PROTOCOL ADX-2191-RP-001

AN OPEN-LABEL, PHASE II STUDY OF ADX-2191 IN SUBJECTS WITH RETINITIS PIGMENTOSA

PROTOCOL VERSION AND DATE: VERSION 1.1 / 7 JULY 2021
IND NUMBER: 155709

ALDEYRA THERAPEUTICS, INC.
131 HARTWELL AVENUE, SUITE 320
LEXINGTON, MA, 02421, U.S.A.

Sponsor Signature:

[redacted]

Conduct: In accordance with the ethical principles that originate from the Declaration of Helsinki and that are consistent with International Council for Harmonisation (ICH) guidelines on Good Clinical Practice (GCP) and regulatory requirements as applicable.

INVESTIGATOR STATEMENT

Protocol Number: ADX-2191-RP-001

Protocol Title: An open-label, phase II study of ADX-2191 in subjects with retinitis pigmentosa

I understand that all information concerning ADX-2191 in connection with this clinical trial and not previously published is confidential. This confidential information includes the Investigator's Brochure, Clinical Trial Protocol, Case Report Form, clinical methodology, and basic scientific data.

I will not initiate this clinical trial without approval from the Institutional Review Board (IRB)/Research Ethics Board (REB)/Independent Ethics Committee (IEC) and I understand that any changes in the protocol must be approved in writing by Aldeyra Therapeutics, Inc., and the Institutional Review Board/Ethics Committee before they can be implemented, except when necessary to eliminate immediate hazards to the subjects.

I will use only the informed consent form approved by Aldeyra Therapeutics, Inc. and by my IRB/REB/IEC and will fulfill all responsibilities for submitting pertinent information to the IRB/REB/IEC responsible for this clinical trial.

By my signature below, I attest that I have read, understand, and agree to abide by all the conditions, instructions, and restrictions contained in Protocol Number ADX-2191-RP-001, and will conduct the trial in accordance with the International Council for Harmonisation (ICH) guidelines on Good Clinical Practice (GCP) and applicable regulatory requirements.

_____
Site Name

_____
Site Address

_____
Investigator's Printed Name

_____ _____
Investigator's Signature Date

SUMMARY OF CHANGES

[redacted]

SYNOPSIS

<table>
<tr><td colspan="2">Name of Sponsor: Aldeyra Therapeutics Inc.</td><td>Investigational Product: ADX-2191 (0.8% intravitreal methotrexate)</td></tr>
<tr><td colspan="3">Title of Protocol: An open-label, phase II study of ADX-2191 in subjects with retinitis pigmentosa</td></tr>
<tr><td>Protocol Number:
ADX-2191-RP-001</td><td>Phase: Phase II</td><td>Indication for Use: Treatment of retinal dystrophy in patients with confirmed rhodopsin mutation</td></tr>
<tr><td colspan="3">Subject Population: Adult subjects with retinitis pigmentosa and confirmed rhodopsin gene mutation (including P23H)</td></tr>
<tr><td colspan="3">Number of Subjects: Approximately 8 (eight) evaluable adult patients</td></tr>
<tr><td colspan="3">Number of Centers: Up to three investigational sites in the United States</td></tr>
<tr><td colspan="3">Duration of Subject Participation: 16 weeks</td></tr>
<tr><td colspan="3">Investigational Product: ADX-2191 (0.8% intravitreal methotrexate) 400mcg in 0.05ml</td></tr>
<tr><td colspan="3">Overall Design: In this open-label Phase II clinical trial, 8 (eight) subjects with retinitis pigmentosa due to rhodopsin mutations (including P23H) will be identified and treated with serial intravitreal injections of ADX-2191 in the worse seeing eye. Ocular structure and function will be evaluated.

Retinitis pigmentosa (RP) is an inherited retinal disease that leads to a progressive degeneration of photoreceptors. Mutations within the rhodopsin gene account for approximately 25% of autosomal dominantly inherited RP cases. The mutation P23H (Substitution of Proline to Histidine at Codon 23 of the rhodopsin protein) is the most common mutation in the rhodopsin protein gene. The P23H mutation is associated with misfolded rhodopsin, which causes endoplasmic reticulum overload, activates the unfolded protein response and triggers apoptosis. Intravitreal methotrexate has been demonstrated in pre-clinical models of retinitis pigmentosa to promote clearance of misfolded rhodopsin without an effect on wild type rhodopsin protein and improves electroretinogram (ERG) responses in P23H mutant rodents. (Liu et al. (2020). Pharmacological clearance of misfolded rhodopsin for the treatment of RHO-associated retinitis pigmentosa. The FASEB Journal, 34(8), 10146–10167).

ADX-2191 is an intravitreal formulation of the active drug substance commonly known as methotrexate. The drug substance is formulated at a concentration of 400 µg/0.05 ml in a sterile, aqueous buffered solution designed for intravitreal delivery. The drug product is supplied in single-dose, 2 mL glass vials with a 0.5 mL fill volume.

In this open label study, the first four subjects (Cohort A) will be treated with monthly intravitreal injections of ADX-2191 for a total of three injections; the last four subjects (Cohort B) will be treated with twice monthly intravitreal injections of ADX-2191 for a total of six injections. All subjects will be followed for a total of 16 weeks following dosing.

Clinical trial procedures to be conducted are outlined in the Schedule of Assessments (Table 1).</td></tr>
<tr><td colspan="3">Study Objective: To evaluate the safety and tolerability of ADX-2191 in patients with retinitis pigmentosa due to confirmed rhodopsin mutations, including P23H.</td></tr>
<tr><td colspan="3">Primary endpoint: The primary endpoints of this study are safety and tolerability.</td></tr>
</table>

Secondary endpoint: The secondary endpoints include:

1) Best Corrected Visual Acuity (BCVA): Change in BCVA will be assessed using a normally illuminated ETDRS (Early Treatment Diabetic Retinopathy) chart.
2) Low-luminance visual acuity (LLVA): Change in Low-luminance visual acuity (LLVA) and the low luminance deficit (LLD) will be assessed. The LLD is defined as the difference between the BCVA and LLVA. LLVA will be assessed using a 2.0 log unit neutral density filter while reading a normally illuminated ETDRS (Early Treatment Diabetic Retinopathy) chart.
3) Macular Integrity Assessment (MAIA) Microperimetry: Central retinal sensitivity will be assessed using MAIA microperimetry using a standard 10-2 test grid with a Goldman size III stimulus of various intensities presented on a mesopic background (4 apostilibs).
4) Full field Electroretinography (ffERG): Change in dark-adapted flash (0.01 $cd \cdot s \cdot m^{-2}$) b-wave amplitudes will be analyzed. Testing will be performed using a standard International Society for Clinical Electrophysiology of Vision (ISCEV) protocol.
5) Dark-Adapted Chromatic Perimetry: Change in dark adapted retinal sensitivity (dB) to the blue and red stimuli on the Medmont Dark-Adapted Chromatic (DAC) perimeter will be assessed.
6) OCT: Change in Central Subfield Foveal Thickness (CSFT) and Change in Ellipsoid Zone (EZ) area/width will be assessed using a spectral domain optical coherence tomograph (SD-OCT) volume scan using the Heidelberg Spectralis.

Safety: The safety and tolerability of ADX-2191 in subjects with retinitis pigmentosa due to confirmed rhodopsin mutations, including P23H, will be evaluated. Adverse Event data from Screening Visit A (Visit 1) until final study visit Visit 10 will be listed (by subject) and tabulated. Any serious adverse events will be described in subject narratives. In addition, any unanticipated adverse events will be separately tabulated. A urine pregnancy test will be performed at screening for women of childbearing age.

Study Population:

Inclusion Criteria - Preoperative

1) Up to 8 adult patients age 18 or older of any gender
2) Diagnosis of retinitis pigmentosa due to rhodopsin gene mutation, including P23H, confirmed previously by a CLIA or an investigational/academic lab
3) Impairment on VF in the opinion of the Investigator, as determined by perimetry
4) Willing to participate in the study as evidenced by signing of an informed consent document
5) Subjects of childbearing potential, both male and female, must agree to use two forms of birth control for the duration of the clinical trial. Males should continue to use the two forms of birth control for a minimum of three months after last injection (30 days after completion of Visit 9/Week 16). Females of childbearing potential must use the two forms of birth control for a minimum of 30 days following their last injection.
6) For females of child-bearing potential, subjects must have a negative pregnancy test at Screening and not lactating

Exclusion Criteria – Preoperative

1) Known allergy or hypersensitivity to methotrexate
2) BCVA worse than LogMAR BCVA 1.0 (Snellen equivalent 20/200) in the worse seeing eye
3) History of severe dry eye, corneal herpetic disease, LASIK, penetrating keratoplasty, or other significant corneal disease

4) Inability or unwillingness to use artificial tears during the study period
5) Pre-existing eye conditions or complicating systemic diseases that could interfere with the interpretation of the study or create inconsistent follow up. Examples may include severe cardiovascular disease, malignancy, profound amblyopia, diabetes or sickle cell disease if manifestation of advanced retinopathy is present, or subjects with immunodeficiency
6) Planned eye surgery during the study period
7) Participation in any clinical study of an investigational product within 90 days prior to enrollment and throughout enrollment period
8) Have previously received Methotrexate intravitreally or systemically within 3 months of Screening 1 visit
9) Patients who may need to use Methotrexate for another medical condition during the study period
10) Any history of systemic or ophthalmic condition or circumstances which, in the opinion of the Investigator, could compromise the subject's ability to comply with the protocol or that could compromise the subject's safety or the interpretation of the clinical trial results

Statistical Methods: Subject baseline characteristics, including demographics, concomitant diseases and medications, will be listed and tabulated. The intent to treat population is defined in the inclusion/exclusion criteria and is all subjects who have had ADX-2191 administered. No formal interim analysis is planned. Prior to database lock, the Sponsor will finalize a statistical analysis plan (SAP) detailing all planned analyses.

Table 1: Schedule of Events and Assessments

Assessment	Visit 1 (V1) Screening Visit A	Visit 2 (V2) Screening Visit B Week 0	Visit 3 (V3) Day 4[1]	Visit 4 (V4) Week 2[2]	Visit 5 (V5) Week 4[2]	Visit 6 (V6) Week 6[3]	Visit 7 (V7) Week 8[3]	Visit 8 (V8) Week 10[3]	Visit 9 (V9) Week 12[3]	Visit 10 (V10) Week 16[3]
Informed consent	X									
Eligibility criteria	X									
Demography, medical and ocular history	X									
Best Corrected Visual Acuity	X[4]	X[4,6]	X[4]		X[4]		X[4]		X[4]	X[4]
Low Luminance Visual Acuity	X[4]	X[4,6]	X[4]		X[4]		X[4]		X[4]	X[4]
Full ophthalmic exam, including intraocular pressure, slit lamp, and dilated funduscopy	X[4]		X[4]		X[4]		X[4]		X[4]	X[4]
Wide-field Color Fundus Photography and Autofluoresence	X[4]								X[4]	X[4]
Macular SD-OCT (Spectral Domain Optical Coherence Tomography)	X[4]				X[7]		X[7]		X[4]	X[4]
Dark-Adapted Chromatic (DAC) Perimetry	X[7]	X[6,7]	X[7]		X[7]		X[7]		X[7]	X[7]
Macular Integrity Assessment (MAIA) Microperimetry	X[7]	X[6,7]	X[7]		X[7]		X[7]		X[7]	X[7]
Full field electroretinography	X[4]								X[4]	
Cohort A (Monthly ADX-2191 injection)										
Corneal Slit Lamp Safety Exam		X[7]								
Urine Pregnancy Test		X			X		X			
Intravitreal injection of ADX-2191		X[5,7]			X[5,7]		X[5,7]			
Concomitant medications	X	X	X		X		X		X	X
Adverse events	X	X	X		X		X		X	X
Cohort B (Bimonthly ADX-2191 injection)										
Corneal Slit Lamp Safety Exam		X[7]		X[7]		X[7]		X[7]		
Urine Pregnancy Test		X		X	X	X	X	X		
Intravitreal injection of ADX-2191		X[5,7]		X[5,7]	X[5,7]	X[5,7]	X[5,7]	X[5,7]		
Concomitant medications	X	X	X	X	X	X	X	X	X	X
Adverse events	X	X	X	X	X	X	X	X	X	X

1: Visit window +/- two days
2: Visit window +/- three days
3: Visit window +/- five days
4: Bilateral evaluation
5: Injection to be performed after all other assessments have been performed.
6: Results from Screening Visit B (V2) will be used for quantitative study analysis purposes. Investigator may repeat any tests of Screening Visit B on a separate unscheduled visit if a difference in results is observed from Screening Visit A, as per investigator assessment
7: Study eye only

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

°C	Centigrade
°F	Fahrenheit
µg	micrograms
AE	Adverse Event/Adverse Experience
BCVA	Best Corrected Visual Acuity
CRF	Case Report Form
CRC	Central Reading Center
CFR	Code of Federal Regulations
CTCAE	Common Terminology Criteria for Adverse Events
CBC	Complete Blood Count
DAC/DACP	Dark Adapted Chromatic Perimetry
DHHS	Department of Health and Human Services
DNA	Deoxyribonucleic acid
DSM	Data Safety Monitor
ETDRS	Early Treatment Diabetic Retinopathy
EDC	Electronic Data Capture
FDA	Food and Drug Administration
GCPs	Good Clinical Practices
IEC	Independent Ethics Committee
ITT	Intent-to-Treat
ICH	International Council on Harmonisation
IND	Investigational New Drug Application
IP	Investigational Product
IOL	Intraocular lens
IOP	Intraocular pressure
IRB	Institutional Review Board
LLVA	Low Luminance Visual Acuity
MAIA/MAIAM	Macular Integrity Assessment (MAIA) Microperimetry
mL	Milliliters

mm	Millimeter
SD-OCT	Optical Coherence Tomography
QID	Four Times Daily
REB	Research Ethics Board
RP	Retinitis Pigmentosa
RR	Relative Risk
SAE	Serious Adverse Event/Serious Adverse Experience
US	United States
VA	Visual Acuity
WOCBP	Women of Childbearing Potential

1. INTRODUCTION

1.1. Background and Therapeutic Rationale

Retinitis pigmentosa (RP) is an inherited retinal disease that leads to a progressive degeneration of photoreceptors. Mutations within the rhodopsin gene account for approximately 25% of autosomal dominantly inherited RP cases. The mutation P23H (Substitution of Proline to Histidine at Codon 23 of the rhodopsin protein) is the most common mutation in the rhodopsin protein gene. The P23H mutation is associated with misfolded rhodopsin, which causes endoplasmic reticulum overload, activates the unfolded protein response and triggers apoptosis. Intravitreal methotrexate has been demonstrated in pre-clinical models of retinitis pigmentosa to promote clearance of misfolded rhodopsin without an adverse effect on wild type rhodopsin protein and improves electroretinogram (ERG) responses in P23H mutant rodents. (*Liu et al. (2020). Pharmacological clearance of misfolded rhodopsin for the treatment of RHO-associated retinitis pigmentosa. The FASEB Journal, 34(8), 10146–10167*).

The clinical pharmacology of ADX-2191 (intravitreal methotrexate) is based on inhibition of dihydrofolic acid reductase. Dihydrofolates must be reduced to tetrahydrofolates before they can be utilized in the synthesis of purine nucleotides and thymidylate. Therefore, methotrexate interferes with deoxyribonucleic acid (DNA) synthesis, repair, and cellular replication. Actively proliferating tissues such as malignant cells, bone marrow, fetal cells, buccal, intestinal mucosa, and cells of the urinary bladder are in general more sensitive to this effect of methotrexate. When cellular proliferation in malignant tissues is greater than in most normal tissues, methotrexate may impair malignant growth without irreversible damage to normal tissues.

Intravitreal injection provides the clinician with a means to bypass the blood retinal barrier and provide more therapeutic doses of the methotrexate to the affected tissues than could otherwise be achieved through oral or systemic dosing.

Aldeyra is proposing to conduct an open-label Phase II clinical trial in subjects with retinitis pigmentosa due to rhodopsin mutations (including P23H) whom will be treated with serial intravitreal injections of ADX-2191 (methotrexate 0.8%) in the worse seeing eye.

1.2. Potential Risks and Benefits

1.2.1. Potential Risks

General risks for intravitreal injection include infection, hemorrhage; precipitated angle closures; elevated intraocular pressure (IOP) with perfusion compromise; wound leak and hypotony; anaphylactic reaction to either the agent or periprocedural materials.

In addition, known risks associated with use of intravitreal methotrexate include:

- keratopathy. Therefore, all subjects will be recommended to use lubricating eye drops at a minimum of four times a day during the study period.

- mild conjunctival hyperemia ("red eye") and slight pain at injection site. This hyperemia and/or slight pain is seen in almost all eyes receiving methotrexate and resolves within several hours.

1.2.2. Potential Benefit

Taking part in the clinical trial may or may not improve the subject's vision. However, information learned from the clinical trial will help doctors understand more about how to improve vision in patients with retinitis pigmentosa.

2. CLINICAL TRIAL OBJECTIVE AND ENDPOINTS

2.1. Clinical Trial Objectives

To evaluate the safety and efficacy of repeated intravitreal ADX-2191 injections in subjects with retinal dystrophy (retinitis pigmentosa) with confirmed rhodopsin mutation (including P23H).

2.2. Clinical Trial Endpoints

2.2.1. Primary Endpoints

The primary endpoint is safety and tolerability of ADX-2191.

2.2.2. Secondary Endpoints

The secondary endpoint include:

1) Best Corrected Visual Acuity (BCVA): Change in BCVA will be assessed using a normally illuminated ETDRS (Early Treatment Diabetic Retinopathy) chart.
2) Low-luminance visual acuity (LLVA): Change in Low-luminance visual acuity (LLVA) and the low luminance deficit (LLD) will be assessed. The LLD is defined as the difference between the BCVA and LLVA. LLVA will be assessed using a 2.0 log unit neutral density filter while reading a normally illuminated ETDRS (Early Treatment Diabetic Retinopathy) chart.
3) Macular Integrity Assessment (MAIA) Microperimetry: Central retinal sensitivity will be assessed using MAIA microperimetry using a standard 10-2 test grid with a Goldman size III stimulus of various intensities presented on a mesopic background (4 apostilibs).
4) Full field Electroretinography (ffERG): Change in dark-adapted flash (0.01 $cd^{\bullet}s^{\bullet}m^{-2}$) b-wave amplitudes will be analyzed. Testing will be performed using a standard International Society for Clinical Electrophysiology of Vision (ISCEV) protocol.
5) Dark-Adapted Chromatic Perimetry: Change in dark adapted retinal sensitivity (dB) to the blue and red stimuli on the Medmont Dark-Adapted Chromatic (DAC) perimeter will be assessed.
6) OCT: Change in Central Subfield Foveal Thickness (CSFT) and Change in Ellipsoid Zone (EZ) area/width will be assessed using a spectral domain optical coherence tomograph (SD-OCT) volume scan using the Heidelberg Spectralis.

3. INVESTIGATIONAL PLAN

3.1. Overall Clinical Trial Design and Plan

In this open-label, non-comparator Phase II clinical trial, 8 (eight) evaluable subjects with retinitis pigmentosa due to rhodopsin mutations (including P23H) will be identified and treated with serial intravitreal injections of ADX-2191 in the worse seeing eye ("**the study eye**"). Ocular structure and function will be evaluated.

The first four subjects (Cohort A) will be treated with monthly intravitreal injections of ADX-2191 for a total of three injections; the last four subjects (Cohort B) will be treated with twice monthly intravitreal injections of ADX-2191 for a total of six injections. All subjects will be followed for a total of 16 weeks following dosing.

3.2. Subject Numbering

Each subject screened for the clinical trial will be assigned a unique subject number that will be used to identify the subject throughout subject participation in the clinical trial.

3.3. Safety Oversight

During the clinical trial, subject safety will be monitored on a continuous basis by the Sponsor Medical Monitor until the last subject completes the last scheduled clinical trial assessment.

3.4. Clinical Trial Duration for Individual Subjects

The clinical trial consists of 10 clinic visits over a period of approximately 16 weeks.

3.5. Clinical Trial Population Selection

The intended population will be adult subjects with retinitis pigmentosa due to rhodopsin mutations (including P23H) will be identified and treated with serial intravitreal injections of ADX-2191 in the worse seeing eye.

3.5.1. Inclusion Criteria

Subjects meeting **ALL** of the following criteria will be considered eligible for clinical trial entry:

1. Subjects age 18 or older of any gender
2. Diagnosis of retinitis pigmentosa due to rhodopsin gene mutation, including P23H, confirmed previously by a CLIA or an investigational/academic lab
3. Impairment on VF in the opinion of the Investigator, as determined by perimetry
4. Willing to participate in the study as evidenced by signing of an informed consent document
5. Subjects of childbearing potential, both male and female, must agree to use two forms of birth control for the duration of the clinical trial. Males should continue to use the two forms of birth control for a minimum of three months after last injection. Females

of childbearing potential must use the two forms of birth control for a minimum of 30 days following their last injection

6. For females of child-bearing potential, subjects must have a negative pregnancy test at Screening and not lactating

3.5.2. Exclusion Criteria

Subjects meeting **ANY** of the following criteria will be excluded from the clinical trial:

1. Known allergy or hypersensitivity to methotrexate
2. BCVA worse than LogMAR BCVA 1.0 (Snellen equivalent 20/200) in the worse seeing eye
3. History of severe dry eye, corneal herpetic disease, LASIK, penetrating keratoplasty, or other significant corneal disease
4. Inability or unwillingness to use artificial tears during the study period
5. Pre-existing eye conditions or complicating systemic diseases that could interfere with the interpretation of the study or create inconsistent follow up. Examples may include severe cardiovascular disease, malignancy, diabetes or sickle cell disease if manifestation of advanced retinopathy is present, or subjects with immunodeficiency
6. Planned eye surgery during the study period
7. Participation in any clinical study of an investigational product within 90 days prior to enrollment
8. Have previously received Methotrexate (intravitreally, systemically, or any other route) within 3 months of Screening Visit A (V1)
9. Patients who may need to use Methotrexate for another medical condition during the study period
10. Any history of systemic or ophthalmic condition or circumstances which, in the opinion of the Investigator, could compromise the subject's ability to comply with the protocol or that could compromise the subject's safety or the interpretation of the clinical trial results

3.6. Strategies for Recruitment and Retention

Subjects will be recruited directly from the investigative sites' patient population or will be referred for treatment by other physicians. Once enrolled, subjects will be provided with the visit schedule and reminders from the clinical trial staff in advance of their visits to assist with compliance to the schedule of events for the clinical trial.

Subjects who discontinue early from investigational product treatment will complete the end-of-study visit (Week 16) at time of discontinuation. The Sponsor and Investigator will make all efforts to retain all subjects in the clinical trial until the end-of-study visit (Week 16) and to follow-up with early-discontinued subjects for safety purposes.

4. SUBJECT DISPOSITION

4.1. Completed Subjects

A completed subject is one who has not been discontinued from the clinical trial.

4.2. Discontinued Subjects

Subjects may be discontinued prior to their completion of the clinical trial due to:

- Subject request/withdrawal
- AEs
- Positive pregnancy results
- Protocol violations
- Administrative reasons (e.g., inability to continue, lost to follow-up)
- Sponsor termination of the clinical trial
- Any sound medical reason, as determined by the investigator
- Others

Notification of a subject discontinuation and the reason for discontinuation will be made to the Sponsor or designee and will be clearly documented on the CRF.

4.3. Subject Withdrawal

Subjects will be removed from the clinical trial if they develop infectious endophthalmitis and/or no light perception vision. Subjects may also be removed at the discretion of the principal investigator or at the wishes of the subject himself/herself.

4.4. Handling of Subject Withdrawals or Subject Discontinuation of Clinical Trial Intervention

Subjects that are dropped from the clinical trial prior to administration of the interventional investigational product will be considered screen failures and will not be included in analysis.

Any subject receiving the investigational product will be followed for safety throughout the scheduled course of the clinical trial, or until the subject has reached a status of un-reachable. To assure the clinical trial meets the required approximate 8 evaluable eyes for analysis, additional subjects may need to be enrolled to meet this goal.

4.5. Premature Termination or Suspension of Clinical Trial

In the event of premature termination or suspension of clinical trial, the subject will continue to follow with his/her physician as part of their usual standard of care.

5. INVESTIGATIONAL TREATMENT

Product Name: ADX-2191 (methotrexate 0.8%) for intravitreal injection.

5.1. Description of Investigational Product

Methotrexate is an antimetabolite and antifolate drug that has been used for cancer treatment since the 1950s. The drug was originally approved in 1959 and is now available generically. It has exceptionally well-understood biological and clinical effects. Methotrexate is currently FDA-approved for oral administration and for intravenous, intramuscular, intrathecal, or intra-arterial injection. Approved indications include psoriasis, rheumatoid arthritis, and certain neoplastic diseases. Other mechanisms of action involve direct effects on inflammatory pathways, in particular neutralization of the effects of TNF- α and IL-1β, though the molecular mechanisms underlying these effects remain largely uncharacterized.

Several case series have been published using intravitreal injections of methotrexate, most commonly for intraocular lymphoma. (Frenkel et al., 2008; Hardwig, Pulido, & Bakri, 2008; Hardwig, Pulido, Erie, Baratz, & Buettner, 2006; Samson, Waheed, Baltatzis, & Foster, 2001)

5.2. Packaging and Labeling

ADX-2191 drug product is formulated as a sterile and aqueous solution for intravitreal delivery. The drug product will be supplied in single-dose, 2 mL glass vials with a 0.5 mL fill volume.

The ADX-2191 drug product is formulated in 7.5% sucrose and phosphate buffered at pH 7-8.

The quantitative composition of the ADX-2191 drug product are presented in Table 2.

Table 2: Composition of ADX-2191 Drug Product

Component	Amount (%w/v)	Grade
ADX-2191 (methotrexate)	0.8%	GMP
Sucrose	7.5%	USP/NF
Sodium Phosphate Dibasic	0.114%	USP
Sodium Hydroxide or Hydrochloric Acid	pH adjustment	USP/NF
Sterile Water for Injection (WFI)	Dilute to volume	USP

5.3. Investigational Product Storage

ADX-2191 drug product (0.8% Methotrexate Solution for Intravitreal Injection) will be supplied to the clinical sites in a clear 2 mL vial as an aqueous solution that does not require reconstitution. ADX-2191 must be stored at 2 to 8ºC (36 to 46ºF) in an upright position. Do not freeze. Protect from light while in storage (this does not apply to time of preparation and administration).

The Investigational Product (IP) must be stored in a secure area accessible only to the investigator and his/her designees. The IP will be administered only to subjects entered into the clinical trial, in accordance with the conditions specified herein.

The subject kit label will include, but not limited to, the following information:

- trial protocol number
- contents
- subject number and initials (manually recorded)
- storage conditions
- Investigational New Drug statement
- Sponsor's name, address, and phone number

The vial label will be included, but not limited to, the following information:

- trial protocol number
- contents
- storage conditions
- Investigational New Drug statement
- manufacturer name and address
- Sponsor name, address, and phone number

5.4. Investigational Product Dosage, Preparation, and Administration

Proposed dosage of ADX-2191: 400 µg in 0.05 mL intravitreal injection

ADX-2191 will be provided as the investigational product to clinical trial sites that have obtained IRB approval. The IP will be supplied in single-use vials. ADX-2191 should be withdrawn from the vial into an appropriate syringe allowing excess volume to accommodate needle fill if the needle will be exchanged. Used vials/syringes should be disposed of appropriately in accordance with local policy which may include use of yellow "chemo waste" bags.

Administration of ADX-2191 is described in Section 6.8. Refer to the Pharmacy Manual for additional details.

5.5. Investigational Product Accountability

The IP is to only be prescribed by the principal investigator or his/her named sub-investigator(s) and is only to be used in accordance with this protocol. The IP must be distributed only to subjects properly qualified under the conditions specified herein.

The investigator or designated qualified site staff is responsible for monitoring the inventory of IP and for the accountability of all used and unused IP received from the supplier. This includes the amount of IP dispensed to subjects, and the amount returned or disposed upon the completion of the trial. Inventories must be carefully and accurately documented according to applicable

state, federal, and local regulations, current Good Clinical Practices (GCPs), including the International Council for Harmonization (ICH) guidelines, and clinical trial procedures. IP accountability will be available for Sponsor's review and will be verified by the clinical trial monitor during site visits and at the completion of the trial.

At the conclusion of the trial, IP reconciliation will be performed, and all remaining IP not returned to the sponsor, will be destructed and disposed of according to clinical site's SOP. Sponsor will be provided with a final accounting of IP for approval prior to destruction.

The investigator or designated qualified site staff will maintain accurate records of receipt and condition of IP, including dates of receipt, and temperature log of storage conditions. In addition, accurate records will be kept of the date of IP administration and the subject to whom IP was administered. Any reasons for departure from the protocol-specified dispensing regimen must also be recorded.

5.6. Investigational Product Retention

IP must be retained until completion or termination of the clinical trial, and written authorization from the Sponsor has been received. All unused and used IP drug should be destroyed at the site or returned to the distributor, as specified by Sponsor. It is the investigator's responsibility to ensure that the Sponsor has provided written authorization prior to return or destruction, and that appropriate records of the disposal are documented and maintained. No used or unused IP may be disposed until fully accounted for by the clinical trial monitor.

5.7. Subject Compliance with Investigational Product Administration

Compliance or product administration will be assessed through accurate medication administration records maintained at the clinical sites. The investigator/or appropriately licensed designee will administer the investigational product as defined in the protocol administration schedule. IP compliance will be assessed through review of the subject's medical record.

5.8. Subject Compliance with Clinical Trial Intervention

Protocol compliance will be assessed at each clinical trial visit. Compliance information will be collected from the medical/research record and reported via an CRF. This information will be reviewed by the clinical trial monitor in accordance with the Monitoring Plan.

5.9. Assessment of Clinician and/or Subject Compliance with Clinical Trial Procedural Intervention

Clinician/subject compliance will be assessed at each clinical trial visit. Compliance information will be collected from the medical/research record and reported via a case report form. This information will be reviewed by the clinical trial monitor in accordance with the Monitoring Plan.

5.10. Concomitant Therapy and Procedures

The use of any concomitant medication, prescription or over the counter, taken within 30 days prior to signing consent, is to be recorded on the source document and corresponding CRF along with the reason the medication was taken.

Concurrent enrollment (within 90 days of enrollment) in another investigational drug or medical device clinical trial is not permitted.

5.11. Prohibited Concurrent Therapies and Procedures

Prohibited medications, treatments, and procedures during the clinical trial are outlined in the Exclusion Criteria (Section 3.5.2).

6. CLINICAL TRIAL PROCEDURES

Clinical trial assessments and evaluations should be performed by the investigator and/or qualified site staff according to Schedule of Events and Assessments (Table 1). Refer to the Clinical Trial Activities Schedule (Section 7).

6.1. Informed Consent

Informed consent forms must be approved for use by the reviewing Institutional Review Board (IRB)/Research Ethics Board (REB)/Independent Ethics Committee (IEC). Informed consent must be obtained for all subjects participating in the clinical trial prior to performing any procedures. The informed consent process must be adequately documented in the source records.

6.2. Eligibility Review

The investigator or qualified site staff will confirm that all inclusion and exclusion criteria have been met.

6.3. Demographics, Medical History, and Social History

Demographic information to be captured include subject initials (where locally permitted), date of birth (alternatively year of birth, if full date of birth is not allowed to be collected for legal reasons), age, sex, race, and ethnicity will be obtained from the subject and recorded in the CRF.

Medical and social history will be recorded in the CRF. Current underlying conditions, including conditions diagnosed within the last 30 days of consent, which may have resolved before screening, must be recorded.

6.4. Concomitant Medications

Concomitant medications used 30 days prior to consent to treat any medical conditions will be recorded in the CRF. Any changes in dosage or new medications added must be recorded in the subject CRF. The Sponsor and investigator or qualified site staff will review and evaluate concomitant medication usage on an ongoing basis.

6.5. Adverse Events

Any serious, unexpected adverse events (AE) believed to be due to the intervention rather than the natural disease process will prompt a filing of an adverse event form with the local IRB office and the FDA.

AEs will be collected from the time the subject signs the informed consent form through 30 days after the last dose of IP. AEs must be recorded in the CRF. The Sponsor and investigator or qualified site staff will review and evaluate AEs on an ongoing basis. See Section 8 for further detail on AE reporting.

6.6. Urine pregnancy testing

For women of childbearing potential (WOCBP), a urine pregnancy test will be collected at the Screening Visit B (Visit 2), and prior to each injection of ADX-2191, through Visit 8 / Week 10. Results must be available and confirmed to be negative before the subject may be enrolled in the clinical trial. IP must be discontinued for any subject with a positive pregnancy test result.

6.7. Ophthalmic Examinations

6.7.1. Best-Corrected Visual Acuity

BCVA will be collected at Screening Visit A (V1), Screening Visit B (V2), and Visits V3, V5, V7, V9, and V10. The BCVA will be measured using ETDRS chart by masked technicians at a standard photopic light level of at least 85 cd/m^2. Both eyes will be examined at all time points.

6.7.2. Low Luminance Visual Acuity

Low-luminance visual acuity (LLVA) will be collected at Screening Visit A (V1), Screening Visit B (V2), and Visits V3, V5, V7, V9, and V10. LLVA will be assessed using a 2.0 log unit neutral density filter while reading a normally illuminated ETDRS (Early Treatment Diabetic Retinopathy) chart. If <20 letters are read at 4 meters, testing should be repeated at 1 meter. Both eyes will be examined at all time points.

6.7.3. Macular Integrity Assessment (MAIA) Microperimetry

Central retinal sensitivity will be assessed using MAIA microperimetry using a standard 10-2 test grid with a 4-2 bracketing threshold strategy and Goldman size III stimulus of various intensities presented on a mesopic background (4 apostilibs). MAIA will be collected at Visits V1, V2, V3, V5, V7, V9, and V10. Only the study eye will be evaluated.

6.7.4. Full Field Electroretinography

A standard full-field electroretinogram (ERG) will be performed. Change in dark-adapted flash ($0.01\ cd^{\bullet}s^{\bullet}m^{-2}$) b-wave amplitudes will be analyzed. Testing will be performed using a standard

International Society for Clinical Electrophysiology of Vision (ISCEV) protocol. Both eyes will be evaluated. ERG will be collected at Visits V1 and V9.

6.7.5. Dark-Adapted Chromatic (DAC) Perimetry

Change in dark-adapted retinal sensitivity (dB) to the cyan (blue) and red stimuli on the Medmont Dark-Adapted Chromatic (DAC) perimeter will be assessed. The test eye will be patched for 45 minutes to allow for dark adaptation. At each test point the stimulus will be presented with either a cyan (505 nm, 75 dB maximum dynamic rage) or a red (620 nm, 50 dB maximum dynamic range) color. 103 points (144 ° horizontally and 72 ° vertically) will be tested using a 1.72° stimulus (equivalent to the Goldman size V) will be presented for 200ms, with a 400ms response time, and fixed interval between stimuli at 1.1 seconds. Retinal sensitivities will be determined using a four-down, two-up staircase threshold strategy. The maximum photopic luminance for the cyan stimuli will be no more than 12.58 cd/m2 with a test range of 0 to 75 dB attenuation. For the red stimuli, the maximum luminance will be no more than 4.64 cd/m2 with a range of 0 to 50 dB. Appropriates lenses should be used for correction of refractive error and age-related accommodation loss. Patient controlled pauses are encouraged as often as needed to reduce to prevent fatigue. Exams with >20% false positives will be excluded. Only the study eye will be evaluated. DAC Perimetry will be collected at Visits V1, V2, V3, V5, V7, V9, and V10. Only the study eye will be evaluated.

6.7.6. Macular Spectral-Domain Optical Coherence Tomography (SD-OCT)

Macular SD-OCT imaging will be done; the Heidelberg Spectralis is preferred. Both eyes will be imaged at Visits V1 and V10. The macular volume scan should be obtained using the preset "Posterior Pole settings: **Scan Extent:** Volume Scan (30° x 25°); **Scan Sections/B-scans:** 61 sections (with a 120 μm spacing); **Resolution:** High Speed with an ART mean of at least 9 frames. SD-OCT will be collected at Visits V1, V5, V7, V9, and V10. Both eyes will be evaluated at visits V1, V9, V10 and only the study eye will be evaluated at V5 and V7.

6.7.7. Full Ophthalmic Clinical Examination

Routine full ophthalmic, dilated exams, including intraocular pressure measurement, slit lamp examination, and dilated funduscopy will be performed on Screening Visit A (V1) and Visits V3, V5, V7, V9, V10. Both eyes will be examined at all time points. The full ophthalmic exam will also serve as the corneal safety assessment on days when a full ophthalmic exam is done prior to an intravitreal injection.

6.7.8. Color and Autofluorescence Fundus Photographs

All subjects will undergo pre-operative color and autofluorescence photographs. Both eyes will be imaged at Visits V1, V9, and V10. The Optos UWF or Zeiss CLARUS are preferred;

appropriate dilation prior to acquiring imaging is recommended. A single ultra-widefield image with minimal artifacts and good exposure is required. High resolution TIF, BMP, JPEG, PNG, or DICOM file formats are accepted. Photographs will be collected at Visits V1, V9, and V10.

6.7.9. Corneal Slit Lamp Safety Exam

An undilated slit lamp only exam of the to-be-treated eye will be performed to evaluate the cornea for significant keratopathy prior to intravitreal injection of the IP. Therefore, the Corneal Slit Lamp Safety Exam will be done for subjects in Cohort A at Week 0 (V2) and for Cohort B at Week 0 (V2), Week 2 (V4), Week 6 (V6), Week 10 (V8).

6.8. Investigational Product Intravitreal Injection

Upon completion of the final screening tests at Visit 2, subjects will receive ADX-2191 400 µg/0.05 mL intravitreal injection to the study eye, in accordance with the schedule of events (Table 1). The study eye will be the subject's worse seeing eye. Subjects who are scheduled to receive an intravitreal injection at Weeks 0 (V2), Week 4 (V5), or Week 8 (V7) following completion of testing.

Intravitreal ADX-2191 injections will be performed aseptically after the topical application or subconjunctival injection of an anesthetic agent and 5% povidone iodine to the conjunctiva. Each patient will receive an intravitreal injection of 400 µg/0.05 mL ADX 2191, 3.0 mm posterior to the limbus with a 33-gauge or similar needle.

6.9. Dispense Artificial Tears and Lubricating Ointment

All subjects receiving the investigational product will be prescribed artificial tears and/or lubricating ointment at a minimum of four times daily (QID) throughout the injection phase of the clinical trial. Artificial Tears will be prescribed for Cohort A at Week 0 (V2), Week 4 (V5), and Week 8 (V7) and for Cohort B at Week 0 (V2), Week 2 (V4), Week 4 (V5), Week 6 (V6), Week 8 (V7), and Week 10 (V8).

6.10. Pre-Injection Assessment

Subjects must have a corneal slit lamp exam performed prior to injection of ADX-2191 to assure the safety and viability of the cornea prior proceeding with the investigational treatment. For women of childbearing potential (WOCBP), a urine pregnancy test will be collected and confirmed to be negative prior to undergoing the investigational treatment.

7. CLINICAL TRIAL ACTIVITIES

Clinical trial activities are summarized in the Schedule of Events and Assessments (Table 1).

NOTE: Event weeks are defined as follows: Week 0 is defined as the second and final screening visit.

7.1. Screening Visit A (Visit 1)

- Obtain written informed consent (Section 6.1)
- Review eligibility criteria (Section 6.2)
- Collect demographic information and document medical and social history (Section 6.3)
- Collect samples for urine pregnancy test, if applicable (Section 6.6)
- Perform BCVA (Section 6.7.1)
- Perform LLVA (Section 6.7.2)
- Collect Macular Integrity Assessment (MAIA) (Section 6.7.3)
- Collect Full-field electroretinography (ERG) (Section 6.7.4)
- Collect Dark-Adapted Chromatic Perimetry (Section 6.7.5)
- Collect Macular Optical Coherence Tomography (Section 6.7.6)
- Perform Full Ophthalmic Examination (Section 6.7.7)
- Collect Wide Field Color and Autofluorescence Fundus Photographs (Section 6.7.8)
- Collect concomitant medications (Section 6.4)
- Collect adverse events (Section 6.5)

7.2. Week 0 Screening Visit B (Visit 2)

- Perform BCVA (Section 6.7.1)
- Perform LLVA (Section 6.7.2)
- Collect Macular Integrity Assessment (MAIA) (Section 6.7.3)
- Collect Dark-Adapted Chromatic Perimetry (Section 6.7.5)
- Perform Corneal Slit Lamp Safety Exam (Section 6.7.9)
- Investigational Product Intravitreal Injection (Section 6.8)
- Prescribe artificial tears and/or lubricating ointment (Section 6.9)
- Collect concomitant medications (Section 6.4)
- Collect adverse events (Section 6.5)

7.3. Day 4 (Visit 3)

- Perform BCVA (Section 6.7.1)
- Perform LLVA (Section 6.7.2)
- Perform Full Ophthalmic Examination (Section 6.7.7)

- Collect Dark-Adapted Chromatic Perimetry (Section 6.7.5)
- Collect Macular Integrity Assessment (MAIA) (Section 6.7.3)
- Collect concomitant medications (Section 6.4)
- Collect adverse events (Section 6.5)

7.4. Week 2 (Visit 4)—Cohort B only

- Perform Corneal Slit Lamp Safety Exam (Section 6.7.9)
- Collect samples for urine pregnancy test, if applicable (Section 6.6)
- Investigational Product Intravitreal Injection (Section 6.8)
- Prescribe artificial tears and/or lubricating ointment (Section 6.9)
- Collect concomitant medications (Section 6.4)
- Collect adverse events (Section 6.5)

7.5. Week 4 (Visit 5)

- Perform BCVA (Section 6.7.1)
- Perform LLVA (Section 6.7.2)
- Perform Full Ophthalmic Examination (Section 6.7.7)
- Collect Macular Optical Coherence Tomography (Section 6.7.6)
- Collect Dark-Adapted Chromatic Perimetry (Section 6.7.5)
- Collect Macular Integrity Assessment (MAIA) (Section 6.7.3)
- Collect samples for urine pregnancy test, if applicable (Section 6.6)
- Investigational Product Intravitreal Injection (Section 6.8)
- Prescribe artificial tears and/or lubricating ointment (Section 6.9)
- Collect concomitant medications (Section 6.4)
- Collect adverse events (Section 6.5)

7.6. Week 6 (Visit 6)—Cohort B only

- Perform Corneal Slit Lamp Safety Exam (Section 6.7.9)
- Collect samples for urine pregnancy test, if applicable (Section 6.6)
- Investigational Product Intravitreal Injection (Section 6.8)
- Prescribe artificial tears and/or lubricating ointment (Section 6.9)
- Collect concomitant medications (Section 6.4)
- Collect adverse events (Section 6.5)

7.7. Week 8 (Visit 7)

- Perform BCVA (Section 6.7.1)
- Perform LLVA (Section 6.7.2)
- Perform Full Ophthalmic Examination (Section 6.7.7)
- Collect Macular Optical Coherence Tomography (Section 6.7.6)
- Collect Dark-Adapted Chromatic Perimetry (Section 6.7.5)
- Collect Macular Integrity Assessment (MAIA) (Section 6.7.3)
- Collect samples for urine pregnancy test, if applicable (Section 6.6)
- Investigational Product Intravitreal Injection (Section 6.8)
- Prescribe artificial tears and/or lubricating ointment (Section 6.9)
- Collect concomitant medications (Section 6.4)
- Collect adverse events (Section 6.5)

7.8. Week 10 (Visit 8)—Cohort B only

- Perform Corneal Slit Lamp Safety Exam (Section 6.7.9)
- Collect samples for urine pregnancy test, if applicable (Section 6.6)
- Investigational Product Intravitreal Injection (Section 6.8)
- Prescribe artificial tears and/or lubricating ointment (Section 6.9)
- Collect concomitant medications (Section 6.4)
- Collect adverse events (Section 6.5)

7.9. Week 12 (Visit 9)

- Perform BCVA (Section 6.7.1)
- Perform LLVA (Section 6.7.2)
- Perform Full Ophthalmic Examination (Section 6.7.7)
- Collect Wide Field Color and Autofluorescence Fundus Photographs (Section 6.7.8)
- Collect Macular Optical Coherence Tomography (Section 6.7.6)
- Collect Dark-Adapted Chromatic Perimetry (Section 6.7.5)
- Collect Macular Integrity Assessment (MAIA) (Section 6.7.3)
- Collect concomitant medications (Section 6.4)
- Collect adverse events (Section 6.5)
- Collect Full-field electroretinography (ERG) (Section 6.7.4)

7.10. Week 16 (Visit 10)

- Perform BCVA (Section 6.7.1)
- Perform LLVA (Section 6.7.2)
- Perform Full Ophthalmic Examination (Section 6.7.7)
- Collect Wide Field Color and Autofluorescence Fundus Photographs (Section 6.7.8)
- Collect Macular Optical Coherence Tomography (Section 6.7.6)
- Collect Dark-Adapted Chromatic Perimetry (Section 6.7.5)
- Collect Macular Integrity Assessment (MAIA) (Section 6.7.3)
- Collect concomitant medications (Section 6.4)
- Collect adverse events (Section 6.5)

8. ADVERSE EVENT REPORTING

AEs will be collected from the time the subject signs the informed consent form through 30 days after the last IP administration.

8.1. Adverse Event

An AE is any untoward medical occurrence in a patient administered an IP, which does not necessarily have a causal relationship with the IP treatment. Therefore, an AE can be any unfavorable and unintended sign, symptom, or disease temporally associated with the use of the IP, whether or not it is considered related to the IP.

Abnormal investigational findings (i.e., physical exam) should not be reported as AEs unless they are associated with clinical signs and symptoms, lead to treatment discontinuation, or are otherwise considered clinically relevant by the investigator. In cases of surgical or diagnostic procedures, the condition/illness leading to such a procedure is considered as the AE rather than the procedure itself. In case of a fatality, the cause of death is considered as the AE, and the death is considered as its outcome.

8.2. Events Not to Be Considered as Adverse Events

Pre-existing medical conditions/signs/symptoms present before the Screening Visit (Visit 1) that do not worsen in severity or frequency during the clinical trial are defined as baseline medical conditions and are not to be considered AEs.

Pregnancies are not considered AEs but must be reported, see Section 8.7.

8.3. Recording Adverse Events

Safety assessments will include but not limited to evaluation of AEs using National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0 2017, or higher, clinical laboratory results, pregnancy testing, and vital sign measurements. If a CTCAE

grading is not listed for a specific event then the AE will be graded as mild, moderate or severe, per Section 8.4.

AEs will be recorded from the time of signing of informed consent up to the final clinical trial visit.

All AEs must be recorded in the site's clinical trial records and the AE CRF. Investigators should use correct medical terminology when recording events and avoid abbreviations.

The investigator should attempt to establish a diagnosis of the AE based on signs, symptoms, and/or other clinical information. The diagnosis, and not the individual signs/symptoms, or laboratory abnormalities, should be documented in the subject's source documents and the CRF unless the etiology of the event is unknown. If signs/symptoms cannot be medically characterized as a single diagnosis or syndrome at the time of reporting, each individual event should be recorded as an AE. If a diagnosis is subsequently established, it should be reported as follow-up information.

An assessment should be made at each visit (or more frequently if necessary) of any changes in severity, the suspected relationship to IP, the interventions required to treat it, and the outcome.

8.4. Assessment of Causality and Severity

For each AE recorded, the investigator will make an assessment of causality and severity as follows:

1. Relationship to IP: The investigator must assess whether they consider an AE to be drug-related. In assessing this relationship, the investigator must use information about the drug as outlined in the Investigator's Brochure, the subject's pre-existent medical conditions/concurrent medication, and chronology of the event relative to drug administration. The following definitions will be used:
    - Definitely Related
    - Probably Related
    - Possibly Related
    - Unlikely to be related
    - Not Related

    For regulatory authority reporting, only "related," or "not related" classification will be used. AEs identified as definitely, probably, or possibly, will be classified as "related." Those AEs identified as unlikely, or not related, will be classified as "not related."
2. Event Severity: The investigator will be asked to use their medical judgment to assess the severity of the AE.
3. The following are guidelines to be used by the investigator to judge the event severity of an AE:
    - Mild (Grade I) - awareness of sign or symptom, but easily tolerated
    - Moderate (Grade II) - discomfort enough to cause interference with usual activity

- Severe (Grade III) - incapacitating with inability to work or perform usual activity
- Very Severe (Grade IV) – Vision or life-threatening; urgent intervention indicated
- Death (Grade V) – death of subject

4. Expectedness: The expectedness of an AE should be determined based on upon existing safety information about the IP using the following explanations:
    - *Unexpected*: an AE that is not listed in the IB or is not listed at the specificity or severity that has been observed.
    - *Expected*: an AE that is listed in the IB at the specificity and severity that has been observed.
    - *Not applicable:* an AE unrelated to the IP.
    - AE events that are mentioned in the IB as occurring with a class of products or as anticipated from the pharmacological/mechanical (or other) properties of the product but are not specifically mentioned as occurring with the particular product under investigation, are to be considered unexpected.
    - The investigator should initially classify the expectedness of an AE, but the final classification is subject to the determination of the Sponsor's Medical Monitor.
5. Duration: Start and end dates and times, or if continuing.
6. Frequency: whether the event is a single episode, recurrent or continuous.
7. Action taken.
8. Whether it constitutes a SAE, per definition below.
9. Outcome: resolved, resolved with sequelae, continuing, death, or unknown (only for subjects that are lost to follow-up).

8.5. Treatment-Emergent Adverse Events

A TEAE is defined as an AE that occurred during the clinical trial after the first dose of IP or was present prior to dosing and exacerbates after the first dose of IP.

8.6. Serious Adverse Events

An SAE is any AE that results in any of the following outcomes:

- death;
- a life-threatening AE;
- Note: An AE is considered "life-threatening" if, in the view of either the investigator or Sponsor/designee, the subject is at immediate risk of death as a result of the AE. "Life-threatening" does not include an AE that, had it occurred in a more severe form, might have caused death.
- inpatient hospitalization or prolongation of existing hospitalization;

- Note: The term "inpatient hospitalization" refers to any inpatient admission (even if less than 24 hours). For chronic or long-term subjects, inpatient admission includes transfer within the hospital to an acute/intensive care inpatient unit. Inpatient hospitalization does not include emergency room visits; outpatient/same-day/ambulatory procedures; or admission to observation/short-stay units, rehabilitation facilities, hospice facilities, nursing homes, or clinical research/Phase 1 units.
- Note: The term "prolongation of existing hospitalization" refers to any extension of an inpatient hospitalization beyond the stay anticipated or required for the reason for the initial admission, as determined by the investigator or treating physician.

- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions;
  - Note: A serious adverse event (SAE) specifically related to visual threat would be interpreted as any potential impairment or damage to the subject's eyes (e.g., hemorrhage, retinal detachment, central corneal ulcer, or damage to the optic nerve).
- a congenital anomaly/birth defect; and,
- other serious (Important Medical Events) events that do not fit other outcomes, where the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in the SAE definition, based upon appropriate medical judgment.

8.6.1. Unexpected Adverse Event

An unexpected adverse event is defined as an AE, the nature or severity of which is not consistent with the information in the Investigator's Brochure.

8.6.2. Reporting Serious Adverse Events

The investigator is responsible for reporting all SAEs, regardless of causality, to the Sponsor designee within 24 hours of learning of the occurrence. The paper SAE form must be sent to the study Medical Monitor, Dr. Tomasz Stryjewski, at tstryjewski@aldeyra.com

The reporting timeframe starts when the subject signs the informed consent or assent form through 30 days after the last IP administration. At a minimum, a description of the event and the Investigator's judgment of causality must be provided at the time of the initial report. These preliminary reports will be followed by detailed descriptions that will include copies of hospital case reports, autopsy reports, and other documents when requested and applicable.

A follow-up SAE Report must be submitted within 24 hours of the investigator receiving the follow-up information (such as information regarding complications, progression or resolution).

An SAE that is considered completely unrelated to a previously reported event should be reported separately as a new SAE.

The original copy of the SAE Report Form and the fax confirmation sheet (or email) must be kept with the source documentation at the clinical trial site.

Follow-up information should be communicated the same way, using a new SAE Report Form stating that it is a follow-up to the previously reported SAE and giving the date of the original report. The follow-up information should describe whether the event has resolved or continues, if and how it was treated, and whether the subject continued or withdrew from clinical trial participation.

The investigator and qualified site staff should institute any supplemental investigations of SAEs based on their clinical judgment of likely causative factors. This may include clinical laboratory tests not specified in the protocol, histopathologic examinations, or consultations with specialists. The Sponsor or their designee may also request the investigator to conduct supplemental assessments.

If the SAE was not previously documented in the Investigator's Brochure and is thought to be related to IP, the Sponsor or their designee may urgently require further information from the investigator for regulatory authority reporting. The Sponsor may need to issue an investigator Notification to inform all investigators involved in any clinical trial with the same drug that this SAE has been reported.

8.6.3. Follow-up of Adverse Events

All AEs will be followed until stabilization/resolution or until clinical database lock. AEs are to be reported to the IRB in accordance with IRB policy.

All SAEs will undergo active follow-up until resolved or the event becomes chronic or stable. Follow-up data for SAEs obtained after clinical database lock will be incorporated into the safety database.

8.6.4. Reporting Serious Adverse Events to Regulatory Health Authorities/ Institutional Review Boards/Research Ethics Boards/Independent Ethics Committees

The investigator is responsible for following all local regulations for the reporting of safety information, including the reporting of SAEs to their local IRB/REB/IEC.

The investigator must promptly report to his or her local IRB/REB/IEC all unanticipated problems involving risks to subjects. This includes death from any cause and all serious adverse events reasonably or possibly associated with the use of IP.

The Sponsor or their designee is responsible for appropriate reporting of relevant AEs, suspected unexpected serious adverse reactions (SUSARs) involving IP, to all regulatory authorities.

In addition, the Sponsor or designee will be responsible for the submission of safety letters (e.g., SUSARs) to the central IRB/REB/IEC and to participating investigators of all SUSARs involving IP according to applicable regulations.

After termination of the clinical trial (determined as last subject, last visit), any unexpected safety issue that changes the risk-benefit analysis and is likely to have an impact on the subjects

who have participated in it, will be reported by the Sponsor as soon as possible to the competent authority (ies) concerned together with proposed actions.

8.7. Reporting Pregnancies

Pregnancies for women will be reported from the time the subject signs the informed consent form through final clinical trial visit or 30 days after the last IP administration, whichever is later. Males will report pregnancy of their partners through 90 days after the last IP administration.

Subjects who become pregnant will be withdrawn from the clinical trial. The pregnancy should be followed up to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications.

Pregnancy should be recorded on a subject's source documents and a Pregnancy Notification and Outcome Form and reported using the same procedure for reporting SAEs detailed in Section 8.6.2. A pregnancy, by itself, is not an SAE. Pregnancy follow-up should also be recorded and should include an assessment of the possible relationship to the IP of any pregnancy outcome. Any pregnancy-related SAE (e.g., spontaneous abortion, birth defect) or any other SAE experienced during pregnancy must be recorded on a separate SAE Report Form and reported per SAE reporting procedures in Section 8.6.2.

9. STATISTICS HYPOTHESES AND METHODS OF ANALYSES

9.1. Clinical Trial Populations

9.1.1. Intent-to-Treat (ITT)

The Intent-to-Treat (ITT) population consists of all subjects who are enrolled and receive the IP. All data will be included, and no subjects will be excluded because of protocol violations.

9.1.2. Per-Protocol Population

The Per-Protocol (PP) population is a subset of the ITT population and includes the subjects who completed the trial with no major protocol violations.

9.1.3. Safety Population

The safety population includes all enrolled subjects who received the test article. The safety population will be analyzed as treated and will be used for the safety analyses. No data will be excluded for any reason.

[redacted]

9.2. Statistical Analysis

9.2.1. General Considerations

[redacted]

Quantitative variables will be summarized descriptively using number of subjects (n), mean, standard deviation, median, minimum, and maximum. Qualitative variables will be summarized using counts and percentages.

All analyses will be two-sided at a significance level of 0.05. The 95% confidence intervals will be provided where appropriate.

The SAP will serve as the final determinant of the statistical procedures, notwithstanding anything herein.

9.2.2. General Imputation Methods

Every attempt will be made to capture all clinical trial data. The method on how to handle the complete and/or partial missing date in the adverse events and medications will be addressed in these individual analysis sections of the SAP.

9.2.3. Multiplicity Consideration

Only one hypothesis will be tested. No multiplicity adjustment will be done in this clinical trial.

9.2.4. Demographic and Baseline Medical History

The demographic and baseline medical history data, obtained at screening, will be summarized descriptively. For quantitative variables, the summaries will include the number of observations, mean, standard deviation, median, minimum, and maximum. Qualitative variables will be summarized using counts and percentages.

9.2.5. Primary Endpoint Analyses

Safety endpoints will be summarized descriptively. Safety endpoints include:

- Slit-lamp evaluation
- Dilated ophthalmoscopy
- Adverse event (AE) query (reported, elicited, and observed)

Adverse events will be summarized descriptively. Frequencies and percentages will be provided per treatment group of subjects with treatment-emergent adverse events (TEAEs), serious TEAEs, and TEAEs causing premature discontinuation. An adverse event is treatment-emergent if it occurs after the first dose of investigational treatment. Furthermore, frequencies will be given of subjects with TEAEs by system organ class, by system organ class and preferred term, by system organ class, preferred term and maximal severity, by system organ class, preferred

term and strongest relationship, and by system organ class, preferred term, maximal severity, and strongest relationship.

9.2.6. Secondary Endpoint Analyses

Secondary endpoints will be analyzed to evaluate for change from Screening Visit B (Visit 2) to the level at Week 16 (Visit 10). Change from Screening level will be the dependent variable. The secondary endpoints include letters of BCVA or LLVA, improvement in visual field area and microperimetry, and increase in dark-adapted flash b-wave amplitudes.

9.2.7. Planned Interim Analyses (if applicable)

No interim analyses are planned for this clinical trial.

9.2.8. Statistical Analyses Plan

Prior to database lock, the Sponsor will finalize a statistical analysis plan (SAP) that will detail all planned analyses. Any analyses conducted in addition to those specified in the SAP will be clearly documented as post hoc. The SAP will serve as the final determinant of the statistical procedures, notwithstanding anything herein.

10. QUALITY CONTROL AND QUALITY ASSURANCE

The Sponsor will implement a system of quality assurance that includes all elements described in this protocol. Within this system, standard operating procedures (SOPs) from the Sponsor and CRO will be implemented to ensure that the clinical trial is conducted in compliance with regulatory requirements and GCP. Quality control will be applied to each stage of data handling to ensure that data are accurate, reliable and processed correctly.

11. CLINICAL TRIAL ADMINISTRATION

11.1. Institutional Review Board / Research Ethics Board / Independent Ethics Committee

The protocol and all protocol amendments must be signed and dated by the investigator and approved in writing by the applicable IRB/REB/IEC in accordance with GCP prior to implementation. In addition, the IRB/REB/IEC must approve the written informed consent and assent forms, any consent or assent form updates, subject recruitment procedures (e.g., advertisements), and any written information to be provided to subjects prior to implementation.

The investigator must provide an annual report to the IRB/REB/IEC on the progress of the clinical trial including number of subjects enrolled, discontinued, and SAEs, unless otherwise specified by the IRB/REB/IEC. It is required that a yearly review of the protocol by the IRB/REB/IEC be documented in a letter from the IRB/REB/IEC. The investigator must provide notification to the IRB/REB/IEC of the completion, termination, or discontinuation of the clinical trial.

The investigator must supply the Sponsor with copies of all written correspondence with the IRB/REB/IEC.

The investigator will make all attempts to ensure that the IRB is constituted and operates in accordance with regulatory requirements, ICH GCP and any local requirements.

11.2. Ethical Conduct of the Clinical Trial

The clinical trial will be carried out in keeping with national and local legal requirements, including in accordance with regulatory requirements outlined in Food and Drug Administration (FDA) Code of Federal Regulations (CFR) Title 21 [Part 50, Part 54, Part 56, Part 312 and Part 11] as well as the ICH GCP E6 Guidelines. This clinical trial was designed and shall be implemented and reported in accordance with the ICH Harmonized Tripartite Guidelines for Good Clinical Practice, with applicable local regulations [including European Directive 2001/20/EC, US CFR Title 21], and with the ethical principles laid down in the Declaration of Helsinki.

11.3. Subject Informed Consent

The Sponsor will provide sample Informed Consent Forms for use in the clinical trial. Any changes to the proposed consent and assent forms suggested by the investigator must be agreed to by the Sponsor before submission to the IRB/REB/EC. The investigator or designee will provide the Sponsor with a copy of the IRB/REB/IEC-approved informed consent and assent forms prior to the start of the clinical trial.

Before each subject is enrolled in the clinical trial, written informed consent will be obtained according to the regulatory and legal requirements of the participating site.

The subjects should sign the current final IRB/REC/EC approved consent form. The process of obtaining informed consent and assent should be documented in the subject source documents. Each investigator must retain the original signed and dated informed consent and assent forms. A copy of the signed and dated informed consent and assent forms will be given to the subject. No subject can enter the clinical trial, or have clinical trial-specific assessments performed before his/her informed consent has been obtained.

The Informed Consent Forms should be revised whenever there are substantial changes to procedures or when new information becomes available that may affect the willingness of the patient to participate. Revisions to the consent forms required during the clinical trial must be approved by the Sponsor, and a copy of the revised consent forms provided to the Sponsor. For any updated or revised forms, the subjects must be re-consented for continued participation in the clinical trial.

11.4. Confidentiality

All clinical trial findings and documents will be regarded as confidential. The investigator and qualified site staff must not disclose such information without prior written approval from the Sponsor. The investigator agrees not to use or disclose protected health information other than as permitted or required by the subject authorization or as required by law.

Subject confidentiality will be strictly maintained to the extent possible under the law. Subject names must not be disclosed. Subjects will be identified on the CRFs and other documents submitted to the Sponsor by their initials (if locally permissible) and/or assigned subject number; not by name. Documents that identify the subject (e.g., the signed informed consent form) must be maintained in confidence by the investigator. All clinical trial documents are provided by the Sponsor in confidence to the investigator and qualified site staff. None of this material may be disclosed to any party not directly involved in the clinical trial without Sponsor's written permission. The investigator must assure that subjects' anonymity will be maintained. The investigator will maintain this for the longest period of time allowed by his/her own institution and, in any case, until further communication from the Sponsor.

11.5. Protection of Subject Data

The collection and processing of personal data from subjects enrolled in the clinical trial will be limited to those data that are necessary to investigate the safety, quality, and utility of the IP) used in the clinical trial and to support the development and interpretation of the trial's clinical outcomes assessments.

The data must be collected and processed with adequate precautions to ensure confidentiality and compliance with applicable data privacy protection laws and regulations. Appropriate technical and organizational measures to protect the personal data against unauthorized disclosures or access, accidental or unlawful destruction, or accidental loss or alteration must be put in place. Sponsor personnel whose responsibilities require access to personal data agree to keep the identity of subjects confidential.

The informed consent obtained from the subject includes explicit consent for the collection and processing of personal data and for the investigator to allow direct access to his or her original medical records for clinical trial-related monitoring, audit(s), IRB/REB/EC review, and regulatory inspection. The consent also addresses the transfer of the data to other entities and to other countries.

11.6. Clinical Trial Monitoring

Prior to initiation of the clinical trial at a site, the Clinical Trial Monitor, who is an authorized individual designated by the Sponsor, will visit the site to verify the qualifications of the investigator and designated site staff, inspect the adequacy of the facilities, and inform the clinical trial team of responsibilities and the procedures to ensure proper conduct of the clinical trial. During the conduct of the clinical trial, the Clinical Trial Monitor will visit the sites to verify adherence to the protocol, assess drug accountability, data integrity, and subject safety. The monitors will conduct 100% source document verification of subject data by comparing the CRFs with the source documents to ensure accuracy and consistency.

All aspects of the clinical trial will be carefully monitored with respect to GCP and SOPs for compliance with applicable government regulations. The Clinical Trial Monitor will have access to all records necessary to ensure integrity of the data and safety of the subject, and will periodically review the progress of the clinical trial with the investigator.

Frequent communication between the clinical site and the Sponsor is essential to ensure that the safety of the clinical trial is monitored adequately.

11.7. Procedures for Training of Clinicians on Procedural Intervention

All procedures required for the control and interventional cohorts are standard practice for the investigators of the clinical trial. To assure that procedures are consistent with the protocol, the investigator's will be trained on the required methods and procedures at site initiation or training Webex.

11.8. Procedures for Training Interventionists and Monitoring Intervention Fidelity

Prior to a site's enrollment of subjects, the investigator and applicable clinical trial team members will be trained on the protocol and associated documentation.

11.9. Case Report Forms and Source Documents

Paper case report forms will be used to collect study and subject data at the research site. After monitoring the data, the information collected from the site will be double entered into a regulatory compliant database for review and analysis.

The investigator will be responsible for the accuracy of the data entered in the case report forms and ensure that the data collected are accurate and complete. The paper CRF will be source data verified by the clinical monitor. Any changes required following monitoring will be made by the investigator or qualified site staff and will be documented with a full audit trail on the CRF, and within the database system.

The investigator or qualified site staff will prepare and maintain adequate and accurate clinical trial documents (e.g., medical records, AE and concomitant medication reporting, source data collection forms, etc.) designed to record all observations and other pertinent data for each subject. It is required that the author of an entry in the source documents be identifiable.

Source data may be directly captured from devices, transferred from third parties (e.g., laboratory data, imaging). This information may be uploaded into secure files for study analysis. The remainder of the data, captured initially on paper, may be entered retrospectively into the database system.

For each subject, CRF and corresponding source records will be maintained at each clinical site. CRFs should be completed in a timely manner, and every effort should be made to have forms completed and current in anticipation of a visit by the Sponsor or designee. Upon clinical trial completion, the monitor will arrange for a final review of the clinical trial files, after which the file should be secured by storage for the appropriate period as specified in Section 12.

The investigator will allow the Sponsor or designee(s), contract designees, authorized regulatory authority inspectors, and the IRB/REB/IEC to have direct access to all documents pertaining to the clinical trial.

11.10. Access to Source Documents and Audits

Regulatory agencies may request access to all clinical trial records, including source documents, for inspection and copying, in keeping with country regulations. The investigator should immediately notify the Sponsor of any announced or unannounced regulatory agency inspections. An auditing inspection may also be conducted by the Sponsor representative or designee. Any aspect of the trial may be subject to audit by the Sponsor and/or inspection by regulatory authorities or the IRB/REB/IEC. Such audits/inspections may take place at the Sponsor's site(s), the CRO, or at the clinical sites, including laboratories, pharmacies and any other facilities used for the clinical trial. The investigator will permit the designated Sponsor representatives and regulatory bodies to have direct access to the source documents to verify data represented in the CRF and database system.

11.11. Protocol Deviations and Violations

Exceptions to the eligibility criteria will not be granted. It is expected that subjects will meet all eligibility criteria. Departures from the protocol should be avoided, unless required for the safety of the subject. Should other unexpected circumstances arise that will require deviation from protocol-specific procedures, the investigator should contact the Sponsor to discuss the appropriate course of action.

The investigator should document all protocol deviations/violations in the subject's CRF and source documents or the investigator Site File, if appropriate. Protocol deviations will be documented by the clinical trial monitor and will be included in the final clinical trial report. Protocol deviations should be submitted to IRB/REB/IEC, in accordance with the site's IRB/REB/IEC requirements.

11.12. Amendments to the Protocol

To alter the protocol, amendments must be written by the Sponsor and approvals must be received from all parties that approved the original protocol (IRB/REB/IEC, and if applicable, the local regulatory authorities) before implementation. Notwithstanding the need for approval of formal protocol amendments, the investigator is expected to take any immediate action required for the safety of any subject included in the clinical trial, even if this action represents a deviation from the protocol.

The Sponsor may make administrative changes (i.e., changes that do not significantly affect subject safety, the clinical trial's scope or scientific quality) without a formal protocol amendment.

11.13. Discontinuation of the Clinical Trial

The Sponsor reserves the right to discontinue the clinical trial under the conditions specified in the clinical trial agreement.

11.14. Investigator Responsibilities

The investigator(s) should be qualified by education, training, and experience to assume responsibility for the proper conduct of the clinical trial, should meet all the qualifications

specified by the applicable regulatory requirement(s), and should provide evidence of such qualifications through up-to-date curriculum vitae and/or other relevant documentation requested by the Sponsor, the IEC/REB/IRB, and/or the regulatory authority(ies).

The investigator is responsible for ensuring that the clinical trial is performed in accordance with the protocol, current ICH guidelines on GCP, and applicable regulatory and local requirements.

GCP is an international ethical and scientific quality standard for designing, conducting, recording, and reporting studies that involve the participation of human subjects. Compliance with this standard provides public assurance that the rights, safety, and well-being of subjects are protected, consistent with the principles that originated in the Declaration of Helsinki, and that the clinical trial data are credible.

11.15. Financial Disclosure

The investigator is required to disclose any financial arrangement during the clinical trial and for one year after, whereby the outcome of the clinical trial could be influenced by the value of the compensation for conducting the clinical trial, or other payments the investigator received from the Sponsor. The following information is collected: any significant payments from the Sponsor or subsidiaries such as a grant to fund ongoing research, compensation in the form of equipment, retainer for ongoing consultation or honoraria; any proprietary interest in investigational product; any significant equity interest in the Sponsor or subsidiaries as defined in 21 CFR 54(2)(b) (1998).

11.16. Registration of Clinical Studies and Disclosure of Results

The Sponsor or designee will register and/or disclose the existence of and the results of clinical trials as required by law.

11.17. Publication and Disclosure Policy

As is customary for multicenter trials, publication by individual clinical sites or investigator/institution will not be allowed without the explicit written permission of the Sponsor. The Sponsor will determine authorship of the principal clinical trial manuscript(s) in conjunction with the investigators, in abiding with current guidelines and requirements of medical journals. For such manuscript(s), masthead roles for investigators will be determined based on subject enrollment and scientific contributions to the clinical trial.

12. RETENTION OF RECORDS

The investigator agrees to adhere to the document retention procedures by signing the protocol. Essential documents, as listed below, must be retained by the investigator for as long as required by national and international regulations. The Sponsor will notify the investigator(s)/institution(s) when the clinical trial-related records are no longer required.

Essential documents include but are not limited to:

- IRB/REB/IEC approvals for the clinical trial protocol and all amendments

- All source documents and laboratory records
- CRF copies
- Subjects' informed consent / assent forms (with clinical trial number and title of trial)
- Form FDA 1572
- Any other pertinent clinical trial documents

All source documents (e.g., informed consent forms, laboratory reports, progress notes, medical histories, physical and diagnostic findings, IP dispensing/disposition records) that support data in the CRFs of each subject must be retained in the files of the responsible investigator.

According to ICH guidelines for GCP, essential documents should be retained for a minimum of two years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. However, these documents should be retained for a longer period (25 years) if required by relevant regulatory or legal authorities.

If the responsible investigator retires, relocates or for any other reason withdraws from the responsibility of keeping the clinical trial records, custody must be transferred to a person who will accept the responsibility. The Sponsor representative must be notified in writing of the name and address of the new custodian, prior to the transfer.

No records should be disposed of without written approval of the Sponsor.

REFERENCES