Supplementary Information

Allogeneic Serum Eye Drops: A Randomized Clinical Trial to Evaluate the Clinical Effectiveness of Two Drop Sizes

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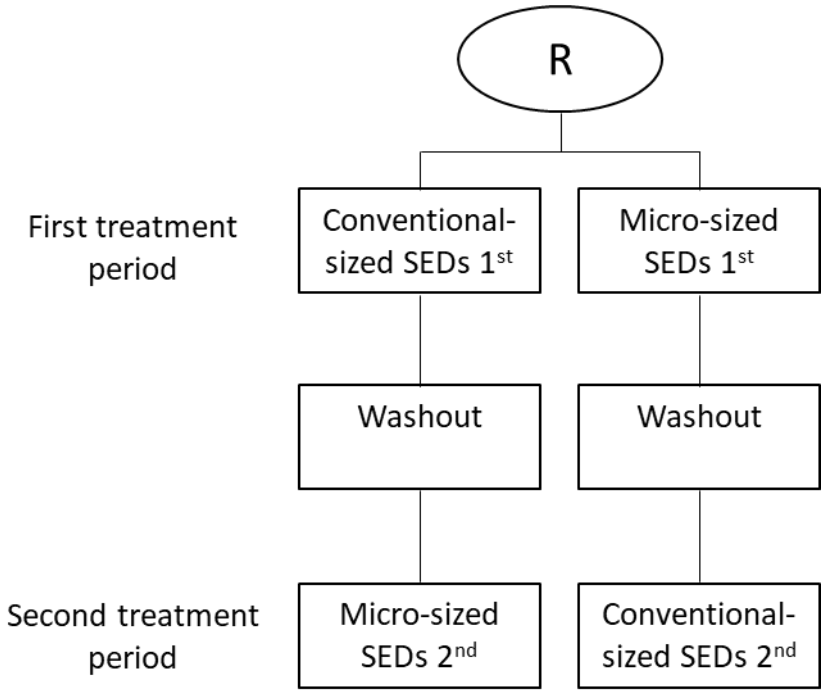
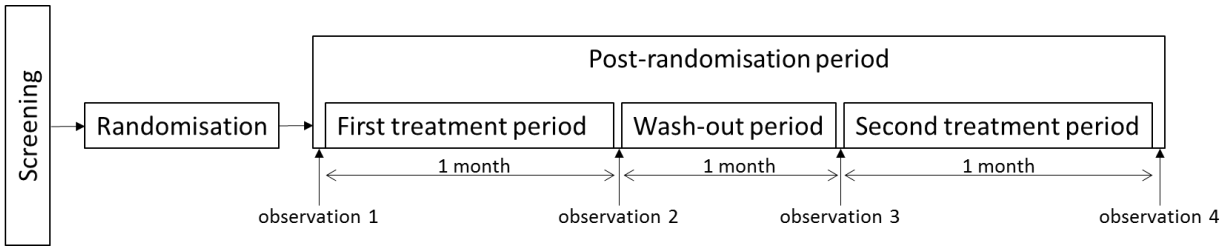
Supplementary Material: Production of allogeneic serum eye drops (SEDs)

For the production of SEDs, blood was drawn and left to clot for 6 to 24 hours at room temperature, then centrifuged twice, after which the serum was expressed into an empty collection container. Serum was rapidly frozen (core <-30°C within 1 hour) and stored at <-25°C until pooling, filtration and aliquoting into portions for packing lines. To reduce differences between serum batches, a pooled product of 8 sera was prepared. Briefly, eight sera were thawed rapidly at 37°C using a thawing device (DH4 Quick Thaw, Helmer, Noblesville, IN, USA), processed and refrozen within 6 h. A pool of 8 sera was sterile filtered using the Opticap XL5 filter assembly (MIL0000L1715633, Merck KGaA, Darmstadt, Germany). Then, the sterile filtered pool was aliquoted in portions of approximately 240 mL each and stored after rapid freezing at ≤ -25°C. Before aliquoting into final packaging system for patient use, one unit of pooled sterile filtered serum was rapidly thawed at 37°C using a thawing device (DH4 Quick Thaw, Helmer, Noblesville, IN, USA). Subsequently the unit was sterile connected to one of the eye drop application systems. To produce micro-sized SEDs, undiluted serum was aliquoted in the mu-Drop system (mu-Drop BV, Apeldoorn, The Netherlands). A mu-Drop vial contains 140 µL serum and is intended for single use, for both eyes. To produce conventional-sized SEDs, undiluted serum was aliquoted in the Augentropf Meise eye drop systems (Heinz Meise GmbH, Schalksmühle, Germany). Each Meise vial contains 1.5 mL serum, which is sufficient for one day. Mu-Drop and Meise vials were stored frozen (≤-25°C until issued to the hospitals). On the day of the patient's visit, allogeneic SEDs were inserted in a validated temperature controlled transport vessel to keep the SEDs frozen until the patients arrived home for further storage at ≤-18°C for a maximum period of 6 months.

Supplementary Figure S1: Randomization scheme



Supplementary Figure S2: Scheme of the sequence, period and treatment crossover.



Patients were randomized to first receive either allogeneic micro sized serum eye drops (SEDs) or allogeneic conventional sized SEDs for one month (= first treatment period). After the first treatment period patients who received micro sized SEDs were switched to conventional sized SEDs and those who received conventional sized SEDs were switched to micro sized SEDs for the second treatment period, according to the study's crossover nature. After the first treatment period all patients underwent a wash-out period of 1 month during which they returned to standard treatment they were on before going on-study.

Per patient, the on-study time was 3 months. Prior to and after each treatment period the following ocular surface measurements were carried out (observation 1 to 4): OSDI questionnaire, tear production, tear break up time, number of corneal punctates and visual acuity. During both treatment periods and the wash-out period, patients were asked to keep a diary on the use of serum eye drops and experience with the applicators. At the end of the study the diary was collected from the patient.

Supplementary Material: Detailed statistical analysis

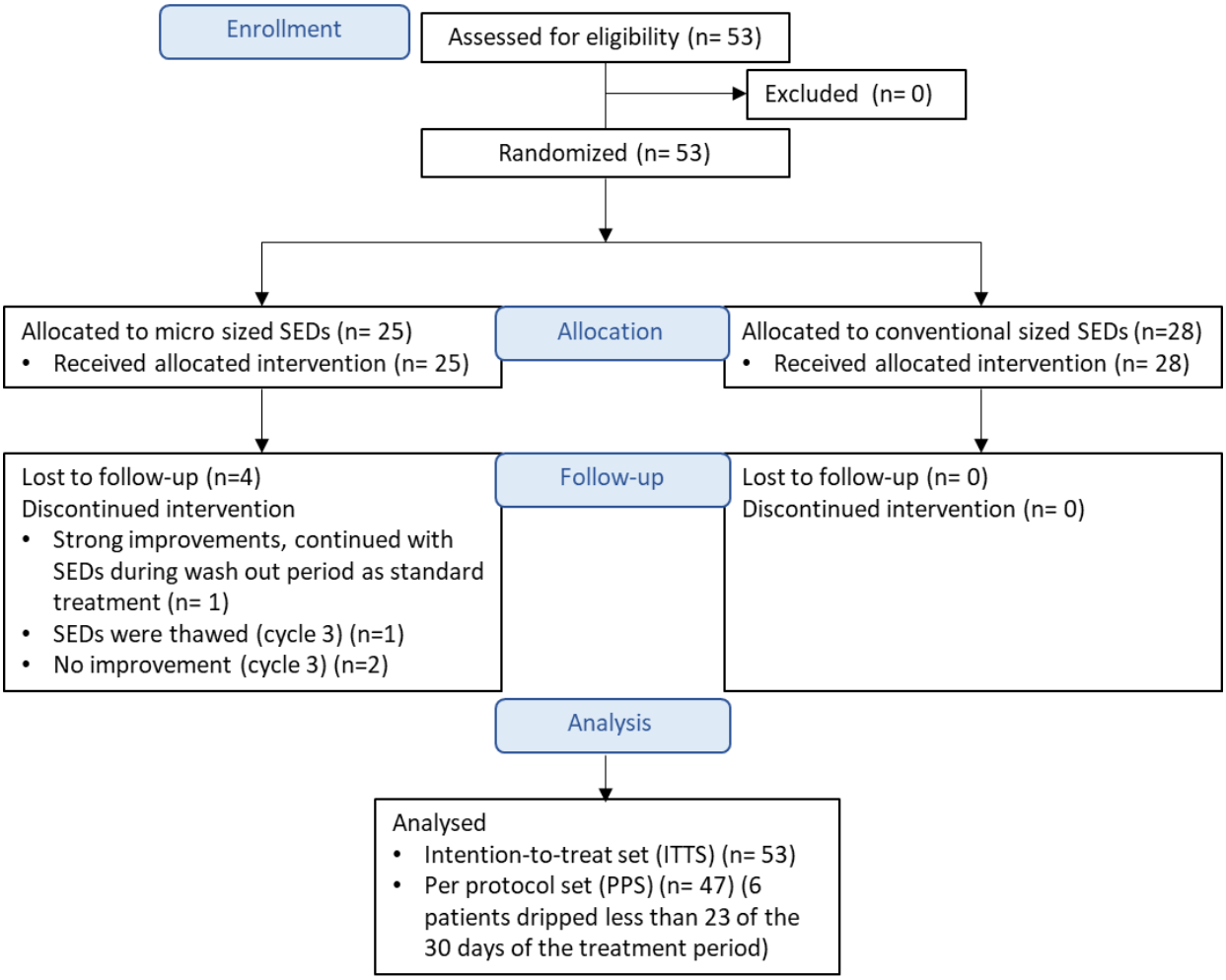
For the analysis of the OSDI score, a linear mixed model (LMM) for cross-over design was used. This model used all observations from each patient, adjusting for order of randomization. The optimal dependency structure was based on AIC and the following covariates were added to the model: observation time (pre or post treatment), treatment (micro-sized SEDs or conventional-sized SEDs), period (first or second treatment period) and their two- and three-way interactions. The main contrast of interest was the interaction between treatment and time. Other interactions were removed if not significant.

For the analysis of TBT, TP and CP, a 3-level random effects LMM for cross-over design was used, including two measurements per time point per patient (left and right eye). For these models, the outcome variables were log transformed to approximate normality. The optimal dependency structure was based on AIC and the following covariates were added to the models: eye (left or right), observation time (pre or post treatment), treatment (micro-sized SEDs or conventional-sized SEDs), period (first or second treatment period), and their two- and three-way interactions. The main contrast of interest was the interaction between treatment and time, other interactions were removed if not significant.

The ease of use of the systems was evaluated using the patients' diaries. To compare the average score and the course over time of ease of use between both eye drop systems, a LMM was used with the optimal dependency structure based on AIC and the following covariates: day since start of treatment period (1-30), treatment (micro-sized or conventional-sized SEDs), period (first or second treatment period), and their two- and three-way interactions. The main contrast of interest was the

interaction between days and treatment. Other interactions were removed if not significant. From the final model, the estimated marginal means over the 30 days were compared between the two treatment groups. Also the estimated time course (slope) for both groups was compared.

Supplementary Figure S3: Flow diagram showing patients inclusion, treatment and analysis.



Supplementary Table S1: Baseline ocular medication specified per patient group (number of patients/total number of patients per group (n) (%)).

Baseline ocular medication used per patient group [number of patients/n (%)]	Patient group (n)		
	Graft versus host disease (10)	Severe dry eye disease (24)	Sjögren's disease (19)
Lubricants (artificial tears and/or gel or ointment)	10/10 (100)	22/24 (92)	19/19 (100)
Cyclosporine	3/10 (30)	6/24 (25)	7/19 (37)
Corticosteroid	1/10 (10)	5/24 (21)	3/19 (16)
Antibiotics	1/10 (10)	3/24 (13)	1/19 (5)
Anti-allergy	-	1/24 (4)	2/19 (11)
Glaucoma eye drops	-	3/24 (13)	-
Pressure reducing eye drops	-	1/24 (4)	-
Pain reducing eye drops	-	1/24 (4)	-

Supplementary Material: Post hoc analysis

In our study, clinical outcomes varied significantly. Therefore, we investigated post hoc whether an underlying common factor for patients that do respond to SED could be noticed.

We found in our cohort that all of the patients diagnosed with graft versus host disease improved in the severity of subjective symptoms experienced, as determined by the OSDI score after the first treatment period with SED, while for the other diagnosis this was less (Supplementary Table S2). The average mean improvement in OSDI score was greater in patients with graft versus host disease compared to other diagnosis. Patients diagnosed with graft versus host disease have the highest baseline OSDI score which could partly explain the observed greater mean improvement in OSDI score. Because the OSDI score in patients with graft versus host disease was very bad at first (average baseline OSDI score ± SD was 73 ± 20), there is more to be gained compared to other diagnoses (Supplementary Table S3).

In summary, post hoc analyses show that in patients with graft versus host disease treatment with SEDs appears to have benefits. In patients with other diagnosis some do respond and other do not respond to SED treatment.

Because our study was not intended nor not powered to conclude that SED treatment works better in patients with graft versus host disease than in other diagnoses, a post-marketing surveillance can give a much better answer to this, provided that data is collected on the OSDI score.

Supplementary Table S2: Improvement in OSDI score per diagnosis. Percentage of patients showing improvement in OSDI score after the first treatment period and the average mean improvement in OSDI score after SED treatment in patients diagnosed with graft versus host disease, severe dry eye disease, Sjögren's disease.

Underlying disease (n)	Improvement in OSDI score after first treatment period with SEDs (% of patients)	Average mean improvement in OSDI score for both SEDs treatments (mean (SD))
Graft versus host disease (10)	100	24 (23)
Severe dry eye disease (24)	75	7 (16)
Sjögren's disease (19)	61	7 (18)

OSDI Ocular Surface Disease Index, *SEDs* serum eye drops, *SD* standard deviation

Supplementary Table S3: Average baseline OSDI score per diagnosis.

Underlying disease (n)	Average baseline OSDI score (mean (SD))
Graft versus host disease (10)	73 (20)
Severe dry eye disease (24)	48 (21)
Sjögren's disease (19)	54 (19)

OSDI Ocular Surface Disease Index, *SD* standard deviation