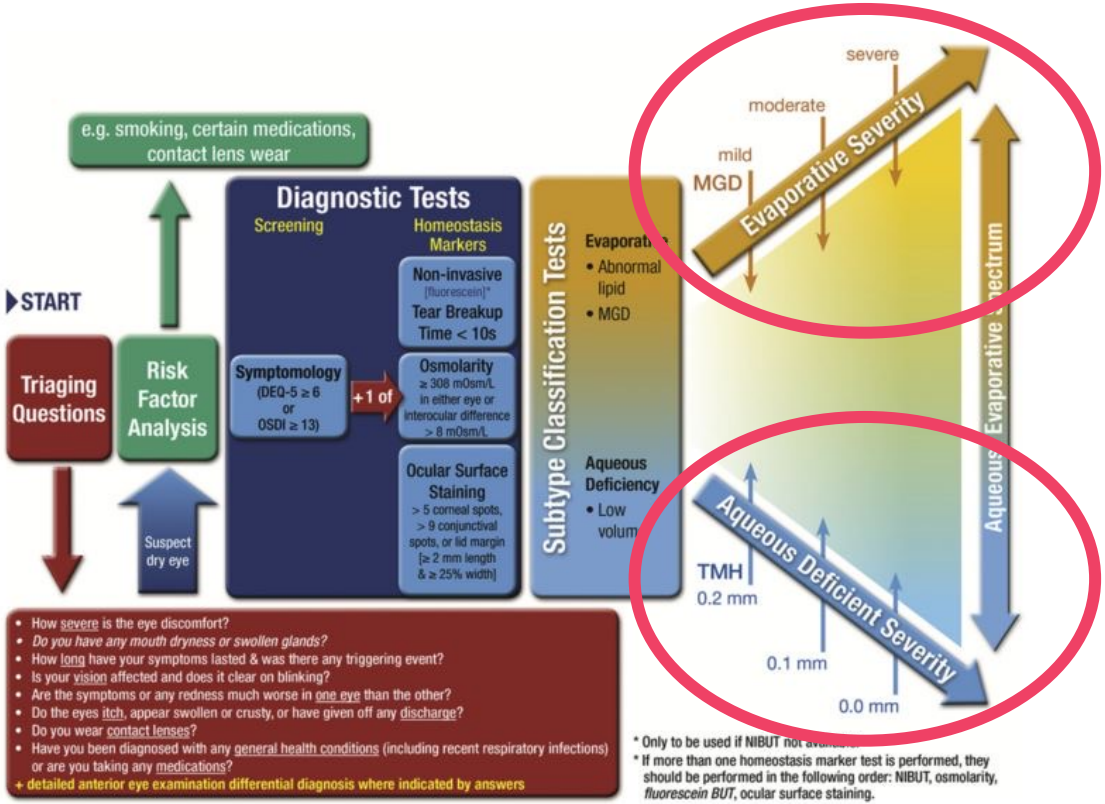



**Fig. 5.** Recommended diagnostic approach for DED. Please see the original report for a complete description of this figure [11].

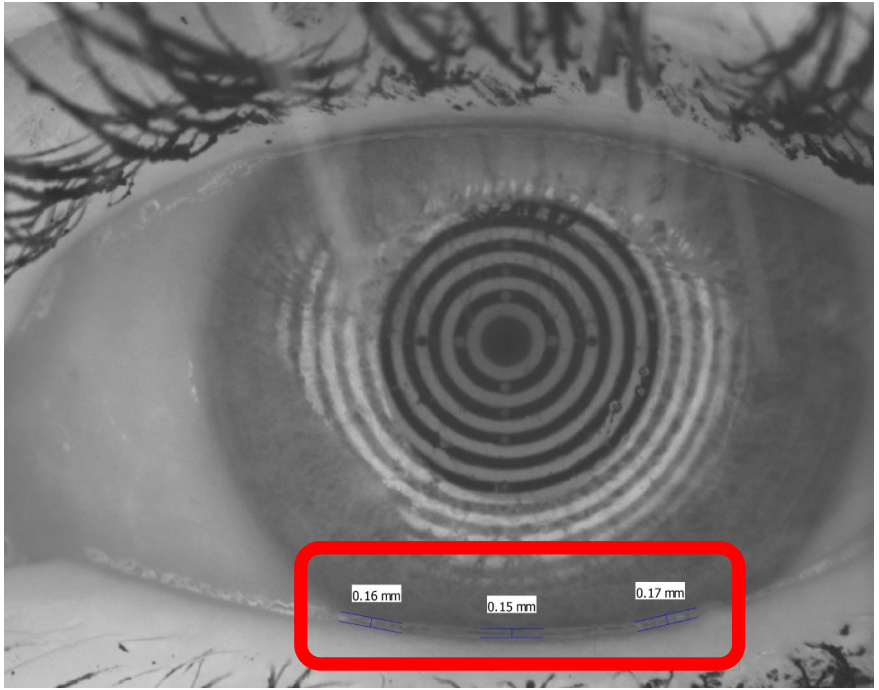
Adapted from Bron et al., 2017

# Diagnostic tests

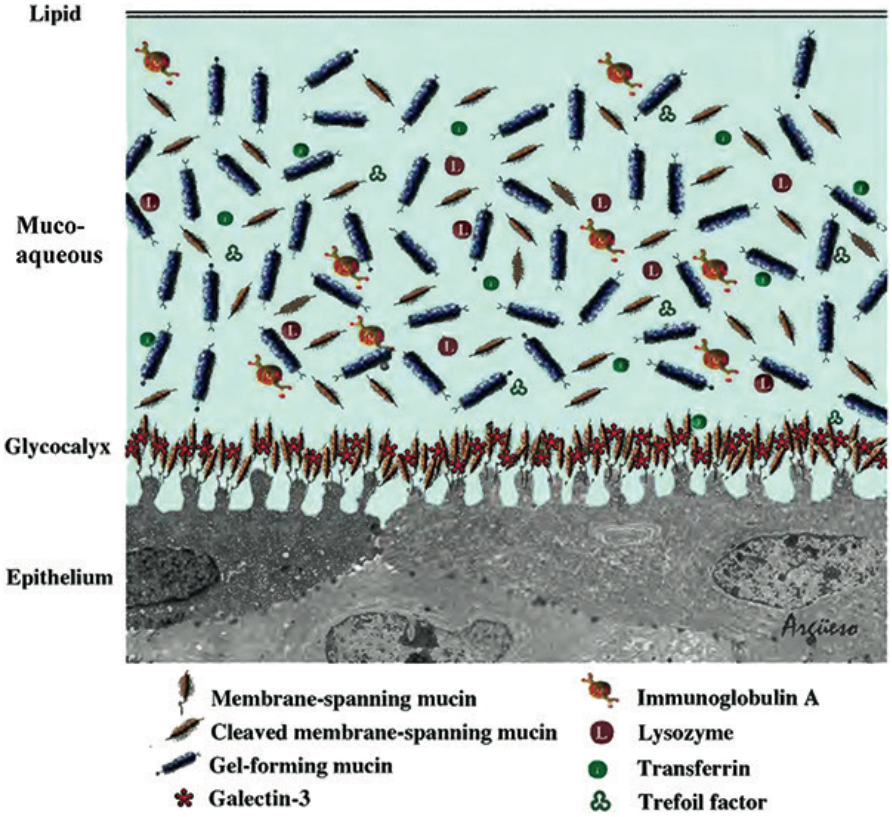
# Schirmer's I test

Photo: Dry Eye Disease, Nordic Guidelines 2016, Steffen Heegaard et al

# Tear meniscus height (TMH)




## Keratograph 5M (Oculus®)




The tear film structure showing the mucins and galectin of the glycocalyx, soluble mucins and proteins in the mucoaqueous layer and the surface lipid layer. Adapted from Craig et al., 2013.

# Fluoresceine tear film break-up time (TBUT)

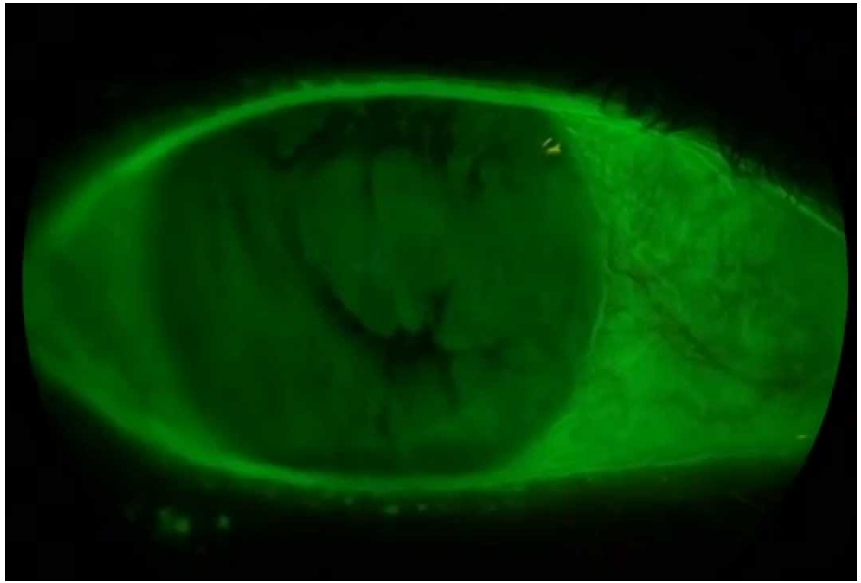
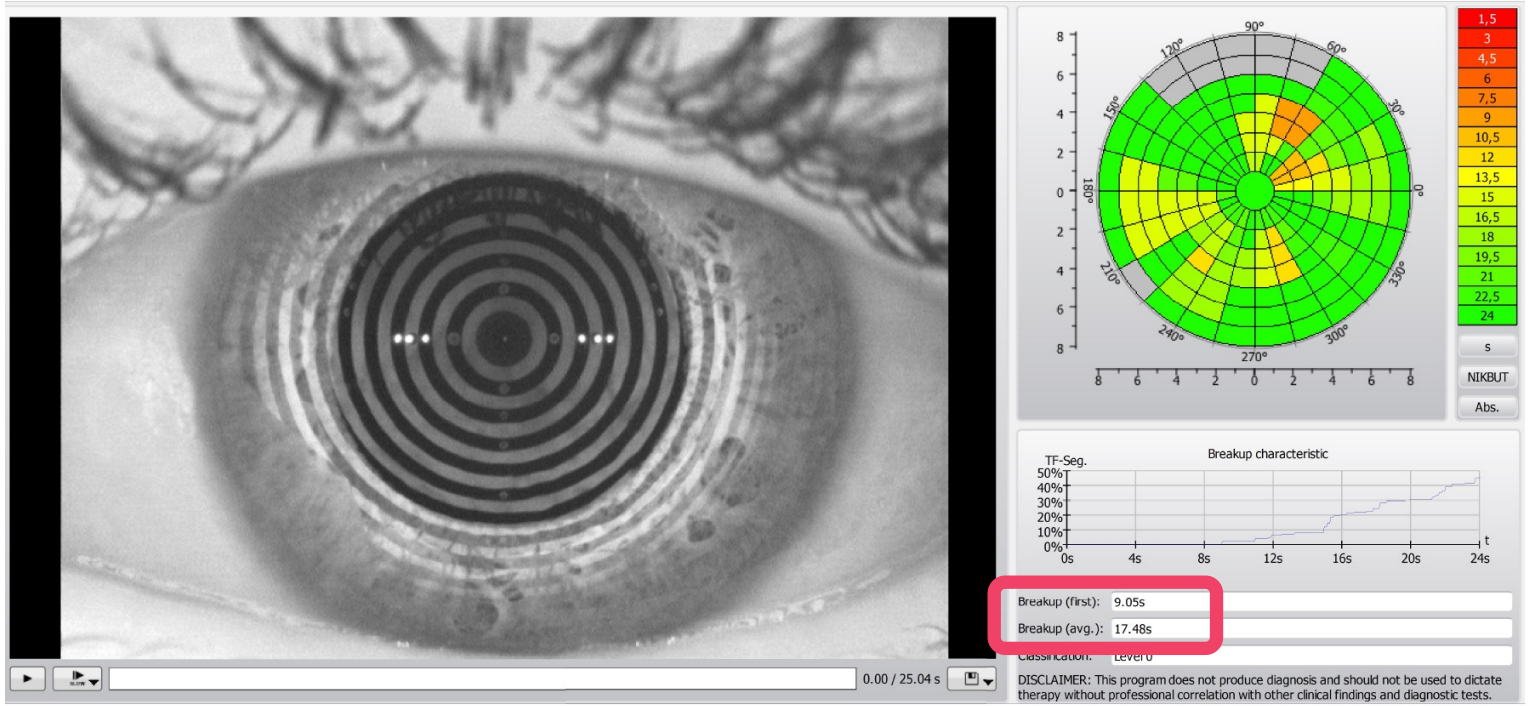


Photo: Dry Eye Disease, Nordic Guidelines 2016, Steffen Heegaard et al

# Non-invasive tear break-up time (NIKBUT)




# Keratograph 5M (Oculus®)


# TearLab® osmolarity system

Photo: www.tearlab.com

# Fluoresceine corneal staining (Oxford scale)

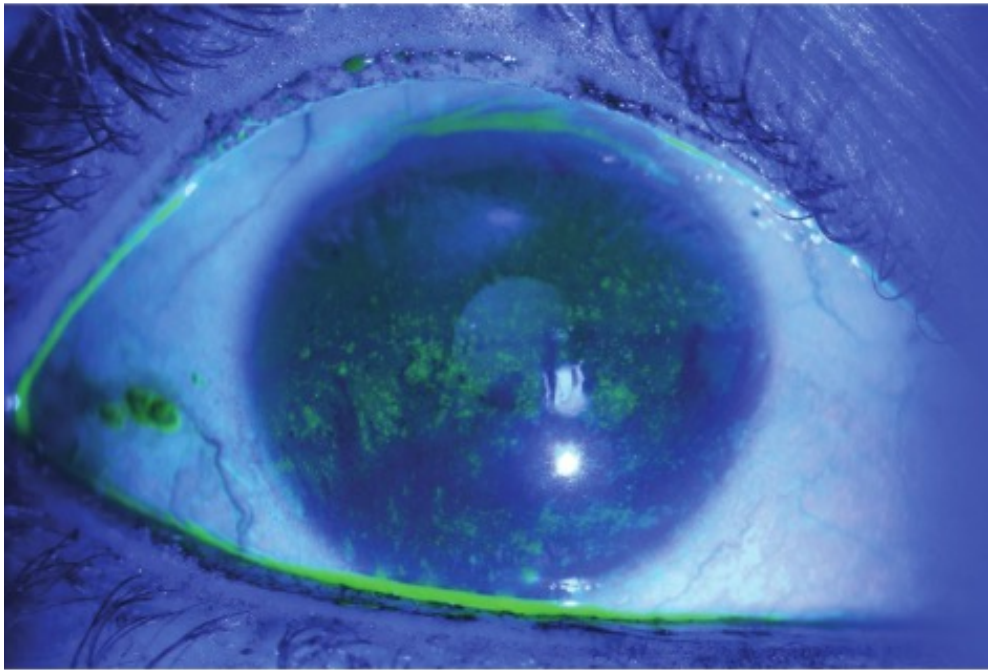


Photo: Dry Eye Disease, Nordic Guidelines 2016, Steffen Heegaard et al

# Dry eye and proteomics

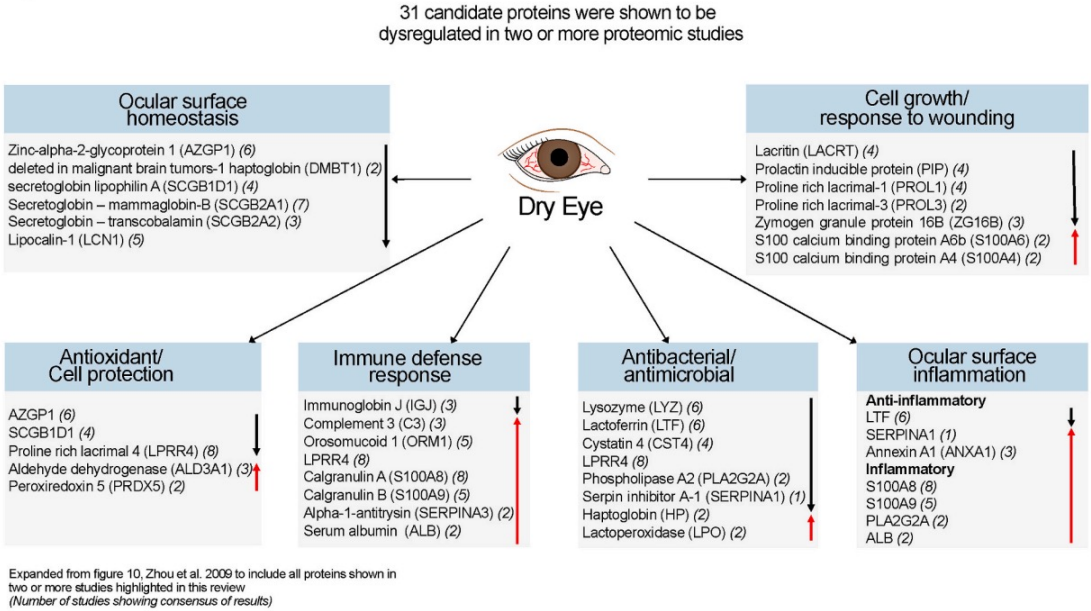
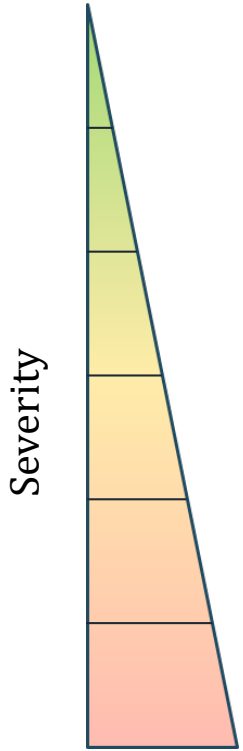



**Fig. 5.** Overview of protein functions shown dysregulated in dry eye disease in two or more proteomic studies.

*C. Jackson et al, 2022*: " Dry eye disease and proteomics"

# Treatment of dry eye disease




- Dietary and local environmental interventions
- Artificial tear substitutes
- Topical anti-inflammatory therapy
- Devices
  - Contact lenses
  - Punctal occlusion
- Biological tear substitutes
  - Autologous or allogeneic serum drops
- Surgical approach
  - Tarsorrhaphy
- Experimental approaches
  - Salivary gland transplantation

Symptomatic

# Sjögren's Syndrome (SS)

SS is a T cell-driven autoimmune disease characterized by focal lymphocytic infiltration of the lacrimal and salivary glands which leads to dryness of the eyes and mouth

**Primary** SS or **secondary** SS (rheumatoid arthritis, systemic lupus erythematosus, systemic sclerosis etc.)

Expression of autoantibodies (SSA/SSB) and increased expression of several proinflammatory cytokines is common

SS affects almost exclusively females (>90%)

Zoukri; Effect of inflammation on lacrimal gland function, Exp Eye Res. 2006 May; 82(5): 885–898.

# Sjögren's Syndrome (SS)

Diagnostic criteria **for primary SS** according to the American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR):

- anti-SSA/Ro antibody positivity +3
- focal lymphocytic sialadenitis +3

- abnormal Ocular Staining Score +1
- Schirmer's test result of ≤5 mm/5 min +1
- unstimulated salivary flow rate of ≤0.1 mL/min +1

A total score of ≥4 for the above items meet the criteria for primary SS

Chiboski et al, 2017; "2016 American College of Rheumatology/European League Against Rheumatism classification criteria for primary Sjögren's syndrome: A consensus and data-driven methodology involving three international patient cohorts"

# The ideal treatment …

A regenerative therapy for treatment of aqueous deficient dry eye disease will ideally restore lacrimal gland function without causing adverse side-effects and be feasible in terms of cost, production and application in the clinic

*Hirayama, 2018*: "Advances in Functional Restoration of the Lacrimal Glands"

# Previous studies

Since 2008, 25 studies have investigated the development of new drug targets and therapies or focused on direct lacrimal gland regeneration using stem cells from various sources

# Previous studies

Investigated cell types include:

- **mesenchymal stem cells (MSCs)**
- ductal cells
- acinar cells
- lacrimal gland stem cells

# Mesenchymal stem cells (MSCs)

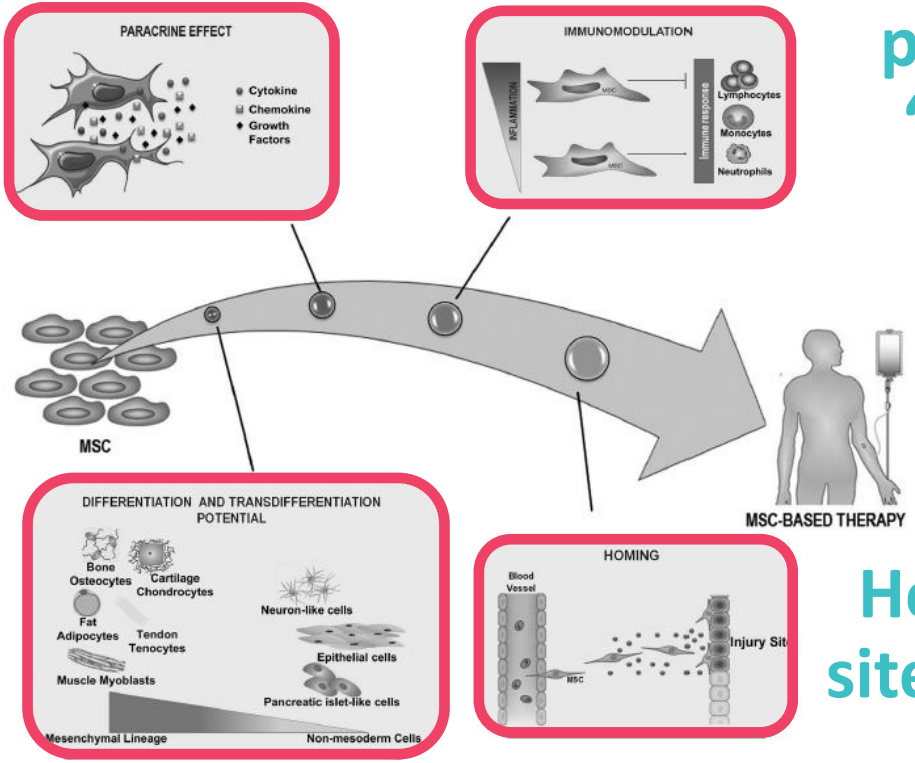
MSCs are multipotent, adult stem cells with a set of characteristic properties defined by The International Society for Cellular Therapy (ISCT):

- Plastic-adherent properties
- Characteristic cell surface marker expression
- Multi-lineage differentiation potential
- Self-renewal capacity

Dominici et al, 2006: Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement

# Secretion of anti-inflammatory cytokines

# Immunomodulating properties via "cell-to-cell"




# Differentiation potential

# Homing to site of injury

**Figure 1.** Biological properties supporting MSC clinical use. The therapeutic potential of MSCs relies on their unique properties as i) the capacity to differentiate into various cell lineage (bottom, left), ii) the ability to secrete soluble factors that are crucial for cell survival and proliferation (top, left), iii) the ability to modulate immune response (top, right), iv) the ability to migrate to the exact site of injury (bottom, right).

Squillaro et al., 2016 "Clinical Trials with Mesenchymal Stem Cells: An Update"

# Pre-clinical studies

MSCs as a treatment of DED in different animal models:

In **dogs**: *A. J. Villatoro et al.*, 2015; *M. K. Bittencourt et al.*, 2016

In **rabbits**: *Xue Li et al,* 2016

In **rats**: *Beyazyildiz et al.*, 2014

In **mice**: *Aluri et al.*, 2017; *Abughanam et al.*, 2019; *Dietrich et al.*, 2019; *Yao et al.*, 2019

# Pre-clinical studies

MSCs derived from different tissue types:

- Lacrimal gland MSCs
- Umbilical cord MSCs
- Bone marrow MSCs
- Adipose tissue MSCs (ASCs)

## Allogeneic Mesenchymal Stem Cell Transplantation in Dogs With Keratoconjunctivitis Sicca

Maura K. W. Bittencourt,*[1] Michele A. Barros,†[1] João Flávio P. Martins,† Jose Paulo C. Vasconcellos,* Bruna P. Morais,† Celine Pompeia,‡ Matheus Domingues Bittencourt,§ Karine dos Santos Evangelho,¶ Irina Kerkis,‡ and Cristiane V. Wenceslau‡

**"MSC transplantation has an effect over a long period (up to 12 months), even after a single administration"**

12 months after MSC transplantation. Our data demonstrate that allogeneic MSC transplantation in KCS dogs is safe since no adverse effects were observed immediately after transplantation and in short- and long-term follow-ups. A statistically significant increase in the STT and ocular surface improvements was found in all eyes studied. In all the eyes with mild–moderate KCS, STT values reverted to those of healthy eyes, while in eyes with severe KCS, although complete reversion was not found, there was improvement in tear production and in other clinical signs. Our study shows that a single dose of a low number of MSCs can be used to treat KCS in dogs. In contrast to immunosuppressive drug use, MSC transplantation has an effect over a long period (up to 12 months), even after a single administration, and does not require daily drug administration.

## Use of Adipose-Derived Mesenchymal Stem Cells in Keratoconjunctivitis Sicca in a Canine Model

Antonio J. Villatoro,[1] Viviana Fernández,[1] Silvia Claros,[1,2] Gustavo A. Rico-Llanos,[1,2,3] José Becerra,[1,2,3] and José A. Andrades[1,2]

**"Sustained effect during 9-month follow-up … These results could reinforce a good effective solution to be extrapolated to future studies in humans"**

our knowledge, this is the first time in literature that implantation of allogeneic Ad-MSCs around lacrimal glands has been found as an effective therapeutic alternative to treat dogs with KCS. These results could reinforce a good effective solution to be extrapolated to future studies in human.

Bittencourt et al., 2016 "Allogeneic Mesenchymal Stem Cell Transplantation in Dogs with Keratoconjunctivitis Sicca"
Villatoro et al., 2015 "Use of Adipose-Derived Mesenchymal Stem Cells in Keratoconjunctivitis Sicca in a Canine Model"

Research Paper

# Mesenchymal stem cell transplantation alleviates experimental Sjögren's syndrome through IFN-β/IL-27 signaling axis

Genhong Yao[1*], Jingjing Qi[1,2*], Jun Liang[1], Bingyu Shi[1], Weiwei Chen[1], Wenchao Li[1], Xiaojun Tang[1], Dandan Wang[1], Liwei Lu[3], Wanjun Chen[4], Songtao Shi[5], Yayi Hou[2✉], Lingyun Sun[1✉]

**MSCs → interferon-β → dendritic cells produce IL-27 → drive naïve T cells to differentiate into regulatory T cells (Tregs) rather than into pro-inflammatory Th17 cells**

**In patients with Sjögren's syndrome, disease severity was correlated with a low level of IL-27 in serum**

glands, saliva flow rate and serum IL-27 were examined. The effects of MSCs on the IL-27 production and Th17/Treg cell in SS patients and mice *in vitro* and *in vivo* were determined for the mechanistic study.

**Results**: This study showed that SS patients had decreased IL-27 level and increased ratio of Th17/Treg cells. Consistently, exacerbated SS-like symptoms were observed in IL-27 deficient NOD mice, along with increased ratio of Th17/Treg cells. Importantly, MSC transplantation alleviated SS-like symptoms by elevating the level of IL-27 to restore Th17/Treg balance in NOD mice. Mechanistically, MSC-secreted interferon-β (IFN-β) promote dendritic cells to produce IL-27.

**Conclusions**: Thus, we have revealed a previously unrecognized function of MSC-mediated IL-27 production by DCs in suppressing SS-like syndrome, which provided evidences for clinical application of MSC in patients with SS.

*Yao et al.*, 2019: "Mesenchymal stem cell transplantation alleviates experimental Sjögren's syndrome through IFN-β/IL-27 signaling axis"

# Adipose-Derived Mesenchymal Stem Cells Reduce Lymphocytic Infiltration in a Rabbit Model of Induced Autoimmune Dacryoadenitis

Xue Li,[1] Xiaoxiao Lu,[1] Deming Sun,[2] Xilian Wang,[3] Liyuan Yang,[1] Shaozhen Zhao,[1] Hong Nian,[1] and Ruihua Wei[1]

**"The ASC-treated rabbits showed decreased autoimmune responses, and the secretory function of their lacrimal gland was restored significantly … Downregulated Th1 and Th17 responses but enhanced Tregs function … Suppressed the expression of MMP-9, MPP-2, IL-1b, and IL-6, and enhanced the expression of the anti-inflammatory cytokine IL-10.**

Adipose-derived mesenchymal stem cells reduce lymphocytic infiltration in a rabbit model of induced autoimmune dacryoadenitis. *Invest Ophthalmol Vis Sci.* 2016;57:5161–5170. DOI:10.1167/iovs.15-17824

**Conclusions.** Our results demonstrated that ADSC administration efficiently ameliorates autoimmune dacryoadenitis mainly via modulating Th1/Th17 responses.

Keywords: adipose-derived mesenchymal stem cells (ADSCs), autoimmune dacryoadenitis, cytokines, Th17 responses, Th1/Th17 cells

*" Xue Li, 2016: "*Adipose-Derived Mesenchymal Stem Cells Reduce Lymphocytic Infiltration in a Rabbit Model of Induced Autoimmune Dacryoadenitis"

The Ocular Surface 19 (2021) 43–52

Contents lists available at ScienceDirect

The Ocular Surface

journal homepage: www.elsevier.com/locate/jtos

# Safety and feasibility of mesenchymal stem cell therapy in patients with aqueous deficient dry eye disease

Michael Møller-Hansen [a,*], Ann-Cathrine Larsen [a], Peter Bjerre Toft [a], Charlotte Duch Lynggaard [b], Camilla Schwartz [c], Helle Bruunsgaard [d], Mandana Haack-Sørensen [e], Annette Ekblond [e], Jens Kastrup [e], Steffen Heegaard [a]

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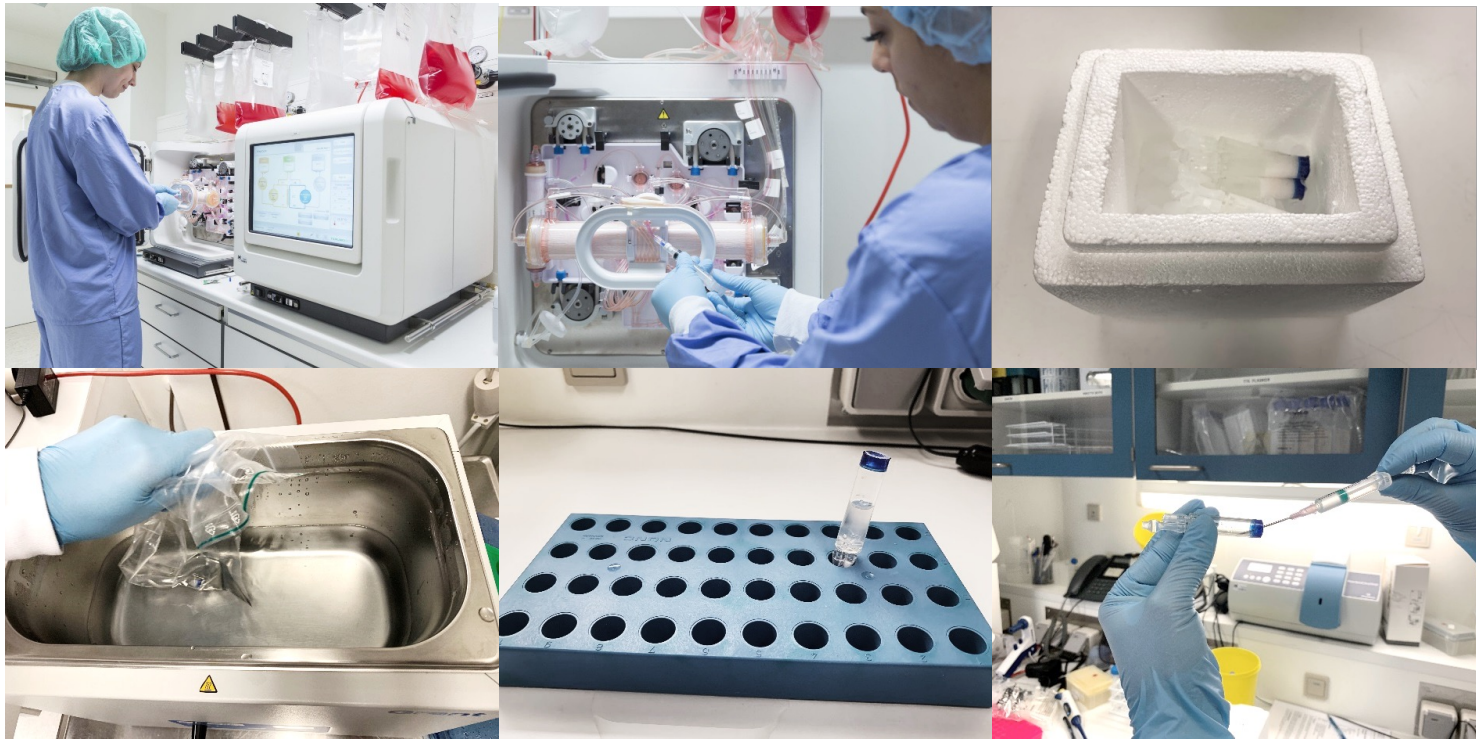
[e] Cardiology Stem Cell Centre, The Heart Centre, Rigshospitalet, University of Copenhagen, Denmark



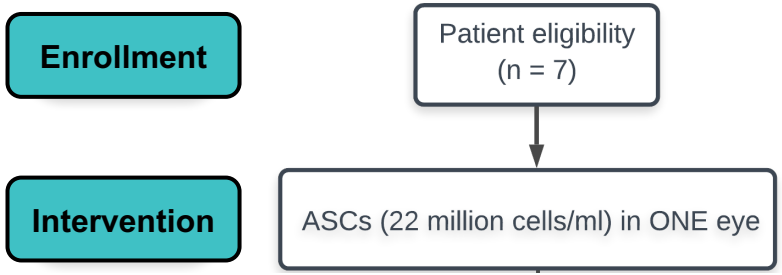
https://copenhagensciencecity.dk/copenhagen-stem-cell-research-enters-next-phase/

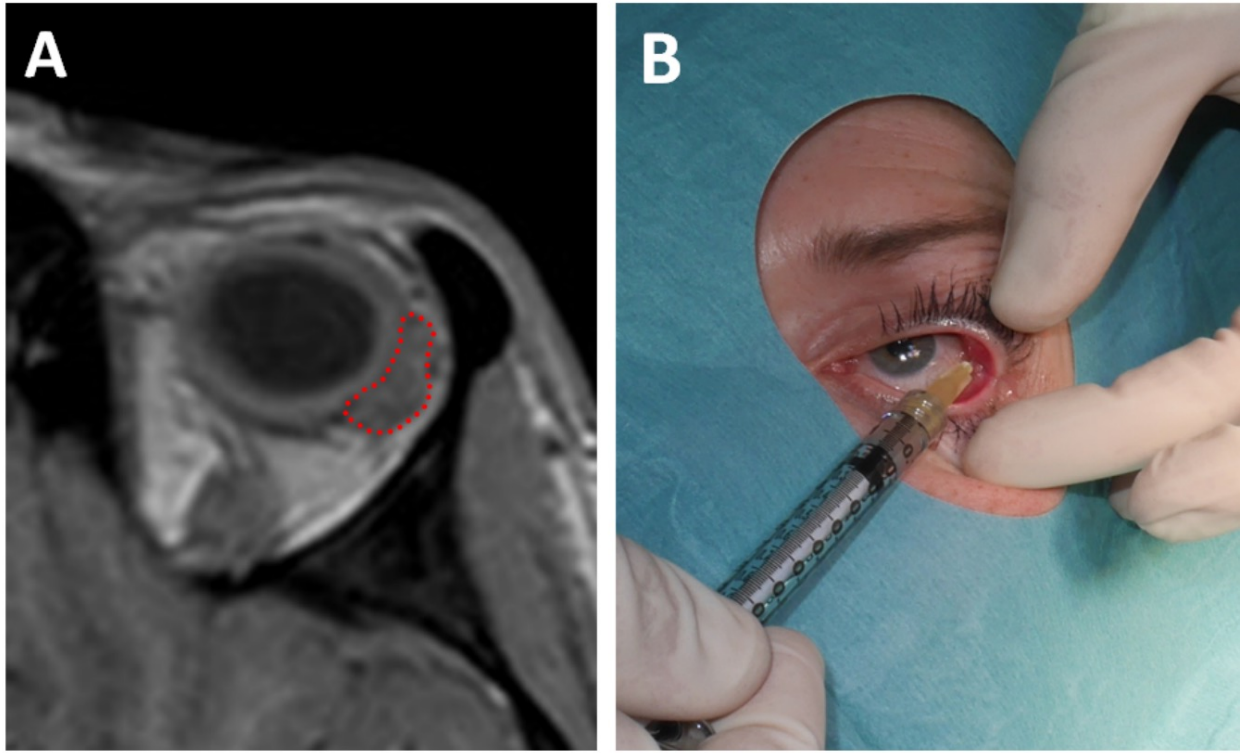
**Enrollment**

Patient eligibility
(n = 7)

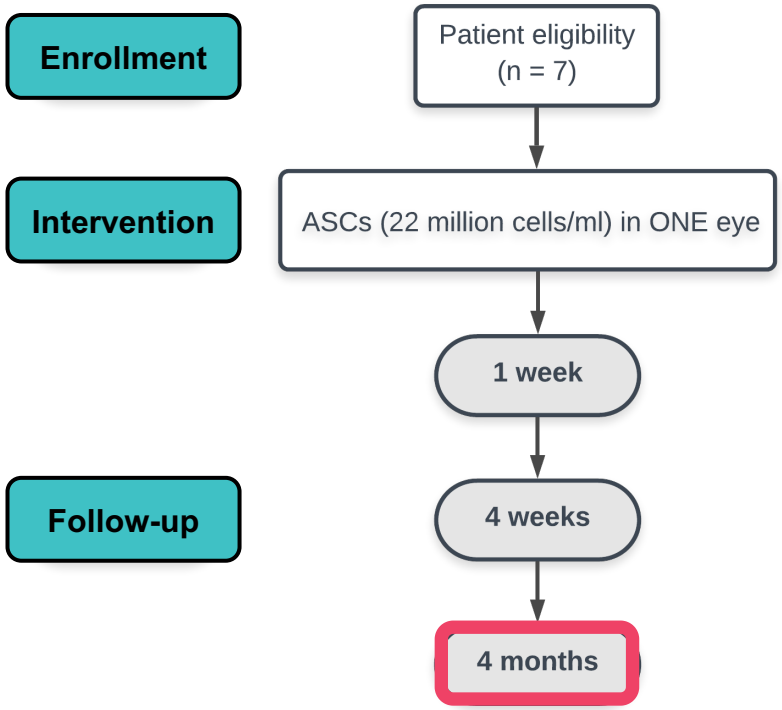
**Inclusion criteria**

- Age ≥ 18 years
- Ocular Surface Disease Index (OSDI) score ≥ 30
- Schirmer's test 2-5 mm/5 minutes
- Flourescein tear break-up time (TBUT) < 10 sec


Enrollment
Patient eligibility
(n = 7)
Intervention
ASCs (22 million cells/ml) in ONE eye




Møller-Hansen et al., 2021 "Safety and feasibility of mesenchymal stem cell therapy in patients with aqueous deficient dry eye disease"


Enrollment
Patient eligibility
(n = 7)
Intervention
ASCs (22 million cells/ml) in ONE eye
1 week
Follow-up
4 weeks
4 months

# Primary outcome:
# Safety
# (Adverse effects)

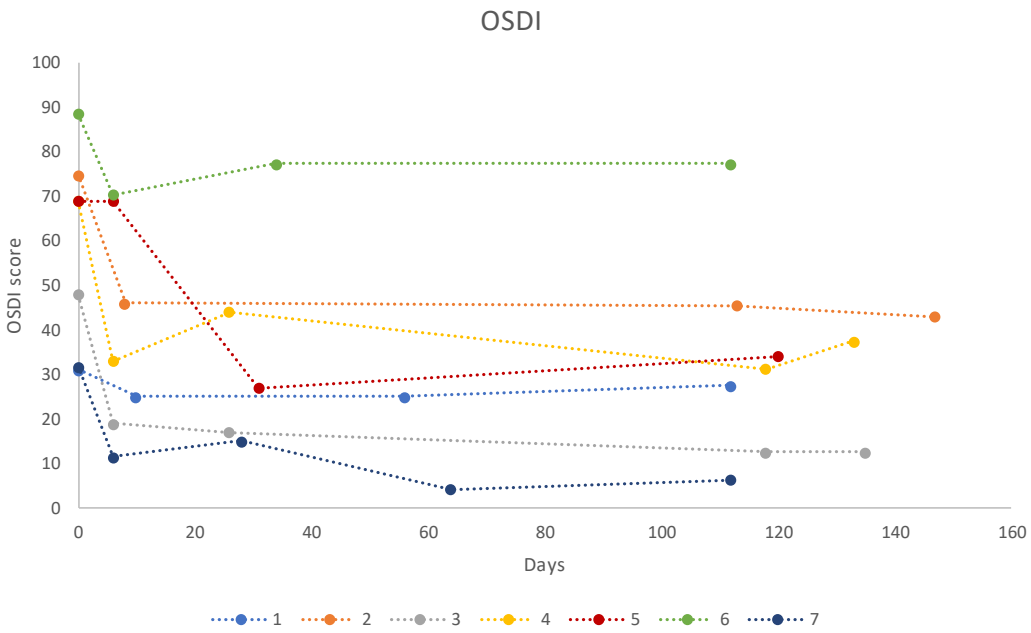
# No serious adverse reactions related to the study treatment

# Most frequent adverse effects observed immediately after treatment:

- Temporary increase in ocular discomfort
- Temporary periorbital edema

# Secondary outcomes

# Ocular Surface Disease Index (OSDI)




Mean descrease of 40% in OSDI-score 4 months after treatment ($p < 0.002$)

**Table 1**

Subject demographic and disease characteristics at baseline and at last follow-up.

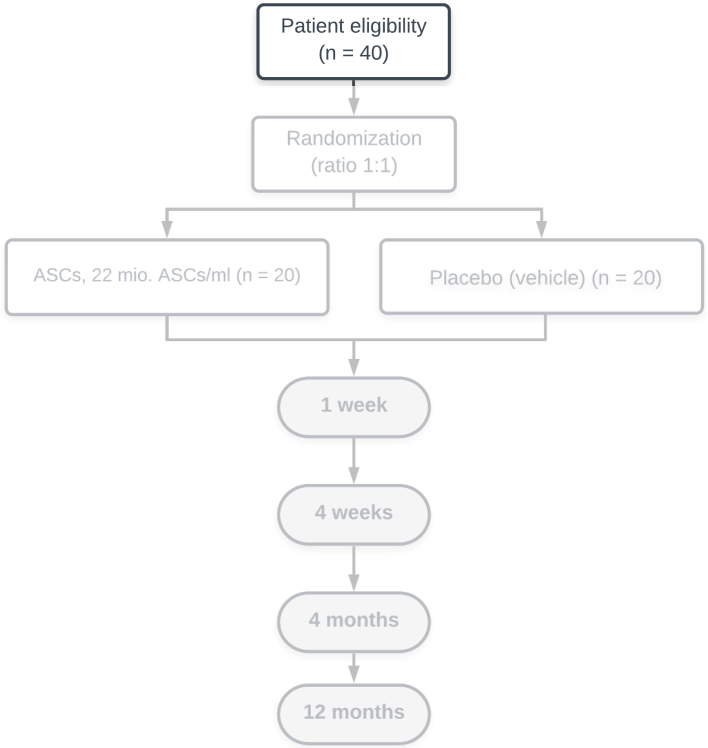
| Characteristic | Value | *P* | | | |
|---|---|---|---|---|---|
| Age, years, mean ± SD | 60 ± 9.3 | | | | |
| Female, *n* (%) | 7 (100%) | | | | |
| LG volume ($cm^3$), mean ± SD | 0.35 ± 0.13 | | | | |
| Injection dose (ml), mean ± SD | 0.14 ± 0.05 | | | | |
| Follow-up (days), mean ± SD | 126 ± 14 | | | | |
| OSDI score | | ***0.002*** † | | | |
| Baseline | 58.9 ± 20.6 | | | | |
| Last follow-up | 34.3 ± 21.7 | | | | |
| | Study eye | *P* | Fellow eye | *P* | *P** |
| Visual acuity (logMar), mean ± SD | | 0.10 | | 0.17 | |
| Baseline | 0.1 ± 0.1 | | 0.09 ± 0.11 | | 0.6 |
| Last follow-up | 0.04 ± 0.13 | | 0.06 ± 0.12 | | 0.36 |
| Tear osmolarity (mosm/l), mean ± SD | | ***0.002*** † | | 0.34 | |
| Baseline | 313.9 ± 10.4 | | 306.3 ± 10.9 | | 0.2 |
| Last follow-up | 291.6 ± 10.9 | | 305.7 ± 11.2 | | ***0.04*** § |
| TBUT (s), mean ± SD | | ***0.002*** † | | 0.34 | |
| Baseline | 3.7 ± 1.5 | | 3.7 ± 0.9 | | 1.0 |
| Last follow-up | 7.1 ± 1.5 | | 4.7 ± 1.6 | | ***0.02*** § |
| Corneal staining (Oxford grade), mean ± SD | | 0.10 | | 0.36 | |
| Baseline | 2.4 ± 0.1 | | 2.3 ± 0.4 | | 0.36 |
| Last follow-up | 1.3 ± 1 | | 1.9 ± 0.8 | | 0.1 |
| Schirmer's I test (mm/5 min.), mean ± SD | | ***0.03*** † | | 0.17 | |
| Baseline | 4.6 ± 0.7 | | 4.6 ± 3.2 | | 1.0 |
| Last follow-up | 8.1 ± 3.1 | | 6.7 ± 4.5 | | 0.25 |

LG, lacrimal gland; OSDI, Ocular Surface Disease Index; TBUT, tear break-up time. † $P < 0{,}05$ from baseline to last follow-up. § $P < 0{,}05$ between study eye and fellow eye. * $P$ of the interocular difference.

# AMASS

A randomized clinical trial evaluating allogeneic Adipose-derived MesenchymAl stem cells as a treatment of dry eye disease in patients with Sjögren's Syndrome

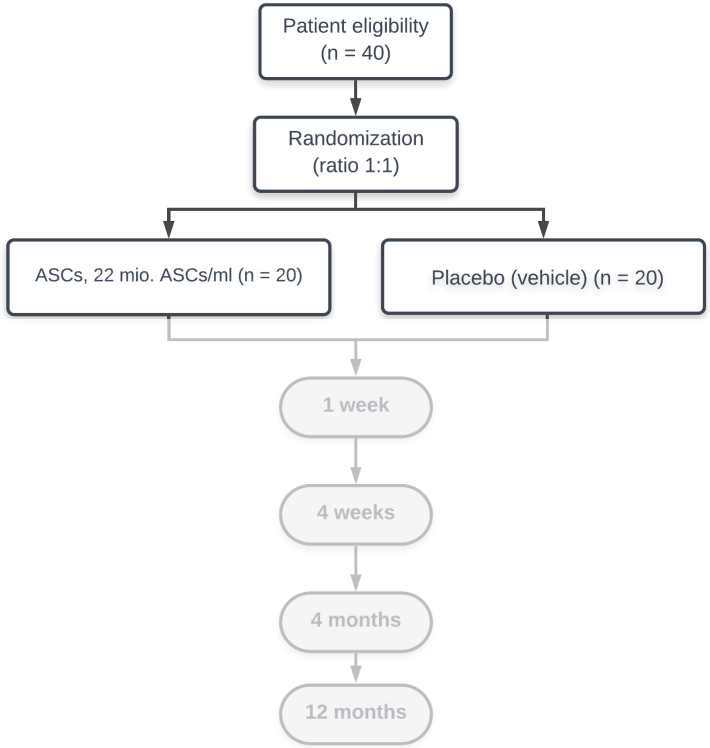
# AMASS


Patient eligibility
(n = 40)
Randomization
(ratio 1:1)
ASCs, 22 mio. ASCs/ml (n = 20)
Placebo (vehicle) (n = 20)
1 week
4 weeks
4 months
12 months

# Inclusion criteria

- Age ≥ 18 years
- Diagnosis of Sjögren's syndrome (ACR/EULAR criteria)
- OSDI-score ≥ 33
- Schirmer's test 1-5 mm/5 minutes
- Non-invasive tear break-up time (NIKBUT) < 10 sec

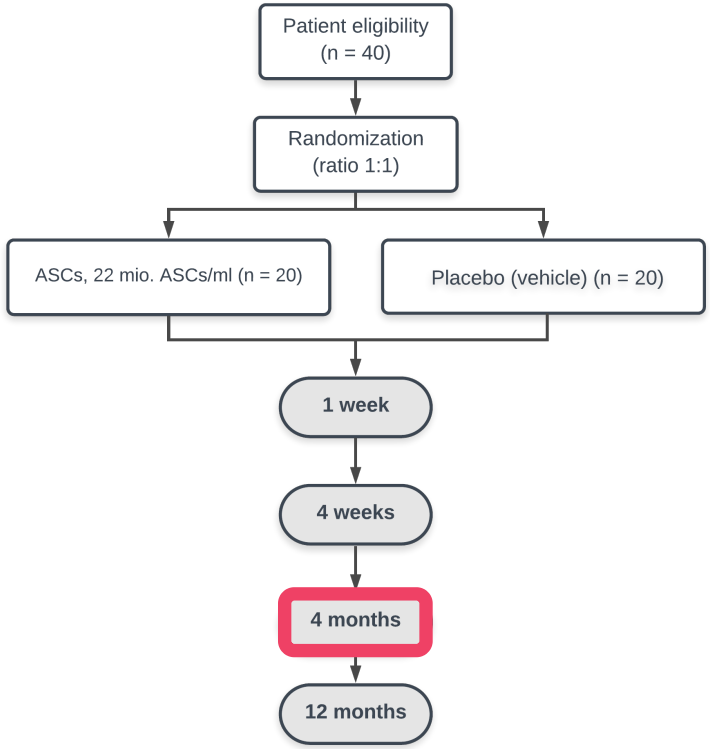
# AMASS


Patient eligibility
(n = 40)
Randomization
(ratio 1:1)
ASCs, 22 mio. ASCs/ml (n = 20)
Placebo (vehicle) (n = 20)
1 week
4 weeks
4 months
12 months

# Injection procedure in AMASS

# Ultrasonic guidance

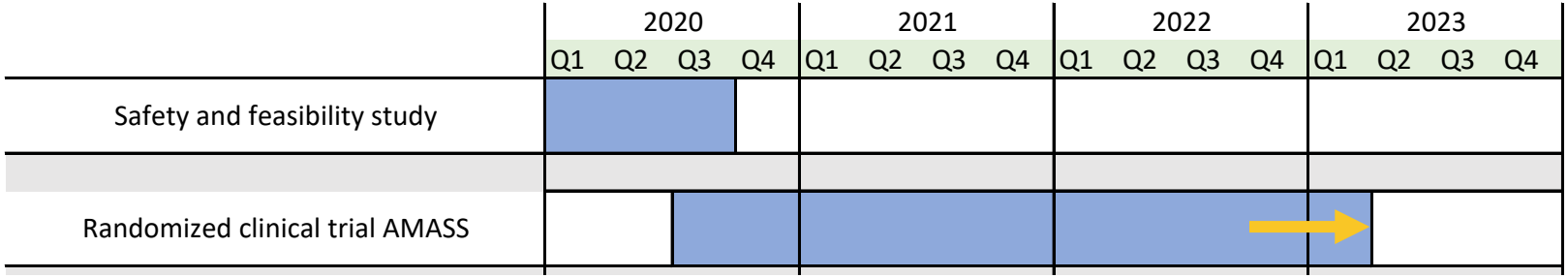
# AMASS


Patient eligibility
(n = 40)
Randomization
(ratio 1:1)
ASCs, 22 mio. ASCs/ml (n = 20)
Placebo (vehicle) (n = 20)
1 week
4 weeks
4 months
12 months

Primary outcome: **OSDI-score**

Secondary outcome measures:

- NIKBUT (K5M,*Oculus*®)
- Tear meniscus height (K5M,*Oculus*®)
- Schirmer's I test
- Tear osmolarity (*Tearlab®)*
- Corneal staining (Oxford score)
- Development of anti-HLA antibodies
- Adverse events


2020
2021
2022
2023
Q1 Q2 Q3 Q4 Q1 Q2 Q3 Q4 Q1 Q2 Q3 Q4 Q1 Q2 Q3 Q4
Safety and feasibility study
Randomized clinical trial AMASS

# Take-home messages

1. **Injection of allogeneic adipose-derived mesenchymal stem cells into the lacrimal gland seems to be a safe and well-tolerated treatment**

2. **This treatment seems to increase tear production and alleviate symptoms of dry eye disease in patients with Sjögren's Syndrome**

3. **The results of a double-blinded randomized clinical trial, AMASS, will be published in 2023.**

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# Next step?

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr

Analysis of lacrimal gland derived mesenchymal stem cell secretome and its impact on epithelial cell survival

Jana Dietrich[a,*], Mathias Roth[b], Simone König[c], Gerd Geerling[b], Sonja Mertsch[a], Stefan Schrader[a]

**"The secretome of MSC harbors proteins, such as Lcn2, prosaposin, Rac1 and STAT1, which positively influence the cell survival of LG epithelial cells and could be useful to induce/enhance LG regeneration in vivo."**

*Dietrich et al, 2019*: "Analysis of lacrimal gland derived mesenchymal stem cell secretome and its impact on epithelial cell survival"

# Next step?

SCIENTIFIC REPORTS
natureresearch
Check for updates

OPEN

## hADSCs derived extracellular vesicles inhibit NLRP3inflammasome activation and dry eye

**"Topical hADSC-Evs treated mice showed decreased corneal epithelial defects, increased tear production, decreased goblet cell loss, as well as reduced inflammatory cytokines production"**

*Yu et al, 2020:* "hADSCs derived extracellular vesicles inhibit NLRP3 inflammasome activation and dry eye"

# Thank you!

Synoptik Fonden

Øjenforeningen

**Afdeling for Øjensygdomme, Rigshospitalet-Glostrup**
**Kardiologisk Stamcellecenter, Rigshospitalet**
Prof. Steffen Heegaard, MD, DMSc
Anne K. Wiencke, MD, PhD
Ann-Cathrine Larsen, MD, PhD
Peter Bjerre Toft, MD, DMSc
Prof. Lene Terslev, MD, PhD
… og gode kolleger på Center for Forskning i Øjensygdomme