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Clinical Trials

A Phase I/II Placebo-Controlled Randomized Pilot Clinical Trial of Recombinant Deoxyribonuclease (DNase) Eye Drops Use in Patients With Dry Eye Disease

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Purpose: To determine whether DNase eye drops have the potential to reduce signs and symptoms of dry eye disease (DED).

Methods: A placebo-controlled, randomized clinical trial was performed to compare the safety and efficacy of DNase eye drops 0.1% four times a day for 8 weeks in patients with severe tear deficient DED. The change in safety outcome measures (drug tolerability and proportion of adverse events) and efficacy outcome measures (Ocular Surface Disease Index [OSDI] score, corneal and conjunctival staining) were analyzed between baseline and week 8.

Results: Tolerability and adverse events were similar in placebo group and DNase group. Within the DNase group (but not placebo group), corneal staining showed a statistically significant and clinically meaningful reduction at week 8 compared with baseline. The OSDI score also showed a significant median reduction of 27.3 at week 8 compared with baseline within the DNase group. The median reduction in corneal staining and mucoid debris/strands was significantly greater in the DNase group as compared with the placebo group. In the DNase group, the median reduction in OSDI (−20.75) was more than placebo group (−8.43); however, the difference between groups was borderline significant.

Conclusions: In this pilot study, treatment of severe tear deficient DED patients with DNase eye drops appears safe, well tolerated, and has the potential to reduce the severity of signs and symptoms.

Translational Relevance: Data from this pilot clinical trial demonstrate the therapeutic potential of DNase eye drops in dry eye disease, possibly due to degradation neutrophil extracellular traps (NETs) from the ocular surface.

Introduction

Dry eye is a multifactorial disease of the ocular surface characterized by a loss of homeostasis of the tear film, and accompanied by ocular symptoms, in which tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities play etiological roles.[1] It is well recognized that ocular surface inflammation plays a prominent role in dry eye disease (DED) pathogenesis[2]; however, the mechanisms that cause inflammation are incompletely understood. This has hindered the development of new treatments. Currently, only two drugs are approved for treating DED.

Our laboratory was the first to discover that numerous neutrophils are present on the ocular surface of patients with severe tear-deficient DED subtypes

(Sjogren's syndrome, ocular Graft-vs.-Host-Disease (GVHD), non-Sjogren's DED, and ocular cicatricial pemphigoid) and that they release their nuclear chromatin complex as a type of biologic "spider's web."[3,4] These extracellular DNA (eDNA) webs are termed neutrophil extracellular traps (NETs).[5,6] Although NETs are part of the innate immune defense, they can cause chronic inflammatory diseases.[7,8] NETs/eDNA accumulate on the ocular surface of DED patients either because of increased formation (due to hyperosmolarity)[9] and/or reduced clearance (due to tear deficiency and consequent nuclease deficiency).[3] Our data suggest that eDNA production and clearance mechanisms are dysregulated in tear deficient DED subtypes. Further, we reported that tear fluid eDNA abundance correlates with signs and symptoms in tear deficient DED subtypes.[10]

Based on our findings, we hypothesized that clinical strategies that reduce the abundance of neutrophils and their extracellular products (eDNA and NETs) on the ocular surface have the potential to reduce signs and symptoms of tear-deficient DED. Deoxyribonuclease I (DNase) is the major extracellular endonuclease that selectively cleaves DNA.[11] DNase is a "waste-management enzyme."[12] It degrades and clears DNA that leaks into the extracellular space due to cell death. Since eDNA forms the backbone of NETs, DNase treatment will degrade the NETs. Therefore, DNase can be used as a therapeutic strategy in tear deficient DED subtypes to clear NETs that accumulate on the ocular surface. DNase is available as Pulmozyme (Genentech, South San Francisco, CA) and is approved by the US Food and Drug Administration (FDA) for use as a daily inhaled solution in conjunction with standard therapies in the management of patients with cystic fibrosis to improve pulmonary function.[13,14] Application of DNase as a nebulizer has not been associated with toxic side effects, perhaps because DNase enzymatic activity is irreversibly neutralized by cytoplasmic G-actin inside the cells.[15]

To assess if DNase therapy is feasible, safe, tolerable, and potentially effective in reducing signs and symptoms of tear deficient DED, we performed a prospective, phase I/II, randomized, placebo-controlled, double-masked pilot clinical trial in these patients using DNase (0.1%) eye drops four times a day. Other than a published case report,[10] there is no data regarding the use of DNase application to the human eye; therefore, our clinical trial was an essential first step toward establishing the safety and therapeutic potential of this therapy.

Methods

The study protocol was approved by the Institutional Review Board of University of Illinois at Chicago (UIC). An investigational new drug (IND) was assigned by the FDA for the study drug (DNase). Informed consent was obtained from all patients after the nature and possible consequences of treatment were explained. Research was conducted in accord with the tenets of the Declaration of Helsinki. This single center pilot study was conducted in the UIC Department of Ophthalmology Clinical Trials and Translational Center.

The key criteria for inclusion were: (1) 18 years or older; (2) capable of giving informed consent; (3) documented DED for at least 6 months; (4) Schirmer I without anesthesia <10 mm; (5) corneal/conjunctival staining >1; (6) and Ocular Surface Disease Index (OSDI) >13. The key exclusion criteria were: (1) active ocular infection or ocular allergies; (2) any history of eyelid surgery or ocular surgery within the past 3 months; (3) corneal epithelial defect larger than 1 mm^2 in either eye; (4) the use of topical cyclosporine or corticosteroids within 2 weeks of enrollment; and (5) current use of contact lenses. Participants were permitted to continue their other chronic treatments, including the use of artificial tears, eyelid massage, or warm compresses. A 2-week washout period was required if topical corticosteroids or topical cyclosporine were discontinued before enrollment.

The drug used in this study was recombinant human deoxyribonuclease I (0.1% DNase). This drug is FDA approved for human use and is marketed as Pulmozyme (Genentech). The placebo used was the drug vehicle. The drug and its vehicle were supplied by the manufacturer for this clinical trial (Genentech). Subjects were randomly assigned to one of two groups (#1, #2). Group #1 was given drug vehicle (placebo) and group #2 was given study drug (DNase). The placebo and DNase eye drops were applied to both eyes four times a day for 8 weeks. We used a computer-based random code generator (Research Randomizer; http://randomizer.org/) to generate one set of nonunique, unsorted numbers with a range from 1 to 2 representing the group number. Based on the randomizer generated table, subjects received either placebo or study drug. Randomization was performed by the UIC Eye and Ear Infirmary (EEI) Pharmacy staff, and neither participants nor research staff were aware of the assigned treatments. The study medications were stored, packaged, and dispensed

from the UIC EEI Pharmacy. The study medications were dispensed in sterile eye droppers of 3 mL volume (each containing 400–500 μL of the study medications), which were used as single-dose applications. One drop of the drug or placebo solution was administered to each eye four times a day. Therefore, four eyedroppers were required per day. At each visit, subjects received 56 sterile multidose eye droppers that were used as single-dose applications. Prepared eye droppers were placed in a dark (brown) colored zip-lock packet before being dispensed to the subject. The medications were to be stored in a refrigerator (4°C), away from direct, strong light.

Efficacy end points include a change in the following measures: (1) OSDI; (2) Clinical Global Impression (CGI); (3) Subject Global Assessment (SGA); (4) Corneal Staining, (5) Conjunctival Staining, (6) Mucoid debris/strands; and (7) Validated Bulbar Redness (VBR) grading scale. Safety end points include the following: (1) tolerability to eye drops at day 1 (postdose) and at weeks 2, 4, 6, and 8; (2) proportion of adverse events; (3) patient retention; and (4) drug adherence.

At all visits, the principal investigator (SJ) conducted a complete undilated examination of the eyes using a slit lamp. The investigator examined the tear film, eye lids, lashes, bulbar and palpebral conjunctiva, upper and lower lid puncta, cornea, anterior chamber, iris, lens, and anterior vitreous and recorded any abnormal findings. Symptoms of ocular discomfort were assessed with OSDI.[16] The OSDI rating scale has 12 questions in three discrete areas, with responses rated on a five-point scale. Subjects completed this scale on day 1 prior to first dose (baseline), week 2, week 4, week 6, week 8, and week 10. Tear production was measured over 5 minutes using a filter paper applied over the lateral 1/3 of the eyelid (Schirmer I without anesthesia). Presence of mucoid debris/strands over the ocular surface was assessed, and the amount graded as absent (0) or present (1+, 2,+ or 3+). Corneal and conjunctival staining were assessed using Rose Bengal dye. Scoring of corneal and conjunctival staining was performed using a slit lamp examination after using the grading system described by the 1995 National Eye Institute (NEI) workshop.[17] Corneal staining was graded in five zones, and conjunctival staining was graded in two zones. Each zone was graded from 0 to 3 based on the density of punctate staining. The final staining score was the sum of individual scores. Ocular surface redness (nasal or temporal) was assessed using the VBR grading scale.[18] The VBR consisted of a set of 10 images illustrating different degrees of ocular surface redness, ranging from normal to severe, and each image was assigned a value in an order of ascending severity.

The CGI was performed as follows[18]: Question (to physician): In general, compared with the patients' dry eye symptoms and signs at baseline, how would you characterize his/ her overall signs and symptoms now? The responses were categorized on a seven-point scale as follows: marked worsening, moderate worsening, minimal worsening, unchanged, minimal improvement, moderate improvement, and marked improvement. At each visit, the subjects were asked to assess their overall change from baseline. The SGA was performed by asking two questions as follows[19]: Question #1 (to subject): Compared with your first visit, how are your dry eye symptoms now? Question #2: Compared with your first visit, how is the mucoid debris/strands from your eyes now? The responses to both questions were categorized on a five-point scale as follows: much worse, worse, about the same, improved, and much improved.

Subjects assessed their tolerance to the administration of the test medication utilizing a visual analog scale (VAS). The VAS is a 100-mm horizontal line with verbal descriptors at either end. The VAS ratings were completed after administration of the test medication on day 1 (postdose), week 2, week 4, week 6, and week 8. Subjects placed a single slash mark across the horizontal line between the end labeled "completely intolerable" (0 mm) and "easily tolerable" (100 mm).

Statistical Analysis

We summarized the data by using the frequency with the percentage for categorical variables. For continuous variables, due to the skewness of the distribution for most of variables, the median with the interquartile range (IQR) were reported instead of mean and standard error to mitigate the influence of any extreme value. Both available data and intent-to-treat analyses were conducted to assess the treatment effect on the differences of efficacy outcomes between baseline and week 8. For the available data analysis, an outcome change after 8 weeks was examined within group and between groups by using Kruskal-Wallis test for separate eyes. Notice that as Kruskal-Wallis test is known as a rank-sum test, which incorporates the rank of each data point, it is possible to show a statistically significant difference, even when median and IQR are the same. For to the intent-to-treat analysis, a quantile linear mixed effect

model at median was fitted on the efficacy outcome, corneal staining, assuming missing at random. The interpretation behind this quantile regression is similar to a general linear regression, but it accesses the influence of each factor on the median instead of mean. Thus, it could avoid bias when the outcome variable does not follow a normal distribution. This model included treatment group (DNase versus placebo), time (week 8 versus baseline), and their interaction as the main predictors in the fixed effect part, and a subject-level random effect to take account of any subject dependence for both eyes. Moreover, as sensitivity analyses, we adjusted for the side of eye and other demographics in additional models, and fitted a linear mixed model with the same variables. The results were similar, and thus were not reported. Finally, for the safety outcomes, we described the value of tolerability over time (baseline, week 2, 4, 6, and 8) and compared two groups by using Kruskal-Wallis test, whereas we reported the proportions of adverse events and compared the distribution between group using Fisher's exact test or χ^2 test. All the statistical analyses were conducted by using R (version 3.4.1; R Core Team, Vienna, Austria). Two-sided *P*-values less than 0.05 were considered as statistically significant.

Results

This is an investigator initiated pilot clinical trial of recombinant human deoxyribonuclease (DNase) versus vehicle (placebo) eye drops in patients with DED. Patients with tear deficient DED ($n = 47$) were enrolled in the clinical trial. Patients had diagnosis of Sjogren's syndrome (53% patients), non-Sjogren's DED (30% patients), and ocular GVHD (17% patients). Of the 47 patients enrolled, 41/47 patients completed all visits of the clinical trials (87% retention) and all of them used the study eye drops for duration of the trial (100% adherence to treatment). Of the six patients who dropped out of the study, two were in the placebo group and four in the DNase group. The reasons for dropout included preference for contact lens and other standard of care treatments. Baseline characteristics of the placebo and DNase groups are shown in Table 1. All data are presented as median with the IQR. The two groups had similar distribution for age, gender, OSDI, corneal and conjunctival staining, mucoid debris/strands, tolerability, and diagnosis. The baseline characteristics of right eye (OD) and left eye (OS) in the two groups are shown in Table 2.

In our analysis, we compared the outcome measures (OSDI, corneal and conjunctival staining, mucoid debris/strands, tolerability, SGA, and CGA) for OD or OS within in each group (first analysis) and between groups (second analysis). Both available data and intent-to-treat analyses were conducted to assess the effect of treatment on the change in efficacy outcomes between baseline and week 8. For the first analysis (within group "available data"), the right eye or left eye outcome measures data at week 8 were compared with corresponding eye data at baseline to determine significant differences. This analysis was done separately for placebo and DNase group. In this placebo group, in both eyes (OD or OS), none of the outcome measures showed a significant change between week 8 and baseline (Table 3). The OSDI score showed a median reduction of 13.9, which is minimal clinically important difference (MCID), but the change between week 8 and baseline showed a borderline significance. In the DNase group, both eyes showed a statistically significant and clinically meaningful reduction in corneal staining at week 8 compared with baseline (Table 4). The OSDI score showed a statistically significant median reduction of 27.3 at week 8 compared with baseline, which is more than MCID. In the right eye, mucoid debris/strands were significantly reduced at week 8 compared with baseline, but showed a borderline significance in the left eye. Since there were three distinct subgroups of patients (Sjogren's syndrome, non-Sjogren's DED, and ocular GVHD), we analyzed whether the response was better in one of the subgroups as compared with others. Only conjunctival staining for OD shows a statistical difference between the three subgroups. Thus, we conducted pairwise comparisons only for conjunctival staining within OD. The results showed that only ocular GVHD versus non-Sjogren's DED had a < 0.05 *P*-value. However, after adjusting for multiple comparisons by using the Bonferroni approach, this *P*-value $= 0.038$ ($>0.05/3$) was no longer significant. Therefore, our analysis showed that the response to DNase was not better in one of the subgroups as compared with the others.

For the second analysis (between groups "available data"), the change in outcome measures (week 8 minus baseline) for DNase group was compared with the change in placebo group, for OD and OS separately, to determine significant differences (Table 5). The median reduction in corneal staining (week 8 minus baseline) was significantly greater in the DNase group as compared with the placebo group for OD as well as OS. The median reduction in mucoid debris/

Table 1. Characteristics at Baseline

	Placebo ($N = 22$)	DNase I ($N = 25$)
Age, median [IQR]	55.5 [50.2–60.8]	57.0 [44.0–64.0]
Gender, *n* (%)		
Female	17 (77.3)	23 (92.0)
Male	5 (22.7)	2 (8.00)
Race, *n* (%)		
American Indian or Alaska Native	1 (4.55)	0 (0.00)
Asian	1 (4.55)	2 (8.00)
Black or African-American	5 (22.7)	5 (20.0)
White	15 (68.2)	18 (72.0)
Ethnicity, *n* (%)		
Hispanic or Latino	5 (22.7)	10 (40.0)
Not Hispanic or Latino	17 (77.3)	15 (60.0)
Diagnosis, *n* (%)[a]		
Sjogren's syndrome	12 (54.5)	13 (52.0)
Non-Sjogren's DED	6 (27.2)	8 (32.0)
Ocular GVHD	4 (18.1)	4 (16.0)
OSDI, median [IQR]	49.0 [36.6–72.9]	50.0 [32.5–62.5]
Corneal staining, median [IQR][a]		
OD	5.00 [4.00–8.00]	5.00 [3.00–7.00]
OS	5.00 [4.00–8.00]	5.00 [3.00–6.00]
Conjunctival staining, median [IQR][a]		
OD	4.00 [3.00–6.00]	4.00 [2.00–4.00]
OS	4.00 [2.00–5.00]	4.00 [2.00–4.00]
Conjunctival injection, median [IQR]		
Temporal OD	45.0 [40.0–60.0]	40.0 [30.0–60.0]
Temporal OS	45.0 [40.0–60.0]	40.0 [40.0–60.0]
Nasal OD	40.0 [40.0–57.5]	40.0 [30.0–50.0]
Nasal OS	45.0 [40.0–60.0]	40.0 [30.0–50.0]
Schirmer I, median [IQR]		
OD	0.75 [0.00–1.00]	2.00 [0.00–4.00]
OS	0.25 [0.00–1.75]	2.00 [0.00–5.00]
Corneal filaments, *n* (%)		
OD	4 (18.2)	0 (0.00)
OS	2 (9.1)	1 (4.0)
Mucoid debris strands, *n* (%)		
OD	17 (77.3)	20 (80.0)
OS	19 (86.4)	16 (64.0)
Tolerability OU, *n* (%)[a]		
90	0 (0.00)	2 (8.00)
100	21 (100)	23 (92.0)
Intraocular pressure (IOP), median [IQR]		
OD	18.0 [16.0–20.0]	17.5 [15.0–20.0]
OS	18.0 [15.5–20.0]	18.5 [16.0–22.0]

[a] Total $N = 46$, due to missing data for one patient.

Table 2. OD and OS Characteristics at Baseline Within Groups

	Placebo, Median [IQR]		DNase I, Median [IQR]	
	OD ($N = 22$)	OS ($N = 22$)	OD ($N = 25$)	OS ($N = 25$)
Corneal Staining[a]	5.00 [4.00–8.00]	5.00 [4.00–8.00]	5.00 [3.00–7.00]	5.00 [3.00–6.00]
Conjunctival staining	4.00 [3.00–6.00]	4.00 [2.00–5.00]	4.00 [2.00–4.00]	4.00 [2.00–4.00]
Conjunctival injection (T^1)	45.0 [40.0–60.0]	45.0 [40.0–60.0]	40.0 [30.0–60.0]	40.0 [40.0–60.0]
Conjunctival injection (N^2)	40.0 [40.0–57.5]	45.0 [40.0–60.0]	40.0 [30.0–50.0]	40.0 [30.0–50.0]
Schirmer I	0.75 [0.00–1.00]	0.25 [0.00–1.75]	2.00 [0.00–4.00]	2.00 [0.00–5.00]
Mucoid debris/strands	1.00 [1.00–2.00]	1.00 [1.00–2.00]	2.00 [1.00–3.00]	1.00 [0.00–2.00]
Intraocular pressure (IOP)	18.0 [16.0–20.0]	18.0 [15.5–20.0]	17.5 [15.0–20.0]	18.5 [16.0–22.0]

[a] $N = 21$ for placebo group, due to missing data for one patient.

Table 3. Comparison of Outcome Measures Within Placebo Group

	OD (Baseline vs. Week 8)		
	Baseline ($N = 22$), Median [IQR]	Week 8 ($N = 20$), Median [IQR]	*P* Value[a]
Corneal staining ($N = 41$)	5.00 [4.00–8.00]	6.00 [4.00–8.00]	0.854
Conjunctival staining ($N = 41$)	4.00 [3.00–6.00]	4.00 [3.75–5.00]	0.912
Conjunctival injection (T^1) ($N = 42$)	45.0 [40.0–60.0]	40.0 [40.0–52.5]	0.605
Conjunctival injection (N^2) ($N = 42$)	40.0 [40.0–57.5]	40.0 [40.0–60.0]	0.531
Schirmer I ($N = 42$)	0.75 [0.00–1.00]	0.25 [0.00–1.25]	0.788
Mucoid debris/strands ($N = 42$)	1.00 [1.00–2.00]	2.00 [0.75–2.25]	0.390
SGA DED ($N = 20$)	—	1.00 [0.75–2.00]	—
SGA mucous ($N = 20$)	—	0.50 [0.00–2.00]	—
CGI ($N = 20$)	—	0.00 [0.00–1.00]	—
OSDI[b] ($N = 42$)	49.0 [36.6–72.9]	35.1 [10.6–54.1]	0.074
Intraocular pressure ($N = 40$)	18.0 [16.0–20.0]	18.0 [16.0–20.0]	0.924

T^1, Temporal; N^2, Nasal.
[a] Kruskal-Wallis test. Comparison between baseline and week 8.
[b] Same value for OD/OS.

Table 3. Extended

	OS (Baseline vs. Week 8)		
	Baseline ($N = 22$), Median [IQR]	Week 8 ($N = 20$), Median [IQR]	*P* Value[a]
Corneal staining ($N = 41$)	5.00 [4.00–8.00]	5.00 [4.00–7.25]	0.812
Conjunctival staining ($N = 41$)	4.00 [2.00–5.00]	4.00 [3.00–4.25]	0.572
Conjunctival injection (T^1) ($N = 42$)	45.0 [40.0–60.0]	40.0 [40.0–60.0]	0.751
Conjunctival injection (N^2) ($N = 42$)	45.0 [40.0–60.0]	40.0 [40.0–60.0]	0.885
Schirmer I ($N = 42$)	0.25 [0.00–1.75]	0.00 [0.00–1.00]	0.244
Mucoid debris/strands ($N = 42$)	1.00 [1.00–2.00]	2.00 [0.75–2.00]	0.958
SGA DED ($N = 20$)	—	1.00 [0.00–2.00]	—
SGA mucous ($N = 20$)	—	0.50 [0.00–2.00]	—
CGI ($N = 20$)	—	0.00 [0.00–1.00]	—
OSDI[b] ($N = 42$)	49.0 [36.6–72.9]	35.1 [10.6–54.1]	0.074
Intraocular pressure ($N = 40$)	18.0 [15.5–20.0]	18.0 [16.2–20.0]	0.978

Table 4. Comparison of Outcome Measures Within DNase I Group

	OD (Baseline vs. Week 8)		
	Baseline ($N = 25$), Median [IQR]	Week 8 ($N = 21$), Median [IQR]	*P* Value[a]
Corneal staining ($N = 46$)	5.00 [3.00–7.00]	3.00 [2.00–5.00]	0.025*
Conjunctival staining ($N = 46$)	4.00 [2.00–4.00]	3.00 [2.00–5.00]	0.679
Conjunctival injection (T^1) ($N = 46$)	40.0 [30.0–60.0]	40.0 [30.0–50.0]	0.778
Conjunctival injection (N^2) ($N = 46$)	40.0 [30.0–50.0]	40.0 [30.0–50.0]	0.586
Schirmer I ($N = 46$)	2.00 [0.00–4.00]	3.00 [1.00–4.00]	0.568
Mucoid debris/strands ($N = 46$)	2.00 [1.00–3.00]	0.00 [0.00–1.00]	0.001*
SGA DED ($N = 21$)	—	2.00 [1.00–2.00]	—
SGA mucous ($N = 21$)	—	1.00 [0.00–2.00]	—
CGI ($N = 21$)	—	2.00 [1.00–2.00]	—
OSDI[b] ($N = 46$)	50.0 [32.5–62.5]	22.7 [17.5–45.0]	0.001*
Intraocular pressure ($N = 39$)	17.5 [15.0–20.0]	17.2 [14.6–19.0]	0.582

[a] Kruskal-Wallis test. Comparison between baseline and week 8.
[b] Same value for OD/OS.

Table 4. Extended

	OS (Baseline vs. Week 8)		
	Baseline ($N = 25$), Median [IQR]	Week 8 ($N = 21$), Median [IQR]	*P* Value[a]
Corneal staining ($N = 46$)	5.00 [3.00–6.00]	3.00 [2.00–5.00]	0.039*
Conjunctival staining ($N = 46$)	4.00 [2.00–4.00]	3.00 [2.00–5.00]	0.893
Conjunctival injection (T^1) ($N = 46$)	40.0 [40.0–60.0]	40.0 [30.0–50.0]	0.822
Conjunctival injection (N^2) ($N = 46$)	40.0 [30.0–50.0]	40.0 [30.0–50.0]	0.964
Schirmer I ($N = 46$)	2.00 [0.00–5.00]	3.00 [0.00–5.00]	0.920
Mucoid debris/strands ($N = 46$)	1.00 [0.00–2.00]	0.00 [0.00–1.00]	0.084
SGA DED ($N = 21$)	—	2.00 [1.00–2.00]	—
SGA mucous ($N = 21$)	—	1.00 [0.00–2.00]	—
CGI ($N = 21$)	—	2.00 [1.00–2.00]	—
OSDI[b] ($N = 46$)	50.0 [32.5–62.5]	22.7 [17.5–45.0]	0.001*
Intraocular pressure ($N = 39$)	18.5 [16.0–22.0]	18.0 [16.6–20.1]	0.621

strands (week 8 minus baseline) was also significantly greater in the DNase group as compared with the placebo group for OD as well as OS. The CGI score in the DNase group was also significantly greater as compared with the placebo group, suggesting greater overall clinical improvement in the DNase group. The median reduction in OSDI (week 8 minus baseline) was greater in the DNase group (−20.75) as compared with the placebo group (−8.43), the change in DNase group being more than MCID, and the difference between DNase and placebo group showed a borderline significance.

The intent-to-treat analysis showed that the DNase group had a significantly lower median of corneal staining (1.95 units; *P*-value <0.001) from baseline to week 8 compared with placebo group (Table 6). Thus, the intent-to-treat result also shows the corneal staining in the same direction as what we observed from the available data analysis.

The safety of drug treatment was assessed by tolerability to drug or placebo application and presence of adverse events during the 8-week treatment duration. Comparison of tolerability between the placebo and DNase group showed no differences

Table 5. Comparison of Change (Week 8–Baseline) in Outcome Measures Between Groups

	Placebo (OD) vs. DNase I (OD)		
	OD Placebo ($N = 20$), Median [IQR]	OD DNase I ($N = 21$), Median [IQR]	*P* Value[a]
Corneal staining	0.00 [−0.25 to 1.00]	−1.00 [−3.00 to −1.00]	0.001*
Conjunctival staining	0.00 [0.00 to 1.00]	0.00 [0.00 to 0.00]	0.461
Conjunctival injection (T^1)	0.00 [−10.00 to 0.00]	−10.00 [−10.00 to 10.0]	0.409
Conjunctival injection (N^2)	5.00 [−10.00 to 10.0]	0.00 [−10.00 to 0.00]	0.057
Schirmer I	0.00 [0.00 to 0.25]	0.00 [0.00 to 0.00]	0.921
Mucoid Debris/strands	0.00 [0.00 to 1.00]	−1.00 [−2.00 to 0.00]	<0.001*
SGA DED	1.00 [0.75 to 2.00]	2.00 [1.00 to 2.00]	0.060
SGA mucous	0.50 [0.00 to 2.00]	1.00 [0.00 to 2.00]	0.220
CGI	0.00 [0.00 to 1.00]	2.00 [1.00 to 2.00]	<0.001*
OSDI[b]	−8.43 [−25.00 to −0.70]	−20.75 [−37.00 to −11.80]	0.078
Intraocular pressure (IOP)	−0.50 [−2.75 to 2.00]	0.50 [−3.00 to 1.50]	0.924

[a] Kruskal-Wallis test.
[b] Same value for OD/OS.

Table 5. Extended

	Placebo (OS) vs. DNase I (OS)		
	OS Placebo ($N = 20$), Median [IQR]	OS DNase I ($N = 21$), Median [IQR]	*P* Value[a]
Corneal staining	0.00 [0.00 to 1.00]	−1.00 [−3.00;−1.00]	<0.001*
Conjunctival staining	0.00 [0.00 to 1.00]	0.00 [0.00 to 1.00]	0.877
Conjunctival injection (T^1)	0.00 [−10.00 to 10.0]	0.00 [−10.00 to 10.0]	0.515
Conjunctival injection (N^2)	0.00 [−2.50 to 10.0]	0.00 [−10.00 to 0.00]	0.332
Schirmer I	0.00 [−0.12 to 0.00]	0.00 [−1.00 to 0.00]	0.605
Mucoid Debris/strands	0.00 [0.00 to 0.25]	0.00 [−1.00 to 0.00]	0.032*
SGA DED	1.00 [0.00 to 2.00]	2.00 [1.00 to 2.00]	0.126
SGA mucous	0.50 [0.00 to 2.00]	1.00 [0.00 to 2.00]	0.220
CGI	0.00 [0.00 to 1.00]	2.00 [1.00 to 2.00]	<0.001*
OSDI[b]	−8.43 [−25.00 to −0.70]	−20.75 [−37.00 to −11.80]	0.078
Intraocular pressure (IOP)	0.50 [−2.50 to 1.25]	0.00 [−3.50 to 2.00]	0.874

(Table 7). Overall, the median tolerability was 100% for both groups. The presence of adverse events (any adverse event or specific adverse events) were similar for placebo and DNase groups. Adverse events were reported by 57.1% of patients in the placebo group and 36% of patients in the DNase group (Table 8). In the DNase group, the most common adverse events were burning (in 20% of patients) and grittiness (16%). Adverse events in both groups were transient and self-limited. No serious ocular or systemic adverse events attributable to treatment occurred during the study, and there were no adverse events suggestive of ocular infection. The safety of drug treatment was also assessed by an adverse change in the clinical examination. In both groups, there were no instances of anterior chamber inflammation (cells or flare) and development or progression of cataract. The median intraocular pressure was similar at baseline between the two groups, and within each group the change in intraocular pressure (week 8 minus baseline) was not significant.

Discussion

This is the first clinical trial in humans using DNase eye drops for treating ocular diseases. Since

Table 6. The Results of Quantile Linear Mixed Effect Model at Median for Corneal Staining

Variable	Estimated Coefficient	Standard Error	95% Confident Interval	*P* Value
Intercept	5.53	0.67	(4.19 to 6.87)	<0.0001
Randomization: DNase vs. Placebo	−0.53	0.82	(−2.17 to 1.11)	0.5194
Time: Week 8 vs. Baseline	0.18	0.25	(−0.32 to 0.67)	0.4706
Interaction: Randomization × Time	−1.95	0.35	(−2.66 to −1.23)	<0.0001

there are no data regarding the use of DNase application to the human eye (other than a published case report),[10] we performed this "investigator initiated" clinical trial to assess the safety and the therapeutic potential of DNase in treating DED. The main findings of this clinical trial are that application of DNase eye drops 0.1% four times a day for 8 weeks results in a significant and clinically meaningful reduction in corneal staining as compared with the placebo treatment. The mucoid debris/strands are also reduced significantly with DNase eye drop treatment as compared with the placebo treatment. The OSDI shows clinically meaningful reduction with DNase eye drop treatment; however, the reduction in OSDI with DNase showed only borderline statistical significance as compared with placebo. Taken together, these data suggest that the severity of the ocular surface disease may reduce with DNase eye drop treatment and the patients may become less symptomatic; thus, providing the scientific justification for proceeding with larger adequately powered clinical trials.

Since there were no differences in adverse events between DNase eye drop and placebo, and there were no serious adverse events or ocular infections, data from this clinical trial do not uncover any safety-related concerns for conducting future clinical trials. Our data also suggest that in the current preservative free formulation (Pulmozyme) DNase eye drops are well tolerated. Given the high patient retention (87%) and adherence to treatment (100%) in this trial, future clinical trials are expected to be feasible. The most frequent adverse event in the DNase group, reported in 20% of patients, was transient burning sensation upon instillation of the eye drop. Since we used the marketed formulation of Pulmozyme, which has a nominal pH of 6.3, it is likely that once an ophthalmic preparation with physiological pH is formulated, the burning sensation may be reduced.

The outcomes of this pilot clinical trial should not be over interpreted for potential benefit because an accurate assessment of therapeutic implications of DNase eye drops will only be possible after adequately powered definitive pivotal trials. Our goal in this phase I/II clinical trial was to assess the safety and preliminary efficacy. One limitation of our study is the small sample size. One practical reason for slow recruitment and small sample size in this clinical trial was that the inclusion criteria necessitated discontinuation of anti-inflammatory eye drops (cyclosporine and steroids). A previous DED clinical trial to assess efficacy of anti-inflammatory eye drops has also used the approach of discontinuing ongoing topical anti-inflammatory treatments.[20] Our experience in this clinical trial shows that patients with symptomatic moderate to severe DED are generally on anti-inflammatory eye drops and are unwilling to discontinue them for participating in this trial. To overcome this hurdle, there has been an interest to perform "real

Table 7. Comparison of Tolerability Between Groups

	OD (Placebo) vs. OD (DNase I)			OS (Placebo) vs. OS (DNase I)		
	OD Placebo ($N = 21$)	OD DNase I ($N = 25$)	*P* Value[a]	OS Placebo ($N = 21$)	OS DNase I ($N = 25$)	*P* Value[a]
Baseline ($N = 46$)	100 [100–100]	100 [100–100]	0.190	100 [100–100]	100 [100–100]	0.190
Week 2 ($N = 45$)	100 [100–100]	100 [100–100]	0.719	100 [100–100]	100 [100–100]	0.719
Week 4 ($N = 43$)	100 [97.5–100]	100 [100–100]	0.282	100 [97.5–100]	100 [100–100]	0.504
Week 6 ($N = 40$)	100 [97.5–100]	100 [100–100]	0.067	100 [97.5–100]	100 [100–100]	0.067
Week 8 ($N = 41$)	100 [100–100]	100 [100–100]	0.850	100 [100–100]	100 [100–100]	0.850

[a] Kruskal-Wallis test.

Table 8. Comparison of Adverse Events Between Groups

	Placebo ($N = 21$), *n* (%)	DNase I ($N = 25$), *n* (%)	*P* Value[a]
Any adverse event	12 (57.1)	9 (36.0)	0.256
Grittiness	2 (9.52)	4 (16.0)	0.673
Blurred vision	3 (14.3)	3 (12.0)	1.000
Light sensitivity	1 (4.76)	1 (4.00)	1.000
Redness	3 (14.3)	1 (4.00)	0.318
Dryness	3 (14.3)	0 (0.00)	0.088
Itching	4 (19.0)	1 (4.00)	0.163
Soreness	3 (14.3)	0 (0.00)	0.088
Burning	2 (9.52)	5 (20.0)	0.428

[a] Fisher's exact test or χ^2 test.

world" trials where all ongoing treatments, including anti-inflammatory treatments, are allowed. One such real world clinical trial is the Dry Eye Assessment and Management (DREAM) study, which reported that patients who use omega-3 supplements despite their current treatments do not have significantly better outcomes than those who receive placebo.[21] Such real world trials, however, can only provide data regarding the "additive" benefit of the test drug, not whether the test drug, in of itself, has a beneficial effect.

We have previously reported that abundant NETs are present over the ocular surface of patients with several tear deficient DED subtypes (Sjogren's syndrome, ocular GVHD, and Ocular Cicatricial Pemphigoid [OCP]).[3] There are two possible reasons for buildup of NETs on the ocular surface of severe DED subtypes: (1) tear fluid hyperosmolarity in these patients may have enhanced the formation of NETs[9]; and/or (2) lack of nuclease due to tear fluid deficiency may have reduced clearance of NETs.[3] Both Sjogren's syndrome and ocular GVHD patients have hyperosmolar tears and tear deficiency,[22] features that favor accumulation of NETs and consequent inflammation.[3,9] Abnormal regulation of NETs (excessive NETosis and deficient nucleases) has been suggested to play a role in other inflammatory conditions as well (e.g., in the pathogenesis of dermatomyositis and polymyositis).[23] Excessive DNA and NETs can cause inflammation; however, eDNA must re-enter a cell and bind its intracellular receptor to stimulate downstream signaling pathways.[24,25] Cathelicidin, an antimicrobial peptide that is a molecular component of NETs, binds eDNA and enhances its intracellular entry.[26] Cathelicidin is localized within neutrophils and NETs on the ocular surface of patients with severe DED, particularly within the mucoid films. Once inside the cell, DNA binds TLR9 to stimulate signaling through MyD88, which initiates a signaling cascade leading to an IFN-type I response.[27,28] Thus, based on our findings and those of others, we have proposed a mechanism for inflammation in severe DED. eDNA and NETs in tear fluid bind cathelicidin and re-enter ocular surface cells to stimulate the TLR9-MyD88 pathway and activate the IFN type I response. The finding in this clinical trial that application of DNase eye drops in DED patients results in a significant reduction in corneal staining builds upon our previous finding that tear fluid eDNA abundance correlates best with corneal staining ($r = 0.55$) and weakly with the Schirmer I test ($r = -0.39$) and OSDI score ($r = 0.35$).[10] Taken together, the implication is that inflammation induced by eDNA may have caused corneal epitheliopathy (which is clinically detected as corneal staining), and degradation of eDNA with DNase eye drops may have reduced eDNA-induced inflammation and consequent corneal epitheliopathy, thus reducing corneal staining. It is also possible that ocular surface disease results from unintended but detrimental bystander damage resulting from epithelial toxicity due to molecular components of NETs. Histones can cause direct cytotoxicity to epithelial cells.[29] Extracellular histones are major mediators of cell death in sepsis.[30] Cathelicidin peptide fragments can cause inflammation, erythema, and telangiectasia, particularly in patients with rosacea.[31] Neutrophil elastase induces epithelial cell apoptosis.[32] Since the molecular components of NETs are decorated on the eDNA backbone, degradation of this eDNA backbone by DNase eye drops may have released these molecules, facilitating their subsequent removal from the ocular surface (e.g., flushing with artificial tears).

Our data suggest that from a therapeutic perspective, reducing the abundance of NETs over the ocular surface is likely to benefit tear-deficient DED subtypes, despite different etiologies. Our data show that the beneficial response to DNase was similar in all subgroups (Sjogren's syndrome, non-Sjogren's DED, and ocular GVHD). However, because NETosis increases exponentially with increasing osmolarity,[9] the contribution of NETs to ocular surface disease is likely to be more relevant in moderate to severe DED (associated with greater hyperosmolarity), as opposed to mild dry eye. Therefore, use of DNase eye drops is also likely to be most relevant to patients with moderate to severe tear deficient DED.

Our data also showed that mucoid debris/strands are reduced significantly with DNase eye drops. We have previously shown that the mucoid debris/strands are composed of exfoliated epithelial cells, neutrophils and NETs, and their molecular components.[3] Therefore, mucoid debris/strands may represent repositories of inflammatory materials over the eye surface. By degrading and clearing them, DNase eye drops may lower this inflammatory material load over the ocular surface. The beneficial mucolytic and NETs degrading effects of DNase are well established for reducing inflammation in cystic fibrosis patients.[33,34]

In summary, data generated in this clinical trial show that DNase eye drops appear to be safe and potentially beneficial in treating severe tear deficient DED. The results of this clinical trial support the further development of DNase eye drops for treating DED.

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