CLINICAL STUDY PROTOCOL

A Phase 1/2a, Randomized, Double-Masked, Vehicle-Controlled Study Evaluating the Safety and Exploratory Activity of Two Concentrations (0.1% and 0.4%) of ALY688 Ophthalmic Solution in Subjects with Dry Eye Disease

Study Phase: 1/2a

Product Name:
ALY688 Ophthalmic Solution

IND Number:
134,869

Formulation:
ALY688 Ophthalmic Solution, 0.1% and 0.4%

Study Number:
ALY688-201

NCT #:
NCT04201574

Date:
June 08, 2020

1. SYNOPSIS

Investigational Drug Product:	ALY688 Ophthalmic Solution
Active Ingredient:	ALY688
Study Title:	A Phase 1/2a, Randomized, Double-Masked, Vehicle-Controlled Study Evaluating the Safety and Exploratory Activity of Two Concentrations (0.1% and 0.4%) of ALY688 Ophthalmic Solution in Subjects with Dry Eye Disease
Study Number:	ALY688-201
Study Phase:	1/2a
Study Objectives:	Primary objective: Evaluate the safety and tolerability of 2 concentrations of ALY688 in subjects with dry eye disease (DED) treated topically twice daily (BID) for 8 weeks. Additional objective: Evaluate the efficacy of 2 concentrations of ALY688 in subjects with DED treated BID for 8 weeks.
Study Design:	After meeting eligibility criteria during screening, qualified subjects will undergo a 14-day run-in period during which they will use Vehicle BID bilaterally. If they meet eligibility criteria at baseline, they will be randomized 1:1:1 to receive ALY688 0.1% (n=40), ALY688 0.4% (n=40), or Vehicle (n=40). Dosing will be BID bilaterally. If there are no clinically significant drug-related adverse tolerability changes on Day 3, subjects will continue dosing BID bilaterally for the remaining dosing period for a total of 8 weeks. The study eye will be designated at randomization. If both eyes have an inferior corneal fluorescein staining score (ICSS) ≥2, the study eye will be the one with the greater ICSS. If both eyes have the same ICSS grade, the eye with the higher overall total corneal staining score will be selected. If both eyes show the same total corneal staining score, the eye with the lower tear volume by Schirmer's test will be selected; if both eyes still score equally, the right eye will be selected. Ocular assessments should be conducted by the same assessor throughout a subject's participation period to reduce observer variability. All efficacy assessments will be carried out consistently either in the morning or the afternoon (preferred time window is ±2 hours) throughout all study visits to minimize intra-subject diurnal variability. During the Baseline visit, all assessments will be carried out before dosing with the study medication in order to serve as pre-study baseline. For on-treatment visits at Weeks 2, 4, and 8, all ocular assessments will take place at least 1 hour after receiving study medication. At Weeks 2, 4, and 8, subjects who arrive in clinic before 1 pm should receive their morning dose administered by clinic staff. Subjects who arrive in clinic after 1 pm should self-administer their morning dose and receive their afternoon/evening dose by clinic staff. A subset of subjects (up to 36) at selected sites will undergo blood collection for pharmacokinetic evaluation of the test article at 3 timepoints (15 and 45 minutes and 3 hours) after final dose of study medication at Week 8.
Investigational Product, Dose and Mode of Administration:	Topical, sterile, non-preserved single-use vials of 0.25 mL ocular eye drop: • ALY688 Ophthalmic Solution 0.1% • ALY688 Ophthalmic Solution 0.4% • Vehicle Ophthalmic Solution (same as ALY688 without active drug)

Study Population:	Subjects with symptomatic DED confirmed by clinical examination
Inclusion Criteria:	1. Males or females who are at least 18 years of age at the Screening visit 2. Subject-reported history of dry eye in both eyes for at least 3 months prior to screening 3. Confirmed use, by either medical records or subject self-reporting, of artificial tear substitute for symptoms of DED ≥3 times per week on average for the 4 weeks prior to screening (including regular or as needed use of prescription or over-the-counter products) 4. Willing to suspend use of artificial tear substitutes for the duration of study participation 5. Eye dryness score (EDS) using 0- to 100-point visual analog scale (VAS) in both eyes between 40 and 90 (inclusive) at screening and between 30 and 90 (inclusive) at baseline 6. One eye (same eye) must meet all of the following criteria at both screening and baseline: a. Total corneal fluorescein staining score between 6 and 17 (inclusive) using the Expanded National Eye Institute (NEI) scoring system (0-20 scale) b. Inferior corneal fluorescein staining score (ICSS) ≥2 (0-4 scale) c. Schirmer's test (without anesthesia) score between 1 and 9 mm/5 min (inclusive) 7. Best-corrected visual acuity (BCVA) of +0.6 logarithm of the minimum angle of resolution (logMAR) or better (using Early Treatment Diabetic Retinopathy Study [ETDRS]); Snellen equivalent score of 20/80 or better) and reading Snellen visual acuity of 20/40 (J3) or better in each eye at screening and baseline 8. An understanding, ability, and willingness to fully comply with study procedures and restrictions 9. Ability to voluntarily provide written, signed, and dated (personally or via a legally authorized representative) informed consent to participate in the study 10. For females of childbearing potential, confirmation of a negative urine pregnancy test at screening and baseline and willing to comply with applicable contraceptive requirements detailed in the protocol
Exclusion Criteria:	***Ocular history – medical***

	1. Confluent (grade 4) fluorescein corneal staining in the superior zone in either eye at screening or baseline 2. Confluent (grade 4) fluorescein corneal staining in 3 or more zones in either eye at screening or baseline 3. Improvement in EDS by more than 25% between screening and baseline (run-in period) 4. Allergic conjunctivitis if active within 4 weeks prior to screening or anticipated to be active during the study 5. Severe meibomian gland dysfunction (MGD) using the Simplified Scheme for Grading MGD (based on central 10 gland orifices of each eyelid) with a score of Grade 3 or greater in the study eye evaluating both the upper and lower eyelids. The subject will be excluded if <u>any</u> of the following are present in either of the evaluated eyelids of the study eye: a. Plugging of meibomian gland: more than 2/3 of glands plugged or >6 glands plugged in either eyelid of the study eye b. Character of secretion: paste or no observable expressate in either eyelid of the study eye c. Expressibility: fewer than 2 glands expressible in either eyelid of the study eye NOTE: Mild or moderate (Grade 1 or 2) posterior lid margin disease (MGD) is allowed if the subject has not received topical or systemic treatment (eg, tetracycline, doxycycline, or azithromycin) or mechanical therapy such as lid scrubs, for at least 4 weeks prior to screening and will not receive any such therapy during the study 6. Glaucoma or ocular hypertension requiring treatment within 6 months of screening 7. Use of an intranasal tear stimulant (device or medication) within 4 weeks prior to screening or planned use during the study 8. Unwillingness to avoid wearing contact lenses starting at least 2 weeks prior to screening and during the study 9. DED secondary to scarring (eg, associated with irradiation, alkali burns, Stevens-Johnson syndrome, cicatricial pemphigoid) or destruction of conjunctival goblet cells (as with vitamin A deficiency) 10. Any other clinically significant ocular condition in the study eye that, in the investigator's judgment, is of a severity that adversely affects vision and/or could affect the results of study assessments, including but not limited to: eyelid margin abnormalities (eg, anterior blepharitis, excessive lid laxity, floppy eyelid syndrome, ectropion, entropion), retinal disease (eg, proliferative diabetic retinal disease [minimal early non-proliferative diabetic retinopathy is permitted], age-related macular degeneration [AMD; mild AMD untreated by intravitreal injection is permitted], or retinal vein occlusion), lagophthalmos, exposure keratitis, limbic keratoconjunctivitis, or active ocular or eyelid inflammation, including uveitis 11. Corneal stromal and/or epithelial defects or anatomic abnormalities that, in the investigator's judgment, adversely affect vision and/or could affect the results of study assessment not due to DED (eg, neurotrophic keratitis recurrent corneal erosions, and filaments) 12. Currently active, or history of, ocular herpes or any other ocular infection within 4 weeks prior to screening (with the exception of mild or moderate

	MGD—see above) or if on medication to suppress herpes simplex virus (HSV) 13. Ocular trauma within 6 months prior to screening that, in the investigator's judgment, is of a severity that could affect results of study assessments ***Ocular history – surgical*** 14. Have undergone laser in situ keratomileusis (LASIK) or other types of refractive surgery within 12 months prior to screening, or have persistent symptoms associated with LASIK surgery (neurotrophic etiology) 15. Have undergone penetrating keratoplasty, Descemet's stripping with endothelial keratoplasty, Descemet's membrane endothelial keratoplasty, or deep anterior lamellar keratoplasty in the study eye 16. Have a dissolvable punctal plug placed in the study eye within 3 months prior to screening 17. Have a non-dissolvable punctal plug placed in the study eye within 2 months prior to screening that is, in the investigator's judgment, considered to be unstable, irritating, likely to fall out (based on prior history of a lost plug), or cannot be replaced immediately if it falls out during the study 18. Have any planned ocular and/or lid surgeries during the study, including placement of new punctal plugs (replacement of non-dissolvable plugs that have fallen out is required) 19. Punctal cauterization within 12 weeks prior to screening 20. Prior ocular surgery or laser treatment within 6 months prior to screening NOTE: Glaucoma laser procedures are permitted up to 3 months prior to screening; yttrium aluminum garnet (YAG) capsulotomy is permitted up to 4 weeks prior to screening 21. Eyelid surgery within 12 months prior to screening ***Prior medications*** 22. Any use within 4 weeks prior to screening of any ocular topical medications (excluding artificial tear substitutes) 23. Planned use of any ocular topical medications during the study (including artificial tear substitutes) 24. Any use within 4 weeks prior to screening or planned use during the study of any oral antibiotics (including tetracycline, doxycycline, or azithromycin) for the treatment of blepharitis or MGD 25. Use of the following therapies prior to or during the study: a. Topical brimonidine within 4 weeks prior to screening b. Topical cyclosporine within 6 weeks prior to screening c. Topical lifitegrast within 4 weeks prior to screening d. Any other topical ocular dry eye treatments 4 weeks prior to screening, with the exception of artificial tear substitutes e. The following treatments for MGD within 4 weeks prior to screening: thermal pulsation (Lipiflow), debridement of lid margin (BlephEx), or thermal application (MeiBoFlo, Tear Care) f. Any systemic (including oral) antibiotic 2 weeks prior to screening

	g. Initiation of oral omega fatty acid supplements within 3 months prior to screening or anticipated changes during the study h. Any use within 2 months prior to screening or planned use during the study of topical retinoids, such as retinol, tretinoin, adapalene, tazarotene, alitretinoin, and bexarotene i. Initiation within 4 weeks prior to screening of any medication (oral or topical) known to cause ocular drying or any anticipated change in dosing of such medication during the study j. Oral aspirin or aspirin-containing products or nonsteroidal anti-inflammatory drugs (NSAIDs) unless the subject is on a stable dose for 4 weeks prior to screening with no dose change anticipated during the study NOTE: Use of aspirin or NSAIDs on an as needed basis is allowed if it is no more than 3 doses per week; acetaminophen is allowed k. Systemic, inhalation, intranasal, or topical ocular corticosteroids within 4 weeks prior to screening and during the study. Dermatologic formulations are allowed l. Antihistamines (oral or ocular) within 4 weeks prior to screening or planned use during study participation m. Systemic immunosuppressive medications within 4 weeks prior to screening and during the study ***Product related*** 26. Known hypersensitivity to investigational product or its components ***Systemically related*** 27. Any known history of alcohol or drug abuse within 12 months prior to screening that, in the investigator's judgment, may interfere with the subject's participation in the study 28. Positive urine pregnancy test or nursing an infant (female subjects only) at screening or baseline 29. Any blood donation or significant loss of blood within 4 weeks prior to screening or planned donation during the study 30. History of immunodeficiency disorder, human immunodeficiency virus, hepatitis B or C, or evidence of acute active hepatitis A or organ or bone marrow transplant 31. History or presence of Sjogren's syndrome treated with systemic immunosuppressants NOTE: mild to moderate Sjogren's is permitted if untreated with medications within 3 months prior to screening and no planned treatment during the trial 32. Any other significant physical or mental illness or condition that, in the investigator's judgment, could interfere with study parameters, including but not limited to: poorly controlled diabetes or hypertension, active inflammatory or infectious condition, unstable cardiac disease, or active malignancy 33. Planned surgical procedure(s) during the study that, in the investigator's judgment, may interfere with the subject's participation in the study 34. Clinically significant abnormalities in laboratory tests at screening based upon the investigator's judgment

	Administrative 35. Are employees, or immediate family members of employees, at the investigational site 36. Are members of the same household 37. Are unable or unwilling to follow instructions and participate in all study assessments and visits 38. Less than 80% compliance with BID bilateral Vehicle dosing during the 14-day run-in period (as determined by counting returned single-use vials at baseline) 39. Have used any investigational product or device within 4 weeks prior to screening or planned use during the study
Description of Study Sites:	Up to 150 subjects are planned to be enrolled across up to 12 sites throughout the United States.
Individual Subject Participation:	Vehicle run-in period: 2 weeks Treatment period: 8 weeks Total: 10 weeks
Duration of Study:	Up to 4 months for recruiting plus 2 months for subject treatment, for a total of 6 months
Study Assessments:	The following study assessments and procedures will be performed: • Informed consent • Medical/Ophthalmic history • Concomitant medications • Vital signs (blood pressure [BP] and heart rate [HR]) • EDS and ocular discomfort score (ODS) assessments by VAS • Symptom Assessment in Dry Eye (SAnDE) score assessment • Additional dry eye symptom assessment by VAS • BCVA assessment • Reading speed assessment • Slit lamp biomicroscopy • Conjunctival hyperemia grading • Tear film break-up time (TFBUT) • Corneal fluorescein staining • Conjunctival lissamine green staining • Schirmer's test (without anesthesia); strips to be retained for inflammatory marker analysis • Intraocular pressure (IOP) • Impression cytology • Multidimensional Psychological Questionnaire • Blood collection for laboratory testing, and pharmacokinetic evaluation in a subset of subjects • Urine pregnancy test (if applicable) • Urinalysis • Dilated fundus exam • Collect study drug and diary card

	• Compliance assessment • Inclusion/exclusion criteria assessment • Randomization • Study drug dispensing • Study drug administration (with post-instillation comfort VAS) • Adverse event assessment
Safety Endpoints:	To evaluate the safety and tolerability of ALY688 administered BID over 8 weeks compared with Vehicle, as assessed by ocular examinations (including any changes from baseline in slit lamp biomicroscopy, dilated fundus exam, visual acuity, IOP, and post-dosing comfort) and systemic evaluations (AEs, BP and HR, clinical laboratory test results, and pharmacokinetic results [in a subset of subjects]) in subjects with DED.
Efficacy Endpoints:	To evaluate the efficacy of ALY688 compared with Vehicle by the following measures: • Mean change from baseline at Weeks 2, 4, and 8 in total corneal fluorescein staining score using the NEI/Industry Workshop scale (0-20 scale). • Mean change from baseline at Weeks 2, 4, and 8 in EDS using a VAS (0-100 scale, both eyes) during the visit. NOTE: This will also be assessed by a daily EDS score card for the 7-day period prior to baseline and the Weeks 2, 4, and 8 visits. The mean of these scores for the 7 days prior to the Weeks 2, 4, and 8 visits will be compared with the mean of the scores for the 7 days prior to baseline. Additional exploratory endpoints: • Mean change from baseline at Weeks 2, 4, and 8 in ICSS (0-4 scale) using the Expanded NEI/Industry Workshop scale (5 zones with 0-4 scale/zone) • Mean change from baseline at Weeks 2, 4, and 8 in ODS using a VAS (0-100 scale, both eyes) o NOTE: This will be assessed in the same manner and at the same timepoints as the EDS score • Mean change from baseline at Weeks 2, 4, and 8 in SAnDE score (frequency and severity) based upon subject recollection for the week prior to visit • Mean change from baseline at Weeks 2, 4, and 8 in conjunctival lissamine green staining score using the NEI/Industry Workshop scale (6 zones with 0-4 scale/zone) • Mean change from baseline at Weeks 2, 4, and 8 in conjunctival hyperemia by clinical scoring (0-4 clinical grading scale using standardized grading photos) • Mean change from baseline at Weeks 2, 4, and 8 in TFBUT using fluorescein • Mean change from baseline at Week 2, 4 and 8 in tear volume as assessed by Schirmer's test (without anesthesia) • Mean change from baseline at Week 8 in the following components of the VAS dry eye symptom index: 1) burning/stinging, 2) itching, 3) foreign body sensation, 4) photophobia, and 5) pain. In addition,

	the symptom that each subject self-identifies at baseline as the most bothersome will be analyzed separately. • Mean change from baseline at Week 8 in reading speed as assessed by New International Reading Speed Texts (IReST) in subjects with adequate near visual acuity (BCVA of 20/40 or better) and appropriate language comprehension • Mean change from baseline at Week 8 in conjunctival goblet and lymphocyte cell densities as assessed by impression cytology • Mean change from baseline at Week 8 in selected inflammatory markers in tears from retained Schirmer's tear strips (post hoc analysis) o NOTE: Selected measures from those listed above will also be undertaken as part of the Screening visit to determine eligibility; refer to the Schedule of Events for specific evaluations
Analysis Populations:	Safety and mITT populations: All randomized subjects who receive at least 1 dose of investigational product Per protocol population: All randomized subjects who meet enrollment criteria and complete efficacy evaluations at week 4 or 8
Sample Size and Statistical Methods:	This is an exploratory, hypothesis-generating study. However, with 40 subjects per group (N=120), there is 80% power to detect an effect size of 0.63 with a 2-sided, two-sample t-test at an alpha level of 0.05 (uncorrected for multiplicity). A repeated measures analysis will be performed using the Wei-Lachin approach. In addition to repeated measures, the Week 8 change from baseline will be tested as a single timepoint measure. An aggregated efficacy measure (specific endpoints TBD) will be analyzed as a means to look for consistent trends and to increase the overall power. For example, an aggregate of ICSS, TFBUT, and Schirmer's test results may be analyzed. The test of the several endpoints involves a comparison of mean change from baseline across visits. A fixed effects longitudinal model will be utilized where the data of both eyes will be used for measures evaluated in both eyes with measures repeated across visits. Baseline score will be a covariate in the model. An unstructured correlation matrix will be utilized for correlation between eyes and across visits. Measures that do not involve both eyes will utilize an unstructured matrix to model the correlation across visits. The randomization will be stratified on study site to achieve a balance among treatment groups within a site. Each site will be preloaded with complete blocks. The site will dispense kits by Interactive Web Response System as subjects qualify for treatment assignment. To the extent possible, study drug resupply to sites will be done with complete block(s). Statistical details will be specified more fully in the Statistical Analysis Plan.