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Semifluorinated Alkane Eye Drops in Chronic Ocular Graft-versus-Host Disease: A Prospective, Multicenter, Noninterventional Study

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Keywords
Ocular graft-versus-host disease · Semifluorinated alkanes · Perfluorohexyloctane · Aqueous deficiency · Dysfunctional tears syndrome

Abstract
Purpose: Ocular graft-versus-host disease (oGvHD) following allogeneic hematopoietic stem cell transplantation develops as severe dry eye disease (DED) and is initially treated with lubricants, although no clinical trials are available using artificial tears in oGvHD. This trial was set up to test perfluorohexyloctane (NovaTears®) as nonpreserved layer-forming agent for the treatment of DED in oGvHD. ***Methods:*** 25 patients with severe DED due to oGvHD received 1 drop perfluorohexyloctane 4 times daily during a prospective, multicenter, observational 12-week study on top of established topical therapy. Clinical parameters included Schirmer test, tear film breakup time, corneal staining, meibum secretion and ocular surface disease index. Adverse events, visual acuity and intraocular pressure were key safety parameters. ***Results:*** From 25 patients recruited, 23 presented for the second visit. Perfluorohexyloctane treatment did not lead to any changes in clinical or safety parameters but led to fast relief in symptoms in 57% of the patients. One adverse reaction occurred. ***Conclusions:*** This study showed no change in clinical signs in severe DED due to oGvHD, which was not unexpected due to the underlying pathomechanisms. However, the study showed improvement of symptoms in individual patients allowing application of perfluorohexyloctane as an additional symptomatic therapy in oGvHD.

Introduction

Allogeneic hematopoietic stem cell transplantation (aHSCT) is an increasingly successful therapy for the treatment of hematological disorders such as leukemia, myelodysplastic syndrome, non-Hodgkin lymphoma and others [1].

Besides the desired graft-versus-tumor effect that is known to control remaining tumor cells, graft-versus-host disease (GvHD) is the main cause of complications and morbidity following aHSCT [2, 3]. Generally, GvHD may affect all organs with the skin showing the highest prevalence [4]. The eyes are the second most affected organs, with up to 60% of the patients suffering from ocular

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GvHD (oGvHD) [5–8]. Besides an acute form, which is reported to affect approximately 10% of the patients within the first months of aHSCT, the chronic form of oGvHD sets in several months to years after aHSCT. Chronic oGvHD shows a clinical phenotype of severe aqueous deficiency and evaporation due to dry eye disease (DED). In contrast to other forms of DED that affect tens of millions individuals worldwide [9], oGvHD is a rare disease. The significance of this disease derives from the fact that although patients have survived their tumor, they may not be able to regain a normal life due to vast restrictions in their visual function. oGvHD is hereby one of the leading causes of limited quality of life [10], as well as a cause of blindness if not treated early and aggressively enough.

The underlying pathomechanism of oGvHD includes the generation of autoreactive allogeneic T cells [11, 12], activation of macrophages [12] and neutrophils [13] that lead to tissue destruction including the lacrimal gland and ocular surface with scarring of corneal and conjunctival tissue [11–15].

Treatment of oGvHD is challenging due to the rapid progression of the disease and the strong underlying immune mechanisms, which cannot solely be controlled by systemic therapy. Therefore, oGvHD therapy includes topically applied substances such as artificial tears, steroids, cyclosporine, autologous serum eye drops together with scleral contact lenses and surgical procedures such as amniotic membrane [16] or corneal transplantation [17–19].

Clinical trials in patient populations of oGvHD are limited. Several studies, mostly retrospective, are available on the use of cyclosporine [20–23], topical corticosteroids [24, 25] and autologous serum eye drops [26–30].

However, studies using artificial tears are not available. Hence treatment suggestions are mostly adopted from trials in DED other than oGvHD.

Recently the substance perfluorohexyloctane (NovaTears®), a so-called semifluorinated alkane [31], has been tested and certified as a nonblurring wetting agent for the ocular surface in evaporative DED. It is preservative-free due to a complete lack of aqueous components and therefore the impossibility of microbial growth. In two previous conducted clinical trials, perfluorohexyloctane has demonstrated significant improvement in signs and symptoms of DED and improvement of meibomian gland dysfunction and blepharitis through its superior spreading abilities [32, 33]. Perfluorohexyloctane's molecular properties allow for an extremely low surface tension of 19.65 mNm and are hereby thought to reduce shearing forces between surfaces and leading to a smaller drop size than water once applied to the ocular surface [31]. However, the substance has not been tested in severe evaporative and aqueous deficient DED.

The objective of this study in 25 patients with evaporative and aqueous deficient DED due to oGvHD was to assess the local safety and tolerability of perfluorohexyloctane and efficacy parameters (signs and symptoms) over a 12-week treatment period under concomitant topical therapy.

Methods

Study Design

A prospective, multicenter, observational, 11- to 13-week study of patients with chronic oGvHD was designed and performed in routine specialized outpatient clinics for patients following hematopoietic stem cell transplantation at the Eye Center, Medical Center, University of Freiburg, and the Competence Center for ocular GvHD of the Division of Dry-Eye and Ocular GvHD, Department of Ophthalmology, University of Cologne, Germany.

The study was reviewed and approved by the ethical committees of the University of Cologne (14-370) and University of Freiburg (521/14), adhered to the tenets of the Declaration of Helsinki and was registered at clinicaltrials.gov (NCT02356328).

After informed consent had been obtained, patients were trained how to use NovaTears® according to the Instructions for Use. Patients were advised to apply 1 drop 4 times daily in both eyes and to report any adverse event immediately either by phone or through scheduling an immediate appointment. Patients returned after 12 (window: 11–13) weeks for follow-up. Data for this study were collected between January 2015 and January 2016.

Patients and Examination Parameters

25 patients with severe chronic oGvHD from specialized outpatient clinics of the two centers (Freiburg and Cologne) were included in the study according to the following inclusion criteria: (1) male or female, age ≥18 years, (2) following aHSCT with DED due to chronic oGvHD, (3) Schirmer I test ≤15 and ≥1 mm, (4) tear film breakup time (TFBUT) ≤10 s, (5) Ocular Surface Disease Index (OSDI©) >20, (6) visual acuity >0.2 (decimal), (7) corneal fluorescein staining ≥ grade 1 and ≤3 (Oxford). Exclusion criteria were: (1) males or females <18 years, (2) known hypersensitivities against the study substance, (3) patients with contact lenses, pregnant or lactating women, (4) patients with DED caused by a systemic disease unlike GvHD, (5) planned surgical ophthalmological intervention during the duration of the study, (6) patients who with the exception of cyclosporine-containing eye drops apply lipid-containing products, (7) lack of ability or readiness to sign the informed consent form, (8) lack of ability or readiness to participate in all required examinations including to fill in the OSDI questionnaire and to present for the follow-up examination.

Clinical efficacy parameters that were assessed both at the baseline and at the follow-up visit were performed. Briefly and in the following sequence: best corrected visual acuity (standardized visual charts) and intraocular pressure (applanation tonometry)

Table 1. Hematological disorders (n = 25; 100%)

Hematological disorder by preferred term	n (%)
Acute lymphocytic leukemia	1 (4.0)
Chronic lymphocytic leukemia	1 (4.0)
Chronic myeloid leukemia	4 (16.0)
Hodgkin's disease	1 (4.0)
Myelodysplastic syndrome	5 (20.0)
Myelofibrosis	1 (4.0)
Acute myeloid leukemia	12 (48.0)
T-cell lymphoma	1 (4.0)

were assessed to monitor safety. Then the Schirmer I test without anesthesia was performed, and patients were allowed to open or close eyes during the 5-min duration. During slitlamp examination blepharitis was graded for each eye differentiating between anterior and posterior blepharitis (if present grade 1–3), meibomian gland occlusion or ridging of the lid margin and redness of the conjunctiva (if present grade 1–3). Then fluorescein sodium was applied to both eyes using mildly wetted fluorescein strips (I-DEW FLO, fluorescein sodium ophthalmic strips, Entod Research Cell UK Ltd., London, UK), and TFBUT as well as corneal staining were measured/graded using a yellow filter (Boston, Bausch + Lomb, Berlin, Germany). Corneal staining was graded according to the Oxford grading scheme [34] from grade 0 to 5. Then, meibum secretion was examined by compressing the entire lower and upper eyelid using a Q-tip and differentiating meibum quality (clear, whitish, thick or none) and quantity (none, a little, normal, a lot). In addition, each patient filled in a symptom questionnaire (OSDI© [35]). All examinations were performed only by the principal investigator of the respective center to minimize interinvestigator variability. Patients were also asked for specific symptoms such as burning, redness, itching, etc., usability and subjective treatment satisfaction. For the safety assessment, patients were asked to report adverse events (AEs) throughout the study, any serious deterioration in health had to be reported immediately. All patients reporting problems were called and interviewed by the physicians and were offered an immediate visit at the study site. On the follow-up examination patients were asked the following questions: (1) Whether and how often had they taken perfluorohexyloctane? (2) Was the application very easy, easy, neutral, sometimes difficult or very difficult? (3) How was the sensation after applying the drop (cold, warm, neutral, velvet-like, watery or oily; very pleasant, pleasant, neutral, unpleasant or very unpleasant)? (4) Was the vision after application of perfluorohexyloctane negatively affected (not at all, a little, very much, somewhat, strongly, very strongly)? (5) How fast did the drops relieve dry eye symptoms (very fast, fast, slow, very slow, not at all)? (6) How satisfied was the patient with the treatment (very satisfied, satisfied, neutral, dissatisfied, very dissatisfied)? (7) How likely is it that the patient would continue using the substance (very likely, likely, neutral, unlikely, very unlikely)?

Statistical Analysis and Study Analysis Populations

Two analysis populations were defined.

The safety analysis population consisted of all patients who were enrolled in the study.

Table 2. Concomitant topical therapy at baseline and follow-up (n = 25; 2 patients were lost to follow-up, n = 23)

Concomitant topical therapy	Baseline/follow-up, n (%)
Topical cyclosporine	
0.05%	10 (40/43)
0.1%	1 (4/4)
0.5%	4 (16/17)
1%	6 (24/26)
Tacrolimus ointment	1 (4/4)
Artificial tears	
1 product	17 (68)/15 (65)
2 products	7 (28/30)
3 products	1 (4/4)

The per-protocol population comprised all patients in the safety analysis set that completed the study per protocol. A patient was excluded from the set, if:

- the patient did not reliably take the study medication throughout the study;
- the patient was lost to follow-up.

Statistical analysis of visual acuity, intraocular pressure, Schirmer I test, TFBUT, OSDI© and corneal staining was performed in order to identify statistically significant differences between baseline and follow-up. For all parameters, except for corneal staining, a paired 2-sided t test testing the null hypothesis that the mean change from baseline equals zero was used. The change in corneal staining was analyzed using the Wilcoxon signed rank test for location shifts between baseline and follow-up. Statistical analysis was performed using SAS® (version 9.3 for Microsoft Windows). A p value <0.05 was used to declare significance.

Results

Demographic Data

In total, 25 patients were included in the study. Duration between aHSCT and inclusion into the trial was 64 months on average (7–227 months). The medical data are presented in Table 1. More male patients were recruited than females (11 females, 14 males, age 49.68 ± 10.68 years). One female and one male patient did not complete the study (lost to follow-up for unknown reasons). Eight patients did not take the study medication reliably. Consequently, the safety analysis population consisted of 25 patients, the per-protocol population consisted of 15 patients. The concomitant topical therapy consisting of cyclosporine, tacrolimus and artificial tears is depicted in Table 2. Artificial tear frequency was once per day up to 30× per day. Topical cyclosporine was applied on average for 13 months, tacrolimus (one patient) for 3 months prior to inclusion in the trial.

Table 3. Visual acuity and intraocular pressure analysis (safety analysis population, $n = 25$)

	Baseline	Follow-up	Change from baseline	*p* value
Best corrected visual acuity				
OD				
Median	0.80	0.90	0.00	0.39
Min.-max.	0.25 to 1.00	0.16 to 1.00	–0.47 to 0.38	
OS				
Median	0.80	1.00	0.00	0.59
Min.-max.	0.40 to 1.00	0.20 to 1.00	–0.30 to 0.20	
Intraocular pressure				
OD				
Mean	13.86	14.09	+1.05	0.12
SD	3.54	2.97	2.89	
OS				
Mean	13.00	13.83	+1.35	0.21
SD	3.74	3.53	4.66	

Table 4. Schirmer I test, tear film breakup time (TFBUT) and corneal fluorescein staining analysis (per-protocol population, $n = 15$)

	Baseline	Follow-up	Change from baseline	*p* value
Schirmer I test without anesthesia				
OD				
Mean	1.93	2.73	+0.80	0.17
SD	1.34	2.46	2.15	
OS				
Mean	2.13	2.53	+0.40	0.56
SD	2.13	3.07	2.61	
TFBUT				
OD				
Mean	3.02	3.58	+0.56	0.37
SD	1.36	2.55	2.31	
OS				
Mean	3.20	2.51	–0.69	0.17
SD	2.05	1.46	1.82	
Corneal fluorescein staining				
OD				
Mean	2.13	2.00	–0.13	0.77
SD	0.83	1.31	0.83	
OS				
Mean	1.93	2.00	+0.07	1.00
SD	1.10	1.25	0.80	

Clinical Findings

Visual Acuity, Intraocular Pressure

As can be seen in Table 3, best corrected visual acuity and intraocular pressure did not change over the study period.

Schirmer I Test, TFBUT and Corneal Fluorescein Staining

In the GvHD study, tear secretion, tear film stability and corneal fluorescein staining did not change over the study period, as can be seen in Table 4. A Wilcoxon rank sum test showed no statistically significant differences between baseline and follow-up.

Blepharitis Assessment

Changes in severity of anterior and posterior blepharitis between baseline and follow-up for both eyes combined are shown in Figure 1 (total sample size = 30 eyes (15 patients)). 9/30 eyes showed improvements of anterior blepharitis.

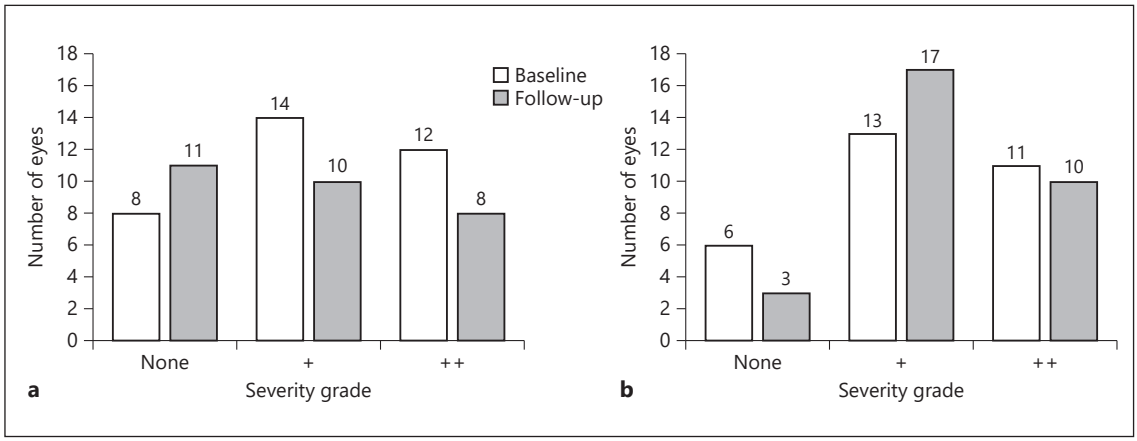

Fig. 1. Changes in severity of anterior (**a**) and posterior (**b**) blepharitis between baseline and follow-up for both eyes combined.

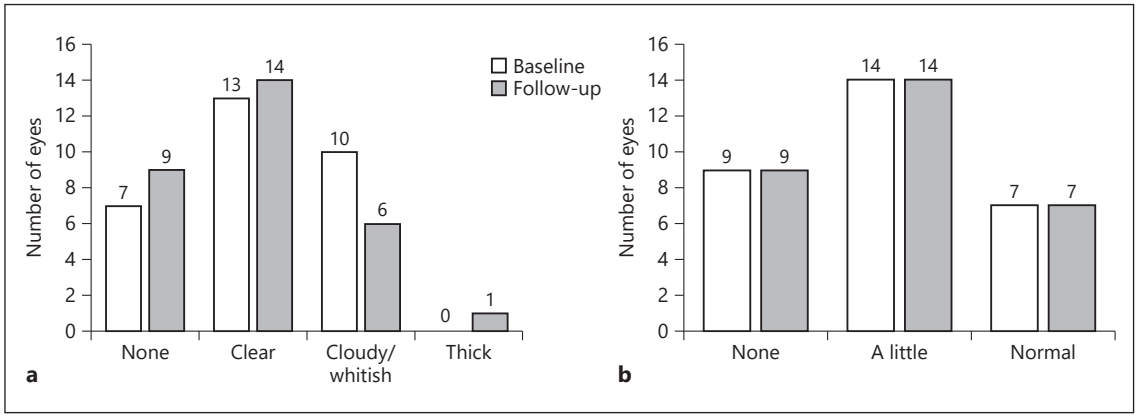

Fig. 2. Changes between baseline and follow-up in the quality (**a**) and quantity (**b**) of meibum for both eyes combined.

8/30 eyes demonstrated improvement for posterior blepharitis. 16/30 and 13/30 eyes showed no change, respectively, and 5/30 eyes for anterior and 9/30 eyes showed worsening of anterior or posterior blepharitis. A Wilcoxon rank sum test (n = 15 each) showed that changes for anterior (OD: p = 0.98; OS: p = 0.59) and posterior blepharitis (OD: p = 0.56; OS: p = 1.00) were not statistically significant.

Meibum Analysis

A shift table of the changes between baseline and follow-up in the quality and quantity of meibum for both eyes combined is shown in Figure 2 (total sample size = 30 eyes [15 patients]).

8/30 eyes showed improvement of meibum quality, and 6/30 eye showed improvement of meibum quantity. 13/30 eyes demonstrated no change in meibum quality, and 18/30 eyes showed no change in meibum quantity. 2/30 patients showed worsening of meibum quality, and 6/30 patients exhibited worsening of meibum quantity. No statistical analysis was applied to this parameter.

Lid Margin Assessment

Most patients showed no change in plugging and ridging of the lid margin. 8/30 patients appeared to have ridging at the follow-up visit. No statistical analysis was applied to these parameters.

Conjunctival Redness Assessment

A shift table of changes in severity of conjunctival redness between baseline and follow-up for both eyes

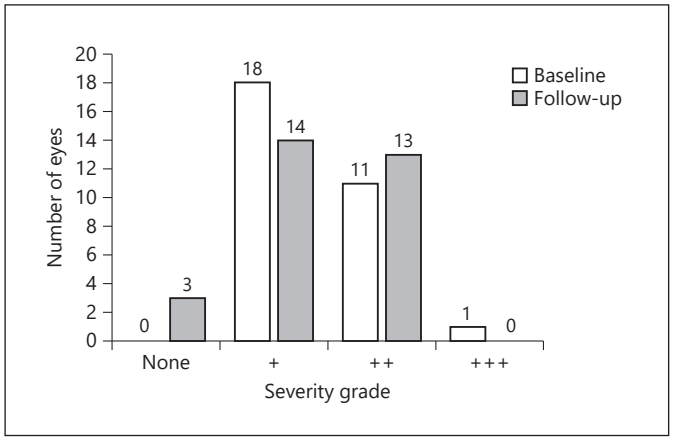

Fig. 3. Changes in severity of conjunctival redness between baseline and follow-up for both eyes combined.

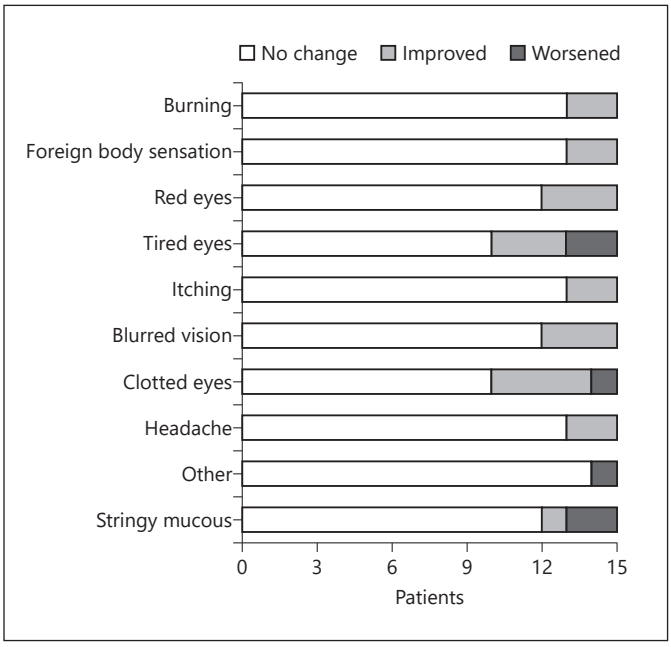

Fig. 4. Symptom change from baseline to follow-up visit.

combined is shown in Figure 3 (total sample size = 30 eyes [15 patients]). 16/30 eyes showed no improvement. 8/40 eyes demonstrated improvement of conjunctival redness, 6/30 patients presented with worsening of conjunctival redness. A Wilcoxon rank sum test (n = 15 each) showed no statistical significance (OD: p = 0.36; OS: p = 1.00).

Subjective Dry Eye Symptom Questionnaire (OSDI©)

The mean OSDI© decreased from 54 to 49 after the use of perfluorohexyloctane over a 11- to 13-week period, indicating a decrease in subjective symptom severity. This change is below the 13-point threshold, which is generally considered to be a significant change in this group of severely affected patients [36]. However, 4 patients showed large improvements of up to 34 points (range 17–34, safety analysis population).

Symptom Assessment Physician

Patients were asked by the physician whether they currently suffer from typical dry eye symptoms both at the baseline and at the follow-up visit. As can be seen in Figure 4, a lower number of DED-associated symptoms were reported after 11–13 weeks of treatment; however, the majority of patients reported no change. Only a few patients reported worsening.

Usability and Overall Satisfaction

Patients' opinion about the usability of perfluorohexyloctane and treatment satisfaction was obtained by a questionnaire at the end of the study (follow-up visit). The majority of patients' responses were positive. The responses of all patients given at the second visit (n = 23) are shown in Table 5.

Adverse Events

A total of 3 AEs was reported during the course of this study; none of them were classified as serious adverse device effects (= incident according to applicable legislation) (Table 6). For AE No. 1, an ocular and nasal allergy was diagnosed by the principal investigator but was considered as being related to pollen and unrelated to the study medication. For AE No. 2, a mild ocular irritation was reported which stopped immediately after discontinuation of the study medication. AE No. 3 was considered unrelated to the study medication and did not lead to any change in dosing.

Discussion

Dry eye in chronic oGvHD is a serious condition that significantly affects patients' quality of life [10]. Treatment recommendations include most of all artificial tears together with topical steroids, cyclosporine, autologous serum and scleral contact lenses [37, 38].

However, regarding artificial tears, these recommendations are solely based on clinical trials in dry eye other than oGvHD. In fact, oGvHD is often listed as an exclu-

Table 5. Usability and overall satisfactory inventory (n = 23)

Q1 (Did the patient constantly apply NovaTears® during the last 11–13 weeks?)	
Daily, recommended dose	15
Daily, less than recommended dose	3
Almost daily	–
Sometimes	5
Not at all	–
Q2 (How easy was the administration of NovaTears® eyedrops into the eyes for the patient?)	
Very easy	6
Easy	9
Neutral	1
Sometimes difficult	5
Very difficult	2
Q3 (How does the patient describe the sensation of NovaTears® eyedrops after application (1)?)	
Cold	–
Warm	1
Neutral	4
Velvety	7
Aqueous	–
Oily	12
Other	1
Q4 (How does the patient describe the sensation of NovaTears® eyedrops after application (2)?)	
Very pleasant	3
Pleasant	10
Neutral	6
Unpleasant	3
Very unpleasant	1

Q5 (Was the patient's vision affected immediately after administration of NovaTears® eyedrops?)	
Not at all	8
A little	12
Somewhat	2
Strongly	1
Very strongly	–
Q6 (How fast did NovaTears® eyedrops relieve dry eye symptoms of the patient?)	
Very fast	1
Fast	12
Slowly	2
Very slowly	1
Not at all	7
Q7 (How satisfied was the patient with NovaTears® treatment?)	
Very satisfied	3
Satisfied	6
Neutral	4
Dissatisfied	8
Very dissatisfied	2
Q8 (How likely is it that the patient will continue using NovaTears®?)	
Very likely	7
Likely	2
Neutral	3
Unlikely	5
Very unlikely	6

Table 6. Adverse events

AE number	Preferred term	Severity	Relationship	Action taken
1	Hypersensitivity	Mild	Not related	Drug withdrawn
2	Application site pain	Mild	Definitely	Drug withdrawn
3	Corneal infiltrates	Mild	Unlikely	Dose not changed

sion criterion in clinical trials with artificial tears. As this disease is based on unique, currently only poorly understood pathomechanisms, clinical trials focusing on oGvHD solely are mandatory. oGvHD is considered to be a rare disease. Current statistics in the European Union state that less than 5 in10,000 individuals (http://ec.europa.eu/health/rare_diseases/policy_de) are affected and US statistics report less than 200,000 individuals affected [39]. Due to the low incidence rate, interest from pharmaceutical companies is limited. Therefore, performing prospective observational trials instead of randomized controlled double-blind studies would be a compromise to cost-effectiveness, despite generating data with a limited level of evidence.

This current observational trial was designed following previous studies in patients suffering from evaporative DED, meibomian gland disease and blepharitis to test semifluorinated alkanes. As a new water-free class

of compounds, these have the ability to form an extremely thin layer on top of the existing tear film components, thereby stabilizing the tear film.

Based on the well-known facts that treatment of chronic oGvHD is difficult and that our study population demonstrated a severe ocular surface disease (average Schirmer I test of 2 mm, TFBUT of 3 s and corneal fluorescein staining of grade 2 together with an average OSDI score of 54), there was limited expectation that perfluorohexyloctane would significantly improve clinical signs on top of a readily established topical therapy. The main aim of this study was rather to observe the usage of a new substance, together with ongoing established topical GvHD therapy and to systematically analyze ocular safety parameters, patient compliance and patient satisfaction, which are equally important factors in treating oGvHD.

According to our results, the application of perfluorohexyloctane is safe and does not cause worsening of clinical signs or symptoms in patients after aHSCT suffering from severe DED due to oGvHD. Although several patients did not apply the study treatment as recommended, 57% of the patients reported a fast or very fast relief of eye symptoms in the patient satisfaction questionnaire, which is one of the central aims in treating these patients with lubricants. Furthermore, 40% of the patients were satisfied or very satisfied with perfluorohexyloctane treatment and were likely or very likely to continue the application beyond the duration of the trial.

Nevertheless, a significant proportion of patients only used the treatment sometimes or discontinued the use ($n = 8$). However, these patients did not all report ocular side effects.

There were only 3 AEs reported, and from these AEs, only 1 was considered related to the use of perfluorohexyloctane. This AE was application site pain, which stopped immediately after discontinuation of usage. This low rate of AEs, in a patient population with a compromised ocular surface, underlines the good safety profile of perfluorohexyloctane in severe dry eye patients.

This study has several limitations. Due to the observational character of the study, the efficacy parameters were compared with baseline measurements, not to a control group, and data were only collected at 2 time points (baseline and follow-up visit). Variations in endpoints and population make a cross study comparison difficult in this indication. Furthermore, the per-protocol population of only 15 patients does not allow to draw extensive conclusions regarding application of perfluorohexyloctane in general.

Overall, perfluorohexyloctane (NovaTears®) seems to be safe in a severely affected population of patients with DED in chronic oGvHD as demonstrated by this prospective observational study. Although no statistical significance was shown, due to the limited number of patients in the per-protocol data set ($n = 15$), improvements occurred in several patients regarding anterior blepharitis and subjective symptoms.

Based on the data presented, we propose the design and conduction of further prospective clinical trials for artificial tears but also applying active compounds such as anti-inflammatory drugs in the population of oGvHD rather than transferring results from trials in other forms of DED.

Statement of Ethics

The study was reviewed and approved by the ethical committees of the University of Cologne (14-370) and University of Freiburg (521/14), adhered to the tenets of the Declaration of Helsinki and was registered at clinicaltrials.gov (NCT02356328).

Disclosure Statement

P.E.: no conflict of interest.
S.K.: employee of Novaliq GmbH.
P.S.: received grants by Novaliq GmbH.

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