DREAM

Dry **E**ye **A**ssessment
and **M**anagement

Manual of Procedures

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The <u>DR</u>y <u>E</u>ye <u>A</u>ssessment and <u>M</u>anagement (DREAM) Study
Manual of Procedures

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CHAPTER 1
BACKGROUND AND SIGNIFICANCE

1.1 Rationale: Summary of Importance of RCT for ω3 PUFAs in Dry Eye Disease

Dry Eye Disease (DED) is one of the most common eye conditions that patients seek care for and cannot be disregarded as a trivial condition. Being more common in women and the graying population, it is a growing public health problem. The costs of treating DED, including artificial tears, cyclosporine, office visits and OTC supplements of unknown efficacy are very high. The average cost of managing DED was estimated at $55.4 billion to the US society overall, taking into account both healthcare costs and loss of productivity costs. Despite the substantial expenditure, DED continues to have significant impact on the quality of life of patients. Hence, there is a definite need to create new innovations to address this problem.

Although the pathogenesis of dry eye disease is not fully understood, it is recognized that inflammation has a prominent role in the development and propagation of this debilitating condition. Irrespective of the etiology, DED eventually leads to inflammation of the ocular surface via various mechanisms such as tear hyperosmolarity and tear film instability. This inflammation in turn leads to ocular surface damage and further exacerbation of DED, thus creating a self-perpetuating vicious cycle of inflammation and DED (Pflugfelder, 2008; Enríquez-de-Salamanca, 2008; Paiva, 2008; Lemp, 2008; DEWS 2007; Nichols, 2011; Stevenson, 2012; Stern 2013, Pflugfelder, 2017). Though DED continues to be divided into two groups, both groups eventually enter this vicious cycle of inflammation that leads to the typical symptoms of DED. Clinical evidence indicates that anti-inflammatory therapies may be able to break this cycle of DED and inflammation, opening new avenues for the treatment of this complex disorder. But as pointed above, despite the currently available treatments, including anti-inflammatory drugs, there is still an unmet need to develop novel anti-inflammatory treatments.

Though there is growing support for the potential anti-inflammatory role of ω3 in treating DED, there is very little substantial evidence of efficacy. The NIH is committed to furthering our understanding of ocular surface disease and immunology, and to the role of supplements in chronic disease.

The Dry Eye Assessment and Management (DREAM) grant utilizes the highest level of evidence; a double masked randomized clinical trial, to answer an important clinical question. This will be one of the first such trials on ω3 in DED, utilizing multiple centers, sufficient study length and with sufficient subjects to provide evidence for the role of ω3 in DED and confidently outline clinical care recommendations. At the same time, DREAM will also provide accurate, prospectively collected information on longitudinal findings in DED in a large well-characterized population. Collection of biomarkers, tear osmolarity, percent HLA DR positive cells and tear cytokines, will provide minimally invasive objective metrics that may provide better methods for classifying severity and outcomes in treatment of DED, as well as contributing to our understanding of the pathology that occurs on the ocular surface with DED. We will also expand our knowledge of the economic impact of DED on society as well as its impact on quality of life and productivity for the patients and the possible impact of ω3 on these issues. The DREAM study addresses a common eye problem and results will have a direct impact on clinical care.

DREAM addresses some of the key priorities of NIH - increased quality evidence on the usefulness of OTC supplements, a disease that affects women more than men, increases with age and improves our knowledge of immunology of the ocular surface.

Utilizing an expert collaborative group, extensive work during the Planning Grant including a Feasibility Study and Symptom Survey Study, all the key needs for a large RCT are in place to answer the outlined Specific Aims of this trial.

1.2 Definition of Dry Eye Disease

The 2007 International Dry Eye Workshop (DEWS) Report presented findings and recommendations prepared by specialized subcommittees and discussed in open forum by over 60 experts from the clinical, research and the drug development fields from Canada, Europe, Japan, and the United States. This is the definitive word on the diagnosis and management of dry eye disease and associated conditions. It defined dry eye as 'a multi-factorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability with potential damage to the ocular surface. It is accompanied by increased osmolarity of the tear film and inflammation of the ocular surface' (DEWS Report, 2007).

Dry eye disease is divided into two categories: aqueous tear deficient and evaporative (DEWS Report, 2007; Lemp, 1995) with several sub groupings. Aqueous tear deficient dry eye can be either Sjögren's syndrome or non-Sjögren's syndrome. Aqueous tear deficient dry eye results from a failure of tear secretion. Evaporative dry eye results from any condition (i.e., meibomian oil deficiency, lid aperture disorders, low blink rate, etc.) that produces an increase in the evaporation rate for the aqueous tears.

Irrespective of the etiology, DED eventually leads to inflammation of the ocular surface via various mechanisms such as tear hyperosmolarity and tear film instability. This inflammation in turn leads to ocular surface damage and further exacerbation of DED, thus creating a vicious cycle of inflammation and DED (Pflugfelder, 2008; Enríquez-de-Salamanca, 2008; Paiva, 2008; Lemp, 2008; DEWS 2007; Nichols, 2011; Barabino, 2012; Stern, 2013). Though DED continues to be divided into two groups, both groups eventually enter this vicious cycle of inflammation that leads to the typical symptoms of DED such as chronic eye pain, eye irritation, foreign body sensation, fluctuating vision, burning, and/or stinging and production of signs such as decreased tear film quantity (lower meniscus—tear-film height at the lid margin), lower tear production (Schirmer's Test), ocular-surface damage demonstrated by breakup of the tear film (TFBUT—tear film breakup time) and staining with vital dyes such as fluorescein and lissamine (DEWS Report, 2007; Lemp, 2008; Nichols, 2011; Stevenson, 2012).

1.3 Public Health and Significance

DED is a widespread, growing problem with serious consequences. There is a need for effective DED treatments. This research addresses NEI's priorities (NEI National Plan for Eye and Vision Research, 2004) by expanding our knowledge of a disease that alters the ocular surface and to relate the signs and symptoms of this common disease to inflammation of the ocular surface through careful evaluation of local biomarkers that would provide further insight into mechanisms of DED, improve our ability to classify its severity and monitor changes with treatment.

1.3.1 Prevalence of DED

Dry eye disease (DED) is one of the most frequently encountered ocular morbidities (Gayton, 2009). Twenty-five percent of patients who visit ophthalmic clinics report symptoms of dry eye, making it a growing public health problem and one of the most common conditions seen by eye care practitioners (Gayton, 2009). In fact, DED is considered one of the top 3 most prevalent chronic eye diseases, together with glaucoma and age-related macular degeneration (AMD) (Hirsh, 2007; Schaumberg, 2003; Lin, 2003; Moss, 2004). An overall summary of data from several United States (US) and international population-based studies (Milajanovic, 2007; Lin, 2003; Chia, 2003; Lin, 2002) suggests that the prevalence of dry eye lies somewhere in the range of 5-30% in the population aged 50 years and older (DEWS Report 2007). These estimates suggest that DED is more prevalent than diabetes (~8% of US population), cancer (~3% of US population), and heart disease (~7% of US population) Galor, 2011). Also, it is more common in women and in the older age group. Based on data from the largest studies of dry eye to date, the Women's Health Study (WHS) and the Physicians Health Study (PHS), it has been estimated that about 3.23 million women and 1.68 million men, for a total of 4.91 million Americans 50 years and older have dry eye. Tens of millions more have less severe symptoms and probably a more episodic manifestation of the disease that is notable only during contact with some adverse contributing factor(s), such as low humidity (DEWS Report, 2007). DED may be more common than even these estimates would suggest, due to under-diagnosis of this condition.

1.3.2 DED is Becoming More Common as the General Population Ages

The number one risk factor for DED is increasing age, with female sex and hormonal changes coming second and third (Friedman, 2010). The incidence of dry eye disease in older women (>80 years old) is 35% higher than the incidence in those aged 48-59 years (Moss, 2008). US Census Bureau estimates suggest that in the period between 2000 and 2050, the number of people in the US aged 65-84 years will increase by 100%, and the number of people aged 85 years and older will increase by 333% (Source: U.S. Census Bureau, 2004, "U.S. Interim Projections by Age, Sex, Race, and Hispanic Origin"). Thus, the high prevalence of dry eye disease in the older age group, combined with aging of the general population and increased life expectancies, raises the public health significance of dry eye disease and has important implications on the cost of providing health care for chronic disease afflicting this age group (also see 1.3.4 below) (DEWS report 2007; Dwyer 2006; Yu, 2011).

1.3.3 DED Substantially Interferes with Quality of Life

It is easy to regard DED as a trivial condition that is merely an irritation to those suffering from it. However, the consequences of dry eye may actually be quite serious. It can have a considerable impact on visual function, daily activities, social and physical functioning, workplace productivity, direct and indirect costs of the disease and quality of life (QOL) (Gayton, 2009; Pflugfelder, 2008; Miljanović, 2007; DEWS report 2007; Yu 2011; Vehof, 2016). In a cohort study of patients with dry eye disease, three quarters of those with non-Sjögren's DED reported that symptoms affected their daily activities despite the use of available treatments. Most frequently affected were the ability to function normally during a variety of daily activities and several indicators of well-being (feeling less-confident, frustrated and unhappy or depressed). Over a quarter noted interference most or all of the time with reading, driving at night, and working with computer screens…activities that most people do every day (see table, next page) (Friedman, 2010; Nelson, 2000). The perceived negative impact of DED symptoms is often discordant with what the clinical signs of DED would predict and is positively associated with lower self-perceived overall health status (Vehof, 2016).

DED is a condition that inflicts chronic pain on those who suffer from it, leading to diminished quality of life and potential loss of livelihood. It has been found that people with Sjögren's and non-Sjögren's dry eye had reduced quality of everyday life relative to people with no dry eye; an effect that increased with increased dry eye severity (Mertzanis, 2005). Vehoff et. al. report that people with chronic pain syndromes (CPS) have higher symptom scores than DED patients without CPS.

The morbidity associated with dry eyes is considerable. Schiffman and colleagues assessed patients with dry eye syndrome with time-tradeoff utility analysis. Utility assessment is a formal method for quantifying patient preferences for health outcomes. They showed that DED reduced quality of life with mean comorbidity-adjusted utility scores ranging from 0.62 for severe DED to 0.78 for mild DED; which compared to utility scores of 0.75 and 0.72 for moderate and moderate to severe angina pectoris (class III/IV), thus underscoring the seriousness with which patients with dry eye view their disease.

Table 4. Effect of Dry Eye Symptoms on Daily Life	
Quality-of-Life Measure[*]	**Patients, %[†]**
Feel less confident	38.6
Need to decrease leisure time	35.7
Get frustrated with daily activities	34.3
Need to change activities	25.7
Feel unhappy or depressed	25.7
Need to decrease work time	11.4
Require help	14.3
Miss outings	12.9
Change work	7.1
Other	22.9
None of the above apply to me	27.1
Vision-Related Activity[‡]	**Patients, %**
Driving at night	32.3
Reading	27.5
Working with computer/bank machine	25.7
Watching television	17.9

[*]Any impact of symptoms.
[†]Each patient indicated as many items as appropriate.
[‡]Symptoms interfered with activities most or all of the time.

The significant impact of DED on diminishing the quality of life has been recorded despite the availability of current treatments for dry eye such as artificial tears, punctual plugs, moisture chamber spectacles, contact lenses and anti-inflammatory drugs (i.e., cyclosporine, corticosteroids and tetracyclines). Tear supplements can minimize the symptoms and alter the composition of the tears in dry eye disease but they do not treat the underlying etiology of the condition; neither do punctual plugs, moisture chamber spectacles or contact lenses. Though anti-inflammatory drugs do target the underlying etiology of DED, most of them are limited due to their risk profile and/or efficacy. Hence, despite the presence of current treatment options, patients are still symptomatic and have significantly diminished quality of life due to DED.

1.3.4 Economic Impact of DED on Healthcare Costs

A review of the economic impact of DED determined that the average cost of managing DED was $55.4 billion to the US society overall, taking into account both healthcare costs and loss of productivity costs (Yu, 2011). DED may impose an economic burden on patients and on society because of the utilization of healthcare resources such as physician visits, medications and surgical procedures. Moreover, DED is associated with decreased productivity and days missed from work (Yu, 2011; Reddy, 2004; Brown, 2009; Pflugfelder, 2008; Enzenauer, 2003; Cross, 2002). In an analysis by Yu et al, survey data were collected from 2171 respondents with DED to estimate both the direct and indirect annual cost of managing dry eye disease in the United States from a societal and a payer's perspective. The direct costs included ocular lubricants, cyclosporine, punctal plugs, physician visits and nutritional supplements. The indirect costs were measured as the productivity lost because of absenteeism and presenteeism. The average annual cost of managing a patient with dry eye was estimated at $783 (variation, $757–$809) from the payers' perspective. When adjusted to the prevalence of DED nationwide, the overall burden of DED for the US healthcare system would be $3.84 billion. From a societal perspective, the average cost of managing DED was estimated to be $11,302 per patient and $55.4 billion to the US society overall. In addition, incomplete efficacy of existing therapies may drive the use of complementary and alternative medicine, and such medicines are generally un-reimbursed expenses and unaccounted for (Reddy, 2004). Reddy (2004) also identified intangible costs such as the monetary value of avoiding pain. Most of the data on the economic impact of DED is derived from survey data and/or review of insurance information, or from a small subset of patients (e.g. those on cyclosporine) rather than large well-characterized DED population. The DREAM study will address this gap in our knowledge of the economic impact of DED by using a very large, well defined DED population in a well-planned RCT and capture important information on the direct and indirect costs of DED in a group that will be representative of the general population and determine if a new treatment will be more cost-effective. In addition, it has been estimated that a new, more effective DED treatment could reduce non-drug direct medical costs by as much as 30% (Pflugfelder, 2008; Alves, 2013), thus stressing the need for further research in understanding DED and its treatment.

1.3.5 Unregulated Use of Over-the-Counter (OTC) Preparations of Polyunsaturated Fatty Acids (ω3 PUFAs) to Treat DED

It has been estimated that the dietary supplement market (including ω3 PUFAs) in the United States is currently $18.5 billion annually (Health Strategy Consulting: http://www.health-strategy.com). Since ω3 PUFAs are available OTC and are not strictly regulated, there is a lack of financial incentive from pharmaceutical companies or companies that produce nutritional supplements to conduct well-controlled rigorous trials. The NIH has called for increased evidence on efficacy and safety of supplements for chronic diseases, including randomized clinical trials (http://www.ahrq.gov/clinic/tp/multivittp.htm). Because Dry Eye Disease is prevalent, increasing in incidence, and has serious negative consequences on patient quality of life, there is a need for effective DED treatments. There has been much interest in nutritional supplements as alternatives to pharmacological treatments (Barnes, 2004). Awareness of benefits of ω3 PUFAs has increased dramatically over the past few years. Though ω3 PUFAs have been reported to have many beneficial effects and have a well-established role in inflammatory diseases, such as rheumatoid arthritis and inflammatory bowel disease as well as cardiac disease (Simopoulos, 2002; Goldberg, 2006; Caughey, 2010; Bahadori, 2010; Calder 2010; Mozaffarian, 2011; www.americanheart.org, April 12, 2011), the same is not true for their role in DED. Although there is generally some basis for believing that ω3 PUFAs are effective at preventing DED or

slowing its progression, there is no substantial evidence to date. Studies evaluating their safety and efficacy are unfortunately fairly limited and have shown mixed results (Bielory, 2003; clinicaltrials.gov identifier, NCT00344721). Nonetheless, many patients as well as doctors believe that they are effective. The Preferred Practice Pattern for Dry Eye Disease by the American Academy of Ophthalmology actually recommends the use of systemic ω3 PUFAs supplements for moderate dry eye disease; however they claim that the use is only **potentially** beneficial, and there have been only a few studies analyzing their efficacy (American Academy of Ophthalmology http://one.aao.org/summary-benchmark-detail/dry-eye-syndrome-summary-benchmark--october-2012). Despite this dearth of evidence, or possibly only anecdotal evidence, many clinicians prescribe and many individuals already take ω3 PUFAs for DED (Alpine, 2006) leading to a massive expenditure of resources without good evidence of efficacy. Hence, the time is ripe for a double masked randomized controlled clinical trial with a sufficient number of subjects to thoroughly investigate the role ω3 PUFAs in the management of DED and provide the highest level of evidence for the same, to address patient needs, clinician needs and also the NIH goals.

1.4 The Role of Inflammation in DED

Inflammation now has a well-established role in DED of varying etiologies such as Sjögren's, non-Sjögren's, evaporative DED, etc. (Stern, 2013; Lisi, 2013). Extensive research in both animal models and DED patients has shown that regardless of the initiating cause, a vicious cycle of inflammation develops on the ocular surface in dry eye that leads to ocular surface disease. The ocular surface and the tear-secreting glands function as an integrated unit. Dysfunction of this unit may develop from aging, a decrease in supportive factors (such as androgen hormones) and ocular surface diseases (HSV), MGD, and systemic inflammatory/ autoimmune disease (e.g. Sjögren's syndrome, rheumatoid arthritis etc.). This dysfunction leads to changes in tear composition such as hyperosmolarity, which stimulate the production of inflammatory mediators on the ocular surface. Inflammation may in turn cause dysfunction or disappearance of cells responsible for tear secretion or retention, further exacerbating DED and the development of a self-perpetuating inflammatory cycle. In addition, inflammation is responsible in part for the chronic irritation and pain symptoms that develop (Chen, 2011; Lee, 2011; Barabino, 2010; Pflugfelder, 2008; Enríquez-de-Salamanca, 2008; Niederkorn, 2007; Preferred Practice Patterns recommended by the American Academy of Ophthalmology 2008; DEWS report 2007).

1.4.1. Evidence of the role of inflammation in pathogenesis of DED

Both animal and human studies on DED have shown that the ocular surface demonstrates increased levels of inflammatory mediators such as cytokines, chemokines, matrix metalloproteinases, increased T cell activation and biomarkers such as HLA-DR.

Cytokines such as IL-1b, IL-7, TNFa and IFN-g, in mouse DED model (Chen, 2011; Song, 2003; Luo 2004; Corrales, 2007; Jin, 2013), and IL-1, TNFa, IL-6 and IL-8 in dry eye patients (Pflugfelder, 1990; Solomon, 2001; Yoon, 2007; Lam, 2009; Wei, 2013; Li, 2013; Hagan, 2013; Lee, 2013) were most frequently elevated in DED. Inflammatory chemokines like IL-8, Macrophage inhibitory protein (MIP) and CCL5 and chemokine receptors such as MIP-2, KC and CCR5 (Goyal, 2009; Gulati, 2006; Yoon, 2007; Lam, 2009; Song, 2003) were also found to be significantly up-regulated on the ocular surface; CCR6/CCL20 were shown to mediate TH17 cell migration to the ocular surface in DED (Dolhman, 2013); T cell (CD3+), as well as subtypes CD4+, CD8, T cell infiltrations are found increased in conjunctiva and lacrimal gland in both Sjögren's and non-Sjögren's dry eye patients (El Annan, 2009; Raphael, 1988; Pflugfelder, 1990;

Stern 2002; Ogawa 2003; Rojas 2005) and are pivotal in the development of cell-mediated immune responses (Perez, 2016). The increased inflammatory mediators found on the ocular surface strongly suggest their active participation in dry eye pathogenesis. Zoukhri (Zoukhri, 2002) confirmed the elevation of IL-1β and IL-1RI in a murine model of Sjögren's syndrome, and showed that exogenous addition of this cytokine inhibited neurally mediated lacrimal gland secretion through a c-Jun NH2-terminal kinase mechanism (Zoukhri, 2006). A synthesized cytokine antagonist based on the structures of IL-1β and IL-1Rα has demonstrated potential to treat DED in mice (Hou, 2013). De Paiva et al (2009) studied the role of TH-17 responses in dry eye and concluded that desiccating stress leads to increased matrix metalloproteinase-9, Th-17-associated genes, IL-6, IL-23, transforming growth factor- β1 and -2, IL-23R, IL-17R,IL-17A, and IFN- γ in cornea and conjunctiva, which in turn led to disruption of the corneal barrier. Antibody neutralization of IL-17 ameliorated experimental DE-induced corneal epithelial barrier dysfunction and decreased the expression of matrix metalloproteinases 3 and 9 (Acera, 2013; Sambursky, 2013). TH-17 related responses were also found to induce autoimmunity in DED and suppression of TH-17 was suggested as a new target for dry eye therapy (Chauhan, 2009, Pflugfelder, 2013). TH17 cells play a principal role in chronic DED (Chen, 2014). Decreased Muc5AC and overly produced IL-6 were found to be correlated with severity in DED patients (Zhang, 2013).

Pathologic changes of ocular surface cells not only are the result of dry eye disease, but also actively participate in the modulation of inflammatory responses. All of the above mentioned proinflammatory chemokines and cytokines are highly expressed in ocular surface tissues as well as in tears. Ocular resident epithelia in DED express increased ICAM-1 (Gao, 2004), which may serve as a signaling molecule for predisposition of ocular surface to inflammation and facilitate potential antigen presentation by epithelial cells (De Saint Jean, 1999). Human Leukocyte Antigen- (HLA-) DR is normally expressed on most immuno-competent cells, such as B and T lymphocytes and antigen presenting cells and is up-regulated in response to signaling such as inflammation. Increased HLA-DR antigen expression by the conjunctival epithelium detected by flow cytometry has been observed as a universal feature of dry eye (De Paiva, 2008). Multiple studies have showed increased HLA-DR expression in dry eye disease (Stern, 2002; Tsubota, 1999; Brignole-Baudouin, 2001, 2004, 2005). The expression of HLA-DR has been found to correlate in a positive fashion with increasing disease severity and has been shown to decrease in correlation with treatment (Brignole-Baudouin, 2001; Epstein, 2013).

In addition to the extensive evidence of inflammation of the ocular surface in DED, current treatment of DED includes use of anti-inflammatory drugs like lifitegrast, steroids and cyclosporine in treating patients across the spectrum of dry eye disease (see section 1.4.2).

To summarize, though the pathogenesis of dry eye disease is likely to be multifactorial, inflammation of the ocular surface has been demonstrated as an important component of dry eye disease, regardless of etiology. Ω3 has been shown to have anti-inflammatory effects, and hence we believe that there is a potential for ω3 PUFAs in treating DED irrespective of the etiology.

1.4.2 Current anti-inflammatory treatment of DED

Following the recognition that inflammation plays a role in the pathogenesis of DED, therapeutic approaches to control inflammation have emerged. The major anti-inflammatory agents currently in use include topical corticosteroids and immunomodulatory agents (Paiva, 2008).

Corticosteroids can effectively diminish DED related signs and symptoms (Marsh, 1999; De Paiva, 2006; Avunduk, 2003; Pflugfelder, 2004). Unfortunately, long term use of topical steroids is associated with complications, such as cataracts and steroid induced glaucoma; hence long term therapy cannot be advocated (Foulks, 2008). They are also associated with decreased bacterial resistance, further adding to the disadvantage of long term use.

Cyclosporine A, an immunomodulator inhibiting T cell activation, approved in 2002 by the FDA for the treatment of ocular conditions, was investigated because of the role of inflammation in the pathogenesis of Dry Eye Syndrome (DES) (McCabe, 2009). Treatment with topical cyclosporine reduces expression of cell surface markers of both inflammation and apoptosis, as well as increases goblet cells associated with clinical improvement in conjunctival biopsies of patients with dry eye disease. There was also a reduction in lymphocytes in these biopsies (Kunert, 2002; Rashid, 2008; Foulks, 2008). In a randomized multicenter clinical trial with 877 dry eye patients with moderate-severe dry eyes, there were significant improvements in corneal fluorescein and rose bengal staining in dry eye patients after treatment with cyclosporine (Sall, 2000). It was also shown to increase tear production as assessed by Schirmer's test, and symptoms of burning and itching decreased from baseline with consistent decrease in clinical signs of dry eye (Sall, 2000; Foulks, 2008). However, not all patients respond well to treatment with cyclosporine. The manufacturer noted that even in the patients who may respond, it may need to be used for 30 days or more before any therapeutic benefit may be observed. So to reduce the treatment duration and also to determine if the patient would respond to cyclosporine, it was suggested that the doctor may initially start the patient on a low-dose steroids (Ridder, 2008). The product information (label) for cyclosporine cites the evidence for efficacy as 15% of cyclosporine-treated patients vs. 5% of vehicle-treated patients having an increase in Schirmer wetting of 10mm or more. Also, it has been found to have some irritating side effects such as burning, stinging, and conjunctival hyperemia, which may contribute to noncompliance and thus poor efficacy (Barber, 2005).

In 2016, the FDA approved lifitegrast ophthalmic solution 5.0% for the treatment of the signs and symptoms of DED. Lifitegrast is an integrin antagonist that decreases T cell-mediated inflammation by blocking the binding of the cell surface proteins lymphocyte function-associated antigen-1 (LFA-1) and intercellular adhesion molecule-1 (ICAM-1) (Tauber, 2015; Perez, 2016). LFA-1 and ICAM-1 are known to have a role in the immune response (Pflugerfeder, 2016; Semba & Gadek, 2016). In a study of 718 adult subjects followed for 90 days, lifitegrast was found to significantly reduce ocular dryness and discomfort as measured on the Visual Analogue Scale compared to subjects on placebo, but no between group differences were noted in non-inferior corneal staining, total corneal staiing or nasal lissamine staining (Tauber, 2015). The effectiveness and acceptability of lifitegrast cannot yet be reported due to the recency of its availability.

Other immunomodulatory drugs (i.e., tacrolimus, sirolimus, MMF, cyclophosphamide, ISA-247/LX-211, etc.) are currently being investigated for ocular inflammation but none are FDA approved for treating DED. These drugs are mainly being investigated in the treatment of uveitis and most are given systemically (Ridder, 2008). ISA-247/LX-211 is an analog of cyclosporin that exhibits greater inhibition of calcineurin than cyclosporin and thus may have a greater immunosuppressive effect and be more effective in the treatment of dry eye than cyclosporin (Anglade, 2007). Topical application of tacrolimus has been shown to increase tear production

in dogs with keratoconjunctivitis sicca (Berdoulay, 2005). Systemically applied methotrexate can relieve the severe dry eye associated with Sjögren's syndrome and has also been used to treat uveitis and scleritis (Cordero-Coma, 2007; Shah, 1992). However, serious side effects such as bone marrow suppression, hepatic and renal toxicity, etc. are very common with systemic immunosuppression (Shanmuganathan, 2005). Current research is directed at identifying new anti-inflammatory molecules with better safety and efficacy profiles, some of which include Resolvins, lymphocyte function associated antigen-1 (LFA-1) antagonists and adenosine receptor antagonists. Future studies are needed to see how these drugs perform in the treatment of dry eye, their long term risk profile and whether effective topical preparations can be produced (Ridder, 2008; Gadaria-Rathod, 2013).

The international workshop on Meibomian Gland Disease (MGD) recommended use of drugs like topical azithromycin and oral doxycycline in DED due to MGD for their anti-inflammatory effects (Geerling, 2011; Foulks, 2010).

Despite the success of anti-inflammatory treatments for DED, most of them are limited by risks or side-effects, therefore new anti-inflammatory treatments are needed. An anti-inflammatory nutritional supplement such as ω3 PUFA is an appealing alternative for many patients.

1.5 ω3 Polyunsaturated Fatty Acids (ω3 PUFAs)

1.5.1 Background information about PUFAs

The ω3 and ω6 PUFAs are derivatives of the essential fatty acids (ω3 PUFAS) alpha-linolenic acid and linoleic acid respectively. The ω3 PUFAs are the 18-carbon polyunsaturated fatty acids. They are essential in the human diet because they cannot be synthesized by the body (Simopoulos, 2009; Wong, 2005; McCowen; Bistrian, 2005; Rosenberg, 2010). Once ingested, the 18-carbon ω3 PUFAs are desaturated and elongated to 20-carbon fatty acid, di-homo-Gamma linolenic acid (DGLA) and arachidonic acid (AA) (ω6 family), or eicosapentaenoic acid (EPA)(ω3 family) and docosahexaenoic acid(DHA)(ω3 family) all of which serve as precursors for eicosanoids. Eicosanoids formed from AA (ω6 family), e.g. PGE2, TXA2 , LTB4 etc., have the potential to increase blood pressure, inflammation, platelet aggregation, thrombosis, vasospasm, allergic reactions and cell proliferation. Those formed from EPA (ω3 family), e.g. PGE3, LTB5 etc., have opposing effects (James, 2000, Calder, 2001). Those from DGLA (ω6), PGE1 and TXA1, are also anti-inflammatory, thus making the effect of ω6 PUFAs on inflammatory response complicated. (Macsai, 2008; Simopoulos, 2009)

1.5.2 ω6 to ω3 ratios

The anti-inflammatory properties of ω3 PUFAs, especially EPA, are due to competition with arachidonic acid as a substrate for cyclooxygenases and 5-lipoxygenase. As shown in table 1, above, PUFAs derived from ω3 and ω6 compete for enzymes involved in their metabolism. Thus, the excessive consumption of foods rich in ω6 fatty acids may compromise the conversion of alpha-linolenic acid to EPA, with adverse effects for health and disease. There is an overproduction of proinflammatory PGE2 and underproduction of anti-inflammatory PGE1 and PGE3 when the ω6 to ω3 FA ratio is high (Calder, 2003). The ideal ω6:ω3 ratio in the diet is approximately 4:1, as is seen in the Mediterranean diet, rich in cold-water fish and natural oils (Simopoulos, 2001). An unfortunate consequence of industrialization may be a disturbance in the ratio of ω3:ω6 fatty acids, with higher consumption of ω6 than ω3. Studies suggest that human beings evolved with a diet that consisted of a 1:1 ratio of ω6 to ω3 fatty acids, but in current Western diets that ratio is closer to 15:1 (Simopoulos, 2002). Increasing systemic levels

of ω3 FAs like EPA and DHA by oral supplementation would thus help in lowering of the ω6:ω3 ratio and hence have an anti-inflammatory effect (Simopoulos, 2009; Milijanovic, 2005).

1.5.3 The effect of ω3 PUFAs on inflammation

ω3 PUFAs have broad anti-inflammatory effects shown in *in vitro*, animal feeding studies and healthy human volunteers. These studies provide an understanding of the mechanism of actions for the therapeutic effects of ω3 PUFAs on inflammatory diseases. Among the most widely reported effects of ω3 PUFA (EPA or DHA) on immune-cell responses is the inhibition of the production of proinflammatory cytokines IL1, IL2 and TNFα (Alnajjar, 2006; Endres, 1989; Calder, 1997; Calder 1998a; Calder 1998b; Khan, 2006; Purasiri, 1997; Venkatraman, 1999) and subsequently the proliferation of T lymphocytes (Calder, 1991; Calder, 1997; Calder 1998a; Meydani, 1991; Purasiri, 1997; Santoli, 1990; Venkatraman, 1999; Yaqoob, 2000; Wu, 1999; Zurier, 1999). This effect is similar to the main mechanism of action of cyclosporine in treating DED. Individuals with DED tend to have increased levels of TNFα and IL-1α in the tear film and hence could benefit from intake of ω3 fatty acids (Roncone, 2008). ω3 PUFAs have additional effects: culture with EPA or DHA inhibited the cytokine-induced cell-surface expression of MHC class II on mouse macrophages and of HLA-DR and HLA-DP on human monocytes (Calder, 1997; Calder, 1998a; Calder, 1998b; Venkatraman, 1999; Zhang, 2005). In accordance with this, the ability of human monocytes cultured with EPA or DHA to present antigen to autologous lymphocytes was diminished (Hughes, 2000). EPA and DHA also inhibited the cytokine-induced upregulation of adhesion molecules on the surface of cultured endothelial cells and decreased binding of leucocytes to endothelial cells (Calder 1997; Calder 1998a; Calder 1998b; Venkatraman, 1999; Wahle, 1999). These ω3 PUFAs have also been shown to inhibit the production of interleukin-1b and TNF-a by human monocytes (Calder, 1992a; Calder 1992b; Calder, 1997; Calder, 1998a; Caughey*,* 1996; D'Souza, 2006; Endres, 1989; Ferrucci*,* 2006; Jaudszus, 2005; La Guardia, 2005; Meydani*,* 1991; Purasiri, 1997; Rossetti*,* 1997; Sundrarjun, 2004; Venkatraman, 1999; Yaqoob*,* 2000; West, 2005; Wu*,* 1999), interleukin-6 by rat macrophages, and superoxide by human neutrophils (Calder, 1998a). Animal feeding and healthy human volunteer study showed similar effects (Calder, 1997; Calder, 1998a; Calder, 1998b; Johnson, 1997; Rossetti, 1997; Venkatraman, 1999; Yaqoob, 2000).

1.5.4 Clinical effectiveness of oral ω3 PUFAs intake on inflammatory diseases

The role of ω3 PUFAs has been evaluated in a variety of inflammatory diseases by several placebo-controlled clinical trials. Nearly all have shown that supplementation with oral ω3 PUFAs has significant beneficial effects with regard to changes in the signs, symptoms and pathophysiology of the disease and also has synergistic action with other anti-inflammatory treatments. Some of the diseases that may benefit from ω3 supplementation are inflammatory diseases such as rheumatoid arthritis, Crohn's disease, ulcerative colitis, psoriasis and lupus, as well as chronic conditions like cardiovascular disease and migraine, as also acute conditions like asthma (Simopoulos, 2002; Calder and Zurier, 2001; Volker, 2000; McCowen and Bistrian, 2005; Calder 2010, Proudman, 2013; Yates, 2013; Oliver, 2013; De Oliveria, 2013).

Inflammation is now considered a part of the pathogenesis of atherosclerosis. Several studies have shown a positive effect of ω3 PUFAs in lowering the incidence of ischemic heart disease and myocardial infarction as well as the risk of atrial fibrillation. Mozaffarian et al 2011 reviewed available evidence for cardiovascular effects of ω3 PUFA consumption and noted that ω3 PUFA consumption lowers plasma triglycerides, resting heart rate, and blood pressure and might also improve myocardial filling and efficiency, lower inflammation and improve vascular function.

They concluded that current data provide strong concordant evidence that ω3 PUFA are bioactive compounds that reduce risk of cardiac death. The American Heart Association recommends intake of approximately 1 g for secondary prevention of coronary artery disease and 2-4 grams daily for people with high triglycerides (www.americanheart.org, April 12, 2011). Goldberg et al (2006) conducted a meta-analysis of 17 randomized, controlled trials assessing the pain relieving effects of ω3 PUFAs in patients with rheumatoid arthritis or joint pain secondary to inflammatory bowel disease and dysmenorrhea and suggested that EPA/DHA supplementation reduces patient assessed joint pain intensity, morning stiffness, number of painful and/or tender joints, and NSAID consumption. Some authors have noted that ω3 supplementation may have a beneficial effect in patients with asthma. Asthma has been associated with a disturbance of the ω3 to ω6 ratio, and supplementation with ω3 may indeed reduce respiratory inflammation in asthma (Wong, 2005; Simopoulos, 2002). In patients with systemic lupus erythematosus (SLE) who are often non-responsive to conventional anti-inflammatory therapies, studies have shown benefit from supplementation with ω3 PUFAs. A double blind, randomized controlled trial of 52 SLE patients revealed that those consuming 3g EPA per day over a 24-week period exhibited significant declines in SLE activity (Pestka, 2010). In a comprehensive review on the role of ω3 PUFAs in inflammatory bowel disease, Calder (2008) noted that though clinical outcomes have been variable in different studies, some trials do report improved gut histology, decreased disease activity, decreased use of corticosteroids and decreased relapse. Besides these, ω3 PUFAs have shown positive effect in infant development, cancer, and more recently, in various mental illnesses, including depression, attention-deficit hyperactivity disorder and dementia. Though the mechanisms of action in these are unclear, it could be partly related to the effect of ω3 FAs in modification of the immune system (Riediger, 2009).

1.5.5 Relationship of PUFAs to DED

Moderate to severe DED is an inflammatory disease (see above); evidence includes increased T cell infiltration, tear inflammatory cytokine, ocular surface HLA-DR, and ICAM expression. ω3 PUFAs have been shown to have anti-inflammatory effects (Matsuyama, 2005; Calder, 2001; Mori, 2001; Jaudszus, 2005; Torres, 2006; Sundrarjun, 2004; Simopoulos, 2002; Calder and Zurier, 2001; Volker, 2000), and all the above inflammatory properties in DED have been reported to be inhibited by ω3 PUFAS. Preferred Practice Patterns recommended by the American Academy of Ophthalmology (2008) enumerated risk factors for dry eye disease, divided into mostly consistent, suggestive and unclear. Low dietary intake of ω3 fatty acids falls under mostly consistent risk factor.

The role of PUFAs, both ω3 and ω6, in dry eye has been studied in several animal and human studies.

Animal Models:

Dietary supplementation of ω6 GLA and ω3 EPA and DHA reduced the increase of PGE1 and PGE2 levels in a rat dry eye model induced with scopolamine in the exorbital lacrimal gland. It also prevented the decrease in mucin production (Viau, 2009). In another study by the same group it was shown that ω3 PUFA deficiency does not increase the severity of dry eye in a rat model of dry eye (Viau, 2011). In a mouse dry eye model, topical administration of ω3 and ω6 resulted in a significant decrease in fluorescein corneal staining, and it was associated with a decrease in the number of inflammatory mediators (Rashid, 2008). lIt has been shown that compounds like Resolvins, that are derivatives of EPA, increased tear flow, promoted a healthy epithelium, and decreased cyclooxygenase-2 (COX-2) and α-smooth muscle actin (α-SMA) as

well as macrophages infiltration in mouse model of DED (Li, 2008) and promoted resolution of inflammation in cultured rat and human conjunctival goblet cells (Dartt, 2011). Treatment with docosahexaenoic acid (DHA) in conjunction with nerve growth factor (NGF) or pigment epithelial derived factor (PEDF) has shown to increase nerve density and corneal epithelial cell proliferation after corneal surgery in rabbits (He, 2010).

ω3 and ω6 combinations

In a multicenter, randomized, controlled trial with 138 patients it was shown that oral 3 and 6 fatty acids for 3 months caused significant reduction in HLA-DR expression in dry eye patients as compared to placebo. However, no significant difference was found for the signs and symptoms, but there was a tendency for improvement in patients receiving the fatty acids treatment (Brignole-Baudouin, 2011). In another study comparing the effect of PUFA supplements alone to the effect of PUFA supplements with cyclosporine drops in the treatment of DED, it was shown that supplementation with ω3 and ω6 PUFAs improved TBUT and relieved patient symptoms. The addition of topical cyclosporine did not convey any statistically significant improvement in TBUT beyond that achieved by the supplement alone (Jackson, 2011). In another double masked randomized study with 181 dry eye patients, it was concluded that ω3 and ω6 PUFAs present an additional therapeutic advantage in patients suffering from ocular dryness who were already treated with lacrimal substitutes (Creuzot-Garcher, 2011). In another study, supplementation with sea buckthorn oil (Hippophae rhamnoides), which is high in ω3 and the ω6 fatty acid linoleic acid attenuated the increase in tear film osmolarity during the cold season and reduced symptoms in patients with dry eye (Larmo, 2010). In a randomized controlled trial of 38 post menopausal women with moderate to severe keratoconjunctivitis sicca at 2 centers, it was shown that a combination of GLA (ω6) with EPA and DHA (ω3) supplementation over a 6 month period led to statistically significant improvements in OSDI scores and surface asymmetry index as compared to the placebo (sunflower oil). Neither group had any improvement in TBUT, tear production or corneal and conjunctival staining. The placebo group showed significantly increased inflammatory markers: HLA-DR and CD11, as compared to the treatment group. The potential limitations of the study included a small sample size, self-reporting of compliance and the fact that the effects of other ingredients in the active supplement, such as Vitamin A, B6, C and E were not evaluated (Sheppard 2013).

ω6 alone

The specific ω6 fatty acid linoleic acid and its product gamma linolenic acid (GLA) are other alternatives. ω6 treatment appeared beneficial in alleviating dry eye symptoms, increasing tear production and improving overall contact lens comfort in patients suffering from contact lens-associated dry eye (Kokke, 2008). GLA and linoleic acid were also found to reduce ocular surface inflammation in patients with Sjögren's syndrome (Aragona, 2005). Oral supplementation of linoleic acid and GLA along with eyelid hygiene has also been shown to improve symptoms and reduce eyelid margin inflammation in meibomian gland dysfunction more than either treatment alone (Pinna, 2007). This effect could be explained by the reduction of inflammatory arachidonic acid products, where the dietary supplementation of linoleic acid and GLA results in the formation of less active prostanoids (Wu, 1999). It is also possible that these fatty acids help normalize the melting point of meibomian secretion.

ω3 alone

It has been shown that the anti-inflammatory effect of PUFAs was related to the balance between ω6 and ω3 PUFA intake. Inflammation can be suppressed when the ratio of ω6:ω3 is less than 4:1 (Calder, 2003; Simopoulos, 2001). A cross-sectional study of 32,470 women showed that women with a higher ω3 fatty acid intake in their diets had 68% less incidence of dry eye (Milijanovic, 2005). In the same study the relationship between the ingestion of ω3 fatty acids as well as the ω3 to ω6 ratio and dry eye syndrome was followed-up for four years. The investigators found that women who ate 5-6 servings of tuna fish per week, which contains high levels of ω3 fatty acids, had a 66% lower incidence of DED than women who ate 2 or fewer servings per week (Milijanovic, 2005). Therefore, several trials were conducted to study the effect of ω3 supplementation alone on DED and MGD (Borner, 2000; Sullivan, 2002; Schaumberg, 2003; Milijanovic, 2005; Macsai, 2008; Wojtowicz, 2011; Oleñik, 2013, Kawakita, 2013; Malhotra, 2015; Bhargava, 2015 and Bhargava, 2015(a)). In a pilot randomized clinical trial to investigate the effects of ω3 fatty acid supplementation (in the form of Flaxseed oil) on lipid composition of meibum, aqueous tear evaporation and tear volume in 36 dry eye patients over 90 days, it was found that the average tear production and tear volume was increased in the ω3 group as shown by Schirmer's test and fluorophotometry, as well as improvement in symptoms as measured by OSDI, but there were no significant effects in meibum lipid composition or aqueous tear evaporation rate or clinical signs of staining (Wojtowicz, 2011). In a prospective randomized placebo-controlled masked trial to study the effect of ω3 PUFAs in simple obstructive MGD and blepharitis, 38 patients received a dose of 3.3g/day of ω3PUFAs or the placebo over a period of 1 year. This trial demonstrated a decrease in the RBC and plasma ratios of ω6 to ω3 in patients taking ω3 dietary supplementation, as compared to controls, and improvements in their overall OSDI score, TBUT and meibum score.   This is the first demonstration of an induced change in the fatty acid saturation content in meibum as a result of dietary supplementation with ω3 fatty acids (Macsai, 2008). More recently, Bhargava & Kumar reported that in a trial of 600 contact lens wearing females who were symptomatic for Dry Eye Disease, 600mg of ω3 daily showed a benefit in alleviating dry eye symptoms, improving lens wear comfort and cytological changes (Bhargava 2015). Similarly, Malhotra reported that among 60 subjects with meibomian gland dysfunction, a daily dose of 1200 mg of ω3 fatty acids for 90 days significantly improved both photopic and mesopic contrast sensitivity, TBUT, ocular surface staining and meibum quality and expressibility (Malhotra, 2015). Epitropoulos and colleages also reported that after 12 weeks, supplementation with ω3 fatty acids yielded significant improvements in tear osmolarity, TBUT, MMP9 and the OSDI symptom scale among 100 patients with meibomian gland dysfunction.(Epitropoulos, 2016). The role of newer families of anti-inflammatory mediators have been studied, specifically resolvins and protectins, both of which are derivatives of ω3 PUFAs EPA and DHA. In animal models, these ω3 derivatives have shown to reverse corneal epithelial damage associated with dry eye, increase tear flow, promote a healthy epithelium, and decrease COX-2 expression and macrophage infiltration (Li, 2010). The synthetic analog of ResolvinE1 (RX-100045) is being tested in a Phase 2 clinical trial for the treatment of chronic dry eye. Preliminary data of a 28-day, randomized, placebo-controlled, 232-patient trial showed dose-dependent and statistically significant improvements in dry-eye patients treated with RX-100045 (Cortina, 2011). The compound also appears to be well tolerated when applied topically. In a study of 66 subjects, DED subjects (DEDG)(n=30) and controls (CG)(n=36) were randomized to receive the placebo (-NS) or the active supplement (S+), consisting of EPA, DHA, vitamins and antioxidants over a 3 month period. Significantly higher expressions of interleukin (IL)-1β, IL6, and IL10 and significantly lower vascular endothelial growth factor expressions were found in the DEDG as compared to the CG. However, levels of IL-1β, IL6, and IL10 in tears were significantly lower in the DEDG+S versus the

DEDG−NS and in the CG+S versus the CG−NS. Subjective symptoms of dry eye significantly improved in the DEDG+S versus the DEDG−NS. The study concluded that supplementation with ω3 and antioxidants help reduce inflammatory biomarkers and improve symptoms of DED (Pinazo-Duran 2013). In another double blind randomized controlled trial of 64 subjects, it was shown that daily supplementation of 360 mg EPA and 240 mg DHA for 1 month led to a statistically significant improvement in TBUT, Schirmers's scores and DED symptom scores as compared to the placebo (Kangari 2013).

In placebo controlled, double blind randomized trial of 264 eyes of patients with DED, it was shown shown that given supplement of (325mg EPA and 175mg DHA) twice a day for 3 months led to significant improvment in both Schirmer's test value and TBUT values in the ω3 group. (Bhargava R 2013). In another small trial, patients with mild to moderate dry eye disease and high tear osmolarity took one of 2 types of ω 3 supplementation (fish oil or krill oil) for 90 days, which found that both types resulted in reduced tear osmolarity and increased tear stability compared with placebo (Deinea, 2017).

Summary of role of ω3 and ω6 PUFAs in DED

The role of ω3 and/or ω6 PUFAs in the treatment of DED is still not completely understood. Though there is increasing evidence that supports their potential use for the treatment of this condition, there are limited randomized controlled trials as described above and most are not double-blinded. Most of the studies that do exist are small studies with data recorded from a single site, with different outcome measures and using varying combinations of PUFAs, ω3 or ω6 or both, with short study duration and contrasting results. Some of the larger studies were epidemiological. Just 1 large scale MGD study (Macsai, 2008) actually recorded changes in blood levels of the PUFAs with treatment to monitor compliance and co-relate treatment effect.

There is no consensus on the dose, composition, length of treatment etc. with ω3PUFAs. The Preferred Practice Pattern for Dry Eye Disease by the American Academy of Ophthalmology (AAO) actually recommends the use of systemic ω3 PUFAs supplements for moderate dry eye disease; however neither the DEWS report nor AAO outlined specific treatment recommendations with respect to dosing. The DREAM study will be the first large scale, multicenter, randomized controlled trial using ω3 PUFAs for DED. The PUFAs that were selected were ω3 PUFAs because 1) derivatives of ω3 are known anti-inflammatory mediators whereas ω6 derivatives are known inflammatory mediators; 2) the ideal ω6:ω3 ratio in the diet should be less than 4:1, but the western diets have a much higher ratio; hence supplementation with ω3 would help lower this ratio (section 1.5.2) and 3) most trials studying the role of PUFAs in various inflammatory diseases have shown anti-inflammatory benefits of supplementation with ω3 PUFAs (Section 1.5.4). Work done in our planning grant, which included a small scale clinical trial with ω3 supplements (DREAM: Feasibility study), extensive review of current literature and collaborations with experts in various fields, helped us identify an ideal composition and dose of the drug, as well as standardized outcome measures and compliance measures like blood tests, which will also help us to objectively co-relate changes in signs and symptoms with the actual levels of ω3 PUFAs in the blood (Gadaria-Rathod N 2013). In addition the DREAM study design of 12 months primary trial and another 12 months of extension study, which is unlike the other brief trials listed above, enables us to have a longitudinal assessment of DED with respect to changes in signs, symptoms and inflammatory biomarkers, in both the placebo and the treatment group and the effect of seasonal variations, if any. The results obtained from this study will help us better understand and describe DED itself and the role of ω3 PUFAs in treating it and thus enable us to confidently outline specific treatment recommendations for using ω3 PUFAs in DED.

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CHAPTER 2
SUBJECT SELECTION

2.1 Study Overview

This Dry Eye Assessment and Management (DREAM) study will include patients with moderate to severe dry eye disease. Patients will be recruited through approximately 20 clinical centers led by an ophthalmologist or an optometrist. The National Eye Institute, National Institutes of Health, Department of Health and Human Services provides funding for DREAM.

2.2 Rationale for Patient Inclusion

This study will include patients with dry eye disease of varying severity levels as we believe that omega-3 (ω3) fatty acids supplementation has the potential to provide benefits to most individuals suffering from this disease. Though the pathogenesis of dry eye disease is likely to be multifactorial, (DEWS 2007), inflammation of the ocular surface has been demonstrated as an important component of dry eye disease, regardless of etiology (Stern, 2004, Rashid, 2008). All dry eye disease, whether evaporative or aqueous-deficient in origin will ultimately result in inflammation of the ocular surface, be it a result of increased tear osmolarity (Gilbard & Farris, 1979; Foulks, 2008) or instability of the tear film. In aqueous-deficient dry eye, tear hyperosmolarity causes a cascade of inflammatory events on the ocular surface. In evaporative dry eye, the volatility of the tear film both leads to and exacerbates existing inflammation on the ocular surface (Pflugfelder SC, 2008; Enríquez-de-Salamanca A, 2008; Paiva CS, 2008; Lemp, 2008; DEWS 2007; Nichols KK, 2011; Stern ME, 2013; Stevenson, 2012).

Accordingly, topical treatment with immunosuppressive agents such as steroids and cyclosporine has been shown to be effective in treating patients across the spectrum of dry eye disease. The presence of HLA-DR expression in conjunctival biopsies and inflammatory cytokines in the tears of dry eye patients of diverse etiologies further speaks to the ubiquity of inflammation in dry eye disease (Epstein SP, 2013; Wei Y, 2013; Eberwein, 2013).

Ω3 and ω6 fatty acids compete with one another for the same desaturation enzymes. As such, high concentrations of one also serve to limit the effect of the other. Since the end-products of ω3 desaturation are known anti-inflammatory mediators, while ω6 fatty acids are used to create pro-inflammatory mediators, systemic intake of ω3 FAs have a pronounced anti-inflammatory effect. Ω3 supplementation has been shown to have therapeutic, anti-inflammatory effects in various other chronic inflammatory diseases (arthritis, Crohn's disease, ulcerative colitis, psoriasis, and lupus) (Simopoulos, 2002; Calder & Zurier, 2001; Volker, 2000; McCowen & Bistrian, 2005; Gadaria-Rathod N, 2013).

Therefore, since inflammation of the ocular surface is part of the pathogenesis of most dry eye disease and ω3 has been shown to have anti-inflammatory effects, subjects with DED symptoms for whom inflammation may play a key role in their disease will be included in this trial.

2.3 Identifying eligible subjects for the Primary Trial

2.3.1 Subject Recruitment and Screening

Patients referred to the Clinical Site for evaluation of dry eye disease will be recruited and contacted by the Coordinator or a participating clinician to begin the process.

Prior to the start of the study, the local clinicians in the region around each Clinical Site will be canvassed with a standardized letter introducing the study, the anticipated start date and requesting referral of potential patients.

2.4 Subject Inclusion Criteria for the Primary Trial

Eligibility criteria have been designed to include a broad spectrum of symptomatic patients with moderate or severe dry eye, typical of the patient population seen in clinical practice.

Each patient must meet the following criteria to be enrolled in this study:

1. Sign and date the informed consent form approved by the IRB

2. $\geq$ 18 years of age

3. Demonstrate at least 2 of the 4 following signs in the same eye at two consecutive visits.   The same signs must be present in the same eye on both visits. ((Screening Visit): 7–21 days prior to randomization, and Visit 00 (Baseline Visit): day of randomization)

    a. Conjunctival staining present $\geq$ 1 (out of possible score of 6 per eye)

    b. Corneal fluorescein staining present $\geq$ 4(out of a possible score of 15 per eye)

    c. Tear film break up time (TFBUT) $\leq$ 7 seconds

    d. Schirmer's test $\geq$ 1 to $\leq$ 7 mm/5min

4. Demonstrate symptoms of dry eye disease (OSDI score of at least 25 ($\geq$ 25 TO $\leq$ 80) at Screening Visit and at least 21 ($\geq$ 21 TO $\leq$ 80) at randomization visit

5. Patient reported dry eye-related ocular symptoms for at least 6 months before the Screening Visit and use or desire to use artificial tears on average 2 times per day in the 2 weeks preceding the screening visit

6. Intraocular pressure (IOP) $\geq$ 5 mmHg and $\leq$ 22 mmHg in each eye

7. Women of child-bearing potential must agree to use a reliable method of contraception during study participation and must demonstrate a negative urine pregnancy test at the Screening Visit

8. Be willing/able to return for all study visits and to follow instructions from the study investigator and his/her staff

9. Be able to swallow large, soft gel capsules

10. Demonstration of compliance with taking softgels as directed during the run-in period ($\geq$ 90% taken, by pill count)

2.5 Subject Exclusion Criteria for the Primary Trial

Patients who meet any of the following criteria will be excluded from the study:

1. Allergic, by patient report, to ingredients of the active or placebo pills (fish, olive oil)

2. Contact lens wear:

   - Discontinuation of use of contact lenses within the last 30 days prior to the Screening Visit.
   - Unwilling to commit to no use of contact lenses for the next year.

3. Pregnant or nursing/lactating

4. Participation in a study of an investigational drug or device within the 30 days preceding the Screening Visit

5. Current diagnosis of any of the following ocular conditions:

    i) acute allergic conjunctivitis

    ii) infection (e.g. bacterial, viral, protozoan or fungal infection of the cornea, conjunctiva, lacrimal gland, lacrimal sac or eyelids)

    iii) inflammation (e.g., retinitis, macular inflammation, choroiditis, uveitis, scleritis, episcleritis, keratitis)

6. History, by patient report, of ocular herpetic keratitis

7. Ocular surgery (including cataract surgery), by patient report, within 6 months of Screening Visit

8. Previous LASIK surgery or any other corneal surgery, by patient report.

9. Use of glaucoma medication or history of filtering surgery for glaucoma

10. Eyelid abnormalities that affect lid function (e.g., lagophthalmos, blepharospasm, ectropion, entropion, severe trichiaisis, etc.)

11. Extensive ocular surface scarring or condition that may compromise ocular surface integrity such as Stevens-Johnson syndrome, prior chemical burn, recurrent corneal erosions, persistent corneal epithelial defects, prior ocular trauma, etc.)

12. Use of EPA/DHA supplements. Cod liver oil is considered an EPA/DHA supplement.

    - Current use of EPA/DHA supplements in excess of 1200 mg/day.
    - Reduction in dose within the past 30 days of EPA/DHA from above 1200 mg/day to under 1200 mg/day.

13. History of liver disease, by patient report.

14. Currently on anti-coagulation therapy such as heparin and warfarin including the novel anticoagulants like dabigatran, apixaban and rivaroxaban. Use of aspirin, clopidogrel (Plavix) or ticagralor and prasugrel (anti-platelets) does not exclude the patient.

15. Patients with hemophilia, thrombocytopenia or other bleeding tendencies, by patient report.

16. History of atrial fibrillation, by patient report.

17. Uncontrolled ocular or systemic disease, by patient report.

18. Cognitive or psychiatric deficit that precludes informed consent or ability to perform requirements of the investigation.

2.6 Eligibility Criteria Regarding Use of Treatments for Dry Eye Disease and Treatments Affecting Dry Eye Disease for the Primary Trial

In general, patients who are on specific treatments for their dry eye disease or systemic treatments that affect dry eye disease must commit to maintaining their current practices for the duration of the Primary Trial (1 year). Criteria for specific treatments are described below.

1. Punctal plugs: Patients who regularly use punctal plugs are eligible if their plugs have been in place for at least two weeks prior to the Screening Visit and they are willing to commit to the same use of plugs for the next year. If a patient has their punctual plugs replaced or removed, they must wait two weeks before their Screening Visit.

2. Lid scrubs and warm soaks of the lids: Patients who regularly use lid scrubs or warm soaks at the time of the Screening Visit are eligible if they are willing to commit to the same use for the next year.

3. Lacriserts: Patients who regularly use Lacriserts at the time of the Screening Visit are eligible if they are willing to commit to the same use for the next year.

4. Artificial tear drops: Patients using artificial tear drops at the time of the Screening Visit are eligible if they are willing to commit to using the same brand for the next year.

5. Anti-histamine eye drops: Patients using antihistamine eye drops are eligible if they have not used topical anti-histamines for 14 days prior to the Screening Visit. Use of these drops during the study will be recorded on study forms.

6. Doxycycline (e.g., Oracea, Vibramycin, Doryx, Monodox): Patients who are using doxycycline at the time of the Screening Visit who want to continue using doxycycline are eligible if they have been using the drug for at least 90 days prior to the Screening Visit and commit to using the drug for the next year. Patients who have discontinued use of doxycycline within the last 30 days are not eligible.

7. Topical cyclosporine (Restasis): Patients who are using topical cyclosporine at the time of the Screening Visit who want to continue using topical cyclosporine are eligible if they have been using the drug for at least 90 days prior to the Screening Visit and commit to using the drug for the next year. Patients who have discontinued use of topical cyclosporine within the last 30 days are not eligible.

8. Topical steroid eye drops or ointment: Patients who are using steroid eye drops/ointment at the time of the Screening Visit who want to continue using them are eligible if they have been using the drops/ointment for at least 90 days prior to the Screening Visit and commit to using the drops/ointment for the next year. Patients who have discontinued use of steroid eye drops/ointment within the last 30 days are not eligible.

9. Chronic use of antibiotic eye drops or ointment: Patients who regularly use antibiotic drops/ointment at the time of the Screening Visit who want to continue using them are eligible if they have been using the drops/ointment for at least 90 days prior to the Screening Visit and commit to using the drops/ointment for the next year. Patients who have discontinued use of antibiotic drops/ointment within the last 30 days are not eligible. Patients who have discontinued use of antibiotic drops/ointment within the last 30 days are not eligible

10. Use of antibiotic eye drops or ointment for an acute infection: Patients who have used antibiotic drops/ointment within 30 days of the Screening Visit for treatment of an acute infection are not eligible.

11. Autologous serum eye drops: Patients who are using autologous serum eye drops at the time of the Screening Visit who want to continue using autologous serum eye drops are eligible if they have been using the drops for at least 90 days prior to the Screening Visit and commit to using the drops for the next year. Patients who have discontinued use of autologous serum eye drops within the last 30 days are not eligible.

12. Eyedrops other than those covered in the above criteria: Patients using eye drops other than those covered in the above criteria at the Screening Visit are not eligible unless they commit to discontinuing them for the next year.

13. Prokera amniotic membrane device: Patients who are using an amniotic membrane device at the time of the Screening Visit who want to continue using an amniotic

membrane device are not eligible. Patients who have discontinued use of an amniotic membrane device within the last 90 days are not eligible.

14. LipiFlow or intense light treatment: Patients who are using these treatments at the time of the Screening Visit who want to continue using one of these treatments are not eligible. Patients who have discontinued use of one of these treatments within the last 90 days are not eligible.

15. Systemic medications known to cause ocular dryness (e.g., isotretinoin (Accutane), anti-depressants): Patients who are using these medications at the time of the Screening Visit who want to continue using one of these treatments are eligible if they have been using them for at least 30 days and commit to using the medications for the next year. Patients who have discontinued use of one of these medications within the last 30 days are not eligible.

16. Systemic corticosteroids or other immunosuppressive agents: Patients who are using these drugs at the time of the Screening Visit who want to continue using these drugs are eligible if they have been using the drugs for at least 90 days prior to the Screening Visit and commit to using the drugs for the next year.

2.7 Eligibility Criteria for the Extension Study

Patients who complete the study visit at 12 months who were assigned to active supplements in the Primary Trial are eligible for the Extension Study if they agree to continue taking study supplements after the randomization to a supplement group for the second year, have a negative urine pregnancy test (women of childbearing potential only), and sign the informed consent statement.

2.8 Inclusion of Women

Gender is not an exclusionary criterion for this study. Every effort will be made to include women in the DREAM trial. If necessary, recruitment of women will be enhanced by targeted enrollment among the participating clinical centers, **however DED is more common in women.**

2.9 Inclusion of Minorities

Race/ethnicity is not an exclusionary criterion. Every effort will be made to recruit a study population that accurately reflects the prevalence of DED in the US population. If necessary, clinicians at the Clinical Site will contact local physicians who serve minorities who could refer patients to the local Clinical Site.

2.10 Exclusion of Children

Children will not be included in the study, since dry eye disease is a problem found primarily in adults.

Exhibit 2-1

Dry Eye Assessment and Management (DREAM) Design Summary

Feature	Criteria
Objective	Evaluate the effectiveness and safety of supplementation with ω3 fatty acids in relieving the symptoms of moderate to severe dry eye disease (DED)
Major Eligibility Criteria	**Primary Trial** ≥ 2 of the following 4 signs in the same eye at screening and baseline visits (Same signs must be present at Screening and Baseline Visits) • Conjunctival staining present ≥ 1 (out of possible score of 6 per eye) • Corneal fluorescein staining present ≥ 4 (out of a possible score of 15 per eye) • Tear film break up time (TBUT) ≤ 7 seconds • Schirmer's test ≥ 1 to ≤ 7 mm/5min Ocular Surface Disease Index (OSDI) score: 25-80 at screening, 21-80 at baseline Symptoms of DED ≥ 6 months Use or desire to use artificial tears ≥2 times/ day in preceding 2 weeks **Randomized Withdrawal (Extension Study)** Assigned to active supplements in Primary Trial and wiling to continue taking supplements
Randomization	Unit is person
Masking	Double masked
Treatments	1)Active supplements: 2000 mg EPA and 1000 mg DHA per day; 2)Placebo (olive oil)
Outcome Measures 1°	Mean of change from baseline in OSDI score at 6 and 12 months (Primary Trial) Mean of change from 12 months in OSDI score at 18 and 24 months (Extension Study)
2°	Compliance with the study treatment protocol as measured by changes in blood levels of fatty acids and pill counts ≥ 10 point change in OSDI-decrease for Primary Trial, increase for Extension Study Change in

Exploratory	Signs of DED (conjunctival and corneal staining, TBUT, Schirmer's test)
	Use of artificial tears and other treatments for DED
	Quality of life as measured by the SF-36
	Score on the Brief Ocular Discomfort Inventory (BODI)
	Cost and incremental cost-effectiveness
	Incidence of ocular and systemic adverse events, changes in VA and IOP
	Contrast sensitivity
	Meibomian gland secretion evaluation
	Signs measured by keratography: TBUT, tear meniscus height, redness, meibography
	Tear osmolality
	Biomarker levels:MMP-9 in tears, tear cytokine levels, expression of HLA-DR and other inflammatory markers on conjunctival cells, and serum antibodies associated with Sjögren's Syndrome and other autoimmune diseases
Sample size	Primary Trial: 579 total; 386 Active supplements, 193 Placebo
	Extension Trial: 190; 95 per group [50% in Active group in Primary Trial choose to enroll]
Follow-up	Primary Trial: Visits at 3,6,12 months; telephone call at 9 months
	Extension Study: Visits at 18, 24 months; telephone calls at 15 and 21 months
	Telephone call 1 month after exit from study

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CHAPTER 3
STUDY SUPPLEMENTS AND TREATMENT

3.1 Treatment Regimen for Study Supplements

Each patient will be instructed to take 5 soft gel capsules per day. Patients will take either active supplement or matching placebo (identical size, shape, color, and taste). Each active supplement soft gel capsule will contain:

400 mg EPA
200 mg DHA

The total daily dose from the 5 capsules will be 3.0 grams:

2000 mg EPA
1000 mg DHA

Active supplements will contain vitamin E to combat potential oxidative effects of EPA. Placebos will contain the same volume of olive oil. The daily dose of supplement or placebo constitutes approximately 45 calories.

3.2 Rationale for Composition and Dosage of Study Supplements

A ratio of EPA to DHA of 2:1 was selected because this ratio is found in many natural foods, and is nearly the same as the ratio (1.86:1) of EPA to DHA used in the Age-Related Eye Disease Study 2 (AREDS 2) clinical trial for age-related macular degeneration and cataract.

The dose of 3 grams was chosen to achieve a maximal therapeutic effect without added risk. Various doses have been used in clinical trials for a wide variety of conditions, with as little as 0.7g for a trial for dry eye to 5.6g for a trial for ulcerative colitis. (Brignole-Baudouin, 2011; Varghese, 2000). The American Heart Association recommends intake of approximately 1 g for secondary prevention of coronary artery disease (Kris-Etherton, 2003) and 2-4 grams daily for people with high triglycerides (http://www.heart.org/HEARTORG/GettingHealthy/NutritionCenter/Vitamin-and-Mineral-Supplements_UCM_306033_Article.jsp accessed December 12, 2013).

Also, in subjects already taking over-the-counter (OTC) ω3 supplementation, the dose of 3 grams is large enough to induce a significant change in ω3 levels and yet safe enough to be within the recommended limit. Currently, there are more than 200 OTC products containing ω3 fatty acids available to consumers and most brands limit supplementation to the 1000 mg dose. The maximum allowed additional supplementation by the inclusion criteria is 1200mg/day of ω3; hence even those patients taking OTC ω3 will be within the recommended limit.

This supplement composition and dosage was utilized in the DREAM: Feasibility study and proved to be effective in raising the RBC membrane levels of ω3 fatty acids (EPA and DHA) while simultaneously lowering ω6 fatty acid levels (arachidonic acid) in subjects taking the active supplement. Conversely, subjects taking placebo did not show a significant change in RBC membrane fatty acid composition.

In summary, selecting a high, yet safe dose maximizes the probability of detecting a treatment effect of ω3 fatty acid supplementation, if one truly exists.

3.3 Distribution of Supplements for the Run-in Period

Upon completing the certification process, each Clinical Center will receive a set of labeled bottles of supplements to distribute to patients for the run-in period. The Coordinator completes a supplement accountability log by entering the identification number and alpha code, date of distribution, and bottle identifier at the time supplements are given to the patient at the end of the first study visit (See Chapter 4.4.8.).

3.4 Assignment and Distribution of Supplements for the Primary Trial

After the study clinician assesses from the screening and baseline examinations and medical history that the patient is eligible for the trial and the patient has signed a consent form, the Clinic Coordinator will enter the required data into the DREAM Data Management system. The data system will check entered responses against all eligibility criteria and will indicate which items, if any, need correction or confirmation. If all required data have been received and the patient is eligible, the Clinic Coordinator opens the data management system's randomization module, answers questions about data collection completeness, and saves the form to generate a randomized treatment assignment for the patient. The system generates a message that confirms that the randomization has been successfully completed and the Clinic Coordinator faxes a prescription for study supplements, signed by the Clinician, to the Investigational Drug Service. The system also generates a message that is transferred to the Investigational Drug Service of the University of Pennsylvania, which mails a supply with the assigned supplements to the patient. The Investigational Drug Service will send additional shipments of supplements to the patient throughout the period of the Primary Trial. The Coordinator prints the follow-up visit schedule. The baseline visit materials and follow-up visit schedule are filed in the patient's Study chart.

Random treatment allocations will be computer generated and stratified by clinical center. A permuted block method of randomization will be used to assure balance over time.

3.5 Assignment and Distribution of Supplements for the Extension Trial

After patients are determined to be eligible for the Extension Trial and the patient has reaffirmed willingness to participate in the Extension Trial, the Clinic Coordinator opens the data management system's randomization module for the Extension Trial, answers questions about completeness of activities required before randomization, and saves the form to generate a randomized treatment assignment for the patient during the Extension Trial. The system generates a message that confirms that the randomization has been successfully completed and the Clinic Coordinator faxes a prescription for study supplements, signed by the Clinician, to the Investigational Drug Service. The system also generates a message that is transferred to the Investigational Drug Service of the University of Pennsylvania, which mails a supply with the assigned supplements to the patient. The Investigational Drug Service will send additional shipments of supplements to the patient throughout the period of the Extension Trial. The Coordinator prints the follow-up visit schedule. The follow-up visit schedule is filed in the patient's Study chart.

Random treatment allocations will be computer generated and stratified by clinical center. A permuted block method of randomization will be used to assure balance over time.

3.6 Administration and Storage of Supplements

Five capsules should be taken each day; the distribution throughout the day can be adjusted to the schedule of individual patients. All five capsules may be taken at one time or the capsules can be distributed across 1-5 different times during the day. Patients should be encouraged to select a daily dosing schedule and adhere to that schedule every day to aid remembering to take all 5 capsules each day. If any doses are missed, the patient should resume taking 5 a day the following day. Each patient is provided with a pill case designed to accommodate dosing for a week as another aid to remembering to take 5 capsules each day.

Supplements containers should be stored in a cool, dry environment. Supplement should not be frozen and should be kept below 77°. Supplements may be stored in a refrigerator, but not the freezer compartment.

3.7 Treatment Reduction or Suspension

Patients who have report upset stomach, burping, fishy aftertaste, or other minor gastrointestinal symptoms and who want to discontinue use of study supplements may have their daily dosage reduced in an effort to relieve symptoms. Patients who develop a condition for which the DREAM dose of 3 gm of DHA and EPA is contraindicated should be instructed to suspend treatment until the condition resolves. Some patients may refuse to take any study supplements. In all of the above cases, patients should be encouraged to continue their follow-up visits. When suspension of taking study supplements is believed to be permanent, the Clinic Coordinator must contact the Coordinating Center to discontinue additional shipments of study supplements to the patient. If the symptoms or conditions resolve, the patient may restart or increase their study supplements up to the original dose of 5 capsules per day. Patients should return all bottles of study supplement to the clinical center.

Whenever subjects reduce or discontinue study treatment, the clinic coordinator or treating clinician must report this in the subject's medical record. The entry should include all instructions given to the subject. Any discontinuation or reduction in study supplements is reported on the Follow-up Health Review Form that is completed at all follow-up visits.

3.8 Concomitant Therapy for Dry Eye Disease

In general, patients will be required to maintain the treatments that they were using at entry into the study throughout the follow-up period. Patients will be instructed to continue using the same brand of artificial tears during the study as they were using at the screening visit. However, if patients are unhappy with their brand of artificial tears, they will be allowed to change their brand after a period of six months. Similarly, patients using punctual plugs will be instructed to continue their use throughout the follow-up period. Usage of artificial tears and punctual plugs will be recorded at baseline and at each follow-up visit.

3.9 Rescue Therapy for Dry Eye Disease

Patients should be encouraged to continue taking study supplements throughout the period of their scheduled participation. Patients who report inadequate relief from symptoms and request additional treatment may be offered treatments for dry eye disease in addition to their study supplements as an alternative to the patient dropping out of the study. Patients who prefer open label ω3 supplements must return their study supplements and use over-the-counter ω3 supplements. These treatments will be recorded on the case report forms.

3.10 Risks Associated with Use of Ω3 Fatty Acids

Ω3 supplements are generally well tolerated. High doses of ω3 may in theory increase the bleeding time by inhibiting the arachidonic acid pathway. However, in a comprehensive review, it was concluded that there was no increased risk of clinically significant bleeding noted with ω3 doses of up to 7 g of combined DHA and EPA per day, even when combined with antiplatelet therapy or warfarin (Harris, 2007; Lavie, 2009; Watson, 2009; Defilippis, 2010; Lee, 2011). Clinically, it does not pose a significantly increased risk of bleeding for patients undergoing coronary artery bypass grafting, carotid endarterectomy, or femoral artery catheterization, even at high doses combined with antiplatelet therapy or warfarin (Harris, 2007; Bays, 2011).

Lovaza is an FDA-approved ω3 fatty acid supplement indicated for the treatment of hypertriglyceridemia in dosing regimens up to 4g/day. Concomitant anticoagulation is not contraindicated with Lovaza. However, the label cautions that "patients receiving treatment with both Lovaza and anticoagulants should be monitored periodically." The American Heart Association recommends that patients taking more than 3 grams of ω3 fatty acids from capsules should do so only under a physician's care. A recent interpretation of these safety considerations in patients taking high-doses of ω3 fatty acids is to use similar general guidelines as applicable to other anticoagulants. This includes discontinuing supplementation if bleeding episodes occur or if the patient is at high risk for bleeding complications as when undergoing major surgery (Bays, 2007).

In this study, all patients on antiplatelet drugs such as aspirin and Plavix (clopidogrel) will be periodically monitored for any history of increased bleeding or bruising. If such an event does occur, the patient will be instructed to discontinue the study supplements. All patients on anti-coagulants such as heparin and warfarin will be excluded from the study.

Ω3 fatty acids may lead to an elevation of liver enzymes in patients with hepatic impairment. Such patients should be monitored periodically for any changes in liver enzymes. In this study, patients with a history of liver disease will be excluded.

The most commonly observed side effects are nausea, gastrointestinal symptoms, fish-scented halitosis, and dysgeusia, which are not considered as major health hazards (Watts, 2007). There is also some concern about ingestion of mercury when fish oil is consumed, however purified fish oils in pharmaceutical grade capsules typically have negligible amounts (Watts, 2011; Saravanan, 2010). The manufacturers of the study supplement and the placebo will use a process to extract the ω3s and purify them and test the refined oil for purity and stability in voluntary compliance with standards set under the guidance of the Council for Responsible Nutrition. Once the study supplement is manufactured, the certificate of analysis documents that all specifications for the content of the supplements have been met.

3.11 Supplement Distribution, Storage, Accounting and Destruction

Federal law requires documentation of receipt, use, and disposition of every dose of investigational medication. The active and placebo supplements are manufactured by Nutrilite and then shipped to the Penn Investigational Drug Service (IDS) for labeling and distribution to the Clinical Centers (run-in supplements) and to patients (randomly assigned supplements). A set of supplement accountability records is used by each clinical center to document distribution and return of run-in supplements and return of supplements sent to the patient by the Investigational Drug Service.

Each Clinical Center will receive an initial supply of run-in supplements when a center has achieved DREAM certification. Subsequent supplies are ordered by the Clinic Coordinator by faxing the DREAM Order Form to the IDS. The IDS uses next day shipping and sends the designated supplement recipient at the center information to track the package. The Clinic Coordinator must track packages not received by the following afternoon and the IDS must be immediately notified about lost shipments. The staff at each receiving site will inspect the condition of each shipment of supplement containers upon arrival, and immediately stores the containers. Supplements are not to be used beyond the date printed on the packaging. Unused or expired supplements must be destroyed at the Clinical Center as specified in Exhibit 3-1.

Study supplements must be stored securely in the clinical center (if a separate pharmacy is not used) or in the local or on-site pharmacy (if one is used). It is important that supplements are not accessible to non-study staff. Supplements must be stored at room temperature or in a refrigerator (not a freezer) in a locked cabinet or other non-transportable locked container.

All run-in supplements received must be logged in and documented on the DREAM Study Supplement Accountability Log. Supplement Accountability Logs are supplied to simplify the accountability of the study supplement from receipt to dispensing or to the return or destruction of unused product. Bottles of supplement used during the run-in period must be returned by the patient at the Baseline Visit. If the patient does not return the bottle to the clinical center, the Clinic Coordinator should contact the Coordinating Center to arrange for IDS to handle return of the bottles. Failure to return the run-in bottle renders the patient ineligible for the study.

When study supplement bottles are dispensed to a patient, the patient's study ID number and alpha code, date dispensed and the dispenser's initials are recorded on the Study Supplement Accountability Log. Finally, when supplements are destroyed on site, an entry must be made on the Supplement Disposal Log.

Exhibit 3-1
Returning or Destroying Expired or Damaged Run-in Supplement and Returned Supplements

Expired or damaged supplements and supplements returned by patients can be destroyed on-site.

- If disposing of run-in supplements that have not been dispensed to study patients, enter the number of containers you removed from inventory, and enter the new balance on the supplement accountability log. Enter the lot # and expiration date of the containers you removed from inventory.

- Complete the DREAM Supplement Return and Disposal Log while performing the steps that follow to document the disposal for each container and/or group of supplements returned not in a container. Enter the date of the disposal and name(s) of the person(s) who conducted the disposal. Disposers need to sign and date the form.

- Empty the supplements into a red (biohazard waste) bag.

- Either remove the labels from the containers or mark out the bottle number with a heavy black marker.

- Dispose of the containers in the regular trash.

- Retain the Log on site and send copies to the Coordinating Center when requested.

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CHAPTER 4
PATIENT VISITS AND EXAMINATIONS

4.1 Introduction

The DREAM Primary Trial consists of four phases: screening, a 14 day run-in period (between screening and baseline visits), a baseline visit, and a 12 month double–masked treatment phase (baseline to 12 months). Each patient enrolled in the Primary Trial is required to complete a total of five in-office visits and one telephone visit. In addition, a subgroup of eligible patients will be enrolled in the Extension Study and will return for 2 more visits at months 18 and 24 and complete two additional telephone visits. Activities to be completed at each visit are specified in Exhibit 4-1. At least one Study case report form must be completed documenting each of the required visits.

Patients are encouraged to call the Clinic Coordinator at any time if they feel that their condition has worsened and that they need to be examined. An unscheduled visit may be performed as required between two scheduled visits (e.g., due to poor tolerance, assessment of adverse events) and the reason for the visit will be recorded on the CRF. The examinations required at this visit will be at the discretion of the investigator.

If any study visit is missed and cannot be rescheduled within the time window printed on the patient's appointment schedule, a Missed Visit Form must be completed and submitted to the Web based DREAM data system.

4.2 Informed Consent

Written informed consent must be obtained prior to initiation of any study-related procedures. No study-related measures shall be undertaken without obtaining written informed consent about the study and the study supplements. The Clinic Coordinator and the enrolling DREAM certified clinician share responsibility for the patient's orientation into the Study. The Clinic Coordinator should be present for the discussion and must make every effort to ensure that all of the patient's questions and those of the family are answered satisfactorily. The patient should not be asked to sign the consent form until either the Clinic Coordinator or the DREAM clinician has answered all questions. It is important that the patient understands the concept of randomization in clinical trials. The informed consent form shall be signed and dated by the patient. Before any information is submitted to the DREAM Web-based Data Entry System, a patient must sign the consent form.

One original consent form will be signed and a copy made. The copy will be given to the patient and the original form filed in the patient's study chart. The patient's study chart must also document the informed consent process, indicating who discussed the protocol with the patient, when the discussion occurred, whether all questions were answered, date consent obtained and whether the patient received a copy of the signed consent form.

4.3 Pre-Randomization/Run-In Period

There are two visits that occur prior to the patient's randomization into the study, the screening visit (SV) and the baseline visit (Visit 00), which occurs approximately 14 days after the SV (time window 7 to 21 days). The period between the SV and Visit 00 is the run-in period. The purpose of the run-in period is to identify and exclude people who are non-compliant with taking supplements and people who cannot take large pills. Potential patients who meet the eligibility requirements at the screening visit will receive a supplement to take over the course of the run-in period. If the patient successfully completes the run-in period by demonstrating appropriate use through pill count and meets the eligibility requirements at the baseline visit, they will be randomized to either active supplement or placebo.

4.4 Screening Visit (SV) Procedures

Screening visit procedures are intended to evaluate the patient for eligibility via slit lamp evaluation (SLE), tests to determine DED (TBUT, corneal fluorescein staining, Schirmer's tear test, conjunctival lissamine green staining) by questioning the patient about medical history and concurrent conditions, and dispensing run-in supplements. **Patients should NOT use any eye drops for two hours prior to their scheduled study visit**.

All procedures performed at the Screening Visit are recommended to be done in the following order. All ocular assessments should be done on both eyes.

1. Obtain informed consent
2. Assign DREAM identification number and alphabetic code
3. Completion of OSDI & BODI Questionnaire
4. Obtain patient demographic, ocular and systemic medical history
5. MMP-9 testing
6. Slit lamp evaluation (SLE)
7. Tear break up time (TBUT)
8. Corneal fluorescein staining
9. Meibomian gland evaluation
10. Lissamine green staining of the interpalpebral conjunctiva
11. IOP
12. Schirmer's tear test (with anesthesia)
13. Urine pregnancy test (for women of childbearing potential)
14. Determine eligibility
15. Dispense 14 day supply of run-in supplements, pill organizer and patient instruction sheet.
16. Schedule patient's baseline visit (Visit 00)

4.4.1 Patient Identification

Each patient will be assigned a permanent identification number and alphabetic code to be used on all study forms and specimens. The patient identification number is a two-part identifier consisting of a two-digit clinic number and three-digit sequence number. Patients will also have a four-letter randomly generated alphabetic code that is not linked to their name. The patient identification number and alpha code are available on pre-printed patient registration logs supplied to the Clinical Centers by the Coordinating Center.

Each patient is also associated with a site within a clinical center. The patient's site is identified by a two-digit clinic number followed by a single digit site number. The patient's site identifies the address that is used for sending all patient specific correspondence, such as edit queries and appointment reminders to the clinical center. At some point in follow-up, a patient may move from one site to another within a clinical center or from one clinical center to another. If the patient moves to another site or clinical center, a Transfer of Patient Form must be completed. The patient's identification number and alphabetic code are permanent and do not change even if a patient is transferred.

4.4.2 Dry Eye Severity Questionnaires

At all visits, including the screening visit, patients will be asked to complete questionnaires (OSDI and BODI) that ask about their dry eye symptoms and the impact of Dry Eye Disease on their daily lives.

These questionnaires must be completed by the patients by themselves. The clinic coordinator or other clinic staff should not ask the patient these questions.

4.4.3 Patient Demographic, Ocular and Systemic Medical History

The Clinic Coordinator and DREAM clinician, as appropriate, should review with the patient those questions on the case report forms that can be answered to ensure that the patient is eligible. Participation in other clinical trials is not an automatic exclusion; however, the Clinic Coordinator must call the Director of the Coordinating Center to discuss the treatment and follow-up required for any study in which the patient is already participating.

4.4.4 MMP-9 Testing

A test for evaluation of inflammation will be performed on each eye of the patient.

4.4.5 Slit Lamp Evaluation (SLE) and Other Tests to Assess Dry Eye Disease (DED)

At the screening visit, a slit lamp evaluation (SLE), evaluation of the meibomian glands and eyelids, and tests to determine signs of dry eye disease (tear break up time, corneal fluorescein staining, lissamine green staining and Schirmer's test with anesthetic) will be performed.

4.4.6 Intraocular Pressure (IOP) Measurement

IOP measurement in both eyes will be performed at the screening visit, using Goldmann applanation tonometry, a TonoPen, or similar device.

4.4.7 Women of Childbearing Potential

All women of childbearing potential must have a negative urine pregnancy test at the first study visit. Women of childbearing potential have not yet reached menopause and have not undergone tubal ligation or hysterectomy. If a sexually active woman of childbearing potential does not agree to use an acceptable method of contraception, she is ineligible for the study and cannot be enrolled. Acceptable methods of contraception include oral contraceptives, hormone patch or implant, intrauterine device (IUD), condom or diaphragm in conjunction with spermicidal gel, partner with vasectomy and celibacy.

4.4.8 Dispensing Run-In Supplements

The study supplies the bottles of the supplements for the run-in period to the Clinical Centers through the University of Pennsylvania Investigational Drug Service (IDS). If the potential study patient has completed all required SV procedures and remains eligible for the study, the Clinic Coordinator will dispense a bottle of run-in supplements to the patient. Each time a run-in bottle of supplements is dispensed, an entry is made on the Run-In Supplement Accountability Log. For additional information on the documentation of the receipt, use and disposition of study supplements, refer to section 4.6.10.

Each patient is also provided with an instruction sheet about how to take the supplements. Patients should begin taking supplements the day after the SV. Coordinators must be sure that the patient completely understands how the capsules are to be taken. A 7-day pill organizer will be given to the patient to assist them in complying with the dosing regimen.

4.5 Baseline Visit (00) Procedures

The baseline visit is intended to complete the assessment of the patient's eligibility for the study by performing procedures to assess dry eye signs and symptoms and to ascertain the patient's ability to comply with the treatment protocol. If the patient is eligible, the patient is issued a randomized assignment to either active study supplements or placebo. Approximately one week before the baseline

visit, the clinic coordinator will contact the patient to remind them to bring with them to the clinic their bottle of Run-In supplements, their pill organizer, and the containers with the labels for all medications and dietary supplements that they are currently taking, including cod liver oil. Also the coordinator will remind the patient not to use drops within 2 hours of the visit.

All Visit 00 procedures are recommended to be done in the order in the following sequence, unless specified otherwise:

1. Collect/count unused run-in supplements (any time during the visit prior to eligibility determination)
2. Completion of OSDI &BODI Questionnaires
3. Completion of WPAI, SF-36, Healthcare Utilization Questionnaires
4. Ocular and Systemic Medical History
5. Concomitant Medications and Dietary Supplements Use
6. Collect Adverse Event Information
7. Complete Patient Information Form (additional contact data)
8. Tear Osmolarity (at centers with TearLab Tear Osmolarity machine)
9. Keratograph non-invasive tear break up time, tear meniscus height, redness score, and meibography (at centers with required equipment)
10. Manifest Refraction and Visual Acuity
11. Mars Contrast Sensitivity Test
12. Tear collection for cytokine analysis (at centers with required equipment)
13. Slit lamp evaluation (SLE)
14. Tear break up time (TBUT)
15. Corneal fluorescein staining
16. Meibomian gland evaluation
17. Lissamine green staining of the interpalpebral conjunctiva
18. IOP
19. Schirmer's tear test (with anesthesia)
20. Assessment of inclusion and exclusion criteria and eligibility determination
21. Impression cytology
22. Blood collection for determination of fatty acids and of antibodies for autoimmune diseases
23. Obtain randomized treatment assignment
24. Schedule 3 month follow-up visit.

4.5.1 Collecting Unused Study Supplements

The run-in period is intended to confirm whether a patient who wishes to enroll in the study is able to comply with the treatment regimen. If the patient fails to bring their bottle of pills to the baseline visit and the baseline visit cannot be rescheduled within the time window, the patient is ineligible for the DREAM study and all subsequent study procedures should be terminated. In this case, the patient should be asked to return their bottle to the clinical center. If the patient does not return the bottle, the Clinic Coordinator should contact the Coordinating Center to arrange for IDS to handle return of the bottle.

The Clinic Coordinator must count the number of unused run-in gelcaps remaining in the bottle and the pill organizer. A calculation of the number of gelcaps that should have been taken by the patient will be made based on the number of days that the patient was supposed to be taking study supplements. This number will be multiplied by 0.90 and subtracted from the number of gelcaps in the bottle when it was dispensed to the patient. All such calculations will be made using the DREAM Compliance Calculator resident on the DREAM Landing Page. If the number of remaining capsules exceeds the calculated number, the patient is considered to be non-compliant and is ineligible for the study. All subsequent study procedures should be terminated. The number of returned pills must be recorded on the study forms and the Compliance Calculator spreadsheet filed in the patient's study binder.

4.5.2 Dry Eye Severity and Health Economics Questionnaires

The patient will again be asked to complete the OSDI and BODI questionnaires. In addition, they will complete the Work Productivity and Activity Impairment (WPAI) questionnaire, SF-36 and Healthcare Utilization Questionnaire. DREAM certified Clinic Coordinators or clinicians may assist the patient in completing the utilization questions.

4.5.3 Use of Dietary Supplements

The patients will be asked to bring their dietary supplement bottles with them to the Baseline Visit so that the Coordinator can review the ingredients. Do not record any non-prescription dietary supplements that are not listed on the Dietary Supplements Form. The use of omega 3 fatty acids only excludes the patient from the Study when the dose exceeds the limit specified in section 2.5.

4.5.4 Use of Concomitant Medications

The Clinic Coordinator must complete the Concomitant Medication Log based on interviewing the patient. Dietary supplements listed on the Dietary Supplements Form should not be included on the Concomitant Medication Log even if taken with a prescription. Dietary supplements that are not listed on the Dietary Supplements Form should be listed on the Concomitant Medication Log only if they are by prescription.

4.5.5 Assessing Possible Adverse Events at the Baseline Visit

At the baseline visit, the Clinic Coordinator must query the patient about possible adverse events (AEs) since the patient's screening visit. All AEs must be recorded on the AE Log. If the DREAM clinician identifies an adverse event as serious, it must also be reported to the Coordinating Center on the DREAM Serious Adverse Event Reporting Form as detailed in Section 5.9 and 5.10 of this manual. Either the Clinic Coordinator or Study clinician may interview the patient about the event.

4.5.6 Additional Patient Contact Information

Clinic Coordinators must complete the Patient Information Form so that the patient can be traced if contact is lost later in follow-up. Completion of this form may be delayed until after eligibility has been established, but it must be completed before requesting a treatment assignment.

4.5.7 Tear Osmolarity Measurement

At the baseline visit at centers with the TearLab Osmometer, tears from both eyes will be analyzed for osmolarity measurement.

4.5.8 Keratography

At centers with the Oculus Keratograph, the following tests will be performed on each eye:

1. Non-invasive Keratograph break-up time (NIKBUT).

2. Tear meniscus height (TMH).
3. Imaging bulbar redness.
4. Meibography of the upper and lower lids.

4.5.9 Visual Acuity & Contrast Sensitivity Testing

Manifest refraction and protocol visual acuity testing will be performed at the baseline visit. In addition, at the baseline visit, the Mars Letter Contrast Sensitivity Test will be performed.

4.5.10 Tear Collection for Cytokine analysis

At centers with the required equipment, tear samples from both eyes will be collected for cytokine analysis and shipped to the Biomarker Laboratory.

4.5.11 Slit Lamp Evaluation (SLE) and Other Tests to Assess Dry Eye Disease (DED)

To be eligible for the study, there must be demonstrated symptoms of DED at both the screening visit and at the Baseline Visit. A slit lamp evaluation (SLE), evaluation of the meibomian glands and eyelids, and tests to determine signs of dry eye disease (tear break up time, corneal fluorescein staining, lissamine green staining and Schirmer's test with anesthetic) will be performed.

4.5.12 Intraocular Pressure (IOP) Measurement

IOP measurement in both eyes will be performed, using Goldmann applanation tonometry, a TonoPen, or similar device.

4.5.13 Impression Cytology

Impression cytology is a non-invasive means of studying cells on the conjunctiva and facilitates the diagnosis of dry eye disease. The cells collected will be sent to the Biomarker Laboratory for analysis.

4.5.14 Blood Collection

At Visit 00, one blood sample will be collected to test for the level of various fatty acids in the blood. A second blood sample will be collected to be tested for antibodies for Sjögren's Syndrome and other autoimmune diseases.

4.5.15 Obtaining Randomized Treatment Assignment

The Coordinating Center is responsible for random assignment of patients to one of the two treatment groups. Random treatment allocations will be computer generated and stratified by clinical center. Ideally, all Visit 00 procedures should be performed on the day of randomization. If this is not possible, then all Visit 00 procedures must be performed within a 7-day period preceding randomization. If more than 7 days have elapsed, the procedure(s) must be repeated.

After the clinician assesses from ophthalmic examination, tests of dry eye severity, medical history and compliance with run-in supplements that the patient is eligible for the trial, and if the patient has signed a consent form, the Clinic Coordinator will enter the data into the DREAM Data Management system. The data system will check entered responses against all eligibility criteria and will indicate which items, if any, need correction or confirmation. If all required data have been received and the patient is eligible, the Clinic Coordinator opens the on-line Request for Randomization form, answers questions about data collection completeness, and saves the form to generate a randomized treatment assignment for the patient. The system generates a message that confirms that the randomization has been successfully completed and the Clinic Coordinator Faxes a prescription for study supplements, signed by the Clinician, to the Investigational Drug Service. The data system also generates a message to the Penn Investigational Drug Service to send a supply of study supplements to the patient at their

home. The Clinic Coordinator will call the patient one week after the visit to verify that the supplements have been received.

4.5.16 Scheduling the 3-Month Follow-up Visit and 1-Week Telephone Call

Before the patient leaves the clinic at the conclusion of the baseline visit, the Clinic Coordinator must schedule the patient's 1-week call and Month 03 follow-up visit. The 1-week call is to confirm that the patient has received the shipment of study supplements and to answer any questions the patient may have about taking the supplements. The Clinic Coordinator must consult the visit window (6 weeks before to 6 weeks after the ideal date) when making the appointment for the next visit. If no visit occurs in the visit window, the scheduled visit is considered a missed visit.

4.6 Regularly Scheduled Follow-up Visits Through 12 Months

Follow-up clinic visits are scheduled on Months 03, 06, and 12 after randomization for a total of 1 year of follow-up. Every effort should be made to begin Follow-up visits within 4 hours of the start of the Baseline Visit. The time windows for Months 03, 06, and 12 are contiguous with the midpoint between ideal patient times as the limits for the windows. The Clinic Coordinator must consult the visit window schedule when making an appointment. A telephone call is scheduled for 9 months after randomization. Also, letters encouraging compliance to study supplements must be distributed to each patient, 1 month after each visit. **Emailing the patient may replace telephoning the patient** for reminder calls when the patient provides permission to use email for communication during the consent process.

4.6.1 Preparing for Follow-Up Visits

The following tasks should be performed before the patient arrives for a scheduled follow-up visit.

- Remind the patient by telephone, about 2 weeks prior to each visit of the scheduled appointment and to bring their bottles from the shipment received near the time of their last visit, pill organizer, and the containers with the labels for all medications and dietary supplements that they are currently taking, including cod liver oil. **Also remind them not to use eye drops within 2 hours of their visit.**

- Retrieve the patient's Study file.

- Log onto the DREAM database and print a packet of all forms and logs required for the specific follow-up visit. Each page of the printed forms will be pre-populated with the patient's Study identification number, alphabetic identification code, and visit code. When printing forms for each visit, the Clinic Coordinator must remember to print the Concomitant Medication Log and Adverse Event Log located in separate "tabs" in the DREAM database. In the rare event the system cannot print the forms required for the visit, the Clinic Coordinator will photocopy the forms from the Forms Notebook resident at the site and must label each page with the identifying information.

- Be sure that any pertinent information received since the last examination is available to the clinicians.

- Put the Patient Information Form in the folder as a reminder to review and update the information.

- Make sure that supplies for all examinations and specimens are available.

4.6.2 Follow-Up Visit Procedures at Month 3

The procedures to be performed at the Month 3 visit are displayed in Exhibit 4-1. Procedures are recommended to be done in the order in the following sequence, unless specified otherwise:

1. Collect/count unused supplements
2. Completion of OSDI &BODI Questionnaires
3. Ocular and Systemic Medical History
4. Concomitant Medications and Dietary Supplements Use
5. Collect Adverse Event Information
6. MMP-9 testing
7. Visual Acuity: Refraction, if VA changes by 10 or more letters
8. Slit lamp evaluation (SLE)
9. Tear break up time (TBUT)
10. Corneal fluorescein staining
11. Meibomian gland evaluation
12. Lissamine green staining of the interpalpebral conjunctiva
13. IOP
14. Schirmer's tear test (with anesthesia)
15. Verify the patient's current mailing address for delivery of study supplements and update and enter the form into the data system if changes have occurred.
16. Schedule patient's 6 month follow-up visit
17. The Clinic Coordinator will call the patient approximately one week after the visit to verify that the new supply of supplements was received.

4.6.3 Procedures at Month 6

The procedures to be performed the Month 6 visit are displayed in Exhibit 4-1. Procedures are recommended to be done in the order in the following sequence, unless specified otherwise:

1. Collect/count unused supplements
2. Completion of OSDI &BODI Questionnaires
3. Completion of WPAI, SF-36, Healthcare Utilization Questionnaires
4. Ocular and Systemic Medical History
5. Concomitant Medications and Dietary Supplements Use
6. Collect Adverse Event Information
7. Complete Patient Information Form (additional contact data)
8. Tear Osmolarity (at centers with TearLab Tear Osmolarity machine)
9. Keratograph non-invasive tear break up time, tear meniscus height, redeness score, and meibography (at centers with required equipment) (comes before VA)
10. Visual Acuity and refraction, if VA changes by 10 or more letters
11. Mars Contrast Sensitivity Test
12. Tear collection for cytokine analysis (at centers with required equipment)
13. Slit lamp evaluation (SLE)
14. Tear break up time (TBUT)
15. Corneal fluorescein staining
16. Meibomian gland evaluation(move after corneal staining)
17. Lissamine green staining of the interpalpebral conjunctiva
18. IOP

19. Schirmer's tear test (with anesthesia)
20. Impression cytology
21. Blood collection for fatty acid determination
22. Verify the patient's current mailing address for delivery of study supplements and update and enter the form into the data system if changes have occurred.
23. Schedule the Month 9 telephone call
24. Schedule the Month 12 follow-up visit.
25. The Clinic Coordinator will call the patient approximately one week after the visit to verify that the new supply of supplements was received.

4.6.4 Procedures at Month 12

The procedures to be performed at the Month 12 visit are displayed in Exhibit 4-1. At month 12, the procedures listed above for the Month 6 visit will be performed, up to and including blood collection for determination of fatty acids and antibodies for autoimmune diseases. The coordinator will enter the results into the DREAM database. After the data have been entered, the coordinator completes the Treatment Disclosure form to ascertain whether the patient was assigned to active supplements or placebo, which is then disclosed to the patient. If the patient was assigned to placebo, they are not eligible for the Extension study and the patient will be exited from the study.

If eligible for the Extension Study, the patient will be reminded of the Extension Study and told that they are eligible to participate. A second informed consent will be reviewed and then signed by the patient if he/she wants to continue in the study. Patients who agree to continue taking supplements and return for two additional visits will be enrolled in the Extension Study. Women of childbearing potential must have a negative urine pregnancy test at this visit. The coordinator then completes the Extension Study Eligibility Review form, and the patient is again randomized to either omega 3 supplements or placebo. Treatment assignments for the Extension Study follow the method of random allocation as described in 4.5.15. The randomization schedules for the Extension Trial are completely independent of the schedules for the Primary Clinical Trial.

4.6.5 Procedures at Months 18 & 24.

All procedures listed for the Month 6 visit will be performed with the exception that the blood sample for determination of antibodies for autoimmune diseases will be collected only at the Month 24 visit. At month 24, after all data have been collected on paper and requisite forms entered into the Dream Data Management System, the coordinator telephones the Coordinating Center to receive the month 12 treatment assignment. The treatment assignment is disclosed to the patient and the patient is exited from the study. The coordinator completes the Month 12 Treatment Assignment and files it in the patient's study binder.

4.6.6 Telephone Call at Months 9, 15 & 21

Telephone calls are scheduled for approximately 9 months after the Baseline Visit and at 15 and 21 months for patients who continued to the Extension Study. The Clinic Coordinator should schedule these telephone calls with the patient prior to the patient leaving the office at the 6, 12 and 18 month visits. The purpose of the calls is to inquire about any changes in the patient's ocular and medical history, side effects if any and also to ensure that the patient understands how to take the supplements, to encourage compliance and to address any concerns that the patient may have. A brief form is completed to document the telephone calls and is entered into the DREAM database by the Clinic Coordinator. Documentation of the telephone calls is also recorded in the patient's medical record and signed and dated by the Clinic Coordinator.

4.6.7 Telephone Call after Month 12 or Month 24

Telephone calls are scheduled 1 month after the Month 12 visit for patients who do not continue in the Extension Study or 1 month after the Month 24 visit for patients who continue to the Extension Study. The Clinic Coordinator should schedule these telephone calls with the patient prior to the patient leaving the office at the 12 or 18 month visits. The purpose of the calls is to inquire about side effects if any. A Telephone Visit form is completed to document the telephone calls and is entered into the DREAM database by the Clinic Coordinator. Documentation of the telephone calls is also recorded in the patient's medical record and signed and dated by the Clinic Coordinator.

4.6.8 Assessing Interim Medical History During Follow-up Visits:

The DREAM General Follow-Up Visit Information Form specifies collection of data from the patient regarding their health, medications and possible adverse events (AEs) since the patient's last study visit. All AEs must be recorded on the AE Log. If the Study clinician identifies an adverse event as serious, it must also be reported to the Coordinating Center on the DREAM Serious Adverse Event Reporting Form as detailed in Section 5.9 and 5.10 of this manual.

4.6.9 Updating Patient Information

At all follow-up visits, the Clinic Coordinator asks the patient if any contact information has changed since the last visit to the clinic and updates the Patient Information form accordingly. The patient's mailing address for the next shipment of study supplements should be confirmed, and noted if different from the address for the last shipment.

4.6.10 Dispensing and Collecting Study Supplements during Follow-up Visits

After the baseline and Month 3 visits, the Investigational Drug Service (IDS) will provide the patient with unopened bottles of supplements for a 90 day supply. IDS will send the bottles to the patient's given address after receipt of the faxed prescription after the baseline visit. Approximately 2 weeks before the target date of the 3 month visit, the Coordinating Center sends a reminder to the IDS to ship another 90 day supply of supplements. Similarly, approximately 2 weeks before the target date of the 6 month visit, the Coordinating Center sends a reminder to the IDS to ship a 180 day supply of supplements. If the patient is enrolled in the Extension Study, the coordinator faxes a new prescription to the IDS after the 12 month visit and a 180 day supply of the newly assigned supplements will be dispensed. Finally, about about 2 weeks before the target date of the 18 month visits, a final 180 day supply will be sent to the patient. Clinic Coordinators are provided with tracking information for each shipment and will call the patients about week after the shipment to verify that the supplements were received and to answer questions. An instruction sheet accompanies each shipment and the patient will be given the opportunity to ask for any needed clarification about the pill regime during visits to the center and during telephone calls.

All patients must bring the bottles of study gelcaps from the shipment received near the time of their last visit and their pill organizer to each study visit. The Clinic Coordinator must count the number of unused gelcaps remaining in the bottles and record this number on the DREAM Supplement and Return and Disposal Log. If the new shipment has not yet been delivered to the patient, the Coordinator should allow the patient to keep a sufficient supply to allow the patient to keep taking supplements until the next shipment arrives. The supplements in the supply retained by the patient should not be counted for this visit. If the patient mistakenly brings the bottles of supplements that were received just prior to the visit that were intended to supply them through their next visit, the coordinator should return those bottles to the patient without counting the number of pills. The pill count may occur after the patient has left the clinical center, but it must occur shortly thereafter.

If the patient fails to bring their pill bottles to the visit, the Clinic Coordinator will remind the patient to return them to the next visit.

4.6.11.1 Additional Supplements for Month 12 Visits occurring >2 weeks after target date

DREAM visit windows are intentionally wide to aid in scheduling follow-up visits and to minimize missed visits. If a month 12 visit occurs beyond 2 weeks after the target date, a compliant patient runs the risk of running out of supplements before the month 12 visit is completed. Whenever a month 12 visit is scheduled 2 weeks beyond the target date, the coordinator must ask the patient if they need additional supplements to last to the scheduled visit. If additional supplements are needed, the coordinator faxes a "Late Month 12 Visit Prescription for 3 Bottles (available on the DREAM Landing Page) to the Investigational Drug Service.

4.6.11 Scheduling Required Visits and Procedures

It is extremely important that both the Clinic Coordinator and the patient adhere to the follow-up appointment schedule. The patient's appointment schedule should be consulted whenever the patient is given an appointment for a follow-up visit. It is especially important to refer to the schedule when an examination date is changed. Each follow-up visit should be scheduled as close as possible to the target date. However, the visit window is wide enough to allow time for rescheduling within the permissible time limits, thereby decreasing the number of missed visits. Whenever a visit is completed near the end of a time window, an attempt should be made to get the patient back on schedule. Visits not completed within the specified time limits are classified as missed.

The Clinic Coordinator plays a crucial role in ensuring that the required procedures and visits occur on schedule. Before the patient leaves the Clinical Center, the Clinic Coordinator schedules the next visit. Thus, whenever a patient leaves a Study visit, he/she should have an appointment card with the date of the next visit.

4.6.12 Follow-up of Patients Unable to Return for Scheduled Visits

Because of poor health or for other reasons, some patients may not be able to return to the Clinical Center for scheduled study visits despite their original intentions to do so. Information regarding unresolved SAEs can be obtained by the Clinic Coordinator through telephone calls or, after obtaining patient consent, records from a non-study clinician whom the patient has seen may be obtained. If the patient cannot be located through family members or friends, a Patient Search Form should be initiated. Coordinators must still complete Missed Visit Forms for these patients.

If the Clinic Coordinator discovers that the patient has died, the Clinic Coordinator follows procedures for reporting a serious adverse event and completes a Patient Death form.

4.6.13 Missed Visits

Any time a patient misses a scheduled visit, the Clinic Coordinator should contact the patient immediately and arrange another appointment. Whenever it is not possible to examine the patient in a DREAM Clinical Center, the following procedures should be followed to provide as much useful information as possible.

- If a Study patient cannot complete a scheduled visit within the time window for that visit, the Coordinating Center should be notified by completion and entry of a Missed Visit Form within one week of the close of the visit window.

- The patient should be contacted by telephone to schedule the next visit or to confirm the appointment for the next visit. The next visit should be scheduled as close to the target date as possible.

4.6.14 Maintaining Contact

Any time a patient misses a scheduled visit, the Clinic Coordinator must contact the patient immediately and arrange another appointment. If the patient cannot be located, an intensive search should be instituted immediately by the Clinic Coordinator. The Clinic Coordinator should use all available resources to locate the patient, including writing or telephoning each contact provided by the patient at time of enrollment or added since then. Because this search may be long and time-consuming, it is important that it be started as soon as any member of the clinic staff is aware that there is a problem. The steps taken to locate the patient should be documented on a Patient Search Form. In extreme cases when the clinic staff has exhausted all avenues and the patient has not been located, the Coordinating Center should be notified. Missed Visit forms must be completed for these patients for each visit the patient missed.

4.6.15 Managing Patients Who Arrive Having Used Artificial Tears Within the Previous 2 Hours

Many of the study evaluations are affected by recent use of artificial tears. If a patient arrives having used artificial tears within the previous 2 hours, the patient should be asked if they can return another day or if they can use the time to have a snack, shop or engage in another activity. If these options are not feasible, time may be used to count supplements, draw blood if required, complete the economic questionnaires (but not the OSDI or BODI), and the medical (but not ocular) history. The OSDI and BODI, as well as all other study evaluations, may not begin until at least 1 hour after instillation of artificial tears.

4.7 Withdrawal of Subjects and Discontinuation of Study Drug:

In general, patients will not be withdrawn from the DREAM trial, regardless of compliance and adverse events. Every effort will be made to keep the patient under follow-up, even if the patient refuses to return to the DREAM center for visits or consistently misses appointments. Telephone contact should be maintained with patients who are unable to return because of new developments in their lives (serious illness of a family member, unrelated illness in the patient, unexpected travel). In the rare event that the patient requests that there be no further efforts by DREAM staff to contact them, the request must be honored. However, the patient should be told that they are welcome to resume participation at any time within the planned follow-up period of the study. If the patient wishes to withdraw from the study all tests of the final month 12 visit must be performed before the patient leaves the clinic.

4.8 Changing the Site for Patient Follow-up:

During the course of their follow-up, some patients may choose to be seen at another DREAM-certified site within the clinical center. A Transfer of Patient Form must be completed so that materials relating to the patient are sent to the correct location. The patient's study chart should be transferred to the new site after the patient signs a medical records release form.

Patients may move to another area of the country. If another DREAM clinical center is located closer to the patient's new home, a permanent transfer may be arranged and documented with a Transfer of Patient Form. The DREAM staff at the new clinical center must accept responsibility for the follow-up of the patient before the patient can be transferred. The Clinic Coordinator from both clinics must sign the form indicating approval of the transfer, and fax the completed form to the Coordinating Center. The clinic at which the patient was originally enrolled should copy the patient's study chart and send it to the receiving clinic.

4.9 Patient Death:

As soon as clinic personnel become aware that a patient has died, the Clinic Coordinator follows procedures for reporting a serious adverse event, requests a death certificate, completes a Patient Death form, and enters the form into the DREAM Web-based data system. The patient will then be removed from later reminders for visits. The coordinator should attempt to collect unused study gelcaps from family members.

4.10 Guidelines For Documentation of DREAM STUDY Activities:

In accordance with good research practice, it is essential that all study patient-related activities be documented so that information at the clinical centers can be compared with the data in the trial database and in source documents by study site visitors and/or outside auditors as necessary.

Information should be included that documents the following information:

- That all reported procedures and tests were conducted according to protocol.

- That all procedures and examinations were performed by the reported personnel on the dates reported.

- That the study run-in supplements were dispensed to and explained to the patient per protocol by the specified personnel or that protocol deviations have been reported.

- That all study supplements were accounted for, documented and destroyed per protocol.

In addition to the DREAM case report forms, other clinical information is valuable for providing complete documentation of study-related procedures. The following section specifies the types of documentation that are recommended.

4.10.1 Information to be Included in the Medical Chart

- Examination notes, dated and signed by the individual(s) performing the examination, and completed at the time of the examination per usual clinical protocol

- Copies of all internal or external patient-related correspondence.

- Signed and dated notes from telephone calls and other contacts with patients, their families, friends and clinicians.

- Signed notes documenting patient education, counseling, and enrollment decisions regarding the DREAM study.

Patient names and other identifiers should be retained on all such documentation so that the identity of the patient and the correspondence of examination results to the reported data may be confirmed. This information need not be retained in the study files but may be kept in separate clinic files for each patient. The structure of these files may vary depending on local guidelines or requirements. However, some Clinic Coordinators find it expeditious to attach copies of all documents from which data were abstracted to the corresponding forms in the study charts.

4.10.2 Maintaining the Patient's DREAM Study File:

All study visit materials are filed in the patient's study chart as is a copy of the follow-up appointment schedule. The following things should be done to keep the patient's study file as complete and up-to-date as possible at all times:

- The patient's contact information, such as telephone numbers, place of employment, persons who can be contacted about the patient's whereabouts, etc., should be reviewed

and updated at each visit. Contacts already listed should be confirmed. If any changes are made, the information should be added to the Patient Information Form.

- Be sure that copies of the forms and all other information submitted to the Coordinating Center and laboratories are in the patient's file.

4.11 Data Collection and Recording:

The Clinic Coordinator plays a major role during data collection and recording, both by questioning and examining the patient directly and, in some cases, by recording responses dictated by the clinician while examining the patient. Whenever data are recorded by someone else, such as by the clinician during an examination or by the patient responding to self-administered questionnaires, the DREAM Clinic Coordinator should check all such recorded information for completeness and consistency. Therefore, it is important that the Clinic Coordinator have a thorough understanding of the procedures that take place for each required examination, the sequence in which these are best performed in the clinical center, the contents of the data collection forms and other forms to be completed, and local conventions that must be followed to maintain the clinical chart for each patient.

When questionnaires are completed by patients, specific questions are asked of patients to complete case report forms, and information from clinical examinations are recorded directly onto the study case report forms, these documents are considered the source documents.

4.12 Submission of Visit Data to the DREAM Coordinating Center:

One major responsibility of the Clinic Coordinator is to gather the data obtained at the study visit and submit it to the Coordinating Center and to the laboratories analyzing the patients' blood and tears. At any point after the patient has signed a patient consent form, the Clinic Coordinator may enter baseline data into the on-line system.

Study forms should be entered in to the data system as soon as possible after the visit; forms entered more than 7 days later will be considered late. Blood and tear samples should be submitted to the respective laboratories.

The Clinic Coordinator should carefully check all data collection forms before entering the data in the web-based data system. This process is extremely important because correcting errors that have entered the data system is far more time-consuming and expensive than taking the appropriate steps to prevent errors. Every response on the forms should be checked for completeness, consistency with other information reported for the patient, and legibility. In addition, the person performing each procedure or taking responsibility for the recorded data should initial or sign the appropriate component of the form, as indicated on the forms.

4.12.1 Submission of Blood to the Peroxisomal Diseases Laboratory:

All patients enrolled in the trial are required to have their blood analyzed as a measure of compliance with the study drug protocol. The blood will be analyzed by the Peroxisomal Diseases Laboratory at the Kennedy Krieger Institute in Baltimore MD. The Clinic Coordinator has the responsibility to see that the blood samples are sent to the laboratory.

4.12.2 Submission of Tear Samples and Impression Cytology to the Biomarker Laboratory

Patients enrolled in the trial at centers having the requisite materials, will have their tears analyzed for cytokines and all patients will have impression cytology samples for HLA-DR expression. The tears and impression cytology samples will be analyzed by the Biomarker Laboratory at the Icahn School of Medicine at Mount Sinai. The Clinic Coordinator has the responsibility to see that the tear and impression cytology samples are submitted to the Biomarker Laboratory at MSSM.

4.12.3 Completeness of Submitted Data:

Each data form must be checked for completeness and to assure that all pages of all components are included and in the correct order. In addition, the Clinic Coordinator should check the data screen before saving the data into the database. Data will be validated during the entry process. Whenever there is doubt about how an item is to be answered, the Protocol Monitor or Director at the Coordinating Center should be contacted. Items for which an answer always is required usually appear on the left-hand side of each page of each form and data entry screen.

4.12.4 Consistency:

Questions that should be answered only for certain patients appear in boxes in the right hand column of each page of each form and data entry screen. An arrow leading from a specific response to a box indicates that whenever that response is checked, the additional information in the box also is required. Otherwise, items in the box should be left unanswered. Dates should be checked for accuracy. In particular, the date of an examination recorded on a data form should be the actual date the patient was examined and not the date when the data are entered into the database.

4.13 Edits and Corrections:

4.13.1 Edit Queries:

The information submitted to the DREAM database is edited for anomalies by means of special computer programs. When a question exists regarding the answer to one or more of the items on a component, the item is flagged by the data system. The Clinic Coordinator should first check for a data entry error by comparing the response on the paper copy of the form against the database response. If the edit query is due to a data entry error, the Clinic Coordinator may immediately correct the error. If the edit query is not due to a data entry error, the Clinic Coordinator should refer to the patient's record and determine the correct answer for each item flagged. The Clinic Coordinator may need to consult with the Study clinician or other technical staff for specific medical information. In this case, whenever a correction to an earlier value on the paper form is required, the Clinic Coordinator corrects the earlier response on the original data collection form filed at the site by striking through it (so that it is still legible), writing the correct response, and initialing and dating the corrected item(s). The original response should not be obliterated with white-out, marker, or by scratching through it. The database is similarly updated. After edit queries are resolved, the responses of the database and paper forms must be the same.

4.13.2 Errors Discovered in Other Ways:

When errors are detected by the Coordinating Center through audits or data summary reports, Coordinating Center staff will have the ability to flag a database response for review by the Clinic Coordinator. A correction to the database and paper form as described above may be required.

4.14 Quality Assurance Responsibilities:

The validity and credibility of the study depends to a large degree on the collection and reporting of high quality, accurate data. Each study staff member should be aware of his/her responsibility for following the protocol, reporting data accurately and promptly, and resolving any problems that occur in trial-related activities. Although the local Principal Investigator bears primary responsibility for the accuracy and integrity of study data, much of the responsibility falls to the Clinic Coordinator.

In addition to the routine procedures described in previous sections, the primary quality assurance mechanisms to be implemented at the Clinical Center are:

- The person completing each examination and taking responsibility for the examination must be identified by initials and certification number at the end of the section where the data from the examination is recorded.

- Documentation of all tests/procedures must be obtained and kept in patients' study files.

- Any errors or discrepancies discovered at the clinical center are corrected, regardless of the time elapsed since the data were collected, and updated in the database system.

Systematic data collection or reporting problems are brought to the attention of the responsible individual, the local Principal Investigator and the Coordinating Center for review and resolution.

Exhibit 4-1

DREAM Primary Trial Schedule of Procedures

Procedure	-2 wks SV	Visit (Month) 00	03	06	09	12
Obtain Informed Consent	X					Y
OSDI & BODI Questionnaires	X	X	X	X		X
Health Economics Questionnaires (SF-36, WPAI, Healthcare Use)		X		X		X
Medical History and Events	X	X	X	X		X
Concomitant Medication Query		X	X	X		X
Adverse Event Query		X	X	X		X
MMP-9 testing	X		X			
Tear Osmolarity		X^2		X^2		X^2
Keratograph: Break-Up Time, Tear Meniscus Height, Redness and Meibomian Gland Evaluation		X^2		X^2		X^2
Manifest Refraction		X				
Best Corrected VA (if change in VA ≥ 10 letters, do refraction)		X	X	X		X
Contrast Sensitivity		X		X		X
Tear Collection for Cytokines		X^2		X^2		X^2
Slit Lamp Evaluation (SLE)	X	X	X	X		X
Tear Break-Up Time (TBUT) [5]	X	X	X	X		X
Corneal Fluorescein Staining [5]	X	X	X	X		X
Meibomian Gland Examination[5]	X	X	X	X		X
Lissamine green staining[5]	X	X	X	X		X
IOP	X	X	X	X		X
Schirmer's Tear Test (with anesthetic)	X	X	X	X		X
Urine Pregnancy Test	X^1					Y^1
Eligibility Determination	X	X				X
Impression Cytology		X		X		X
Blood Collection (Mon-Thurs) for fatty acid determination		X		X		X
Blood Collection (Mon-Thurs) for antibody determination		X				X
Collection of Unused Study Supplements		X	X	X		X
Randomization		X				Y
Dispense Run-in Supplements	X					
Reminder Calls (2 weeks before each visit)		X	X	X		X^3
"Check-In" Telephone Call					X	X^4
Letter to Encourage Compliance (sent 1 month after each visit)		X	X	X		Y

LEGEND:

SV: Denotes screening visit; 00: Denotes baseline visit

[1] Only women of childbearing potential

[2] Only at centers with required equipment

[3] Call should include information about the Extension Study

[4] Call for final adverse event assessment if not in Extension Study

[5] Do all 4 procedures OD, then restart for OS

Y: Only patients in the Extension Study

Exhibit 4-1

DREAM Extension Study Schedule of Procedures

Procedure	Visit (Month)			
	15	18	21	24
OSDI & BODI Questionnaires		Y		Y
Health Economics Questionnaires (SF-36, WPAI, Healthcare Use)		Y		Y
Medical History and Events		Y		Y
Concomitant Medication Query		Y		Y
Adverse Event Query		Y		Y
Tear Osmolarity		Y[1]		Y[1]
Keratograph Break-Up Time, Tear Meniscus Height, Redness, and Meibomian Gland Evaluation		Y[1]		Y[1]
Best Corrected VA (if change in VA ≥ 10 letters, do refraction)		Y		Y
Contrast Sensitivity		Y		Y
Tear Collection for Cytokines		Y[1]		Y[1]
Slit Lamp Evaluation (SLE)		Y		Y
Tear Break-Up Time (TBUT) [3]		Y		Y
Corneal Fluorescein Staining[3]		Y		Y
Meibomian Gland Examination[3]		Y		Y
Lissamine green staining[3]		Y		Y
IOP		Y		Y
Schirmer's Tear Test (with anesthetic)		Y		Y
Impression Cytology		Y		Y
Blood Collection (Mon-Thurs) for fatty acid determination		Y		Y
Blood Collection (Mon-Thurs) for antibody determination				Y
Collection of Unused Study Supplements		Y		Y
Reminder Calls (2 weeks before each visit)		Y		Y
"Check-In" Telephone Call	Y		Y	Y[2]
Letter to Encourage Compliance (sent 1 month after each visit)		Y		

LEGEND:

[1] Only at centers with required equipment

[2] Call for final adverse event assessment

[3] Do all 4 procedures OD, then restart for OS

CHAPTER 5
SAFETY AND ADVERSE EVENTS

5.1 Medical Monitoring in the DREAM

Medical monitoring in the Study is the responsibility of the DREAM Data & Safety Monitoring Committee (DSMC). A Medical Safety Monitor, who holds an MD, will review reports of serious adverse events (SAEs) as they occur.

5.2 Independent Data and Safety Monitoring Board

The Data and Safety Monitoring Committee (DSMC) will be appointed by the National Eye Institute and is comprised of ophthalmologists and optometrists with expertise in Dry Eye Disease, biostatistician/epidemiologists, a nutritionist and a patient advocate as voting members. The NEI Project Officer serves as an ex officio member. The committee is responsible for the review of performance, safety, and efficacy data. At the first DSMC meeting, the Committee will review the study protocol, offer advice to the Study Executive Committee, and approve the study design. A detailed description of the operations of the DSMC is provided in the DREAM Data and Safety Monitoring Charter.

A Medical Safety Monitor will monitor reports of serious adverse events as they occur and will be available to report to the DSMC as needed.

5.3 Overview of Adverse Events Definitions and Reporting System

Because the DREAM Study is examining the treatment effect of a therapeutic dose of a nutritional supplement, the study is operating under an IND. Hence this study will comply with the adverse events definitions and reporting requirements for clinical trials established by the Food and Drug Administration (FDA) in 21 CFR 312 and the Guidance for Industry on Safety Reporting Requirements for INDs and BA/BE Studies issued December 2012. In this chapter, use of term "sponsor" refers to the Study Chair (IND holder) and the Coordinating Center, which is operating on behalf of the Study Chair with regard to the collection of data about adverse events.

5.4 Definition of Adverse Events and Suspected Adverse Reactions

An adverse event (AE) is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. These include AEs that emerge during the reporting period that were not previously observed in the patient, complications that occur as a result of protocol-mandated interventions or preexisting medical conditions that are judged by the investigator to have worsened in severity or frequency, or have changed in character during the adverse event reporting period.

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug. The sponsor is

responsible for making the causality judgment. Additional information on suspected adverse reactions are in 21 CFR 312.32(c)(1)(i).

5.5 Definition of Serious Adverse Events

Adverse events are classified as serious or non-serious. Determinations of whether an event meets the definition are made in the view of either the investigator or sponsor. A serious adverse event is any AE that is:

- fatal

- life-threatening

- requires or prolongs inpatient hospital stay

- results in persistent or significant disability or incapacity or substantial disruption of the ability to conduct normal life functions

- a congenital anomaly or birth defect in a neonate or infant born to a mother exposed to the investigational product

- considered to be an important medical event (e.g., events that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the patient, and may require intervention to prevent one of the other serious outcomes noted above.)

Important medical events that may not result in death, be life threatening, or require hospitalization may be considered a serious adverse event when, based upon medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Keep in mind that the definition of a SAE focuses on the "outcome" of the event, and the SAE may involve only one, or possibly more, of the above criteria. All adverse events that do not meet any of the criteria for serious events should be regarded as non-serious adverse events.

5.5.1 Severity vs. Serious

The terms "serious" and "severe" are not synonymous. The term "severe" is often used to describe the intensity (severity) of a specific event (e.g., a mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as a severe headache). This is **not** the same as "serious", which is based on patient or event **outcome or action** criteria, usually associated with events that pose a threat to a patient's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations. When recording AEs and SAEs, severity and seriousness must be independently assessed.

5.5.2 Unexpected Events

An adverse event is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan.

5.5.3 Preexisting Conditions

Preexisting conditions should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period. If there is a question as to whether a medical development should be reported as an adverse event, the Investigator or Clinic Coordinator must contact the Study Chair for guidance.

5.5.4 Worsening of Symptoms and Signs of Dry Eye Disease

Developing symptoms and signs that are consistent with the natural history of Dry Eye Disease (DED) are not considered reportable adverse events. Such developments are, however, recorded on the study data collection forms but are not reportable adverse events.

Worsening of symptoms and signs of DED should be recorded as an AE or SAE only if judged by the investigator to have unexpectedly worsened in severity and/or frequency or changed in nature at any time during the study. When recording an unanticipated worsening of dry eye disease, it is important to convey why the development was unexpected. For further guidance see 5.5.6., below.

5.5.5 Abnormal Laboratory Values

Abnormal laboratory results will generally not be recorded as an AE. A clinical laboratory abnormality should be documented as an adverse event if <u>any one of the following</u> conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality

- The abnormality suggests a disease and/or organ toxicity

- The abnormality is of a degree that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.

- The abnormality results in study withdrawal.

5.5.6 Hospitalization, Prolonged Hospitalization or Surgery

With the exception explained in the next paragraph, any adverse event that results in a hospitalization should be documented and reported as a SAE. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for an adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event when the hospitalization or prolonged hospitalization was for diagnostic or elective surgical procedures for a preexisting condition. Surgery should not be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.

5.5.7 Deaths

All deaths that occur during the AE reporting period (section 5.6), regardless of attribution to study intervention, must be recorded on a DREAM Patient Death Form, entered into the DREAM database and immediately reported to the Coordinating Center and local IRB as an SAE.

5.6 Adverse Event Reporting Period

The reporting period during which adverse events must be reported is the period from the screening visit to the end of the study follow-up. All unresolved adverse events must be followed by the Investigator until the events are resolved, the patient is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled study visit, the Investigator will instruct each patient to report any subsequent event(s) occurring within 30 days that the patient, or the patient's personal physician, believes might reasonably be related to prior study treatment. Patients who withdraw early from the study will be contacted by the Clinic Coordinator 30 days after their last visit to ascertain whether any AEs have occurred.

5.7 Collecting Adverse Event Information

During each study visit, investigators and clinic coordinators will assess the occurrence, status change and resolution of AEs and SAEs by examination and by questioning the patient. Complete reporting information includes the following:

- Specific condition or event (MedDRA code and preferred term)
- Grade/severity
- Event type
- Dates of onset and (if applicable) resolution
- Outcome
- Whether event necessitated a change in study treatment
- Abnormal laboratory value (SAEs only)
- Attribution to study drug by investigator and Medical Monitor (SAEs only)

5.8 Assessment of Adverse Events

All events will be MedDRA coded by the Clinic Coordinator using an on-line version of the Common Terminology Criteria for Adverse Events (CTCAE) developed by the National Cancer Institute (National Cancer Institute, 2006). The CTCAE version 4.0 provides definitions for a large subset of adverse event terms and a grading (severity) scale for each adverse event. The CTCAEv4.0 and its associated grading criteria are very specific, providing an adverse event term and grade that precisely describes the event. (Refer to the DREAM Oracle Clinical™ Training Manual for instructions in accessing and using the CTCAEv4.0.) If the Clinic Coordinator cannot find a suitable term using the CTCAEv4, she/he will contact the Coordinating Center for assistance as Coordinating Center DREAM staff have access to the full set of MedDRA 10 terms.

5.8.1 Grading the Severity of Adverse Events

All events must be graded for severity by the Investigator, using a 5 point scale:

1 = Mild: Awareness of sign or symptom, but easily tolerated
2 = Moderate: Interference with normal daily activities
3 = Severe: Inability to perform normal daily activities
4 = Life threatening or disabling: Immediate risk of death or disablement
5 = Death

5.8.2 Attributing the Causality of Serious Adverse Events

FDA believes that the sponsor is better positioned than the individual investigator to assess the overall safety of the investigational drug because the sponsor has access to serious adverse event reports from multiple study sites and is able to aggregate and analyze these reports. Moreover, the sponsor is more familiar with the drug's mechanism of action, class effects, and other information. For these reasons, investigators must immediately report any serious adverse event to the sponsor, whether or not the investigator considers the event to be drug related (21 CFR 312.64(b)).

However, in the report to the sponsor, the investigator must include an assessment of causality (i.e., whether there is a reasonable possibility that the drug caused the event) (21 CFR 312.64(b)). The investigator's view is important for the sponsor to consider when assessing the safety of the drug and determining whether to report an event expeditiously to FDA, because the investigator, who monitors the subject's response to the drug, is knowledgeable about the subject's clinical state (e.g., medical history, concomitant medications) and thus may be sensitive to distinctions between events that may be related to the drug versus those due to the underlying disease process and/or concomitant therapies.

In assessing causality, the investigator will respond either yes or no to the question "Was there a reasonable possibility that the drug caused the adverse event?").

5.8.2.1 Attribution of Causality by the Medical Safety Monitor

The Medical Safety Monitor will evaluate all reported SAEs against accumulating knowledge of omega 3 fatty acids to identify and communicate new safety findings to investigators and to the FDA. In all cases, the Monitor's assessment will prevail with regard to causality and filing MedWatch reports.

5.9 Recording of Adverse Events

The Investigator at the Clinical Center is responsible for ensuring that all AEs and SAEs that are observed during the study are recorded on the DREAM Adverse Event Log and in the patient's clinical record. All SAEs are also reported on the DREAM Serious Adverse Event Initial Reporting Form (or SAE Follow-up Reporting Form) and submitted to the on-line DREAM database. The information recorded on the DREAM Adverse Event Log should be based on the signs or symptoms detected during the clinical evaluation of the patient and on information obtained from the patient. The AE Log is included in the on-line DREAM database, and with the submission of each log entry the computer performs an automatic data check to ensure that all required reporting elements have been entered into the system.

Reports of all SAEs and all accompanying documentation will be electronically sent to the Medical Monitor by the Coordinating Center.

All adverse events occurring during the study period must be recorded. The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Adverse events that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any AE that occurs within 30 days after the study period should be recorded and reported immediately to the Coordinating Center.

5.9.1 Diagnosis vs. Symptoms

If a disease is known at the time an AE is reported, this diagnosis should be recorded on the Adverse Event Log and (if appropriate) on the SAE Reporting Form rather than listing individual symptoms. However, if a cluster of symptoms cannot be identified as a single diagnosis, each individual event should be reported separately. If a diagnosis is subsequently known, it should be reported as follow-up information.

5.10 Reporting of Serious Adverse Events

The investigator must immediately report to the Coordinating Center all serious adverse events, regardless of whether the investigator believes that they are drug related, including those events listed in the protocol as anticipated to occur in the study population independent of drug exposure or in the investigator brochure as predicted to occur with the drug (21 CFR 312.64(b)).

The FDA and the DREAM Study leadership recognize that it may take the investigator a short period of time (i.e., a day) to compile information about the event, but then expects the information to be immediately reported to the sponsor. Investigators are not required to determine whether an event is "unexpected", as this is a sponsor responsibility.

5.10.1 Reporting Serious Adverse Events to the Coordinating Center

When DREAM Clinical Center staff becomes aware of a serious adverse event, the clinic coordinator enters the data into the DREAM database within one day. Copies of all forms and medical records must be maintained in the patient's study folder at the site.

In turn, the Coordinating Center will send an electronic copy of the Serious Adverse Event Report Form and other supporting documentation to the DREAM Medical Monitor and DREAM Study Chair within 5 days of notification by the Clinical Center.

5.10.2 IND Safety Reports

The DREAM Study Chair holds the IND for the use of omega 3 fatty acids in this trial. She, or at her direction, the Principal Investigator of the Coordinating Center is responsible for notifying the FDA and all participating DREAM Investigators of any suspected adverse reaction that are associated with the study supplements that are both serious and unexpected. Follow-up information to a safety report will be submitted as soon as the relevant information is available.

5.10.3 Written IND Safety Reports

The DREAM Study Chair or, at her direction, the Principal Investigator of the Coordinating Center will notify the FDA and all participating DREAM investigators in a written IND safety report of any suspected adverse reaction associated with the use of the study supplements that is both serious and unexpected. Notification will occur as soon as possible, but no later than 15 calendar days after notification of the event. Reports of unexpected fatal or life threatening adverse reactions will be submitted within 7 days. In each written IND safety report, the Chair will identify all safety reports previously filed with the IND concerning a similar adverse experience and will analyze the significance of the SAE in light of the previous similar reports.

5.10.3.1 Telephone/Faxed Transmission of IND Safety Reports

The Study Chair or at her direction, the Principal Investigator of the Coordinating Center, will notify the FDA by telephone or fax of any unexpected fatal or life-threatening event that is associated with the use of the study drugs. Notification will occur as soon as possible, but no later than 7 calendar days after notification of the event.

5.10.4 IRB Notification of SAEs Occurring at Their Center

21 CFR 312.66 requires investigators to report to their IRB all unanticipated problems that pose a risk to human subjects or others," including adverse events that should be considered unanticipated problems. In determining the need to report an event to the IRB, note that any event that meets the criteria for reporting in an IND safety report should also be considered an "unanticipated problem" and reported to the IRB by the investigator.

It is important to note that some events that would not meet the criteria for reporting in an IND safety report would be considered unanticipated problems involving risk to human subjects (e.g., informed consent or privacy issues, certain adverse events that could not be caused by the investigational drug, such as events that occur prior to test article administration as a result of a washout period or due to a screening procedure). All such unanticipated events must be reported immediately to the Coordinating Center as well as to the local IRB, in accordance with local IRB rules. The Clinic Coordinator indicates on the Serious Adverse Event Report Form the status of this notification. Until she/he indicates that the IRB has been notified or that the event does not meet the criteria for IRB notification, the Protocol Monitor will contact the Clinic Coordinator on weekly basis until she/he submits documentation to indicate that IRB notification has been made. During site visits to the clinical centers, the Protocol Monitor will ensure that documentation exists to confirm that the local IRB was notified of all reportable SAEs that occurred at the site.

5.10.5 IRB Notification of SAEs Occurring at Other Centers

Upon receipt of an <u>IND Safety Report</u>, each Clinical Center is responsible for copying the IND report and submitting the copy to their local IRB within 10 working days (or shorter if the local IRB requires a shorter reporting period). The original report and <u>dated</u> documentation of IRB submission (via cover letter) must be maintained at the clinical center. During site visits to the clinical centers, the Protocol Monitor will ensure that documentation exists to confirm that the local IRB was notified of all reportable SAEs.

5.10.6 DSMC Notification of Serious Adverse Events by the Coordinating Center

The Director of the Coordinating Center informs the full DSMC in writing of all serious adverse event reports at semi-annual committee meetings. The Medical Monitor may, at her/his discretion, instruct the Coordinating Center to notify the full DSMC immediately of a serious adverse event, and may request a meeting or teleconference of the committee prior to its next scheduled meeting.

The following information will be provided to the DSMC by the Coordinating Center:

- Clinical Center
- Patient ID Number
- Description of event (MedDRA code)
- Date of onset
- Severity of the event
- Whether study treatment was discontinued
- Medical Monitor's assessment of association between SAE and study drug

5.11 Annual Reports

Every year, within 60 days of the anniversary date that the IND, the Study Chair, or at her direction, the Principal Investigator of the Coordinating Center, will submit to the FDA a report that includes a status report for the study as well as annual summary information that includes:

- Tables of the most frequent and serious SAEs by body system
- Summary of all IND Safety Reports
- A list of deceased patients and causes of death
- Drops-out due to adverse events
- (If relevant) a description of new understanding of the study supplement's actions

5.12 Reporting and Analysis of Serious Adverse Events

Biostatisticians at the Coordinating Center will, on an annual basis, report to the DSMC, the NEI and the FDA their analysis of all cumulative serious adverse events. The analysis will include:

- Number of events
- Frequency of each type of event
- Severity of events
- Attribution of event
- Number of patients who had study supplements stopped
- Whether study supplements could be reinstituted
- Number of patients requiring medication after stopping study supplements at one and two years
- Number of deaths

5.13 Managing Adverse Events

When a patient enrolled in the study experiences an adverse event, the Investigator at the Clinical Site will manage the patient with the best medical treatment protocol for the condition or, if appropriate, will refer the patient to a specialist or to the patient's personal physician.

CHAPTER 6
DATA ANALYSIS, STATISTICAL ISSUES, AND DATA MONITORING

6.1 Study Design Characteristics Affecting Data Analysis and Statistical Issues

The DREAM study consists of three components: 1) the Primary Clinical Trial is a prospective, randomized, double-masked, superiority clinical trial involving an active supplement group and a placebo group; 2) the Extension Study is a prospective, randomized, double-masked withdrawal clinical trial for the patients who were assigned to active supplements in the Primary Clinical Trial and are willing to continue participation for another year; and 3) the patients receiving placebo supplements during the Primary Clinical Trial provide data for a 12-month longitudinal assessment of dry eye disease. The design of the DREAM clinical trials is provided in Exhibit 6-1. The longitudinal assessment is based on the variables measured at baseline, and 3, 6, and 12 months after randomization.

Key aspects of the design and rationale that have major bearing on the approach to data analysis, statistical issues, and data monitoring are noted below:

- The unit of randomization is person. At least one eye of a person must meet the DREAM eligibility criteria for dry eye disease.

- There are 2 treatments in the Primary Clinical Trial, active supplements containing of ω3 fatty acids and placebo supplements. The ratio of the number of patients assigned active supplements to the number assigned placebo supplements is 2:1; i.e., 2/3 to active supplements and 1/3 to placebo supplements.

- The duration of the Primary Clinical Trial is 12 months: from the time of randomization to the 12-month visit.

- The primary outcome measure for the Primary Clinical Trial is the mean change from baseline in the Ocular Surface Disease Index (OSDI) at 6 months and 12 months.

- At 12 months, the patients assigned to the active supplement group in the primary trial will be offered enrollment in the Extension Study and randomized to continue with active supplements or with placebos. The ratio of the number patients assigned active supplements to the number assigned placebo supplements is 1:1; i.e., ½ to active supplement and ½ to placebo supplement.

- The duration of the Extension Study is 12 months: from the time of a second randomization at the 12-month visit to the 24-month visit.

- The primary outcome measure for the Extension Study is the mean change from 12 months in the OSDI at 18 months and 24 months.

- Secondary outcome measures for both the Primary Clinical Trial and the Extension Study are:

  - Compliance with the study treatment protocol as measured by changes in blood levels of essential fatty acids and pill counts;

  - A change of 10 or more points on the OSDI. For the Primary Trial, the change must be a decrease and for the Extension Study, the change must be an increase.

- Change in signs of dry eye disease: corneal fluorescein staining, lissamine green staining of the interpalpebral conjunctiva; tear break-up time (TBUT), Schirmer's test;

- Change in the frequency of use of artificial tears and other treatments for DED;

- Change in quality of life as measured by the Medical Outcome Study - Short Form (SF-36);

- Change in the Brief Ocular Discomfort Inventory (BODI) score;

- Cost and incremental cost-effectiveness of using ω3 fatty acids;

- Incidence of ocular and systemic adverse events;

- Changes in visual acuity and intraocular pressure;

- Exploratory outcome measures for both the Primary Clinical Trial and the Extension Study are:

- Change in contrast sensitivity;

- Change in meibomian gland secretion and lid status;

- Change in signs assessed by keratography : tear break-up time, tear meniscus height, redness, meibography;

- Change in tear osmolarity;

- Change in biomarker levels including cytokine levels in tears and HLA-DR expression from impression cytology.

6.2 Choice of Primary Outcome Measure

6.2.1 Choice of symptoms versus signs as the primary outcome

Although there is some consensus among clinicians regarding the diagnosis and staging of dry eye disease (International Dry Eye Workshop,2007a) there is little correlation between signs and symptoms on a cross-sectional basis and little correlation between changes in signs and changes in symptoms over time (Lemp, 1995; Bron, 2001; Nichols, 2004; Narayanan, 2005; Turner, 2005; Vitale, 2004; Pult, 2011). In 1995, one of the conclusions from the NEI/Industry Workshop on Dry Eyes was that evaluation of subjective symptoms, as measured through a well-designed and validated questionnaire, may be the best way to determine clinical efficacy of treatments. (Lemp, 1995) Twelve years later, the same conclusion was reached by the International Dry Eye WorkShop (DEWS): "Dry eye is a symptomatic disease, and, at the present time, symptom questionnaires are among the most repeatable of the commonly used diagnostic tests. They may provide a more integrated view of the clinical condition over time. Irritative symptoms are largely responsible for the public health burden and for the care-seeking behavior of dry eye patients and their desire for therapy" (DEWS Epidemiology Subcommittee, 2007). For these reasons, decreasing symptoms in patients was chosen as the primary outcome for judging the effectiveness of supplementation with ω3 fatty acids.

6.2.2 Choice of the OSDI for the measure of symptoms

During the planning stage of DREAM, many different questionnaires that assess symptoms and the impact of symptoms of dry eye disease were evaluated for their suitability as an outcome measure for the DREAM clinical trials. At the start, the 15 questionnaires on symptoms or quality

of life that were identified by the DEWS Epidemiology Subcommittee were reviewed (International Dry Eye Workshop – Epidemiology, 2007). Most of the questionnaires are aimed at diagnosis only, rather than evaluating changes over time, and few had undergone any psychometric assessment (McMonnies 1987a, 1987b, Schiffman, 2000). At the time of the DEWS review, there were no questionnaires with assessments of responsiveness to change in severity of symptoms over time.

6.2.2.1 Description of the OSDI

The OSDI is a 12-item patient-reported outcomes questionnaire designed by staff of a pharmaceutical company (Allergan, Inc.) to provide a rapid assessment of the range of ocular surface symptoms, including symptoms related to chronic dry eye, their severity, and their impact on the patient's ability to function.(Walt, 2000; Walt, 2004). In addition to an overall score, there are three subscales of the OSDI: ocular symptoms, vision-related function, and environmental triggers. The initial OSDI items were generated from multiple sources including comments from clinical investigators, several existing quality-of-life instruments, and both symptomatic and functional complaints from multiple dry eye disease clinical trials consisting of over 400 patients. This list was then modified based on comments from patient focus groups throughout the United States.

A series of studies was conducted by Allergan to reduce the number of items on the list and to assess the validity and reliability of different versions of the questionnaire. (Walt, 2004) The final evaluation resulted in a questionnaire of 12 items. (See Exhibit 7-2) The recall period for all items is one week. Each item is scored on a 0-4 ordinal (Likert-type) scale where 0 indicates "none of the time" and 4 indicates "all of the time". Test scores are computed as (Sum of Scores for All Questions Answered x 100) / (Total Number of Questions Answered x 4). The overall score, and each subscale score, ranges from 0 to 100, where a score of 100 corresponds to complete disability, while a score of 0 corresponds to no disability.

6.2.2.2 Measurement properties of the OSDI on administration at one point in time

The measurement properties of the 12-item OSDI were first evaluated in a study of 109 patients with dry eye disease and 30 controls (Schiffman, 2000). Internal consistency, as rated by Cronbach's α, and test-retest reliability, as measured by the intraclass correlation coefficient ρ, were high for both the total score and the score for each of the subscales, as seen in the table below.

	Reliability Measures	
	Cronbach α (95% Confidence Interval)	Test-retest ρ (95% Confidence Interval)
Total Score	0.92 (0.89-0.94)	0.82 (0.73-0.88)
Subscale scores		
Vision-related function	0.88 (0.84-0.92)	0.70 (0.56-0.80)
Ocular symptoms	0.92 (0.89-0.94)	0.74 (0.62-0.83)
Environmental triggers	0.78 (0.71-0.84)	0.81 (0.71-0.87))

Discriminant validity was assessed by comparing OSDI scores to measures of disease severity. When the mean OSDI total scores were compared among patient groups classified by the examining clinicians as no dry eye disease, mild to moderate disease, and severe disease, the mean scores increased from 10 to 21 to 36 ($p < 0.05$). Approximately 25% of control subjects without disease and 10% of those with mild to moderate disease had a score of 0 (floor) and

1% of diseased patients had a score of 100 (ceiling). Correlation of the total score and subscale scores with specific signs and specific test results was low, generally 0.20 or less. Correlation with patient reported outcomes were moderately high (patient global perception of symptoms, r=0.67; McMonnies dry eye questionnaire, r=0.67; NEI-Visual Functioning Questionnaire (VFQ), r=-0.77) for the total score and slightly lower for the subscale scores. When a receiver-operating characteristic (ROC) curve was constructed from the sensitivity and specificity of varying thresholds for identifying patients as having dry eye disease or not (based on the physician's rating), the area under the curve was 0.73 and the threshold score that maximized the sum of sensitivity (60%) and specificity (83%) was a total score of 15. An alternative approach to classifying patients as having dry eye disease, using a composite score based on results of grading of signs and specific tests and the patient's global perception of symptoms, resulted in a threshold total score of 6 to maximize the sum of sensitivity (80%) and specificity (79%). In practice, the following classification of OSDI scores has been adopted: (Miller, 2010; Katz, 2010; Luchs, 2010).

0-12	Normal
13-22	Mild
23-32	Moderate
33-100	Severe

6.2.2.3 Measurement properties of the OSDI on repeated administrations over time

There are two main sources of information on the properties of repeated administration of the OSDI over time, a study sponsored by Allergan involving patients participating in the Restasis Review of Efficacy and Safety vs. Tears in the Relief of Dry Eye (RESTORE) observational registry and a study conducted by the planning group for the DREAM study (Miller, 2010). The study conducted by the planning group is referred to as the DREAM Questionnaire Study.

In the RESTORE study, 310 patients completed the OSDI on two patient visits. The patients and their eye doctors completed global assessments of the change in their signs (eye doctor only) and symptoms between the first and second visits. The goal of the study was to estimate the minimal clinical important difference (MCID) for the OSDI to meet the Food and Drug Administration (FDA) guidelines for defining a responder in clinical trials using patient reported outcome measures (US DHHS FDA, 2009). The average time between visits was approximately 1 year. When considering all patients regardless of their severity level at the first visit, the mean (SD) change in patients reporting no change in symptoms on the global assessment was 2.2 (23.0) and the mean change for patients reporting a minimal change in symptoms was 9.7 (16.4). The mean (SD) change when clinicians reported no change in signs and symptoms on the global assessment was 1.2 (21.7) and the mean change for physicians reporting a minimal change in signs and symptoms was 11.9 (16.4). However, the mean change in score associated with no change or minimal change varied by the severity level at baseline; the mean change associated with a minimal change in symptoms for patients in the Severe category was 22.0 while it was only 4.7 in for patients in the Mild category. The authors of the paper also provided regression-based estimates of the MCID of 9.9 based on the patient-reported global assessment of change and of 7.0 based on the physician-reported global change.

In the DREAM Questionnaire Study, 216 patients completed 2 or more visits in which the patients completed the OSDI and two other questionnaires on symptoms, as well as global assessments of change similar to those in the RESTORE study. Clinicians also completed global assessments

of change. Patients were under the care of the clinician for dry eye disease; however there were no restrictions on the treatments or changes in treatments between the visits. The mean (SD) days between the first and second visits was 55 (60) days. The table below displays the mean (SD) change in scores when all patients, regardless of their severity level at the first visit, were considered. Whether the assessment was made by the patient or the clinician, the mean decrease in OSDI score decreased with the assessment of improvement with a substantial increase between the categories of slight change and much change.

Change in OSDI Scores
by Global Assessment of Change

	N	Mean	SD
Patient Assessment			
No change	92	-0.4	12.6
Slight change	66	-6.0	16.8
Much change	37	-19.4	23.2
Very much change	11	-24.4	25.4
Clinician Assessment*			
No change	89	-1.6	14.6
Slight change	94	-3.5	21.3
Much change	27	-12.9	20.1

* Only 3 with very much change; not included

Using another approach to describing the relation of change in OSDI score to the patient's perception of global change in symptoms in the DREAM Questionnaire Study, an ROC curve was constructed based on identifying patients with a perceived global change in symptoms using different threshold values for the change in OSDI score. The area under the curve was 0.75 and the thresholds of OSDI score change with the highest summed sensitivity and specificity were over the range of OSDI score 10 (sensitivity 67%, specificity 81%) to 13 (sensitivity 59%, specificity 88%).

6.2.2.4 Past use of the OSDI

The OSDI has been used frequently as a secondary outcome measure in investigations of dry eye and ocular surface disease and as a correlate to signs of dry eye disease (Sall, 2000; Stevenson, 2000; Vitale, 2004; Davitt, 2010; Luchs, 2010; Opitz, 2011; Wojtowicz, 2011; Yoon, 2011) Recently, the OSDI has been used as a primary outcome measure in randomized clinical trials of treatments of dry eye disease both by individual investigators and in large, FDA-regulated, Phase III trials by pharmaceutical companies such as Allergan, Alcon, and Galderma (Shin, 2010; Katz, 2010; clinicaltrials.gov NCT00938704, NCT00514852, NCT00761319, and NCT00560703) Although there are 3 subscales for the OSDI, the overall score has been used most commonly in these previous studies.

6.2.2.5 Comparison of OSDI with other questionnaires

Two other candidate questionnaires, the Brief Pain Inventory and the Symptom Bother module of the Impact of Dry Eye on Everyday Living (IDEEL) questionnaire, were evaluated in the DREAM

Questionnaire Study for their suitability to provide an alternative to the OSDI as a primary outcome measure (Cleeland, 1989; Rajagoplan, 2005).

Because the symptoms of dry eye disease share many of the same features as chronic pain, the Brief Pain Inventory (BPI) was considered. The BPI is a well-established questionnaire on the severity and impact of pain, distributed through the MD Anderson Cancer Institute. The expert panel convened to address measurement of pain, the Initiative on Methods, Measurement, and Pain Assessment in Clinical Trials (IMMPACT) group, endorsed use of the two subscale scores from the BPI for assessment of pain and recommended that the two domains of severity and impact on functioning should be included as outcomes in **"all chronic pain clinical trials"** (Dworkin, 2005). The instrument was originally developed to assess pain in patients with cancer, but has been used in patients with pain from a wide variety of conditions including depression, neuropathy, fibromyalgia, and osteoarthritis.

The IDEEL questionnaire was developed by Alcon Pharmaceutical for use in clinical trials of treatments for DED. One of the 3 modules, the Symptom Bother module, focuses on symptoms. The module has been shown to differentiate among those without DED, with mild DED, and with severe DED and to respond to change in disease status as reported by patients (Fairchild, 2008).

Key results from a Rasch Analysis (Tennant, 2007), summarized in the table below, show that all 3 questionnaires had good reliability and internal consistency (Cronbach's α). However, the

	OSDI	BODI	IDEEL
Reliability	0.88	0.94	0.92
Cronbach's α	0.91	0.95	0.93
Category Function	Good	Poor	Good
Dimensionality Component (<2 is ideal, lower is better)	2.17	2.36	2.37
Targeting (lower means more room for worse patients)	-0.62	-1.51	0.20

BODI had poor category function, meaning that the 11 categories for response for each question had overlaps and that fewer response categories would be sufficient. The IDEEL scores were higher (see also table below), with less capacity to accommodate worse patients than the OSDI and BODI.

When all tests are scaled to a 0-100 range, comparison of the mean scores by level of severity as rated by the patient (0-10, 10 most severe) shows a similar range of scores between lowest and highest severity as assessed by patients and by clinicians. The range was approximately 40 for the patient assessment and 12 for the clinician assessment.

Patient Assessment	N	Mean Score		
		OSDI	BODI	IDEEL
<4	64	21	11	34
4-7	133	39	30	54

>7		61	60	53	73

				Mean Score		
	Clinician					
Correlation with Change	N	OSDI	BODI	IDEEL	DI	IDEEL
Score difference vs. Patient 's global assessment of change	206	0.42	0.47	0.43	27	49
					30	55
Score difference vs. Clinician's global assessment of change	212	0.18	0.27	0.36	38	57

Responsiveness to change was evaluated by assessing the correlation between changes in the questionnaire scores and the global assessments of change from one visit to the next by the patient and by the clinician. The correlation coefficients involving the patient's global assessment for the OSDI (0.42), BODI (0.47), and the IDEEL (0.43) were similar (p>0.35 for all pairwise comparisons). The correlation coefficients involving the clinician's global assessment was significantly higher (p=0.01) for the IDEEL (0.36) than the OSDI (0.18) and the correlation coefficient for the BODI (0.27) was not significantly different from either of the other 2. The area under the ROC curve for identifying patients with a perceived global change in symptom was also similar among the 3 questionnaires: 0.76 for OSDI, 0.79 for BODI, and 0.80 for IDEEL. When a change of 10 points was used as a cutoff for the OSDI, the sensitivity was 67% and the specificity 81%. When a change of 12 points was used as a cutoff for the IDEEL, as recommended above by Fairchild, the sensitivity was 64% and specificity was 90%.

6.2.2.6 Choice of the Primary Outcome Variable

The OSDI is the most widely used questionnaire for outcome assessment in clinical trials of DED and deviating from using it as the primary measurement tool for DREAM would require better performance by either the BODI or IDEEL. Because neither the BODI nor the IDEEL was uniformly better than the OSDI, the OSDI has been chosen as the primary measurement tool for DREAM. Because of the widespread use of the BODI in clinical research on chronic pain, the BODI is retained as a secondary outcome measure.

The original primary outcome variable for each of the DREAM clinical trials was a change in OSDI score of 10 or more points between baseline and 12 months. A change in score of 10 was identified in the RESTORE study as the minimal clinically important difference. In the DREAM Survey Study a 10-point change was associated with nearly the maximum sum of sensitivity and specificity (see 6.2.2.3 above)

Upon recommendations from the NEI study section and the Data and Safety Monitoring Committee prior to the initiation of patient recruitment, the definition of the primary outcome variable was changed to the mean change from baseline in the Ocular Surface Disease Index (OSDI) at 6 months and 12 months for the Primary Trial. For the Extension Study, the primary outcome variable is the average of the changes from the 12 month visit in ODSI score at 18 and 24 months. The use of the mean change in scores generally has greater statistical power than use of a proportion with a specific level of change. Similarly, the average at 6 and 12 months generally has a lower variance than either single value, resulting in increased power. The additional following points were considered in choosing the average of the changes at 6 and 12 months:

- If there is a lag between the initiation of supplementation and the effect of supplementation, the estimated difference between groups is decreased if groups are compared prior to the time of full impact of treatment. This reduction decreases the power to detect a treatment group difference. However, effects of ω3 supplementation on triglyceride blood levels are apparent within 8 weeks of initiation in patients with high triglycerides and increase little, if at all, through 12 months; symptoms of rheumatoid arthritis decrease within 3-4 months; some sources for patients note that supplementation may take 6 months for relief of pain

  (https://www.fammed.wisc.edu/sites/default/files//webfm-uploads/documents/outreach/im/handout_omega3_fats_patient.pdf).

- There may be seasonal variation in the OSDI score due to questions related to air conditioning, windy conditions, and low humidity. Even though patients may mark questions concerning environmental triggers as "not applicable", the OSDI score can be affected by seasonal changes. Seasonal effects are expected to be balanced between treatment groups so that the estimated <u>difference</u> in mean change from baseline between the 2 groups would not be biased but the mean changes may be affected by seasonal effects.

- In both the RESTORE and DREAM data, patients with the most severe disease required larger changes in OSDI score to respond that their symptoms had changed than did patients with the mildest disease. The DREAM eligibility criteria exclude patients with scores at the extremes of the ODSI score range to mitigate this heterogeneity.

6.3 Sample Size Considerations

The primary aim of DREAM is to assess the effectiveness and safety of supplementation with ω3 fatty acids for relieving symptoms of DED. The Extension Study provides a starting point for determining whether supplementation must continue indefinitely or may be suspended after a year of continuous use. The sample size is sufficient to provide high power to detect a clinically meaningful difference between supplementation with ω3 fatty acids and placebo. The 2:1 ratio of patients assigned to active supplements versus placebo in the Primary Trial has been selected to increase the power, relative to a 1:1 ratio for the Primary Trial, of the Extension Study with only a small increase in the overall sample size.

The sample size for DREAM was initially calculated for the grant proposal of DREAM, and re-calculated following the recommendation of NEI study section and the Data Safety Monitoring Committee. All calculations were performed using PS software (Dupont, 1990).

6.3.1 Assumptions and calculation of the sample size for the Primary Clinical Trial in the grant proposal for DREAM

The sample size for the study as described in the grant proposal was based on the following assumptions:

- *The statistical test used to compare the two treatment groups at 12 months is a two-sided, chi-square test of equality of 2 proportions.*

- *Type I (α) error rate of 0.05.*

- *Statistical power of 90%.* DREAM is likely to be considered the definitive clinical trial of ω3 fatty acids; therefore, power is set higher than the traditional 80% level because missing a true treatment effect would be a serious error.

- *The proportion of patients assigned to placebo with a 10 point decrease or more on the OSDI at 1 year will be approximately 40%.* In previous clinical trials in DED, response in patients assigned to placebo has been observed for both patient-reported symptoms and signs assessed by masked clinicians. (Foulks, 2003; International Dry Eye Workshop. Design and Conduct of Clinical Trials, 2007). "Placebo response" has been attributed to regression to the mean, an expectation of patients and their clinicians for improvement, and increased compliance with lubricant regimens when participating in a research study. In addition, as noted above, even when patients report no change in symptoms on a global scale, their OSDI score may improve by 10 or more points (1.00 – specificity = 24% in DREAM, mean (SD) OSDI score change in patients reporting no global change = 2.2 (23.0) implying 37% above a 10 point improvement if the score changes are normally distributed in the RESTORE patient group). The eligibility criteria requiring elevated OSDI scores at both the screening visit and the randomization visit are intended to dampen the regression to the mean effect. Repeating the OSDI at interim visits before the 1-year visit may dampen the effect of expectations for improvement. Considering the combined influences contributing to improvement in OSDI scores over 1 year, the proportion of patients assigned placebo with a 10-point improvement is estimated to be 40%.

- *The minimal clinically meaningful difference between active and placebo supplementation is 15%.* Patients who are already using artificial tears and who are still seeking relief from their dry eye symptoms have few choices for treatment. Cyclosporine 0.05% emulsion (Restasis) is the only FDA-approved drug for DED. The product information (label) for cyclosporine cites the evidence for efficacy as 15% of cyclosporine -treated patients vs. 5% of vehicle-treated patients having an increase in Schirmer's wetting of 10mm or more. Cyclosporine, administered twice a day, costs approximately $300 per month. The dose of ω3 fatty acids (e.g., 5 softgels per day of Nutrilite Ocean Essentials –Heart Health), purified to minimize contaminants such as mercury, costs approximately $70 per month. Thus, if the proposed dose of ω3 fatty acid supplementation relieved symptoms for 15% more of patients than placebo, the benefit would be comparable or greater than that of cyclosporine at approximately 25% of the cost. This rationale allows for non-compliance in that the response in non-compliant patients is likely to be the same in both treatment groups (identical placebo and low incidence of unmasking from side effects); therefore, the low expected level of non-compliance will not contribute to a difference in response between treatment groups.

The table below shows the sample size required <u>for analysis</u> for different combinations of placebo response rate and statistical power. The total sample size of 519 provides 90% power or more for any background rate of placebo response (for placebo rates > 50%, the calculations are identical as those for 1-placebo rate) and for a difference of 15% or more.

Sample Size Calculations - Primary Trial

	Improvement		Power = 0.80			Power = 0.90		
	Placebo	Active	Active	Placebo	Total	Active	Placebo	Total
Difference	0.20	0.35	211	106	**317**	279	140	**419**
0.15	0.25	0.40	231	116	**347**	306	153	**459**
	0.30	0.45	246	123	**369**	327	164	**491**
	0.35	0.50	255	128	**383**	340	170	**510**
	0.40	0.55	259	130	**389**	346	173	**519**
	0.45	0.60	258	129	**387**	345	173	**518**
	0.50	0.65	255	128	**383**	340	170	**510**

- *The loss-to-follow-up percentage will be 10%.* Few deaths are expected in the 12 months following enrollment. However, because DED is neither sight-threatening nor life-threatening, patients may choose to discontinue their participation because of competing demands on their time. The 1-year missing data rate in the Age Related Eye Disease Study (AREDS), which involved dietary supplements as a preventive therapy, was 5% and in the Complications of Age-related Macular Degeneration {AMD} Prevention Trial (CAPT), which involved prophylactic laser treatments, the rate was 2%.(AREDS, 2001; CAPT, 2006). These AMD populations were not undergoing direct treatment for a serious condition, but were aware of the need for early detection of vision-threatening choroidal neovascularization. The same intensive methods to avoid missing data will be applied in DREAM, but there is likely to be some erosion of the rates from the AREDS and CAPT levels.

Inflating the total sample size needed for analysis for a 10% loss-to-follow-up rate yields **a total sample size of 579**; 386 in the active supplement group and 193 in the placebo group. The 2:1 treatment allocation ratio provides higher power for the randomized withdrawal study with only 67 patients (13%) more in the Primary Trial than with equal allocation of 256 to each treatment group.

6.3.2 Recalculation of sample size for the Primary Clinical Trial

Upon recommendations from the NEI study section and the Data and Safety Monitoring Committee prior to the initiation of patient recruitment, the definition of the primary outcome variable was changed to the mean change from baseline in the Ocular Surface Disease Index (OSDI) at 6 months and 12 months. The minimal clinically meaningful difference in means between active and placebo was set at 6 representing a small to moderate effect size of 0.24 to 0.30, based on estimates of the standard deviation of change from RESTORE Registry and the DREAM feasibility study of 20 to 25.

In June 2016, the DREAM Data and Safety Monitoring Committee reviewed the sample size calculation and the accumulated data from DREAM. Based on the magnitude of the observed standard deviation of the changes from baseline during follow-up and the observed missed visit rate, a standard deviation of 18 was assumed for the primary outcome variable and a 15% missed visit rate was assumed. Retaining a Type I error rate of 0.05 and power of 0.90 yielded a sample size goal of 505. This goal was met at the end of June 2016; however, recruitment

remained open until July 31, 2016 to accommodate the patients who had already started the recruitment process. A total of 535 patients were enrolled.

6.3.3 Power considerations for the Extension Study in the Grant Proposal for DREAM

Only patients initially assigned to active supplements and who respond by having an improvement (i.e., decrease) in OSDI score ≥ 10 points at the 1-year visit are eligible for the Randomized Withdrawal Trial (Extension Trial).

- The response rate in the Primary Trial will not be known until the Primary Trial is completed; therefore, plausible values must be assumed. If the response rate in the active supplement group in the Primary Trial is 55%, then 55% of the 346 patients are anticipated to complete the 1-year visit, or 190 will be eligible for the second randomization. If the response rate in the Primary Trial is 65%, 225 patients will be eligible.

- An attrition rate of 10% accounts for patients who refuse to continue in the Randomized Withdrawal Trial or who are lost to follow-up during the second year. By definition, all of the patients in the Randomized Withdrawal Study will have had a decrease in their symptoms and their participation rate is anticipated to be high.

- The primary outcome measure for this Extension Study is a worsening (increase) in OSDI score ≥10 points at the 2-year visit relative to the score at the 1-year visit.

- The statistical test used to compare the two treatment groups at 2 years is a two-sided, chi-square test of equality of 2 proportions.

- A significance level 0.05 is considered statistically significant.

- If the active supplements are responsible for the improvement in OSDI score, then "most" of the patients who continue on with active supplement are likely to have no substantial decrease in their OSDI score. Even if the active supplements are responsible for all of the improvement observed in Year 1, the percentage with worsening in the group assigned to placebo for the second year is unlikely to approach 100% because of the possibility of long-term effects of one year of supplementation and carryover of the "placebo effects".

The table below shows that if the difference (Δ) in the Extension Study between the treatment groups is 25% or higher, there is excellent statistical power (≥90%) to detect the difference. There is good statistical power (≥80%) if Δ is 20% and the worsening rate in the active supplementation group is 20% or less.

Power Analysis for the Randomized Withdrawal Trial (Extension Study)

55% Response Rate in Primary Trial Worse* in Year 2				65% Response Rate in Primary Trial Worse* in Year 2			
Active	Placebo	Δ	Power	Active	Placebo	Δ	Power
0.15	0.30	0.15	0.66	0.15	0.30	0.15	0.73
0.15	0.35	0.20	0.86	0.15	0.35	0.20	0.91
0.15	0.40	0.25	0.96	0.15	0.40	0.25	0.98

0.20	0.35	0.15	0.60	0.20	0.35	0.15	0.67
0.20	0.40	0.20	0.82	0.20	0.40	0.20	0.88
0.20	0.45	0.25	0.94	0.20	0.45	0.25	0.97
0.25	0.40	0.15	0.56	0.25	0.40	0.15	0.63
0.25	0.45	0.20	0.79	0.25	0.45	0.20	0.85
0.25	0.50	0.25	0.93	0.25	0.50	0.25	0.98
0.30	0.45	0.15	0.53	0.30	0.45	0.15	0.60
0.30	0.50	0.20	0.77	0.30	0.50	0.20	0.83
0.30	0.55	0.25	0.92	0.30	0.55	0.25	0.95
0.40	0.55	0.15	0.50	0.40	0.55	0.15	0.57
0.40	0.60	0.20	0.75	0.40	0.60	0.20	0.82
0.40	0.65	0.25	0.91	0.40	0.65	0.25	0.96

Shading   denotes power ≥ 0.80 or more

*Worse = a 10-point increase in OSDI

 1:1 ratio of Active and Placebo

10% lost in Year 2 due to refusal and loss to follow-up

6.3.4 Revision of sample size for the Extension Study

Recruitment for the Primary Clinical Trial required more time and resources than originally planned. To remain within budget for DREAM, recruitment for the Extension Study was ended at the same time as recruitment for the Primary Clinical Trial, which was approved by the Data and Safety Monitoring Committee. As of July 31, 2016, 43 (39.4%) of 109 patients completing the Month 12 visit and assigned to Active supplements were enrolled in the Extension Study.

6.4 Data Analysis

For the majority of analyses, the Primary Trial and the Extension Study will be considered as independent clinical trials. Some descriptive longitudinal models will include the data from both trials. In addition, data for the assessment of patients assigned to placebo in the Primary Trial during the first 12 months will include longitudinal models.

6.4.1 Statistical Methods to be Applied

Data analysis for the clinical trials will be conducted using standard statistical techniques for comparing two independent groups: chi-square test for equality of proportions, independent t-test for equality of means, Wilcoxon rank sum test, multiple logistic and linear regression, and proportional hazards modeling. The distribution of continuous variables will be assessed by measures of normality and graphical displays so that non-parametric methods or data transformations may be applied when appropriate. For eye-specific measures, statistical techniques for correlated data that appropriately accommodate measurements on both eyes of a patient will be used.

6.4.2 Assessment of Baseline Comparability of Treatment Groups

Tables will be generated and inspected to compare, by treatment group, the distribution of key baseline variables having descriptive and prognostic importance. These variables will include, but not be limited to, patient age, race, ethnicity, gender, frequency of use of remedies for DED

(artificial tears, lubricants, cyclosporine, punctual plugs, Lacriserts, lid scrubs), use of dietary supplements, Schirmer's wetting, tear break-up time, corneal staining, conjunctival staining tear osmolarity, and signs of blepharitis. For the OSDI score and the results of assessment of the signs of dry eye disease involved in the eligibility criteria, the mean (median for measurements on an ordinal scale) of the values from the Screening Visit and the Baseline Visit will be used as baseline values.

6.4.3 Data Analyses of the Primary Outcome Variable

The primary statistical analyses will be performed on an intent-to-treat basis. For each patient in the Primary Trial, the difference in OSDI score between baseline and 6 months and the difference between baseline and 12 months will be averaged and the mean of the averaged values will be compared between treatment groups. If a patient has only an OSDI score from either 6 months or 12 months but not both time points, the OSDI change from baseline to this single time point will be used for analysis. For each patient in the Extension Study, the difference in OSDI score between 12 months and 18 months and the difference between baseline and 24 months will be averaged and the mean of the averaged values will be compared between treatment groups. Although the distribution is likely to be skewed, the sample sizes of the treatment groups are sufficiently large that the means should be normally distributed so that p-values and confidence intervals based on independent t-tests are accurate. If the baseline OSDI score is imbalanced ($p < 0.10$) between active supplement and placebo groups, the baseline OSDI score will be used as a covariate in a linear regression model. Similarly, if the baseline EPA or DHA level is imbalanced ($p<0.10$) between active and placebo supplement groups, the baseline EPA or DHA levels, respectively, will be used as a covariate.

Analyses will be performed to assess the robustness of the results with respect to dropouts and non-compliance with the eligibility criteria and the treatment protocol. In addition to the above-described analysis of results from all patients who complete the examinations 1 year after randomization (completed cases) with their treatment group assignment classified as assigned at randomization ("intent-to-treat"), an intent-to-treat analysis will be performed using multiple imputation methods (Rubin, 1987; Heyting, 1992; Lavori, 1995). Both predictive model based methods and propensity score methods will be used to evaluate the impact of missing data. Analyses will also be stratified by quartiles based on the compliance measures of pill counts and change in EPA and DHA as measured through blood tests. Further sensitivity analyses will be conducted using pattern mixture models for missing data if there are indications that data are not missing at random (Carpenter, 2013).

Additional analyses to more fully characterize the relation of change in OSDI score to treatment group over time will be performed using longitudinal data analysis methods. A mixed effects model with a random intercept for each patient will be used to account for the correlation from the repeated measurements of change in OSDI. Time (3, 6, 12 months) will be modelled as a categorical variable to allow for a non-linear relationship between change in OSDI score and time. An interaction term between time and treatment group will be included in the model and will be dropped if the interaction is not statistically significant. Both the relation of OSDI change from baseline with follow-up time and the influence of possibly prognostic factors will be evaluated using these models.

6.4.4 Pre-specified subgroup analyses

Four subgroup analyses are planned as pre-specified analyses; analyses of other subgroups will be considered as exploratory analyses. Different effects of treatment within the subgroups will

be assessed through a test of interaction between the subgrouping variable and treatment group.

- Severity of symptoms as measured by the baseline OSDI score. Severe symptoms are considered as an OSDI score (average of Screening and Baseline Visit scores) ≥40

- Severity of signs based on the 4 signs used for eligibility determination. Severe signs are considered present if one or both eyes of a patient had scores (average from the Screening and Baseline Visits) meeting each of the 4 criteria below:

  - Conjunctival staining ≥2

  - Corneal staining ≥4

  - TBUT < 5 sec

  - Schirmer ≤ 7 mm/ 5 min

- High DHA/EPA level. People with both their DHA level and EPA level above the mean value from the Kennedy Krieger adult control group [N=147; mean age = 49.5 +/- 17.0] (DHA 3.7%, EPA: 0.6%)

- Inflammation status as measured by the percent of HLA-DR positive epithelial cells from impression cytology at baseline. High inflammation status is considered as a percentage of HLA-positive cells greater than the median from the DREAM study population.

6.4.5 Data Analyses of Secondary Outcome Variables

Specific secondary outcome variables for the two DREAM trials are compliance with the study treatment protocol as measured by changes in blood levels of essential fatty acids and pill counts; ≥ 10 point change in OSDI(decrease for Primary Trial, increase for Extension Study); change in signs of dry eye disease: corneal fluorescein staining, lissamine green staining of the interpalpebral conjunctiva; tear break-up time (TBUT), and Schirmer's test; change in the frequency of administration of artificial tears and other treatments for DED; change in quality of life as measured by the Medical Outcome Study - Short Form (SF-36); change in the Brief Ocular Discomfort Inventory (BODI); incidence of systemic adverse events and changes in visual acuity, and intraocular pressure; and cost and incremental cost-effectiveness of using ω3.

6.4.5.1 Compliance with the Study Treatment Protocol

Both pill counts and red blood cell (RBC) membrane total lipid FA profiles are measures of compliance with the taking study supplements. Patients who return bottles with the number of remaining pills indicating that 75% or more of the pills were taken as directed will be considered as compliant, a level of compliance used in AREDS (AREDS, 2001). Patients assigned to active supplements should have increases in the % total FA of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and decreases in arachidonic acid (AA). Comparisons of the distributions of change in the levels of these fatty acids between patients taking active and placebo supplements will aid in identification of patients in the active group who are not taking their supplements (e.g., outlier values indicating less increase in % EPA than the majority of patients in the active group) and patients assigned to placebo who may be taking high, unreported doses of over-the-counter supplements (e.g., outlier values indicating larger increases in % EPA than the majority of patients in the placebo group). A secondary data analysis of the proportion with response on the OSDI will be performed excluding non-compliant patients to gain insight on the effect of active supplementation when patients are compliant with the dosing regimen.

6.4.5.2 Change of ≥10 Points on the OSDI

The proportion with a change (decrease for the Primary Clinical Trial and increase for the Extension Study) of 10 or more points on the OSDI between the average of months 6 and 12 and baseline will be compared between the group of patients assigned active supplement and the group assigned placebo. A change in score of 10 was identified in the RESTORE study as the minimal clinically important difference (Miller, 2010) The chi-square test without continuity correction will be used to determine the significance level associated with the comparison. A 95% confidence interval will be calculated for the difference in proportions using the method by Wilson (Brown, 2001).

6.4.5.3 Change in Signs of DED

Changes in corneal fluorescein staining, lissamine green staining of the interpalpebral conjunctiva; tear break-up time (TBUT), and Schirmer's test are important secondary outcome measures because they provide more objective indications of change in the severity of DED. Corneal and conjunctival staining are scored on an ordinal scale, rather than an interval scale, so that subtraction of scores may not yield comparable change scores across patients. The gradings from baseline and follow-up will be categorized as better, the same, and worse and compared between groups through an analogue of the chi-square test for trend in proportions for clustered data (Liang, 1993). TBUT and Schirmer's test are measured on an interval scale. The distribution of the change in these test scores over time will be assessed for symmetry and if the distributions are skewed, the non-parametric Wilcoxon rank sum test for clustered data (1 or two eyes per patient) will be used for comparing treatment groups; otherwise, an analogue of the independent t-test for clustered data will be used (Rosner, 2006; Liang, 1993). The primary analyses for these signs will include only eyes meeting the eligibility criteria at baseline.

6.4.5.4 Change in Frequency of Administration of Artificial Tears and Other DED Treatments

Changes from baseline in the frequency of administration of artificial tears and lubricants {allowed under the protocol} as well as the insertion of punctual plugs, Lacriserts, or initiation of Restasis {not allowed by the protocol} can modify the OSDI score of patients independent of the treatment effects. Change in frequency of use of artificial tears or other lubricants will be categorized as more, the same, or less and the two treatment groups compared with a chi-square test for trend. Patients will also be stratified into groups with respect to the intensity of other measures taken to relieve symptoms (more intense {e.g., greater frequency of artificial tears or insertion of punctual plugs} the same as baseline, and less intense {e.g., reduction or cessation in use of artificial tears}) and the difference in proportions between groups compared with chi-square tests for trend..

6.4.5.5 Change in Quality of Life – MOS-Short Form (SF-36)

The SF-36 is a widely used quality of life instrument with two composite scores, the mental and physical scores. Scoring of the responses will be performed using the SAS code provided by one of the developers of the questionnaire (http://gim.med.ucla.edu/FacultyPages/Hays/util.htm). Changes in the SF-36 scores will be assessed with either the independent t-test if the distributions are symmetrical or the Wilcoxon Rank Sum test if the distributions are skewed. This SF-36 scores are used as a measure of quality of life with meaning of its own, in addition to serving as an input into the cost-effectiveness analysis.

6.4.5.6. Change in Score on the Brief Ocular Discomfort Inventory (BODI)

The Brief Ocular Discomfort Inventory (BODI) is modeled after the Brief Pain Inventory (BPI), a well-established questionnaire on two domains of pain, severity and impact on functioning. The intensity score is the arithmetic average of the first 4 items on the worst, least, average, and current intensity of pain and the interference score is the arithmetic average of the responses on 7 items concerning the impact of pain on function. The scores may be computed and used for assessment if 50% or more of the items in a subscale are available. Differences in change in BODI scores between treatment groups will be assessed with either the independent t-test if the distributions are symmetrical or the Wilcoxon Rank Sum test if the distributions are highly skewed.

6.4.5.7 Incidence of Systemic Adverse Events

Systemic adverse events and Serious Adverse Events, such as hospitalizations for any condition, are expected to occur at a relatively low rate and for conditions not usually associated with high doses of $\omega3$ fatty acids. Patients will be questioned specifically about bleeding episodes at each clinic visit. Comparisons across treatment groups of the rates of all adverse events, all Serious Adverse Events, and of bleeding events will be made using survival analysis techniques – Kaplan-Meier curves with differences assessed with the logrank test. If the number of a specific type of adverse event is low (< 6 per group), the proportion of patients in each treatment group at one-year after randomization will be compared using Fisher's exact test.

6.4.5.8 Change in Visual Function and Intraocular Pressure

Visual acuity is a sensitive measure of toxicity in the eye and intraocular pressure supplies another indicator of possible adverse ocular effects of $\omega3$ fatty acids. Because ETDRS visual acuity charts are used for testing, the letter scores are already on the LogMAR scale. Changes in visual acuity scores and in intraocular pressure will be assessed for symmetry and if the distributions are skewed, the non-parametric Wilcoxon rank sum test for clustered data (1 or two eyes per patient) will be used for comparing treatment groups; otherwise, an analogue of the independent t-test for clustered data will be used (Rosner, 2006; Liang,1993). Analyses will include all eyes, regardless of their eligibility status at baseline, because both eyes of the patient are exposed to the contents of the supplement.

6.4.5.9 Cost and Incremental Cost-effectiveness of Using $\omega3$ Fatty Acid Supplements

Economic analyses focus on three main measures 1) workplace productivity losses; 2) healthcare, including medications and utilization of physician visits; and 3) quality of life and clinical outcomes to calculate the incremental cost-effectiveness ratio associated with adding $\omega3$ to the dry eye disease treatment regimen. The first analyses use data only from the Primary Trial.

A second set of analyses incorporate the data collected at 18 and 24 months from the Extension Study. If symptoms remain the same for the patients assigned to placebo and active supplements, the gains in effectiveness associated with $\omega3$ estimated at one year can be projected forward without having to project forward the cost. If there is a differential change in the second year between those assigned to active and placebo supplements, both the changes in utility and the additional cost of the supplements need to be incorporated into the estimate of the cost-effectiveness of two years of a regimen of $\omega3$.

6.4.5.9.1 Workplace Productivity Losses

The Workplace Productivity and Activity Impairment Specific Health Problem (WPAI:SHP) instrument is completed at baseline, 6 months, and 12 months. The WPAI is a six question instrument that ascertains whether an individual is working and, if so, how much time was worked in the past week, how much time was missed because of specific health conditions, and how much time was missed for other reasons. Respondents are also asked about their experience with lost productivity while at work because of the health condition (in this case, dry eye). The instrument is accompanied by an algorithm to use the data to estimate the combined lost workplace productivity due to the combination of absenteeism and presenteeism. The instrument also asks about a lack of productivity for other regular daily activities outside the workplace. A 12-month period of experience is estimated by assuming that the baseline data apply for the month 1 to 3, 6-month data apply to months 4 to 9 and the 12-month data apply for months 10 to 12.

6.4.5.9.2 Healthcare Utilization

Clinic coordinators question patients about healthcare utilization in the past 4 weeks, It will be assumed that any 4-week period is representative of the time periods surrounding the interview as given above for the WPAI:SHP. Lead in questions with a simple yes or no response to "any utilization of this type in the last four weeks" are administered first and transition to more open-ended responses once it is established that a patient has had at least some utilization. Eye care related to management of DED (such as visits with an ophthalmologist, optometrist, complementary health provider; plug surgeries; medications (e.g. cyclosporine, ophthalmic solutions); and any medical encounter due to dry eyes, as deemed by patients and their clinician) is captured only at the baseline visit because after that time, management is prescribed by the study protocol and deviations are captured as part of the general data collection. Eye care professional visits are coded for the different levels of care. Pharmaceutical products are assumed to be taken as directed; prices are obtained from the Red Book of Drug Topics. Because the average wholesale price in the Red Book may be an overestimate of the price many customers pay, average sale price data will be substituted when available. Outpatient services will be valued based on the Medicare fee schedule and the clinical diagnostic laboratory fee schedule. The DREAM Medical Monitor judges whether hospitalizations, which are also recorded as Serious Adverse Events, are related to the dry eye condition or management of DED. Prices are obtained from the average costs of days of hospitalization and from the University Health Consortium, an alliance of academic hospitals which provides a central repository of financial and clinical data for each individual patient treated in one of its member institutions. The prices of complementary and alternative care providers and herbal supplements are obtained from online sites. Sensitivity analyses can be performed using only those costs that are judged to be related and comparing the results with those obtained when using all costs.

6.4.5.9.3 Incremental Cost Effectiveness of $\omega 3$

To measure the relationship between dry eye and health related quality of life, we will use a generic health related quality of life measure: the SF-36. (Luo, 2012; Rajagoplan, 2005; Schiffman, 2003) The SF-36 asks 36 questions and provides measures for eight domains and two summary scales—a physical component score and mental component score. These can be converted into health utility scores (Le, 2011; Hanmer, 2006). Health utility scores range from 1, no problems, to potentially less than zero, where zero is a health state as bad as being dead, based on societal preferences. Health utility scores allow calculation of quality adjusted life years (QALYs) and performance of a cost-utility analysis. Changes in health utility can be used in cost-effectiveness analyses. The values obtained at baseline, six months, and twelve months will all

be included in the analysis. Changes in QALYs for the group randomized to the active supplement are compared with changes in QALYs for the group randomized to placebo. A generalized estimating equations (GEE) approach is used to estimate the difference in the change between the two groups over time.

The incremental cost-utility ratio is calculated as the difference in costs divided by the change in outcome to indicate how many extra dollars are spent to gain 1 QALY when ω3 is added to the treatment regimen. In the United States the most commonly cited figure is that $50,000/QALY is considered a good buy and more than $100,000/QALY to gain more health is considered expensive. Bootstrapping statistical methods are used to provide estimates of the uncertainty in the incremental cost and incremental effectiveness estimates and interpreting the distribution of the results by asking what proportion of replications imply different ratios. Uncertainty surrounding the incremental cost utility ratio will be also represented using a cost acceptability curve. This will show the probability that the intervention is cost effective compared with usual care, for a range of maximum monetary values that a decision maker might be willing to pay for a unit improvement in outcome. (Fenwick, 2001) The cost-utility analysis is supplemented with a cost-effectiveness analysis in which the presence of a clinically significant change in the OSDI is used as the outcome measure and the amount that must be spent per person who achieves a clinically significant change in OSDI is calculated. Lost productivity is also included in secondary cost-effectiveness analyses.

In addition to sampling variation, some degree of uncertainty can be attributed to the imprecision of the cost inputs and to the methodology employed to derive utility weights from the SF-36 scores. To assess the robustness of the results, the influence of uncertain parameters in the base case analysis will be tested through sensitivity analysis. Costs inputs will be varied over plausible ranges. For utility values, the 95% CI of the mean predicted utility, will define the uncertainty range to be tested in sensitivity analysis. Using one way sensitivity analysis, different variables will be rank ordered for the magnitude of their overall influence on the results. Furthermore, the effect of simultaneously varying all parameters will be tested using Monte Carlo simulation.

6.4.6 Data Analyses of Exploratory Outcome Variables

Specific exploratory outcome variables for the two DREAM trials are contrast sensitivity, meibomian gland secretion and lid status; and signs measured by keratography including TBUT, tear meniscus height, redness and meibography; tear osmolarity; cytokine levels in tears; and HLA-DR expression from impression cytology. Although changes in the levels will be assessed between treatment groups, the main emphasis will be to determine how each is related to the presence and severity of DED.

6.4.6.1 Change in Contrast Sensitivity

Contrast sensitivity measurement may provide a sensitive measure of the effects of ω3 fatty acids on DED. The letters on the Mars contrast sensitivity chart have equal decrements in log contrast. Changes in contrast sensitivity scores will be assessed for symmetry and if the distribution is skewed, the non-parametric Wilcoxon rank sum test for clustered data (1 or two eyes per patient) will be used for comparing treatment groups; otherwise, an analogue of the independent t-test for clustered data will be used (Rosner, 2006; Liang,1993).

6.4.6.2 Data Analyses of Signs of DED

A new device, the Oculus Keratograph, provides measures of TBUT and redness and photographic images to allow measurement of tear meniscus height and characteristics of the meibomian glands. Characterization of the meibomian gland secretions may provide information on the presence and severity of DED. Use of these data is relatively new in DED and their usefulness in diagnosis, staging, and tracking of DED over time is not established. Analyses of these assessments will be similar to the analyses of the biomarkers described below.

6.4.6.3 Data Analyses of Candidate Biomarkers

MMP-9, cytokine (IL-1β, IL-6, IFN-γ, TNF-α) levels in tears, HLA-DR expression and levels of other inflammatory markers on conjunctival cells, tear osmolarity, and levels of antibodies for autoimmune diseases are candidate biomarkers for DED. Biomarkers can be mere indicators of the presence or absence of a condition, or related to the severity of disease, and/or responsive to treatment. A first analysis is to compare levels in patients at follow-up visits who no longer meet the clinical definition of DED with the levels in patients that do meet the definition. A second set of analyses is to classify patients according to their ODSI score (see 6.2.2.2 above) and assess whether levels increase or decrease monotonically (cytokines, tear osmolarity, and inflammatory markers). Patients can also be classified on the DEWS severity scale (International Dry Eye Workshop, 2007a). The third set of analyses is to assess whether changes in severity are associated consistently with changes in the biomarker levels. The distributions of the biomarker levels are first examined for departures from normality and, if necessary, transformations are applied to achieve normality and stable variance over the range of the severity (cytokines and HLA expression only). Groups are compared with regression models that account for the correlation among multiple observations per person and the correlation between eyes (Heitjan, 1997; Diggle, 1994).

6.4.7 Handling Missing Data

Major efforts will be made by the entire DREAM group to avoid loss to follow-up and subsequent missing data. However, despite these efforts some data for the primary and secondary outcome measures may be missing. The percentage of data missing for major analyses will be tabulated. The characteristics at baseline, and during follow-up, of patients who ultimately are unavailable for follow-up will be assessed by comparing distributions between those under follow-up to those who are lost to follow-up. When available, the reasons for loss to follow-up will be reviewed. If missing data from living patients account for more than a small percentage of expected data (>5%), key analyses will be performed not only with the actual observed data on patients under follow-up, but also, using multiple imputation methods (Rubin, 1987; Heyting, 1992; Lavori, 1995). Both predictive model based methods and propensity score methods will be used to evaluate the impact of missing data on the key analyses of the DREAM. Multiple imputation methods have better statistical properties than alternatives such as complete case analyses or single imputation. In addition, as noted above in 6.4.3, pattern mixture models will be used for sensitivity analyses for the primary outcome variable if there are indications that data are not missing at random.

6.4.8 Data Analyses for the Longitudinal Assessment of the Placebo Group

The patients treated with placebo supplements during the one-year period of the Primary Clinical Trial provide new information on the course of DED over an extended period of time. Both the entire placebo group and the subgroup of patients using only artificial tears and lubricants for symptom relief will be considered. The interim data from the clinical center visits at 3, 6, and

12 months provide information on the variability of the disease as measured by the full DREAM battery of assessments of signs, symptoms, and biomarkers (not available at 3 months). Each of the measures will be assessed for constancy over time and correlation with season of the year. Characteristics at baseline will be assessed as risk factors for both improvement and worsening of signs and symptoms between the baseline and one-year examination (no seasonal effect). The correlation among signs and symptoms will be assessed with longitudinal data analysis methods that account for the both the correlation of measurement over time and within person, specifically, mixed effects regression models and multi-level regression using generalized estimating equations (Diggle, 1994; Ten Have, 1999).

6.4.9 Identification of outliers, incorrectly collected data, and possibly fraudulent data

With each freeze of the database, a set of statistical and data analytic algorithms will be applied to detect data warranting further investigation and/or action. True values of data that are very different from the majority of values are known as outliers and may have undue influence on such statistical procedures as estimating the mean and variance and regression analyses. However, apparent outliers are often attributable to error: data recording error, data entry error, error in recoding in computer programs, error in the way in which the measurement is performed or the question asked. Another source of outliers is fraud.

As part of the preparation for any of the data analyses above, continuous variables, including dates, are subjected to the techniques of exploratory data analysis in order to fully understand the distribution of the variable. SAS, which is the main software package for data analysis, has built in procedures to flag and list values that meet certain criteria for outliers based on the median and interquartile range. The identification number of the patient can be attached to the extreme value. The Principal Investigator and Senior Biostatistician will review the exploratory analyses and determine whether an investigation of the accuracy of the value should begin. If the outlier values are valid, statistical methods that minimize the impact of outliers will be used.

Other data patterns will also be explored. Dates of clinical procedures will be examined by day of the week to identify the unlikely occurrence of procedures on weekends. Clusters of data values near cutoff values will be investigated. An inordinate percentage of 0 change values may indicate that the values from the last examination were merely copied. When such data patterns are identified, they will be brought to the attention of the Director for further investigation.

6.4.10 Software for Statistical Analysis

SAS/STAT software (SAS Institute, Inc., 100 SAS Campus Dr., Cary, NC, 27513-2414) is used for performing most statistical analyses. SAS Procedures are available for the vast majority of analysis methods described above, including the multiple imputation methods. Additional software packages are resident on the computer system for the Coordinating Center to handle specialized applications including Winsteps Rasch Model Computer Program (JM Linacre., Beaverton, Oregon: Winsteps.com), Confidence Interval Analysis (CIA) for Windows (University of Southampton School of Medicine (www.medschool.soton.ac.uk/cia/)). When the application can be accommodated more easily by other software packages, Stata (StataCorp, 4905 Lakeway Drive, College Station, Texas 77845) and R (www.R-project.org) are available.

6.5 Data Monitoring

The DREAM Data and Safety Monitoring Committee (DSMC) will follow "NIH Policy For Data And Safety Monitoring" - release date: June 10, 1998) and the "National Eye Institute Guidelines for Data and Safety Monitoring of Clinical Trials" NOTICE: EY-01-002, release date March 2001. The NEI guidelines provide explicit guidelines on responsibilities of the Committee, membership, meeting format, recommendations, release of data, and conflict of interest will be incorporated into the DREAM DSMC Charter and will not be repeated.

Exhibit 6-1 DREAM Design Summary for Clinical Trials

Feature	Criteria
Objective	Evaluate the effectiveness and safety of supplementation with ω3 fatty acids in relieving the symptoms of moderate to severe dry eye disease (DED)
Major Eligibility Criteria	**Primary Trial** ≥ 2 of the following 4 signs in the same eye at screening and baseline visits (Same signs must be present at Screening and Baseline Visits) • Conjunctival staining present ≥ 1 (out of possible score of 6 per eye) • Corneal fluorescein staining present ≥ 4 (out of a possible score of 15 per eye) • Tear film break up time (TBUT) ≤ 7 seconds • Schirmer's test ≥ 1 to ≤ 7 mm/5min Ocular Surface Disease Index (OSDI) score: 25-80 at screening, 21-80 at baseline Symptoms of DED ≥ 6 months Use or desire to use artificial tears ≥2 times/ day in preceding 2 weeks **Randomized Withdrawal (Extension Study)** Assigned to active supplements in Primary Trial and wiling to continue taking supplements
Randomization	Unit is person
Masking	Double masked
Treatments	1)Active supplements: 2000 mg EPA and 1000 mg DHA per day; 2)Placebo
Outcome Measures 1°	Mean of change from baseline in OSDI score at 6 and 12 months (Primary Trial) Mean of change from 12 months in OSDI score at 18 and 24 months (Extension Study)

2°	Compliance with the study treatment protocol as measured by changes in blood levels of fatty acids and pill counts
	≥ 10 point change in OSDI-decrease for Primary Trial, increase for Extension Study
	Change in
	   Signs of DED (conjunctival and corneal staining, TBUT, Schirmer's test)
	   Use of artificial tears and other treatments for DED
	   Quality of life as measured by the SF-36
	   Score on the Brief Ocular Discomfort Inventory (BODI)
	Cost and incremental cost-effectiveness
	Incidence of ocular and systemic adverse events, changes in VA and IOP

Exploratory	Contrast sensitivity Meibomian gland secretion evaluation and lid status Signs measured by keratography: TBUT, tear meniscus height, redness; meibography Tear osmolality Biomarker levels:MMP-9 in tears, tear cytokine levels, expression of HLA-DR and other inflammatory markers on conjunctival cells, and serum antibodies associated with Sjogren's Syndrome and other autoimmune diseases
Sample size	Primary Trial: 535 Extension Study: 43

Follow-up   Primary Trial: Visits at 3,6,12 months; telephone call at 9 months
                   Extension Study: Visits at 18, 24 months; telephone calls at 15 and 21 months

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