**DETAILED STUDY PROTOCOL**

**TITLE**: **Omega-3 Fatty Acids for MDD with High Inflammation: A Personalized Approach**

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**I. BACKGROUND AND SIGNIFICANCE** (including progress report and preliminary studies).

**a. Historical background**

Major depressive disorder (MDD) is common and disabling, with a lifetime prevalence of 16.2% (Kessler et al, 2003), and an annual cost over $80 billion in the United States (Greenberg et al, 1990, 2003), exceeding that of many other diseases. The lifetime risk for suicide is over 4-fold greater among the depressed than the non-depressed (Bostwick et al, 2000), and mortality from all causes is 1.5 to 2 times greater in depressed individuals (Hirshfeld et al, 1994). Despite the growing number of marketed antidepressants, between 19-34% of patients with MDD still do not respond to acute antidepressant treatment, 29-46% may fail to achieve and sustain a full remission (Fava and Davidson, 1996), and between 15-50% will have a recurrence of depression despite continuous antidepressant treatment (Fava, 2000). For the relatively large proportion of individuals that does not respond or is intolerant to antidepressants, alternative treatment options are very important.

For the significant proportion of patients who are non-responsive and/or intolerant to standard antidepressants, alternative therapies are compelling. Many individuals may abandon FDA-sanctioned medications in favor of natural products (Mischoulon, 2004). A survey from 1999 revealed that 34% of psychiatric outpatients with MDD were using alternative therapies (Knaudt et al, 1999). Ernst et al (1998) reported that 20% of mostly US-based depressed patients had used a CAM therapy for their depression. More than 70% of the world's population may use some sort of complementary therapy (NIH, 1997), and there are more visits to alternative practitioners per year in the US than to primary care physicians (Eisenberg et al, 1998). Given the growing interest in CAM, continued research in this area is of major importance.

Major depression is projected to be the second leading cause of disability-adjusted life years worldwide by the year 2020 (Murray and Lopez, 1999). Many individuals with depression seek complementary and alternative medicine (CAM) therapies (Druss et al., 1998; Kessler et al., 2001, Unutzer et al., 2000) not only because of their intrinsic appeal but because more than 50% of depressed individuals do not remit on a standard antidepressant (Nelson, 2003; Fava and Davidson, 1996) or have difficulty tolerating side-effects and, therefore, are obliged to seek innovative treatments, including CAM approaches. Widely recognized among the more promising potential CAM therapies for major mood disorders in recent years are the fish oils or omega-3 fatty acids (Freeman et al, 2006; Sublette et al, 2011). Despite encouraging evidence for clinical effectiveness, very little is known about the mechanisms that may account for their link with their putative antidepressant effects.

Convergent clinical and epidemiological data have implicated omega-3 fatty acids in the pathophysiology, prevention, and therapy of common and disabling conditions such as major depressive disorder (MDD) and cardiovascular disease. Over the past century, the intake of omega-3 fatty acids in the western diet has decreased dramatically, while intake of omega-6 fatty acids has increased. The resulting omega-6-to-omega-3 ratio (n-6:n-3) favors omega-6 in the U.S., as opposed to countries with higher fish consumption, and may have contributed in part to the rise in prevalence of depression and heart disease in western societies. The urban lifestyle of the 21st century, notable for high stress, little rest, and high intake of processed (i.e. omega-6-rich) foods, has been

postulated to create a baseline "pro-inflammatory" state in the average person. This pro-inflammatory state has been demonstrated to play a role in the pathogenesis of cardiovascular disease and is hypothesized to play a role in the development of mood disorders.

**b. Previous pre-clinical or clinical studies leading up to, and supporting the proposed research**

There is growing evidence of antidepressant efficacy for several popular natural products, including omega-3 (PUFA) fatty acids (Freeman et al, 2006; Sublette et al, 2011). There is much variability in individual study findings, as with most antidepressants (Khan et al, 2003). No single study can definitively prove or disprove the efficacy of any psychotropic; meta-analyses and systematic reviews have therefore been especially helpful in clarifying our understanding of this natural agent.

**1. Role of PUFAs in Signal Transduction and Central Nervous System Function**

The PUFA content can affect the structure and function of membranes and associated membrane-bound proteins (e.g., receptors, ion channels and enzymes) (Salem et al., 1986; Salem and Niebylski, 1995). This can affect signal transduction in at least four ways. First, PUFAs can alter the biophysical microenvironment and affect ion channels (Lundbaek and Anderson, 1994). Second, PUFAs can modify the binding affinities of a wide range of neurotransmitter receptors (Fong and McNamee, 1986; Malnoe et al., 1990; Witt and Nielsen, 1994; Miller et al., 1992). Third, PUFAs contribute to the regulation of cellular function by acting as a source of second messengers that are involved in cellular signal transduction (Hudson et al., 1993; Graber et al., 1994; Mathews and van Hold, 1996). Fourth, PUFAs are the precursors of prostaglandins, thromboxanes and leukotrienes, which collectively are called eicosanoids. The eicosanoids mediate the acute phase response to infection or cellular injury. They also have a variety of other physiological effects, including possibly serving as retrograde messengers in the processes underlying long-term changes in synaptic plasticity (Wainwright et al., 1997), and possibly in the adaptation to pharmacologic agents such as antidepressants.

As described above, PUFAs play numerous roles in signal transduction, affecting ion channels, receptors and second messengers, and the data indicate that they are of critical importance in the central nervous system (CNS). PUFAs are found in high concentration in the CNS. For example, arachadonic acid (AA) and DHA are selectively concentrated in gray matter, and together account for approximately 20% of the synaptosomal membrane fatty acids (Salem, 1989). Preclinical studies have shown that each step in the biogenic amine process, including neurotransmitter synthesis, binding, uptake and degradation, can be influenced by membrane fatty acids (Salem, 1989; Hibbeln and Salem, 1995).

PUFA deficiency also leads to a reduced synthesis of dopamine, as well as the decreased storage of newly synthesized dopamine in cytoplasmic vesicles (Zimmer et al., 1998). Hibbeln and colleagues (1998) found that there was a correlation between PUFA levels in plasma, and metabolism of 5–HT and dopamine in the CNS. It has been hypothesized that PUFAs also might affect the uptake of neurotransmitter precursors into the brain. Horsten and colleagues (1997) suggested that because fatty acids and tryptophan (the precursor of 5-HT) compete for a binding site on human albumin, an increase in serum fatty acids will allow less tryptophan to be bound to albumin. This would result in more uptake of tryptophan into the brain, and consequently an increase in 5-HT synthesis.

PUFA-deficient diets can affect behavior in laboratory animals. Mice fed a PUFA deficient diet exhibited lower motivation to escape, a commonly used measure of emotional reactivity in animal models (Frances et al., 1995). The behavioral effects of PUFA deficiency in rodents include changes in attention, motivation, and reactivity to stimuli and rewards, all of which might be indicative of deficits in function of prefrontal dopamine pathways (Wainwright et al., 1997). Carrie and colleagues (2000) demonstrated that behavioral disturbance induced by a PUFA deficient diet can be reversed by PUFA supplementation. These data suggest that PUFA intake and PUFA levels also might influence behavior in humans.

## 2. Evidence for a Link between Omega-3 Fatty Acids and Depression

Confluent epidemiological data have supported a link between omega-3 fatty acids and depression. Analysis of data from the Cross-National Collaborative Group (1992), which showed marked variation in MDD prevalence across countries, revealed a strong inverse relationship between fish consumption and depression (Hibbeln, 1998; Hibbeln, 1999). An increasing prevalence of major depression in western societies since the mid-20th century (Klerman, 1988; Klerman and Weissman, 1989) also bears a postulated relationship to dietary shifts (Leaf and Weber, 1987; Eaton and Kanner, 1985; Taylor et al., 1979) favoring a higher ratio of omega-6:omega-3 fatty acids (Adams et al., 1996; Hibbeln and Salem, 1995). Suicidal ideation (Tanskanen et al., 2001a), as well as depressive symptoms on the Beck Depression Inventory (Tanskanen et al., 2001b), were significantly lower among frequent lake fish consumers in Finland (n = 3024), after adjustment for relevant demographic variables, general health, and habits. Similarly, an earlier census study in Japan (n = 265,000) showed a decreased rate of suicide among subjects with daily vs. less frequent fish consumption (Hirayama, 1990). A recent study by Frasure-Smith and colleagues (2004) showed that depressed patients had lower concentrations of total omega-3 and docosahexanoic acid (DHA), higher ratios of arachidonic acid (AA) to DHA, AA to eicosapentanoic acid (EPA), and n-6:n-3 than controls.

Supporting evidence for a role for omega-3 fatty acids in depression has also come from studies of depressed patients and healthy controls. While early studies involving heterogeneous samples (including bipolar and other forms and severity of depression) reported increases in EPA and DHA in serum and RBC membranes of depressed individuals (Ellis and Sanders, 1977; Fehily et al., 1981), more recent studies in well-characterized subjects with unipolar MDD have reached opposite conclusions. Thus, patients with MDD were found to have a reduction of total omega-3 fatty acids as well as alpha-linolenic acid (ALA) and eicosapentenoic acid in serum cholesteryl esters compared with adults with minor depression or healthy controls (n = 74) (Maes et al., 1996). Of particular note, in a similar cohort of depressed subjects (n = 34) these abnormalities and reduced omega-3 fatty acids in phospholipids persisted despite acute treatment of depression with fluoxetine 20 mg/day with or without adjuncts (trazodone 100 mg or pindolol 7.5 mg) (Maes et al., 1998) suggesting that changes in omega-3 fatty acids did not appear to mediate response to this selective serotonin reuptake inhibitor (SSRI).

In a small sample (n = 24), depressed subjects had lower RBC membrane levels of omega-3 fatty acids than healthy controls and severity of depression correlated with both levels and dietary intake of omega-3 fatty acids (Edwards et al., 1998). The omega-3 fatty acid composition of RBC membrane phospholipids, and particularly DHA content, were significantly depleted among depressed compared with control subjects in a similar study (Peet et al., 1998). In another sample of depressed adults (n = 20), depression severity pre- and post-treatment with antidepressants (mainly tricyclics) was related not to abnormal absolute levels of PUFA but rather to the AA:EPA ratio in plasma and RBC membrane phospholipids, suggesting that imbalance rather than an absolute deficiency may be a crucial factor (Adams et al., 1996).

The etiology of diminished omega-3 fatty acid levels or imbalance of omega-6:omega-3 fatty acids among depressed individuals remains uncertain. While epidemiological studies have suggested decreased consumption, this has not been easy to demonstrate within smaller clinical samples (Adams et al., 1996), and other explanations have been advanced including insufficient capacity for elongation and desaturation of omega-3 fatty acid precursors or increased catabolism of omega-3 fatty acids, including degradation via peroxidation in the setting of hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis in depression (Hibbeln and Salem, 1995). Similarly, the relationship, if any, between omega-3 fatty acid status and response to standard antidepressant therapy has also been insufficiently studied to draw any meaningful conclusions.

## 3. Clinical Evidence for Efficacy of Omega-3 Fatty Acids in Depression

Motivated by studies in community and clinical samples suggesting imbalance of omega-3 fatty acids in depression, Peet and Horrobin (2002) conducted a randomized, placebo controlled, dose finding study of ethyl-eicosapentanoate (EPA) as monotherapy for 70 adults (ages 18-70) with persistent depression (Hamilton

Depression Rating Scale [HAM-D] 17-item score $\geq$ 15) despite treatment with a standard antidepressant. Subjects receiving 1g/day EPA showed significantly higher response rates than subjects receiving placebo (53% vs. 29%), with notable improvement of depressed mood, anxiety, sleep disturbance, lassitude, diminished libido and suicidality. The 2g/day group showed little evidence for a drug: placebo difference and the 4g/day group showed a non-significant trend toward improvement. Mischoulon and colleagues (2008) have recently obtained evidence for a similar inverted U-shaped dose-response curve using DHA in adults with MDD.

In a small sample of subjects with MDD and at least mild recurrent depressive symptoms (24 item HAM-D $\geq$ 18) on antidepressant therapy, Nemets and colleagues (2002) found a statistically significant separation of adjunctive EPA 1g/day from placebo by week 2 with a clinically important difference in the mean reduction of the 24 item HAM-D by the study endpoint at week 4 (12.4 vs. 1.6). An NCCAM funded, randomized, placebo controlled study of EPA 1 g/day vs placebo monotherapy for outpatients with MDD (n = 57) found a non-significant advantage for EPA (Mischoulon et al, 2009). Regarding DHA, a placebo controlled study showed lack of efficacy of 2 g/day DHA for depression (Marangell et al., 2003). In retrospect, the use of a higher dose of DHA may have accounted for the negative result, in view of the Mischoulon et al study (2008). A recent small study by Silvers (2005) suggested that 8 grams of "fish oil" was not more effective than 8 grams of "olive oil" but this under-powered study is marred by problems with attrition, dosage, and choice of rating scales. A recent 3-armed comparison of EPA, DHA, and placebo in adults with MDD (Mischoulon et al, 2015) found no significant advantage for either omega-3 against placebo, though an ancillary investigation of inflammatory biomarkers suggested a subpopulation that may be more likely to benefit from EPA, as will be discussed later (Rapaport et al, 2015).

Omega-3 fatty acids may have efficacy for bipolar as well as unipolar mood disorders. Using high doses of an omega-3 fatty acid mix (6.2 g EPA + 3.4 g DHA) vs. placebo over a 4 month trial, Stoll and colleagues (1999) found that among 30 patients with bipolar I or II disorder, a Kaplan-Meier survival analysis revealed a significantly longer duration of remission for those receiving adjunctive omega-3 fatty acid mix vs. placebo along with their current mood stabilizing regimen. Further investigation is needed to determine whether bipolar disorder actually requires higher doses of omega-3 fatty acids than unipolar illness and to unravel the respective contributions of EPA and DHA. However, Keck and colleagues were unable to replicate this result in a larger-scale study. In their double-blind, placebo-controlled trial of adjunctive EPA 6 g per day for 4 months in patients with bipolar depression (N=59) or rapid cycling (N=62), EPA did not separate from placebo (Keck et al., 2002).

Groups also have studied the relationship between omega-3 FA levels and a range of other psychiatric syndromes including borderline personality and schizophrenia (Zanarini and Frankenburg, 2003, Mellor et al., 1996; Vaddadi et al., 1989; Emsley et al., 2002, Fenton et al., 2001, Maidment, 2000) and postpartum depression (Feeeman et al, 2006, Marangell et al, 2004). These have tended to be very small studies and their conflicting results reflect this limitation.

Taken together, the literature suggests efficacy of omega-3 fatty acids for mood dysregulation. In particular, preliminary studies have suggested that low doses of omega-3 fatty acids may be efficacious and well-tolerated as monotherapy or adjunctive therapy among adults with depressive symptoms. If effective for depression and other psychiatric disorders, the omega-3 fatty acids may have broad appeal, as well as being particularly well-suited for treatment of specific patient populations such as pregnant or lactating women for whom antidepressants are used with caution (Chiu et al., 2003), for elderly people who may not tolerate side effects of conventional antidepressants agents, and for those with medical comorbidity, particularly cardiovascular disease and possibly autoimmune conditions, for which there may be dual benefits.

To date there are two published omega-3 fatty acid treatment studies comparing the efficacy of EPA vs. DHA, one as monotherapy (Mischoulon et al, 2015), previously discussed, and one as augmentation (Mozaffari-Khosravi et al, 2013). In the latter, 12 weeks of adjunctive 1 g/day EPA produced significantly greater reductions in HAM-D-17 scores than 1 g/day DHA in antidepressant-treated subjects with MDD. Apart from our recent study (Rapaport et al, 2015) there had been no published studies that elucidate mediators and moderators of

treatment response or mechanisms of action for the omega-3 fatty acids. Bridging and clarifying these knowledge gaps is the aim of this project, which will focus on treatment efficacy, immune function, and PUFA and lipid metabolism, which seem particularly likely to yield important information about the link between omega-3 fatty acids and depression. While this knowledge is crucial to the elaboration of rational guidelines for the use of omega-3 fatty acids in depression, the results should further our understanding of the pathophysiology of depression and its interrelationship with other medical disorders and contribute to the development of novel therapies.

## 4. Evidence for a Link between Omega-3 and Immune Function

Some of the most compelling and best understood physiological effects of PUFAs are the conversion of these 20-carbon length PUFA into eicosanoids, a series of oxygenated metabolites many of which have a prominent role in immune and inflammatory processes (Meydani, 1993; Calder, 2001; Grimble, 1998). Eicosanoids include prostaglandins, leukotrienes, lipoxins, thromboxane, and prostacyclin. Those derived from the omega-6 fatty acids (arachidonic acid) are generally strongly proinflammatory, while those derived from omega-3 fatty acids are typically less inflammatory (e.g., series 3 vs. 2 prostaglandins, series 5 vs. 4 leukotrienes) and platelet aggregatory (thromboxane A3 vs. thromboxane A2) and may therefore counter the impact of omega-6 fatty acid-derived eicosanoids. The balance downstream between omega-3 fatty acids and omega-6 fatty acids may also influence the balance between anti-inflammatory cytokines such as interleukin (IL) 4, IL-10, and IL-13, and proinflammatory cytokines such as IL-1, IL-6 and TNF-a (Meydani, 1996; Grimble et al., 2002). Epidemiological data clearly demonstrate that a higher omega-6:omega-3 fatty acid ratio is associated with increased levels of the pro-inflammatory acute phase protein C-reactive protein (CRP) and IL-6, and an increased risk of developing cardiovascular disease (Madsen et al., 2001; Ross 1999; Libby 1995). Omega-3 fatty acids decrease the expression of pro-inflammatory cytokines and CRP in both in vivo and in vitro human and animal models. Studies demonstrate that omega-3 fatty acids can decrease plasma levels of CRP as well as mitogen-stimulated and autologous lymphocyte-stimulated pro-inflammatory cytokine production (James et al., 2000; Phillips et al., 2003; Loukianos et al., 2003; Meydani et al., 1993; De Caterina et al., 1994; Ernst et al., 1991; Khalfoun et al., 1997a,b,c; Khalfoun et al., 1998; Purasiri et al., 1997; Kelly, 1991; Lee et al., 1985).

## 5. Evidence for a Link between Depression and Immune Function

Several lines of evidence support the hypothesis that a subset of individuals with major depressive disorder also manifests signs of chronic mild inflammation. Some of the most exciting recent work has been stimulated by epidemiological findings demonstrating that major depressive disorder is an independent risk factor for cardiovascular and cerebral vascular disease (Ford and Erlinger 2004; Pratt et al., 1996; Ford et al., 1998; Mendes et al., 1998; Glassman and Shapiro, 1998; Ruguilies, 2002; Larson, 2001; Jonas and Mussolino, 2000; Musselman et al., 1998). This led investigators to search for common dysfunctional processes observed in cardiovascular and cerebrovascular disease and major depressive disorder. Recent work in cardiology demonstrated that mild chronic inflammation, as manifested by elevations of acute phase proteins such as CRP, is a significant risk factor for subsequent cardiovascular disease (Grundy et al., 2001; Ross, 1999; Danesh et al., 2000; Ford and Erlinger, 2004). Subtle but significant increases in proinflammatory acute phase proteins and cytokines represent a plausible biological explanation for the linkage between depression and the risk of cardiovascular disease.

A small group of investigators began to publish data in the early 1990s suggesting that individuals in an acute episode of major depressive disorder manifested signs of immune activation (Leonard, 2001; Maes, 1999a). Although this literature is not unequivocally positive, the current consensus in the field is that a some patients with non-melancholic major depressive disorder have increased serum/plasma levels of positive acute phase proteins (CRP, haptogloblin, and alpha-1-acid glycoprotein), pro-inflammatory cytokines (IL-1, IL-6, IFN-G and TNF-alpha) and mitogen-stimulated pro-inflammatory cytokine production (Maes et al., 1990-91, 1993a,b,1997a,b,c,1998; Muller et al., 1993; Licinio and Wong, 1999; Musselman et al., 2001a; Owen et al., 2001; Rothermundt et al., 2001a,b,c; Leonard, 2001, Maes, 1999a; Mikova et al., 2001; Anisman, 1999; Kahl et al., 2005; Penninx et al., 2003; Thomas et al., 2005). Recently published work supports the linkage between

increased levels of CRP with major depressive disorder, particularly in men (Ford and Erlinger, 2004; Kop et al., 2002; Lesperance et al., 2004). Plasma levels of sICAM also were elevated in depressed individuals recovering from an acute myocardial event (Lesperance et al., 2004). Another less direct line of evidence for the association between mild inflammation and major depressive disorders is a series from preclinical studies suggesting that antidepressants have immunosuppressive effects (Danzer et al., 1999; Maes et al., 1999b; 2000; Xia et al., 1996). The clinical studies in depressed subjects, designed to complement the animal studies demonstrating immunosuppressive effects of antidepressants, have been less robust (Lin et al., 2000; Kubera et al., 2000). In summary, the confluence of data suggests that some people with major depressive disorder do manifest signs of mild chronic immune activation and inflammation.

**c. Rationale behind the proposed research, and potential benefits to patients and/or society**

**The Importance of Studying Overweight Subjects with MDD and Elevated CRP Levels**

The biological heterogeneity of the subjects who fall within the DSM 5 definition of major depressive disorder (MDD) has been a significant source of variance that has led to problems with evaluating the effects of experimental treatment interventions, including complementary and alternative treatments. Thus it is imperative that the field identifies biomarkers that allow us to begin to rationally subdivide this heterogeneous syndrome. There is a growing body of evidence that one subgroup of patients with MDD have increased peripheral markers of inflammation and that this sub-group is less responsive to conventional treatment and, in at least 2 published reports, more responsive to anti-inflammatory therapy (Raison et al., 2013; Rapaport et al., 2015).

We believe studying these patients is critical to the consistent success of any study in MDD of EPA-enriched omega-3 (n-3) fatty acids. A recent meta-analysis (Grosso et al., 2014) of studies of omega-3 fatty acids in MDD has concluded that the use of omega-3 PUFA is modestly and inconsistently effective in patients with diagnosis of MDD and on depressive patients without diagnosis of MDD, which is likely to be due to the fact that none of these studies enriched their population based on inflammatory status and/or obesity. In fact, in a recent study from our group (Rapaport et al., 2015) of 155 subjects with MDD randomized to 8 weeks of double-blind treatment with eicosapentaenoic acid (EPA)-enriched n-3 1060 mg/day, docosahexaenoic acid (DHA)-enriched n-3 900 mg/day or placebo, although overall treatment group differences were negligible (ES=-0.13 to +0.04), subjects with any 'high' inflammation improved more on EPA than placebo (ES=-0.39) or DHA (ES=-0.60). For subjects with 2-3 “high” biomarkers of inflammation the ES for EPA versus placebo increased to -0.59 and if subjects had more than 3 “high” biomarkers of inflammation, the ES increased to -1.11 versus placebo. Subjects with 'high' biomarkers of inflammation were also less placebo-responsive than subjects with low levels of this biomarker. This replicates a finding reported by Raison and colleagues (2013). These findings support the importance studying the patients defined for inclusion in the proposed trial.

Two other lines of research support the idea of employing an approach that investigates the efficacy of EPA specifically in a cohort of MDD subjects who meet criteria for inflammation. A recent study by Su and colleagues (2014) focused on subjects treated with interferon-alpha (IFN-α) therapy for chronic hepatitis C virus infection, therapy frequently associated with the emergence of depression. They conducted a 2-week, double-blind, placebo-controlled trial comparing EPA, DHA, and placebo for the prevention of IFN-α-induced depression. A total of 162 patients consented to participate and were randomized to the study. All of the patients completed the 2-week trial of either 3.5 g/day EPA, 1.75 g/day DHA or oleic acid placebo; 152 participants were followed throughout the 24 weeks of IFN-α treatment and were included in the analysis. Compared with placebo, the incident rates of IFN-α-induced depression were significantly lower in EPA-treated but not in DHA-treated patients (10% and 28%, respectively, versus 30% for placebo, p =.037). Two weeks of 3.5 g/day EPA was also associated with a statistically significant increase in RBC EPA and DHA levels. In a second study, Ferguson and colleagues (2014) reported that 8-weeks of treatment with 3,600 mg/day of EPA+DHA attenuated *all* of the LPS-induced plasma markers of endotoxemia, as well as fever (p=0.03). Although the plasma cytokine and hs-CRP findings did not reach statistical significance in this small study in a heterogeneous cohort of normal volunteers,

the pattern is suggestive that pre-treatment with EPA+DHA did decrease the inflammatory response to in vivo exposure to LPS.

In summary, we believe the proposed enrichment will increase our ability to detect the biosignature of EPA-enriched omega-3 fatty acids, while reducing placebo response and enhancing the likelihood of antidepressant benefit from such treatment.

**How EPA-enriched Omega-3 Fatty Acid Treatment May Lead to Reduction in the Peripheral Levels of interleukin-6 (IL-6) in Overweight Depressed Patients**

One of the most consistently replicated findings in investigation of peripheral biomarkers and mood disorders is that IL-6 levels are elevated in some acutely ill patients with MDD (for review see Schmidt et al., 2011; Miller at al., 2009). IL-6 is unique amongst the cytokines measured in the periphery in that it is more stable and less evanescent. There are very reliable ELISA assays for plasma IL-6 and our group has over a decade of experience with these assays. Furthermore, in a recently submitted meta-analysis of all studies investigating the blood cytokine levels in acutely ill patients with schizophrenia, bipolar disorder and MDD before and after treatment, we found that IL-6 was elevated in subjects with MDD (ES =0.76; CI 0.56- 0.95, P<0.02) (Miller, Goldsmith, Rapaport, submitted, Mol Psychiatry). We also found that IL-6 levels decreased in response to conventional antidepressant treatment of the episode of MDD (ES -0.36, CI -0.62 to -0.09, P=0.01).

There is little data from the depression literature to inform us about the amount of change IL-6 levels we would anticipate observing with EPA monotherapy in subjects with MDD and thus it is important to look to other studies investigating the impact of EPA on IL-6 levels. In studies of obesity, as the central obesity increased, the level of adipocyte synthesis of pro-inflammatory cytokines like tumor necrosis factor alpha (TNF-α) and IL-6 were increased, indicating a state of chronic inflammation, with the level of C reactive protein (CRP) synthesized from hepatocyte being increased by the influence of IL-6 (Ellulu et al., 2015). In a study by Tousoulis and colleagues (2014), a 19% reduction in IL6 levels from 0.26 to 0.19 pg/ml on omega-3 fatty acids compared to a reduction in IL6 levels from 0.29 to 0.27 pg/ml at 4 weeks was associated with markedly significant improvement of endothelial function and reduction in arterial stiffness among adults with metabolic syndrome.

In molecular studies, omega-3 fatty acids show direct effects on reducing the inflammatory state by reducing IL-6 and TNF-α (Ellulu et al., 2015). Itariu and colleagues (2012) have shown that long chain omega-3 PUFA reduce the concentration of inflammatory markers such as IL-6 in obese subjects. Numerous studies have consistently shown that omega-3 PUFA reduce systemic and adipose tissue inflammation, induce anti-inflammatory gene expression in circulating mononuclear cells and improve metabolic control in severely obese, overweight and elderly subjects. (Ellulu et al., 2015; Itariu et al., 2015; Bakker et al., 2010; Bouwens et al., 2009). For all these reasons, we believe we should see a clear biosignature of EPA-enriched omega-3 fatty acid treatment through a reduction in the peripheral levels of IL-6 in overweight depressed patients and we would expect a clinically significant 15% change in IL-6 levels with EPA-enriched omega-3 fatty acid treatment compared to placebo.

**How EPA-enriched Omega-3 Fatty Acid Treatment May Lead to a Reduction in the Tumor Necrosis Factor-α Response to LPS Stimulation in Depressed Patients**

We propose performing complementary assessments of inflammation by measuring both peripheral plasma cytokine levels as well as mitogen-stimulated PBMC cytokine expression and production. This allows us the opportunity to directly measure the impact of EPA therapy for overweight subjects with increased inflammation on PBMC, key functional components of the immune system involved in modulating acute and chronic inflammatory responses.

A large body of preclinical evidence strongly supports the notion that EPA-enriched omega-3 fatty acid treatment should lead to a reduction in the TNF-α response to LPS stimulation in overweight depressed patients. In a study from our group (Wang et al., 2009), relative to control, macrophages exposed to EPA resulted in lower levels of

TNF-α in the culture medium after stimulation with LPS. Of note, the relationship between inflammatory factor secretion and their mRNA levels was consistent for cells treated with EPA relative to the other cells. EPA has also been shown to down-regulate TNF-α secretion in human adipose tissue and adipocytes cultures (Murumalla et al., 2012) and this is highly relevant to the population of overweight patients with MDD of the proposed study. In addition, male rats receiving a high n-3/n-6 PUFA ratio (1:1, PUFA) diet showed a significant reduction in TNF-α levels compared to control diets (Liu et al., 2013). Fish oil has been shown to attenuate the activation of the HPA axis induced by LPS challenge by decreasing the production of brain/peripheral proinflammatory cytokines through inhibition of TLR4 and down regulating the mRNA expressions of TNF-α receptor-associated factor 6 (Liu et al., 2013). A recent study in mice by Liu YH et al. (2015) has confirmed that omega-3 PUFA intervention can suppress LPS-induced inflammation via, at least in part, down-regulation of pro-inflammatory targets of the TLR4 signaling pathway. Treatment with omega-3 has also been shown to result in significant down-regulation of LPS-induced production of TNF-α by PBMCs (Chitenjali et al., 2015). Similarly, EPA in vitro effectively reduced LPS-induced TNF-α expression significantly when compared with arachidonic acid (Hao et al., 2010). Itoh et al. (2007) found that treatment with EPA at doses of 100 and 200 mol/L significantly suppresses the co-culture-induced increase in TNF-α mRNA expression. Finally, after LPS challenge, pigs fed a diet supplemented with omega-3 fatty acids had significantly lower serum concentrations of TNF-α (Upadhaya et al., 2015).

Several studies in healthy human volunteers involving supplementation of the diet with fish oil have demonstrated decreased production of TNF-α and IL-6 by LPS-stimulated monocytes or PBMC (Caughey et al., 1996; Meydani et al., 1991; Baumann et al., 1999; Trebble et al., 2003; Abbate et al., 1996). More specifically, PBMCs isolated from 9 subjects supplemented with 18 g/d of fish oil concentrate secreted reduced levels of TNF-α in response to stimulation with LPS (Endres et al., 1989). In addition, 9.4 g/d EPA + 5 g/d DHA for 10 weeks resulted in decreased neutrophil chemotaxis to LTB4 (Sperling et al., 1993). Supplementation with 1.62 g/d EPA + 1.08 g/d DHA for 4 weeks in 28 healthy volunteers reduced secretion of TNF-α by PBMCs, in response to LPS compared to secretion prior to dietary supplementation; the reduction in cytokine secretion inversely correlated with the EPA content of mononuclear cells (Caughey et al., 1996). It has been found that 2.3 g/d omega-3 PUFAs for 3 months reduced ex vivo TNF-α secretion by PBMCs in response to concanavalin A (ConA) or phytohaemagglutinin (PHA) (Meydani et al., 1991). Trebble et al. (2003) demonstrated that supplementing 16 healthy men with increasing concentrations of EPA + DHA over 12 weeks (0.3–2.0 g/d) inhibited TNF-α production in response to LPS. Based on these studies, we would expect a clinically significant 15% change in TNF-α production in response to LPS with EPA-enriched omega-3 fatty acid treatment compared to placebo.

In view of the above, it is reasonable to suggest that imbalances in polyunsaturated fatty acids (PUFA) lead to the chronic low level stimulation of pro-inflammatory components of the immune system (acute phase proteins and cytokines) that may be either moderators and/or mediators of depressed states, and may play a role in the reversal of depression when omega-3s are administered. There is a notable lack of information investigating this hypothesis. A better understanding of the relationship between the omega-3 fatty acids and depression is likely to significantly extend our understanding of the pathophysiology and treatment of depression, as well as its increasingly appreciated interconnections with other disorders such as cardiovascular illness.

We propose as a primary goal, to evaluate whether a dose-response relationship exists between dose of polyunsaturated fatty acids (PUFA), delivered as eicosapentaenoic acid (EPA), and change in markers of inflammation, and whether these effects differ from placebo. A key secondary aim is to evaluate the antidepressant effectiveness of EPA in overweight adult outpatients with current major depressive disorder (MDD). To address these aims, the project will use a four-arm, randomized, parallel-group, placebo-controlled design comparing placebo versus three doses of EPA (1 gm/day, 2 gm/day, or 4 gm/day) administered over 12 weeks. The study is to be conducted at two sites: Emory University School of Medicine, and Massachusetts General Hospital. Eligible participants will be between the ages of 18-80 who have current MDD, are overweight, and who demonstrate peripheral inflammation, defined as an high sensitivity C-reactive protein level $\geq$ 3 mg/L. The primary outcome will be change in plasma IL-6 levels and/or mitogen-stimulated peripheral blood mononuclear cells (PBMC) TNF-α expression levels in EPA- versus placebo-treated participants.

The results of this investigation are intended to be used to design and power a larger definitive test of the efficacy and biological effects of EPA in patients with major depressive disorder. Our proposal has relevance to understanding the clinical and biological mechanisms of action of a widely used, effective, and safe natural antidepressant, as well as providing insight into the interrelationship between depression, stress, obesity, and immune function.

## II. SPECIFIC AIMS

### a. Specify objectives and hypotheses to be tested in the research project

We plan to test our central hypotheses and accomplish the overall objective by pursuing the following specific aims:

**Specific Aim 1 (Primary Aim):** To evaluate whether a dose-response relationship exists between dose of EPA and decrease either in plasma IL-6 levels or in mitogen-stimulated PBMC TNF-α expression and secretion, when compared with placebo.

*Hypothesis 1: Overweight subjects with MDD and hs-CRP levels ≥3.0 mg/l treated with 1g/day, 2g/day or 4g/day of EPA will demonstrate a ≥0.40 effect size at both week 8 and week 12 for decrease in plasma IL-6 levels and/or mitogen-stimulated PBMC TNF-α expression and secretion when compared with placebo-treated subjects. The dose-response relationships on the two inflammatory markers will be explored.*

**Specific Aim 2:** To evaluate whether EPA treatment produces a decrease in ratings of depression severity, when compared with placebo-treated subjects; and whether the changes in IL-6 or mitogen-stimulated PBMC TNF-α expression will mediate changes observed in ratings of depression.

*Hypothesis 2a: Overweight subjects with MDD and hs-CRP levels ≥ 3mg/l treated with 1g/day, 2g/day or 4g/day of EPA will demonstrate a ≥0.35 effect size at both week 8 and week 12 for-decrease in ratings of depression severity, as measured by the Inventory of Depressive Symptoms, Clinician-Rated version, when compared with placebo-treated subjects. The dose-response relationships on the depression score will be explored.*

*Hypothesis 2b: Changes in IL-6 and mitogen-stimulated PBMC TNF-α expression will mediate changes observed in ratings of depression.*

**Exploratory Aim:** To evaluate whether EPA treatment produces decreases in plasma TNF-α, leptin, hs-CRP, and IL-1ra levels and mitogen-stimulated PBMC IL-6 expression, as well as in the expression of inflammation pathway-related genes.

*Hypothesis 3a:* Overweight *subjects with MDD and hs-CRP levels ≥ 3mg/l treated with 1g/day, 2g/day or 4g/day of EPA will demonstrate a decrease in plasma TNF-α, leptin, hs-CRP, and IL-1ra levels and mitogen-stimulated PBMC IL-6 expression when compared with placebo-treated subjects.*
*Hypothesis 3b): Overweight subjects with MDD and hs-CRP levels ≥ 3mg/l treated with 1g/day, 2g/day or 4g/day of EPA will demonstrate a decrease in the PBMC expression of genes involved in the inflammatory pathway when compared with placebo-treated subjects.*

## III. SUBJECT SELECTION

### a. Inclusion/exclusion criteria

**Inclusion Criteria:**
To be included in the study, participants must meet all of the following:

1. Ability to provide informed consent for study participation.
2. Men or women aged 18-80 years old.
3. Have a current primary psychiatric diagnosis of major depressive disorder (MDD), as defined by DSM-5 criteria using the MINI v.7.0.
4. A Screening and Baseline visit Inventory of Depressive Symptoms, Clinician rated (IDS-C30) total score $\geq$ 25.
5. Overweight at screening, defined as BMI > 25 kg/m$^2$**.**.
6. Screening visit high-sensitivity C-reactive protein concentration $\geq$ 3 mg/L.
7. Willing to not significantly modify their diet from the time they sign consent through the end of study participation.

**Exclusion Criteria:**
Potential participants will be excluded if they meet any of the following criteria:

1. Use of any psychotropic agents within 2 weeks of the baseline visit, with the exception of prescription hypnotics (eszopiclone, zaleplon, zolpidem, suvorexant, ramelteon), diphenhydramine, or a stable daily dose of a benzodiazepine.
2. Breastfeeding or pregnant women, women intending to become pregnant within 6 months of the screening visit, or women of child bearing potential who expect to engage in heterosexual sex during trial participation and are not using a medically accepted means of contraception (defined as oral contraceptive pill or implant, condom, diaphragm, IUD, status-post tubal ligation, or partner with vasectomy)
3. Patients who, in the investigator's judgement, pose a current, serious suicidal or homicidal risk.
4. Serious or unstable medical illness that in the investigator's opinion could compromise response to treatment or interpretation of study results.
5. History of seizure disorder, except for childhood febrile seizures.
6. Meeting DSM-5 criteria at any point in their lifetime, for any of the following:
    a. Neurocognitive Disorder
    b. Psychotic Disorder
    c. Bipolar disorder
    d. Anorexia Nervosa
7. Meeting DSM-5 criteria in the 3 months prior to the screening visit for any Substance Use Disorder (except for nicotine or caffeine use disorder).
8. Meeting DSM-5 criteria at screening for current obsessive compulsive disorder or bulimia nervosa.
9. Presence of psychotic features at any time during the current major depressive episode.
10. Any conditions or medications (within 1 week of baseline or during the trial) that might confound the biomarker findings, including:
    a. Regular ingestion of NSAIDs or COX-2 inhibitors, or any use of oral steroids, immunosuppressants, interferon, chemotherapy, or anticoagulants.
        i. NOTE: Patients will be instructed not to take an NSAID or COX-2 inhibitor in the 24 hours prior to a biomarker assessment visit.
    b. Malignancy not in remission for at least 1 year.
    c. Active autoimmune disorder or inflammatory bowel disease.
    d. Insulin-dependent diabetes mellitus.
11. History of severe sensitivity to soy products, fish products, or PUFA supplements.
12. Laboratory evidence of undiagnosed hypothyroidism or any change in treatment for hypothyroidism in the 3 months prior to screening.
13. Patients who have failed to respond during the course of their current major depressive episode to >4 adequate antidepressant trials, defined as six weeks or more of treatment with the FDA-defined minimally effective dose.

14. Patients who have taken a supplement of at least 1 g/day of omega 3 fatty acids for at least 6 weeks during the current major depressive episode.
15. Patients who have had electroconvulsive therapy (ECT) during the current depressive episode or within 6 months of the screening visit.
16. Patients who have taken supplements enriched with omega-3 fatty acids (see Appendix A for list of products) within sixty (60) days of the screening visit.
17. Patients who, at baseline, are consuming a diet that contains more than 3g/day of omega-3 FA, or who consume more than 3 meals of fatty fish per week.
18. Patients who have a history of a bleeding disorder.
19. Patients who have participated in another clinical trial of an investigational medication within 1 month of the screening visit.
20. Patients who are currently in psychotherapy that was initiated within 90 days prior to the study screening visit.

**b. Source of subjects and recruitment methods**

1)Patients in the Depression Clinical and Research Program (DCRP) at MGH and Emory University clinical practices and studies; 2) Referrals from members of the MGH and Emory Psychiatry Departments (over 600 and 250 clinicians, respectively) and primary care physicians who will receive informational talks about the study; 3) Referrals from the MGH Weight Center and the Emory Nutrition and Metabolism Support Service, the Emory University Hospital Center for Clinical and Molecular Nutrition, and the Emory Bariatric Service (family members of patients), and primary care practices at Brigham and Women's Hospital; 4) IRB-approved flyers, web, newspaper, TV, radio and MBTA advertisements; and 5) MGH Patients in the Research Patient Data Registry (RPDR) system who have consented to be contacted through the Research Opportunities Direct to You (RODY) program**.** We will also seek approval to recruit study participants at Grady Health System in Atlanta, GA. Emory will also utilize an existing study participant pool as a recruitment strategy from existing Emory IRB-approved studies (IRB 00062830, PI: C.Gillespie and IRB 0078593, PI: T. Jovanovic).

*Retention feasibility:*
In our previous clinical trials our attrition rates were < 20% overall. We expect a comparable or lower attrition rate in this study for several reasons: 1) The focus on overweight individuals will allow us to expand our usual recruitment base, and these subjects should be particularly invested in the study, given the severity of being overweight and its impact on mood, and 2) All subjects will be offered free three-month follow-up treatment at the conclusion of the study.

**IV. SUBJECT ENROLLMENT**

**a. Methods of enrollment, including procedures for patient registration and/or randomization**

The subjects will be recruited through advertisements and clinical referrals from psychiatrists and general physicians who are treating outpatients with MDD. Participants must agree not to significantly modify their diet during the 12 weeks of the study.

Approximately 400 adult MDD patients (ages 18-80) will be enrolled in the study, and approximately 100 of these participants will be randomized to enter the 12-week double-blind treatment period. Because the primary statistical analysis is based on the analysis of 80 per-protocol completers, the actual number enrolled may differ from 100, based on the participant early termination rates. Each of the four study arms (3 EPA arms and one placebo arm) will have 25 patients, with the expectation of 20 completers per arm, based on a 20% early termination rate.

The target sample of 80 per-protocol completers will be based on the following criteria:

- Complete 12 weeks of treatment and the week 12 biomarker and clinical assessments within the protocol-defined visit window.
- Adhere to the study treatment, defined as taking between 80-125% of the study medication
- Do not report engaging in any major protocol violations during the trial that would threaten the validity of the clinical or biological data, including:
    - Taking any antidepressant or mood stabilizing medication.
    - Taking any immune-modulating drugs, other than those permitted in the protocol.
    - Taking any illicit drugs
    - Starting a new course of an evidence-based psychotherapy for depression.

**b. Procedures for obtaining informed consent**

The subjects for this study must be capable of understanding the nature of this study as well as the discomforts and potential benefits. These will be explained in full by licensed physician investigators, and subjects interested in participating will then be asked to sign a consent form.

**c. Treatment assignment, and randomization**

Patients screened for the study and found to be eligible will return for their baseline visit after one week, during which no psychotropic medication or PUFAs will be administered. Patients must have an IDS-C30 total score ≥ 25 at the baseline visit in order to be eligible for randomization. The baseline IDS-C30 may be administered either by telephone 48 hours prior to V3, or in person at V3.

The double-blind treatment phase starts with the baseline visit and ends with the Week 12 visit (V9). Participants will be seen every two weeks during this phase to assess change in depressive symptoms, adverse events, and to assess changes in biomarkers. Fasting blood draws will occur at visits 3, 5, 7, and 9. We will ask participants to fast from the midnight prior to the visit until after the blood draw. We will ask them to not eat any food or drink anything besides coffee, tea, or water until after the blood draw is complete. They can take any normal medications they take every morning as well.

Randomization will be in randomly permuted blocks of 4 or 8 with separate randomization schedules for each site. The randomization schedule for each site will be created by a statistician who has no other role in the study data collection or analysis. The randomization schedules will be sent directly from the statistician to each site's Research Pharmacy which will maintain the list. Qualifying study subjects will be assigned the next sequential treatment assignment on the randomization schedule, in the order they are randomized.

If a study participant is unable to attend their scheduled V4, V6, or V8 appointments, all assessments can be completed over the phone and the visit should continue as usual. Vitals will not be collected for a visit completed over the phone. The participant will be directed to take pills from their extra blister pack until new study medication can be mailed overnight to the participant's home. The study participant will have the opportunity to complete their self-report forms online, or they will have their self-report forms mailed overnight to their home, along with a return envelope so that the participant can either mail back or scan in their completed self-report forms for the visit. Compensation for this visit will be issued at the following scheduled visit. Completion of the visit over the phone may not take place if the participant is ill and may not occur more than two times with each study participant.
Patients will be randomized to one of four treatment arms: 1) EPA 1 g/day; 2) EPA 2 g/day; 3) EPA 4 g/day; or 4) Placebo capsules that are matched to the EPA capsules in terms of appearance, odor, and taste. At the time of randomization, the study coordinator will contact the Research Pharmacy to complete the coded treatment assignment described below.

Blinding of randomized treatment will be ensured as follows: The randomization list maintained by the Investigational Research Pharmacies will contain the actual treatment assignment, along with a letter code (A, B,

C, or D) corresponding to one of the 4 treatment groups - with this correspondence being decided and communicated between the 2 pharmacies at the start of the study. Throughout the study period and continuing until the major hypotheses have been tested and reported, the correspondence of letter-coded treatment assignment to actual treatment (one of 3 EPA doses or placebo) will be known only by pharmacy staff. The study PI, study coordinators, clinicians conducting outcome assessments, and study statistician, as well as research assistants interacting with patients or entering/editing study data and laboratory technicians performing and reporting biomarker and PUFA assays, will have no access to any subject's actual treatment assignment. Conversation among study staff, or between subjects and staff concerning speculated assignment will be strongly discouraged. Only after the study statistician has completed and reported analyses by coded treatment assignment for study Aim 1 (primary hypothesis, concerning impact of treatment on plasma IL-6 levels or in mitogen-stimulated PBMC TNF-α expression and secretion) and Aim 2a (secondary hypothesis, concerning impact of treatment on IDS-C scores) will the correspondence between letter codes and actual treatment be revealed to study personnel. The only exception to this blinding procedure is that a pharmacist with access to actual treatment assignment may unblind a subject in order to establish the proper follow-up treatment for any subject who develops a serious adverse event that is possibly related to PUFA treatment; if this occurs, follow-up of the subject will be handled by a physician who is not involved in the study.

**d. Subject Compensation**

At Emory, participants will receive $30 for each completed screening visit (V1 and V2). Beginning with Visit 3, Emory participants will receive $40 for each completed study visit with fasting blood draws (V3, V5, V7, and V9) and $20 for each completed study visit without blood draws (V4, V6, and V8). Participants at MGH will receive $20 for the first screening visit and $25 for the second screening visit. After randomization, MGH participants will receive $40 for every visit they attend with a fasting blood draw (visits 3, 5, 7, and 9) and $25 for the visits without fasting blood draws (visits 4, 6, and 8). If participants do not finish the study, we will compensate them for the visits they have completed. At both sites, if participants complete all study visits, they will receive $280 total. Parking vouchers will be provided for the MGH garages and patient transportation costs of up to $25 per study visit will be remunerated as to relieve the burden of travel expenses. Study participants coming from Grady Health System to Emory University may be reimbursed for transportation or their transportation expense may be paid with an Emory-owned credit card. In case study visits are performed at Grady Health System, study participants will be remunerated for their study visit as well as for parking (vouchers preferred). Parking at Emory's main study location is free, so travel expenses will not incur for study participants coming to the main study's location.

## V. STUDY PROCEDURES

**a. Study visits and parameters to be measured**

Approximately 400 patients will enter the 12-week four-arm, randomized, parallel-group, placebo-controlled design, which includes randomization of 25% of the patients to 1g/day of EPA, 25% of the patients to 2g/day of EPA, 25% to 4g/day of EPA, and 25% of the patients to placebo. Each arm will have 100 patients, with the goal of achieving 80 completers per arm, and assuming a 20% early termination rate. The subjects will be drawn through primary care physicians, the MGH Weight Center, the Emory Nutrition and Metabolism Support Service, the Emory University Hospital Center for Clinical and Molecular Nutrition, the Emory Bariatric Service, Grady Health System and IRB-approved flyers, newspaper, TV, radio, MBTA advertisements, and clinical referrals.

i. SCREENING PHASE

Due to the need for participants to have a hs-CRP $\geq$ 3 mg/L to be eligible for the study, two screening visits will be used to minimize expenses associated with screening. However, elements of the screening visits (V1 and V2) may be combined as needed (i.e., if a participant prefers to complete screening in one office visit due to time/transportation constraints). The screening period may extend up to 28 days prior to the baseline visit (V3) if necessary to allow for time need for participant scheduling, washout of any psychotropic medications, and allow

for any required repeat laboratory testing.

For study participants at the Emory sites, a portion of the assessments may be performed at the Atlanta Clinical Translational Science Institute (ACTSI), an Emory and Grady based entity, providing research space and resources for Emory and Grady faculty.
Study participants can choose whether to have all study visits done at Emory or Grady.

Visit 1:

The goal of this visit is to allow identification of easily identified exclusionary criteria, and to establish that the participant meets the inclusion criteria necessary to proceed to Visit 2.

The first screening visit, Visit 1, will require approximately 90 minutes during which the following procedures will be performed:

1. Informed consent process
2. Collection of demographic information
3. Establish that the participant is in a current major depressive episode, using the MINI Mood module
4. Establish that the severity of depressive symptoms is sufficient for eligibility, using the IDS-C30
5. Establish that subject is overweight, using height and weight
6. Establish fatty fish consumption frequency, to ensure participant consumes no more than three meals of fatty fish per week.
7. Vital signs assessment
8. Urine drug screen
9. Blood draw for hs-CRP
10. Review of concomitant medications and supplements used by the participant
11. Give copy of Food Record to the participant to complete for the three days prior to V2

Visit 2:

The goal of Visit 2 is to confirm all eligibility criteria are met for the participant to proceed to the baseline visit. Visit 2 will occur approximately 3 days after Visit 1. The washout period, if necessary, will begin during this visit and will vary according to subject's current medication(s), dose, and study physician's discretion, and will be completed at least 2 weeks prior to the baseline visit. This visit will require approximately 90 minutes, during which the following procedures will be performed:

1. Collect Food Diary and complete Food Processor Report to ensure participant does not have a diet that involves consumption of >3 g/day of omega-3 FA
2. Completion of MINI to identify excluded and comorbid psychiatric disorders
3. Confirmation of psychiatric diagnosis by interview by psychiatrist
4. Medical history and physical exam
5. 12-Lead electrocardiogram (EKG)
6. Laboratory screening, including:
    a. Complete blood count
    b. Comprehensive metabolic panel (blood chemistry), including liver function tests
    c. Thyroid function tests
    d. Urinalysis
    e. Urine pregnancy test for women of child bearing potential
7. Columbia Suicide Severity Rating Scale, lifetime version

ii. DOUBLE-BLIND TREATMENT PHASE

The double-blind treatment phase starts with the baseline visit and ends with the Week 12 visit (V9). Participants will be seen every two weeks during this phase to assess change in depressive symptoms, adverse events, and to assess changes in biomarkers.

Visit 3 (Baseline Visit):
Participants completing screening will proceed to the baseline visit. This visit will require approximately 120 minutes and will involve confirmation of continued eligibility, biomarker collection, randomization, instruction on study medication, and completion of self-reports. The baseline IDS-C30 may be administered either by telephone 48 hours prior to V3, or in person at V3. The following procedures will be performed at this visit:

1. Vital signs and weight
2. Assessment of adverse events
3. Review of concomitant medications
4. Tobacco History
5. Clinician-rated instruments, including:
    a. IDS-C30 to confirm total score ≥25 to permit randomization
    b. CSSRS
    c. CGI-S
    d. Hamilton Anxiety Rating Scale
6. Urine pregnancy test for women of child-bearing potential
7. Phlebotomy for biomarker samples including:
    a. Plasma for IL-1ra, IL-6, TNF-α, and leptin
    b. Plasma for C-reactive protein
    c. PBMC for mitogen-stimulated PBMC TNF-α and IL-6 expression and secretion levels
    d. Plasma for Specialized pro-resolving lipid mediators (SPMs)
8. Self-reports, including:
    a. Childhood Trauma Questionnaire (CTQ)
    b. Perceived Stress Scale (PSS)
    c. Life Experiences Survey (LES)
    d. Berlin Questionnaire
    e. Patient Health Questionnaire-15 (PHQ-15)
    f. Symptoms of Depression Questionnaire (SDQ)
    g. United States Index of Deprivation (USiDep)
    h. Sheehan Disability Scale (SDS)
    i. Quality of Life Satisfaction Questionnaire (Q-LES-Q)
    j. Cognitive and Physical Functioning Questionnaire (CPFQ)
9. Randomization and dispensing study medication

Visit 4 (Week 2):
This visit will occur 14 (±3) days after V3. This visit will require about 60 minutes, during which the following procedures will be performed:

1. Assessment of adverse events
2. Review of concomitant medications
3. Clinician-rated instruments, including:
    a. IDS-C30
    b. CSSRS
    c. CGI-S
    d. CGI-I
4. Self-reports, including:
    a. PSS
    b. SDQ
5. Dispensing of study medication and collection of unused study medication

Visit 5 (Week 4):
This visit will occur 14 (±3) days after V4. This visit will require about 90 minutes, during which the following procedures will be performed:

1. Vital signs and weight
2. Assessment of adverse events
3. Review of concomitant medications
4. Clinician-rated instruments, including:
    a. IDS-C30
    b. CSSRS
    c. CGI-S
    d. CGI-I
    e. HAM-A
5. Self-reports, including:
    a. PSS
    b. PHQ-15
    c. SDQ
    d. SDS
    e. Q-LES-Q
    f. CPFQ
6. Phlebotomy for biomarker samples (same as Visit 3)
7. Dispensing of study medication and collection of unused study medication

Visit 6 (Week 6):
This visit will occur 14 (±3) days after V5. This visit will require about 60 minutes, during which the following procedures will be performed:

1. Assessment of adverse events
2. Review of concomitant medications
3. Clinician-rated instruments, including:
    a. IDS-C30
    b. CSSRS
    c. CGI-S
    d. CGI-I
    e. Assessment of Blinding
4. Self-reports, including:
    a. PSS
    b. SDQ
    c. Assessment of Blinding
5. Dispensing of study medication and collection of unused study medication

Visit 7 (Week 8):
This visit will occur 14 (±3) days after V6. This visit will require about 90 minutes, during which the following procedures will be performed:

1. Vital signs and weight
2. Assessment of adverse events
3. Review of concomitant medications
4. Clinician-rated instruments, including:
    a. IDS-C30
    b. CSSRS
    c. CGI-S
    d. CGI-I
    e. HAM-A
5. Self-reports, including:

a. PSS
b. PHQ-15
c. SDQ
d. SDS
e. Q-LES-Q
f. CPFQ

6. Phlebotomy for biomarker samples (same as Visit 3)
7. Dispensing of study medication and collection of unused study medication

Visit 8 (Week 10):

This visit will occur 14 (±3) days after V7. This visit will require about 60 minutes, during which the following procedures will be performed:

1. Assessment of adverse events
2. Review of concomitant medications
3. Clinician-rated instruments, including:
    a. IDS-C30
    b. CSSRS
    c. CGI-S
    d. CGI-I
4. Self-reports, including:
    a. PSS
    b. SDQ
5. Dispensing of study medication and collection of unused study medication

Visit 9 (Week 12) [ALSO EARLY TERMINATION VISIT]:

This visit will occur 14 (±3) days after V8. This visit will require about 90 minutes, during which the following procedures will be performed:

1. Vital signs and weight
2. Assessment of adverse events
3. Review of concomitant medications
4. Clinician-rated instruments, including:
    a. IDS-C30
    b. CSSRS
    c. CGI-S
    d. CGI-I
    e. HAM-A
    f. Assessment of Blinding
5. Self-reports, including:
    a. PSS
    b. PHQ-15
    c. SDQ
    d. SDS
    e. Q-LES-Q
    f. CPFQ
    g. LES
    h. Assessment of Blinding
6. End of Treatment urine pregnancy test for women of child-bearing potential. Women of child-bearing potential will be advised to continue their method of contraception for at least one week after the last dose of study medication.
7. Physical Exam
8. Phlebotomy for biomarker samples (same as Visit 3)

9. Collection of unused study medication

**Safety Data**

The routine laboratory tests (complete blood count, urinalysis and clinical chemistry tests) will be performed at each site. Blood will be collected and analyzed for the screening tests and biomarker analyses. Urine samples will be collected and analyzed with a toxicology screen and urinalysis. We will collect physical records in the form of questionnaires, phone screenings, and psychiatric interviews. We will request access to participants' medical records only for reasons related to patient safety. Participant case report forms will be kept in locked file cabinets in the offices of each study site.

Biological specimens are linked to the individual patient only through a unique research code. All documents that directly reveal the participant's identity, such as signed consent forms, are stored in charts that are marked on the outside only with the participant's code number.

At each visit, an experienced clinician will perform assessment of suicide risk and safety status using the C-SSRS and a clinical evaluation.

**Washout Out Period**

If study participants are currently on a medicine for psychiatric symptoms (except medicines for sleep or anxiety problems) they will need to stop taking them 2 weeks before their baseline visit (Visit 3). Safety monitoring will occur per standard-of-care at each site. If symptoms of major depression worsen significantly during this washout period, including thoughts about suicide or homicide, study participation can be terminated by any study physician and other alternatives will be discussed.

**b. Drugs to be used**

1. Study capsules containing EPA-enriched omega-3, 500 mg tabs, supplied by Nordic Naturals.
2. Placebo capsules matching the EPA 500 mg tabs, also to be supplied by Nordic Naturals.

Blister packs with EPA- and placebo-tablets will be stored at each site's investigational pharmacy. The blister packs will be prepared by each site's investigational pharmacy. The investigational pharmacy will keep the randomization list and dispense the blister packs containing the study medication for each visit during the randomized phase. Participants will be given two blister packs per week. The patient will take one row of four pills at each dosing period totaling 8 pills per day. There will be two additional blister packs dispensed at the beginning of the study, along with a replacement blister pack if needed (7 extra days in case of scheduling issues for the entire trial).

All groups will be instructed to take four capsules of the study medication (or placebo) every morning and another four capsules every afternoon or evening for a total of 8 capsules per day.

c. Devices to be used
None

d. Procedures/surgical interventions, etc.
None

e. Data to be collected and when the data is to be collected

| Study Phase | Screening | | Double-blind Treatment | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Visit Number** | **V1** | **V2** | **V3** | **V4** | **V5** | **V6** | **V7** | **V8** | **V9** |
| **Week** | **-3 - -2** | **-1** | **0** | **2** | **4** | **6** | **8** | **10** | **12** |
| **Day** | **-28 - -10** | **-7** | **0** | **14** | **28** | **42** | **56** | **70** | **84** |
| Informed consent | X | | | | | | | | |
| Demographics | X | | | | | | | | |
| Fatty fish consumption | X | | | | | | | | |
| Vital signs | X | | X | | X | | X | | X |
| Height | X | | | | | | | | |
| Weight | X | | X | | X | | X | | X |
| Psych/Medical History | | X | | | | | | | |
| Physical examination | | X | | | | | | | X |
| 12-lead EKG | | X | | | | | | | |
| MINI v.7.0 | | X | | | | | | | |
| MINI Depression Module | X | | | | | | | | |
| Tobacco History | | | X | | | | | | |
| Dispense Study Drug | | | X | X | X | X | X | X | |
| Blinding Evaluation | | | | | | X | | | X |
| **Laboratory Tests** | | | | | | | | | |
| hs-CRP - Screening | X | | | | | | | | |
| Complete blood count | | X | | | | | | | |
| Comprehensive Metabolic Panel | | X | | | | | | | |
| Urinalysis | | X | | | | | | | |
| Urine drug screen | X | | | | | | | | |
| Urine Pregnancy | | X | X | | | | | | X |
| Thyroid function test | | X | | | | | | | |
| Biomarker Testing | | | X | | X | | X | | X |
| n-3, n-6, and Ratio | | | X | | X | | X | | X |
| **Psychiatric Measures** | | | | | | | | | |
| IDS-C30 | X | | X | X | X | X | X | X | X |
| CGI-S | | | X | X | X | X | X | X | X |
| CGI-I | | | | X | X | X | X | X | X |
| C-SSRS | | X | X | X | X | X | X | X | X |
| HAM-A | | | X | | X | | X | | X |
| CTQ | | | X | | | | | | |
| SDQ | | | X | X | X | X | X | X | X |
| PSS | | | X | X | X | X | X | X | X |
| PHQ-15 | | | X | | X | | X | | X |
| Food Diary | X | | | | | | | | |
| Food Processor Report | | X | | | | | | | |
| SDS, Q-LES-Q, CPFQ | | | X | | X | | X | | X |
| Life Experiences Survey | | | X | | | | | | X |
| USiDep | | | X | | | | | | |
| Berlin Questionnaire | | | X | | | | | | |
| **Safety Measures** | | | | | | | | | |
| Concomitant Meds | X | X | X | X | X | X | X | X | X |
| Adverse Events | | X | X | X | X | X | X | X | X |

Data collection and visit schedule are summarized in the table below:

CGI-I, CGI-S: Clinical Global Impression-Improvement, -Severity; CPFQ: Cognitive and Physical Functioning Questionnaire; CSSR: Columbia Suicide Severity Rating Scale; CTQ: Childhood Trauma Questionnaire; HAM-A: Hamilton Anxiety Rating Scale; IDS-C30: 30-item Inventory of Depressive Symptoms-Clinician rated; LFTs: Liver function tests; MINI: Mini-International Neuropsychiatric Interview; PHQ-15: Patient Health Questionnaire-15 item; PSS: Perceived Stress Scale; Q-LES-Q: Quality of Life Satisfaction Questionnaire; SDS: Sheehan Disability Scale; SDQ: Symptoms of Depression Questionnaire; USiDep: United States Index of Deprivation.

*Please see Appendix B for a description of scales.

Biomarker Sample Collection

Whenever possible all blood samples for biomarker analysis will be collected between 7:30 am and 11 am, after 30 minutes of rest, or at the discretion of the principal investigator. This time restriction does not apply to the screening visit laboratory tests, which may be performed at any time of day.

**g. Laboratory Procedures**

Blood collection: Whole blood for plasma and peripheral blood mononuclear cells (PBMCs) will be collected into EDTA vacutainer tubes. Plasma will be separated by centrifugation at 1000 x g for 20 minutes at 4ºC, aliquoted into chilled siliconized polypropylene tubes and stored at -80ºC until batch assayed for plasma cytokine and hs-CRP analyses. Plasma will be replaced by saline for the isolation of PBMCs on a ficoll-hypaque gradient using CPT tubes. PBMCs will be aliquoted to be used fresh for stimulation assays and stored in freezing serum (90% fetal bovine serum, 10% DMSO) at -80ºC for nuclear extraction or mRNA isolation. We will also collect a 10cc plasma sample to send to Dr. Charles Serhan at Brigham and Women's Hospital, Boston for measurement of Specialized Pro-Resolving lipid mediators (SPMs). These will be run using lipid mediator metabololipidomics analytical methods, as described by Dalli & Serhan (2012) and Colas et al (2014). Briefly, deuterium-labeled internal standards (0.5 ng) are added to plasma aliquots [$d_5$-RvD2, $d_8$-5-hydroxyeicosatetraenoic acid ($d_8$-5-HETE), $d_4$-leukotriene $B_4$ ($d_4$-$LTB_4$), $d_5$-lipoxin $A_4$ ($d_5$-$LXA_4$) and $d_4$-prostaglandin $E_2$ ($d_4$-$PGE_2$) to facilitate quantification of mediator recovery. Samples are extracted using SPE columns, eluted with methyl formate, and organic solvent evaporated using a nitrogen stream. Samples are suspended in methanol for analysis by liquid chromatography coupled with tandem mass spectroscopy (LC-MS/MS), using QTrap ABI 5500 (ABSciex, Framingham, MA).

1. The following procedures will be performed at Emory University

Plasma cytokines: Customized Fluorokine MAP Multiplex Human Biomarker Panels (R&D Systems, Minneapolis, MN) will be used to measure plasma TNF-alpha and IL-6. These inflammatory markers were chosen based on their reliable changes in depression and previous studies demonstrating their change in response to anti-inflammatory therapies. Each determination requires 50-100µl, and all samples will be assayed in duplicate according to manufacturer's instructions. Quality control plasma of both low and high cytokine concentrations will be included with every assay. The mean inter- and intra-assay coefficients of variation for control samples are reliably 10% or less. Cytokines will be expressed in pg/ml. Plasma samples from each subject will be assayed together to avoid inter-assay variability.

C-reactive protein (CRP): Plasma CRP will be assessed with a high sensitivity turbidimetric assay as previously described.(Raison et al, 2013) Sensitivity of the assay is rated at 0.18 mg/L, range of measure is 0.2 to 80 mg/L, and functional sensitivity (at 20% CV) is 0.2 mg/L. Plasma samples from each subject will be assayed together to avoid inter-assay variability.

LPS Stimulation: For stimulated cytokine production and gene expression, 100ul of 2.5 X 106 cells/ml freshly isolated PBMCs will be plated with either media alone or media + 10 ng/ml LPS and incubated at 37°C in 5%

CO2 for 5 hours. Supernatant and cells will then be collected separately and stored at -80°C for analysis of cytokine protein and gene expression as described above.

2. The following procedures will be performed at Tufts-NEMC.

LPS Stimulation: For stimulated cytokine production and gene expression, 100ul of 2.5 X 106 cells/ml freshly isolated PBMCs will be plated with either media alone or media + 10 ng/ml LPS and incubated at 37°C in 5% CO2 for 5 hours. Supernatant and cells will then be collected separately and stored at -80°C for analysis of cytokine protein and gene expression as described above.

Gene Expression: RNA will be isolated from stored PBMCs using an RNeasy mini kit (Qiagen, Valencia, CA). cDNA will be synthesized from RNA using a Reverse Transcription System (Promega, Madison,WI) according to the manufacturer's instructions. Real Time PCR will be performed using SYBR green and Quantitect primer assays (Qiagen,Valencia,CA) for human TNF-alpha (QT00029162) and IL-6 (QT00083720) actin (QT00095431) and glyceraldehyde3-phosphate dehydrogenase (GAPDH) (QT00079247) on a real-time PCR 7300 (Applied Biosystems,Foster City, CA). Relative quantification (ΔΔCt) will be used to assess expression of target genes, using actin or GAPDH as an endogenous control.

The investigators of the proposed research have extensive experience in running the proposed assays including expertise in plasma cytokine determinations (Raison et al, 2009; Raison et al, 2010a; Raison et al, 2010b, Raison et al, 2013), measurement of CRP (Raison et al, 2013), and conducting tissue culture experiments involving cell stimulation.(Kaori et al, 2015; Rapaport et al, 2010; Rapaport et al, 2012; Tsunoda et al, 2015).

3. The following procedures will be performed at Brigham and Women's Hospital-Harvard (Dr. Serhan's lab)

Dr. Serhan's Lab is located at the Center for Experimental Therapeutics and Reperfusion Injury at Harvard Medical School. He will use de-identified plasma samples to run lipid mediator metabololipidomics analyses. Specifically, he will analyze SPMs which include lipoxins, resolvins, protectins, and maresins. These SPM's are enzymatically biosynthesized during resolution of self-limited inflammation, thus providing a biological rationale for a potential reduced CRP-level during and after DHA/EPA treatment.
Targeted SPMs and their precursors, among others, are EPA lipid mediators 18-HEPE. 5-HEPE, 12-HEPE, 15-HEPE, RvE1 and RvE2 as well as DHA lipid mediators 17-HDHA, 14-HDHA, RD1 and PD1.
Changes in Leukotrienes (LTB-4), prostaglandins (PGE-2, PGD-2, PGF2a) as well as thromboxane (TXB-2) will also be identified.

## VI. BIOSTATISTICAL ANALYSIS

### a. Specific data variables being collected for the study

The primary outcome is the change in plasma IL-6 levels or mitogen-stimulated PBMC TNF-α expression levels. Additional biological outcomes include changes in plasma TNF-α, leptin, hs-CRP, and IL-1ra levels and mitogen-stimulated PBMC IL-6 levels.

For the secondary aim of assessing antidepressant efficacy, change in IDS-C30 score will be the key outcome measure. Additional secondary measures of antidepressant efficacy will include change in IDS-C30, CGI-S, and CGI-I ratings, IDS-C30 response rate (defined as ≥50% reduction from baseline score), QIDS-C remission rate (defined as endpoint score ≤5; score extracted from IDS-C30 scale), and attainment of a CGI-I score of 2 or less by the end of treatment.

### b. Study endpoints

Endpoints will be defined as change from baseline to Weeks 4, 8, and 12 for the inflammatory biomarkers, and change from baseline to bi-weekly depression scores; when possible these measures will be obtained at the termination/final visit for patients who choose to (or have to) end participation in the study prematurely.

Patients may choose to withdraw from the study at any time.

Participants may be withdrawn by the investigator should any of the following occur:

(1) severe, persistent intolerance to study medication
(2) worsening of depressive symptoms such that the subject's safety is endangered (e.g. suicidality)
(3) development of mania or psychotic symptoms
(4) a serious adverse event (SAE) that is either:
    i) considered by the investigator to be possibly, probably, or definitely related to the study medication, or
    ii) places the subject at increased risk of harm if she were to continue in the study
(5) persistent non-adherence to the study medication, defined as not taking between 80-120% of the study medication pills for two consecutive visits
(6) development of pregnancy

**a. Suicide risk among patients treated with antidepressant**

Patients with MDD may experience worsening of their depression and/or the emergence of suicidal ideation and behavior (suicidality), whether or not they are taking antidepressant medications, and this risk may persist until MDD remission occurs. This guidance is consistent with global class labelling for antidepressants. Although there has been a long-standing concern that antidepressants may have a role in inducing worsening of depression and the emergence of suicidality in certain subjects, a causal role for antidepressants in inducing such behaviors has not been established. Nevertheless, subjects being treated with study medication will be observed closely for clinical worsening and suicidality, especially at the beginning and end of the course of treatment. Consideration will be given to possibly discontinuing the investigational product in subjects whose depression is persistently worse or whose emergent suicidality is severe or abrupt in onset or was not part of the subject's presenting symptoms. To assess suicidal ideation and behaviors, the CSSRS will be used in this trial.

The following symptoms, anxiety, agitation, panic attacks, insomnia, irritability, hostility, impulsivity, akathisia (psychomotor restlessness), hypomania, and mania, have been reported in patients being treated with antidepressants for MDD. Consideration will be given to possibly discontinuing the study medication in subjects for whom such symptoms are severe, abrupt in onset, or were not part of the subject's presenting symptoms.

<u>Operationalized Discontinuation Criteria for Suicidality:</u>

Research participants are exited from the study should any of the following occur:

- Any emergent CSSRS defined suicidal behavior
- A suicidal ideation score of 5 (indicating active suicidal ideation with specific plan and some level of intent) on the CSSRS
- In the absence of a CSSRS suicidal ideation score of 5 or CSSRS-defined suicidal behavior, the investigator determines the patient to have a significant short-term risk for a suicide attempt.

**b. Pregnancy**

Any participant who becomes pregnant during the study will be withdrawn from the study. The investigator will collect pregnancy information, record it on the Pregnancy Form, and submit it to the lead site PI, Mark Rapaport, MD, via email within 2 weeks of learning of a participant's pregnancy. The participant will also be followed to determine the outcome of the pregnancy. Follow-up is expected to end approximately 8 weeks following the estimated delivery date. Any premature termination of the pregnancy will be reported. While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy for medical reasons will be recorded as an AE. A spontaneous abortion is always considered an SAE and will be reported as such.

**c. Procedures for Early Terminating Participants**
All randomized participants who terminate the trial prior to the Week 12 visit will be asked to return for an early termination visit. The study team should complete all the Week 12 assessments at the study termination visit, with the exception of collecting blood samples. All subjects who complete the trial or discontinue because of withdrawal of consent, lack of response, side effects, or investigator decision will receive treatment as clinically appropriate and will then be referred for appropriate follow-up care.

**c. Statistical methods**
**Preliminary Data Analyses:**

Data Completeness and Quality: During the study, every reasonable effort will be made to minimize potential missing data. Self-report data provided by study subjects will be reviewed by study staff for completeness and quality (such as multiple answers when one response is allowed) and any problems will be resolved prior to the subject leaving the premises. Any incomplete data, illegible, or questionable provided by clinicians will be resolved through feedback with the clinician, within a day following the study visit. The REDcap system will flag any remaining data quality problems (such as data values outside the expected range) during data entry; any problems will be resolved before the record is added to the database. Extensive quality control procedures for handling and assaying laboratory samples will be carried out to ensure complete and valid biological data. Early in the study and periodically throughout the period of data collection, data will be reviewed by the study Statistician for completeness, consistency, and other indicators of data quality in a blinded fashion. Missing data will be described but will be inexplicitly imputed in all analyses by using only available data.

Baseline Characteristics: Demographic and key baseline clinical characteristics will be tabulated and compared by treatment group. While randomized groups are expected to be similar on these characteristics, any variable with a $P \leq 0.10$ difference across the four groups will be considered as a candidate covariate, as described below.

Early Termination: Early termination information for randomized subjects by reasons and time in study - will be fully described by treatment. To maximize the power to detect biologically and clinically meaningful treatment effect sizes, enrollment will continue until there are 80 per-protocol study completers.

Data Normality: Assessment of normality of the primary and secondary endpoints will be evaluated and appropriate data transformation will be performed as needed.

**Analysis Population:** The modified intent-to-treat (mITT) evaluable sample is defined as all randomized subjects who take the assigned treatment and have baseline and at least one post-baseline assessment of the relevant outcome measures. The per protocol analysis population (PP) will include all data in the mITT population until the experience of major protocol violations. The PP population will be defined in a blinded fashion before database lock. The study will make every effort to follow up patients who have been removed from the tested treatments due to major protocol violations and collect outcome data.

The primary analysis for all study aims will be based on the PP analysis population, as the inflammatory marker and depression data may be sensitive to potential protocol violation. The mITT analysis population will be used for sensitivity analyses.

**Specific Aim 1 (Primary Aim):** To evaluate whether a dose-response relationship exists between dose of EPA and decrease either in plasma IL-6 levels or in mitogen-stimulated PBMC TNF-α levels, when compared with placebo.

> Hypothesis 1: Overweight subjects with MDD and hs-CRP levels $\geq 3.0$ mg/l treated with 1 g/day, 2 g/day or 4 g/day of EPA will demonstrate a $\geq 0.40$ effect size at both week 8 and week 12 for decrease in plasma IL-6 levels and/or mitogen-stimulated PBMC TNF-α levels when compared with placebo-treated

subjects. *The dose-response relationships on the two inflammatory markers will be explored.*

**Analysis:**

The primary analyses to test this hypotheses will be carried out by mixed model repeated measures (MMRM analysis of change in plasma IL-6 and mitogen-stimulated PBMC TNF-α from baseline to each post-baseline assessment (at week 4, 8, and 12). Baseline variables that have potential relationship to the endpoints (change in plasma IL-6 or mitogen-stimulated PBMC TNF-α) will be evaluated and entered into the MMRM model as appropriate, and will be evaluated as potential covariates -- such as baseline level of plasma IL-6 or mitogen-stimulated PBMC TNF-α, age, gender, level of perceived life stress, and presence/absence of early childhood trauma. Only those baseline variables that differ among randomized treatment groups at $P \leq 0.10$ and are significantly correlated with the primary outcome variable for the entire sample (change in plasma IL-6 or mitogen-stimulated PBMC TNF-α) will be entered into the model as covariates. From the MMRM analysis we will report the overall test of the significance of treatment-by-time across the four treatment groups, with appropriate covariates, as well as least-square means (and standard errors of means) per treatment group, for change from baseline to each time point. Tests of paired comparisons (each EPA dose vs. placebo, plus pairwise comparison across EPA doses) will be reported for each time point. These results will provide essential data for designing an adequately powered antidepressant efficacy study (phase 2 of the project) comparing EPA to placebo at an EPA dose which has been demonstrated to have a clinically meaningful impact on plasma IL-6 or mitogen-stimulated PBMC TNF-α, or both. They will also provide new data for the field concerning the duration of treatment needed to initiate change in inflammatory biomarkers within various EPA monotherapy dose groups, as well as amounts of change compared to the placebo group, by EPA dose and time in treatment, for depressed overweight subjects with high hs-CRP.

While significance tests are always of interest, a major purpose of this first-phase study is to determine whether there is sufficient evidence of EPA collectively in moving the two inflammatory markers as well as the depression score so that a large-scale study can be conducted, as well as to identify an optimal dose of EPA to use in the larger, adequately powered trial of EPA antidepressant efficacy. For this reason, adjustments in computation of probability values for multiple dose comparisons to EPA will not be made. Results will be interpreted primarily in terms of effect size for change over time in plasma IL-6 and mitogen-stimulated PBMC TNF-α.

**Specific Aim 2:** To evaluate whether EPA treatment produces a decrease in ratings of depression severity, when compared with placebo-treated subjects, will be observed; and whether the changes in IL-6 or mitogen- stimulated PBMC TNF-α expression will mediate changes observed in ratings of depression.

Hypothesis 2a: Overweight subjects with MDD and hs-CRP levels ≥ 3mg/l treated with 1 g/day, 2 g/day or 4 g/day of EPA will demonstrate a $\geq 0.35$ effect size at both week 8 and week 12 for decrease in ratings of depression severity, as measured by the Inventory of Depressive Symptoms, Clinician-Rated version, when compared with placebo-treated subjects. *The dose-response relationships on the depression score will be explored.*

**Analysis:**

Analyses to test this hypothesis will be performed by means of MMRM analysis of change in clinician-rated IDS (IDS-C30) depression ratings based on the per-protocol sample as defined above. As for Aim 1, potential covariates will be evaluated and entered into the MMRM model as appropriate. Results will be presented in terms of mean change (and standard error of mean) in IDS-C scores by treatment group at the end of 12 weeks of treatment, with effect size and overall significance of the slopes of change by treatment, along with mean change, effect size, and significance of change between each pair of treatment groups as of each study visit (Week 2, 4, 6, 8, 10, and 12). This will make it possible to identify the influence of both dose and treatment duration on the antidepressant benefit of EPA. These results will provide essential data for designing an adequately powered antidepressant efficacy study (phase 2 of the project) comparing EPA to placebo at an EPA dose that has been

demonstrated to have a clinically meaningful impact on depression as well as on plasma IL-6 or mitogen-stimulated PBMC TNF-α, or both, for depressed overweight subjects with high hs-CRP. MMRM results will be supplemented with analysis of rates of response and remission by treatment group, based on IDS-C30 depression severity scores (all evaluable subjects, and study completers). Similar analyses will be carried out for additional measures such as self-reported depression ratings, clinician-rated anxiety, and CGI ratings of severity and improvement.

As for Aim 1, adjustments in computation of probability values for multiple dose comparisons to EPA will not be made. Results will be interpreted primarily in terms of effect size for change over time in IDS-C30 depression severity scores.

Hypothesis 2b: Changes in IL-6 and mitogen-stimulated PBMC TNF-α expression will mediate changes observed in ratings of depression.

**Analysis:**
Two analyses will be conducted to test this study hypothesis. We recommend that these be based on study completers (the 80 subjects completing 12 weeks of treatment). First, change in plasma IL-6 and mitogen-stimulated PBMC TNF-α will be correlated, within treatment group, with change (and percent change) in IDS-C30 scores over 12 weeks. A correlation of $r \geq 0.50$ will be considered an indicator of at least moderate association of change between either biomarker and improvement in depression. Second, change in each of the two primary biomarkers of change will be compared between responders and non-responders in each treatment group (responders being those with at least 50% reduction in IDS-C depression severity scores).

**Exploratory Aim:** To evaluate whether EPA treatment produces decreases in plasma TNF-α, leptin, hs-CRP, and IL-1ra levels and mitogen-stimulated PBMC IL-6 levels, as well as in the expression of inflammation pathway-related genes.

Hypothesis 3a: Overweight subjects with MDD and hs-CRP levels ≥ 3mg/l treated with 1 g/day, 2 g/day or 4 g/day of EPA will demonstrate a decrease in plasma TNF-α, leptin, hs-CRP, and IL-1ra levels and mitogen-stimulated PBMC IL-6 levels when compared with placebo-treated subjects.

**Analysis:**
MMRM analysis (incorporating relevant covariates) will be conducted to assess treatment group differences in change other inflammatory markers over 12 weeks of treatment, in a manner parallel to that described for change in plasma IL-6 or mitogen-stimulated PBMC TNF-α, study Hypothesis 1. Results will broaden the understanding of EPA effects at different doses on an array of inflammatory biomarkers that may be considered targets of study in the second-phase study.

Hypothesis 3b): Overweight subjects with MDD and hs-CRP levels ≥ 3 mg/l treated with 1 g/day, 2 g/day or 4 g/day of EPA will demonstrate a decrease in the expression of genes involved in the inflammatory pathway when compared with placebo-treated subjects.

**Analysis**:
Exploratory analyses of potential predictors and targets of antidepressant response to EPA at different doses will be examined by analysis of differential gene expression in peripheral blood mononuclear cells from responders vs. non-responders to various doses of EPA and placebo over 12 weeks of treatment. A two-stage analysis will be conducted: In the first stage, gene transcripts will be identified that are associated with at least a 20% difference (1.2 fold change) in response to a given dose of EPA but not response to placebo. Following the methods described in Mehta et al. (2013), the predictive values of these transcripts will then be subjected to pathway analysis to identify specific mechanisms or pathways that appear to be associated with response to target doses of EPA.

d. **Additional Analysis Notes:**

Decision Rule: The formal decision rule for moving to the large-scale UH3 antidepressant efficacy study is that results of the UG3 study must show the following: *Overweight subjects with MDD and hs-CRP levels ≥3.0 mg/l treated with 1g/day, 2g/day or 4g/day of EPA must demonstrate a ≥0.40 effect size at both week 8 and week 12 for decrease in plasma IL-6 levels and/or mitogen-stimulated PBMC TNF-α expression and secretion, when compared to placebo-treated subjects in one of the three EPA doses (positive result for Hypothesis 1), as well as a ≥0.35 effect size at both week 8 and week 12 for decrease in ratings of depression severity as measured by IDS-C30 scores, when compared to placebo-treated subjects in one of the EPA doses (positive result for Hypothesis 2a).* Multiplicity issues due to multiple doses and multiple endpoints, as well as multiple time points (weeks 8 and 12), have been considered in the development of the decision rule for moving forward to the next phase of large scale study, by controlling false positive error and false negative error rates in this preliminary study. A simulation study has been conducted to evaluate this decision rule by NCCIH and shows that the false positive error can be controlled under 7% under various valid correlation structures under the assumption that EPA has no effect on the two specified inflammatory markers and the depression score. In the event that more than one EPA dose meets both of the above criteria, selection of an EPA dose for the efficacy study will be based on additional considerations such as study retention rates by dose, as well as amounts and patterns of change in the primary and secondary outcomes by dose.

Sensitivity Analyses: Two types of sensitivity analysis may be conducted on the mITT analysis population: (1) In case of non-normality of any outcome variable for this study, non-parametric analyses will be performed using rank order statistics (Kruskal-Wallis and Wilcoxon Rank Sum tests for multiple group and pairwise comparisons, respectively). (2) If any randomized subject has a major protocol violation during the course of the study that warrants exclusion of data for specific visits (agreed upon by NCCIH and the study investigators), secondary analyses of the study aims may be conducted that include data collected for visits after the major protocol violation has occurred. Consistency of outcomes of the primary and secondary analyses would lend support to the study conclusions.

## VII. RISKS AND DISCOMFORTS

### a. Complications of surgical and non-surgical procedures, etc.

Blood-drawing for laboratory tests may cause mild pain, discomfort and bruising or, rarely, dizziness, fainting or infection. Subjects with unstable medical conditions will not be enrolled in this study. All subjects will be closely monitored with vital sign checks at each visit.

### b. Drug side effects and toxicities

Omega-3 fatty acids have a benign side effect profile, with a dose-related gastrointestinal upset as its main side effect. The more moderate risks of nausea, diarrhea, bloating, unpleasant belching, and thinning of the blood (which could result in longer bleeding times when cuts or other abrasions) are less likely to occur (1-9% of subjects).The dose of EPA used in this study are well within the range recommended for cardiovascular benefit (Kris-Etherton et al., 2002).. Over the past 20 years, several thousand research subjects have participated in clinical trials of omega-3 fatty acids, using a range of dosages (up to 10g/day) and trial lengths. No serious adverse reactions have been reported. Further supporting the safety of high-dose omega-3 fatty acids is the safety of the traditional Arctic people's diet, which may contain more than 16 grams per day of omega-3 fatty acids (Simopulos et al., 1999). Dietary supplementation with marine omega-3 fatty acids has been shown to prolong bleeding time in humans, and may decrease thrombotic potential. However, little else is known about the direct effects of dietary fatty acids on hemostatic and fibrinolytic activities (Tracy, 1999). For this reason, patients taking anticoagulants, or having a history of coagulopathy will be excluded from the study. Subjects are advised that problems and side effects not listed above may occur and will be informed of any new risks to which they may be exposed. According to standard procedure within the 2 research program sites (MGH and Emory/ Grady

Health System), subjects who show persistent worsening during the course of a clinical trial or develop unstable psychiatric symptoms (e.g., suicidality, homicidality, psychosis) will be withdrawn from the study and referred for appropriate treatment. Subjects on double-blind treatment may be unblinded in an emergency.

Subjects will also be informed that there is a potential risk of worsening of depression if the patients were to discontinue their antidepressant medication prior to study participation.

**c. Device complications/malfunctions**

Not applicable.

**d. Psychosocial (non-medical) risks**

Psychological or emotional discomfort may arise due to the screening procedure, interviews and questionnaires. The instruments administered during the course of the study involve no specific risks or discomforts beyond those of a standard clinical interview situation such as upset feelings at a review of current health status or boredom. However, we will always remind subjects that participation in research is voluntary and that subjects may withdraw consent at any time.

**e. Radiation Risks**

Not applicable.

## VIII. POTENTIAL BENEFITS

**a. Potential benefits to participating individuals**

It is hoped that subjects who participate may experience at least a 50% amelioration of their depression from treatment with the omega-3 fatty acid and may experience fewer side effects than the medicine doctors can prescribe. The information obtained in the study may also benefit other individuals with depression and high inflammation, as it may widen treatment options for depression, as well as our understanding of the physiology of depression.

**b. Potential benefits to society**

Particularly at this time, where there is growing public concern about the safety of traditional antidepressant medication, it is important to know whether CAM alternatives may serve as a safe and effective treatment for those patients with major depressive disorder who have high peripheral inflammation. The results of our study will markedly increase our knowledge about mechanisms of n-3 fatty acids' effects on lipid and immune function in relationship to treatment of major depressive disorder. It may greatly enhance our understanding of a potential mechanism by which n-3 fatty acids palliate major depressive disorder. Thus, this study has significant potential public health benefits.

## IX. MONITORING AND QUALITY ASSURANCE

**a. Independent monitoring of source data**

Westat, a global research monitoring corporation, will provide independent monitoring of the data collected in this study. Westat will perform site visits at each site prior to the enrollment of the first study subject. Follow-up monitoring visits at each site are scheduled to occur after the trial has been on-going for one year or after 50% of the participants have been enrolled. More frequent monitoring may occur if requested by NCCIH. Westat will also conduct a close-out visit soon after the last patient has completed their trial participation.

**b. Safety monitoring**

The Emory Departments of Psychiatry and Neurology Data Safety Monitoring Board (DSMB) will conduct twice-yearly review of the trial safety data, retention rates, and any breaches of confidentiality that occur during the study. Reports will be submitted to the DSMB with the relevant data, broken out by each site individually, and with both sites combined. Data will be submitted in a blinded manner, though the DSMB may request unblinded data to be reported if the DSMB has cause for concern. In this case, an independent statistician who has no other role on the study will be consulted to prepare the unblinded report, which will be submitted directly to the DSMB. The data to be included in each DSMB report will include all study information collected up until 30 days prior to the report submission due date.

The DSMB will review all serious adverse events that occur during the trial. SAEs will be reported to the DSMB within 10 days of learning of their occurrence, and any deaths will be reported within 24 hours. The DSMB includes physicians who are experienced in clinical research, research coordinators, and administrative support. A statistician (Dr. Guthrie) will be a standing member of the DSMB for this study. Study clinicians who currently serve on the DSMB will recuse themselves when the current studied is being reviewed by the DSMB. A copy of the DSMB's study reviews will be provided to each site's IRB at the time of study renewal.

**c. Outcomes monitoring**

No interim unblinded assessment of outcomes is planned for this study. Patient completion, early termination (along with reasons for early termination) and remission rates by site will be provided to the DSMB twice yearly as part of the DSMB monitoring report.

**d Adverse event reporting guidelines**

**(i) Classification of AE Severity**

Any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research. Adverse events will be coded using Medical Dictionary for Regulatory Activities (MedDRA) terms:

Grade 1 Mild AE (does not have a major impact on the patient)
Grade 2 Moderate AE (interferes with some function but not day-to-day activities)
Grade 3 Severe AE (interferes with activity and daily living)

Documentation of the presence of any side-effect or adverse event (AE) will be completed by one of the treating psychiatrists at every visit by recording all spontaneously reported AEs, which will be classified as either mild, moderate, or severe. Patients shall be allowed to contact the investigator or a member of his staff at any time between visits concerning adverse events or worsening of symptoms.

**(ii) AE Attribution Scale**

AEs will be categorized according to the likelihood that they are related to the study intervention. Specifically, they will be labeled definitely unrelated, definitely related, probably related, or possibly related to the study intervention.

The DSMB will monitor the study for any adverse events that arise over the course of the project; however, the following adverse events have been identified by the co-PIs as events that may occur during the course of the trial:

1) Psychological and/or emotional discomfort arising from the clinical interview and questionnaires
2) Blood draw complications
3) worsening of depressive symptoms
4) diarrhea
5) nausea
6) fish breath/fish taste in mouth

**(iii) AE Reporting and Follow-up**

Information on new AEs and continuation of AEs are collected at every study visit. In addition, tables (see Appendix 1) will be utilized for data collection and reporting. If an SAE occurs, the respective IRB's will be notified within 10 days of the notification of the event as per the respective University IRB guidelines. The site IRB's will review the SAE and its implications for the safety of subjects continuing in the study. The site IRB will determine if the circumstances merit any modification, suspension, or termination of the study. The report of the IRB will be sent to NCCIH.
Non-serious adverse events will be reviewed in summary format by the DSMB during the regularly scheduled DSMB reviews.

**e Serious Adverse Events reporting guidelines**
Expedited review will occur for all events meeting the FDA definition of Serious Adverse Events (SAEs) – i.e., any fatal event, immediately life-threatening event, permanently or substantially disabling event, event requiring or prolonging inpatient hospitalization, or any congenital anomaly. This also includes any event that a study investigator or the DSMB judges to impose a significant hazard, contraindication, side effect, or precaution. For purposes of this study, all SAEs will be required to be reported to the DSMB, regardless of any judgment of their relatedness to the study drug. All relevant information will be reported to the DSMB for each SAE including information about the event and its outcome, dosing history of all study drugs, concomitant medications, the subject's medical history and current conditions, and all relevant laboratory data. Notification by e-mail, and FAX transmittal of all related study forms shall be made to the DSMB within 2 days of the occurrence of any SAE. Information will be reviewed and a determination made of whether there was any possible relevance to the study drug. Additional reporting to local IRBs will be completed in accordance with the local IRB policies; reporting to NIH, and FDA will be made according to their respective regulations governing SAE reporting.

Unexpected fatal or life-threatening AEs related to the intervention will be reported to the NCCIH Program Officer within 10 days. Other serious and unexpected AEs related to the intervention will be reported to the NCCIH Program Official within 30 days.

Anticipated or unrelated SAEs will be handled in a less urgent manner but will be reported to the Independent Monitor(s), IRB, NCCIH, and other oversight organizations in accordance with their requirements. In the annual AE summary, the Independent Monitor(s) Report will state that they have reviewed all AE reports.

## VIII. MULTIPLE PI LEADERSHIP PLAN

This study will be administered as a two-site Collaborative UG3.

Given the multidisciplinary aspects of the proposed research project, a multiple PI option is proposed, with Drs. Rapaport, Fava and Mischoulon acting as co-PIs. A detailed description of the leadership plan follows.

1) Roles/areas of responsibility of the PIs

Dr. Rapaport will be responsible for the oversight and coordination of all aspects of the proposed clinical study at Emory University/Grady Health System, whereas Drs. Fava and Mischoulon at the Depression Clinical and Research Program (DCRP) at MGH will be responsible for the oversight and coordination of all aspects of the proposed clinical study at MGH. Dr. Rapaport will also be responsible for the oversight and coordination of all aspects of the data management and data analyses, as well as of the measurements of the biomarkers carried out at Emory University, whereas Drs. Fava and Mischoulon will be responsible for the oversight and coordination of all aspects of the measurements of those biomarkers carried out at Tufts University. The three PIs will jointly oversee and be responsible for the overall project, and have a track record of successful collaborations for more than two decades, which involved similar procedures and tasks as the ones proposed in the current application. The Emory University and MGH PIs will be responsible for their own fiscal and research administration. As stipulated by SF424 guidelines, a Principal Investigator Assurance will be retained for all named PIs. Dr. Rapaport will serve as contact PI and be responsible for submission of progress reports to NIH and all communications to NCCIH.

2) Fiscal and management coordination & communication between PIs

The three PIs will form a Steering Committee, which will involve the three PIs, and key personnel of both sites (Drs. Schettler, Dunlop, and Kinkead at Emory University and Drs. Papakostas, Alpert, and Nierenberg at MGH). The goal of this committee will be to manage the oversight and coordination of the projects, resource allocation, publications and data sharing. This Committee will also oversee decisions on minor changes in research direction and have the authority to reallocate funds between PIs, as required. Drs. Rapaport and Fava will serve as Co-Chairs of the Steering Committee. The Steering Committee will meet monthly virtually (via teleconference). The goal of the monthly Steering Committee meetings will also be to allow more in-depth reviews of the (a) progress of the study, (b) collaborations between the sites; and (c) resolution of any issues that might arise. The steering committee and key study personnel will have twice-yearly face-to-face meetings with personnel from NCCIH representatives.

To ensure a smooth coordination, each PI will share progress and research results with the other PIs and key personnel. The PIs already have established a weekly teleconference to discuss issues related to study implementation, experimental design, data analysis, and administrative responsibilities. We will communicate more frequently by telephone or email on an as needed basis.

3) Publication and intellectual property policies

A publication policy will be established based on PIs' and key personnel's scientific contributions. Publication plans will be reviewed and approved by the Steering Committee. An Intellectual Property Committee composed of representatives from each institution will be formed to ensure that intellectual properties are protected according to agreed policies.

4) Conflict Resolution

In case of disagreement, the PIs as well as Dr. Wendy Weber (NCCIH Program Scientist), will meet virtually (via videoconference) and try to resolve it. If the disagreement is not resolved, the dispute will be referred to an Arbitration Committee, which will include one impartial senior executive from each institution (Emory University: Dr. Frank Brown; MGH: Dr. Jordan Smoller). Members of the arbitration committee will not be directly involved in the proposed research.

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## APPENDIX A: Prohibited Supplements

Any Supplement with “Omega 3” or “Fish Oil” in the product name is prohibited. In addition, the following listed supplements also contain Omega 3 fatty acids and are therefore prohibited. Patients should bring the bottles of all supplements they are using to the screening visit for review by study staff.

<u>Additional prohibited Omega-3 containing supplements:</u>

- Bausch and Lomb Ocuvite
- GNC Triple Cod Liver Oil
- MET-Rx Triple Omega
- MRM Smart Blend
- Nature Made Cod Liver Oil

- New Chapter Wholemega
- Nordic Naturals Ultimate Omega D3
- Nordic Naturals Baby's DHA
- Omax3 Ultra-Pure
- WHC UnoCardio 1000 + Vitamin D 1000

**APPENDIX B: Description of Structured Interviews and Scales**

Berlin Questionnaire
The Berlin Questionnaire (Netzer 1999) is a 10-item self-report scale that estimates a person's risk for obstructive sleep apnea.

Clinical Global Impressions - Severity (CGI-S) and Improvement (CGI-I)
The CGI assessments (Guy, 1976) are ratings of severity of the disorder and of global improvement since beginning of the study. These two instruments are completed by the clinician based on assessment of the patient's clinical status. They measure, based on history and scores on other instruments: a) CGI-S (severity): the current condition of the patient on a scale of 1-7 (1 being normal, and 7 being among the most severely ill patients); b) CGI-I (improvement): the degree of improvement, as perceived by the clinician, since the start of treatment on a scale of 1-7, 1 being very much improved, and 7 being very much worse. Improvement in CGI ratings is used to determine the degree of response over time with a given treatment.

Food Processor 7.8 Questionnaire
(ESHA Research) Patients will fill out the questionnaire for three consecutive days between screening and baseline visit to assess their dietary intake of omega-3 PUFAs. Data from this questionnaire will be analyzed at the baseline visit prior to randomization. Using an average daily intake of 3.0 gm of total omega-3 PUFA as the cut-off value (as determined by a three day dietary intake analysis), subjects with high dietary intake of omega-3 PUFA (and EPA) will not be included in the study. The average value of 3.0 gm/day (for the past three days) was decided upon so as not to exclude a patient for the occasional intake of larger quantities of omega-3 PUFA shortly before the initiation of the screening process. Subjects will be asked not to modify their routine diet through the period of the study and to advise the investigators if they do.

Childhood Trauma Questionnaire (CTQ)
The CTQ (Bernstein, 2003) is a 28-item self-report instrument that assesses childhood trauma in the following areas: physical, sexual and emotional abuse and physical and emotional neglect. Each item is rated on a scale of 1 (never true) to 5 (very often true). The 5 subscales are then totaled, with scores ranging from 5-25 for each traumatic category.

Cognitive and Physical Functioning Questionnaire (CPFQ)
The CPFQ (Fava et al., 2009) is an 8-item self-report form that assesses difficulties with energy, alertness and cognition.
Columbia Suicide Severity Rating Scale (CSSRS)
The CSSRS (Posner et al., 2007) is a brief, standardized, clinician-administered measure that assesses the essential information (behavior, ideation, lethality and severity) and distinguishes between suicidal occurrences and non-suicidal self-injury. The CSSRS is composed of 5 questions addressing suicidal behavior and 5 questions assessing suicidal ideation, and is endorsed by the FDA for clinical trials. This brief instrument systematically tracks suicidal ideation and behavior (e.g., suicide attempts, wish to die, thoughts of suicide, plan and intent), and classifies events according to the following categories*: **Suicidal events** – completed suicide, suicide attempt, preparatory acts toward imminent suicidal behavior and suicidal ideation. **Non-suicidal events** – self-injurious behavior, no suicidal intent and other, no deliberate self-harm. **Indeterminate or potentially suicidal events** – self-injurious behavior, suicidal intent unknown*.

Hamilton Anxiety Rating Scale (HAM-A)

The HAM-A (Hamilton, 1959) is a 14-item clinician-administered scale that assesses psychic and somatic symptoms of anxiety in the previous week.

Inventory of Depressive Symptomatology, Clinician-Rated (IDS-C30)
The IDS-C30 (Rush et al., 1986) is designed to assess severity of depression. Thirty questions focus on neurovegetative and other depressive symptoms experienced over the past 7 days. Higher scores indicate more severe pathology. A decrease of 50% or more in the IDS-C score is considered to be a positive response to treatment, while a final score of 11 or less is considered typical of remission.

Life Experiences Survey (LES)
The LES (Sarason et al., 1978) is a 43 item scale that lists numerous events which individuals may experience and call for social readjustment. Subjects are asked to indicate events which they have experienced during the previous reporting period and whether these events were perceived as positive or negative. Additionally, subjects are asked to rate on a 7-point scale the degree of impact these events have on their lives. From these responses it is possible to derive three life change scores: positive, negative, and total.

Mini-International Neuropsychiatric Interview, Version 7.0 (MINI)
The MINI (Sheehan et al., 1998)) is a short structured diagnostic interview for DSM 5 and ICD-11 psychiatric disorders. It is easier and faster to administer than the Structured Clinical Interview for DSM-5 and provides an accurate structured psychiatric interview for multicenter clinical trials.

Patient Health Questionnaire (PHQ-15)
The PHQ-15 (Kroenke et al., 2002) contains the most prevalent symptoms found in US primary care, and covers the past four weeks. It measures symptom severity with a 3-point scale, and is recommended for studies that focus on common somatic symptoms.

Perceived Stress Scale (PSS)
The PSS (Cohen et al., 1998) is a 14-item self-rated scale that measures subjective experience of stress, specifically the degree to which situations in one's life are appraised as stressful, unpredictable, uncontrollable, and overloading.

The Quality of Life Satisfaction Questionnaire-short form (Q-LES-Q)
The Q-LES-Q (Endicott et al., 1993)) is a 14-item patient-administered instrument that rates quality of life satisfaction on a 1-5 scale, with 13 specific areas of life, as well as medication, and overall life satisfaction over the past week.

Sheehan Disability Scale (SDS)
The SDS (Sheehan, 2000) is a patient-rated instrument designed to assess the impact of perceived problems on work productivity, social/leisure activities, and family life/home responsibilities. The Sheehan Disability Scale consists of 3 questions rated on a visual analog scale (0 to 10). Higher scores represent greater impairment of activity.

Symptoms of Depression Questionnaire (SDQ)
The SDQ (Pedrelli et al., 2014) is a validated self-rating instrument has 43 items on a scale of 1-6, measuring multiple depressive symptom domains. The time frame for this scale is the past 24 hours.

United States Index of Deprivation (USiDep)
The USiDep is an 8-item self-report questionnaire derived from the New Zealand Index of Deprivation (Salmond et al., 2006). The scale assesses a patient's economic stress by inquiring about ability to pay for such things as heating, clothes and food items.