Dry Eye Assessment and Management

Manual of Procedures
# The DRy Eye Assessment and Management (DREAM) Study
Manual of Procedures

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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). 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 (Miljanovic, 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). 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).

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).

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>
<thead>
<tr>
<th>Quality-of-Life Measure*</th>
<th>Patients, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feel less confident</td>
<td>38.6</td>
</tr>
<tr>
<td>Need to decrease leisure time</td>
<td>35.7</td>
</tr>
<tr>
<td>Get frustrated with daily activities</td>
<td>34.3</td>
</tr>
<tr>
<td>Need to change activities</td>
<td>25.7</td>
</tr>
<tr>
<td>Feel unhappy or depressed</td>
<td>25.7</td>
</tr>
<tr>
<td>Need to decrease work time</td>
<td>11.4</td>
</tr>
<tr>
<td>Require help</td>
<td>14.3</td>
</tr>
<tr>
<td>Miss outings</td>
<td>12.9</td>
</tr>
<tr>
<td>Change work</td>
<td>7.1</td>
</tr>
<tr>
<td>Other</td>
<td>22.9</td>
</tr>
<tr>
<td>None of the above apply to me</td>
<td>27.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vision-Related Activity†</th>
<th>Patients, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Driving at night</td>
<td>32.3</td>
</tr>
<tr>
<td>Reading</td>
<td>27.5</td>
</tr>
<tr>
<td>Working with computer/bank machine</td>
<td>25.7</td>
</tr>
<tr>
<td>Watching television</td>
<td>17.9</td>
</tr>
</tbody>
</table>

*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 recent 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 a recent 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 unreimbursed 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 recent 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; Enriquez-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-1β, 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). 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-DR (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 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).

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).

Recently, 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, LTBS 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-1β and TNF-α 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). Recently, it 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). Recently, 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 recent 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 recent 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 (Borner, 2000; Sullivan, 2002; Schaumberg, 2003; Milijanovic, 2005; Macsai, 2008; Wojtowicz, 2011). 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). Recently 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 recent 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 and the CG+S versus the CG−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, Schirmer’s test value 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 that given supplement of (325mg EPA and 175mg DHA) twice a day for 3 months led to significant improvement in both Schirmer's test value and TBUT values in the ω3 group. (Bhargava R 2013).

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).

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. ≥ 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 ≥ 1 (out of possible score of 6 per eye)
   b. Corneal fluorescein staining present ≥ 4(out of a possible score of 15 per eye)
   c. Tear film break up time (TFBUT) ≤ 7 seconds
   d. Schirmer’s test ≥ 1 to ≤ 7 mm/5 min
4. Demonstrate symptoms of dry eye disease (OSDI score of at least 25 (≥ 25 TO ≤ 80) at Screening Visit and at least 21 (≥ 21 TO ≤ 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) ≥ 5 mmHg and ≤ 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 (≥ 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, entropion, entropion, severe trichiasis, 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.
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.
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 anti-histamine 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**

<table>
<thead>
<tr>
<th>Feature</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Objective</strong></td>
<td>Evaluate the effectiveness and safety of supplementation with ω3 fatty acids in relieving the symptoms of moderate to severe dry eye disease (DED)</td>
</tr>
<tr>
<td><strong>Major Eligibility Criteria</strong></td>
<td><strong>Primary Trial</strong>&lt;br&gt;≥ 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)&lt;br&gt;• Conjunctival staining present ≥ 1 (out of possible score of 6 per eye)&lt;br&gt;• Corneal fluorescein staining present ≥ 4 (out of a possible score of 15 per eye)&lt;br&gt;• Tear film break up time (TBUT) ≤ 7 seconds&lt;br&gt;• Schirmer’s test ≥ 1 to ≤ 7 mm/5min&lt;br&gt;Ocular Surface Disease Index (OSDI) score: 25-80 at screening, 21-80 at baseline&lt;br&gt;Symptoms of DED ≥ 6 months&lt;br&gt;Use or desire to use artificial tears ≥ 2 times/day in preceding 2 weeks</td>
</tr>
<tr>
<td><strong>Randomization</strong></td>
<td>Unit is person</td>
</tr>
<tr>
<td><strong>Masking</strong></td>
<td>Double masked</td>
</tr>
<tr>
<td><strong>Treatments</strong></td>
<td>1) Active supplements: 2000 mg EPA and 1000 mg DHA per day; 2) Placebo (olive oil)</td>
</tr>
<tr>
<td><strong>Outcome Measures</strong></td>
<td><strong>1°</strong>&lt;br&gt;Mean of change from baseline in OSDI score at 6 and 12 months (Primary Trial)&lt;br&gt;Mean of change from 12 months in OSDI score at 18 and 24 months (Extension Study)</td>
</tr>
</tbody>
</table>
|                    | **2°**<br>Compliance with the study treatment protocol as measured by changes in blood levels of fatty acids and pill counts<br>≥ 10 point change in OSDI-decrease for Primary Trial, increase for Extension Study<br>Change in Signs of DED (conjunctival and corneal staining, TBUT,
Exploratory

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
CHAPTER REFERENCES


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 $\omega_3$ 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 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.

3.8 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.

3.9 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 anticoagulants 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.

3.10 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.

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 a Supplement 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.
CHAPTER REFERENCES


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 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 regime.

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. 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.

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 an electronic 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, redness 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 and will request an eligibility determination for the patient for the Extension Study. If the patient is not eligible, no further supplements will be dispensed 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 child-bearing potential must have a negative urine pregnancy test at this visit. 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.

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 brief form is completed to document the telephone calls and is entered into the
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 will provide the patient with unopened bottles of supplements for a 90 day supply. At the 6 month follow up, bottles for a 180 day supply will be provided. If the patient is enrolled in the Extension Study, a 180 day supply of the newly assigned supplements will be dispensed after the Month 12 and Month 18 visits. Clinic Coordinators will call the patients a week after the visit 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 a case report form. 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. 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 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 into 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.
## DREAM Primary Trial Schedule of Procedures

<table>
<thead>
<tr>
<th>Procedure</th>
<th>-2 wks SV</th>
<th>00</th>
<th>03</th>
<th>06</th>
<th>09</th>
<th>12</th>
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<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Medical History and Events</td>
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<td>X</td>
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<td>X</td>
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<td>X</td>
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<td>X²</td>
<td>X²</td>
<td></td>
<td></td>
<td></td>
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<tr>
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<td>X²</td>
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<td>X</td>
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<tr>
<td>Contrast Sensitivity</td>
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<td></td>
<td></td>
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<tr>
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<td>Collection of Unused Study Supplements</td>
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<td>X</td>
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<td>X</td>
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<tr>
<td>&quot;Check-In&quot; Telephone Call</td>
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<td>X</td>
<td>X</td>
<td>X</td>
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<td>Y</td>
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<td>Letter to Encourage Compliance (sent 1 month after each visit)</td>
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<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
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</tr>
</tbody>
</table>

**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
## DREAM Extension Study Schedule of Procedures

<table>
<thead>
<tr>
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<th>18</th>
<th>21</th>
<th>24</th>
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<td>Y</td>
<td>Y</td>
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<tr>
<td>Health Economics Questionnaires (SF-36, WPAI, Healthcare Use)</td>
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<td>Y</td>
<td>Y</td>
<td></td>
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</tr>
<tr>
<td>Medical History and Events</td>
<td></td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Concomitant Medication Query</td>
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<td>Y</td>
<td>Y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adverse Event Query</td>
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<td>Y</td>
<td>Y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tear Osmolarity</td>
<td></td>
<td>Y¹</td>
<td></td>
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</tr>
<tr>
<td>Keratograph Break-Up Time, Tear Meniscus Height, Redness, and Meibomian Gland Evaluation</td>
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<td>Y¹</td>
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<td>Y</td>
<td></td>
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<td>Contrast Sensitivity</td>
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<td>Y</td>
<td>Y</td>
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<td>Y¹</td>
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<td></td>
<td></td>
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<td>Y</td>
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<td></td>
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<td>Y</td>
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<tr>
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<tr>
<td>Meibomian Gland Examination</td>
<td></td>
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<td>Y</td>
</tr>
<tr>
<td>Lissamine green staining</td>
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<td>Y</td>
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<td>Y</td>
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<td>Y²</td>
<td></td>
</tr>
<tr>
<td>Letter to Encourage Compliance (sent 1 month after each visit)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Y</td>
</tr>
</tbody>
</table>

**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 any one of the following 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 a 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 IND Safety Report, 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 dated 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 reinstated
- 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 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 $\omega_3$ 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 meibomian gland secretion and lid status;
  - Change in signs assessed by keratography: tear break-up time, tear meniscus height, redness, meibography;
  - Change 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 $\omega_3$ 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 $\alpha$, and test-retest reliability, as measured by the intraclass correlation coefficient $\rho$, were high for both the total score and the score for each of the subscales, as seen in the table below.

<table>
<thead>
<tr>
<th>Reliability Measures</th>
<th>Cronbach $\alpha$ (95% Confidence Interval)</th>
<th>Test-retest $\rho$ (95% Confidence Interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Score</td>
<td>0.92 (0.89-0.94)</td>
<td>0.82 (0.73-0.88)</td>
</tr>
<tr>
<td>Subscale scores</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vision-related function</td>
<td>0.88 (0.84-0.92)</td>
<td>0.70 (0.56-0.80)</td>
</tr>
<tr>
<td>Ocular symptoms</td>
<td>0.92 (0.89-0.94)</td>
<td>0.74 (0.62-0.83)</td>
</tr>
<tr>
<td>Environmental triggers</td>
<td>0.78 (0.71-0.84)</td>
<td>0.81 (0.71-0.87)</td>
</tr>
</tbody>
</table>
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. When an alternative approach to classifying patients as having dry eye disease, a composite score based on results of grading of signs and specific tests and the patient’s global perception of symptoms, was used the threshold score that maximized the sum of sensitivity (80%) and specificity (79%) was a total score of 6. 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 at 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 for 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.

<table>
<thead>
<tr>
<th>Change in OSDI Scores</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patient Assessment</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No change</td>
<td>92</td>
<td>-0.4</td>
<td>12.6</td>
</tr>
<tr>
<td>Slight change</td>
<td>66</td>
<td>-6.0</td>
<td>16.8</td>
</tr>
<tr>
<td>Much change</td>
<td>37</td>
<td>-19.4</td>
<td>23.2</td>
</tr>
<tr>
<td>Very much change</td>
<td>11</td>
<td>-24.4</td>
<td>25.4</td>
</tr>
<tr>
<td><strong>Clinician Assessment</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No change</td>
<td>89</td>
<td>-1.6</td>
<td>14.6</td>
</tr>
<tr>
<td>Slight change</td>
<td>94</td>
<td>-3.5</td>
<td>21.3</td>
</tr>
<tr>
<td>Much change</td>
<td>27</td>
<td>-12.9</td>
<td>20.1</td>
</tr>
</tbody>
</table>

* 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

<table>
<thead>
<tr>
<th></th>
<th>OSDI</th>
<th>BODI</th>
<th>IDEEL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reliability</td>
<td>0.88</td>
<td>0.94</td>
<td>0.92</td>
</tr>
<tr>
<td>Cronbach’s α</td>
<td>0.91</td>
<td>0.95</td>
<td>0.93</td>
</tr>
<tr>
<td>Category Function</td>
<td>Good</td>
<td>Poor</td>
<td>Good</td>
</tr>
<tr>
<td>Dimensionality Component (&lt;2 is ideal, lower is better)</td>
<td>2.17</td>
<td>2.36</td>
<td>2.37</td>
</tr>
<tr>
<td>Targeting</td>
<td>-0.62</td>
<td>-1.51</td>
<td>0.20</td>
</tr>
</tbody>
</table>

BODI had poor category function, meaning that the 11 categories for response for each question had overlapped 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.

<table>
<thead>
<tr>
<th>Patient Assessment</th>
<th>N</th>
<th>OSDI</th>
<th>BODI</th>
<th>IDEEL</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;4</td>
<td>64</td>
<td>21</td>
<td>11</td>
<td>34</td>
</tr>
<tr>
<td>4-7</td>
<td>133</td>
<td>39</td>
<td>30</td>
<td>54</td>
</tr>
<tr>
<td>&gt;7</td>
<td>61</td>
<td>60</td>
<td>53</td>
<td>73</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Clinician Assessment</th>
<th>N</th>
<th>OSDI</th>
<th>BODI</th>
<th>IDEEL</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;4</td>
<td>82</td>
<td>33</td>
<td>27</td>
<td>49</td>
</tr>
<tr>
<td>4-6</td>
<td>107</td>
<td>40</td>
<td>30</td>
<td>55</td>
</tr>
<tr>
<td>&gt;6</td>
<td>69</td>
<td>46</td>
<td>38</td>
<td>57</td>
</tr>
</tbody>
</table>

### Responsiveness to change

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 higher 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 primary outcome variable is the average of the changes from baseline in OSDI score at 6 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 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. [Note: Effects of \( \omega_3 \) 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 difference in mean change from baseline between the 2 groups would not be biased but the mean changes may be affected by seasonal effects.

- If the variance of the distribution of change in OSDI score is the same at both 12 and 6 months, then the gain in power from averaging is offset by the loss in power from reducing the size of the treatment effect when the 6-month treatment effect is 41.8% of the 12-month effect. In other words, averaging provides better power to detect an overall difference as long as the difference at 6 months is at least 42% of the difference at 12 months.

- 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 \( \omega_3 \) 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 \( \omega_3 \) 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. Calculations were performed using PS software (Dupont, 1990).

#### 6.3.1 Assumptions and calculation of the sample size for the Primary Clinical Trial

Several assumptions must be made in order to calculate sample size:
• **The statistical test used to compare the two treatment groups is a two-sided, t-test of equality of means.**

• **Type I (α) error rate of 0.05.**

• **Statistical power of 89%.** 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 standard deviation of the distribution of change in OSDI score is 25, both at 6 months and at 12 months.** Based on longitudinal data from the RESTORE registry and the DREAM feasibility study, the standard deviation of the distribution of change in OSDI score between visits may be assumed to be 20 to 25. The higher estimate was chosen to decrease the risk of the study having statistical power below the desired level.

• **The mean change from baseline in OSDI score is 6 points different between treatment groups at 12 months and 4.5 at 6 months.** 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. 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. The mean OSDI score in both treatment groups may decrease; however, if ω3 supplementation has an effect, the mean decrease in score should be greater than in the placebo group. The average of the treatment group differences in means at 6 and 12 months is 5.25. The detectable effect size of 5.25/25 = .21 is small; however, a small effect size for a relatively inexpensive treatment with few expected side-effects is important to identify.

• **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. Although loss to follow-up has been higher in other clinical trials of DED at 6 months, many of the studies did not follow non-compliant patients or those who stopped taking medications because of adverse events. 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 allocation ratio provides higher power for the
Extension Study with only 67 patients (13%) more than with equal allocation of 256 to each treatment group.

The table below shows the sample size required for analysis for different combinations of treatment effect and standard deviation of the distribution of change in OSDI score.

<table>
<thead>
<tr>
<th>Difference in Change from Baseline</th>
<th>Power Analysis: 579 Enrolled, 519 Analyzed</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 Mo.</td>
<td>% at 6 Mo.</td>
</tr>
<tr>
<td>5</td>
<td>1.00</td>
</tr>
<tr>
<td>5</td>
<td>0.75</td>
</tr>
<tr>
<td>5</td>
<td>0.50</td>
</tr>
<tr>
<td>5</td>
<td>0.42</td>
</tr>
<tr>
<td>5</td>
<td>1.00</td>
</tr>
<tr>
<td>5</td>
<td>0.75</td>
</tr>
<tr>
<td>5</td>
<td>0.50</td>
</tr>
<tr>
<td>5</td>
<td>0.42</td>
</tr>
<tr>
<td>6</td>
<td>1.00</td>
</tr>
<tr>
<td>6</td>
<td>0.75</td>
</tr>
<tr>
<td>6</td>
<td>0.50</td>
</tr>
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<td>6</td>
<td>0.42</td>
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<tr>
<td>6</td>
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<tr>
<td>6</td>
<td>0.75</td>
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<tr>
<td>6</td>
<td>0.50</td>
</tr>
<tr>
<td>6</td>
<td>0.42</td>
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</table>

### 6.3.2 Power considerations for the Extension Study

Only patients assigned to active supplements in the Primary Trial (N=386) who complete the 12-month visit (90%; N=347) are eligible for the Extension Study. A high percentage of patients (45%) are assumed to refuse continuation in the Extension Study because of no perceived benefit from the supplements, unwillingness to be randomized to placebo supplements, unwillingness to commit to 2 more additional visits, or unwillingness to continue taking as many softgels per day as required by the Study. Applying a 45% refusal rate, yields 190 patients enrolling in the Extension Study. If a 10% loss to follow-up rate is applied, 172 patients will be available for analysis.
If similar assumptions are made for the Extension Study and the 12-month OSDI score is the new baseline for the Extension Study, there are substantial gains on power from using the average of the 18 and 24 month OSDI scores. If the true mean difference between groups at 24 months is 8 points, the standard deviation of changes is 20, and 75% of the 24-month treatment effect is present at 18 months and alpha error is 0.05, the Extension Study has 90% power to detect a difference between treatment groups. It is reasonable to assume a lower standard deviation than for the Primary Trial because a more homogeneous group of patients (all willing to continue supplements presumably because of perceived benefit) is expected.

<table>
<thead>
<tr>
<th>24 Mo.</th>
<th>% at 18 Mo.</th>
<th>18 Mo. effect</th>
<th>Average of 18 &amp; 24 Mo.</th>
<th>SD of Change</th>
<th>24 M Only</th>
<th>Average of 18 &amp; 24Mo.</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>1.00</td>
<td>6.00</td>
<td>6.000</td>
<td>20</td>
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<td>0.79</td>
</tr>
<tr>
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<td>4.50</td>
<td>5.250</td>
<td>20</td>
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<td>0.50</td>
<td>0.55</td>
</tr>
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<td>2.51</td>
<td>4.254</td>
<td>20</td>
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<td>0.50</td>
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</tr>
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<td>0.49</td>
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<tr>
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<tr>
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<td>5.67</td>
<td>25</td>
<td>0.55</td>
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</tr>
</tbody>
</table>
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-squared tests 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.

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. 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. A t-test for independent means with a robust “sandwich” variance estimator will be used to determine the significance level associated with the comparison. (Liang, 1986) 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 with robust variance estimation. 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.

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 (Liang, 1986). OSDI scores will be used from all visits when the OSDI was administered. Both the relation of OSDI with follow-up time and the influence of possibly prognostic factors will be evaluated using these models. Subgroup analyses will be performed to assess the consistency of the treatment effect across clinics and the levels of important baseline covariates such as OSDI score, EPA blood level, DHA blood level, age, and gender.

### 6.4.4 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.

#### 6.4.4.1 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.

#### 6.4.4.2 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 one year after randomization will be compared between the group of patients assigned active supplement and the group assigned placebo. A change in score of 10 has been 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).

### 6.4.4.3 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 all eyes because the initially unaffected fellow eye may have changes in signs. Secondary analyses will include only those eyes meeting the eligibility criteria at baseline.

### 6.4.4.4 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.

### 6.4.4.5 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.

### 6.4.4.6 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.

**6.4.4.7 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 $\omega_3$ 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.

**6.4.4.8 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 $\omega_3$ 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.

**6.4.4.9 Cost and Incremental Cost-effectiveness of Using $\omega_3$ 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 $\omega_3$ 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 Extension Study. If symptoms remain the same for the patients assigned to placebo and active supplements, the gains in effectiveness associated with $\omega_3$ 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 $\omega_3$.

**6.4.4.9.1 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.

6.4.4.9.2 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.

6.4.4.9.3 Incremental Cost Effectiveness of ω3
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; Rajagopalan, 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.

6.4.5 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.

6.4.5.1 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).

6.4.5.2 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.

6.4.5.3 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 merely 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).

6.4.6 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.

6.4.7 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 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. Characteristic 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).

6.4.8 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 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.

6.4.9 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 S-Plus (1700 Westlake Avenue North, Suite 500, Seattle, WA 98109-3044) 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

<table>
<thead>
<tr>
<th>Feature</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Objective</strong></td>
<td>Evaluate the effectiveness and safety of supplementation with ω3 fatty acids in relieving the symptoms of moderate to severe dry eye disease (DED)</td>
</tr>
</tbody>
</table>
| **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 |
| **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: 579 total; 386 Active supplements, 193 Placebo  
Extension Study: 190; 95 per group                                                                                                                                                                       |
| **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                                                                 |
6.6 REFERENCES:

Allergan, Inc. Restasis Product Information. 


Yoon KC, Im SK, Kim HG, You IC. Usefulness of double vital staining with 1% fluorescein and 1% lissamine green in patients with dry eye syndrome. *Cornea*. 2011 Sep;30:972-6.
CHAPTER 7
TESTING PROTOCOLS

7.1 Standardization of exam:
To decrease variation in exam findings, the following actions should be taken:

- Ask subjects to return within two hours ( +/- 2 hours of ) of the original baseline visit time for all other visits, since there is likely some diurnal variation in DED signs and symptoms;
- Ask subjects to refrain from using topical artificial tears for at least 2 hours prior to exam;
- Use standardized approach to lid evaluation with use of the Meibomian Gland Evaluator to determine if there is abnormality of lid secretions, since device allows for giving a standard pressure to the lower lid and eliminates the variable pressure if a Q-tip is used or pressure with the examiners finger on the lid.
- Use the standardized photos of corneal staining, lissamine staining and meibomian gland secretion to help standardize the grading of findings at the slit lamp examination.

7.2 Ocular Surface Disease Index (OSDI) Questionnaire and BODI (Brief Ocular Discomfort Inventory)
The clinic coordinator will give these two questionnaires to the patient at each scheduled visit and instruct the patient to complete the OSDI first and the BODI second. The patient should use a pen to complete the form. If patients ask for help in completing the form, the coordinator may read the instructions that are on the questionnaire to the patient and note that some of the questions have a “Not applicable” option. Patients must choose the response for each item without input from the coordinator or people accompanying the patient. After the patient has filled out the questionnaires, the coordinator reviews each questionnaire to be sure each item has been completed. If some items are blank, the coordinator should ask the patient to complete the items. Patients should be asked to date and initial changes that they make.

7.3 Health Economics Questionnaires: SF-36, Work Productivity and Activity Impairment Questionnaire (WPAI), & Healthcare Utilization Form
The clinic coordinator will give these questionnaires to the patient at the baseline visit and all other follow-up visits except at Month 03. The patient should use a pen to complete the form. If patients ask for help in completing the form, the coordinator may read the instructions that are on the questionnaire to the patient. For the WPAI and SF-36, patients must choose the response for each item without input from the coordinator or people accompanying the patient. For the Healthcare Utilization Form, the patient may ask people accompanying the patient for assistance in remembering their healthcare utilization. After the patient has filled out the questionnaires, the coordinator reviews each questionnaire to be sure that the skip patterns have been followed and all required items have a response. If the forms have errors in completion, the coordinator should ask the patient to revise the form. Patients should be asked to date and initial changes that they make.
7.4 MMP-9
MMP-9 testing will be performed at the SV and 03 months visits. Patients who are allergic to cornstarch or Dacron are excluded from testing. In addition, patients who have had an ocular infection within the last 90 days are excluded from testing.

The test must be done before any of the diagnostic drops are used. Perform test on both eyes following the detailed instructions in Appendix 7-1.

7.5 Manifest Refraction
Manifest refraction will be performed at the baseline visit (00) and at later visits if visual acuity changes by 10 or more letters. A DREAM Clinician or Technician certified for this task may perform the refraction. The local refraction technique may be used.

7.6 Best Corrected Visual Acuity (BCVA) & ETDRS
BCVA should be measured at 00, 03, 06, 12 (18 and 24) month visits. The refractive correction from the most recent manifest refraction should be used. A DREAM Clinician or Technician certified for this task may perform visual acuity testing. The procedure is summarized in Appendix 7-2.

- ETDRS Chart 1 is used for the right eye; ETDRS Chart 2 is used for the left eye.
- The distance from the patient’s eyes to the chart should be 3.2 meters (10 feet 6 inches).
- Use a light meter to measure the light hitting the chart. Hold the meter with the round sensor disc facing toward you and hold it directly in front of the center of the chart. Be careful not to have your shadow falling onto the area being measured. The level should be 189-377 Lux.
- The subject should be positioned according to the elevation of the chart (seated or standing), so the chart is at a comfortable viewing angle.
- Occlude the eye not being tested.
- The subject should attempt to read each letter. Maximum effort should be made to identify each letter on the chart. Encourage subject to guess.
- The subject is asked to read each letter on each line, starting with the top line from left to right.
- Circle letters read correctly on the form.
- When the subjects say they cannot read a letter, they should be encouraged to guess. If the subjects identify a letter as one of two or more letters, they should be asked to choose one letter and, if necessary, to guess. When a subject misses all 5 letters on a line or it becomes evident that no further meaningful readings can be made (usually with the participant being unable to guess at a letter, despite urgings to read or guess), the examiner should stop the test for that eye.
- Write the number of letters read correctly on each line onto the form. Sum the numbers (a calculator is suggested). If the total score has changed by 10 or more letters for an eye from the previous visit, perform a manifest refraction and redo the visual acuity testing using a new form.
7.7 Mars Letter Contrast Sensitivity Test

The Mars Letter Contrast Sensitivity Test should be administered according to the directions below (summarized in Appendix 7-3) at 00, 06, 12 (18 and 24) month visits. A DREAM Clinician or Technician certified for this task may perform the contrast sensitivity testing. The instructions below are from the manufacturer.

Illumination: The chart should be illuminated uniformly. The lamp on the standard ophthalmic equipment stand will generally provide sufficient and sufficiently uniform illumination. It is essential that glare and visible reflections from the chart surface (e.g. of the illuminating lamp) be avoided as these may interfere with the measurements. Testing should not be conducted through any coatings, laminations, or coverings on the chart, even if these are transparent or translucent. Use a light meter to measure the light hitting the chart. Hold the meter with the round sensor disc facing toward you and hold it directly in front of the center of the chart. Be careful not to have your shadow falling onto the area being measured. The level should be 189-377 Lux.

Viewing distance and correction: The patient's viewing distance to the chart is by design 50 cm (20 inches), but may range from the standard near refraction distance of 40 cm (15.75 inches) to 59 cm (23 inches). Patients should wear their refractive correction used for ETDRS testing with an add of +2.00 D, and an occluder or patch on the untested eye. The test is quite tolerant of small refractive errors since the letters are large (20/480 equivalent at 50 cm). Testing, however, should be performed with the eyes undilated. For patients with very low visual acuity who cannot easily read the highest contrast letters, test distance may be shortened to 25 cm (increasing the add, if necessary, to +4.00 D); in this case care must be taken not to allow the patient's head to occlude the light source illuminating the chart.

Charts: Use Form 1 for the right eye and Form 2 for the left eye.

Instructions to the patient: Ask the patient to read the letters from left to right across each line of the chart. If the patient responds with a letter other than C, D, H, K, N, O, R, S, V, or Z, do not score the response as incorrect. Instead, inform the patient of the restricted letter set, and ask for another response. This is in order to support the assumption that the probability of a guess is 1/10. Encourage the patient to guess even when the letters seem too faint.

Recording responses and scoring: On the score sheet, mark in the grid corresponding to the chart form used, a 1 for a correct response and a 0 for an incorrect response. Terminate testing only when the patient makes two consecutive errors or reaches the end of the chart. Do not terminate the test because the patient has given up and has stopped responding. If this happens, encourage the patient to guess, and score the guesses as ordinary responses. This will help to insure that the score is based on what the patient can see and not on what the patient believes he or she can see.

7.8 Tear Osmolarity

Centers that have the TearLab Osmometer will perform tears osmolarity at the 00, 06, 12 (18 and 24) month visits. A DREAM Clinician or Technician certified for this task may perform tear osmolarity.
• The TearLab Osmometer consists of:
  o two collection pens (can use either or both)
  o a reader into which the collection pens are docked
  o single-use disposable test cards (one per eye)
  o two electronic check cards (reusable)

• Ensure that the supplies are not influenced by outside heating or cooling and are stored at controlled room temperature (20-25 °C / 68-77°F)

• The TearLab osmolarity test measures tear osmolarity by determining the electrical impedance of an electrical current passed through an in-vitro tear sample.

• Tear osmolarity should be done before any eye drops are placed in the eye.

7.8.1 Tear Osmolarity Calibrations

Osmolarity Control Solution and Test Cards
• Upon receipt of each new shipment of test cards, calibrate each Pen with a High control solution ampoule and a test card, and record results on the TearLab Quality Control Log.
  
  Note: If the test results are not within the expected range listed in the Control Solution instructions, do NOT test study subjects and contact the Coordinating Center immediately.

• Each control solution ampoule should be used only once per calibration session and discarded.

• Four days before you run out of High control solution ampoules, fax the TearLab Quality Control Log to TearLab per instructions on the log. This will instruct TearLab to send your site new High control solutions.

Calibrations Before Measurements on DREAM Patients
• Perform once on each day a patient will be seen;

• Following the TearLab instruction guide, test each Pen with the Electronic Check Card and record results on the TearLab Quality Control Log and on the Tear Osmolarity Assessment Case Report Form;

• Following the TearLab instructions guide, calibrate each Pen with a High control solution ampoule and a test card and record results on the TearLab Quality Control Log.
  
  Note: If any test result is not within the expected range listed in the TearLab Quality Control Log, do NOT test study subjects and contact the Coordinating Center immediately.

7.8.2 Osmolarity Tear Testing
• Tear Testing must precede all other diagnostic examinations, testing and staining, and before any eye drops are placed in the eye;

• Subject must refrain from administrating any tear supplements within two hours prior to the tear osmolarity test;

• Following the TearLab Quick Reference Guide, test the right eye and the left eye of the study subject. Record all results on the form.
7.9 Tear Collection for Cytokines

This will be performed at the 0, 6, 12 (18 and 24) month visits ONLY at the sites that have a -80°C freezer, liquid nitrogen or dry ice available for temporary storage of samples until they are shipped to the Biomarker Laboratory at Mount Sinai. A DREAM Clinician or Technician certified for this task may perform the tear collection. Detailed instructions for collection, storage, and shipping can be located in Appendix 7-4.

- Remember to fill out a Tear Cytokine Registration Form for EACH collection.
- Remember to update Tear Cytokine Site Log after each collection.

Note: Samples must be shipped to the Biomarker Laboratory at Icahn School of Medicine at Mount Sinai within 6 months of collection (2 times/year). For detailed shipping instructions, see Appendix 7-4.

7.10 Keratograph:

At the sites that have the Keratograph machine available, examinations will be performed at visits: 00, 06, 12 (18 and 24). The test should be done before any drops are used. The right eye must be examined first. For detailed instructions on each procedure, please refer to Appendix 7-5. A DREAM Clinician or Technician certified for this task may perform keratography.

7.10.1 Evaluation of non-invasive tear film break-up time:
The non-invasive tear film break-up time (NIK BUT) measures tear film stability. The NIKBUT is automatically measured within seconds, without fluorescein application. The data field will show tear film break-up time (NIKBut) in seconds. The First Tear Break Up time should be recorded on the form.

7.10.2 Tear Meniscus Height:
Tear meniscus will be evaluated along the eyelid with the built-in ruler. Different magnification levels facilitate measurement. Tear meniscus height should be measured at three points: directly below the pupil, below the 5 o’clock position of the cornea, and below the 7 o’clock position of the cornea. All three values should be recorded in the form.

7.10.3 Eye Image
This is an objective method to classify the bulbar and limbal redness automatically by using the Eye-Image function. The Eye-Image function detects vessels in the conjunctiva and evaluates the degree of redness. Redness images will be submitted to the Scheie Image Reading Center and then sent to Oculus USA for analysis (see Appendix 7-5). The redness results will not be available on the imaging screen for recording on the Keratography Assessment form as originally planned. (Leave the Bulbar Redness question on the case report form blank).

7.10.4 Meibography of the upper and lower eyelid:
The upper and lower eyelid will be inverted to assess the Meibomian glands. Morphological changes in the gland tissue will be visible using the Meibo-Scan. The resulting image can be evaluated by the person taking the picture and the grade is recorded on the form. An image of the grading scale has been provided as a reference. Meibography results will be downloaded and sent to the Coordinating Center.

The results of each test will be saved in the Keratograph for future use.
7.11 Slit Lamp Examination

All slit lamp examination findings should be evaluated by certified Clinicians (7.11-7.16). They will be performed at every visit, right eye examined first and then the left eye. Record all results on the Ocular Evaluation form.

- External examination and biomicroscopy should be performed using a slit lamp
  - Magnification should be consistent with standard clinical practice except for the Meibomian Gland Evaluation, which is described below.
- Grading should be done as follows:

**Conjunctiva - Erythema (bulbar)**
- 0= None (normal)
- 1= Mild (a flush reddish color)
- 2= Moderate (more prominent red color)
- 3= Severe (definite redness)

**Conjunctiva - Edema (bulbar)**
- 0= None (normal)
- 1= Mild (slight localized swelling)
- 2= Moderate (moderate/medium localized swelling or mild diffuse swelling)
- 3= Severe (severe diffuse swelling)
- 4= Very Severe (very prominent/protruding diffuse swelling)

**Anterior Chamber Cells (Slit beam - 0.3 mm wide, 1.0 mm long)**
- Grade 0= (<1 cells in the field)
- Grade 0.5= (1-5 cells in the field)
- Grade 1+= (6-15 cells in the field)
- Grade 2+= (16-25 cells in the field)
- Grade 3+= (26-50 cells in the field)
- Grade 4+= (>50 cells in the field)

**Anterior Chamber Flare Slit beam - 0.3 mm wide, 1.0 mm long)**
- Grade 0 = (None)
- Grade 1+= (Faint)
- Grade 2+= (Moderate: iris & lens, details clear)
- Grade 3+= (Marked: iris & lens, details hazy)
- Grade 4+= (Intense: fibrin or plastic, aqueous)

**Tear Film Debris**
- 0= None (absent)
- 1= Mild (present in inferior tear meniscus)
- 2= Moderate (present in inferior tear meniscus and in tear film overlying cornea)
- 3= Severe (present in inferior tear meniscus and in tear film overlying cornea. Presence of mucus strands in inferior fornix or on bulbar conjunctiva)
**Lid Margin Debris (evaluate upper and lower eyelid)**
- Normal 0 collarettes
- Mild 1-5 collarettes
- Moderate 6-20 collarettes
- Severe 21-40 collarettes, 1-2 clumps. Very Severe 40+ collarettes, >3 clumps

**Eyelid margin - Erythema (redness & neovascularization of lid margin upper and lower eyelid)**
- Normal
- Mild (redness localized to a small region of the lid margin OR skin)
- Moderate (redness of most or all lid margin OR skin)
- Severe (redness of most or all lid margin AND skin)
- Very Severe (marked diffuse redness of both lid margin AND skin)

**Lid foam (soapy look along the lower eyelid margin)**
- 0 = No
- 1 = Yes

**Lashes**
- 0 = Normal
- 1 = Abnormal (specify)

**Eye lid skin - Edema**
- Normal
- Mild (localized to a small region of the lid)
- Moderate (diffuse, most or all lid but not prominent/protruding)
- Severe (diffuse, most or all lid AND prominent/protruding)
- Very Severe (diffuse AND prominent/protruding AND reversion of the lid)

**Chalazion present on any eyelid?**
- 0 = No
- 1 = Yes

**Facial skin rosacea:**
- 0 = No
- 1 = Yes

### 7.12 Fluorescein Instillation
Fluorescein must be instilled in each eye separately, **starting with the right eye**. Use 2% fluorescein (preservative free), a sterile petri dish, and an Eppendorf pipette to instill drops by placing a drop of the dye into the petri dish and then pipetting the drop with the Eppendorf pipette. Do not instill drops in the left eye until the TBUT, corneal staining, MG evaluation and conjunctival staining with lissamine green for the right eye has been completed.
- The fluorescein bottle should never touch the eye or come into contact with the patient. If the bottle does become contaminated, discard the bottle after the examination and order a new bottle immediately.

7.13. Tear Break-Up Time (TBUT)
This should be done at every visit.

- Use the Eppendorf micropipette and tip to draw up 5μl of fluorescein 2% from a sterile petri dish and place in the inferior cul de sac of the eye while avoiding touching the tip to the eye, eyelid or skin;

- **Wait 30 seconds** (have patient blink several times) and then examine tear film on the slit lamp. Use a broad beam cobalt blue illumination on the cornea and yellow barrier filter hand held in front of the objective;

- Using a stop watch, measure the time between the last blink and the appearance of the first randomly distributed discontinuity in the fluorescein stained tear film.

- Record results on the ocular assessment form.

**Note:** The TBUT should be measured *three times* during the first minute, beginning 30 seconds after instillation.

7.14 Corneal Fluorescein Staining
This should be done at every visit:

- Corneal staining should be graded using the cobalt blue filter of the slit lamp;

- Grading is performed **approximately 2.5 minutes** after fluorescein instillation, ensuring the dye does not diffuse into the stroma, blurring the discrete margins of any staining defects.

- Use a broad beam cobalt blue illumination on the eye and yellow barrier filter hand held in front of the objective to observe corneal staining.

- Score (0-3) each of the 5 areas of the cornea as shown below

- Record results on the ocular assessment form.
7.15 **Meibomian Glands** (evaluation of the Meibomian gland openings of the lower eyelid):

To standardize the exam, the Meibomian Gland Evaluation (MGE) will be utilized; a handheld instrument used to standardize the force applied to the lower lid to evaluate the meibomian gland secretions:

- The MGE is used under a slit lamp biomicroscope, with a 10 to 16x magnification;
- Have the patient look up and away, and then place the MGE on the skin directly below the lash of the lower eyelid;
- When it touches the skin, rotate the shaft of the instrument down about 15 to 45 degrees so the face is tangential to the eyeball;
- Press the shaft of the instrument mid-way (approximately 3mm) so there is a force over the glands and then adjust the instrument so the flat surface of the lower eyelid margin rolls slightly outward to see the gland orifices;
- Wipe the gland orifices along the eyelid margin with dry cotton immediately after putting pressure while the instrument is in place and keep pressure;
- The MGE must be held in place for a minimum of 10 and a maximum of 15 seconds in central region of the lower lid;
- Grade the secretion and record the grading for the glands in the CRF;
- Disinfect the device with alcohol wipe after each use.
- Record results on the ocular assessment form.

**Meibomian gland evaluation - USE MGE - Plugging** (evaluate 5 of the lower eyelid Meibomian gland openings in the central mid-portion of the lower eyelid):

- None plugged
- Mild (1-2 glands plugged)
- Moderate (3-4 glands plugged)
- Severe (All 5 are plugged)

**Lid secretion Grading (USE MGE):**

- Clear liquid oil
- Mild haze/cloudy liquid
- Paste (toothpaste-consistency)
- Obstructed (no secretion, including capped orifices)

7.16 **Lissamine Green Staining of the Interpalpebral Conjunctiva**

This should be done at every visit after evaluating TBUT, corneal staining and MG evaluation:

- Without flushing the eye to remove any fluorescein, place one drop of lissamine into a sterile petri dish. Take 5 μL of 1 % lissamine green from the petri dish using an Eppendorf micropipette and tips and place the dye into the lower conjunctival sac;
• Grade the lissamine green staining after 1-2 minutes have elapsed following instillation;

• Using white light of moderate intensity, grade the nasal-bulbar and temporal-bulbar conjunctiva for staining using the scale provided on the form.

• Score (0-3) each of the temporal and nasal areas of conjunctiva as shown below

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<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No coloration</td>
</tr>
<tr>
<td>1</td>
<td>Some punctations</td>
</tr>
<tr>
<td>2</td>
<td>Well defined punctations</td>
</tr>
<tr>
<td>3</td>
<td>Many punctations</td>
</tr>
</tbody>
</table>
```

• Record results on the ocular assessment form.

• The fluorescein bottle should never touch the eye or come into contact with the patient. If the bottle does become contaminated, discard the bottle after the examination and order a new bottle immediately.

7.17 **Tonometry for measurement of intraocular pressure (IOP)**

This should be done at SV, 0, 3, 6, 12 (18 and 24) month visits. The test can be done by a DREAM Clinician or Technician certified for this task.

• IOP should be assessed using Goldman applanation tonometry or TonoPen after administration of topical anesthetic;

• Record results on the ocular assessment form.

**Note:** The same type of equipment must be used throughout the course of the study for each subject.

7.18 **Schirmer’s Test**

The test can be done by a DREAM Clinician or Technician certified for this task. This should be done at every visit using an exam room:

• Free of draft;

• Moderate lighting;

• No glare in the field of vision

The test should be performed bilaterally, using Schirmer’s test strips:

• Use topical anesthesia: one drop in each eye

• Wait 5 minutes after putting in the drops (if anesthetic is administered before IOP, 5 min from that time)
• Fold Schirmer’s test strips back at indentation;
• Subject is instructed to look straight ahead;
• Test strips are then hung onto the lower conjunctival sac in the temporal one-third of each lid, avoiding contact with the corneal surface;
• Test strips should remain in place for five minutes (measured with a stop watch), during which time the subject should keep eyelids closed;
• After five minutes the Schirmer’s test strips are removed
• The distance from the fold to where each strip is wetted is recorded in millimeters. Record results on the ocular assessment form.

7.19 Impression Cytology
The test can be done by a DREAM Clinician or Technician certified for this task. This should be done at the 0, 6, 12 (18 and 24) month visits. Wait 20 minutes after any conjunctival staining procedures before performing conjunctival cell collection. Two samples per eye will be collected. Detailed instructions for materials, storage, collection and shipping can be located in Appendix 7-6.

• Remember to fill out the Impression Cytology Registration Form.
• Remember to update Impression Cytology Site Log.
• Record results on the ocular assessment form.

Note:
• Do not touch the filter membrane to anything but the conjunctiva.
• Avoid personal contact with the solution in the Impression Cytology tube (contains paraformaldehyde).
• Store samples in the refrigerator (4-8°C) until shipping. Do NOT freeze samples. Maintain the Impression Cytology Sample Storage Temperature Log Monday through Friday.
• Samples must be sent to the Biomarker Lab at Mount Sinai within two weeks of collection (2 times/month). For detailed shipping Instructions, see Appendix 7-6.

7.20 Blood Collection for Fatty Acids Testing
The procedure must be done by personnel trained in phlebotomy.

• Use an EDTA-Lavender top vacutainer tube such as Becton Dickenson Catalog #DB367863. This tube will be provided in the blood specimen kit;
• Collect 3-5 ml of blood and invert the tube 6-10 times;
• Each tube should be labeled with pre-printed labels provided by the Coordinating Center:
  o Subject ID# and alphabetic ID code
  o Visit label, such as 00, 06, 12, etc
  o Date the blood was drawn
• Complete a DREAM Blood Sample Requisition form to be included with the shipped blood;

• Blood samples will preferably be shipped to the Kennedy Krieger Institute within 24 hours of collection, though samples are viable for up to 48 hours. (Appendix 7-7):
  - Samples must be collected only Monday-Thursday*
  - The whole EDTA blood can remain at room temperature until it is packaged for shipping

*Please call the laboratory if, because of extraordinary circumstances, a blood sample is to be delivered to the laboratory on a Saturday.

7.21 Blood Collection for Inflammatory Markers Testing (Sjö Testing)
The procedure must be done by personnel trained in phlebotomy.

• Affix to the red marbled phlebotomy tube the pre-printed Sjö™ testing label supplied by the Coordinating Center. Enter the date of the visit on the label in the space provided.

• Have personnel trained in phlebotomy collect 4-10 ml of blood (approximately ¾ of the serum separator tube).

• Gently invert the serum separator tube 5-6 times to activate the clot activator and blood.

• Prepare tube for shipping according to packaging and shipping instructions in the Sjö™ Blood Vial Collection Kit. If specimen cannot be sent on day of collection, please refrigerate and ship as soon as possible (within 5 days).

• Place tube in specimen collection bag.

• Place Test Requisition Form in the outside pocket of specimen bag.

• Fold and roll specimen bag and contents into white mailer box and place into Orange FedEx Clinical Pak.

• Call FedEx for pick up at 1-800-463-3339. Write FedEx tracking number in patient’s chart.
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MMP-9 Testing Instructions - To Be Completed for Each Eye

- Before performing this test the first time review the 4 minute instructional video located at http://www.rpsdetectors.com/en/products/inflammadry/practice-tools/how-to-use-inflammadry/.

- Check that you have kit contents
  - A foil pouch containing a sterile sample collector, a foil pouch containing a test cassette, and a buffer vial

- Collect the sample
  - Open the pouch with the sample collector; do not touch the sampling fleece
  - Instruct patient to look up
  - Gently expose the **palpebral conjunctiva** (lid, NOT eye)
  - Dab sampling fleece in along the conjunctiva
  - Release the lid after every 2-3 dabs & allow the patient to blink
  - After completing a **minimum of 6-8 dabs**, allow sampling fleece to rest on conjunctiva for an additional 5 seconds to ensure saturation
  - Inspect sampling fleece – when adequate tear sample is collected, fleece will glisten. If not glistening, repeat.

- Assemble the test
  - Open the pouch with the test cassette
  - Snap the test sample into the cassette
  - **Press firmly** when assembling the test to allow specimen transfer. A double click means the test is properly assembled.

- Run the test
  - Immerse the absorbent tip into the buffer solution for a **minimum of 20 secs** until a purple fluid wave is observed moving across the cassette’s result window.
  - Replace the protective cap and lay the test flat on a horizontal surface.

- Read the test
  - Wait a full **10 minutes** before reading result. Must be read within **1 hour**.
  - Read any form of a red line, whether faint, broken, or shadow, as a positive test result.
DREAM Visual Acuity Testing

- Use refractive correction from last manifest refraction
- ETDRS Charts at 3.2 m:
  - Right Eye = Chart 1 
  - Left Eye = Chart 2
- Measure the light hitting the center of the chart (189-377 lux). Be careful of your shadow.
- Occlude the eye not being tested.
- Tell patient to read each letter, starting from top line, left to right.
- Encourage patient to guess when unsure of letter.
- Circle letters read correctly on the form.
- Stop testing when patient subject
  - Misses all 5 letters on a line
  - OR
    - It becomes evident that no further meaningful readings can be made (usually with the participant being unable to guess at a letter, despite urgings to read or guess),
- Record the number of letters read correctly on each line.
- Sum the numbers (a calculator is suggested).
- If the total score has changed by 10 or more letters for an eye, perform a manifest refraction and redo the visual acuity testing using a new form.
DREAM Mars Contrast Sensitivity Testing

- Use refractive correction from last manifest refraction with +2.00 add

- MARS Charts at 50cm (20 inches):
  - Right Eye = Form 1
  - Left Eye = Form 2

- Measure the light hitting the center of the chart (189-377 lux). Be careful of your shadow.

- Occlude the eye not being tested.

- Tell patient to read each letter, starting from top line, left to right.

- Encourage patient to guess when unsure of letter.

- Place a “1” on the form for letters read correctly. Place a “0” on form for letters read incorrectly.

- Accept only letters C, D, H, K, N, O, R, S, V, or Z. Ask patient to try again if another letter is given.

- Stop testing when the patient misses 2 consecutive letters
Chapter 1: Materials for Tear Cytokine Collection and Storage at Site

- Tear Cytokine Registration Form (Picture 1);
- Drummond disposable 20 µL micro capillary tubes with rubber head squeezer (Picture 2);
- Pre-printed temperature and moisture resistant label w/ Patient ID (Picture 3);
- Moisture resistant fine-tip Sharpies (Picture 4);
- 1.5 ml Eppendorf collecting tube, single packed sterile and low retention with safe-lock (Picture 5);
- Scissors (Picture 6);
- Parafilm (Picture 6);
- Ice bucket w/ regular ice (Picture 7);
- Dry ice box w/ dry ice (Picture 7);
- Pre-labeled sample storage box (Picture 8);
- Ultra cold lab freezer (range: -80°C ± -3°C), or Dry Ice (-79°C), or Liquid Nitrogen (-196°C) (Picture 8);
- Gloves (optional);
- Scotch Tape;
- Tear Cytokine Site Log.
Chapter 2: Procedures for Tear Collection

1. Key points prior to start:
   - No anesthesia or eye drops before/after collection.
   - Arrange to collect tears +/- 2 hours of the baseline visit time.
   - To ensure non-stimulated tear collection, minimize any unnecessary contact between the capillary tube and patient’s conjunctiva.
   - Make sure all personnel have completed Certification forms.

2. Prepare a Tear Cytokine Registration Form.

3. Check labels and patient ID to ensure they match. Write the date with Sharpie on the assigned pre-printed label provided by Mount Sinai Study Chair.

4. Open an individually packed tear collecting tube (picture 5), close the lid, stick and wrap the pre-printed label around tube. Note: In ONE movement, seal the label so that the clear part of the label overlaps the QR code. Do NOT try to reseal to adjust alignment as doing so will damage the QR code.

5. Put the tube on ice.

6. Allow patient to seat comfortably on a chair without rolling wheels, open both eyes naturally;

7. **Begin with the right eye (OD).** Carefully lay a new disposable 20 µL micro capillary tube on the lower lid margin, hold still for approximately 5 min until a quarter of the capillary tube is filled with tears (approximately 4-5 µL). Advise patient to blink as gently as possible during the tear collecting process to minimize unnecessary contact with the capillary tube;

   a. *Tip:* Tear collection technician can lift the patient’s upper eye-lid wider with one hand if patient is blinking rapidly.

8. Insert the micro capillary tube with tears into the tear collecting tube, carefully attach a rubber-head squeezer to the end without tears, and gently expel to let all tears run down.
to the bottom with one squeeze. Failure to do so might result in tears being lost in the rubber squeezer.

9. Tightly close the attached lid, lock the lid with Safe-Lock bud, set back on ice;

10. Take another new 20 µL micro capillary tube, repeat the procedure in Step-7 on the left eye (OS);

11. Repeat Step-8, use the SAME tear collecting tube to pool tears from both eyes of the same patient;

12. Press hard to close the lid tight and make sure Safe-Lock bud is on the locked position;

13. Note: for sites storing tear samples in a liquid nitrogen tank, an additional clip is provided and should be slid onto the top of the lid to tighten the Safe-Lock.

14. Using one half of one square of parafilm, seal the lid of the tube so that it is wrapped a maximum of two times. Immediately set the tube back on ice.

15. Transfer sample to ultracold freezer, liquid nitrogen, or dry ice for storage within 30 minutes of collection.

**Chapter 3: Procedures for Tear Cytokine Storage at Site**

1. Within 30 minutes of collection, complete one of the following:

   - If a site has a -80°C freezer, put the properly labeled and sealed collecting tubes with tears to a pre-labeled storage box into the freezer for storage at the collection site (no more than 6 months);

   - If a site doesn’t have a -80°C freezer immediately available, but has a liquid nitrogen service, store the tear collecting tubes in liquid nitrogen tank until shipping;

   - If a site doesn’t have a -80°C freezer immediately available nor a liquid nitrogen service, the tear sample tubes can be kept inside the dry ice container, covered with enough dry
ice bullets (5 lbs can last for 5 days) and lid, and transfer to a nearby site with -80°C freezer as soon as possible;

2. Record all the sample/patient information with precise collecting date and times into Tear Registration Form

3. Update your confidential Tear Cytokine Site Log.

4. Ship samples to Biomarker Lab at Mount Sinai within 6 MONTHS of collection (typically sites will ship twice/year).
Chapter 4: Tear Cytokine Shipment to Biomarker Lab at MSSM

Part-1: Materials and Supplies Needed for Tear Sample Shipment:

1. International Air Transport Association (IATA)-certified INFECION-5000 Shipper kits, will be sent to you about TWO WEEKS before actual shipping date.

Specificities of INFECION-5000 Shipper kit:
1) A liquid-tight 0.5L polyethylene pressure vessel able to withstand up to 95 kPa pressure;
2) Temperature protection exceeding 60 hours with only 5.6 lb of dry ice;
3) Complete and ready-to-use packaging system with all required labels and markings
4) Compact size and shipping weight for ease of handling and cost-effective shipments;
5) INF-9875 Return Label for convenient return and reuse;
6) Packing instructions printed on shipper box flap, ideal for international shipping as well
7) Inside dimensions: 7.5 x 4.25 x 8.5 in. (19.1 x 10.8 x 21.6cm) with 0.5L vessel;
8) Outside dimensions: 11 x 8 x 12.25 in. (27.9 x 20.3 x 31.1cm);

2. Dry Ice, 5 lb/bag, you will order from local supplier about ONE WEEK before the actual shipping date

3. Parafilm;

4. Standard-Grade Packaging Tape, Clear, 1.89" x 54.7 yds.;

5. Scotch tape
Part-2: Tear Sample Shipment Procedures:

- Shipping MUST be done by IATA certified personnel

Samples must be shipped to Biomarker lab at Mount Sinai within 6 months of collection. Sites will typically send shipments twice a year.

1. You must order dry ice 1 week before the shipment date;
2. Prepare a Shipping Log (Form C) to be included in the package.
3. Make a copy of each sample’s Registration form (Form A) to be included in the package.
4. Remove intermediate vessel assembly from the IATA Packing Instruction 602-certified shipper system as per IATA liquid regulations;
5. Remove self-sealed bubble bag from the vessel;
6. Insert sample tubes into bubble bag cells, taking care to separate samples from each other;
   - It is recommended to place one tube per bag cell but each cell can hold up to 6 collecting tubes per bubble bag cell. If placing more than one, regulations require that tubes are wrapped in such a way as to not be in direct contact with each other (use cotton, tape, etc.);
   - There are 6 cells/bubble bag, thus a maximum of 30 collecting tubes per Shipper kit.
7. Roll bubble bag lengthwise with pockets facing upward. Place bubble bag containing samples into the vessel so that the bubble bag’s pockets face upward towards the mouth of the container.
8. Place the lid on the vessel. Firmly and evenly push the lid down seating it over the O-ring.
9. Turn the cap clockwise to close the vessel.
10. Check that the cap is screwed on correctly to provide a proper seal.
11. Place the properly closed intermediate vessel back into the Shipper kit making certain it is seated in the die-cut foam insert, then fill the container with dry ice.
12. Include papers (Tear Sample Registration forms, Shipping Log, and Shipping Declaration Form) in the Shipper Kit in the space between the inner and outer cardboard boxes.
13. Close the box flaps: (the two smallest flaps first followed by the two larger)
14. Seal the box with shipping tape.
15. Properly fill out shipping labels.
16. Secure shipping labels and all appropriate hazardous labels to outside of outer box.
17. Call UPS for package Pick-up or send to nearest UPS store.
18. **Shipping Address:**

   Yi Wei/Seth Epstein/Ines Lashley  
   Department of Ophthalmology, Box 1183  
   Annenberg Building, Rm 22-11  
   Icahn School of Medicine at Mount Sinai  
   New York, NY 10029  
   Tel: (212)864-7644 (Office); (212)241-6795 (Lab);  
   Fax: (212)241-4550  
   Email: yi.wei@mssm.edu

23. Send email to yi.wei@mssm.edu, giving notification of shipment, number of samples, date of shipment, your site ID.

Reminders:
- After the FIRST collection of tears, send an email to yi.wei@mssm.edu to ensure arrival of the shipper kit within 6 months.
- You do not need a long-term, large space to store INFECONE SHIPPER kit, we will send you **ONE every 6 months** (after your first tear sample is collected), **TWO WEEKS before your scheduled shipping date**;
- Instructions related to the shipping and packaging with the INFECON Shipper kit will be included;
- **You will be expected to order dry ice from a local provider ONE WEEK prior to expected shipping date**;
- Always arrange shipping out dates for Mondays or Tuesdays; avoid any holidays or long weekends.
APPENDIX 7-5
Keratography

1. Before starting, collect cotton-tipped applicator and alcohol swabs.
2. Connect and turn on the machine
3. Open **Oculus Keratograph software**.
4. To add a new patient: click on **New** and enter patient information with following format:
   - Last Name – fill in the DREAM ID number (XX-XXX),
   - First Name – fill in the DREAM 4-letter ID alphabetic code (AAAA);
   - Date of birth – fill in the date of visit
   - ID Number – fill in the visit number (00, 06, 12, 18, 24).
   - Click **Save**.
   For each patient, use the same DREAM ID and alpha code for each visit. Change only the date of visit and visit number to match the patient’s visit.

5. Once a patient is created/selected, press **Keratograph**
Under Examination tab select **New**.

7. Make sure the patient is comfortable and the chin and the forehead are touching the handles. Perform the tests in low light conditions.

On the left hand side of the screen you will see all the tests available.

The tests that will be conducted include:

- **TF-Scan**
  - Tear Meniscus Height
  - NIKBUT
- **Eye-Image**
  - Eye-image
- **Meibo-Scan**
  - Meibography Upper/Lower Lid

When performing exams, you do not need to choose right eye or left eye. The machine detects movement of the keratograph camera from one eye to the next for each test and registers accordingly.

Take as many photos as necessary for each examination, but please review all photos taken during an examination and select the best quality images so that only one photo of each type is submitted to the Scheie Image Reading Center.
NIKBUT (Non-invasive keratograph tear break up time):
1. On the left-side, Under TF-Scan, Click on NIKBUT
2. Adjust the machine so that the red circle is on the patient’s pupil. Move the machine in and out slowly until red arrows appear.
3. Follow the arrows as directed and when the machine is in place, screen will notify you to instruct patient to blink twice.
4. Instruct patient to: blink twice and then keep their eyes open for as long as they can. Recording will begin automatically
5. During the recording, continue to follow the arrows as directed. Recording terminates at 23 seconds or when the patient blinks.
6. The result is successful when first tear break up time appears (number in the lower right corner).

4. Sometimes the result might say “measurement is too short”. If this occurs, repeat the steps. Record the Breakup time (first) time on the Keratography Assessment form.
5. Repeat for the other eye. Record the breakup time on the Keratography Assessment form.
APPENDIX 7-5
Keratography

**Bulbar Redness Eye-Image:**

1. Under Examination, select **New**.
2. Let the patient know that the light is going to be very bright. Have patient close their eyes.
3. On the left-side, Under **Eye-Image**, Click on **Eye-Image**.
4. Focus the shaded circle over where you suspect the iris/cornea of the patient will be. Zoom in and out to obtain the most focused picture you can. Ask the patient to open their eyes and keep their eyes as wide open as possible.
5. Refocus the image if needed. Click **Image** or use the pedal to capture an image.
6. Repeat with the other eye.
7. There will be no bulbar redness scores shown on the screen. Leave the responses for Bulbar redness score blank on the Keratography Assessment form.

**Tear Meniscus Height**

1. Under Examination, select **New**.
2. On the left-side, Under TF-Scan, Click on **Tear Meniscus Height**.

3. Focus on lower eye lid. In particular, try to get the tear meniscus to look as focused as possible. Move in and out to achieve good clarity and turn the knob to move up and down.

4. Once picture is focused, Click **Image** on the right side of the screen or use the pedal to take a picture.

5. Once picture appears, click ruler button on the left (last button) and measure the height in three locations.

1) directly below the pupil, 2) 5’oclock position, 3) 7’oclock position

6. If you need to delete the previous measurement due to error, then click single x (second button) or all x (third button) to remove all measurements. The image will stay – only the measuring tool gets deleted.

7. Record the three tear meniscus heights on the Keratography Assessment form.

8. Repeat for other eye and record the heights on the Keratography Assessment form.
Meibomian Glands:

1. Under Examination, select **New**.

2. On the left-side, Under Meibo-Scan, Click on **Meibography Upper/ Lower Lid**.

3. With patient in the machine, move up and down by turning the knob to align the red box on the screen to where you expect the upper eyelid to be after inversion. Slide the machine to the side to allow access to the lid.

4. Invert the upper eye lid. (see below for suggested procedures)
   a. Upper Lid → Ask the patient to look down and to relax their eyelids. Using a cotton tip applicator, press gently at the middle of the lid. Grab the eyelashes and invert the lid, using the applicator as the point of rotation. When inverted, switch the q-tip to holding the inverted lid by the lashes.

5. Slide the machine back in its place, make sure the eyelid is within the red box, and focus the image.

6. Click **Image** or use the pedal to capture the image.

7. Return the patients upper eye lid to normal position.

8. A SECOND red box will appear on the screen again for lower lid imaging. Repeat procedures of placing the red box in the expected position of lower lid after inversion. Slide the machine to the side to allow access to the lid.

9. Invert the lower eye lid. (see below for suggested procedures)
   a. Lower lid → Press underneath the lower eyelid with cotton tip applicator. Press in and slightly up to expose lower lid.

10. Slide the machine back in its place, make sure the eyelid is within the red box, and focus the image.

11. Click **Image** or use the pedal to capture the image.

12. Screen will automatically process the images and the below screen will appear

---

**Tip:** This is easier if done by two people. One will invert and hold the lid while the other focuses the image and captures it.
APPENDIX 7-5
Keratography

Grade the dropout using the scale provided and record on the Keratography Assessment form. Repeat for other eye.

Exporting Keratograph Photos

1. In patient management screen click on the patient name once to highlight:

   ![Patient Management Screen](image)

2. Mac: Hold down the ‘fn’ key at lower left, ‘shift’ key at lower right and the hit the F11 key
   PC: Hold down “shift” key at lower right and hit the F11 key

3. Click OK

   ![Call-All with selected patients](image)

4. Click OK

   ![Automatic Examination Call](image)
APPENDIX 7-5
Keratography

Locating the Keratograph Photos

During Keratograph assessments all images are being saved into one computer directory or folder named Topo. **PLEASE DO NOT DELETE ANY IMAGES FROM THE TOPO FOLDER.**

There will be a standard set of ten images for each patient’s visit which are described below. These descriptors are embedded in the image file names.

<table>
<thead>
<tr>
<th>Test</th>
<th>Eye</th>
<th>Lid</th>
<th>Image type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meibography</td>
<td>OD</td>
<td>Lower</td>
<td>Enhanced</td>
</tr>
<tr>
<td>Meibography</td>
<td>OD</td>
<td>Lower</td>
<td>Normal</td>
</tr>
<tr>
<td>Meibography</td>
<td>OD</td>
<td>Upper</td>
<td>Enhanced</td>
</tr>
<tr>
<td>Meibography</td>
<td>OD</td>
<td>Upper</td>
<td>Normal</td>
</tr>
<tr>
<td>Meibography</td>
<td>OS</td>
<td>Lower</td>
<td>Enhanced</td>
</tr>
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<td>Meibography</td>
<td>OS</td>
<td>Lower</td>
<td>Normal</td>
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<tr>
<td>Meibography</td>
<td>OS</td>
<td>Upper</td>
<td>Enhanced</td>
</tr>
<tr>
<td>Meibography</td>
<td>OS</td>
<td>Upper</td>
<td>Normal</td>
</tr>
<tr>
<td>Redness</td>
<td>OD</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Redness</td>
<td>OS</td>
<td>n/a</td>
<td>n/a</td>
</tr>
</tbody>
</table>

On a Windows computer, all the keratograph images are stored under the directory C:\Topo. Use Windows Explorer to locate the directory. Below is an example of the Topo directory.

![Topo directory example]

On Apple computers all the keratograph images are stored in a Topo folder. Use Finder to locate the folder.
All the following instructions will be for a Windows computer.

In the Topo directory, please set up a directory for each patient’s study visit with the study id, alpha code, visit date, and visit code. The following directories are for subjects 25-998 who had a baseline visit on October 10, 2014 and 25-999 who had a baseline visit on October 15, 2014.

Once a patient’s directory is created, locate the images for that session and copy the images into the directory.

Below is an example of copying the images for the 25-998 October 10th session. First select the image set to be copied. Click on the first image, hold the shift key down, and click on the last image. This will select all images between the first and last image.

From the Windows Explorer Menu select Edit Copy (or Ctrl+c).
Open the directory that you want to copy to and from the Windows Explorer menu select Edit Paste (or Ctrl+v).

Once you have copied the patient’s session images into a directory, you may want to review and delete from this folder any extra images from this patient’s session folder only.
If you make a deletion error, you will need to return to the main Topo directory to copy and paste images. This is why we ask that you do not delete any images from the Topo directory.

![Image of file explorer window]

**Uploading Keratograph Images**

When you have all images ready to upload, please follow the instructions in the “Keratograph Image Web Submission Application User’s Guide” which is available on the DREAM landing page, [http://rt5.cceb.med.upenn.edu/public/dream_home.html](http://rt5.cceb.med.upenn.edu/public/dream_home.html).
Dry Eye Assessment and Management Study
Scheie Image Reading Center

Keratography Image Web Submission Application User's Guide

Version 1
09/19/2014
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Introduction
For the DREAM Study, keratography images will be submitted online through a web browser. Only .png images may be submitted. Images for a study patient should be stored in a directory that identifies the images by study id, alpha code, and visit code.
Only technicians who are certified for DREAM study keratography may submit images. The certification website address is accessible from the DREAM landing page at http://rt5.cceb.med.upenn.edu/public/dream_home.html.

System Requirements
- Windows PC
- Internet Connection
- At least one required Internet Browser:
  - Google Chrome (free download) or
  - Mozilla Firefox (free download)

Google Chrome may be downloaded from:

Mozilla Firefox may be downloaded from:

IMPORTANT NOTE: Microsoft Internet Explorer may not work properly for this application.

Accessing the Web Submission Application
The Keratograph Image Submission Application is accessible from the DREAM Landing Page at http://rt5.cceb.med.upenn.edu/public/dream_home.html. Click on the link Keratograph Image Submission Application.

Login Screen
For the DREAM Keratograph Submission Application, the username is the same as the DREAM certification username and the password is the same as the DREAM Certification Number. If you do not know or remember your certification username number, it can be found by logging into your account on the DREAM certification website. If you need assistance with your Username or password, please contact Mary Brightwell-Arnold at maryba@mail.med.upenn.edu. To log in, type in your Username and password and click on Submit.
Chapter 8 Application Links

After successfully logging in, a selection of links will appear on the left side of the screen. These include Home, Load Image, Show All Images, and Logout. Clicking on Home will return to the below screen.

Study: Dry Eye Assessment and Management Study (DREAM)

Home

Load Image

Show All Images

Logout
Loading Image
To upload images to the Scheie Image Reading Center, click on Load Image. A data entry screen will appear.

- **Clinic**
  - Use the pull down list to select the DREAM clinic.

- **Subject #**
  - Enter the DREAM study id without a dash. This will be a 5 digit code. This id is available from the Study Clinic Coordinator.

- **Alpha Code**
  - Enter the DREAM study alpha code. This will be 4 alphabetic characters. This alpha code will be checked by the system to validate the Subject #. This alpha code is available from the Study Clinic Coordinator.

- **Visit Code**
  - Use the pull-down list to select the DREAM visit code.

- **Keratography Image Date**
  - Enter the date of the visit at which the keratography was performed.

- **Keratography Imager last name**
  - Enter the last name of the imager

- **Keratography Imager certification number**
  - Enter the 4 digit DREAM certification number of the imager

Below is an example of a completed data entry screen.
Selecting files for submittal
Click on the Choose Files button. This will bring up a screen that resembles a Windows Explorer screen.
Navigate to the file folder where the DREAM images are located.

Select all the images that are to be submitted and click on the open button. Scroll down and you will see the list of images that were selected.

Choose files

Submit to Reading Center
Click on the upload button and wait until the upload process is complete. In the lower left corner will be an indicator of % complete while uploading. If the upload is successful, a list of the images uploaded will be presented on the screen.

Image upload completed. This batch has been marked as 99900_2014_09_18_10_20_user1

PID# 99900 - 99900_AAAA_Left_2014_08_21_10_29_51_Tearfilm overview.png loaded successfully
PID# 99900 - 99900_AAAA_Left_2014_08_21_10_34_48_Camera Image.png loaded successfully
PID# 99900 - 99900_AAAA_Left_2014_08_21_11_02_41_Meibo Overview.png loaded successfully
PID# 99900 - 99900_AAAA_Left_2014_08_21_11_06_12_Meibo Overview.png loaded successfully
PID# 99900 - 99900_AAAA_Right_2014_08_21_10_23_53_Tearfilm overview.png loaded successfully
PID# 99900 - 99900_AAAA_Right_2014_08_21_10_31_45_Camera Image.png loaded successfully
PID# 99900 - 99900_AAAA_Right_2014_08_21_10_58_29_Meibo Overview.png loaded successfully
PID# 99900 - 99900_AAAA_Right_2014_08_21_11_05_03_Meibo Overview.png loaded successfully

After the upload you will be able to submit another DREAM patient’s images by completing the data entry screen, selecting images, and uploading. Pressing the home link will return you to the main screen. From this screen you may select Load Image, Show All Images, or Logout.

Home
Load Image
Show All Images
Logout
Show all images
If you click on the link Show All Images, this will take you to a page that will list all images submitted by your clinic to the Scheie Image Reading Center. You may scroll to find a set of images submitted. In the example below, the black lines are server folder names; the blue lines are clickable links to individual images.

When you click on one of the image’s links, this will allow the image to be browsed.

A click on the web browser back button will return to the image set list.

Chapter 9 Error Message Examples
If there is an error during the upload process, there may be a pop-up message. Click on OK and correct the error before attempting to upload the image set. Below are examples of some of the error messages.
Clinic Missing

Please check the following:
Clinic No. is blank.

Invalid combination of Subject # and Alpha Code

Please check the following:
The combination of clinic, pid and alpha is invalid
## Missing data

Keratography Image Date: MM/DD/YYYY, must be greater than 1980.01.01

1. Keratography Imager last name: 
2. Keratography Imager certification number: 

Please check the following:
- Image date is blank.
- Imager last name is blank.
- Imager certification number is blank.

OK

## A dash was inserted in the Patient ID

Please check the following:
- PID is invalid.

99-99
Subject #

OK
Invalid date - date was typed in without /

Keratography Image Date: 09182014

Please check the following:
Photography date is invalid

OK

Invalid certification number

Keratography Imager certification number: 99

Please check the following:
Photographer certification number is invalid. Must be 4 digits and numeric only

OK

No image files has been selected

Please check the following:
No file has been selected.

OK

Invalid File Type or Image Exceeds Size Limit

Only .png files may be uploaded.

PID# 99903 - IMG_2184.JPG - file type not allowed - file skipped
Validation Post Submission
After images have been submitted to the Scheie Image Reading Center, the data recorded in the online form and the images will undergo further validation and review. If there are any discrepancies in the submission, the Reading Center will contact the photographer. In some cases, a resubmittal of images may be required. *Please retain study images* so that these may be retrieved by the study id and visit code. Remember to back up the study images frequently.
Example of How to Export U12 Files from OCULUS Software

Step 1 - Choose one patient by clicking once and highlight blue

Step 2 - Click on export button at bottom left
Step 3 – Click on Single File (U12)

Step 4 – Click on three dots at end of window

Step 5 – Choose where to save the (U12) file...to be determined...possible Dropbox account link
APPENDIX 7-5
Keratograph

Step 6 – Leave file name as date and time already created and click Save button bottom right.

Step 7 – Click Export button bottom left
Chapter A: Materials for Impression Cytology Collection and Short Term Storage at Site

1. For Collection:
   - Impression Cytology Registration Form
   - Supor-6 sterile filter membranes (see picture below)
   - 2 Ophthalmology Forceps (see picture below)
   - Scissors
   - Pre-printed temperature and moisture resistant label with Patient ID
   - Moisture resistant fine-tip Sharpie
   - Parafilm (see picture below)
   - Ice bucket with regular ice
   - Scotch Tape
   - Impression Cytology Site Log
   - Impression Cytology Collection Tubes with Solution (see picture below)
   - Antibiotic Ophthalmic Solution (Polytrim)
   - Anesthetic Eye Drops (Proparacaine)
   - Gloves – optional

1. For Storage:
   - Refrigerator (range: 4-8°C)
   - Pre-labeled sample storage box
   - Impression Cytology Sample Storage Temperature Log
Chapter B: Procedure for Impression Cytology Collection

1. Key points prior to start:
   a. Make sure all personnel have completed Certification forms.
   b. Minimize any unnecessary contact between patient’s conjunctiva and forceps.
   c. Arrange to collect sample at baseline and then +/- 2 hours of the baseline visit time at follow-up exams.
   d. Ensure that at least 20 minutes has passed since fluorescein and lissamine staining.
   e. Instill a drop of anesthetic (Proparacaine) in each eye prior to starting.
   f. Do not touch the filter membrane to anything but the conjunctiva.
   g. Avoid personal contact with the solution in the Impression Cytology tube (contains paraformaldehyde).

2. Prepare an IC Registration Form.

3. Write the date and R or L with Sharpie on two assigned pre-printed labels.

4. Remove two tubes from the refrigerator and adhere the pre-printed labels. Note: In ONE movement, seal the label so that the clear part of label overlaps the QR code. Do NOT try to reseal to adjust alignment as doing so will damage the QR code.

5. Set the tubes on ice.

6. Carefully hold a Supor-6 filter membrane (circle) with the forceps and cut it in half with scissors.
   a. Do not touch filter membrane to anything but the patient’s eye. We only want to collect cells from patient’s conjunctiva.
   b. Tip: Filter membranes are sticky and can be hard to pick up with one forceps at the designated corner. Use both forceps provided for easier handling.
   c. Tip: Cut membrane filters in half PRIOR to the visit to minimize examination time for patient.
   d. Tip: For patients with smaller eyes, cut membrane filters into slightly smaller pieces based on eye size to ensure it will fit.
APPENDIX 7-6
Impression Cytology

e. Tip: Prior to starting, you may ask the patient to hold the collecting tube.

7. Samples will only be collected from the temporal (outer) side of the eye. See drawing below.

8. Collect samples from right eye (OD) first. For **Superior (upper) Temporal** sample:
   a. Ask the patient to fixate on object DOWN and to the LEFT.
   i. Fixation keeps patient’s eyes steady otherwise they may have a tendency to look at the filter and you won’t be able to collect cells efficiently.
   b. Hold the patient’s upper eye-lid open with one hand then place the membrane half onto the corneal conjunctiva. Hold the filter on the conjunctiva for 1-2 seconds, and then lift the filter membrane off. Do NOT let go off the filter paper.
   c. Examine membrane half for an adequate amount of cells (~75% glistening cells) and insert the filter membrane half into the collection tube labeled “R”. Each tube will contain samples from only one eye. (see picture, R and L samples will be in different tubes)
      i. Do NOT try to push the membrane into the liquid with your forceps. Forceps are not to touch the liquid.
      ii. **Tip:** If filter membrane doesn’t immediately submerge in the liquid, secure Cap and Invert tubes to submerge the filter in the liquid.
      iii. NOTE: Avoid the solution in collection tube (contains paraformaldehyde).

9. For **Inferior (lower) Temporal** sample:
   a. Ask the patient to fixate on an object UP and to the LEFT.
   b. Repeat steps 8b and 8c.

10. When both samples from the right eye have been collected, close the tube, pressing hard to close the lid tightly.

11. Repeat the procedures in Step-8 and 9 on the left eye (OS).
   a. For Superior Temporal sample, have patient look DOWN and to the RIGHT
   b. For the Inferior Temporal sample, have patient look UP and to the RIGHT.
   c. Place both samples from the left eye into the collection tube labeled “L”.

12. Wrap each collection tube (right and left) with one half of one square of parafilm, and immediately place them back on ice.

13. Place a drop of Antibiotic Ophthalmic Solution (Polytrim) in both eyes.
APPENDIX 7-6
Impression Cytology

14. Transfer the samples to the refrigerator. Do NOT freeze IC samples.

Chapter C: Procedures for IC Short-Term Storage at Site

1. Within 30 minutes after collection, put the properly labeled and sealed collection tubes with the samples into a pre-labeled storage box in a refrigerator (4-8°C) for temporary storage at your site.
2. Record all the sample/patient information with precise collecting date and times into the Impression Cytology Registration Form.
3. Update your confidential IC Site Log.
4. Update your Impression Cytology Sample Storage Temperature Log Monday through Friday.
5. Ship to Biomarker lab at Mount Sinai within 2 weeks of collection. (typically site will ship twice/month).
Part-1: Impression Cytology Sample Shipment Materials:

1. International Air Transport Association (IATA)-certified INFECONE-3000 Shipper kits includes a compartmental bubble pouch that can hold up to six vials, absorbent sheets with a 50mL (1.7oz.) capacity, a Mini Super Label, and an outer box with all required markings.

2. Cold pack – Insert into freezer (-20°C) of a conventional refrigerator at least 24 hours before the actual shipping date.

Key Points:

- Ship to Biomarker Lab for arrival Monday- Friday by overnight shipping; avoid shipping that would involve weekends or holidays.
- When Biomarker lab personnel receive a shipment from your site, they will place an order for a new Shipper kit to be delivered to your site.
- Biomarker lab personnel will also monitor the amount of cold packs and collecting tubes a satellite center has and will replenish with new supplies when needed.
- Impression cytology collecting tubes are viable for 1 year, thus your site will receive a shipment of new tubes for replacement annually; then discard the unused old tubes.

Part-2: Impression Cytology Sample Shipment Procedures:

- Shipping MUST be done by IATA certified personnel

Samples must be shipped to Biomarker lab at Mount Sinai within 2 weeks of collection. Sites will typically send shipments twice per month.

1. Insert cold pack into freezer (-20°C) of a conventional refrigerator at least 24 hours before the actual shipping date.
2. Prepare a Shipping Log.
3. Make a copy of each sample’s Registration form that will be included in the package.
2. Remove cover from the IATA Packing Instruction 602-certified shipper system as per IATA liquid regulations.

3. Remove the cold pack from the -20°C freezer and insert it into the Shipper kit. (do NOT use dry ice; only cold packs).

4. Carefully check each impression cytology sample tube, making sure all labels are properly wrapped with scotch tape the caps are pushed down firmly, and the caps are sealed with Parafilm;

5. Remove the sample tubes from the refrigerator, one by one, wrapping each with its own separate bubble bag, and then place them into the Shipper vessel. A max of 30 tubes per shipper.
   a. Regulations require that tubes are wrapped in such a way as to not be in direct contact with each other (use cotton, tape, etc), if there is more than one tube per cell. (Recommended up to 2 tubes per bubble bag cell).

6. Seal the bubble bag.

7. Place the cover onto the Shipper vessel.

8. Place Shipment Log and Sample Registration Forms into carton and close inner flaps.

9. Close tuck-top flaps with the full width flange inside the carton.

10. Secure Shipper with packing tape.

11. Properly fill out shipping labels.

12. **Shipping Address:** Yi Wei/Seth Epstein/Ines Lashley
    Department of Ophthalmology, Box 1183
    Annenberg Building, Rm 22-11
    Icahn School of Medicine at Mount Sinai
    New York, NY 10029

    Tel: (212)864-7644 (Office); (212)241-6795 (Lab);
    Fax: (212)241-4550
    Email: seth.epstein@mssm.edu

15. Secure shipping labels and all appropriate hazardous labels to outside of outer box.

16. Call UPS for package Pick-up or send to nearest UPS store.

17. Send email to seth.epstein@mssm.edu, giving notification of shipment, number of samples, date of shipment, your site ID.
Blood Sample for Fatty Acids Testing Shipping Instructions

- Wrap the lavender-top Vacutainer in the absorbent cloth.
- Place it securely in the tube storage container.
- Close the container and tape it closed.
- Place the container in the cardboard box.
- Put the cardboard box in the ziplock plastic bag.
- Put the DREAM Blood Sample Requisition form into the bag.
- Place the bag into the FedEx shipping bag for biological Substances. If another shipping bag is used, apply a UN 3373 label so that it is visible when the box is placed in the shipping bag. Put the box and sample requisition form, in the FedEx diagnostic specimen plastic envelope and seal.
- Fill out the “From” section of the preprinted FedEx label and using the self-stick adhesive on the back of the form; attach the preprinted label to the appropriate place on the FedEx outer wrap.
- Call FedEx, 800-463-3339, for the nearest “pickup or drop box”.

Peroxisomal Diseases Laboratory
ATTN: Richard Jones PhD
Kennedy Krieger Institute
707 North Broadway, Room 530
Baltimore, MD  21205

Telephone: 443-923-2788
Fax: 443-923-2755
Chapter 8
Laboratory Procedures and Sample Analysis

The sample analyses for DREAM studies will be performed by (1) The Biomarker Laboratory located at Icahn School of Medicine at Mount Sinai in New York City and (2) Central Fatty Acid Laboratory located at Kennedy Krieger Institute.

All laboratories will be run according to our established standardized operating procedures (SOPs), including appropriate tracking of the sample as they are received by the Laboratory; appropriate storage for all samples; backup plans for emergencies: backup storage facilities, backup generators and computers; and temperature logs for appropriate storage during analysis and secure data storage.

8.1 Biomarker Laboratory:

- The Biomarker Laboratory will analyze multiple inflammatory cytokines in tear samples and HLA-DR+cells in Impression Cytology samples of conjunctiva, as per our established SOPs.1,2

8.2 Impression Cytology

Impression cytology samples will be taken binocularly at baseline, visits 06 months and 12 months. For patients participating in the Randomized Withdrawal Study, additional samples will be taken at 18 and 24 months. After receipt, the tubes will be logged in and transferred to storage (1 - 7º C) until analysis within 30 days of sample receipt (see Appendix 7-6 in the Manual of Procedures).

8.2.1 Processing/Immunostaining of Samples:

Cells will be isolated from the samples and stained via a technique adapted from previously established methods by Baudouin et al (1997) and subsequently personalized.2-4 Briefly, the samples will be preserved in 2mL cold (1-7ºC) 0.05% paraformaldehyde in 0.5% BSA-PBS buffer immediately after collection. The cells will be mechanically separated from the filters by gentle agitation and brief centrifugation; the filters will then be removed without appreciably disturbing the cell pellets. The cells will be stained for surface HLA-DR expression using an anti-HLA-DR antibody directly conjugated with an immunofluorescent dye. Cells will then be collected by centrifugation and resuspended in 400uL BSA (0.5%) in PBS buffer in preparation for acquisition by flow cytometer.

8.2.2 Flow Cytometry Calibration, Controls and Data Collection:

After immunostaining, the resuspended cells will be analyzed by flow cytometry. The calibration of the instrument will be confirmed each day before use and the instrument will be recalibrated once per week with manufacturer recommended “quality control” methodology. New lots of antibodies will be compared and normalized to preexisting lots before being used to confirm calibration of the instrument.
8.2.3 Analysis of Flow Cytometric Data:

Data collected on the cells will be plotted for analysis. Briefly, dot plots for cell size (forward scatter) vs. cellular granularity (side scatter) and fluorescence channel (FL-1) vs. cellular granularity (side scatter) as well as a histogram (FL-1 vs. event count) will be plotted to elucidate the cell populations. Analytic gates will be set around this area of interest in the form of a standardized template so as to exclude cellular debris and aggregates. The relative number of cells highly expressing HLA-DR\textsuperscript{+} antigen as a percentage of the total number of cells will then be obtained from a cytogram, yielding the dot plots’ and histogram’s statistics, indicating the mean intensity of cell fluorescein which will be proportional to HLA-DR expression. In each sample, a minimum of 1,000 cells will be analyzed; samples containing less than 1,000 cells will be discarded. All specimens will be examined by a masked observer.

8.2.4 Transfer of Data to the Coordinating Center:

Data will be sent to MSSM server and logged into the secure database on the computer, which will also be backed up and then will be sent to the Coordinating Center for analysis and correlation with other findings.

8.3 Tear Cytokines:

Tear samples will be taken at baseline, the visits at 06- and 12-months. For patients participating in the Randomized Withdrawal Study, additional samples will also be taken at visits 18- and 24-months from eligible and randomized patients. After the sample is taken, it will be stored at -80°C freezer on the sites and be shipped to the Biomarker Laboratory following the procedures in Appendix 7-6 in the Manual of Procedures. After receipt by the Biomarker Laboratory, the samples will be logged in and transferred to storage in -80°C freezer for a short period of time before assay. The raw data will be processed in the Biomarker lab and the results will be sent, in a timely fashion, to the coordinating center for decoding and statistical analysis.

8.3.1 Tear sample processing:

Tear cytokines will be measured by MILLIPLEX-MAG kit (High Sensitivity Human Cytokine Kit, Cat # HSCYT MAG-28SK, Millipore Corporation, Billerica, MA 01821) based on the Luminex\textsuperscript{®} xMAP\textsuperscript{®} technology, as per the manufacturer’s manual. The levels of inflammatory cytokine/chemokine expression in tear will be measured according to the established SOPs and the manufacturer’s recommendations.\textsuperscript{5} One day before each assay, about 60 tear samples will be thawed on ice and tear volumes and IDs on the label will be recorded and re-confirmed.\textsuperscript{6,7} After individual dilution factors are calculated, the assay buffer provided by manufacturer will be added to each sample to make the total volume equal to 50 µL. Each diluted tear sample will be promptly sealed and stored on dry ice and transferred to -80°C freezer to prevent evaporation. Next day, the diluted tear samples will be directly transferred to the designated wells in a specially designed 96-well assay plate, plus fresh-made various diluted standards and internal quality controls. Each well is preloaded with antibody-immobilized internally color-coded microsphere beads. The beads will capture the cytokine molecules of the tear sample and a biotinylated detection antibody during the incubation. The resultant beads will then react with Streptavidin PE conjugate to develop the secondary color reaction on the surface.
8.3.2 Analysis:

Plates will be read on a Luminex 200™ System, where the microsphere beads will rapidly pass through two laser beams, one to excite the internal dyes and the other to excite the PE fluorescent dyes on the beads. The mean fluorescence intensity is recorded, analyzed and converted into a known concentration in comparison with the assay standards using the Milliplex Analyst Software (Cat # 40-013, Millipore Corporation, Billerica, MA 01821).8,9

8.3.3 Transfer of Data to the Coordinating Center:

Data will first be validated by the Biomarker Laboratory and then uploaded into a secure MSSM intranet data base server. These data will be backed up and will then be transferred to the Coordinating Center for further decoding and then analysis and correlation with other findings.

8.4 Central Fatty Acid Laboratory:

- The Central Fatty Acid Laboratory at the Kennedy Krieger Institute will quantitatively analyze the total fatty acids and the cis- versus trans- fatty acids partition in the red blood cell samples of the patients.

Blood samples will be taken at baseline, and at the visits at 06-and 12-months. Blood samples will preferably be shipped to the Kennedy Krieger Institute withing 24 hours of collection, though samples are viable for up to 48 hours. For patients participating in the Randomized Withdrawal Study, additional blood samples will be taken at the 18- and 24-month visits. After the sample is taken, sites will follow the procedure in Appendix 7-7 in the Manual of Procedures for shipping instructions.

8.4.1 Methods of Analysis:

The red blood cells are prepared by centrifugation and two washes with saline. The packed washed red blood cells are stored in freezer vials under nitrogen (N₂) gas at -80°C until analysis.

8.4.1.1 Measurement of plasmalogens as dimethylacetals

Duplicate 100ul aliquots of fresh or frozen red blood cells are transferred into 13 x 100 mm glass screwed capped tubes and the lipids are extracted with 3ml isopropanol: hexane 2:3.10,11 After removal of the precipitated proteins by centrifugation, the solvent containing the lipids is evaporated under N₂ gas. The first set of duplicates is used for the gas chromatographic analysis of the vinyl ether phospholipids, plasmalogens, measured as dimethylacetals (Plasmalogens are reduced in patients with peroxisomal disorders of plasmalogen synthesis and also in some diseases of aging.) To this set of dried red blood cell total lipids is added 1.5ml 1N methanolic HCl and the tubes are flushed with N₂ gas and capped with Teflon-lined 12 mm silicone discs before placing in a 75°C oven overnight. The tubes are cooled to room temperature and the fatty acid methyl esters and dimethylacetals are extracted with 3ml hexane. After separation of the hexane layer by centrifugation, the hexane layer is collected, dried and solubilized in 100ul hexane, transferred to gas chromatographic glass injection vials and analyzed by capillary gas chromatography, an Agilent 5970 GC equipped with a flame detector. The dimethylacetals are separated on a 50 meter non-polar column, such as a DB-1, identified by retention time and measured against the internal standard fatty acids C19:0 and C27:0 that are added at the lipid extraction step.
8.4.1.2 Measurement of total fatty acids

The second set of extracted red blood cell total lipids is used for the preparation and analysis of the total lipid fatty acids by capillary gas chromatography mass spectroscopy. Briefly stable isotope internal standards are added for quantitation. In the presence of the antioxidant, BHT, the fatty acids are released from the total lipids by 0.6N HCl in acetonitrile, heated for 45 minutes at 104°C. After cooling to room temperature, the samples are treated with the addition of 2ml of 1.0 N NaOH and heated again at 104°C for 45 minutes. (This step completes the release of all fatty acids from the complex lipids.) The tubes are cooled and neutralized with the addition of 350ul 6N HCl. The fatty acids are extracted with 2 ml hexane. After centrifugation, the hexane layer is transferred to a fresh 13 x 100mm glass screw capped tube and the hexane layer is dried under N2 gas. To the dried fatty acids is reconstituted in 10ul triethylamine and 50ul of 10% pentafluorobenzyl bromide in acetonitrile. This reaction takes place at room temperature over 20 minutes and prepares the fatty acid pentafluorobenzyl bromide esters. At the end of reaction, 150ul of 0.1N HCl and 1 ml hexane are added, the tubes mixed and centrifuged. The hexane layer containing the purified fatty acid pentafluorobenzyl bromide esters is transferred to a GC vial for injection and analysis by a GCMS.

We use an Agilent 6890/5973 GC/MS operating in the negative chemical ionization mode using ammonia as the reagent gas. The capillary column is a polar, special order, Supelco SP2560 50m x 0.25mm x 0.2um. Using a polar column permits the separation of the *trans* fatty acid from the *cis* fatty acids.

The fatty acids are identified against their known mass as well as retention time and are measured against standard curves prepared for each fatty acid measured. The recovery of the isotope deuterium labeled fatty acid is determined for absolute quantitation. All results are reviewed by the chromatographers, compared with concurrent normal and abnormal controls and other quality procedures such as trend analysis for each fatty acid measured, before the results are accepted.

8.4.2 Statistical Analysis:

Final results of the plasmalogens as dimethylacetals and the total lipid fatty acids by GCMS are electronically combined into one report and transferred into the PDL database for generation of written reports. Excel spreadsheet reports for each sample analyzed are also prepared by electronic transfer and then sent to the coordinating center for analysis.
REFERENCES:


CHAPTER 9
QUALITY ASSURANCE ACTIVITIES

9.1 Overview
The Coordinating Center has primary responsibility for assuring that the quality of the data collected and reported in the study are of consistently high quality. Many factors contribute to the quality of the data, from the design and procedures of the trial to the analytic methods employed. The Coordinating Center works with the Drug Distribution Service to implement a quality assurance program for tracking study drug inventory and with the CRCU to ensure quality assurance regarding the study data.

9.2 General Quality Assurances
The major quality assurance features of the study are:

- Standard data collection forms and procedures;
- Common protocol for eligibility, examination, and follow-up of all patients in all clinical centers;
- Computerized treatment allocation with eligibility review preceding enrollment;
- MASKED assessment of the primary outcome measure and secondary outcome measures;
- Direct data entry into the study database at the Clinical Centers;
- Central, computer driven data editing for missing, invalid, and suspect responses;
- Regular reporting on performance of all Clinical Centers;
- Monitoring visits to all centers;
- Specific data analyses to identify incorrect or fraudulent data collection processes;
- Certification of clinic staff;
- Regular meetings of the Investigative Group to review methods and discuss problems.

Staff at the Coordinating Center and Chairman’s Office participate in the design of all data collection forms, coordinate modifications to existing forms, and develop new forms as needed. Because the Coordinating Center supplies all centers with master copies of forms, it ensures that the current versions of all forms and components are available to the clinical centers in the rare event that the on-line system is temporarily unavailable.

The members of the DREAM Planning Committee played a major role in developing the DREAM protocol and preparing the Manual of Procedures. Coordinating Center personnel update the chapters of the Manual of Procedures and are responsible for periodically distributing updates to all centers.
Biostatisticians and the Systems Analyst at the Coordinating Center prepare the treatment allocation schedules for each clinic. Treatment group allocations are issued only after verification of eligibility via the on-line database management system.

Coordinating Center staff members are responsible for ensuring that all data processing activities in the study proceed smoothly, as described in Chapter 14, and for timely editing, resolution of problems, and reporting. Concurrent data processing and editing are important for providing feedback to each individual involved in data collection and submission and to those involved in patient care to ensure that the procedures specified in the protocol are properly interpreted and applied.

Protocol Monitors of the Coordinating Center have primary responsibility for visiting the Clinical Centers to ensure protocol adherence and to assist in identifying and resolving problems. Other Coordinating Center staff assist with these visits as necessary. Staff at the Coordinating Center provides information to the Director to facilitate the activities of overseeing clinical center operations. The Study Chair may also visit Clinical Centers, as needed, to address specific performance issues.

Biostatisticians at the Coordinating Center develop a set of data analytic routines meant to identify patterns in the data that might indicate incorrect or fraudulent data collection processes. Further investigation of these findings will be conducted. Guidelines set by the NEI and the Office of Research Integrity will be followed.

The Director of the Coordinating Center is responsible for the certification program for the study (see Chapter 14). In addition to the initial training of Clinic Coordinators, the Director and Protocol Monitor also organize and chair sessions for the Clinic Coordinators at the annual meetings. Problems and issues related to following the protocol, handling of study medications and submission of data and blood are reviewed and discussed to identify methods for resolving problems and improving or easing operations.

The yearly meeting of the Investigative Group is an important component of quality assurance. These meetings provide a mechanism of sharing information among DREAM investigators and other personnel. The Coordinating Center staff, with input from the Chairman's Office and Operations Committee, plays a major role in organizing these meetings and preparing reports and presentations to be made to the Investigative Group.

9.3 Clinical Center Monitoring Committee

The Clinical Center Monitoring Committee has responsibility for the quality assurance activities required to maintain standardization of procedures and adherence to the DREAM protocol. Membership and specific functions may be found in Section 10.9.1. Problems in Clinical Center performance or adherence to the protocol are normally resolved by the Director and Protocol Monitor working directly with the staff of the clinic. When these efforts fail, the problem is referred to the entire committee. If necessary, the Clinical Center Monitoring Committee reports failure to resolve the issue to the Operations Committee or the Executive Committee.
9.4 Site Visits to Clinical Centers

Periodic site visits by an independent observer are necessary to ensure that there is standardization of procedures, that clinic personnel have been trained adequately, that the clinic facilities meet standards, and that patients and their data are being managed as specified in the protocol. The site visitor also provides assistance in solving logistical problems by conveying efficient, accurate solutions used in one clinical center to other clinical centers. All sites will be visited within a few months of the initiation of patient recruitment and will then be re-visited annually. Clinical Centers may be visited more frequently if the Clinic Monitoring Committee deems it necessary due to problematic performance or clinic staff turnover.

9.4.1 Scheduling and Preparation

The site visit should be scheduled so that the clinic staff members may arrange their day appropriately, usually a month or more in advance. A copy of the site visit agenda is sent to the Principal Investigator of the clinic and to the Clinic Coordinator. The site visitors re-arrange the agenda to meet the scheduling constraints of the clinical center.

Site visitors prepare for the visit by reviewing previous site visit reports, notes from recent quarterly telephone calls, clinic report cards issued by the Clinical Center Monitoring Committee and any comments or concerns from the laboratories regarding the Clinical Center. The data processing staff prepares data to be checked against clinic forms and original source materials.

The Clinic Coordinator prepares by making sure that patients and staff are available for the site visitor to observe the Clinic staff perform the entire set of study protocols. The site visitors may ask the Clinic Coordinator to assist in making arrangements for local lodging and transportation.

9.4.2 Conduct of the Visit

Site visits will begin early in the morning and will generally require 1-2 days. Strict adherence to the protocol is stressed throughout the visit. If clinical center staff view some part of the protocol as unreasonable or difficult to implement, the clinic personnel are instructed to follow the protocol. The site visitors bring the issue to the Operations Committee, Executive Committee, Director of the Coordinating Center, Study Chairman or other person as warranted by the particular issue.

General areas of review during the site visit are listed below:

- Clinic staff, facilities and equipment
- Storage and access to study medications and drug accounting procedures
- Flow of patients through the clinic during study visits
- Up-to-date study documentation including the Manual of Procedures, data collection form masters, protocol memoranda, study medication inventory and tracking documentation, documentation confirming reports of serious adverse events to the local IRB and other regulatory documents.
- Review of signed consent forms for 100% of patients during the enrollment period (some of the signed consent forms may be reviewed via remote monitoring, using a webcam and Skype-like software.)
• Review of a sample (approximately 5%) of data collection forms for comparison with data in the DREAM database and source documents
• Observation of the Clinic Coordinator during at least one patient visit
• Storage and access to study patient files, including proper storage of signed consent forms and handling of edit messages
• Discussion of individual patients with follow-up problems
• Meeting with the Principal Investigator of the clinic to discuss recruitment, follow-up, and areas of concern

9.4.3 Site Visit Reports
A written summary prepared by the site visitor will be sent to the Clinic Coordinator, Principal Investigator and members of the Clinical Center Monitoring Committee. A copy of the report is also maintained in the Coordinating Center library of study documentation.

9.5 Regularly Scheduled Telephone Calls/Web Meetings
A telephone call or on-line video meeting is scheduled once every 4 months (unless a site visit has recently occurred) between the Protocol Monitor and clinic coordinator to ensure that changes (if any) in study personnel, facilities, and equipment have been communicated and that progress is being made in any problem areas of performance. The Clinic Coordinators bring any problems, either within the clinical center, or with the Coordinating Center, to the attention of the Protocol Monitor or Director.

9.6 Preventing Drop-outs and Missed Visits
Each Clinical Center must make visits as pleasant as possible by minimizing wait time, and providing comfortable waiting and examination facilities. The Coordinator and the PI for each Clinical Center will continually educate the patient as to the nature of the Study, the need for the patient’s continued participation, and answer questions concerning DED and the use of Omega 3 fatty acids.

The Coordinator will contact patients to remind them of a follow up visit (and to bring their empty study bottles and unused supplements to the appointment) within the week of the appointment. The contact can be by telephone or email (if previous consent was received) but a patient response is necessary. To ensure patient compliance for scheduled visits, extended office hours may be provided. Every effort must be made by the Clinical Center to remain in contact with patients, even if they do not want to return to be examined or follow the protocol.

9.7 Quality Assurance Related to Drug Storage and Accountability
Kits of run-in supplements will be supplied to each center by the Investigational Drug Service of the University of Pennsylvania. Each Clinical Center will store the kits in their local pharmacy or in locked areas at the Clinical Center in a manner consistent with their standard clinical practice. All kits must be stored at room temperature away from moisture, heat and direct sunlight or refrigerated. When dispensing bottles of run-in supplements, the clinic coordinator will record
the bottle number dispensed onto the drug inventory logs. Outdated supplies will be recalled by the Investigational Drug Service or destroyed on site and replacement supplies provided.

At each visit after the screening visit, the clinic coordinator will collect unused gelcaps from the patient. Returned gelcaps must be stored in the returned bottle. The date the bottle was returned to the Clinical Center must be clearly marked on the bottle label and stored with the other bottles designated for the patient.

All drug storage facilities and medication dispensing and collection records will be made available to the site visitor for inspection during site visits.

9.8 Ensuring Data Integrity and Quality
Ensuring the integrity and quality of data collected in the DREAM Study is critical. Refer to Section 14.3.7 for DREAM Quality Assurance activities related to data management.
CHAPTER 10
ORGANIZATIONAL STRUCTURE OF THE STUDY

10.1 Introduction
The functional units in the trial are the Chair’s Office, Coordinating Center, Clinical Centers, the Drug Distribution Service, and the Central Laboratory for Analysis of Fatty Acids. The administrative organization consists of an Executive Committee, an Operations Committee, a Clinic Monitoring Committee, a Data and Safety Monitoring Committee, the Investigative Group and other committees as required. See Exhibits 10-1 and 10-2 for a schematic of the organizational structures within the DREAM.

10.2 Chair’s Office
The Chair’s Office provides the scientific and medical guidance for directing the Executive and Operations Committees and the Investigative Group. The Chair provides steady leadership for the overall performance of all aspects of the study. Interactions with the study Consultants are managed predominantly through the Chair’s Office (See Chapter 13 for more details on the role of the Chair’s Office.) In addition, the Study Chair oversees the laboratories for determination of HLA-DR and cytokine levels.

10.2.1 Location and Staff
The DREAM Study Chair’s Office is located in New York City, New York within the Department of Ophthalmology at the Mt. Sinai School of Medicine. The Study Chair is assisted by a Project Manager and an Administrative Assistant. The laboratories are staffed by Co-Directors and a Lab Technician/ Sample Coordinator.

10.2.2 Responsibilities
The Chair is responsible for overseeing the scientific direction of the Study, maintaining effective study committees, and keeping an operative working group of investigators. Specific responsibilities may change during the course of the study to meet changing needs. Responsibilities will include, but not be limited to, the following:

- Monitoring of study progress. During the study, monthly documentation on recruitment at each clinic is used by the Chair to encourage and assist each site to reach recruitment goals. The Chair also notes the most prolific recruiting clinics and encourages other investigators to use their techniques to improve recruitment. Recruitment for all clinic sites will be discussed during all Executive and Operations Committee meetings until recruitment is closed. Monitoring of follow-up rates is handled in a similar way as recruitment.

- Responding to all inquiries from news agencies, professional groups, and other organizations about the study. The Office of the Chair responds whenever dissemination will be of greater than local scope. Clinical Center Principal Investigators may supply information only when the request is from a local organization and the question is factual in nature.
• Oversight of the laboratories for determination of HLA-DR and cytokine levels. Responsibilities include inventory receipt of all blood and cell samples received, perform the testing to determine levels of either HLA-DR or selected cytokines, and transfer the results to the Coordinating Center for incorporation into the study database. See Chapter 8 for a detailed a summary of the laboratory procedures.

10.3 Coordinating Center

The Coordinating Center contributes to the success of the trial in fully evaluating the effects of the treatment regimens through leadership, organization, communication, and facilitation of the execution of the trial protocol. It is the Coordinating Center’s responsibility to ensure that the provisions of the Manual of Procedures (the operational version of the study protocol) are carried out by all participating units. The Coordinating Center provides expertise on study design, statistical analysis, data processing and management, and coordinates the selected activities needed to carry out the study.

10.3.1 Location and Staff

The DREAM Coordinating Center is located in Philadelphia, Pennsylvania within the Department of Ophthalmology at the University of Pennsylvania. Statistical, epidemiologic, and data processing expertise are provided by Coordinating Center staff through the Department of Ophthalmology. Professional and support personnel are employed to collect, process, and analyze the data for DREAM.

In general, the Coordinating Center is responsible for coordinating and/or organizing all study activities involving the Coordinating Center, the Central Laboratory for Analysis of Fatty Acids, the Clinical Centers, the Executive Committee, the Operations Committee, the Clinic Monitoring Committee, and the Data and Safety Monitoring Committee.

10.3.2 Responsibilities

A detailed description of the responsibilities and procedures related to the Coordinating Center may be found in Chapter 14. In general the activities of the Coordinating Center are to:

• Work with the other members of the study to further refine the study design;
• Provide the infrastructure necessary to support the conduct and monitoring of the study;
• Create and maintain the study database through design of data collection forms, data capture and processing, and data editing;
• Monitor all Clinical Centers for adherence to the study protocol;
• Serve as a resource to Clinical Center staff for issues concerning study protocol and procedures;
• Check on the completeness and quality of all data and to periodically distribute reports to participating clinics on delinquent forms, incomplete forms, etc.;
• Provide timely, regular reports to the Clinical Centers, Central Laboratory for Analysis of Fatty Acids, Executive Committee, Operations Committee, and Data and Safety Monitoring Committee concerning study progress and performance;
• Serve as a liaison between the Drug Distribution Service and the Clinical Centers;
• Provide interim and final statistical analysis of the accumulated data;
• Design and implement a full program of quality assurance activities;
• Participate actively with the preparation of scientific reports;
• Administer Purchased Services Agreements with the Clinical Centers;
• Site visit each of the Clinical Centers at regular intervals;
• Assist in training and certifying Clinic Coordinators in study procedures;
• Prepare and distribute patient recruitment and retention aids for use at the Clinical Centers;
• Prepare annual reports on the status of the study for the National Eye Institute.

10.4 **Central Laboratory for Analysis of Fatty Acids**

The DREAM Central Laboratory for Analysis of Fatty Acids is responsible for the measurement of fatty acids in red blood cells at baseline and selected points in follow-up.

10.4.1 **Location**

The Central Laboratory for Fatty Acids is located in Baltimore, Maryland within the Peroxisomal Disease Laboratory, The Kennedy Krieger Institute of the Johns Hopkins University.

10.4.2 **Functions**

Some of the specific functions of the Central Laboratory are to inventory receipt of all blood samples received, measure selected fatty acid levels in red blood cells, and transfer the results to the Coordinating Center for incorporation into the study database. See Chapter 8 for a detailed a summary of Central Laboratory procedures.

10.5 **Drug Distribution Center**

The Investigational Drug Service of the University of Pennsylvania serves as the DREAM Drug Distribution Center. The Drug Distribution Center is responsible for labeling and distributing the supplements in a masked fashion. The Drug Distribution Center provides bottles of study run-in supplements to the clinical centers and, after randomization, sends the study supplements directly to the subject’s home. The Drug Distribution Center maintains records of all shipments and returns of unused supplements and reports these transactions to the Coordinating Center, as needed.

10.6 **Clinical Centers**

Each center is responsible for enrolling and treating patients in the study is known as a Clinical Center and is supported by a separate Purchased Services Agreement with the Coordinating Center through a grant from the National Eye Institute.
10.6.1 Clinical Center Staff and Resources

Each Clinical Center is headed by a Principal Investigator who is an eye care clinician (ophthalmologist or optometrist) and who represents the clinical center at meetings of the Investigative Group. Additional eye care clinicians may be certified to recruit, enroll, and follow study patients. Each Clinical Center has at least one person designated as the Clinic Coordinator who is responsible for having a thorough knowledge of the protocol, keeping changes in protocol and procedures up-to-date, ensuring that all non-protocol events within the clinical center are properly documented, maintaining patient interest and participation in the study, seeing that the proper forms are accurately completed and the correct complement of required examinations and tests are performed and submitted, and handling communications regarding data collection and submission with the staffs of the Coordinating Center, Central Laboratory, and Office of the Study Chair. Each Clinical Center also has a study certified Technician who is responsible for performing or assisting the eye care clinician with specific examination procedures.

10.6.2 Clinical Center Functions

The function of each of the clinical centers is to implement the provisions of the Manual of Procedures at the local level. Each clinical center is responsible for recruitment of an adequate number of patients and for follow-up of all patients. See Chapter 4 for additional operational aspects of Clinical Center staff.

10.7 Executive Committee (EC)

The Executive Committee has overall responsibility for directing the activities of the study. The Executive Committee is responsible for the major scientific leadership of the study; providing approval for all ancillary studies, abstracts, presentations, and papers; making changes in the study protocols, and advising on matters of publicity and recruitment. The committee meets twice a year - once in conjunction with the Investigative Group. Other meetings may be by teleconference or in-person.

10.7.1 Membership

The EC is composed of the Study Chair (who also serves as Chair of the Executive Committee), the Principal Investigator of the Coordinating Center, the Project Manager in the Office of the Study Chair, the Director of the Coordinating Center, four of the principal investigators of participating Clinical Centers, one of the clinic coordinators of a participating Clinical Center, and the NEI Project Officer. The Study Chair may at her discretion, and with the approval of the Operations Committee, appoint additional members to the committee, whose expertise would enhance the work of the EC. Other study personnel or individuals may be invited to attend one or more Executive Committee meetings at the discretion of the Committee or Study Chair.

10.7.2 Functions

Some specific functions of the Executive Committee are:

- To approve such changes or modifications in the specifications of treatment techniques as may be necessary or desirable;
- To approve major changes in the DREAM Manual of Procedures;
• Through subcommittees and individuals, to advise and assist the Coordinating Center on operational matters;

• To resolve operating problems brought to the Executive Committee by investigators, the Coordinating Center, and the Reading Centers;

• To approve management plans developed by the DREAM Study Chair and Director of the Coordinating Center for conflict of interest when DREAM Investigative Group members have a significant financial interest;

• To review appeals by investigators concerning the decisions by the DREAM Study Chair and Director of the Coordinating Center concerning significant financial interests;

• To monitor the performance of all participating centers. In this regard, the committee utilizes information provided by the Coordinating Center to evaluate the quality of data collected by the individual centers and their adherence to protocol. Any clinic that is behind schedule in meeting its recruitment goals, submits blood and tear samples that are consistently judged unsuitable, or fails to adhere to protocol according to reports of the Clinic Monitoring Committee is reviewed by the Executive Committee as to whether that clinic should continue to participate in the Study;

• To ensure enforcement of the editorial policy specified in Chapter 11.

• To approve ancillary studies and to monitor the progress of those approved.

• To supervise the dissemination of study results;

• To appoint subcommittees as necessary.

10.8 Operations Committee

The Operations Committee has responsibility for handling study issues in a timely manner between meetings of the Executive Committee. Issues regarding overall study progress, areas of particular concern with respect to performance of any of the study centers, and publicity are typically addressed by this committee. In general, changes to the protocol will not be made without convening the Executive Committee.

10.8.1 Membership

The members of the Operations Committee are the Study Chair, the Principal Investigator of the Coordinating Center, the Project Manager in the Office of the Study Chair, the Director of the Coordinating Center, the Project Director in the Coordinating Center and the NEI Project Officer. Other people may be asked to participate on a consultative basis at the discretion of the Study Chair on an as needed basis. The Study Chair will chair the Operations Committee.

10.8.2 Meetings

Early in the study period, meetings of the committee will be scheduled on a weekly basis. Later, the meetings may be less frequent, but at least monthly. Most meetings will be held by teleconference. The agendas for the meetings will be provided by the Coordinating Center with input from the Chair’s Office.
10.9 Clinic Monitoring Committee

The Clinic Monitoring Committee is responsible for the quality assurance activities required to maintain standardization of procedures and adherence to the study protocol in the clinical centers. The Committee will act in accord with Good Clinical Practices and with established standards for certification of clinic staff and timeliness of activities. The Committee implements the quality assurance programs described in Chapter 9.

10.9.1 Membership

The Director of the Coordinating Center chairs the Clinic Monitoring Committee. Other members include the PI of the Coordinating Center, the Project Manager of the Office of the Study Chair, the Protocol Monitors, the Systems Analyst and other individuals, as needed, with special expertise in clinic management and quality assurance methodology. The Study Chair is an *ex officio* member of this committee.

10.9.2 Functions

Some of the specific functions of the Clinic Monitoring Committee are:

- To visit each clinical center early in the enrollment phase to ensure that all required equipment and facilities meet study criteria and that the required staff members have been recruited and trained in the study protocol;
- To visit each clinical center periodically during subsequent years to review operations, to certify new staff and to review any special problems and explore ways to correct them;
- To monitor study data for unexpected patterns that suggest problems in measuring or recording the data;
- To maintain the certification program for clinic staff, following the criteria approved by the Executive Committee;
- To communicate with each Clinic Coordinator tri-annually to review staff changes and clinic problems;
- To schedule and organize training sessions for participating clinicians, Clinic Coordinators as required;
- To place on the agenda of the Executive Committee clinic problems for which corrective action is required or to which extraordinary resources of the Coordinating Center have been diverted;
- To place on the agenda of the Data and Safety Monitoring Committee any clinic problems that may compromise the accuracy or the quality of data reported.
- To develop and distribute individualized Clinic Report Cards highlighting the clinical center’s performance in key areas.

10.9.3 Meetings

The Clinic Monitoring Committee meets quarterly. Telephone calls, emails, and written communications are used to transact committee business between meetings.
10.9.4 Protocol Monitor

The Protocol Monitors at the Coordinating Center are responsible for reviewing adherence to the study protocol and evaluating each clinical center’s effectiveness in attaining study goals. The Protocol Monitors observe clinic operations during regularly scheduled site visits, prepare written reports, and discuss observations with the Executive Committee as well as with the clinic staff. These individuals are key members of the Clinic Monitoring Committee.

10.10 Investigative Group

The Investigative Group represents all of the operational units participating in the study and is responsible for maintaining a protocol that is specific, practical, and well-understood by all participants.

10.10.1 Membership

Members include the Principal Investigators, certified Clinicians, Clinic Coordinators Technicians at the clinical centers; all members of the Executive Committee, personnel and consultants in the Chair’s Office and Coordinating Center, and the representative of the NEI.

10.10.2 Meetings

The Investigative Group meets once each year to review the progress of the study and to solve problems that have arisen in implementing the protocol. In general, the Clinic Coordinator and Principal Investigator from each clinical center are required to attend; other members of the Investigative Group may attend. Separate sessions for Clinic Coordinators are usually part of the Investigative Group meetings. Separate meetings of other clinic personnel are scheduled as necessary. Individuals not associated with the study may be invited by the Chair, but only if exceptional circumstances arise requiring their attendance for the benefit of the study. These meetings are an essential part of the quality assurance program in maintaining good communications among all study components, reinforcing difficult aspects of the protocol, and emphasizing the importance of the study.

10.11 Data and Safety Monitoring Committee

The responsibility for reviewing the ethical conduct of the study and for monitoring the data for evidence of adverse or beneficial treatment effects is assigned to the Data and Safety Monitoring Committee (DSMC). The DSMC is advisory to the NEI. The DSMC will follow the guidelines put forth by NEI in the “National Eye Institute Guidelines for Data and Safety Monitoring of Clinical Trials” dated March 26, 2001 (NOTICE: EY-01-002).

Results of all data analyses involving comparisons of treatment groups are first presented to the Data and Safety Monitoring Committee unless this committee has given other instructions. Results are not available to the participating clinicians who are treating patients until the Data and Safety Monitoring Committee decides to release the information.

10.11.1 Membership

The Data and Safety Monitoring Committee (DSMC) is appointed by NEI officials. The DSMC voting membership consists of ophthalmologists and optometrists, biostatistician/epidemiologists, a nutritionist with expertise in the health effects of ω3 supplementation and a patient advocate. The NEI representative serves as an ex officio member.
DSMC voting members may not be involved in the study, nor have a vested interest in its outcome, have ties to the study investigators (e.g., no history of extensive collaboration), nor financial ties to any commercial concerns likely to be affected by the study's outcome. If at any time a DSMC member perceives that he/she or another member of the Committee has a potential conflict of interest, he/she is obligated to bring the issue to the attention of the full DSMC for open discussion and resolution. DSMC members will complete a conflict of interest disclosure form and a statement of confidentiality before their first meeting. The Chair of the Data and Safety Monitoring Committee may appoint additional members as appropriate, who have additional expertise in patient safety, confidentiality, and medical ethics.

Executive sessions of the voting members only may be held as deemed necessary by the Chair of the Data and Safety Monitoring Committee.

10.11.2 Responsibilities
NEI guidelines provide specific responsibilities for the DSMC. The DSMC is responsible for assuring that study patients are not exposed to unnecessary or unreasonable risks and that the study is being conducted according to high scientific and ethical standards. Specifically, the DSMC will:

- Assess the performance of the trial with respect to patient recruitment, retention and follow-up, protocol adherence, and data quality and completeness, to help ensure the likelihood of successful and timely trial completion.
- Monitor interim data regarding the safety and efficacy of the study treatments, so that the trial will be concluded as soon as there is convincing evidence of the treatment effects.
- Review and consider any protocol modifications or ancillary studies proposed by the Study investigators after the main trial begins to ensure that these do not negatively impact on the main trial. Addition of an ancillary study could burden the study patients so much that they are apt to discontinue participation in the trial. Protocol modifications will be considered in the context of their potential impact on scientific integrity and subject safety.
- Advise the NEI and the study investigators as to whether a protocol should continue as scheduled or undergo a modification due to a finding from the monitoring process.

10.12 Medical Safety Monitor
The Medical Safety Monitor is responsible for ongoing monitoring of reports of SAEs submitted by the clinical centers to ensure good clinical care and to quickly identify safety concerns. The Medical Safety Monitor is a physician with expertise in the health effects of ω3 supplements. The Medical Safety Monitor may suggest measures to prevent the occurrence of particular adverse events, and with assistance from the Coordinating Center, will prepare regular reports concerning Serious Adverse Events. (For more information on SAE reporting, see Chapter 5). In the event of unexpected SAEs or an unduly high rate of SAEs, the Medical Safety Monitor will promptly contact the Study Chair and the NEI representative, who will notify the DSMC Chair. The DSMC may convene a meeting or teleconference of the Committee to consider the concerns and plan appropriate action.
EXHIBIT 10-2
ORGANIZATIONAL STRUCTURE OF THE DREAM STUDY

National Eye Institute

- Medical Monitor
- Data & Safety Monitoring Committee
- Chair’s Office

Coordinating Center

- Laboratories For HLA-DR & Cytokines
- Clinical Centers
- Drug Distribution Center

Central Laboratory for Fatty Acids
CHAPTER 11
STUDY POLICIES

11.1 Introduction
This protocol is formally approved by the Data and Safety Monitoring Committee (DSMC). It is essential to the success of the study that every member of the DREAM Investigative Group adheres to the procedures outlined herein. If any DREAM Study investigator finds that, for whatever reason, adherence to these procedures is difficult or not possible, he or she should discuss the problem with the Study Chairman.

11.2 Protection of Human Subjects
The protection of patients participating in the DREAM Study has been paramount in the design and implementation of the study. This includes consideration of the risks and benefits of participation, plans for the consent process, and inclusion and exclusion criteria.

11.3 Institutional Review Board Review and Informed Consent
Each patient must provide written informed consent in order to participate in the Study. The consent forms are prepared locally based on a prototype provided by the DREAM Coordinating Center and are submitted to the local IRB for approval. (See Exhibit 11-1 for the template consent form.) All consent forms must be fully HIPAA compliant. Each participating Clinical Center must provide the Coordinating Center with a copy of the approved form before the site is certified to enroll patients into the Study.

Consent must be obtained from each patient prior to performing any study-specific procedures. Informed consent must be documented through the signature of the participating patient on the locally approved consent form. A copy of the signed/dated consent must be provided to the patient and the original will be maintained at each Clinical Center. The signed consent form must be available for inspection during site visits.

Investigators at each center are responsible for conducting the consent process, describing study procedures, discussing the risks and benefits and alternatives to participation, and discussing the voluntary nature of participation with the potential subject. The patient should be asked to sign the consent form only after the patient has been introduced to the study and had all questions answered to his or her satisfaction.

All investigators and clinic staff must complete training programs in ethics and maintaining the safety of human subjects in clinical research and in complying with HIPAA regulations prior to becoming eligible for DREAM certification. Training may be provided by the individual institutions’ approved training program or by the NIH or CITI websites. If the local institution requires additional training for those engaged in human research at their institution, this too must be completed before commencing with any DREAM procedures. Certificates documenting the successful completion of ethics and patient safety programs must be submitted to the Coordinating Center by all members of the investigative group prior to DREAM certification.
11.3.1 Patient Confidentiality

Participating Clinical Centers must take all appropriate measures to protect the confidentiality of DREAM study patients. All blood samples, tear samples and study forms that leave the Clinical Center do not identify the patient by name. Patients are assigned a unique numeric and letter code that is not related to their birth date, social security number or name. All study materials are kept in locked file cabinets at the Coordinating Center, the Central Laboratory for Analysis of Fatty Acids and at the MSSM laboratories for HLA-DR and cytokine levels. Patient identities are not be revealed in any publication that may result from this Study. The participating Clinical Centers maintain a log of patients’ names and assigned patient ID numbers, which are kept in a locked cabinet. Clinical information is not to be released without written permission of the patient, except as necessary for monitoring by the IRB, FDA, the NEI, the OHRP, and Protocol Monitors.

11.4 Patient Costs

Patients do not pay any charges for study supplements. The Study Supplements are supplied by the Study at no cost to the clinical center or patient. Charges for Study office visits are the responsibility of the patient, Medicare, or the patient’s other insurance. The frequency of visits and procedures within the DREAM Study are within the norms of standard care for patients with Dry Eye Disease or Meibomian Gland Dysfunction. In addition, insurance companies will be charged for any treatment for side effects that may occur as a result of participation in the study.

11.5 Publicity

All publicity and press releases on behalf of the DREAM Study are to have prior approval of the Executive Committee. DREAM investigators who are approached by the press for information concerning the study should refer these inquiries to the Study Chairman. It is recognized that when information is sought from an individual investigator by the local press in his or her own community, it is sometimes necessary or desirable for the investigator to handle the request him/herself. In such an event, the participating investigator who gives information should speak as an individual and not as the official representative of the DREAM Trial. This fact should be made clear to the press; however, the information given should be accurate and reflect the general policy and views of the group.

During the recruitment phase of the study, announcements (pre-approved by the DREAM Operations Committee) may be placed in local media (newspaper, radio, television). The Coordinating Center will also prepare for each clinical center a set of slides to present at local professional society meetings to aid in recruitment and study visibility. On a national level, study publicity will be increased by posting on the ClinicalTrials.gov web site, and by mailings to DED patient organizations.

11.6 Publication Plan

DREAM study papers are defined as those that use data, documents, or other information collected during the course of the Study. Publication of the results of the DREAM Study will be governed by the policies and procedures developed by the Executive Committee. The Executive Committee reviews all written reports prepared for publication.
A subcommittee of the Executive Committee ensures that the preparation of the results for abstract presentation or publication complies with NIH policies and guidelines, and appropriate analysis and conclusions are reached.

11.6.1 Authorship
All reports from the Dry Eye Evaluation and Management Group that involve comparison of treatment groups and/or the major outcome measures of the DREAM Study will list the “Dry Eye Evaluation and Management Group (DREAM)” as author. All professional participants of the Group are listed at the end of each paper and are considered as contributors. In addition, all DREAM Study personnel, past and present, may be listed with the approval of the principal investigator for whom they have worked. With the approval of the Executive Committee, publications may list members of the writing team in a footnote on the title page.

11.6.2 Manuscript Writing Teams
The DREAM Operations Committee will determine potential manuscript topics based on interim analyses and hypotheses. Investigative Group members are invited to volunteer for writing assignments and to suggest additional topics where appropriate. The Coordinating Center solicits members for the writing committees for DREAM papers from among the DREAM Investigative Group. Final designation of the writing committee will be made by the chair of the writing committee. The Executive Committee may recommend particular members of the Investigative Group for inclusion in the writing committee of specific papers. Along with the Operations Committee, each writing team will select the journal to receive the submission.

11.6.3 Manuscript Pre-Submission Review
Papers prepared for publication must be sent to the DREAM Chairman or to the Coordinating Center Director for review and advance approval by the Executive Committee. If approved by the Executive Committee, the manuscript is then sent to the Data and Safety Monitoring Committee (DSMC) for review and approval.

Oral presentations of more than local scope must be approved in advance by the Executive Committee. Abstracts to be printed must be approved by the Executive Committee. The DSMC may also decide to mandate their review of oral presentations and abstracts in advance. No unpublished study results may be used for oral presentations, local or otherwise, unless the Executive Committee grants a specific exception. The above restrictions do not apply to local presentations on the design of the DREAM study provided these presentations contain no unpublished Study results. Such presentations are encouraged to stimulate recruitment.

Copies of Study papers are sent to all Principal Investigators as well as members of the Executive Committee and the Data and Safety Monitoring Committee (DSMC) for information before publication. Reprints of published papers are mailed to members of the DSMC and to each center for distribution among the staff.

Manuscripts emanating from ancillary studies must be sent to the Executive Committee for review before submission for publication. See also Section 11.8.6.
11.6.4 Acknowledgements

Each publication must acknowledge support from the National Eye Institute (NEI).

11.7 Data Sharing

NIH released “Final NIH Statement on Sharing Research Data” (NOT-OD-03-032) on February 26, 2003 which modified “NIH Announces Draft Statement on Sharing Research Data” (NOT-OD-02-035). In accord with NIH guidelines, a summary, de-identified data set will be made available through the DREAM website at the time of publication and through direct inquiries to the Study Chair or Coordinating Center. The DREAM data sets will be largely self-documenting in that an item identifier is embedded within the label for each variable. In addition, key derived variables will also be contained in the data sets.

The rights and privacy of people who participated in the Study will be protected at all times by stripping the data from all identifiers that could lead to disclosing the identity of individual research participants. This commitment to privacy-protected data sharing will be incorporated in all levels of database design.

By the end of the funding period, de-identified SAS data sets and form images corresponding to all data collection forms used, as well as key derived variables, will be put on file with a data repository such as the National Technical Information Service (NTIS).

The full SAS databases (not de-identified) associated with DREAM will be kept on secured computer systems maintained by the Study Chair and by the Director of the Coordinating Center. Researchers may request limited access data sets and will need to enter into a data sharing agreement. Guidelines for the process of requesting such data sets and their content have been put forth recently by NHLBI (Geller, 2004). Access to the DREAM database will be similar to these guidelines. Researchers requesting limited access data sets will bear the cost of their preparation.

11.8 Ancillary Studies

Individual investigators who wish to carry out ancillary studies are encouraged to do so. It is believed that such ancillary studies may greatly enhance the value of the DREAM and ensure the continued interest of many capable investigators. However, to protect the integrity of the DREAM, such ancillary studies must be reviewed and approved by the Executive Committee and Data and Safety Monitoring Committee before their execution, whether or not they involve the need for supplementary funds.

11.8.1 Definition of a DREAM Ancillary Study

An ancillary study is a research study that requires either

- Supplementary observations or procedures to be performed upon all or a subgroup of DREAM patients according to a set protocol, or,
- Additional effort or activity by the Coordinating Center staff beyond the current scope of DREAM.
11.8.2 Reasons for Requirement of Approval

Everyone concerned with DREAM is entitled to prior assurance that no ancillary study will:

- Complicate the interpretation of the DREAM results;
- Adversely affect patient cooperation;
- Jeopardize the public image of DREAM;
- Create a serious diversion of DREAM resources locally or at the Resource Centers.

11.8.3 Preparation of Request for Approval of a DREAM Ancillary Study

The request for approval of an ancillary study should be in narrative form. It should contain a brief description of the objectives, methods, and significance of the study. Full details should be given concerning any procedures to be carried out on any DREAM patients, such as visual function tests, psychiatric interviews, psychological testing, radiological procedures, venipuncture, etc. Mention should be made of any substances to be injected or otherwise administered to the patients. Any observations to be made or procedures to be performed on a patient outside of the Clinical Center should be described. Mention should be made of the extent to which the ancillary study will require extra clinic visits by the patient or will lengthen the patient’s usual clinic visits.

11.8.4 Procedures for Obtaining Ancillary Study Approval

The investigator concerned should send the ancillary study request to the Principal Investigator of the Coordinating Center for distribution to all members of the Executive Committee. Within a reasonable time, the Principal Investigator will summarize any questions and/or objections raised by members of the Executive Committee and send this summary to the applicant so that he/she may amplify, clarify, and/or withdraw the request. The members of the Executive Committee will then have another opportunity to review the request. The Principal Investigator of the Coordinating Center then prepares a statement of the Executive Committee consensus, including any remaining reservations or objections. This statement is forwarded to the investigator who requested approval for the ancillary study. After Executive Committee approval is obtained, the information is then forwarded to the DSMC for its approval.

11.8.5 Funding of Ancillary Studies

If no additional funds are required, the investigator may proceed with the ancillary study as soon as the Executive Committee and Data and Safety Monitoring Committee approve it. If additional funds are needed, the investigator may prepare and submit a new research grant application to the Division of Research Grants, National Institutes of Health, or any other potential sponsor, for review in the same manner as any other new research grant application. It is understood that the investigator is not to activate the ancillary study until approval has been received from the DREAM Executive Committee and DSMC.

11.8.6 Publication of Ancillary DREAM Results

All manuscripts or presentations for scientific meetings based on ancillary study data must be reviewed and approved by the DREAM Executive Committee before publication or presentation. Such review will pertain to the expected impact on DREAM Study objectives and not to scientific merit alone. Appropriate acknowledgment of DREAM resources used—whether data, patients, or DREAM investigators—should be included.
11.8.7 Progress Reports to Executive Committee

The investigator of each approved ancillary study is required to provide a written progress report for review by the Executive Committee at each scheduled meeting. The Coordinating Center reminds the investigators of the deadline and collects progress reports for distribution to the Executive Committee.

11.9 Related Studies

Individual DREAM investigators who carry out studies related to ongoing, completed, or proposed DREAM studies should be aware that their conclusions and interpretations might be viewed by non-DREAM investigators as reflecting the position of the DREAM Group. The study may be related because of types of patients included, types of treatment evaluated, or similarity of methods to those used in the DREAM Study. Therefore, investigators are encouraged to submit reports from related studies to the Executive Committee for review prior to presentation or submission for publication in order to assure that the goals of the DREAM Study are not jeopardized.

11.10 APPROVAL OF CHANGES IN PROTOCOL

All significant changes to the DREAM protocol must be approved by the DREAM Operations Committee, Executive Committee and the DSMC. In some circumstances, approval from NEI may also be required. When a change in protocol is implemented, a protocol memorandum will be issued to clinical center staff. All DREAM clinical center staff will be required to acknowledge receipt of the protocol memorandum and that they understand its contents by signing and dating a form that is sent to the Coordinating Center. During site visits, the Protocol Monitor reviews whether all DREAM protocols, forms and other documents are up to date.

11.10.1 Changes to the Manual of Procedures

The DREAM Manual of Procedures will be revised to reflect changes to the protocol. All revised manual chapters are distributed by the Coordinating Center. Any revisions to chapters that originate in other DREAM resource centers (i.e., the Study Chairman’s Office) must be sent to the Coordinating Center for distribution to the clinical centers.
EXHIBIT 11-1
Research Subject Informed Consent Form For Primary Study

Protocol Title: DREAM: Dry Eye Assessment and Management Trial

Principal Investigator: Insert PI Name Here
                  INSERT Address

Emergency Contact: Insert Emergency Contact
                  Insert Phone Number/ Pager, etc.

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Why am I being asked to volunteer?
You may qualify to take part in this research study because you have been diagnosed with dry eye disease by your eye care provider. This is a study that will test an over-the-counter nutritional supplement in people who have Dry Eye Disease.

Funds for conducting this research are provided by the National Eye Institute, which is part of the National Institutes of Health.

You are being invited to participate in a research study. Your participation is voluntary which means you can choose whether or not you want to participate. If you choose not to participate, there will be no loss of benefits to which you are otherwise entitled. Before you can make your decision, you will need to know what the study is about, the possible risks and benefits of being in this study, and what you will have to do in this study. The research team is going to talk to you about the research study, and they will give you this consent form to read. You may also decide to discuss it with your family, friends, or family doctor. You may find some of the medical language difficult to understand. Please ask the study doctor and/or the research team about this form. If you decide to participate, you will be asked to sign this form.

What is the purpose of this research study?
The purpose of this study is to test omega-3 fatty acids supplement, an over-the-counter nutritional supplement, vs. placebo (olive oil) in people with dry eye disease. Omega-3 fatty acids are used to treat hypertriglyceridemia (high levels of fat in the blood.) Their usefulness in treating dry eye disease has not been established and is experimental in this study.
How long will I be in the study? How many other people will be in the study?

Your participation in this research study is expected to last either five visits over one year or 7 visits over two years. After the first year-long study, you may be asked, if you are eligible, to continue with a one-year extension study.

The number of people expected to take part in this research study at this site is up to 50 people. The total number of people throughout the United States expected to take part in this research study is 600 people at 21 centers.

What am I being asked to do?

If you decide to take part in this research study you will be responsible for the following things:

- Coming in for all visits (5 visits in total) and if eligible for the extension study, following up for one additional year with two visits.
- Taking the 5 omega-3 or placebo capsules each day.
- Women who are able to have children and who are sexually active must use an effective means of birth control throughout the study.
- If you agree to participate in this research study, the following information describes what will be involved.

Study Drug

Your consent to participate in this study means that you agree to receive and take study pills. These pills will either be omega-3 supplements (containing 400 mg EPA and 200 mg DHA) or a placebo (containing olive oil). You will be asked to take 5 of these pills a day for at least one year.

Run-In (test) Period

At the end of the first visit (the screening visit), we will give you study pills to take until your next visit in about 2 weeks. During this first screening visit, your eye care provider will talk with you about when to take the 5 pills during each day. We want you to try out taking the pills every day during this period. Some people may find it hard to swallow large pills every day and we want to see how you do with it.

After the Run-In Period

After your second visit, if you are still eligible for the study, you will receive a supply of either omega-3 supplements (containing 400 mg EPA and 200 mg DHA) or a placebo (containing olive oil) in the mail. The type of pills (omega-3 supplements or a placebos) you receive will be determined randomly, like the flip of a coin, rather than having your treatment selected by your study eye care provider or you. You have a 67% (2 out of 3) chance of getting the omega-3 supplements and a 33% (1 out of 3) chance of getting the placebos. You will be asked to take five pills a day until the conclusion of the study (one year). If you are eligible to participate in the study for the second year, your pills during the second year will again be determined randomly. You have a 50% chance of getting the omega-3 supplements and a 50% chance of getting the placebo. Neither you nor the study eye care provider will know what study treatment you get. Information on the drug that you are taking could be obtained through your
Study Visits
Study visits will occur today after you sign this consent form, in about two weeks from now, and then 3, 6, and 12 months later. If you continue in the extension study, there will be study visits at 18 and 24 months. At one or more of the study visits the following tests will be performed: questionnaires about your dry eyes and general health, vision exam, tear collection, slit lamp examination, Schirmer’s test for tear production, eye pressure measurement, pregnancy test, impression cytology (examination of the cells on the surface of the white part of the eye), photographs of the eye and blood collection. All of these tests are described below.

Medical History
You will be asked questions about your medical history and any current medications you are taking. This is to ensure that you meet all of the necessary requirements for the study and do not have any conditions that would make participating unsafe for you. Questions asked during a medical history typically cover current and past medications or therapies, illnesses, conditions or symptoms, and allergies. You will be asked about any new medical events and/or medications at each visit.

Vision Exam
Your vision will be tested and you will be asked to read eye charts. We will try to improve your vision by adjusting the prescription in your eyeglasses by placing different lenses in front of your eyes.

Questionnaires
At all visits you will be asked to fill out two questionnaires that will take about 10 minutes to complete. Each one will ask questions about how your dry eye disease affects your daily life.

At your next visit and every six months afterwards, you will also be asked to fill out several questionnaires that will help us determine the economic and physical and emotional health impact of dry eye disease. None of the procedures described below are experimental, which means that they are part of standard medical practice or that they have been approved by the Food and Drug Administration (FDA).

Tear Collection [OMIT THIS SECTION IF THE CLINICAL CENTER IS NOT COLLECTING TEARS].

A small amount of your tears will be collected by placing a small tube (like a tiny straw) on the edge of your lower eyelid to collect the tears that pool along the lower eyelid. The collected tears will be tested for the presence inflammatory substances.

Keratograph [OMIT THIS SECTION IF THE CLINICAL CENTER IS NOT PERFORMING KERATOGRAPHY].

Pictures of your eyes and eyelids will be taken to evaluate the tear film, the eye redness and the glands in your eyelids, which make some of the tear film. Your identity will not be revealed in the pictures because these are close up photographs of the eye only.
Tear Osmolarity  [OMIT THIS SECTION IF THE CLINICAL CENTER IS NOT PERFORMING TEAR OSMOLARITY].

To determine how much salt is in your tears (tear osmolarity), a very small amount of tears will be collected from your lower eyelid margin using a small instrument.

Eyelid gland evaluation
To check the glands in the eyelid that make part of the tear film, your eye care provider will apply light pressure to your lower eyelid with a small paddle to study the fluid that comes out of the glands.

Slit lamp Examination
The study eye care provider will look closely at your eyes and eyelids through a microscope (magnifying lens) to assess the health of the surface of your eyes, eye lashes, lids, and lens. The study eye care provider will then apply a small drop of dye (2 types) on the surface of your eye to evaluate effects of dry eye on the ocular surface.

Schirmer’s test
To measure the amount of tears your eyes make, we will first numb your eyes with an eye drop so you feel no pain. Then we will place a small piece of sterile filter paper on your lower eyelid and leave it in place for 5 minutes while your eyes are closed. Then the strip of filter paper will be removed.

Eye pressure measurement
Your eye pressure will be measured after placing a numbing drop on the eye so you feel no pain, as is done in most standard eye examinations.

Pregnancy Test
Women who are able to have children will be asked to provide a urine sample for a pregnancy test at their first visit. To take part in this study, women who are able to have children and who are sexually active must use an effective means of birth control throughout the study. The study staff will discuss with you what acceptable methods of birth control can be used to prevent pregnancy while participating in this trial. If you are pregnant or planning to have a baby in the next year, you should not participate in this study. However, if you do become pregnant during the trial, notify your study eye care provider immediately.

Blood Collection
About 2 tablespoons of blood will be drawn for laboratory testing. This procedure will be performed either by the study eye care provider or study staff. This sample will be sent to a laboratory to check the level of fatty acids in your blood to make sure the pills are being taken properly. A second sample of about 1 teaspoon of blood will be taken during the same blood draw at certain visits. This sample will be used to test for antibodies that may be associated with Sjögren’s Syndrome or other autoimmune diseases (conditions that occur when your body tissues are attacked by your own immune system and that can sometimes cause dry eyes). The blood will be analyzed at the end of the study and you will receive the results of the test after your last DREAM visit.
Impression Cytology
In this test, a numbing eye drop is placed on each eye so you feel no pain and then a piece of filter paper is gently touched to the white part of the eye using blunted forceps. Blunted forceps are like dull tweezers. After 1-2 seconds on the eye, the paper is removed placed into a tube, and sent to a laboratory to test if there are signs of inflammation.

MMP-9
At this screening visit and at the 3-month visit, a fleece strip will be gently dabbed on the inside of your lower eyelid for a couple of seconds. Tears that are absorbed by the strip will be then evaluated for the presence of inflammation due to the dry eye.

Study phone calls and letters
During your participation in the study, Dr. NAME OF PI or study staff will call you about two weeks before each visit to remind you of your appointment. We will also call you about a week after you receive your study pills to check how you are doing once you start taking the study pills. Between the visits that are 6 months apart, we will call you to check how you are doing and to confirm your next appointment. If you forgot to bring your bottle of pills at your previous visit, we will call you the day before your next scheduled visit to remind you to bring your bottle of pills to the next visit. Finally, we will call you one last time one month after your last visit. We will also mail you postcards or letters with reminders about taking study pills.

If you give us permission (below), we will leave messages on your telephone answering machine when no one answers our telephone call. Also, if you give us your permission and prefer contact by email, we will send the reminders to you by email.

If you notice any pain, new eye irritation or change of vision after the tests, contact the eye care provider conducting this study, Dr. NAME OF PI or associates, immediately at (212) 824-7644.

A description of this clinical trial is available on http://www.ClinicalTrials.gov. This Web site does not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

What are the possible risks or discomforts?
The most common side effect of omega-3 supplementation for approximately 5% of the population is upset stomach, burping, or fishy aftertaste. Some studies have shown that patients taking omega-3 and an anticoagulant or other drug affecting the clotting of blood might have prolonged bleeding time (cuts you receive may take longer to stop bleeding). However, other studies have suggested no relationship between them. If you notice excessive bleeding, you should stop taking your study pills and notify the study clinician immediately.

Some studies have suggested that omega-3 could be a risk factor for prostate cancer. However, other studies have suggested no relationship between them. Because prostate cancer is a disease that is associated with many genetic and other factors, further research will be required to clarify the role of these fatty acids.
Large doses of heavy metals such as (arsenic, cadmium, lead, mercury) may be linked to prostate cancer. However the DREAM pills don't have a large dose of heavy metals and the amount of these elements in DREAM pills are within the safety dosage limits.

Fluorescein and lissamine dye eye drops are safe, but rarely cause an allergic reaction. Side effects from their use on the eye include redness, watering, itchiness, eye discoloration, irritation of the eye and swelling of the eyelids. These side effects usually go away in about an hour after the drops are put on the eye. These side effects are rare.

Impression cytology using small pieces of sterile filter paper placed on the numbed eye may cause mild eye irritation that usually subsides within a few minutes. There is a very small risk of eye infection (pink eye) but only sterile equipment is used and right afterwards a drop of eye antibiotic will be placed on the eye to decrease the risk of infection even further.

The study eye care provider or study staff will take your blood by sticking a small needle through your skin, as is done in routine blood sampling for a blood test. The risks of blood drawing include bleeding, pain, bruising, and the slight possibility of infection at the place where the needle goes in. Some people feel dizzy or may faint during or after a blood drawing, so we will ask you to sit for a few minutes after taking the blood sample.

If you become injured during the study, you should inform the treating doctor or nurse that you are participating in a research study.

**What if new information becomes available about the study?**

During the course of this study, we may find more information that could be important to you. This includes information that, once learned, might cause you to change your mind about being in the study. We will notify you as soon as possible if such information becomes available.

**What are the possible benefits of the study?**

It is important to know that you may not get any benefit from taking part in this research. Others may not benefit either. However, possible benefits may be relief from your symptoms associated with dry eye disease, such as irritation, foreign-body sensation, itching, burning, and blurred vision.

Your participation in this study will help eye care clinicians diagnose and monitor dry eye disease more effectively and will help future dry eye research and future treatment for patients with dry eyes.

**What other choices do I have if I do not participate?**

You may decide not to take part in this research study without any penalty. The choice is up to you. Participation in this study is optional and the alternative to participation is not to participate and to continue managing your dry eyes as you are now doing. You may discuss any alternatives with your eye care clinician.

**Will I be paid for being in this study?**

If you agree to take part in this research study, we will pay you $25.00 for each visit in appreciation of you making the effort to complete the visit. For the visit at 12 months, you will receive an additional $75.00. For this study, the maximum payment to you would be $200.
Please note: In order to be compensated for your participation in this study, you must provide your Social Security Number. Additionally, please note that the Name of Practice is required to report to the IRS any cumulative payments for participation in research studies that exceed a total of $600 in a calendar year.

**Will I have to pay for anything?**

There will be no extra charge to you or your insurance company for study related activities that are not part of standard eye care. You and/or your health insurance will be billed for standard eye care and visits that are part of regular eye care.

You are still responsible for any deductibles or applicable co-pays for routine office visits. Please talk to your eye care clinician and study team about putting you in touch with a financial counselor to determine exactly what the deductible and co-pay will be for you; this is highly variable depending on your type of insurance.

**What happens if I am injured from being in the study?**

In the event of injury resulting from your participation in this research study, we will offer you the care you need. We may bill your insurance company or other third parties, if appropriate, for the costs of the care you get for the injury, but you may also be responsible for some of them.

There are no plans for the Name of Practice to pay you or give you other compensation for the injury. You do not give up your legal rights by signing this form.

If you think you have been injured as a result of taking part in this research study, you should contact Dr. (Fill in name) at telephone number (212) 824-7644 or contact the Institutional Review Board at telephone number (215) 898-2614.

**When is the Study over? Can I leave the Study before it ends?**

This study is expected to end after all participants have completed all visits, and all information has been collected.

This study may also be stopped at any time by your physician, the study Sponsor, or the Food and Drug Administration (FDA) without your consent because:

- The Primary Investigator feels it is necessary for your health or safety. Such an action would not require your consent, but you will be informed if such a decision is made and the reason for this decision.
- You have not followed study instructions.
- The Sponsor, the study Principal Investigator, or the Food and Drug Administration (FDA) has decided to stop the study.

If you decide to participate, you are free to leave the study at any time without any penalty. Withdrawal will not affect your ability to receive medical care at Name of Practice or to receive any benefits to which you are otherwise entitled.
If you decide to stop being in the research study, please contact the Principal Investigator listed on first page or the research staff.

You may withdraw your permission for the use and disclosure of any of your protected information for research, but you must do so in writing to the Principal Investigator at the address on the first page. Even if you withdraw your permission, the Principal Investigator for the research study will still use the information that was already collected if that information is necessary to complete the study. Your health information may still be used or shared after you withdraw your authorization if you should have an adverse event (a bad effect) from participating in the study.

If you stop being in the research study, information already collected will not be removed from the research study database and will continue to be used to complete the research analysis.

Who can see or use my information? How will my personal information be protected?
As you take part in this research project it will be necessary for the research team and others to use and share some of your private protected health information. Consistent with the federal Health Insurance Portability and Accountability Act (HIPAA), we are asking your permission to receive, use and share that information.

What protected health information is collected and used in this study, and might also be disclosed (shared) with others?
As part of this research project, the researchers will collect your:

- Name
- Address
- Telephone number
- Ocular Medical History (includes current and past medications or therapies, illnesses, conditions or symptoms, allergies, etc.)
- Physical and Emotional Health Information

During the study the researchers will gather information by:

- taking a medical history (includes current and past medications or therapies, illnesses, conditions or symptoms, allergies, etc.)
- completing the tests, procedures, questionnaires and interviews explained in the description section of this consent.

Why is your protected health information being used?
Your personal contact information is important to be able to contact you during the study. Your health information and the results of any tests and procedures being collected as part of this research study will be used for the purpose of this study as explained earlier in this consent form. The results of this study could be published or presented at scientific meetings, lectures,
or other events, but would not include any information that would let others know who you are, unless you give separate permission to do so.

The research team and other authorized members of **NAME OF PRACTICE** may use and share your information to ensure that the research meets legal, institutional or accreditation requirements. For example, the **Institutional Review Board at the University of Pennsylvania** is responsible for overseeing research on human subjects, and may need to see your information. If you receive any payments for taking part in this study, the **NAME OF PRACTICE Finance Department** will need your name, address, social security number, payment amount, and related information for tax reporting purposes. If the research team uncovers abuse, neglect, or reportable diseases, this information will be disclosed to appropriate authorities.

**Who, outside NAME OF PRACTICE might receive your protected health information?**

As part of the study, the Principal Investigator, study team and others in the **Name of Practice workforce** may disclose your protected health information, including the results of the research study tests and procedures, to the following people or organizations: (It is possible that there may be changes to the list during this research study; you may request an up-to-date list at any time by contacting the Principal Investigator.)

- Research data coordinating office and/or their representative(s) at the University of Pennsylvania who will be responsible for sending out the study supplements, collecting results and findings from all the centers.
- The National Eye Institute, the sponsoring government agency and/or their representative who need to confirm the accuracy of the results and the use of government funds.
- The United States Food and Drug Administration.
- The United States Department of Health and Human Services and the Office of Human Research Protection.
- Outside laboratories who will be performing laboratory analysis for all the research centers involved in this clinical trial (Ocular Biomarker Laboratory at Icahn School of Medicine at Mount Sinai and Kreiger Laboratory at Johns Hopkins Medical Center).
- A Data Safety Monitoring Board or other committee that will monitor the study on an ongoing basis for safety.

The Coordinating Center at the University of Pennsylvania will receive your name, address, and telephone number so that they can send study supplements directly to your home. In all other disclosures outside of **NAME OF PRACTICE**, you will not be identified by name, social security number, address, telephone number, or any other direct personal identifier, unless disclosure of the direct identifier is required by law. Some records and information disclosed will be identified with a unique code number. The Principal Investigator will ensure that the key to the code will be kept in a locked file, or will be securely stored electronically. The code will not be used to link the information back to you without your permission, unless the law requires it, or rarely if the Institutional Review Board allows it after determining that there would be minimal risk to your privacy. Monitors, auditors, the IRB, the Food and Drug Administration will be granted direct access to your medical records for verification of the research procedures and
data. By signing this document you are authorizing this access. We will publish the results of this research. However, we will keep your name and other identifying information confidential.

**For how long will NAME OF PRACTICE be able to use or disclose your protected health information?**

Your authorization for use of your protected health information for this specific study does not expire.

**Will you be able to access your records?**

During your participation in this study, you will not be able to access your study records. This is to prevent the knowledge of study results from affecting the reliability of the study. Your information will be available should an emergency arise that would require your treating physician to know this information to best treat you. You will have access to your medical record and any study information that is part of that record when the study is over or earlier, if possible. The investigator is not required to release to you research information that is not part of your medical record.

**Can you change your mind?**

You may withdraw your permission for the use and disclosure of any of your protected information for research, but you must do so in writing to the Principal Investigator at the address on the first page. Even if you withdraw your permission, the Principal Investigator for the research study will still use your protected information that was already collected if that information is necessary to complete the study. Your health information may still be used or shared after you withdraw your authorization if you should have an adverse event (a bad effect) from being in the study. If you withdraw your permission to use your protected health information for research that means you will also be withdrawn from the research study, but standard medical care and any other benefits to which you are entitled will not be affected. You can also tell us you want to withdraw from the research study at any time without canceling the Authorization to use your data.

It is important for you to understand that once information is disclosed to others outside Name of Practice, the information may be re-disclosed and will no longer be covered by the federal privacy protection regulations. However, even if your information will no longer be protected by federal regulations, where possible, Name of Practice has entered into agreements with those who will receive your information to continue to protect your confidentiality.

### Electronic Medical Records and Research Results

**What is an Electronic Medical Record?**

An Electronic Medical Record (EMR) is an electronic version of the record of your care within a health system. An EMR is simply a computerized version of a paper medical record.

If you are receiving care or have received care within the **Name of Health System affiliated with Practice or the Name of the Practice** (outpatient or inpatient) and are participating in a **Name of Practice** research study, results of research-related procedures (i.e. laboratory tests, imaging
studies and clinical procedures) may be placed in your existing EMR maintained by Name of Practice.

If you have never received care within the Name of Health System affiliated with Practice or the Name of the Practice and are participating in a Name of Practice research study that uses the Name of Health System affiliated with Practice services, an EMR will be created for you for the purpose of maintaining any results of procedures performed as part of this research study. The creation of this EMR is required for your participation in this study. In order to create your EMR, the study team will need to obtain basic information about you that would be similar to the information you would provide the first time you visit a hospital or medical facility (i.e. your name, the name of your primary doctor, the type of insurance you have). Results of research procedures performed as part of your participation in the study (i.e. laboratory tests, imaging studies and clinical procedures) may be placed in this EMR.

Once placed in your EMR, these results are accessible to appropriate the Name of Health System affiliated with Practice workforce members that are not part of the research team. Information within your EMR may also be shared with others who are determined by the Name of Health System affiliated with Practice to be appropriate to have access to your EMR (e.g. health insurance company, disability provider, etc).

**Who can I call with questions, complaints or if I’m concerned about my rights as a research subject?**

If you have questions, concerns or complaints regarding your participation in this research study or if you have any questions about your rights as a research subject, you should speak with the Principal Investigator listed on page one of this form.

This research has been reviewed and approved by an Institutional Review Board. If a member of the research team cannot be reached or you want to talk to someone other than those working on the study, you may contact the Office of Regulatory Affairs at the University of Pennsylvania with any question, concerns or complaints by calling (215) 898-2614.

When you sign this form, you are agreeing to take part in this research study. This means that you have read the consent form, your questions have been answered, and you have decided to volunteer. Your signature also means that you are permitting the Name of Practice to use your personal health information collected about you for research purposes within our institution. You are also allowing the Name of Practice to disclose that personal health information to outside organizations or people involved with the operations of this study.

A copy of this consent form will be given to you.
Signature Block for Capable Adult

Your signature or mark below documents your permission to take part in this research and to the use and disclosure of your protected health information. A signed and dated copy will be given to you.

DO NOT SIGN THIS FORM AFTER THIS DATE

______________________________  __________________________
Signature or mark of subject     Date and Time

______________________________
Printed name of subject

PERMISSION TO LEAVE A MESSAGE ON VOICEMAIL OR A TELEPHONE ANSWERING MACHINE

By signing below, I authorize you to leave a message on the voicemail or telephone answering machine of the phone number listed below if I am not available to answer the phone. I understand that the messages will be about future study visits, instructions to bring study pills to the office visit and reminders to take the study pills as directed.

______________________________
Signature or mark of subject

______________________________
Printed name of subject

Number to call to leave a message if no answer (with area code): __________________________

PERMISSION TO SEND STUDY REMINDERS BY EMAIL

By signing below, I authorize you to send me emails at the address below about future study visits, instructions to bring study pills to the office visit and reminders to take the study pills as directed.

______________________________
Signature or mark of subject

______________________________
Printed name of subject

Email address: __________________________

DREAM Manual of Procedures  11-18  October 2014
Person Explaining Study and Obtaining Consent

Signature of person obtaining consent

Date and Time

Printed name of person obtaining consent
Exhibit 11-2
Research Subject Informed Consent Form For Extension Study

Protocol Title: DREAM: Dry Eye Assessment and Management Trial

Principal Investigator: Insert PI Name Here
INSERT Address

Emergency Contact: Insert Emergency Contact
Insert Phone Number/ Pager, etc.

Why am I being asked to volunteer?

You qualify to take part in this research study because you have been diagnosed with dry eye disease by your eye care provider, you participated in the DREAM Study and were taking Omega-3 supplements.

Funds for conducting this research are provided by the National Eye Institute, which is part of the National Institutes of Health.

You are being invited to participate in a research study. Your participation is voluntary which means you can choose whether or not you want to participate. If you choose not to participate, there will be no loss of benefits to which you are otherwise entitled. Before you can make your decision, you will need to know what the study is about, the possible risks and benefits of being in this study, and what you will have to do in this study. The research team is going to talk to you about the research study, and they will give you this consent form to read. You may also decide to discuss it with your family, friends, or family doctor. You may find some of the medical language difficult to understand. Please ask the study doctor and/or the research team about this form. If you decide to participate, you will be asked to sign this form.

What is the purpose of this research study?

The purpose of this study is to test for how long people with dry eye disease need to take omega-3 fatty acids supplements, an over-the-counter nutritional supplement. Omega-3 fatty acids are used to treat hypertriglyceridemia (high levels of fat in the blood.) Their usefulness in treating dry eye disease has not been established and is experimental in this study.

How long will I be in the study? How many other people will be in the study?

Your participation in this research study is expected to last one year.

The number of people expected to take part in this research study at this site is up to 20 people. The total number of people throughout the United States expected to take part in this research study is 190 people.
What am I being asked to do?

If you agree to participate in this research study, the following information describes what will be involved.

**Study Drug**

Your consent to participate in this study means that you agree to receive and take study pills. These pills will either be omega-3 supplements (containing 400 mg EPA and 200 mg DHA) or a placebo (containing olive oil). These are the same two types of pills that were used in the first year of the DREAM Study. You will be asked to take 5 of these pills a day for at least one year. This is the same amount of pills you were asked to take in the first year of the DREAM Study.

You will receive a supply of either omega-3 supplements (containing 400 mg EPA and 200 mg DHA) or a placebo (containing olive oil) in the mail. These pills will be mailed to you within a few days after completing the 12 month visit in the original DREAM Study. It is important that you take the new pills and do not take any of the old pills if you have any left. The type of pills (omega-3 supplements or a placebos) you receive will be determined randomly, like the flip of a coin, rather than having your treatment selected by your study eye care provider or you. You have a 50% (1 out of 2) chance of getting the omega-3 supplements and a 50% (1 out of 2) chance of getting the placebos. You will be asked to take five pills a day until the conclusion of the study (one year). Neither you nor the study eye care provider will know what study treatment you get. Information on the drug that you are taking could be obtained through your eye care provider if it would affect the care you receive in an emergency. After your last visit, we will tell you whether you were taking omega-3 supplements or placebos.

**Study Visits**

If you sign this consent form, there will be 2 study visits. The first visit will occur approximately 6 months from now and the second visit will occur 6 months later (approximately one year from now). At both of these study visits the following tests will be performed: questionnaires about your dry eyes and general health, vision exam, tear collection, slit lamp examination, Schirmer's test for tear production, eye pressure measurement, impression cytology (examination of the cells on the surface of the white part of the eye), photographs of the eye and blood collection. These are the same tests that were done in the first year of the DREAM Study and are described below.

**Medical History**

You will be asked questions about your medical history and any current medications you are taking. This is to ensure that you meet all of the necessary requirements for the study and do not have any conditions that would make participating unsafe for you. Questions asked during a medical history typically cover current and past medications or therapies, illnesses, conditions or symptoms, and allergies. You will be asked about any new medical events and/or medications at each visit.

**Vision Exam**

Your vision will be tested at each visit and you will be asked to read eye charts. We will try to improve your vision by adjusting the prescription in your eyeglasses by placing different lenses in front of your eyes.
Questionnaires

At all visits you will be asked to fill out two questionnaires that will take about 10 minutes to complete. Each one will ask questions about how your dry eye disease affects your daily life. You will also be asked to fill out several questionnaires that will help us determine the economic and physical and emotional health impact of dry eye disease.

None of the procedures described below are experimental, which means that they are part of standard medical practice or that they have been approved by the Food and Drug Administration (FDA).

Tear Collection [OMIT THIS SECTION IF THE CLINICAL CENTER IS NOT COLLECTING TEARS].

A small amount of your tears will be collected by placing a small glass tube on the edge of your lower eyelid. The collected tears will be tested for the presence inflammatory substances.

Keratograph [OMIT THIS SECTION IF THE CLINICAL CENTER IS NOT PERFORMING KERATOGRAPHY].

Pictures of your eyes and eyelids will be taken to evaluate the tear film, the eye redness and the glands in your eyelids, which make some of the tear film. Your identity will not be revealed in the pictures because these are close photographs of the eye only.

Tear Osmolarity [OMIT THIS SECTION IF THE CLINICAL CENTER IS NOT PERFORMING TEAR OSMOLARITY].

To determine how much salt is in your tears (tear osmolarity), a very small amount of tears will be collected from your lower eyelid margin using a small instrument.

Eyelid gland evaluation

To check the glands in the eyelid that make part of the tear film, your eye care provider will apply light pressure to your lower eyelid with a small paddle to study the fluid that comes out of the glands.

Slit lamp Examination

The study eye care provider will look closely at your eyes and eyelids through a microscope (magnifying lens) to assess the health of the surface of your eyes, eye lashes, lids, and lens. The study eye care provider will then apply a small drop of dye (2 types) on the surface of your eye to evaluate effects of dry eye on the ocular surface.

Schirmer’s test

To measure the amount of tears your eyes make, we will first numb your eyes with an eye drop so you feel no pain. Then we will place a small piece of sterile filter paper on your lower eyelid and leave it in place for 5 minutes while your eyes are closed. Then the strip of filter paper will be removed.
Eye pressure measurement

Your eye pressure will be measured after placing a numbing drop on the eye so you feel no pain, as is done in most standard eye examinations.

Pregnancy Test

Women who are able to have children will be asked to provide a urine sample for a pregnancy test before they enroll in this study. To take part in this study, women who are able to have children and who are sexually active must use an effective means of birth control throughout the study. The study staff will discuss with you what acceptable methods of birth control can be used to prevent pregnancy while participating in this trial. If you are pregnant or planning to have a baby in the next year, you should not participate in this study. However, if you do become pregnant during the trial, notify your study eye care provider immediately.

Blood Collection

About 2 tablespoons of blood will be drawn for laboratory testing. This procedure will be performed either by the study eye care provider or study staff. This sample will be sent to a laboratory to check the level of fatty acids in your blood to make sure the pills are being taken properly.

At this visit, a second sample of about 1 teaspoon of blood will be taken during the same blood draw. This sample will be used to test for antibodies that may be associated with Sjögren’s Syndrome or other autoimmune diseases (conditions that occur when your body tissues are attacked by your own immune system and that can sometimes cause dry eyes). The blood will be analyzed at the end of the study and you will receive the results of the test after your last DREAM visit.

Impression Cytology

In this test, a numbing eye drop is placed on each eye so you feel no pain and then a piece of filter paper is gently touched to the white part of the eye using blunted forceps. Blunted forceps are like dull tweezers. After 1-2 seconds on the eye, the paper is removed, placed into a tube, and sent to a laboratory to test if there are signs of inflammation.

Study phone calls and letters

During your participation in the study, Dr. NAME OF PI or study staff will call you about two weeks before each visit to remind you of your appointment. We will also call you about a week after you receive your study pills to check how you are doing once you start taking the study pills. Between the visits, we will call you to check how you are doing and to confirm your next appointment. If you forgot to bring your bottle of pills at your previous visit, we will call you the day before your next scheduled visit to remind you to bring your bottle of pills to the next visit. Finally, we will call you one last time one month after your last visit. We will also mail you postcards or letters with reminders about taking study pills.
If you give us permission (below), we will leave messages on your telephone answering machine when no one answers our telephone call. Also, if you give us your permission and prefer contact by email, we will send the reminders to you by email.

If you notice any pain, new eye irritation or change of vision after the tests, contact the eye care provider conducting this study, Dr. NAME OF PI or associates, immediately at (212) 824-7644.

A description of this clinical trial is available on http://www.ClinicalTrials.gov. This Web site does not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

**What are the possible risks or discomforts?**

The most common side effect of omega-3 supplementation for approximately 5% of the population is upset stomach, burping, or fishy aftertaste. Some studies have shown that patients taking omega-3 and an anticoagulant or other drug affecting the clotting of blood might have prolonged bleeding time (cuts you receive may take longer to stop bleeding). However, other studies have suggested no relationship between them. If you notice excessive bleeding, you should stop taking your study pills and notify the study clinician immediately.

Some studies have suggested that omega-3 could be a risk factor for prostate cancer. However, other studies have suggested no relationship between them. Because prostate cancer is a disease that is associated with many genetic and other factors, further research will be required to clarify the role of these fatty acids.

Large doses of heavy metals such as (arsenic, cadmium, lead, mercury) may be linked to prostate cancer. However the DREAM pills don't have a large dose of heavy metals and the amount of these elements in DREAM pills are within the safety dosage limits.

Fluorescein and lissamine dye eye drops are safe, but rarely cause an allergic reaction. Side effects from their use on the eye include redness, watering, itchiness, eye discoloration, irritation of the eye and swelling of the eyelids. These side effects usually go away in about an hour after the drops are put on the eye. These side effects are rare.

Impression cytology using small pieces of sterile filter paper placed on the numbed eye may cause mild eye irritation that usually subsides within a few minutes. There is a very small risk of eye infection (pink eye) but only sterile equipment is used and right afterwards a drop of eye antibiotic will be placed on the eye to decrease the risk of infection even further.

The study eye care provider or study staff will take your blood by sticking a small needle through your skin, as is done in routine blood sampling for a blood test. The risks of blood drawing include bleeding, pain, bruising, and the slight possibility of infection at the place where the needle goes in. Some people feel dizzy or may faint during or after a blood drawing, so we will ask you to sit for a few minutes after taking the blood sample.

If you become injured during the study, you should inform the treating doctor or nurse that you are participating in a research study.
What if new information becomes available about the study?
During the course of this study, we may find more information that could be important to you. This includes information that, once learned, might cause you to change your mind about being in the study. We will notify you as soon as possible if such information becomes available.

What are the possible benefits of the study?
It is important to know that you may not get any benefit from taking part in this research. Others may not benefit either. However, possible benefits may be relief from your symptoms associated with dry eye disease, such as irritation, foreign-body sensation, itching, burning, and blurred vision.

Your participation in this study will help eye care clinicians diagnose and monitor dry eye disease more effectively and will help future dry eye research and future treatment for patients with dry eyes.

What other choices do I have if I do not participate?
You may decide not to take part in this research study without any penalty. The choice is up to you. Participation in this study is optional and the alternative to participation is not to participate and to continue managing your dry eyes as you are now doing. You may discuss any alternatives with your eye care clinician.

Will I be paid for being in this study?
If you agree to take part in this research study, we will pay you $25.00 for each visit in appreciation of you making the effort to complete the visit. For the visit at 24 months, you will receive an additional $30.00. For this study, the maximum payment to you would be $80. Please note: In order to be compensated for your participation in this study, you must provide your Social Security Number. Additionally, please note that the Name of Practice is required to report to the IRS any cumulative payments for participation in research studies that exceed a total of $600 in a calendar year.

Will I have to pay for anything?
There will be no extra charge to you or your insurance company for study related activities that are not part of standard eye care. You and/or your health insurance will be billed for standard eye care and visits that are part of regular eye care.

You are still responsible for any deductibles or applicable co-pays for routine office visits. Please talk to your eye care clinician and study team about putting you in touch with a financial counselor to determine exactly what the deductible and co-pay will be for you; this is highly variable depending on your type of insurance.

What happens if I am injured from being in the study?
In the event of injury resulting from your participation in this research study, we will offer you the care you need. We may bill your insurance company or other third parties, if appropriate, for the costs of the care you get for the injury, but you may also be responsible for some of them.
There are no plans for the University of Pennsylvania to pay you or give you other compensation for the injury. You do not give up your legal rights by signing this form.

If you think you have been injured as a result of taking part in this research study, you should contact Dr. (Fill in name) at telephone number (212) 824-7644 or contact the Institutional Review Board at telephone number (215) 898-2614.

When is the Study over? Can I leave the Study before it ends?

This study is expected to end after all participants have completed all visits, and all information has been collected.

This study may also be stopped at any time by your physician, the study Sponsor, or the Food and Drug Administration (FDA) without your consent because:

• The Primary Investigator feels it is necessary for your health or safety. Such an action would not require your consent, but you will be informed if such a decision is made and the reason for this decision.
• You have not followed study instructions.
• The Sponsor, the study Principal Investigator, or the Food and Drug Administration (FDA) has decided to stop the study.

If you decide to participate, you are free to leave the study at any time without any penalty. Withdrawal will not affect your ability to receive medical care at Name of Practice or to receive any benefits to which you are otherwise entitled.

If you decide to stop being in the research study, please contact the Principal Investigator listed on first page or the research staff.

You may withdraw your permission for the use and disclosure of any of your protected information for research, but you must do so in writing to the Principal Investigator at the address on the first page. Even if you withdraw your permission, the Principal Investigator for the research study will still use the information that was already collected if that information is necessary to complete the study. Your health information may still be used or shared after you withdraw your authorization if you should have an adverse event (a bad effect) from participating in the study.

If you stop being in the research study, information already collected will not be removed from the research study database and will continue to be used to complete the research analysis.

Who can see or use my information? How will my personal information be protected?

As you take part in this research project it will be necessary for the research team and others to use and share some of your private protected health information. Consistent with the federal Health Insurance Portability and Accountability Act (HIPAA), we are asking your permission to receive, use and share that information.
What protected health information is collected and used in this study, and might also be disclosed (shared) with others?

As part of this research project, the researchers will collect your:

- Name
- Address
- Telephone number
- Ocular Medical History (includes current and past medications or therapies, illnesses, conditions or symptoms, allergies, etc.)
- Physical and Emotional Health Information

During the study the researchers will gather information by:

- taking a medical history (includes current and past medications or therapies, illnesses, conditions or symptoms, allergies, etc.)
- completing the tests, procedures, questionnaires and interviews explained in the description section of this consent.

Why is your protected health information being used?

Your personal contact information is important to be able to contact you during the study. Your health information and the results of any tests and procedures being collected as part of this research study will be used for the purpose of this study as explained earlier in this consent form. The results of this study could be published or presented at scientific meetings, lectures, or other events, but would not include any information that would let others know who you are, unless you give separate permission to do so.

The research team and other authorized members of NAME OF PRACTICE may use and share your information to ensure that the research meets legal, institutional or accreditation requirements. For example, the Institutional Review Board at the University of Pennsylvania is responsible for overseeing research on human subjects, and may need to see your information. If you receive any payments for taking part in this study, the NAME OF PRACTICE Finance Department will need your name, address, social security number, payment amount, and related information for tax reporting purposes. If the research team uncovers abuse, neglect, or reportable diseases, this information will be disclosed to appropriate authorities.

Who, outside NAME OF PRACTICE might receive your protected health information?

As part of the study, the Principal Investigator, study team and others in the Name of Practice workforce may disclose your protected health information, including the results of the research study tests and procedures, to the following people or organizations: (It is possible that there may be changes to the list during this research study; you may request an up-to-date list at any time by contacting the Principal Investigator.)
• Research data coordinating office and/or their representative(s) at the University of Pennsylvania who will be responsible for sending out the study supplements, collecting results and findings from all the centers.

• The National Eye Institute, the sponsoring government agency and/or their representative who need to confirm the accuracy of the results and the use of government funds

• The United States Food and Drug Administration

• The United States Department of Health and Human Services and the Office of Human Research Protection.

• Outside laboratories who will be performing laboratory analysis for all the research centers involved in this clinical trial (Ocular Biomarker Laboratory at Icahn School of Medicine at Mount Sinai and Kreiger Laboratory at Johns Hopkins Medical Center).

• A Data Safety Monitoring Board or other committee that will monitor the study on an ongoing basis for safety.

The Coordinating Center at the University of Pennsylvania will receive your name, address, and telephone number so that they can send study supplements directly to your home. In all other disclosures outside of NAME OF PRACTICE, you will not be identified by name, social security number, address, telephone number, or any other direct personal identifier, unless disclosure of the direct identifier is required by law. Some records and information disclosed will be identified with a unique code number. The Principal Investigator will ensure that the key to the code will be kept in a locked file, or will be securely stored electronically. The code will not be used to link the information back to you without your permission, unless the law requires it, or rarely if the Institutional Review Board allows it after determining that there would be minimal risk to your privacy. Monitors, auditors, the IRB, the Food and Drug Administration will be granted direct access to your medical records for verification of the research procedures and data. By signing this document you are authorizing this access. We will publish the results of this research. However, we will keep your name and other identifying information confidential.

For how long will NAME OF PRACTICE be able to use or disclose your protected health information?

Your authorization for use of your protected health information for this specific study does not expire.

Will you be able to access your records?

During your participation in this study, you will not be able to access your study records. This is to prevent the knowledge of study results from affecting the reliability of the study. Your information will be available should an emergency arise that would require your treating physician to know this information to best treat you. You will have access to your medical record and any study information that is part of that record when the study is over or earlier, if possible. The investigator is not required to release to you research information that is not part of your medical record.
Can you change your mind?
You may withdraw your permission for the use and disclosure of any of your protected information for research, but you must do so in writing to the Principal Investigator at the address on the first page. Even if you withdraw your permission, the Principal Investigator for the research study will still use your protected information that was already collected if that information is necessary to complete the study. Your health information may still be used or shared after you withdraw your authorization if you should have an adverse event (a bad effect) from being in the study. If you withdraw your permission to use your protected health information for research that means you will also be withdrawn from the research study, but standard medical care and any other benefits to which you are entitled will not be affected. You can also tell us you want to withdraw from the research study at any time without canceling the Authorization to use your data.

It is important for you to understand that once information is disclosed to others outside Name of Practice, the information may be re-disclosed and will no longer be covered by the federal privacy protection regulations. However, even if your information will no longer be protected by federal regulations, where possible, Name of Practice has entered into agreements with those who will receive your information to continue to protect your confidentiality.

Electronic Medical Records and Research Results

What is an Electronic Medical Record?

An Electronic Medical Record (EMR) is an electronic version of the record of your care within a health system. An EMR is simply a computerized version of a paper medical record.

If you are receiving care or have received care within the Name of Health System affiliated with Practice or the Name of the Practice (outpatient or inpatient) and are participating in a Name of Practice research study, results of research-related procedures (i.e. laboratory tests, imaging studies and clinical procedures) may be placed in your existing EMR maintained by Name of Practice.

If you have never received care within the Name of Health System affiliated with Practice or the Name of the Practice and are participating in a Name of Practice research study that uses the Name of Health System affiliated with Practice services, an EMR will be created for you for the purpose of maintaining any results of procedures performed as part of this research study. The creation of this EMR is required for your participation in this study. In order to create your EMR, the study team will need to obtain basic information about you that would be similar to the information you would provide the first time you visit a hospital or medical facility (i.e. your name, the name of your primary doctor, the type of insurance you have). Results of research procedures performed as part of your participation in the study (i.e. laboratory tests, imaging studies and clinical procedures) may be placed in this EMR.

Once placed in your EMR, these results are accessible to appropriate the Name of Health System affiliated with Practice workforce members that are not part of the research
team. Information within your EMR may also be shared with others who are determined by the Name of Health System affiliated with Practice to be appropriate to have access to your EMR (e.g. health insurance company, disability provider, etc).

Who can I call with questions, complaints or if I’m concerned about my rights as a research subject?

If you have questions, concerns or complaints regarding your participation in this research study or if you have any questions about your rights as a research subject, you should speak with the Principal Investigator listed on page one of this form.

This research has been reviewed and approved by an Institutional Review Board. If a member of the research team cannot be reached or you want to talk to someone other than those working on the study, you may contact the Office of Regulatory Affairs at the University of Pennsylvania with any question, concerns or complaints by calling (215) 898-2614.

When you sign this form, you are agreeing to take part in this research study. This means that you have read the consent form, your questions have been answered, and you have decided to volunteer. Your signature also means that you are permitting the Name of Practice to use your personal health information collected about you for research purposes within our institution. You are also allowing the Name of Practice to disclose that personal health information to outside organizations or people involved with the operations of this study.

A copy of this consent form will be given to you.

**Signature Block for Capable Adult**

Your signature or mark below documents your permission to take part in this research and to the use and disclosure of your protected health information. A signed and dated copy will be given to you.

DO NOT SIGN THIS FORM AFTER THIS DATE

Signature or mark of subject ___________________________ Date and Time

Printed name of subject ___________________________
PERMISSION TO LEAVE A MESSAGE ON VOICEMAIL OR A TELEPHONE ANSWERING MACHINE

By signing below, I authorize you to leave a message on the voicemail or telephone answering machine of the phone number listed below if I am not available to answer the phone. I understand that the messages will be about future study visits, instructions to bring study pills to the office visit and reminders to take the study pills as directed.

____________________________________
Signature or mark of subject

____________________________________
Printed name of subject

Number to call to leave a message if no answer (with area code): ______________________________

PERMISSION TO SEND STUDY REMINDERS BY EMAIL

By signing below, I authorize you send me emails at the address below about future study visits, instructions to bring study pills to the office visit and reminders to take the study pills as directed.

____________________________________
Signature or mark of subject

____________________________________
Printed name of subject

Email address: ______________________________

**Person Explaining Study and Obtaining Consent**

____________________________________
Signature of person obtaining consent  Date and Time

____________________________________
Printed name of person obtaining consent
Chapter 12
CLINICAL CENTER STAFF RESPONSIBILITIES AND CERTIFICATION

12.1. INTRODUCTION
Each Clinical Center in DREAM Study is directed by the local DREAM Principal Investigator, who is certified as a DREAM Clinician. Each clinical center must have one or more people who fill the following roles:

- **Clinician**: Responsible for enrolling, treating, and following DREAM patients;
- **Clinic Coordinator**: Responsible for supervising activities related to the DREAM study and integrating these with clinic operations and entering all data.
- **Technician**: Responsible for performing some or all of 9 clinical tests (manifest refraction, contrast sensitivity testing, MMP-9 testing, collecting impression cytology samples, IOP measurement, Schirmer’s testing, tear osmolarity measurement, keratography and collecting tears for cytokine analysis) and recording the findings on study CRFs.

In addition, each clinical center must be fully certified with respect to having the required space, equipment, resource materials and staffing, including an IATA-certified individual responsible for the shipping of blood, impression cytology samples and tear samples.

12.2. RESPONSIBILITIES OF CLINICAL CENTERS
The responsibilities of each DREAM Clinical Center team include the following:

- To recruit a sufficiently sized staff such that the Clinical Center will have at least one person certified for each required DREAM role at all times during the trial;
- To assure that all DREAM staff are trained and certified as required.
- To assess eligibility of patients for the DREAM Study.
- To enroll the number of eligible participants per month required by the study design;
- To maintain up-to-date informed consent documents that meet DREAM and local institutional review board (IRB) standards;
- To maintain all equipment and supplies required for DREAM;
- To properly store and account for all bottles of study drug received from the Investigational Drug Distribution Service;
- To arrange for each patient enrolled in DREAM to be examined in the DREAM clinic according to the schedule established.
- To ensure that all required procedures are conducted at each study visit;
- To complete the proper data collection forms and obtain the other materials required at each scheduled clinic examination.
- To maintain DREAM participant records in an easily accessible but confidential manner;
- To transmit all forms, documents, and materials to the Coordinating Center and study laboratories as expeditiously as possible after each clinic visit.
- To respond promptly to requests from the Coordinating Center and laboratories.
• To provide representation (Principal Investigator and Clinic Coordinator) at all meetings of the DREAM Investigative Group.
• To cooperate in scheduling and conduct of the site monitoring visits.

12.3. CLINICAL CENTER PRINCIPAL INVESTIGATOR RESPONSIBILITIES

Clinical Center Principal Investigator responsibilities include:

• To have a thorough understanding of the DREAM design and methods;
• To ensure that local institutional review board requirements are satisfied and approvals obtained and maintained;
• To ensure that the appropriate DREAM-certified staff, facilities, and equipment are in place and available for study procedures when needed;
• To oversee the maintenance of study documentation at the clinical center;
• To open clinic and study files of participants evaluated for the DREAM and otherwise assist site visitors during clinic monitoring visits;
• To notify the Coordinating Center promptly if any systematic data collection or reporting problems are discovered or suspected;
• To notify the Coordinating Center promptly when there are personnel changes that affect the study;
• To attend scheduled meetings of the DREAM Investigative Group and committees on which the Center Principal Investigator may serve.

12.4. DREAM Clinician Responsibilities

Responsibilities of clinicians who are participating in DREAM include:

• To have a thorough understanding of the DREAM design and methods;
• To participate in the informed consent process for DREAM participants;
• To perform ocular examinations of study patients;
• To ensure that the patient understands the treatment regimen;
• To assess the severity of Dry Eye signs and symptoms at each visit.

12.5. CLINIC COORDINATOR

12.5.1. Qualifications

Because day-to-day responsibility for most clinical center tasks falls to the Clinic Coordinator, it is important that this individual be selected carefully, thoroughly trained in the DREAM protocol, and recognized as the local DREAM "expert" in the clinical center. It is essential that the Clinic Coordinator is recognized by the center Principal Investigator and local institutional administration as a collaborating member of the DREAM research team. It is also important that the Clinic Coordinator allocates sufficient time to the myriad of activities required for the project.

The Clinic Coordinator has extensive contact with DREAM patients; therefore it is important that this individual have the ability to deal well with people. The rapport that frequently develops between a patient and the Clinic Coordinator is extremely important to assuring the continued cooperation of a patient throughout the course of a study. Patients frequently turn to the Clinic Coordinator for clarification or confirmation of their discussions with the clinicians; therefore, it is
mandatory that the Clinic Coordinator be a mature, responsible person with a thorough understanding of the DREAM protocol, design, and rationale. In addition, the Clinic Coordinator must have excellent organizational skills and attention to detail.

12.5.2. Responsibilities

The responsibilities of the DREAM Clinic Coordinator include, but are not limited to the following:

- To coordinate clinical center activities related to DREAM;
- To assist the Center Principal Investigator in obtaining and maintaining IRB approval for DREAM;
- To maintain the clinic organization as a well-coordinated unit for evaluating, treating, and following DREAM patients.
- To have a thorough understanding of the DREAM design and methods;
- To serve as a resource for other clinic personnel concerning the details of the protocol and decisions requiring notification of or approval from the Executive Committee.
- To assist other clinic personnel with DREAM certification;
- To participate in the informed consent process during participant recruitment;
- To schedule and coordinate patient examinations;
- To remind patients of upcoming study visits and to bring their bottles of unused medication.
- To dispense bottles of run-in study supplements and collect unused gelcaps/bottles
- To account for all study medication
- To ensure that DREAM patients understand the study treatment regimen
- To store and ship blood, Impression Cytology and tear (for sites collecting tears) samples to the appropriate laboratories
- To obtain hospital records regarding serious adverse events experienced by DREAM patients and to report SAEs to the local IRB if required.
- To review all forms and materials for completeness and accuracy before submitting data to the Coordinating Center;
- To record and enter from completed case report forms into the DREAM database through the web-based data system;
- To promptly respond to queries from the Coordinating Center and central laboratories regarding information, documents and samples required for DREAM;
- To coordinate use of DREAM patient education and recruitment materials, including slides, brochures, and exhibits provided by the Coordinating Center.
- To be the primary interface between the clinical center and the Coordinating Center by being the primary recipient of incoming mail from the US Postal Service, express carriers such as Federal Express, FAX communications, voice mail, and e-mail;
- To distribute materials and information to the appropriate DREAM team members.
- To maintain required DREAM documentation including:
  - Up to date DREAM Manual of Procedures
- Recruitment logs
- Patient Log, containing DREAM identifiers, patient name, and enrollment date.
- Patient study file, containing the signed consent forms, follow-up schedule, completed CRFs and the patient information sheet for individual study patient.
- Current addresses and other information required to maintain contact with each participant
- Copies of any paper study forms, including signed copies of the consent documents
- Up-to-date study drug dispensing and return logs
- Logs of tear, impression cytology and blood samples sent to the central laboratories

- To coordinate local arrangements for clinic monitoring visits so that all DREAM-certified personnel are available.
- To communicate with the Coordinating Center concerning problems with maintaining data quality;
- To notify the Coordinating Center concerning personnel changes that affect local DREAM operations.
- To inform the clinical center Principal Investigator of any problems with clinic management and to suggest ways to resolve them.
- To ensure that all budgetary items required for subcontracting are prepared and submitted in an accurate and timely manner.
- To attend scheduled meetings of the DREAM Research Group.

12.5.3. Internal Communication

The Clinic Coordinator should make certain that each person certified for the DREAM study understands the role of the Clinic Coordinator. Telephone numbers and work schedules should be exchanged.

The Clinic Coordinator should explore benefit programs for patients within the local institution and learn how to facilitate patient participation in these programs. These may include reduced parking charges, low cost meals, special arrangements for local transportation, etc. Clinic Coordinators from other studies are useful contacts for learning about local resources.

The Clinic Coordinator should arrange with the clinical center’s Principal Investigator to meet regularly and to organize time for DREAM study clinic staff meetings particularly during the clinic start-up and patient enrollment phases.

12.5.4. Interaction with Coordinating Center

The Coordinating Center staff works closely with the Clinic Coordinator to resolve any problems that arise at DREAM clinical centers. The Coordinating Center staff has primary responsibility for training candidates for certification as DREAM Clinic Coordinators, with the assistance of the Chairman’s office when required.
The Coordinating Center staff provides the following materials to the Clinic Coordinator:

- A copy of the *DREAM Study Manual of Procedures* and revisions as they become available.
- A copy of the *DREAM Study Forms Book*, which includes copies of all forms used in the DREAM, whether for data collection, clinic management, or study management. These paper copies will serve as a back-up for the rare instance that the web-based materials are not accessible.
- A patient log for assigning DREAM Study patient identification numbers. A copy of the *DREAM Address Registry* that includes the name, address, telephone numbers, FAX, and e-mail address of personnel in all DREAM clinical centers and members of DREAM committees.
- Recruitment aids, such as copies of informational brochures for patients and referring clinicians and slides for presentations at professional meetings.
- Other clinic and patient management aids, such as individual patient follow-up schedules, visit reminders, and printed labels for blood, impression cytology and tear samples, supplement accountability logs, and pill counting.

Study forms are available online and are also provided in a Forms Notebook by the Coordinating Center. Whenever DREAM forms have been revised, the Clinic Coordinator is responsible for seeing that all old versions in the clinical center are destroyed so that they are not used by mistake. Under no circumstances should outdated forms be used. The Clinic Coordinator is responsible for explaining to other clinic staff any changes in procedures that are required by form revisions. He/she should consult personnel at the Coordinating Center whenever uncertain about such changes.

To supplement information in the Manual of Procedures and to communicate new procedures and policy expeditiously between updates to the Manual, numbered DREAM protocol memoranda are sent from the Coordinating Center or central laboratories. One copy of each memorandum should be filed in numeric order in a binder or file folder set up specifically for this purpose. A second copy should be inserted in the Manual of Procedures with the appropriate chapter and retained until the information is incorporated into the next revision of the chapter. Additional copies are made for any DREAM staff member who is affected by the new or revised information.

12.5.7. Interaction with Central Laboratories

Staff at the central laboratories are available to resolve problems or address inquiries that arise regarding obtaining, storing and submitting blood and tear samples. Clinic coordinators must maintain meticulous records about all materials submitted to the laboratories, including recording tracking numbers of all shipments.

12.5.8. Interaction with Study Patients

One of the most important duties of the Clinic Coordinator is maintaining good rapport with all DREAM patients and assuring that each patient’s whereabouts are known at all times. Thus, it is essential that all requested contact information at the Screening Visit be recorded in full and updated regularly. Patients who die before follow-up in the DREAM is completed are reported to the Coordinating Center as soon as the Clinic Coordinator learns of the death. A Patient Death Report is submitted immediately.
12.5.9. Workspace

With so many responsibilities, it is important that the Clinic Coordinator have adequate workspace. Private office space is necessary for obtaining patient histories, talking with patients and family members, making telephone calls to patients and physicians, and labeling samples. The room should be large enough for the Clinic Coordinator’s desk, file space for DREAM records and patient charts and seating for the patient and family member(s). The doorway and floor space should accommodate a wheelchair. Ideally, the office should be near the DREAM Principal Investigator’s office or primary examination room.

12.5.10 Coordinator Activities during Follow-up Visits

The Clinic Coordinator’s activities during follow-up visits fall within 4 categories: 1) ensuring that all required procedures are conducted during the visit 2) reporting serious adverse events (if necessary), 3) submitting data to the DREAM Coordinating Center and central laboratories, and 4) scheduling the next study visit. Refer to Chapter 4 for additional information on coordinator duties during patient visits.

12.6 Technician (or Person Responsible for Performing the Technician-Specific Tasks):

12.6.1. Qualifications

Because the DREAM Technician will be performing many of the procedures not performed by the clinician, it is vital that this person be an experienced ophthalmic technician. A DREAM clinician or clinic coordinator may also be dually certified as the technician.

12.6.2. Responsibilities

The responsibilities of the technician will vary across Clinical Centers. There are 9 activities (listed below) that are assigned to the DREAM technician; however the clinicians at some centers will perform some of these tasks. The Technician at each site, after consultation with the DREAM PI, will identify which of the following procedures they will perform according to DREAM protocol and record results on study forms:

- MMP-9 testing
- Collecting tears for cytokine analysis (at centers with a -80° C freezer or liquid nitrogen)
- Performing impression cytology
- Maintaining temperature logs for storage of biomarker samples
- Measuring Tear Osmolarity and performing Quality Control of Tear Osmolarity machine (at centers with the TearLab Osmometer)
- Keratography and uploading of results to Coordinating Center (at centers with the Oculus Keratograph)
- Measuring IOP
- Schirmer’s Tear Testing
- Visual acuity testing
- Contrast Sensitivity Testing
12.7 OVERVIEW OF CERTIFICATION PROCEDURES

It is important that all procedures in DREAM be standardized and that all individuals who are part of the DREAM Investigative Group understand the protocol to the degree necessary for them to fulfill their responsibilities. Clinic facilities must also meet specific standards in order to follow the DREAM protocol.

There are specific roles in the clinical centers that must be filled with certified personnel. These roles are DREAM Clinician, Clinic Coordinator and Technician (or person certified to perform the technician-specific roles). The person certified to perform the technician roles can be the same person as the Clinician or Clinic Coordinator. Most aspects of the certification process will be accomplished on-line via the DREAM certification web site.

12.8 CERTIFICATION CRITERIA FOR ALL MEMBERS OF THE INVESTIGATIVE GROUP

Everyone engaged in DREAM must have core knowledge about DREAM so that questions from patients and others may be answered accurately. All members of the Investigative Group must complete a General Knowledge Assessment about DREAM that requires knowledge of such basic facts as the name of the study, the definition of and implications of Dry Eye Disease, the study rationale and treatment, and the primary outcome measure. In addition, individuals are required to complete role-specific assessments, as described below.

Knowledge assessments must be completed by the original DREAM group and by all new personnel at the time of hiring. The Coordinating Center Director and Protocol Monitor are responsible for reviewing the certification materials completed by Clinic Coordinators and contacting the respondents if there are areas of misunderstanding. The study Chairman or her designees at the Coordinating Center is responsible for reviewing materials submitted by clinicians for DREAM certification. The Coordinating Center Director and Protocol Monitor maintain a log of all people who have successfully completed certification requirements.

Prior to becoming eligible for DREAM certification, all investigators and clinic staff must have training in protecting the rights and welfare of human subjects involved in clinical research, and in complying with HIPAA regulations. Current certificates documenting the successful completion of a Human Subjects Training program must be submitted to the Coordinating Center by all members of the investigative group prior to DREAM certification.

Everyone certified for the DREAM study must also submit to the Coordinating Center a signed Affirmation of Integrity in the Submission and Handling of Clinical Trials Research Data.

12.9. CERTIFICATION REQUIREMENTS FOR DREAM CLINICIANS

All DREAM Clinicians are required to be either ophthalmologists or optometrists specializing in diseases of the cornea. Clinicians must have specific knowledge of the major eligibility criteria, examination protocols, treatment regimen and procedures for managing patients during follow-up.

12.9.1. Specific Certification Requirements for Clinicians in the DREAM Study

The certification process entails fulfillment of several criteria to demonstrate knowledge and proficiency in Study procedures. To be certified, the DREAM clinician must:

- Read specific sections of the DREAM Manual of Procedures
• Attend a DREAM Training meeting; or review the slide presentation on performing study procedures according to DREAM protocol (available on the DREAM Certification website and the DREAM Landing Page (http://rt5.cceb.med.upenn.edu/public/dream_home.html)

• Complete the on-line DREAM Study General Knowledge Assessment

• Complete the on-line Clinician's Knowledge Assessment

• Submit documentation of training in the Protection of Human Subjects

• Submit signed Affirmation of Integrity in the Submission and Handling of Clinical Trials Research Data.

• Submit a signed CV and license.

• Submit a Financial Disclosure Form

Upon successful completion of all certification requirements, a valid certification number for each ophthalmologist will be issued by the Coordinating Center.

12.10. CERTIFICATION OF CLINIC COORDINATORS

All DREAM Clinic Coordinators should be experienced in coordinating clinical research. All DREAM Clinic Coordinators must have specific knowledge of the major eligibility criteria, treatment protocols, and procedures during follow-up visits.

12.10.1 Specific Requirements for Clinic Coordinator Certification for DREAM

The certification process entails fulfillment of several criteria to demonstrate knowledge and proficiency in Study procedures. To be certified, the Clinical Coordinator must:

• Read specific chapters of the DREAM Manual of Procedures.

• Attend a DREAM Training meeting; or review of the relevant slide presentations from the training meeting.

• Complete a DREAM Study General Knowledge Assessment.

• Complete a Clinic Coordinator-specific Knowledge Assessment.

• Complete DREAM database training and demonstrate proficiency in understanding the DREAM Case Report forms by completing and submitting a set on paper using a mock scenario

• Submit documentation of training in the Protection of Human Subjects

• Complete a telephone discussion with the Director of the Coordinating Center to assess the Coordinator's knowledge of the DREAM protocol and provide an opportunity to discuss study logistics and answer any remaining questions.

• Submit signed Affirmation of Integrity in the Submission and Handling of Clinical Trials Research Data.

• Submit a signed CV

• Submit a Financial Disclosure Form

A valid certification number will be issued by the Coordinating Center after the knowledge assessments have been completed, database proficiency has been demonstrated, and the telephone call has been successfully conducted.
12.11. CERTIFICATION OF DREAM TECHNICIANS

All DREAM Technicians should be experienced ophthalmic technicians.

12.11.1 Specific Requirements for Technician Certification for DREAM

As stated earlier, the specific tasks performed by the technician will vary by site. To be certified to perform a specific technician role, the candidate must:

- Read specific sections of the DREAM Manual of Procedures
- Complete a DREAM Study General Knowledge Assessment.
- View the relevant training slideshow available from the DREAM certification website and in the DREAM Procedures Toolbox on the DREAM Landing Page (http://rt5.cceb.med.upenn.edu/public/dream_home.html)
- Complete the Procedure-specific Knowledge Assessment
- Submit an Attestation of Training form signed by the Principal Investigator
- Submit documentation of training in the Protection of Human Subjects
- Submit signed Affirmation of Integrity in the Submission and Handling of Clinical Trials Research Data.

12.12 INITIAL CERTIFICATION OF A CLINICAL CENTER

In addition to the individual role specific certifications, the Coordinating Center will also certify the clinical centers. The Clinic Coordinator at each site is responsible for completing the online DREAM Site Certification Checklist. The checklist identifies the requirements to be completed at each clinical center before patient recruitment can be initiated at the site. Application for and receipt of Institutional Review Board approval of the clinical trial, acquisition of the testing equipment required for the DREAM protocol, appropriate facilities to store study medications, receipt of the required Study documents, and certification of at least one staff member in each role are among the items listed. All clinical centers must have the equipment and staffing required by the DREAM protocol available on the days that DREAM patients are scheduled for clinic visits to the site.
CHAPTER 13
OPERATIONS AND PROCEDURES OF THE STUDY CHAIR’S OFFICE

13.1 Responsibilities of Study Chair
The Chair is responsible for the overall conduct of the Study throughout all phases of the study. To facilitate discussion, the responsibilities of the DREAM Study Chair’s Office are organized according to phase of the clinical trial. The phases are categorized as initial design and protocol development, final preparation for trial initiation, patient recruitment, patient treatment and follow-up, patient closeout, and final termination of the trial.

13.1.1 Initial Design Phase and Protocol Development
During the initial design phase of the trial, the Study Chair plays a major role in the following activities:
- Developing the study design with the Planning Committee and Coordinating Center;
- Maintaining the current IND from the Food and Drug Administration (FDA);
- Developing the guidelines for treatment with study drugs;
- Determining eligibility and examination criteria for entry and exclusion to the trial in collaboration with the DREAM Operations Committee;
- Outlining examination, testing, and safety procedures;
- Determining primary and secondary outcomes;
- Actively participating in the selection of DREAM Clinical Centers;
- Developing procedures for training and certifying DREAM clinicians at each clinical center;
- Reviewing procedures for Biomarker Laboratory and sample acquisition, and site training;
- Refining and editing chapters of the Manual of Procedures.

13.1.2 Final Preparation for the Initiation of the Trial
Prior to initiating the Study, a number of activities will be performed by the Chair to begin the trial with a fully developed protocol. These activities include:
- Finalize the protocol details;
- Review all case report forms;
- Review plans for grading of corneal and conjunctival staining, TBUT, MGD evaluation, Tear osmolarity measurements, Tear and Impression Cytology collection and shipping to Biomarker Laboratory;
- Establish agenda for training meetings for key DREAM clinical staff;
- Review patient consent and human subject considerations for local IRB submission;
• Participate in the development of and review of patient recruitment materials including patient information brochures, brochures for referring ophthalmologists, and design slides for presentation by DREAM clinicians at local professional meetings;
• Participate in a meeting of the Data and Safety Monitoring Committee to review the protocol.

13.1.3 Patient Recruitment and Treatment and Follow-up Phase
Activities during this phase can generally be categorized as administrative, problem management, data interpretation and presentation, and planning for future trials. The Study Chair’s responsibilities are summarized for each category.

Study Administration
• To review all requisite reports submitted by the Coordinating Center to meet FDA requirements associated with operating under an IND;
• Participate in the affairs of each of the standing committees, chairing the Operations and Executive Committees;
• Participate in planning and lead the annual Investigative Group meeting;
• Participate in open sessions of the Data and Safety Monitoring Committee (DSMC);
• Serve as the primary source on questions arising concerning ophthalmologic eligibility criteria and patient care;
• Serve as the spokesperson for public relations about the Study beyond local scope, delegating activities to others within the study group deemed appropriate.

Problem management
• Regularly address recruitment issues with the principal investigator and staff at clinical centers;
• Discuss the treatment protocol with study clinicians;
• Address safety issues that arise during the trials;
• Address poor follow up at clinic centers;
• Travel to clinical centers having problems with study performance with the site visitors from the Coordinating Center.

Data interpretation and presentation
• Participate in drafting all primary study publications;
• Establish writing committees for all study publications;
• Reporting the primary results of the study to appropriate audiences.
Planning for future trials

- Keep abreast of scientific developments and industry efforts in the area of new treatments for DED;
- Develop, in conjunction with the Executive Committee, the research design for additional clinical trials for DED.

13.1.4 Patient Closeout Phase

- Help develop a plan for subsequent care of patients completing the study;
- Inform all principal investigators of clinical centers of the requirements for storage and retention of study documents and patient records to be in compliance with NIH, FDA, IRB, and HIPAA guidelines;
- Review all IND amendments and annual reports to the NIH and FDA, prepared by the Coordinating Center, before submission.

13.1.5 Termination Phase

- Develop and maintain a plan for sharing of DREAM data;
- Disseminate DREAM results to medical and news organizations and the public.

13.2 Organization of the Study Headquarters

13.2.1 Internal Organization

The staffing of the Office of the Study Chair includes the following:

- Chair (Principal Investigator);
- Project Manager;
- Administrative Assistant;
- Biomarker Laboratory personnel: Co-Directors, Laboratory Technician/Manager.

13.2.2 Personnel Responsibilities

The responsibilities of the Chair have been described above in detail. Responsibilities of the other members of the Office of the Study Chair follow:

The responsibilities of the **Project Manager** include:

- To assist the Study Chair in all of the above responsibilities and as necessary to meet the needs of the Study;
- To participate as a member of the Planning Committee, Executive Committee and the Operations Committee;
- To serve as the central point of contact to the Office of the Chair, coordinating issues related to patient management such as recruitment, eligibility criteria, follow-ups, medical management, etc.;
- To provide agendas for meetings from the Chair’s office with inputs from the Coordinating Center;
- To record minutes of the meetings;
The **Administrative Assistant** will provide administrative support to the Study Chair. Specific responsibilities include:

- To maintain an up-to-date study resource materials for the Office of the Study Chair in both printed and electronic from including the study telephone, address, FAX, and e-mail directory (distributed by the Coordinating Center), *Manual of Procedures*, protocol memoranda, recruitment materials, and materials provided in support of study meetings;
- To prepare documents written by the Study Chair and transfer them to the Coordinating Center for distribution or directly to specific DREAM committees;
- To monitor grant expenditures and prepare materials for the annual non-competing grant continuations;
- To make travel arrangements;
- To maintain office supplies;
- To assist the Chair and Project Manager as necessary to meet the needs of the Study.

The **Co-Directors** of the Biomarker Laboratory will oversee smooth operations of all laboratory procedures, under the direction of the Study Chair:

- To ensure that all procedures follow GLP;
- To oversee the Technician / Sample Coordinator to ensure all samples are tracked on receipt at MSSM, stored appropriately, and analysis data entered into computer files and transmitted to the Coordinating Center and computer backup in place to ensure no lost data;
- Perform analyses, as outlined in MOP: HLA DR expression of ocular surface cells and tear cytokine concentrations;
- Keep clear and complete records of all laboratory activities;
- Provide regular reports to the Study Chair and the CC of laboratory activities.

The **Lab Technician/ Sample Coordinator** assists the Biomarker Laboratory Co-Directors:

- Tracks all samples received at Biomarker Laboratory;
- Places samples in appropriate storage on arrival;
- Assists in analysis procedures;
- Enters final data into computer files;
- Transmits data to CC;
- Keeps clear and complete records of all activities.
CHAPTER 14
COORDINATING CENTER OPERATIONS AND PROCEDURES

14.1 Responsibilities of the Coordinating Center
The Coordinating Center’s responsibilities are varied and change as the study progresses. To facilitate discussion, the responsibilities of the DREAM Coordinating Center are organized according to the phase of the clinical trial. The phases are categorized as initial design and protocol development, final preparation for trial initiation, patient recruitment, patient treatment and follow-up, patient closeout, and final termination of the trial.

14.1.1 Initial Design Phase and Protocol Development
During the initial design phase of the trial, the Coordinating Center staff plays a major role in the following activities:

- Developing the study design, including sample size calculations;
- Outlining the data collection schedule;
- Outlining plans for data analysis;
- Drafting chapters of the Manual of Procedures;
- Outlining the data collection forms;
- Planning the design of a World Wide Web-based data capture system;
- Customizing a web-based certification system for staff at DREAM Clinical Centers
- Outlining data management procedures, including the division of labor and responsibility between data management staff within the Center for Preventive Ophthalmology and Biostatistics (CPOB) and staff within the Clinical Research Computing Unit (CRCU);
- Planning the supply of supplements and placebos with Nutrilite
- Collaborating with the Investigational Drug Distribution Service in developing plans for drug repackaging, distribution and destruction;
- Implementing communication plans between the Coordinating Center, CRCU, Drug Distribution Service, Central Laboratories, Reading Centers, and the Study Chair’s Office;
- Initiating the Purchased Services Agreements with DREAM clinical centers;
- Developing procedures to report serious adverse events;
- Developing quality assurance procedures for all aspects of the DREAM.
14.1.2 Final Preparation for the Initiation of the Trial

Prior to initiating the Study, a number of activities will be performed by the staff of the Coordinating Center to begin the trial with a fully developed protocol and well trained staff for all aspects of the study. These activities include:

- Finalizing protocol details;
- Drafting, pilot testing, and finalizing the data collection forms;
- Generating treatment allocation schedules for patients;
- Providing the CRCU and Investigational Drug Service with treatment allocation schedules for incorporation into the randomization process;
- Finalizing the data collection and data management system to integrate all Study activities including laboratory data;
- Preparing other materials to be used by clinical center staff, such as patient logs and other auxiliary forms;
- Completing beta testing of the web-based DREAM data system for baseline forms and the randomization module;
- Completing beta testing of the web-based DREAM data system with follow-up visit forms;
- Completing beta testing of the web-based clinic staff certification system;
- Collaborating with the Study Chair in organizing the agenda for orienting, training, and certification of clinic staff at a kickoff Investigative Group training meeting;
- Refining the patient consent and developing the IRB protocol for local IRB submission;
- Training and certifying DREAM Clinic Coordinators;
- Distributing the Manual of Procedures and Address Registry to all clinical centers;
- Supplying each clinical center with a set of study data collection form masters;
- Developing and distributing Power Point presentations for clinical center use to address local physician groups for enrollment;
- Developing a newsletter for clinic staff to enhance communication and study recruitment;
- Establishing an electronic and paper repository for DREAM study documents, such as minutes, manuals, etc.;
- Ensuring that each clinical site has the required equipment, supplies and study run-in supplements;
- Establishing clear communications between the Coordinating Center and the Investigational Drug Distribution Service at the University of Pennsylvania;
• Establishing clear communications between the clinical centers and all DREAM resource centers (Coordinating, Central Laboratory for Fatty Acids, Office of the Study Chair and laboratories, and Drug Distribution Center);

• Participating in a meeting of the Data and Safety Monitoring Committee to review the protocol;

• Developing and implementing procedures for certifying DREAM clinical centers;

• Finalizing procedures for site visits to clinical centers;

• Initiating site visits by Protocol Monitors;

• Collaborating with the laboratories to finalize the quality control program for laboratory testing;

• Developing an informational study website for use by DREAM personnel at the clinical centers and resource centers;

• Preparing and distributing minutes of meetings of the Data and Safety Monitoring Committee and the Clinic Monitoring Committee;

• Establishing and maintaining an electronic roster of certified study personnel.

14.1.3 Patient Recruitment and Enrollment, Treatment and Follow-up Phase
Activities during this phase can generally be categorized as administrative, data collection and management, data analysis and reporting, quality assurance, and planning for future phases. Coordinating Center responsibilities are summarized for each category.

Study Administration

• Participating in the affairs of each of the standing committees;

• Coordinating and providing the necessary materials in support of all study meetings;

• Coordinating communications among the various functional units and committees;

• Assisting the staff of each clinical center to interpret and follow the protocol and procedures documented in the Manual of Procedures and triaging medical issues to the Office of the Study Chair;

• Managing the Purchased Services Agreements with clinical centers;

• Supplying the clinical centers with new and revised data collection forms, template consent forms and other printed materials;

• Maintaining accurate study archives, including study history and proceedings of committee meetings;

• Preparing and distributing to clinical centers reminders of upcoming patient visits and materials overdue;

• Coordinating the annual meetings of the Investigative Group in collaboration with the Office of the Study Chair.
- Maintaining an accurate DREAM telephone, address, fax, and e-mail directory;
- Publishing and distributing study newsletters for patients and clinical center staff;
- Developing and implementing an informational study website for study patients.

**Data Collection and Management**
- Receiving data electronically submitted from the clinical centers via the web-based data capture system;
- Assisting clinical center staff with patients for whom follow-up is a problem;
- Receiving and maintaining SAS datasets of all data records created by laboratories;
- Providing on-going support and reference to the Clinic Coordinators regarding data collection and adverse event reporting procedures;
- Performing edit checks to ensure high quality data.

**Data Analysis and Reporting**
- Preparing reports for the Investigative Group concerning the status of patient recruitment, patient follow-up, and adherence to the protocol;
- Preparing study-wide adverse event reports for the DREAM Medical Safety Monitor and FDA;
- Preparing periodic reports for the Data and Safety Monitoring Committee concerning adverse and beneficial treatment effects;
- Preparing and submitting all reports needed to meet FDA requirements associated with operating under an IND;
- Developing analytic methods appropriate to the study design, in conjunction with the Data and Safety Monitoring Committee;
- Preparing all analyses to be reported in publications from the study;
- Participating in the drafting of all study publications;
- Performing other analyses deemed appropriate by the Executive Committee, Data and Safety Monitoring Committee, or other Study participants as time permits;
- Monitoring the accumulating data to determine whether the assumptions used to calculate sample size requirements are met and recommending modifications to the study design if these appear to be necessary;
- Reporting to appropriate audiences statistical and methodological innovations developed during the course of the study.

**Quality Assurance**
- Conducting initial training sessions for clinic personnel to review study design, data collection methods, adverse event reporting, and procedures for interfacing
with the Drug Distribution Service, the Central Laboratory for Fatty Acid, the laboratories on HLA-DR and cytokine determination, the Office of the Study Chair, and the Coordinating Center;

- Visiting each clinical center to review procedures, verify data entered from source medical records, and to “troubleshoot” in any area in which the clinical site may require it;
- Preparing monthly reports summarizing patient recruitment in each clinical site,
- Preparing quarterly reports on data quality and protocol adherence in the clinical centers for the Clinic Monitoring Committee;
- Maintaining documentation of all procedures and operations at the Coordinating Center;
- Maintaining the data files in a secure manner to assure their integrity and adherence with HIPAA requirements;
- Backing up the data files to assure that data are not lost;
- Reporting periodically on the quality of the data accumulated at the Coordinating Center;
- Cooperating with any individual or group assigned to review operations at the Coordinating Center.

Planning for Future Phases

- Maintaining an up-to-date summary of the development status of alternative treatments for dry eye disease;
- Developing procedures for closing out patient follow-up at the appropriate time;
- Implementing a data sharing plan consistent with NIH guidelines;
- Planning for permanent, accessible storage of study records and data.

14.1.4 Patient Closeout Phase
As with earlier phases of the study, the primary responsibilities of the Coordinating Center staff during the Patient Closeout phase are centered on coordination, developing, testing, and refining procedures, and data management and analysis. Specific responsibilities during this period are:

- Familiarizing clinic staff with closeout procedures;
- Coordinating patient closeout;
- Monitoring adherence to established procedures for patient closeout as specified by NIH, FDA, and local IRB, HIPAA, and institutional guidelines;
- Developing plans for final data editing and storage;
- Completing plans for final analysis and preparation of publications;
- Participating in paper writing activities;
• Providing a mechanism for continuing communications among investigators and performing additional analyses;
• Monitoring of archiving of images and destruction of excess study medication.

14.1.5 Termination Phase
During the last phase of the study, communications with the investigators at the clinical centers will continue to be important. The Coordinating Center anticipates engaging in the following activities during this period:

• Completing scheduled data analyses;
• Placing data files, documentation, and other materials in the selected archives;
• Distributing draft manuscripts and reprints of publications to the other investigators;
• Preparing Power Point presentations summarizing results from publications for use by DREAM investigators;
• Serving as the communications center for the study.

14.2 Organization of the Coordinating Center

14.2.1 Internal Organization
Staffing at the Coordinating Center may change as the study progresses. The staffing of the Coordinating Center includes the following roles as organized in Exhibit 14-1:

• Principal Investigator
• Director
• Associate Project Director/Protocol Monitor
• Systems Analyst
• Administrative Assistant
• Administrative Coordinator
• Senior Biostatistician
• Biostatistician
• CRCU Project Director
• Financial Administrator

14.2.2 Personnel Responsibilities
The Coordinating Center Principal Investigator has responsibility for providing leadership and guidance to the study in areas related to study design, administration, and implementation. The Principal Investigator also has overall responsibility for all functions of the Coordinating Center and works closely with the Director to determine the general approach and methods to be used in each area of Coordinating Center operations. Specific responsibilities include:
• To serve as a voting member of the DREAM Operations Committee with responsibility contributing to the agenda for each meeting prepared by the Office of the DREAM Study Chair;

• To serve as a voting member of the DREAM Executive Committee with responsibility for contributing to the agenda for each meeting prepared by the Office of the DREAM Study Chair;

• To organize and plan for meetings for the Investigative Group, in collaboration with the DREAM Chair;

• To lead internal meetings of the Coordinating Center staff;

• To provide advice and guidance to the Coordinating Center staff on methods consistent with the standards of good practice for multicenter clinical trials.

• To consult with the Systems Analyst, CRCU Project Director, and Biostatisticians in the refinement of the data management system and development of new subsystems;

• To work with the Biostatisticians to design analyses of the study data for treatment monitoring and performance monitoring;

• To collaborate with the other study investigators to prepare study findings for publication;

• To assist with planning and preparation of Data and Safety Monitoring Reports;

• To serve as a resource in problem solving for the clinical centers;

• To identify and summarize emerging information on treatments for dry eye disease that might have impact on the DREAM protocol;

• To work with the Financial Administrator on the budgetary matters for the Coordinating Center and the business arrangements with the drug distribution center;

• To administer, with the Financial Administrator, the Purchased Services Agreement with the Clinical Centers.

The **Director** is responsible for the daily operations of the the Coordinating Center. Specific responsibilities include:

• To have a thorough knowledge of the study protocol and the rationale behind the key design points, as well as knowledge of the key principles of clinical trials design and practice;

• To serve as a voting member of the DREAM Operations Committee;

• To serve as a voting member on the DREAM Executive Committee;

• To serve as chair of the DREAM Clinic Monitoring Committee;

• To serve as chair of the Data Forms Development Committee;

• To develop new data collection forms, in consultation with the Data Forms Development Committee;
• To supervise quality assurance activities at the Coordinating Center and Clinical Centers with input from the Principal Investigator;
• To oversee the certification process including implementation of the web-based knowledge assessment system for all study staff;
• To supervise the day-to-day Coordinating Center activities in the areas of data collection, data management, data reporting, data analysis, quality assurance and administrative support activities;
• To develop, in association with the Principal Investigator, the Coordinating Center budget for annual continuation applications;
• To develop annual progress reports for the NEI;
• To prepare correspondence and progress reports for the Penn IRB;
• To coordinate activities for staff recruitment with the university personnel office;
• To perform some site visits to the clinical centers, as needed and write summary reports;
• To maintain a Log of Extraordinary Events for exceptional circumstances and significant deviations from the protocol;
• To continually review and update the Study Manual of Procedures
• To critically review all interim reports for consistency and accuracy;
• To supervise the production of the periodic reports required by the Data and Safety Monitoring Committee, Investigative Group, and Clinic Monitoring Committee;
• To supervise the development of an informational study web site for the Investigative Group and study patients;
• To plan and present the initial training for all DREAM Clinic Coordinators;
• To collaborate with other DREAM investigators to prepare Study findings for publication.

The **Associate Project Director/ Protocol Monitor**, in conjunction with the Director, is the first line contact with clinical center staff with regard to issues of certification and questions concerning study protocol and good clinical research practices. Because of the Monitor’s thorough knowledge of DREAM procedures and close contact with clinical staff, the Director will delegate all or portions of specific projects to the Protocol Monitor. Specific responsibilities include:

• To provide support to clinical center staff with questions regarding the study protocol and to refer appropriate questions to the Principal Investigator or Director of the Coordinating Center, the Director of the Central Laboratory for Fatty Acids, the HLA-DR and cytokine laboratories, the Investigational Drug Service, the Study Chair, or the Executive Committee;
• To keep in touch with the staff at each clinical site through quarterly telephone interviews with each Clinic Coordinator and to bring areas of concern to the attention of the Director and/or Clinic Monitoring Committee;
• To serve on the Data Forms Development Committee;
• To serve on the Clinic Monitoring Committee;
• To develop a comprehensive checklist for activities to be completed during each site visit;
• To develop a template for site visit reports;
• To schedule and conduct site visits to all clinical sites or assign visits to other site visitors;
• To query clinical center staff about missing and delinquent data;
• To oversee the production of the study newsletters for patients and clinic staff;
• To develop new data collection forms, in consultation with the Data Form Development Committee;
• To maintain the database on study certified personnel;
• To follow-up on identified problems until they are resolved;

Because the Coordinating Center serves as the data reporting and analysis arm of the study, data management staff members are crucial to the successful operation of the Coordinating Center. Data management is a collaborative effort among staff of the Center for Preventive Ophthalmology and Biostatistics (CPOB) and the Clinical Research Computing Unit (CRCU) of the University of Pennsylvania. Specific responsibilities of the CPOB **Systems Analyst** are:

• To collaborate with the CRCU Project Director on the overall design of the DREAM data management system, taking the lead role in the specifications and project management aspects while the CRCU Project Director leads on the development and deployment of the web-based data capture system.
• To generate randomization schedules for the clinical centers and provide the CRCU and Investigational Drug Service with files of the randomization schedules to be incorporated into the randomization process;
• To develop documentation for users of the study SAS datasets;
• To develop an internal SAE monitoring database;
• To develop and maintain a quality control and reporting system that meets the needs of the study;
• To serve on the Data Forms Development committee;
• To oversee the preparation of data reports for review by the Data and Safety Monitoring Committee at least twice each year;
• To oversee the preparation of performance monitoring reports in support of the Clinic Monitoring Committee;
• To advise the Principal Investigator and Director on hardware, software, and personnel requirements;
• To do any necessary programming for data analysis under the supervision of the Principal Investigator and Biostatisticians;
• To prepare and maintain documentation of programs, procedures, and file structures;
• To assure that adequate documentation of the data reporting system is available at all times;
• To assure that adequate procedures have been established and maintained for preserving the integrity and security of the data files extracted from the CRCU database;
• To advise the investigators on all activities that interface with the data reporting system;
• To develop a reporting system of outstanding forms and materials for clinical sites;
• To develop a reporting system of outstanding gradings for the Central Laboratory for Fatty Acids and the HLA-DR and cytokine laboratories.

The Administrative Assistant is responsible for providing the DREAM Study staff with administrative and back-up data entry support. The Administrative Assistant responsibilities are:

• To maintain an electronic registry of staff names, addresses, telephone numbers, FAX numbers, and e-mail addresses for all study personnel;
• To fax/email appointment reminders and notices to Clinic Coordinators each month;
• To file study-related forms and reports in a secure location;
• To photocopy forms, the Manual of Procedures, and other materials when requested;
• To print mailing labels for all DREAM personnel;
• To assist with the preparation and assembly of reports for Study committees and the Investigative group;
• To assist all Coordinating Center staff members as necessary to meet the needs of the Study.

The Administrative Coordinator provides high-level administrative support to the Coordinating Center. Specific responsibilities include:

• To identify meeting sites and negotiate contracts for sleeping rooms, meeting rooms, and transportation (as needed) for meetings of the Investigative Group and Data and Safety Monitoring Committee;
• To perform word processing for highly-formatted data collection forms, documents, and reports;
• To maintain the DREAM Coordinating Center Handbook of Policy and Procedures;
• To maintain a current version of the Manual of Procedures and distribute updates to all centers;
• To maintain an electronic and paper history file of all versions of the Manual of Procedures;
• To place orders for materials and track their status;
• To develop layout and final copy for special study materials such as patient and clinical center staff newsletters;
• To develop slides and other materials for study-related presentations;
• To make travel arrangements for Coordinating Center personnel and for members of various DREAM committees;
• To oversee preparation and assembly of Study documents and reports;
• To perform final formatting and distribute minutes of the meetings of the Data and Safety Monitoring Committee, Clinic Monitoring Committee, and Investigative Group;
• To maintain office supplies for the Coordinating Center,
• To maintain up-to-date records of cumulative Coordinating Center expenditures and unobligated funds;
• To assist the Principal Investigator and Director with budget preparation for annual continuation applications;
• To type DREAM correspondence as necessary;
• To format agendas, site visit reports, manuscripts, slide images, and other materials required for study meetings;
• To make arrangements for study meetings, including contacting hotels, reserving rooms and equipment, identifying participants, and authorizing payment of bills.

The Senior Biostatistician and Biostatisticians work closely with the Principal Investigator in activities related to data analysis and interpretation. The Biostatistician is largely responsible for carrying out analyses designed by the Senior Biostatistician and Principal Investigator.

Specific responsibilities of the Senior Biostatistician include:

• To consult with the Principal Investigator and Systems Analyst in the refinement of the data management system and development of new subsystems;
• To assist with planning and preparation of Data and Safety Monitoring Reports;
• To develop analyses of the data required for adequate monitoring of all aspects of treatment benefit or harm, in consultation with the Principal Investigator;
• To perform analyses aimed at detection of outliers and data patterns that may indicate irregularities in data collection procedures;
• To develop, document, test and maintain statistical analysis programs for study outcome data;
• To assist the Systems Analyst in incorporating appropriate statistical summary measures and tests into routine reports;
• To support the implementation of the statistical stopping guidelines associated with interim data analyses as approved by the Data and Safety Monitoring Committee;
• To support the needs of the study writing committees by preparing accurate and timely analyses of the data, as requested;
• To lead writing committees on selected topics that involve a high amount of statistical analysis;
• To develop new statistical methodology as indicated and to present and publish such methodology appropriately;
• To perform other data analytic tasks as directed by Principal Investigator and Study Chair.

Clear communication between the Coordinating Center and the CRCU are essential to providing an efficient and responsive data management system. Specific responsibilities of the CRCU Project Director are:

• To collaborate with the Coordinating Center Systems Analyst on the overall design of the DREAM data management system, taking the lead role on the development and deployment of the web-based data capture system while the Systems Analyst leads in the specification and project management aspects;
• To supervise the CRCU project team of systems analysts and programmers who support the web-based data capture system;
• To ensure that the detailed logical and content checking for each data collection form, as specified by the Systems Analyst, are implemented in a timely and accurate way;
• To oversee the changes to the data capture system required to accommodate changes in the data collection forms;
• To ensure that personnel who staff the CRCU Help Desk are fully knowledgeable about the study and any trial-specific customizations to the data capture system;
• To develop a User’s Manual for the clinical centers;
• To provide training sessions on the data entry system for the Clinic Coordinators;
• To provide updates and clarifications on use of the data entry system during the course of the trial to the Director for distribution to the clinical center staff.

The Financial Administrator is a member of the sponsored projects administrative unit within the Department of Ophthalmology, University of Pennsylvania. The administration of the approximately 20 purchased services agreements for the DREAM Clinical Centers requires considerable effort beyond the usual administration of a grant. Specific responsibilities include:
To collaborate with the Principal Investigator and the University Office for Sponsored Research in developing a template Purchased Services Agreement for all clinical centers;

To maintain records on the funds paid to each center;

To respond to questions from clinical center staff regarding payments;

To issue payments on a monthly basis to clinical centers for accomplishing milestones that signal completion of specific work;

To issue payments on a quarterly basis to clinical centers for completion of study visits.

14.3 Data Management

14.3.1 Overview of DREAM Data Management

The DREAM data management system captures data through several data streams, creates a single unified data base, allows generation and distribution of alerts and reports to clinical center staff and to the staff of the laboratories, and the generation of monitoring and statistical analysis reports. See Exhibits 14-2 and 14-3 for schematic view of the system. Because all of the patients in the Randomized Withdrawal Trial (Extension Study) are participants in the Primary clinical trial, DREAM will have one database for both trials.

The data streams in DREAM consist of:

- Data collected at the clinical centers by the Clinic Coordinator and Clinicians. Data are collected at a screening visit, a baseline visit, and follow-up visits. The software used for this component of the data system is Oracle Clinical Remote Data Capture (RDC), implemented by the CRCU staff. Data from each the clinical centers are uploaded on a daily basis into the SAS database.

- Data from the Central Laboratory for Fatty Acids provide the levels of fatty acids of interest at baseline and selected follow-up visits. The Central Laboratory transfers electronic files in the form of spreadsheets. Data from the Laboratory are uploaded monthly basis.

- Data from the laboratories for HLA-DR and cytokine levels provide measurement values from the testing at baseline and selected follow-up visits. The laboratories transfer electronic files in the form of spreadsheets. Data from the laboratories are uploaded on a monthly basis.

- Data from the Reading Centers on keratography images, data from laboratories measuring markers in the blood for inflammatory disease.

14.3.2 Data Management System

The DREAM data capture system is a collaborative effort between the Center for Preventive Ophthalmology and Biostatistics and the Clinical Research Computing Unit, both at the University of Pennsylvania. The CRCU has a large staff with expertise in hardware technology and data management systems dedicated to providing state-of-the art support services. Database security, to protect from both internal (user) breaches and external breaches, has
been an area of particular development. CRCU procedures conform to FDA Bioresearch Monitoring Program and International Conference on Harmonization (ICH) guidelines. The CPOB has staff members who are very familiar with the subject area and take the lead on development of data collection instruments and providing the logic specifications for real-time checking of data during capture. The CPOB staff members combine the data captured at the clinical centers with the data transferred from the laboratories into one SAS database and are responsible for generating reports and data analysis. See Exhibits 14-2 and 14-3.

The database management system for the clinical centers is developed as sets of applications using Oracle Clinical™, which resides on the Oracle Relational Database Management System. The CRCU staff develops project specific standard operating procedures. All programming is performed according to written Standard Operating Procedures and specifications that describe the features and functions of the system. Prior to deployment and use by Clinical Center and Coordinating Center personnel, the electronic systems are subjected to extensive testing. This testing is conducted according to a written test plan and is intended to validate the proper functioning of the system. Once the system has been tested and validated, it is migrated from a ‘development’ environment and is deployed in a ‘production’ environment. Modifications to the system are requested using standard operating procedures, resulting in development of written specifications that explicitly document programming requirements. Following modification to the system, the system is re-subjected to testing and validation before being deployed in production mode.

Training users is an essential part of the deployment process. Webinars for clinical coordinators are held during the study start up phase to familiarize them with the Oracle Remote Data Capture system. These sessions are recorded for use in the DREAM educational and certification program of new staff. Users’ manuals on data entry procedures and troubleshooting are prepared by CRCU and distributed by Data Coordinating Center staff. A CRCU Help Desk provides telephone and email support services for connectivity and access issues for the clinical center staff and low vision service providers. A parallel version of the data system is available for training and certification activities.

14.3.3 Management of Data from Clinical Centers
The first data on a participant in DREAM is collected by the clinical center staff (see Exhibit 14-2). Entry of completed data collection forms on participants who have signed the consent form initiates the creation of a master record for the participant. Thereafter, all data from all data streams are checked against the master record with regard to ID number and ID alphabetic code before acceptance into the DREAM database.

14.3.3.1 Design of Clinical Center Data Collection Forms
Design of clinical center data collection forms and administrative forms is the responsibility of the Data Forms Development Committee, led by the Director, with the Systems Analyst and Associate Project Director/ Protocol Monitor as members. Draft forms are circulated to the Principal Investigator, Study Chair, and selected members of the Executive Committee for review and comment. Near the time of finalization, the draft forms are also provided to a few Clinic Coordinators for review and comment.
The DREAM data collection forms are designed to facilitate accurate completion and data entry. The layout of the forms generally consists of two columns; the left column consisting of items required for all patients and the right column consisting of items that are answered conditional on the responses to the items in the left column. The correct logical flow is conveyed through use of directional arrows. Multiple choice and check-off responses are used as much as possible; however, unusual findings may be recorded in comment fields that are keyed in their entirety. Key instructions on additional actions to take or forms to complete are included in the form items.

Logical sections of the forms are divided into different form sections or components, with numbering of items specific to the component. The component concept allows for modularity of form design and therefore minimizes the impact of form revisions.

### 14.3.3.2 Data Recording and Entry

Clinic Coordinators prepare for each visit by accessing the DREAM data management website and requesting a printout of all case report forms and logs that may be needed for a particular visit. Patient identification numbers and alpha codes are available on pre-printed patient registration logs supplied to the Clinical Centers by the Coordinating Center. The numerical and alphabetical identification codes for a patient may be entered into the system to have all forms printed with the identification information on each page. If the DREAM data management system is not available, each clinical site has a notebook of paper copies of the forms, organized by visit, to use as photocopy masters. Most of the clinical examination data and all of the questionnaire data are recorded directly onto the DREAM forms during the clinic visit and are considered the source documentation.

Data entry of case report forms completed in the clinical center is performed by the Clinic Coordinators. Each item entered is checked for valid codes, legitimate ranges, legal dates, etc. Invalid entries are flagged. Sections of the forms that are complete may be data entered and sections that are incomplete may be data entered later. Each screen is reviewed by the Clinic Coordinator before saving. Validation of data occurs during and after the data entry session. Electronic logs of the completion status of each form and assessment are tracked by the Coordinating Center to determine the status of data collection for each registered patient. (Refer to the DREAM Data Management User’s Manual for additional information about the data management system.) Form revisions and additions are accommodated by having data management staff modify or create the appropriate data definition.

The DREAM database is extracted from CRCU servers to the CPOB server on a daily basis. The additional tasks of performance and intervention monitoring, further quality assurance measures, and analysis are the responsibility of the CPOB staff.

### 14.3.3.3 Data Edits for Clinical Center Data Collection Forms

Data that have been entered into the Web-based system are subject to additional consistency checking involving more complex logic than implemented during data entry checking. Often these post-entry checks involve several different forms and visits. The CPOB Systems Analyst develops the logic for these study-specific checks and the comments to be associated with the
resulting data queries. These instructions are incorporated into the CRCU data system by CRCU staff.

The Clinic Coordinator reviews online edit messages and the corresponding data forms. If necessary, the Clinic Coordinator corrects the paper form, initials and dates the form, and updates the online database.

The updated data records are again subjected to the entire data checking system. When extraordinary circumstances arise in which the query may never be able to be resolved to meet the requirements of the edit logic, the Systems Analyst may, with the approval of the Director, flag specific items on specific forms as exempt from further edit.

The CRCU Oracle Clinical system generates electronic transaction records after every record correction so that a fully verifiable audit trail is created.

14.3.4 Management of Data from Laboratories

Data from each of the DREAM laboratories follow a similar process (Exhibit 14-3). Samples are sent to the laboratory labeled with the patient’s numerical and alphabetical identification codes along with the visit identifier. Laboratory staff members log receipt into an electronic inventory. After the laboratory testing is complete, the results are entered into an electronic spreadsheet. Both the inventory and the results are transferred to the Coordinating Center on a regular basis. The full dataset is sent with each transfer rather than incremental datasets so that all changes in the laboratory datasets made by laboratory personnel are reflected in the transferred dataset. The Systems Analyst runs a program that compares the inventory to the entries on the clinical case report forms to identify samples that were not received by the laboratory and alerts the clinical centers. Also, the inventory and the file of results are compared to identify the backlog of samples yet to be tested by the laboratory and the report is provided to the laboratory. The laboratory is also alerted to any duplication, errors on numerical or alphabetical identification codes, or logical discrepancies with respect to dates of acquisition in the center, receipt in the laboratory, and testing.

14.3.5 Integrated SAS Database

Full extracts of the Oracle Clinical database that resides on the CRCU servers are downloaded on a daily basis to the DREAM SAS database on the CPOB server. The files from the laboratories are received monthly. The study database consists of SAS system files. One SAS system file corresponds to each type of case report form, questionnaire record, laboratory result, etc. The majority of DREAM reports, tables, and analyses access the integrated SAS database.

14.3.6 Backup of the DREAM Database

The DREAM database, data management system, and data analysis system represent the efforts of the entire Investigative Group over the duration of the study. All study data entered through the Oracle Clinical system are backed up on the CRCU servers and their offsite back-up storage servers. The clinical database is extracted daily to the CPOB servers. Each laboratory maintains its own back-up system locally. Data files reside on the CPOB file server.
and are backed up nightly. A rotation of backup tapes is maintained so that the database can be restored as of the most recent day of the week, week, month, or quarter. A copy of the monthly backup tapes is also stored off site. In addition, all DREAM CPOB personnel are required to keep copies of key documents such as forms, correspondence, and reports on the file server, which is on an automatic backup schedule. Files of the data system as of the time of each freeze and for each publication are also archived.

14.3.7 Quality Assurance Activities Related to Data Management

The overall quality assurance program for the study is described in Chapter 9. Specific quality assurance features related to data management are:

- Standard data collection forms and procedures;
- Training and certification in data collection and data entry;
- Explicit instructions with release of new data collection forms about new/revised questions and instructions.
- Central concurrent processing of data to detect problems early and provide feedback to the clinical centers;
- Data edits for missing, invalid, and suspect responses;
- Regular reporting on performance of all centers;
- Checking a random sample of all entered data against original data collection forms after data editing has been completed. If this procedure identifies an unacceptably high residual error rate (more than 15 errors per 10,000 keystrokes) all aspects of data management will be reviewed with special attention to data entry procedures at the particular clinical center;
- Checking a random 10% sample of original data collection forms against medical records during site visits.

14.4 Randomized Treatment Allocations

The Coordinating Center generates schedules of randomly assigned treatment allocations that will be computer generated and stratified by clinical center. Schedules are generated for the Primary Clinical Trial and for the Randomized Withdrawal Trial (Extension Study). A permuted block method of randomization will be used to ensure balance over time and a randomly selected block size will be used to further thwart any possible attempts to determine the next treatment allocation based on perceived knowledge of previous allocations. The schedules are provided to the CRCU for incorporation into the randomization modules of the DREAM database management system and to the Investigational Drug Service.

14.5 Enrolling the Patient into the Primary Clinical Trial

Patients are evaluated for eligibility beginning with a Screening Visit. Candidate patients are assigned a numerical and an alphabetic identification code from a registration log provided by
the Coordinating Center. Data from patients who sign the consent form are entered into the DREAM data management system. During the Baseline Visit, additional procedures are performed and the forms needed to establish eligibility are entered into the system by the Clinic Coordinator. The Coordinator invokes a randomization module. The program checks each of the eligibility criteria and alerts the Coordinator of any missing data or eligibility criteria not met. If all required data have been received and the patient is eligible, the system generates a message that confirms that the randomization has been successfully completed. An appointment schedule customized to the patient’s randomization date is also made available at the time of randomization. The Clinic Coordinator places copies of the schedule in the patient’s study file, the Patient Log and/or follow-up schedule notebook. A copy is given to the patient. The Coordinator faxes a prescription for the patient to the Drug Distribution Center, so the study supplements can be sent directly to the patient’s home.

14.6 Enrolling the Patient into the Randomized Withdrawal Trial (Extension Study)

Patients who were assigned to the active supplements in the Primary Clinical Trial are eligible for the Extension Study. During the Month 12 Follow-up Visit, the Coordinator enters the forms needed to establish eligibility. Similar to the initial randomization described above, the Coordinator invokes a randomization module for the Extension Study. If eligibility is confirmed, an appointment schedule customized to the patient is made available at the time of randomization. The Clinic Coordinator places copies of the schedule in the patient’s study file the Patient Log and/or follow-up schedule notebook. A copy is given to the patient. The Clinic Coordinator faxes a prescription for the patient to the Drug Distribution Center, so the study supplements can be sent directly to the patient’s home.

14.7 Reports Developed by the Coordinating Center

The Coordinating Center provides reports based on available information to support the clinical centers, the laboratories, the quality assurance activities of the study (see Chapter 9), and the periodic meetings of the Operations Committee, Executive Committee, Clinic Monitoring Committee, Investigative Group, and Data and Safety Monitoring Committee.

14.7.1 Creation of Data Sets for Reporting

Certain reports that are designed to check the completeness of activities in the clinical centers, laboratories and Coordinating Center are run on the current database, usually involving the master files and auxiliary files and programs that identify and count specific data collection forms without analyzing the content of the data record. Other reports geared to a comprehensive summary of the study data require a significant amount of preparation. Therefore a data cutoff date must be chosen (usually the end of the month 30 to 60 days before the report is needed) so that the data files are not continually changing while work on the report is ongoing. When the cutoff date arrives, a “snapshot” of the data files is created.

Before proceeding with the freeze, checks are run to verify the completeness of available information. Clinic Coordinators are encouraged to resolve any known data entry backlogs. The frozen copy of the data is then used as input to the numerous programs that perform the functions necessary to produce the tables for the report.
14.7.2 Creation of Data Extracts
The frozen datasets consist of the full complement of SAS system files in the integrated database. Specific summary files are created that contain important data that will be used for many reports/tables such as visits completed, changes in OSDI score, changes in signs and adverse events.

14.7.3 Database of Tables
During the course of the study, hundreds of tables will be used for the various committee meetings. Some tables are used in reports to several committees. To keep generation of the tables efficient and organized, a database on the tables is maintained (here “table” is used loosely, and may refer to a formatted listing or graph). Each table is assigned a working number. The Systems Analyst maintains a master list of working tables. For a particular report, the working tables may be put into any order.

14.8 Other Data Analysis
In addition to scheduled reports, the Coordinating Center staff members are responsible for performing all data analysis tasks. Such tasks may be associated with preparation of publications and presentations from the DREAM investigators, with funding renewals or initiatives, or with continuing data monitoring.

14.9 Management of Serious Adverse Event Reports
The Coordinating Center receives notification of Serious Adverse Events (SAEs) via a specific form that is submitted by the Clinic Coordinator. An immediate assessment of all incoming SAEs is made to determine whether the event needs to be reported to the FDA. See Chapter 5 for a full description of handling adverse events within DREAM. The Coordinating Center sends an electronic copy of each Serious Adverse Event Report Form, baseline medical history, medications reported at baseline and follow-up, and other supporting documentation such as hospital records to the DREAM Medical Monitor within 7 days of notification by the Clinical Center. The Associate Director tracks the receipt of the Medical Monitor’s assessment of the coding (preferred term, seriousness, severity, expected or not, related to the study supplements). As follow-up information is received for each SAE, this information is also provided to the Medical Monitor, as well as a form for indicating any needed change in the coding of the SAE.

14.10 Certification of DREAM Personnel

14.10.1 Overview of Certification
An important function of the Coordinating Center is participating in the development and administration of the DREAM certification program. The program is described in detail in Chapter 12 and an overview is provided here. Careful adherence to all protocols in every aspect of the study, including examination procedures and the data collection and data entry process, is essential to the success of the trial. The quality of study data depends upon uniformity of procedures and data collection and careful data entry. Therefore, standardized training on all protocols and data collection procedures, along with certification of DREAM personnel, are important components of quality assurance.

The DREAM Operations Committee oversees the design and implementation of the training program and sets the certification requirements for all DREAM personnel. DREAM Study
personnel must be certified before performing any study procedures. Before a center can begin enrolling participants, at least one person must be certified for each role and center-specific criteria, such as IRB approval and required equipment, must be fulfilled. All people seeking certification receive a checklist of certification requirements that must be met for each study-specific role prior to any interaction with study patients. The Coordinating Center is responsible for reviewing completed certification requirements, developing the on-line system for completing knowledge assessments, providing customized feedback, issuing certification numbers, and maintaining the electronic roster of all certified study personnel.

14.10.2 Certification System
The certification of clinical center staff is primarily web-based. All individuals seeking certification in any study role must first register on the DREAM certification website. Once registered, the individual seeking certification can read the requirements for certification and begin the certification process. (See Chapter 12 for more details regarding the certification system and certification requirements.) Knowledge assessment tests are taken on-line, with interactive feedback on incorrectly answered items. Staff in Coordinating Center review submitted materials and conduct telephone interviews as required for certification and update the completion status on the candidate’s checklist. The checklists are automatically updated with successful completion of the online knowledge assessments or review of education modules. For roles that require review of submitted materials or telephone interviews, individual members of the Coordinating Center have restricted access to change the status flags for the associated items on the checklist. These people are alerted via email when the candidate indicates that materials have been submitted (date provided) so that submitted materials may be tracked.

Summary information on the certification status of all registered candidates from a center may also be displayed to the Clinic Coordinator. The status of additional requirements for certification of a center, such as verification that all required equipment is on site, are also displayed on the center-specific summary view. After satisfactory completion of all certification requirements, the certification website notifies the newly certified staff member and the Clinic Coordinator at the site. When a Clinical Center achieves certification, a letter is sent to PIs and Clinical Coordinators listing all certified personnel (and their certification numbers) at their clinical center.

The names of all certified personnel are entered on the roster of certified personnel. The Director of the Coordinating Center provides certification update reports to the DREAM Operations Committee.

14.11 Administration of Subcontracts with Clinical Centers and Administering the Payment Plan for Patient Care Costs
The Principal Investigator of the Coordinating Center holds responsibility for administration of the Purchased Services Agreements with the clinical centers. The departmental Financial Administrator and staff members of the Office of Research Services of the University of Pennsylvania provide the subcontracting documents after receiving specifications of the terms from the Principal Investigator. These staff members produce the documents, distribute the documents to the clinical centers, respond to queries regarding the terms of the contract, and
negotiate changes in language. The Financial Administrator executes procedures to provide payment, and resolves accounting discrepancies, and tracks the payment through the University accounting process to the clinical centers. In addition, the Financial Administrator solicits and follows-up on the materials needed each year to fulfill requirements for non-competing renewals of grant awards and the execution of new agreements each year. The Principal Investigator reviews paperwork for all payments to clinical centers and intervenes when administrative processes appear to be stalled at either the University of Pennsylvania or one of the clinical centers.

Costs associated with patient care are reimbursed on a per visit basis; patient care payments will be made only after all the required materials (case report form, laboratory samples) are submitted from the clinical center.

14.12 Preparations for Study Meetings

A major factor in ensuring protocol adherence and good communications among study personnel in the various functional units is the Investigative Group meeting held once each year. The Coordinating Center staff members play a major role in preparing for these meetings. Similarly, Coordinating Center personnel provide materials and support for meetings of the Operations Committee, Clinic Monitoring Committee, Data and Safety Monitoring Committee and SAE reviews by the Medical Safety Monitor. The Administrative Coordinator identifies meeting sites and negotiates contracts for sleeping rooms, meeting rooms, and transportation (as needed) for meetings of the Investigative Group and Data and Safety Monitoring Committee.

The Principal Investigator of the Coordinating Center and the Study Chair prepare study meeting agendas. The agendas guide the assembly and preparation of materials to be discussed at the various meetings. Meeting notebooks are prepared by the Coordinating Center to facilitate the discussions of the Executive Committee and Investigative Group.

All logistical support for Study in-person meetings and teleconferences is provided by Coordinating Center staff. The Administrative Coordinator bears responsibility for most of these functions.

14.13 Study Library

The Coordinating Center is responsible for maintaining a record of study progress and activities. The Library contains both paper and/or electronic copies of the documents. Responsibility for maintaining a Study Library is assigned to the Administrative Coordinator. The following documents are kept in the DREAM Study Library for reference by Coordinating Center staff and other study investigators:

- Minutes of meetings:
  - Operations Committee
  - Executive Committee
  - Investigative Group
  - Clinic Monitoring Committee
- Investigative Group Progress Reports
• Site visit reports
• Reports from scheduled telephone calls to Clinic Coordinators
• Previous and current versions of Manual of Procedures
• Copies of reports from site visits to the Coordinating Center
• Protocol memoranda
• Archive of previously used versions of data collection forms
• Log of Extraordinary Events
• Reprints of study publications.

Other materials may be added to the Library as directed by the Coordinating Center Principal Investigator. Copies of confidential data reports and meetings of the DSMC are kept in locked filing cabinets in individual staff offices.

14.14 Coordinating Center Handbook of Procedures
The Coordinating Center Administrative Coordinator and other personnel designated by the Principal Investigator are responsible for developing a Handbook of Procedures as a reference document for Coordinating Center staff. The descriptions of procedures included in the Handbook are much more detailed than those included in this chapter and provide step by step instructions for many of the activities of the Coordinating Center.

14.15 Meetings of the Coordinating Center
Meetings of all members of the Coordinating Center occur monthly, or more frequently as required. Meetings of the statistical team, project management team, or data management team are scheduled on an as-needed basis. Such meetings allow staff members to remain up-to-date on study progress and discuss all aspects of a problem and ways to resolve it.

14.16 DREAM Websites
The Coordinating Center maintains a website to provide information about the trial to the public and prospective patients, as well as to study staff. The website is a component of the CPOB website (http://www.med.upenn.edu/cpob/). The Associate Director also collaborates with the Coordinating Center Principal Investigator to initiate and maintain DREAM on www.clinicaltrials.gov.

Visitors to the website will have access to a description of the study that includes information on dry eye disease, a listing of DREAM clinical centers and resource centers with complete contact information, the Manual of Procedures, links to websites on dry eye disease and addressing participation in clinical trials, copies of previously distributed DREAM patient newsletters, and DREAM publications.
EXHIBIT 14-1
DREAM Coordinating Center Organizational Chart

Principal Investigator

Senior Biostatistician

Biostatistician

Director

CRCU Project Director

CRCU Staff

Assoc. Project Director/Protocol Monitor Monitors

Systems Analyst

Administrative Assistant

Administrative Coordinator
EXHIBIT 14-2
Data from Clinical Centers

**Clinical Sites**

- Data enter baseline eligibility forms
- Receive randomization assignment and appointment schedule
- Data enter other baseline and follow-up forms
- Review queries and update data

**CRCU Data Management**

- Process eligibility forms and determine eligibility
- Display randomization assignment and appointment schedule
- Validate and store incoming data
- Generate edit queries
- Maintain history of updates
- Backup databases

**CPOB Project Management**

- Extract data and convert to SAS datasets
- Create monitoring and analysis reports as needed
EXHIBIT 14-3
Data from Laboratories

**Clinical Sites**
- Acquire samples during patient visits
- Transmit samples to Laboratory

**Laboratories**
- Log in receipt
- Analyze samples
- Maintain databases

**CPOB Project Management**
- Receive data extracts
- Convert data to SAS datasets
- Generate error list
- Create monitoring and analysis reports as needed
- Backup databases
- Maintain history of updates