Protocol Title: An Investigation of Vitamin A Palmitate Supplementation in Patients with Age-Related Macular Degeneration (and without reticular pseudodrusen) and Delayed Dark Adaptation

Abbreviated Title: DA VitA AMD

Protocol Number: 18-EI-0067

Date of This Submission/Version: September 6, 2023/v9.0

Principal Investigator

[redacted]

Coordinating Center: [redacted]

Total Requested Accrual:

18 Cohort 1 Participants:

Eight participants were enrolled, including 3 screen failures. Five participants were included in the primary analysis.

Cohort 2 Participants:

Up to 5 participants will initially be enrolled in the second cohort. Up to an additional 5 participants may be enrolled in order to obtain 5 participants for primary outcome analysis.

Total Participants:

A total of 18 participants (8 participants in Cohort 1 and up to 10 participants in Cohort 2) may be enrolled in the study. A total of 10 participants (5 in each cohort) may be included in the primary analysis for the study.

0 Healthy Volunteers

Project Uses Ionizing Radiation: ☒ No ☐ Yes

IND/IDE: ☐ No ☒ Yes

Drug/Device #: 138, 192

Sponsor: [redacted]

Durable Power of Attorney: ☒ No ☐ Yes

Multi-institutional Project: ☒ No ☐ Yes

Data and Safety Monitoring Board: ☒ No ☐ Yes

NEI SAE Review Committee: ☐ No ☒ Yes

Technology Transfer Agreement: ☒ No ☐ Yes

Samples Being Stored for Future Research: ☒ No ☐ Yes

Covered Protocol Requiring DEC Clearance (NIH Policy 102) ☐ No ☒ Yes

Approved for Short Form Consent Process for Non-English Speakers ☐ No ☒ Yes

Consent and Assent

- **Flesch-Kincaid Reading Level of Consent:** 8.7

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LIST OF ABBREVIATIONS

Abbreviation	Term
BCVA	Best-corrected visual acuity
CC	Clinical Center
CD	Clinical Director
CFP	Color Fundus Photography
CFR	Code of Federal Regulations
CRIS	Clinical Research Information System
CTCAE	Common Terminology Criteria for Adverse Events
Emmes	The Emmes Company, LLC
EMR	Electronic Medical Record
ETDRS	Early Treatment Diabetic Retinopathy Study
FAF	Fundus Autoflourescence Imaging
FDA	Food and Drug Administration
HRPP	Human Research Protection Program
IND	Investigational New Drug
IOP	Intraocular Pressure
IP	Investigational Product
IRB	Institutional Review Board
IU	International Unit
LLQ	Low luminance questionnaire
LLVA	Low luminance visual acuity
LORD	Late-onset retinal disease
NEI	National Eye Institute
NEI QA	National Eye Institute Quality Assurance
NEIS	National Eye Institute Support
NIH	National Institutes of Health
OCT	Optical Coherence Tomography
PI	Principal Investigator
RIT	Rod Intercept Time
RPD	Reticular Pseudodrusen
RPE	Retinal Pigment Epithelium
SAE	Serious Adverse Event
SOP	Standard Operating Procedures
USP	United States Pharmacopeia
YAG	Yttrium-Aluminum Garnet

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PRÉCIS

Objective: The objective of this study is to investigate the potential efficacy and safety of vitamin A palmitate dosing in improving dark adaptation in participants with age-related macular degeneration (AMD) and abnormal dark adaptation.

Study Population: The first cohort consists of five participants with AMD who meet the eligibility criteria. The second cohort will consist of five participants with AMD who meet the eligibility criteria. Up to five additional participants may be accrued in the second cohort to account for participants who withdraw from the study prior to receiving one month of study supplementation for a reason unrelated to an adverse reaction. Up to 18 participants may be enrolled in this study.

Design: This is a prospective, uncontrolled, single center, pilot study to investigate the potential efficacy and safety of vitamin A palmitate dosing in improving dark adaptation in participants with AMD and abnormal dark adaptation. Participants in the first cohort were instructed to take 16,000 IU of vitamin A palmitate daily for two months. Enrollment for Cohort 1 ended on May 24, 2019. Participants in the second cohort will be instructed to take 48,000 IU of vitamin A palmitate daily for one month. Participants in both cohorts will continue in the study for one month after ending Vitamin A supplementation. Participants in Cohort 1 may enroll into Cohort 2 as long as their last intake of vitamin A palmitate was greater than two months prior to their enrollment into Cohort 2.

Outcome Measures: For each cohort, the primary outcome is the measurement of dark adaptation parameters (thresholds and kinetics) by the following: dark adaptation times as measured by the AdaptDx comparing before and after vitamin A palmitate supplementation and dark adaptation parameters as measured by the Medmont comparing before and after vitamin A palmitate supplementation. The primary outcome will be assessed at Month 2 in the first cohort and Month 1 in the second cohort. For both cohorts, the secondary outcomes include changes in low luminance visual acuity (LLVA) and changes in patient reported outcomes as measured by the low luminance questionnaire (LLQ). The secondary outcomes also include measurement of dark adaptation parameters (thresholds and kinetics) comparing baseline and one month after completing supplementation (Month 3 in Cohort 1 and Month 2 in Cohort 2).

1.0 INTRODUCTION/SCIENTIFIC RATIONALE

Age-related macular degeneration (AMD) has been the leading cause of central vision loss in people age 65 or older in developed countries[1,2]. Decreases in central vision from late AMD is well-established, and even intermediate AMD can display small but statistically significant reductions in central acuity compared with those without AMD[3-5]. However, earlier cell changes accompanying AMD have direct links to additional measures of retinal function. Histopathological examination of eyes from patients with AMD has demonstrated preferential loss of rods in the photoreceptor layer of the retina with cones persisting as the last surviving photoreceptors[6-8]. Studies employing multiple approaches to measure rod and cone function have documented preferential reduced rod function in eyes with AMD[9-14]. A focal dark adaptometer able to focus on areas 0.5 to 3 mm from the fovea, areas thought to have earliest rod loss, has demonstrated impairments in eyes with non-advanced AMD compared to older eyes without AMD, even when visual acuity varied little between severity groups[11]. Increasing AMD severity was associated with increased rod intercept time (RIT), an outcome of dark adaptation, with eyes having reticular pseudodrusen (RPD) demonstrating the most significant delays[11].

Interpreting the data on dark adaptation across different phenotypes of macula, there are several possible hypotheses that have been proposed in the literature. A testable hypothesis proposed in the literature is that the sub-RPE (retinal pigment epithelium) deposits act as diffusion barriers for entry of sufficient vitamin A into the photoreceptors. This would lead to a local deficiency or a form of nutritional deprivation at the level of the photoreceptor. Vitamin A deficiency has long been known to cause night blindness with rod dysfunction and while these patients are not deficient based on serum levels, there still could be a local deficiency at the level of photoreceptors. Without available vitamin A (due to a serum deficiency or local deficiency) to combine with opsin, photoreceptors cannot regenerate functional visual pigment at the normal rate after light exposure leading to a slowing of dark adaptation.

A first step in understanding the pathophysiology identified by abnormal dark adaptation would be to supplement eyes with abnormal dark adaptation with vitamin A palmitate. Restoration or improvement of visual function while on supplemental vitamin A palmitate would provide data to support this hypothesis in the setting of AMD. Jacobson et al[15] tested this hypothesis in patients with Sorsby's fundus dystrophy and demonstrated that vitamin A palmitate

supplementation could restore dark adaptation in these patients using 50,000 IU vitamin A palmitate for one month, but was also able to show maintenance of some (but not all) aspects of dark adaptation with 5,000 IU vitamin A palmitate[15]. Ayyagari and Sieving et al[16] demonstrated that the dark adaptation of patients with late-onset retinal disease (LORD) with mutations in CTRP5 could be shifted with 15,000 IU vitamin A palmitate within two months of supplementation. Owsley et al[17] initiated investigation into this question in older adults with early AMD and the results demonstrated that a short-term, high-dose course of preformed vitamin A increased the rate of rod-mediated dark adaptation. However, this hypothesis could be investigated with greater depth using more refined fundus phenotyping and psychophysical testing.

The preliminary analysis of the data from Cohort 1 in the DA VitA study shows that 16,000 IU of vitamin A palmitate per day was well tolerated with no adverse events requiring discontinuation of the study product. Given the previously demonstrated efficacy of vitamin A palmitate supplementation in the treatment of Sorsby's fundus dystrophy[15] and eyes with early AMD[17], the introduction of a second cohort that will take an increased dose of vitamin A palmitate (48,000 IU per day) in the DA VitA study is necessary since the data show that Cohort 1 did not present significant improvement in dark adaptation after taking 16,000 IU per day. The knowledge gained would provide further insights into pathogenesis of age-related macular degeneration.

2.0 STUDY OBJECTIVES

The study objective is to investigate the potential efficacy and safety of vitamin A palmitate dosing in improving dark adaptation in participants with AMD and abnormal dark adaptation.

3.0 PARTICIPANTS

Five participants with AMD who met the eligibility criteria were enrolled into the first cohort. Enrollment for Cohort 1 was halted on May 24, 2019 to focus on enrollment for Cohort 2. For the second cohort, up to five participants with AMD who meet the eligibility criteria may be enrolled in this protocol. Up to five additional participants may be accrued in the second cohort to account for participants who withdraw from the study prior to receiving one month of study supplementation for a reason unrelated to an adverse reaction. Participants in Cohort 1 may enroll

into Cohort 2 as long as their last intake of vitamin A palmitate was greater than two months prior to their enrollment into Cohort 2.

3.1 Participant Eligibility Criteria

3.1.1 Inclusion Criteria

To be eligible, the following inclusion criteria must be met, where applicable.

1. Participant must be 50 years of age or older.
2. Participant must understand and sign the protocol's informed consent document.
3. Any participant of childbearing potential (see Appendix 1 for definition) must be willing to undergo urine pregnancy tests throughout the study.
4. Any participant of childbearing potential (see Appendix 1 for definition) and any participant able to father children must have (or have a partner who has) had a hysterectomy or vasectomy, be completely abstinent from intercourse, or must agree to practice at least one acceptable method of contraception throughout the course of the study and for one week after study supplement discontinuation. Acceptable methods of contraception include:
    - Hormonal contraception (i.e. birth control pills, injected hormones, dermal patch or vaginal ring),
    - Intrauterine device,
    - Barrier methods (diaphragm, condom) with spermicide, or
    - Surgical sterilization (tubal ligation).
5. Participants must agree to notify the study investigator or coordinator if any of their doctors initiate a new prescription medication during the course of this study.
6. Participant must agree to not take ≥8,000 IU vitamin A palmitate outside the study supplementation.
7. For supplementation eligibility, participant must have normal liver function as demonstrated by the Chemistry 20 panel or have mild abnormalities not above grade 1 as defined by the Common Terminology Criteria for Adverse Events v4.0 (CTCAE).
8. Participant must not be pregnant or breast-feeding and must have a negative urine pregnancy test within 24 hours prior to initiation of study medication.

3.1.2 Exclusion Criteria

A participant is not eligible if any of the following exclusion criteria are present.

1. Participant is in another investigational study and actively receiving study therapy.
2. Participant is unable to comply with study procedures or follow-up visits.
3. Participant is already taking vitamin A palmitate supplements ≥ 8,000 IU.
4. Participant has a history of vitamin A deficiency.
5. Participant has a condition that, in the opinion of the investigator, would preclude participation in the study (e.g., unstable medical status including blood pressure and glycemic control).
6. Participant has a history of hepatitis or liver failure.
7. Participant has chronic gastrointestinal disease.
8. Participant will be excluded if the participant has serologic evidence of an active hepatitis infection.
9. Participant was in Cohort 1 and took his/her last dose of vitamin A palmitate less than two months prior to enrolling in Cohort 2.

3.2 Study Eye Eligibility Criteria

The participant must have at least one eye meeting all inclusion criteria and none of the exclusion criteria listed below.

3.2.1 Study Eye Inclusion Criteria

1. The eye must have a best-corrected ETDRS visual acuity score better than or equal to 20/80 (i.e., equal to or better than 54 letters).
2. Participant must have at least one large druse.
3. Abnormal dark adaptation, which is defined as having an AdaptDx test with a RIT of 16 minutes or more at the screening visit. This is at least one standard deviation greater than the average normal RIT and includes room to account for variability in testing. If at any point during current testing or under a previous NEI protocol, a participant has exceeded the 40 minute test ceiling, they will have satisfied the inclusion criteria.

3.2.2 Study Eye Exclusion Criteria

1. Presence of advanced macular degeneration with central geographic atrophy or choroidal neovascularization.

2. Presence of definite reticular pseudodrusen.
3. An ocular condition is present (other than AMD) that, in the opinion of the investigator, might alter visual acuity during the course of the study (e.g., vein occlusion, uveitis or other ocular inflammatory disease, neovascular glaucoma, Irvine-Gass Syndrome, etc.).
4. Substantial cataract that, in the opinion of the investigator, is likely to be decreasing visual acuity by three lines or more (i.e., cataract would be reducing acuity to 20/40 or worse if eye was otherwise normal).
5. History of major ocular surgery (e.g. cataract extraction, scleral buckle, any intraocular surgery, etc.) within three months prior to study entry.
6. History of YAG (Yttrium-Aluminum Garnet) capsulotomy performed within two months prior to study entry.

3.3 Choice of Study Eye in Cases of Bilateral Disease

If both eyes meet the study eye eligibility criteria described above, the following criteria will be used to select the study eye for the purposes of this investigation:

1. The eye with the better visual acuity will be chosen.
2. If both eyes are equal acuity, the right eye will be arbitrarily chosen as the study eye.

4.0 STUDY DESIGN AND METHODS

This is a prospective, uncontrolled, single center, pilot study to investigate the potential efficacy and safety of taking 16,000 IU (Cohort 1) or 48,000 IU (Cohort 2) of vitamin A palmitate in improving dark adaptation in participants with AMD and abnormal dark adaptation.

4.1 Recruitment

Participants will be recruited from the NEI clinic. Participants will be initially recruited from the following protocols: the NEI screening protocol (08-EI-0102), the Ocular Natural History protocol (16-EI-0134), DA Extension (17-EI-0112), Rod Function (16-EI-0024), or DA_AMD (11-EI-0147). Eligibility will be determined by the investigator. The participants must have the presence of one large druse. Self-referral and referral from outside physicians will be permitted. If any recruitment materials are utilized, IRB-approval will be obtained prior to distribution. The projected recruitment time for five participants in the second cohort is approximately 12 months.

4.2 Screening Visit

After the consent form is signed, all potential participants will undergo a screening evaluation in the NEI ophthalmology clinic to determine whether they meet the full eligibility criteria for vitamin A supplementation. A screening visit will be used to understand the level of dark adaptation impairment and to assess the appropriateness of the participant for inclusion. The screening and baseline examinations are outlined in Appendix 2 and listed in section 4.3.1. The investigator will review the assessments and serologic testing conducted at the screening visit prior to inviting the participant to return for continuation and vitamin A supplementation.

4.3 Study Design and Procedures

The study duration will be three months for the first cohort during which participants will be instructed to take vitamin A palmitate 16,000 IU daily by mouth for two months. In the second cohort, the study duration will be two months and the participants will be instructed to take vitamin A palmitate 48,000 IU daily by mouth for one month. In addition, there will be a screening visit between 60 days and one day prior to the baseline visit. For the first cohort, the primary outcome will be assessed at baseline versus Month 2 and the secondary outcomes will be assessed at baseline versus Month 2 and baseline versus Month 3. For the second cohort, the primary outcomes will be assessed at baseline versus Month 1 and the secondary outcomes will be assessed at baseline versus Month 1 and baseline versus Month 2. For both cohorts, visits will occur at baseline, and then monthly and as clinically indicated. The study will require five visits for the first cohort and four visits for the second cohort. All visits must be conducted within a window of ± 14 days from the target day. At each visit, the participant will undergo a review of systems, such as an assessment of headache frequency and severity, as well as an assessment of safety variables. The tests scheduled at each visit may be split and completed within seven days (including the 7^{th} day) from the visit date. A complete ophthalmologic examination will be performed at each visit to measure outcome variables. Participants will be screened with serum for hepatitis or hepatic dysfunction. However, for caution, we will monitor liver function tests. If participants in either cohort cannot tolerate the prescribed dose of vitamin A palmitate, they will be instructed to stop taking the supplement and they will be removed from the study.

If participants are on Coumadin® (Warfarin) at enrollment, they must visit their prescribing physician to check their Coumadin® status within two to four weeks after initiating IP to have their

Coumadin® regimen adjusted if necessary (as taking vitamin A and Coumadin® can increase the chances of bruising and bleeding.)

4.3.1 Ocular and Systemic Evaluations

The following examinations will be performed at the study visits as indicated in the study flowsheet (Appendix 2):

1. Medical/Ophthalmic History
2. Supplement Accountability
3. Review of Systems
4. Concomitant Medication Assessment
5. Adverse Event Assessment
6. Best-Corrected Visual Acuity (BCVA) (ETDRS) (Short and full testing)
7. Manifest Refraction
8. Slit Lamp Examination
9. Dilated Fundus Examination
10. Intraocular Pressure (IOP)
11. Color Fundus Photography on both eyes*
12. Dark Adaptation using the AdaptDx in the study eye
13. Dark Adaptation using the Medmont in the study eye
14. Fundus Autofluorescence Imaging (FAF) on both eyes*
15. Optical Coherence Tomography (OCT) on both eyes
16. Low Luminance Visual Acuity (LLVA) in both eyes
17. Low Luminance Questionnaire (LLQ)
18. Hepatitis Screening (Hepatitis C antibody (HCV Ab) test and Hepatitis B surface antigen (HBsAg) test)
19. Hepatic Panel (Checks liver function testing)
20. Serum Vitamin A Levels
21. Pregnancy Test for Participants of Childbearing Potential (See Appendix 1 for definition)

*Imaging may be done under this or another protocol if done within six months of baseline.

4.4 Study and Concomitant Therapies

4.4.1 Study Supplement Formulation

The vitamin A palmitate supplement will be supplied as 8,000 IU pills verified by United States Pharmacopeia (USP).

4.4.2 Dosage, Administration, and Storage

For both cohorts, participants are considered to have complied with the protocol if they have consumed at least 80% of the total doses over the treatment period. The first cohort was instructed to take two 8,000 IU pills by mouth once per day (16,000 IU) with a meal. The preliminary analysis of the data from the first cohort shows that 16,000 IU of vitamin A palmitate per day was well tolerated with no adverse events requiring discontinuation of the study product. The data also show that the first cohort did not present significant improvement in dark adaptation after taking 16,000 IU of vitamin A palmitate per day. A higher dose (50,000 IU) has been previously used in other studies of monogenic (inherited) retinal degeneration with good effect and good tolerance.[26] It has even been explored in the context of individuals ≥ 50 years old with AMD, albeit a different study design, but the higher dose of vitamin A was well-tolerated.[16] Therefore, for the second cohort, administration of three 8,000 IU pills will occur by mouth twice per day (48,000 IU) and should be taken with a meal. The vitamin A palmitate supplement should be stored at room temperature (15-30°C).

4.4.3 Concomitant Therapy

Participants in both cohorts may continue to receive all medications and standard treatments administered for their conditions at the discretion of their treating physician, except for high-dose vitamin A palmitate supplementation (≥ 8,000 IU per day). A participant will be withdrawn from the study if placed on a daily dose of ≥8,000 IU of vitamin A palmitate.

There are no pharmacologic interactions known to occur between vitamin A palmitate and tetracyclines, but there is the potential for additive side effects as each drug has the potential to cause intracranial hypertension. One case report in a patient receiving high doses of vitamin A palmitate combined with minocycline reported the development of benign intracranial hypertension.[24] For this reason, regular ophthalmic examinations during study visits is the

recommended care for participants receiving the drug combination. Specific questions regarding headaches and attention to optic nerve for presence of papilledema will be noted at each visit.

If participants are on Coumadin® at enrollment, they must visit their prescribing physician to check their Coumadin® status within 2-4 weeks after initiating study supplementation to have their Coumadin® regimen adjusted if necessary (as taking vitamin A palmitate and Coumadin® can increase the chances of bruising and bleeding.)

All participants are eligible to receive standard-of-care treatment for AMD while enrolled in this study. However, if a participant develops neovascular AMD in the study eye during the course of the study, the participant may receive standard-of-care treatment for neovascular AMD according to the recommendations of the participant's treating retina specialist. The participant will be withdrawn from this study and the last observation will be carried forward for data analyses.

4.4.4 Study Supplement Accountability

The NIH pharmacy is responsible for the accountability of all undispensed study supplement. Adequate supplement accountability records include documentation of all study supplement and supplies shipped, received, dispensed and returned to the NIH pharmacy. The NIH pharmacy will destroy returned study supplement and supplies following reconciliation by the Coordinating Center. The investigator is responsible for the accountability of all dispensed study supplement. Adequate supplement accountability records include documentation of all study supplement administered and disposed of by the investigator.

4.5 End of Participation

At the conclusion of the study, the participants will no longer be able to receive the study supplement under this protocol. Follow-up care will be arranged with an outside ophthalmologist or the participant will continue to be seen at the NIH under another protocol, if available and the participant is eligible. The participants and their physicians, with written consent, will be informed of their disease status during this study. Clinical data obtained during participation may be shared with the participants and with written permission from the participants, their private physicians. Results from the overall study may be shared once the study team has analyzed the data from all participants.

4.6 Storage of Samples and Data

No samples will be stored for this study. The clinical data will be stored in the NEI's electronic medical record, the Clinical Research Information System (CRIS) and Emmes' database. All individual data will remain confidential.

Data may also be shared with collaborating laboratories at NIH or outside of NIH and/or submitted to NIH-designated repositories and databases if consent for sharing was obtained. Repositories receiving data from this protocol may be open-access or restricted access.

Data will be stripped of identifiers and may be coded ("de-identified") or unlinked from an identifying code ("anonymized"). When coded data is shared, the key to the code will not be provided to collaborators, but will remain at NIH. Data may be shared with investigators and institutions with an FWA or operating under the Declaration of Helsinki (DoH) and reported at the time of continuing review. Sharing with investigators without an FWA or not operating under the DoH will be submitted for prospective IRB approval. Submissions to NIH-sponsored or supported databases and repositories will be reported at the time of continuing review. Submissions to non-NIH sponsored or supported databases and repositories will be submitted for prospective IRB approval.

Required approvals from the collaborating institutions will be obtained and materials will be shipped in accordance with NIH and federal regulations.

5.0 RISKS/DISCOMFORTS

There are risks associated with the procedures required for participants in this study. However, these are all standard procedures that are performed as part of a normal eye and medical examination.

Some of the discomforts associated with the ocular examination include the following:

1. Dilating drops or anesthetic drops may sting. They can cause an allergic reaction, or if contaminated, can cause an infection, but neither of these problems is very likely to occur. Dilating drops can also cause a sudden increase of pressure (acute glaucoma) in eyes that are already predisposed to develop this condition. There is little risk of glaucoma being triggered in this way, but if it is, treatment is available. The participant's intraocular

pressure will be obtained at each ophthalmic examination to determine whether there is an increased risk of developing glaucoma.

2. Color fundus photographs involve a bright flash to take pictures of the retina. This brief flash may cause temporary discomfort, but does not damage the eye.
3. In rare instances, the cornea may be scratched during measurement of intraocular pressure or use of a funduscopic contact lens. A corneal abrasion of this sort may be painful, but it heals quickly with no lasting effects.
4. OCT, color fundus photography and fundus Autofluorescence (FAF) imaging are non-invasive tests used to document and analyze retinal pathology and have no known medical risks.

Possible risks and discomforts associated with non-ocular examinations include:

1. Blood draws can cause discomfort and bleeding/bruising at the site of venous puncture. There is a remote risk of fainting or local infection. If any of these conditions arise, they will be treated.
2. The medical/ophthalmic history, questionnaires, and pregnancy tests entail no medical risk. No additional risks or discomforts are expected for the other clinical procedures.
3. There may be a risk to confidentiality. All precautions will be employed at the clinical sites and Coordinating Center.

5.1 AdaptDx™ Risks

The dark adaptation protocol as outlined in Appendix 3 entails no medical risk. Other than the discomforts noted above regarding the dilating eye drops and bright flash of light, there may be fatigue and loss of motivation or attention due to the length of the procedure. There is no risk of photosensitivity associated with the AdaptDx™.

5.2 Medmont Dark Adapted Perimetry

5.2.1 Kinetics of Dark Adaptation

Eye drops will be used to dilate the pupil. Participants will view a background light in a Ganzfeld dome for five minutes. The intensity of the background light will vary and calculated to bleach between 20 to 90% of rhodopsin. The background light will then be turned off and recovery of retinal sensitivity to Medmont blue and red stimuli will be measured. Sensitivity at each time point will be measured by the participant pressing a button when a blue or red light is observed.

Testing time will typically be up to one hour. The procedures for testing with the Medmont dark adapted perimeter are described further in Appendix 4.

5.2.2 Dark Adapted Retinal Sensitivity

Eye drops will be used to dilate the pupil and the participant will be dark adapted for 30 minutes. Dark adapted sensitivity will be measured across the visual field by the participant pressing a button when a blue or red light is observed within the field being tested. Testing time will typically take 10-15 minutes.

5.3 Study Supplement-Related

While long-term vitamin A supplementation has been associated with hip fractures, short term vitamin A supplementation appears to have no effect on bone turnover. [18, 19, 25]

Vitamin A and other retinoids as well as beta carotene, a carotenoid, have been candidate therapies in several chemoprevention studies for tobacco-related cancers. Several clinical trials have now demonstrated a surprising increase in cancer risk in participants taking beta carotene supplements.[20,21] Vitamin A supplementation has not been associated with a higher risk of lung cancer in tobacco based trials, and additional prevention trials are ongoing.

A study of pharmacokinetic metabolites over 20 days in adult men who received vitamin A at doses equivalent to 50,000 IU found these doses consumed over this length of time did not lead to hypervitaminosis A.[29] Similar doses of 50,000 IU vitamin A have been explored previously in a similar population of patients with AMD with no significant side effects.[17]

High doses of vitamin A supplementation has also been administered in accordance with the World Health Organization (WHO) guidelines to decrease childhood mortality.[30] Some countries, such as South Africa, implemented a “routine periodic high -dose Vitamin A supplementation” program which provided six monthly high dose (100,000 IU to 200, 000 IU) vitamin A retinyl palmitate capsules to children without significant side effects.[30]

There are small risks associated with chronic high-dose vitamin A toxicity.[28] The effects of toxicity may include hepatomegaly, elevated blood lipid levels, bone changes, and increased intracranial pressure.[28]

A study investigating high dose vitamin A in molar pregnancy used 200,000 IU vitamin A in adult women for up to 60 days and found no significant toxic effects.[31] Other studies show there may be a possible risk of teratogenicity in a fetus when a participant of childbearing potential consumes high doses (i.e., > 10,000 IU/day) of preformed vitamin A palmitate.[27] There have been reports of malformations in children when their mothers consumed high doses (>25,000 IU/day) of preformed vitamin A during pregnancy.[27]

5.3.1 Drug Interaction-Related

Vitamin A palmitate should be used in caution when combined with blood thinning medication. If participants are on Coumadin® at enrollment, they must visit their prescribing physician to check their Coumadin® status within two to four weeks after initiating the study supplement to have their Coumadin® regimen adjusted if necessary (as taking vitamin A palmitate and Coumadin® can increase the chances of bruising and bleeding.)

Additional information can be found in Section 4.4.3.

6.0 ADDITIONAL CONSIDERATIONS

6.1 Research with Investigational Drugs

This study is being conducted under an IND 138,192.

6.2 AdaptDx and Medmont Dark Adaptation Devices

On May 1, 2017, the IRB determined that the use of the AdaptDx and the Medmont dark adaptation perimeter devices meet the criteria for non-significant risk (NSR) devices under FDA guidelines (21 CFR 812.3(m)). These devices are/do not:

1. Intended as an implant and presents a potential for serious risk to the health, safety or welfare of a subject;
2. Purported or represented to be for use supporting or sustaining human life and presents a potential for serious risk to the health, safety or welfare of a subject;
3. For a use of substantial importance in diagnosing, curing, mitigating or treating disease, or otherwise preventing impairment of human health and presents a potential for serious risk to the health, safety or welfare of a subject; or
4. Otherwise presents a potential for serious risk to the health, safety or welfare of a subject.

Per this determination, no investigational device exemption (IDE) is needed. The AdaptDx dark adaptation device is commercially available.

7.0 PARTICIPANT SAFETY MONITORING

Participants will be monitored for adverse events by the study investigators after IP initiation.

7.1 Participant Withdrawal Criteria

Participation in the study is strictly voluntary. Participants may choose to withdraw from this study for any reason at any time without penalty, without loss of benefits or prohibition from enrolling in other clinical protocols. All participants will be included in the analyses at the end of the study.

The investigator may withdraw a participant at his/her discretion for the following reasons:

- Investigator determination that study continuation is not in the best medical interest of the participant;
- Findings in the course of the trial that may affect willingness to participate;
- Participant requiring additional medicines that will interfere with the study supplement;
- Serious suspected adverse reaction;
- Visual acuity loss of ≥ 15 letters from baseline;
- Any other safety concerns;
- Participant unable to meet the criteria required for continued participation;
- Participant stops taking the IP before the primary outcome is assessed;
- Participant is taking daily dose of ≥ 8,000 IU of vitamin A palmitate in addition to study supplement;
- Participant receives standard-of-care treatment for neovascular AMD in the study eye;
- Participant is unable to complete the AdaptDx™ test;
- Participant was in the first cohort and had his/her last intake of vitamin A palmitate less than two months prior to enrolling in the second cohort.

7.2 Pregnancy Monitoring

If an investigator becomes aware that a study participant has become pregnant while taking any study supplement and for one week after their last dose of study supplement, the investigator will advise the participant to stop taking the study supplement immediately.

If an investigator becomes aware that a study participant has impregnated their partner while taking any study supplement and for one week after their last dose of study supplement, the investigator will remind the participant of the potential risks to the unborn fetus.

In either case of reported pregnancy, the participant (and/or partner) will be referred to the NIH OB/GYN consultation service for evaluation and counseling. The investigator must follow the participant (or partner) through the pregnancy outcome.

8.0 OUTCOME MEASURES

8.1 Primary Outcome

The primary outcome for the first cohort is the measurement of dark adaptation parameters (thresholds and kinetics) by the following:

- Dark adaptation times as measured by the AdaptDx comparing baseline and two months after vitamin A palmitate supplementation.
- Dark adaptation parameters as measured by the Medmont comparing baseline and two months after vitamin A palmitate supplementation.

The primary outcome for the second cohort is the measurement of dark adaptation parameters (thresholds and kinetics) by the following:

- Dark adaptation times as measured by the AdaptDx comparing baseline and one month after vitamin A palmitate supplementation.
- Dark adaptation parameters as measured by the Medmont comparing baseline and one month after vitamin A palmitate supplementation.

8.2 Secondary Outcomes

The secondary outcomes include changes in LLVA and patient reported outcomes as measured by the LLQ comparing baseline to the following timepoints:

- Completion of supplementation (Month 2 in Cohort 1 and Month 1 in Cohort 2)
- One month after completing supplementation (Month 3 in Cohort 1 and Month 2 in Cohort 2)

The secondary outcomes also include the measurement of dark adaptation parameters (thresholds and kinetics) by the following:

- Dark adaptation times as measured by the AdaptDx comparing baseline and one month after completing supplementation (Month 3 in Cohort 1 and Month 2 in Cohort 2).
- Dark adaptation parameters as measured by the Medmont comparing baseline and one month after completing supplementation (Month 3 in Cohort 1 and Month 2 in Cohort 2).

9.0 STATISTICAL ANALYSIS

9.1 Primary Outcome Analysis

In this prospective, pilot study, analyses will be primarily descriptive and by participant. The study does not lend itself to a formal sample size calculation. This is a pilot investigation targeted as understanding changes in dark adaptation within a given participant before and after vitamin A palmitate supplementation. The number of participants with changes in dark adaptation parameters will be summarized. Changes in rod-intercept time on the AdaptDx will be calculated for each participant. Similarly, pre-bleach thresholds and kinetic parameters will be compared before initiating supplementation to after completing supplementation for Cohort 1 (Month 2) and Cohort 2 (Month 1).

9.2 Secondary Outcome Analysis

Changes in LLVA and LLQ responses will be presented by the participant and summarized from before initiating supplementation to after completing supplementation in Cohort 1 (Month 2) and Cohort 2 (Month 1). In addition, LLVA and LLQ will be summarized from before initiating supplementation to one month after completing supplementation in Cohort 1 (Month 3) and Cohort 2 (Month 2).

The number of participants in the second cohort with changes in dark adaptation parameters one month after completing supplementation (Month 3 in Cohort 1 and Month 2 in Cohort 2) will be summarized and changes in rod-intercept time will be calculated for each participant. Pre-bleach thresholds and kinetic parameters will be compared before initiating supplementation to one month

after completing supplementation (Month 3 in Cohort 1 and Month 2 in Cohort 2). The outcomes will be monitored throughout the study period.

Safety outcomes, including the number and severity of systemic and ocular toxicities, adverse events and infections will be summarized by severity, type and assessed relatedness to the study therapy throughout the study period.

10.0 HUMAN SUBJECTS PROTECTION

10.1 Equitability

Accrual for this study will be equitable among participants meeting the enrollment criteria. Participants less than 50 years of age are excluded because persons younger than 50 rarely present with AMD. Pregnant and lactating individuals are also excluded from the study based on the teratogenicity of vitamin A.

10.1.1 Justification for Exclusion of Children

Children are not eligible for this study as children rarely present with AMD.

11.0 BENEFITS

While enrolled in this study, all participants are eligible to receive standard-of-care treatment for AMD. Some benefit may occur if the vitamin A supplementation improves vision. The study will lead to generalizable knowledge about AMD.

12.0 CONSENT DOCUMENTS AND PROCESS

The informed consent document will be provided as a physical or electronic document to the participant or consent designee as applicable for review prior to consenting. A designated study investigator will carefully explain the procedures and tests involved in this study, and the associated risks, discomfort and benefits. In order to minimize potential coercion, as much time as is needed to review the document will be given, including an opportunity to discuss it with friends, family members and/or other advisors, and to ask questions of any designated study investigator. A signed informed consent document will be obtained prior to any research activities taking place.

The initial consent process as well as re-consent, when required, may take place in person or remotely (e.g., via telephone or other NIH approved remote platforms used in compliance with

policy, including HRPP Policy 303) per discretion of the designated study investigator and with the agreement of the participant/consent designee(s). The remote consent option will allow the consent designee and participant to engage in the informed consent process in a way that is similar to what it would be if it were conducted in-person when a participant is unable to be present at the study site. Whether in person or remote, the privacy of the subject will be maintained. Consenting investigators (and participant/consent designee, when in person) will be in a private area (e.g., clinic consult room). When consent is conducted remotely, the participant/consent designee will be informed of the private nature of the discussion and will be encouraged to relocate to a more private setting if needed. If the consent process is occurring remotely, participants and investigators will view individual copies of the approved consent document on screens at their respective locations; the same screen may be used when both the investigator and the participant are co-located but this is not required.

Note: When required, the witness signature will be obtained similarly as described for the investigator and participant below.

Consent will be documented with required signatures on the physical document (which includes the printout of an electronic document sent to the participant) or on the electronic document. The process for documenting signatures on an electronic document is described below.

When a hand signature on an electronic document is used for the documentation of consent, this study will use the following electronic platform to obtain the required signatures:

- iMedConsent platform (which is 21 CFR Part 11 compliant)

Both the investigator and the participant will sign the electronic document using a finger, stylus or mouse. Electronic signatures (i.e., the “signature” and a timestamp are digitally generated) will not be used.

Study investigators with consenting privileges will obtain informed consent. All participants will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures and potential risks of the study. The participants must have the ability to understand and sign an informed consent form, which must be signed prior to enrollment. The participants will have an opportunity to carefully review the consent and ask questions regarding this study prior to signing,

and they will be informed that they may withdraw from the study at any time without prejudice to themselves or benefits lost. If the participant requires the consent to be in larger font in order to read it well, this will be provided. If participants are visually impaired to the point of being unable to read the consent, they can take the consent back with them to read it over with a family member or with the use of magnifying devices. If the participant chooses, the investigator can also read the consent verbatim to the participant and answer any questions that may arise.

An investigator present during the consent process will document the consent process in the participant's medical record. A signed copy of the informed consent form will be provided for the participant to keep.

13.0 DATA AND SAFETY MONITORING

The NEI Serious Adverse Event (SAE) Review Committee is responsible for monitoring data and safety and will exercise oversight of the clinical investigation independently from the study investigators.

13.1 Coordinating Center

The Emmes Company, LLC has been assigned as the coordinating center for this trial to conduct data collection, protocol monitoring, data analysis and reporting. The coordinating center provides routine monitoring of study participants' data. Monitoring visits will occur on a schedule depending on the status of the study. More frequent monitoring visits will be performed at the beginning of the study when enrollment is open. Monitoring will decrease as enrollment closes and as participant follow-up continues.

Although Emmes advises the NEI Clinical Director and Principal Investigator on data and statistical activities, the coordinating center staff does not have direct access to or interaction with participants.

13.2 NEI Adverse Event Review Committee

The NEI SAE Review Committee, which consists of the NEI Clinical Director and three other NEI physicians, will be responsible for reviewing any reported serious safety events, if they occur under this protocol. The Committee will review accumulating data on a semiannual basis to determine whether the study should continue. The study PI will recuse herself from discussions of this

protocol. If changes to the protocol are indicated, recommendations will be made to the NEI Director and Institutional Review Board (IRB) who will consider and act on such recommendations in a timely manner. Should any suspected serious adverse reactions occur, the NEI Clinical Director may, at his discretion, assemble the Review Committee before the scheduled date to consider if the study should go forward. In addition, if three or more participants experience non-serious suspected adverse reactions that require temporary or permanent cessation of the IP, the PI shall report this to the NEI Clinical Director. The NEI Clinical Director may convene the Review Committee before the scheduled time to consider the cessation of the study as a whole.

13.3 Criteria for Stopping the Study

The NEI SAE Review Committee may recommend temporarily suspending or closing enrollment or stopping the study at any time due to safety concerns, demonstration of efficacy or lack of efficacy or slow recruitment.

14.0 QUALITY ASSURANCE

The NEI and Emmes maintain quality control by adhering to standard operating procedures (NEI QA program and NEIS standard operating procedures). These procedures cover the full protocol cycle beginning with staff credentialing and training, and protocol development and approval, through database development, data collection, monitoring and analysis, and finally manuscript preparation at the conclusion of the study. Data quality assurance is of the utmost importance to the NEI and Emmes. The two groups use a quality assurance system that relies on real-time data checks and reports throughout the course of a study to ensure the accuracy of information. This system is a secure and confidential data management system that stores data and provides quality assurance and reporting. Emmes has developed a number of routine reports specifically designed for monitors (e.g., listings of serious adverse events, etc.). Data will be reviewed for quality at least annually.

Additionally, Emmes has developed summary reports of discrepancies, as well as reports of the exceptions databases, which include requests and reasons for exceptions. The results of the reports are communicated back to site staff, and, along with protocol compliance issues, to the NEI SAE Review Committee (if applicable).

Following the monitoring plan for this study, Emmes will perform monitoring activities, including on-site audits, review of database entries and the resolution of study issues. In addition to monitoring, Emmes performs various detailed automated and manual data quality checks. The results from these checks and any protocol compliance issues are communicated back to site staff and to the NEI Project Officer, NEI Clinical Director and applicable regulatory bodies.

15.0 REPORTABLE EVENTS

Reportable events will be tracked and submitted to the IRB as outlined in Policy 801.

16.0 ALTERNATIVE THERAPIES

There are currently no alternative therapies for treating AMD or dark adaptation deficits associated with them.

17.0 PRIVACY

All research activities will be conducted in as private a setting as possible.

18.0 CONFIDENTIALITY

No blood, tissue or other samples will be stored in this study. All medical records will be kept confidential and will only be reviewed by the participating investigators. Data will be kept in password-protected computers held at the NEI and the coordinating center. Only study investigators and authorized coordinating center staff will have access to the study data. The participants' names will not appear on any of the data forms reported to the coordinating center. A unique identifier will identify the participant if their information is shared with the coordinating center for research purposes. The participants' names will not appear in any publication of the study results. Participants' personal information will be kept as private as possible. However, records can be inspected by organizations for quality assurance and data analysis. These include the members of the IRB and NEI SAE review committee.

19.0 CONFLICT OF INTEREST

The NIH guidelines were distributed to all the investigators and none of the investigators had any conflicts of interest.

20.0 TECHNOLOGY TRANSFER

There are no technology transfer agreements in place for this protocol. The AdaptDx dark adaptometer was provided to the NEI as a gift from Genentech, Inc.

21.0 COMPENSATION

For this study, there is no compensation for participation. This protocol includes reimbursement for travel and subsistence. Participants needing financial assistance will be able to receive supplemental reimbursement based upon need. Requests for supplemental reimbursement will be evaluated on a case-by-case basis for valid financial and/or medical need through a standardized process.

22.0 REFERENCES

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APPENDIX 1: DETERMINING CHILDBEARING POTENTIAL

A participant who is considered non-childbearing due to a medical condition (i.e., participant has previously undergone a hysterectomy) does not need a pregnancy test, Follicle-stimulating Hormone (FSH) test or contraception.

If a participant is considered non-childbearing due to menopause, it must be in accordance with the IRB/NIH OB-GYN guidance on the definition of menopause. This guidance defines menopause as:

- Participants over age 55 who have not had a period for one year will be considered menopausal and do not need a pregnancy test, FSH test or contraception.
- Participants between 50 and 55, who have not had a period for one year, should have an FSH test. If their FSH level is ≥ 20 mIU/mL, they will be considered menopausal and do not need pregnancy testing or contraception. If their FSH level is < 20 mIU/mL, they will need pregnancy testing and contraception as required by the protocol.
- Participants between 45 and 50 who have not had a period for one year will need both an FSH test and a pregnancy test. If they are not pregnant and their FSH level is ≥ 20 mIU/mL, they will be considered menopausal and will not require contraception or additional pregnancy testing. If their FSH test is < 20 mIU/mL, they will need pregnancy testing and contraception as required by the protocol.

APPENDIX 2: STUDY FLOWSHEET

Study Visit[7]	Screening	Baseline	Month 1	Month 2	Month 3[8]
Visit Number	-001	000	001	002	003
Target Day from Baseline Visit	-60 to -1	0	30	60	90
Visit Window	N/A	N/A	–14 days/ +14 days	–14 days/ +14 days	–14 days/ +14 days
Cohort 1: Treatment					
Vitamin A palmitate 16,000 IU dispensed		X	X		
Cohort 2: Treatment					
Vitamin A palmitate 48,000 IU dispensed		X			
General Assessments					
Medical/ Ophthalmic History	X	X			
Supplement Accountability			X	X[9]	
Concomitant Medications Assessment	X	X	X	X	X
Adverse Event Assessment			X	X	X
Review of Systems		X	X	X	X
Ophthalmic Assessments					
BCVA (ETDRS)[1]	X[1]	X	X	X	X
Manifest Refraction[2]		X		X	
Slit Lamp Examination	X	X	X	X	X
Dilated Fundus Examination	X	X	X	X	X
Intraocular Pressure (IOP)	X	X	X	X	X
Color Fundus Photography†		X		X	X
AdaptDx dark adaptation testing	X[4]	X	X	X	X
Medmont dark adaptation testing	X[4]	X	X	X	X
Fundus Autofluorescence†[3]		X		X	X
Optical Coherence Tomography (OCT)	X	X	X	X	X
Low Luminance Questionnaire (LLQ)		X	X	X	X
Low Luminance Visual Acuity (LLVA)		X	X	X	X
Laboratory Assessment					
Hepatitis Screening (HCV Ab and HBsAg)	X[5]				
Hepatic panel (checks liver function testing)	X[5]		X	X	X
Serum Vitamin A levels	X[5]		X	X	X
Pregnancy Test	X[6]	X	X	X	X

† The color fundus photography and fundus autofluorescence results may be utilized from any NEI protocol if performed within six months of baseline.

[1] The option to use the BCVA (ETDRS) short testing can only occur during the screening visit. The BCVA (ETDRS) full testing will occur at the baseline and subsequent visits

[2] BCVA with manifest refraction must also be performed when scheduled and when there is a change in visual acuity of ≧10 ETDRS letters (≧ 0.20 logMAR) from the last study visit.

[3] Fundus Autofluorescence may include multi-spectral imaging.

[4] If AdaptDx and/or Medmont have been performed within 6 months under any other protocol, they do not have to be done at the screening visit. For participants who have exceeded the AdaptDx test ceiling during any NEI protocol, their data will be accepted regardless of their test date.

[5] Screening labs can be performed under this protocol or another NEI protocol (08-EI-0102, 11-EI-0147, 16-EI-0134, 17-EI-0112, or 16-EI-0024) within 60 days of projected baseline visit.

[6] For participants of childbearing potential only, participants must have a negative pregnancy test within 24 hours prior to dispensation of study supplement. Pregnancy tests will only be conducted at each visit if the participant is determined to be of childbearing potential. See Appendix 1 for guidance on determining whether a participant is considered to be of childbearing potential.

[7] The tests scheduled at each visit may be split and completed within seven days (including the 7th day) from the visit date.

[8] The study visit, treatment, and assessments at Month 3 are to be completed for Cohort 1 only.

[9] This assessment will be completed for any participants who return the study supplement at Month 2.

APPENDIX 3: DARK ADAPTATION PROTOCOL

Dark adaptation is the primary exposure of interest. Rod-mediated dark adaptation will be measured with an AdaptDx™ dark adaptometer manufactured by MacuLogix. Participants will view the test target with their best optical correction for the test distance. To control for pupil size prior to dark adaptation testing, pupils of the study eye will be dilated to ≥ 6 mm with 1% tropicamide and 2.5% phenylephrine hydrochloride. Pupil size will be measured before each dark adaptation measurement to confirm adequate dilation. Based on prior studies of dark adaptation, it is expected that up to 5% of participants will be unable to adequately complete the test due to poor motivation or poor attention. Participants who are unable to complete the test will be considered withdrawals and additional participants may be accrued.

The AdaptDx™ provide the rod intercept time as a characterization of dark adaptation speed. The protocol for testing with a computer–automated dark adaptometer is described in detail in the User Manuals. Briefly, the dilated test eye is exposed to a standard photographic flash and the speed of visual sensitivity recovery in the dark is measured. Sensitivity recovery is measured by repeatedly estimating absolute threshold (least amount of light detected) about once per minute until recovery reaches a criterion sensitivity, typically in 40 minutes or less. Sensitivity recovery is measured at 5˚ in the inferior visual field on the vertical meridian. The test target is a 500 nm, 2˚ diameter circular spot. Participants are given a 15-second rest break between each measurement. The resulting recovery function is characterized by a measure of visual sensitivity recovery called the rod intercept. The rod intercept is the time in minutes at which the visual sensitivity recovery crosses the criterion sensitivity.

APPENDIX 4: EVALUATION OF LIGHT

Medmont Dark Adapted Chromatic (DAC) Perimeter

The DAC perimeter uses blue and red LEDs and has been specifically designed to measure dark adapted retinal sensitivity. Sensitivity is calculated from the inverse of light thresholds. Due to the sensitivity of dark adapted photoreceptors, light thresholds are extremely low (ca. 10^{-5} cd/m^2). Therefore, maximum light intensity of the blue and red LEDs is quite low (ca. 10 cd/m^2) so that photoreceptor thresholds can be achieved at maximum attenuation of the LEDs. By comparison a typical perimeter (e.g. Humphrey or Octopus) used in the eye clinic has a maximum intensity of 3200 cd/m^2. As such, the light intensities used by the Medmont DAC at more than 100 times lower than used in commercially available conventional perimeters and as such do not represent a light hazard to the patient.

Light Intensity for Rhodopsin Bleaching

We are proposing to bleach 20% to 90% of retinal rhodopsin by having the participant view a background light from 210 to 4250 cd/m^2 respectively for five minutes. These background levels are at least 40% lower than the 7000 cd/m^2 used by the only currently commercially available full--field dark adaptometer (Roland Consult, Germany) which the patient also views for five minutes. In addition, the maximum background light level we are proposing is only 14% of the light level (30 000 cd/m^2) used by other dark adaptation studies.[15] The background intensities we are proposing are 40%-85% lower than used previously and as such do not represent a light hazard to the patient.

Procedures:

1. The participant is placed in the dark for 30 minutes.
2. Sensitivities on the Medmont perimeter are tested to obtain level of “pre-bleach” sensitivities.
3. Participant is asked to view a background light from 210 to 4250 cd/m^2 respectively for five minutes.
4. Sensitivities at various locations in the macula are tested using the Medmont perimeter over a time frame not to exceed 60 minutes.