Age-related Macular Degeneration (AMD) in the Vitamin D and Omega-3 Trial (VITAL)

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Protocol: AMD in the VITamin D and OmegA-3 TriaL (VITAL)

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I. BACKGROUND AND SIGNIFICANCE

Age-related macular degeneration (AMD) is the leading cause of blindness in the US. An estimated 1.75 million US men and women suffer from the advanced forms of geographic atrophy or neovascular AMD, and another 7 million are at substantial risk of this outcome.[1] Despite advances in photodynamic[2] and antivascular endothelial growth factor therapy[3, 4] for patients with neovascular AMD, and the demonstration of benefit from a supplement of antioxidants plus zinc for people with intermediate to advanced AMD,[5] vision loss from AMD remains commonplace. ***VITAL-AMD*** will use the resources of the ***VIT***amin D and Omeg***A***-3 Tria***L*** (***VITAL***) **(U01 CA138962; JoAnn Manson, PI)** to test the efficacy of two promising preventive agents in delaying the incidence and progression of AMD among 20,000 older men and women. Vitamin D3 and omega-3 fatty acids share anti-inflammatory, antioxidant, antiproliferative, and antiangiogenic properties, and evidence supports potential benefits of both agents as strong candidates for prevention of AMD. ***VITAL-AMD*** will have strong statistical power to address the primary (incidence) and secondary (progression to advanced stages) prevention of AMD. Findings from this study will clarify whether these agents reduce incidence and progression of AMD, and will provide important data for both public health and clinical guidelines for the primary and secondary prevention of AMD.

Role of DHA+EPA in AMD - Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are marine omega-3 polyunsaturated fatty acids that can be synthesized from alpha-linolenic acid, though the major source is through diet. DHA and EPA are important in the developing eye and visual processing. DHA is found in neural and vascular cell membranes and is a major structural component of photoreceptor outer segments.[6] DHA is required for normal photoreceptor function,[7] and it accounts for the permeability and fluidity of outer segment membranes.[8, 9] DHA protects the vascular and neural retina from a range of stressors including reactive oxygen, ischemia, and inflammation, and helps promote survival of photoreceptor and RPE cells during phagocytosis.[10] The distal tips of photoreceptor outer segments are intermittently shed and renewed, necessitating a constant supply of DHA and EPA. DHA insufficiency is associated with a delay in photoreceptor response and alterations in retinal structure and function that could influence the onset and progression of AMD.[11] Supplementation with DHA and EPA appears to reverse these deficits[12] and could have a similar beneficial effect on AMD.

EPA is concentrated in the retinal vascular endothelium and in blood components[13] and has potent anti-inflammatory properties. EPA regulates lipoprotein metabolism and inhibits expression of several inflammatory mediators (e.g. intercellular adhesion molecule (ICAM)-1, monocyte chemotactic protein (MCP)-1, vascular endothelial growth factor (VEGF) and interleukin (IL)-6).[14, 15] EPA also inhibits nuclear factor κB (NF-κB) activation, and thus expression of a number of inflammatory compounds that can damage the Bruch membrane and lead to AMD.[16] NF-κB is a central regulator of IL-6, a critical regulator of CNV.[15] EPA is the precursor to a group of

eicosanoids (e.g. series-3 prostaglandins and series-5 leukotrienes) that counteract series-2 prostaglandins derived from arachidonic acid that promote inflammation, cell proliferation, and angiogenesis and interfere with immune cell function.[13] EPA and DHA also produce resolvins, which stop infiltration and transmigration of polymorphonuclear leukocytes, block the production of pro-inflammatory mediators and regulate the trafficking of leukocytes, cells and mediators to sites of inflammation.[17] Further, EPA depresses VEGF-specific tyrosine kinase receptor activation and expression,[18] and with DHA may reduce neovascularization independent of VEGF.[19] Recent work has shown that omega-3 fatty acids attenuate choroidal neovascularization in mice,[15] and reduce or reverse retinal lesions in the Ccl2-/-/Cx3cr1-/- mouse model of AMD.[20] Such findings are in line with epidemiological evidence in humans.

Some cross-sectional studies have reported significant inverse associations between higher fish intake and risk of early[21] or late AMD,[22-26] while others found no association.[27-29] Among studies examining omega-3 fatty acids, 2 studies found no significant relationships,[21, 30] whereas 4 studies showed reduced risks of neovascular AMD[22-24] or central geographic atrophy,[25] although in 2 studies the benefit was restricted to those with low dietary intake of linoleic acid.[22, 23] Prospective studies include a report on 567 incident cases of AMD from the Nurses' Health Study (NHS) and Health Professionals Follow-up Study (HPFS) that showed a significant 30% reduction in AMD incidence with high DHA intake (RR not significant after adjustment for other fats).[31] High intake of EPA was associated with a non-significant 23% lower risk of AMD. In the Blue Mountains Eye Study, higher intake of omega-3 fatty acids lowered risk of early AMD by 60% after 5 y.[32] At 10 y, the reduction was attenuated and not significant.[33] In the Melbourne Collaborative Cohort Study, higher omega-3 fatty acid intake reduced early AMD risk by 15%.[34] In the Age-related Eye Disease Study (AREDS), after 6.3 y of follow-up, risks for central geographic atrophy were 45% & 56% lower in those with high intake of DHA & EPA, respectively.[35] Higher intakes of DHA & EPA were also associated with ~25% lower risks for progression to advanced AMD after 8 y follow-up in AREDS.[36] At 12 y, there continued to be a significant ~30% reduction in risk of advanced AMD for EPA & DHA,[37] as well as total intake of omega-3 fatty acids.[38] A meta-analysis showed that a high intake of omega-3 fatty acids conferred a 38% risk reduction for advanced AMD, and fish intake ≥2x/wk was associated with 25% and 33% lower risks of early and advanced AMD, respectively.[39]

We recently examined dietary intake of omega-3 fatty acids and AMD among 38,022 women in the Women's Health Study (WHS) who completed a dietary questionnaire at baseline and were free of a diagnosis of AMD. A total of 235 cases of visually significant AMD were confirmed during 10 y of follow-up. Women in the highest third of DHA intake had a 38% lower risk of AMD (RR, 0.62; 95% CI, 0.44-0.87) compared to those in the lowest third of intake. High intake of EPA was also associated with a reduced risk of AMD (RR, 0.66; 95% CI, 0.48-0.92). These prospective data from a large population of women appear to be the strongest evidence to date to support a role for DHA and EPA in the primary prevention of AMD.

Possible Mechanisms for Vitamin D in AMD - A plausible role for vitamin D3 in AMD prevention can be postulated due to its immunomodulatory, anti-inflammatory, and antiangiogenic properties. 1,25(OH)2D interferes with the activation and signaling of NF-κB.[40, 41] 1,25(OH)2D decreases production of proinflammatory cytokines IL-2,[42] IL-6,[43] IL-8,[44] and IL-12,[45] increases production of the anti-inflammatory cytokine IL-10,[46] and regulates expression of matrix metalloproteinase-9.[47] Vitamin D also lowers blood levels of C-reactive protein, a marker of systemic inflammation that we have prospectively linked to elevated risks of AMD in the WHS[48] and others have shown to be associated with AMD.[49-52] Vitamin D is a potent stimulator of mechanisms for pathogen elimination, which could be relevant given the possible involvement of chronic infection in AMD.[53] In addition, vitamin D regulates the renin-angiotensin-aldosterone system (RAAS) via suppression of renin gene expression and biosynthesis,[54] and could exert an indirect beneficial effect on AMD through improved blood pressure control.[55] Favorable effects of vitamin D on glucose tolerance and insulin sensitivity[56, 57] could also mediate a beneficial effect in AMD as suggested by the association between dietary glycemic index and AMD.[58-62]

In vitro and in vivo studies have demonstrated that 1,25(OH)2D profoundly inhibits growth of a variety of tumors by directly impacting the proliferation and activity of endothelial cells (ECs) and inhibiting angiogenesis, actions mediated by VDR.[63-65] In the mouse oxygen-induced ischemic retinopathy (OIR) model, 1,25(OH)2D-treated animals demonstrated a dose-dependent reduction in retinal neovascularization, and inhibition of retinal EC capillary morphogenesis without a significant inhibitory effect on EC proliferation and migration.[66]

To date, there are 3 studies of vitamin D and AMD, all supportive. Circulating 25(OH)D reflects all sources of vitamin D exposure, has a half-life of 2-3 weeks, and is the most comprehensive and stable indicator of vitamin D status.[67] Data from the 3rd NHANES indicated that serum levels of 25(OH) D were inversely associated with the prevalence of early AMD (OR for extreme quintiles, 0.64, 95%CI, 0.5-0.8), but not advanced AMD (OR, 1.16, 95%CI, 0.5-3.1).[68] Data from the Carotenoids and Age-Related Eye Disease Study indicated that high serum 25(OH)D status may be protective against intermediate AMD in women <75 y (OR for extreme quintiles, 0.57; 95% CI, 0.33-0.99; p=0.02).[69] Recent data from a monozygotic twin study with discordant AMD phenotypes found higher dietary intake of vitamin D in the twin with less severe AMD.[70]

II. SPECIFIC AIMS

The primary aims of this proposal are:

1. Does fish oil supplementation (EPA+DHA, 1g/d), compared to placebo, reduce the incidence and/or progression of AMD?

2. Does vitamin D3 supplementation (2000 IU/d), compared to placebo, reduce the incidence and/or progression of AMD?

The primary aims will be addressed in analyses involving the entire ***VITAL*** cohort of n=20,000 older men and women.

The secondary aims of this proposal are:

3. Are there synergistic or antagonistic effects of fish oil and vitamin D3 supplementation on the primary and secondary AMD endpoints?

This secondary aim will be addressed in analyses involving the entire ***VITAL*** cohort of n=20,000 older men and women.

4. Does baseline circulating vitamin D status modify the effect of vitamin D3 supplementation on AMD?

5. Does baseline circulating omega-3 fatty acid status modify the effect of EPA+DHA supplementation on AMD?

Secondary aims 4 and 5 will be addressed in the subgroup which provides a baseline blood specimen (N~16000).

6. Are there associations of polymorphisms in CYP24A1, vitamin D receptor, desaturase genes, or other genes with functional relevance to vitamin D and omega-3 fatty acid pathways with incidence of AMD?

7. Do polymorphisms in CYP24A1, the vitamin D receptor, complement factor H, ARMS2, C3, C2/BF, RORA, or other functionally relevant genes modify the effects of vitamin D3 on the AMD endpoints?

8. Do polymorphisms in desaturase genes (FADS1, FADS2, FADS3), elongase of very long fatty acids 2 (ELOVL2), complement factor H, ARMS2, C3, C2/BF, RORA, or other functionally relevant genes modify the effects of EPA+DHA on the AMD endpoints?

Secondary aims 6, 7, and 8 will be addressed in the subgroup which provides a baseline blood specimen using a nested case-control sample of all AMD cases (incident AMD cases and progression to advanced AMD) and a set of age- and sex-matched controls remaining free of AMD.

III. SUBJECT SELECTION/ENROLLMENT

VITAL-AMD is an ancillary study of the NIH-funded ***VITAL*** trial. All persons randomized in ***VITAL*** are included in this ancillary study.

Individuals are eligible for ***VITAL*** trial participation if they meet the following criteria: (1) are age ≥60 (men) or ≥65 (women); (2) have at least a high school education (to complete mail-based questionnaires); (3) have no history of cancer (except non-melanoma skin cancer), MI, stroke, TIA, angina pectoris, CABG, or PCI; (4) have none of the following safety exclusions: history of kidney stones, renal failure or dialysis, hypercalcemia, hypo-or hyperparathyroidism, severe liver disease (cirrhosis), or sarcoidosis or other granulomatous diseases such as active chronic tuberculosis or Wegener's granulomatosis; (5) have no allergy to fish (for EPA+DHA); (6) have no other serious illness that would preclude participation; (7)

are consuming no more than 800 IU of vitamin D from all supplemental sources combined (individual vitamin D supplements, calcium+vitamin D supplements, medications with vitamin D [e.g., Fosamax Plus D], and multivitamins), or, if taking, willing to decrease or forego such use during the trial (to ensure the overall dose is well below the no-observed-adverse effect level (NOAEL) of 4000 IU specified by the European Commission Scientific Committee on Food (ECSCF); (8) are consuming no more than 1200 mg/d of calcium (the RDA) from all supplemental sources combined, or, if taking, willing to decrease or forego such use during the trial; (9) are not taking fish oil supplements, or, if taking, willing to forego their use during the trial; and (10) are willing to participate, as evidenced by signing the informed consent form.

IV. STUDY PROCEDURES

The procedures used to ascertain and document self-reports of AMD in ***VITAL-AMD*** are modeled on those used for more than two decades by the PIs in Physicians' Health Study I and II, Women's Health Study, and Women's Antioxidant Folic Acid Cardiovascular Study. All information is obtained masked to randomization status. Baseline and follow-up questionnaires are sent to study participants annually to obtain reports of diagnoses of AMD. Non-respondents are sent additional requests and are finally called to ensure complete follow-up. Reports of AMD may also be received through letters or telephone calls. Following the report of AMD, we obtain written consent to identify the diagnosing eye doctor(s) and obtain medical record information. We will contact the eye doctor(s) by mail and request they complete an AMD questionnaire or forward a complete copy of the patient's medical records pertaining to the diagnosis of AMD.

AMD questionnaire - The AMD questionnaire asks about the date of initial diagnosis of AMD, the best-corrected visual acuity (VA) at the time of diagnosis, the date when VA reached 20/30 or worse, the most recent VA, and information about the pathological findings observed on exam (drusen, RPE hypo- &/or hyperpigmentation, geographic atrophy, RPE detachment, subretinal neovascular membrane, or disciform scar). The questionnaire will inquire about drusen size (small, intermediate, large) as well as information on the location of geographic atrophy (central versus noncentral). We will obtain information on treatments for AMD (laser, photodynamic, or anti-VEGF therapy). We will also obtain information about whether there are other ocular abnormalities that could explain or contribute to visual loss. If so, the eye doctor is asked to provide his/her judgment as to whether AMD, by itself, is significant enough to cause VA of 20/30 or worse. Once obtained, the medical record information will be evaluated by a single reviewer (Dr. Christen), masked to the treatment assignments, to determine conformity with uniform diagnostic criteria. In cases of uncertainty, Dr. Schaumberg will also review the records. Reports for which doctors indicate diagnostic uncertainty will not be included as cases. When other ocular pathology is present, we will not include the case in our visually-significant AMD case group unless the examining eye doctor indicates their judgment that the AMD by itself would be sufficient to result in VA of 20/30 or worse. We define neovascular AMD by presence of RPE detachment, subretinal neovascular membrane, or disciform scar, either from clinical exam,

photos, or OCT if available, and will document the date of diagnosis of neovascular AMD.

Fundus Photographs - In order to obtain the highest quality endpoints possible in ***VITAL-AMD***, we will obtain standard fundus photographs (e.g. 30° color photographs centered on the macula) of each eye from all participants for whom we confirm a diagnosis of AMD by review of medical records. In our recent experience, nearly all cases of neovascular AMD have photographs, angiograms, or OCTs available as part of routine care, however, dry AMD cases may not have photos. We will take the following steps to obtain photos for each prevalent case at baseline and for all incident cases: 1) When we obtain the medical record information, we will request copies of fundus photos centered on the macula for both eyes if available, or if not available we will ask whether the doctor would be willing and able to obtain a new set of photos for ***VITAL-AMD***. 2) Study participants who do not already have photos in their medical records will be asked whether they would be willing to have photos taken (we have received funds to reimburse clinics for taking and sending photographs for ***VITAL*** participants with AMD), 3) If the participant's own doctor is not willing or able to take a set of photographs (e.g. s/he doesn't have the equipment), we will assist participants to obtain photographs from a doctor near their home. We will use databases (e.g. the Academy of Ophthalmology maintains a list by subspecialty) to search for retina specialists or other eye doctors with offices near the study participant and ascertain their willingness to take a set of photos for ***VITAL-AMD***. 4) At the end of the trial, we will obtain a 2nd set of photos from all cases who remain alive and for whom we have not already documented progression to advanced AMD. 5) We will use the simplified AREDS scale to evaluate the photographs (grading will be done masked to randomization status).

Assessment of AMD progression. - We will send an annual follow-up questionnaire to all participants with medical-record confirmed AMD to ascertain progression to advanced AMD. The questionnaire will ask about changes in the participant's vision, and any updates on their diagnosis of AMD (e.g. the development of the "wet" form, any treatments received [e.g. anti-VEGF therapy], etc.). We will obtain follow-up medical records from all cases in which we suspect progression may have occurred. We will also routinely obtain follow-up records every two years from all participants with confirmed AMD that has not yet progressed to advanced AMD to help ensure no cases of progression are missed. Finally, we will also obtain a second set of retinal photographs at the end of the trial and grade these using the simplified AREDS scale. Progression along the simplified AREDS scale will be ascertained during analysis by comparison of the final grade with the baseline grade.

Validity of medical record-confirmed incident AMD - At initiation of ***VITAL-AMD*** we will conduct a pilot study to further validate our methodology for collection of photographs and to estimate sensitivity of case detection in the ***VITAL*** population. We will immediately seek to obtain informed consent for review of eye exam records and retinal photographs from a sample of 500 randomly selected ***VITAL*** participants at baseline. We will review medical records and photos masked to both randomized treatment assignments as well as the participants' response to the question about a prior diagnosis of AMD. We will estimate sensitivity based on the proportion of true

positives, that is the proportion of true AMD cases (identified by reviewing medical records and photos) that we identified using our methodology for AMD case identification (i.e. those that reported AMD on the baseline questionnaire that were confirmed by review of medical records). With estimated baseline prevalence of AMD=0.07 we will be able to estimate sensitivity with a 95% CI of ±15%. If sensitivity is found to be too low (e.g. <65%), we will consider strategies for increasing case detection in ***VITAL-AMD***, for example by increasing participant awareness of AMD and the importance of eye exams for its detection, e.g. through participant newsletters.

V. BIOSTATISTICAL ANALYSIS

Assuming annual event rates for AMD observed in our previous trials of men and women, and an average follow-up of 6 years (expected for ***VITAL***), ***VITAL-AMD*** will have good (≥80%) to excellent (≥95%) power to detect reductions of ≥20% in the primary AMD endpoint.

The study design is a 2x2 factorial randomized trial. With the large sample size, randomization should assure an equal distribution of known and unknown confounders between treatment groups, but we will control in analysis for any chance imbalances that may occur for AMD risk factors or conditions requiring more frequent medical attention that could increase the likelihood of AMD diagnosis. We will estimate the main treatment effects using the intention-to-treat principle based on randomized treatment assignment. Initial analyses of primary and secondary AMD endpoints will include contingency tables in which the rate of each endpoint (number of events per person-year of observation) among participants allocated to active treatment will be compared with the rate among those allocated to its placebo; controlling by stratification for the other treatment assignment. In addition, Kaplan-Meier survival estimates, the logrank test, and proportional hazards regression models201 will be used to determine whether there is a difference in time to an AMD event. Because an extended exposure to the study agents may be required to observe an effect, we will also conduct analyses which exclude AMD events that occur during the early years after randomization. To address the issue of AMD ascertainment, we will also test associations in models where the at-risk population during each 1 y follow-up period is restricted to those who report having had an eye exam during that year.

We will examine effect modification by the other randomized intervention, by race/skin pigmentation (for vitamin D), and by other risk factors by including an interaction term in proportional hazards models, as well as using methods for testing additive interactions. Stored blood samples will be used to measure genetic polymorphisms and analyses will examine the effect of these polymorphisms on the AMD endpoints, as well as modification of the treatment effects. These analyses will be analyzed as a nested case-control study using multivariable conditional logistic regression analyses and will also include testing of interactions on the additive scale.

VI. RISKS AND DISCOMFORTS

The only possible risk in this study involves the social/psychological risk that could result from inadvertent disclosure of confidential information from the questionnaires or blood tests. However, we have many safeguards in place to avoid this possibility, and we have never had an inadvertent breach of confidentiality in any of our trials. Confidentiality of participants is secured via locked file cabinets, use of participant ID numbers, and restrictions on access to computerized records and use or release of both individual and aggregate ***VITAL*** participant data, including randomized treatment assignments, in publications and presentations. Access to the ***VITAL*** databases and certain network files is restricted to essential staff only, and is protected by passwords and restricted access accounts. All blood specimens sent to the laboratories for analysis will be labeled with an ID number only, so that no individual identifying information will be made available in any form to these sites. In addition, employees involved with the proposed study will be asked to sign a form agreeing not to disclose any information to which they might have access, regardless of their personal perception about its confidentiality.

There is no additional risk to participation in ***VITAL-AMD*** beyond that incurred by participants as part of their regular activities in ***VITAL***. All participants will have agreed by signing their ***VITAL*** consent form that their blood and other relevant data will be stored for future analyses and that they would not learn of the results of those analyses.

VII. POTENTIAL BENEFITS

For the majority of participants, there will be few direct benefits from participating in this primary prevention study other than the awareness of being involved in a large endeavor to answer relevant and timely questions regarding the possible benefits of vitamin D and marine omega-3 fatty acids. The potential benefits to society relate to the increasing use of both vitamin D and fish oil for many health purposes, with data not yet clearly indicating either clear benefit or harm. This study will provide a wealth of data on the effects of these supplements on risk of AMD, the leading cause of blindness in the United States, and will help guide individual decisions, clinical recommendations, and public health guidelines.

VIII. MONITORING AND QUALITY ASSURANCE

As Co-PIs, Dr. William Christen and Dr. Debra Schaumberg will be responsible for monitoring data collection and assuring the validity and integrity of the data and adherence to the IRB-approved protocol.

A ***VITAL*** Data and Safety Monitoring Board (DSMB) has been created as an independent body charged with ensuring that the safety of participants is protected and that the scientific goals of the study are being met. The ***VITAL*** DSMB is charged with monitoring differences by treatment agent of ancillary study outcomes, including AMD, and will be empowered to terminate the trial based on evidence of substantial harm or benefit. To support those purposes, the DSMB will review any proposed amendments to the study protocol, examine the progress of the trial and the unblinded data on study endpoints, perform expedited review of all serious adverse

events (i.e., events meeting the FDA definition of Serious Adverse Events, such as any fatal event including suicide, immediately life-threatening event, or permanently or substantially disabling event), perform ongoing monitoring of drop-outs and non-serious adverse events, determine whether study procedures should be changed or the study should be halted for reasons related to the safety of participants, and perform periodic review of the completeness and validity of data to be used for analysis of safety and efficacy. The DSMB will also ensure participant privacy and research data confidentiality. The DSMB will employ monitoring rules[71, 72] that will serve solely as guidelines in decisions regarding continuation or stopping of treatment arms. While these rules are intended for the primary endpoints of ***VITAL*** (primary prevention of cancer and CVD), the DSMB will also consider secondary and ancillary ***VITAL*** endpoints (including AMD) in assessing the overall balance of benefits and risks of the two agents. All decisions must be made after examining the totality of evidence, including other trial data, on these agents. All decisions will be made after examining the totality of evidence, including other trial data, on these agents.

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