Investigating N-3 Fatty Acids to prevent Neonatal Tobacco-related outcomeS (INFANTS)

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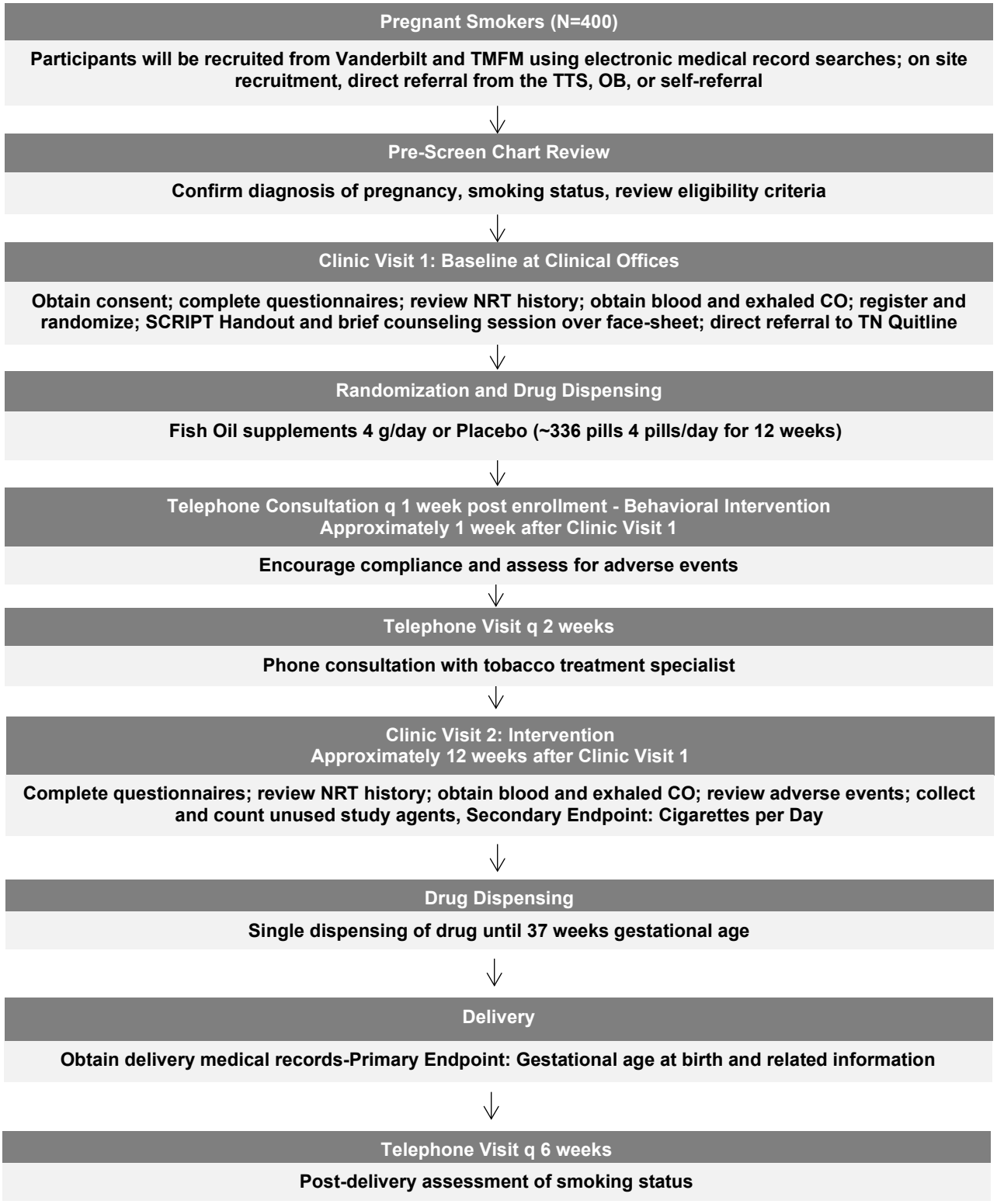
Pregnant Smokers (N=400)
Participants will be recruited from Vanderbilt and TMFM using electronic medical record searches; on site recruitment, direct referral from the TTS, OB, or self-referral
Pre-Screen Chart Review
Confirm diagnosis of pregnancy, smoking status, review eligibility criteria
Clinic Visit 1: Baseline at Clinical Offices
Obtain consent; complete questionnaires; review NRT history; obtain blood and exhaled CO; register and randomize; SCRIPT Handout and brief counseling session over face-sheet; direct referral to TN Quitline
Randomization and Drug Dispensing
Fish Oil supplements 4 g/day or Placebo (~336 pills 4 pills/day for 12 weeks)
Telephone Consultation q 1 week post enrollment - Behavioral Intervention
Approximately 1 week after Clinic Visit 1
Encourage compliance and assess for adverse events
Telephone Visit q 2 weeks
Phone consultation with tobacco treatment specialist
Clinic Visit 2: Intervention
Approximately 12 weeks after Clinic Visit 1
Complete questionnaires; review NRT history; obtain blood and exhaled CO; review adverse events; collect and count unused study agents, Secondary Endpoint: Cigarettes per Day
Drug Dispensing
Single dispensing of drug until 37 weeks gestational age
Delivery
Obtain delivery medical records-Primary Endpoint: Gestational age at birth and related information
Telephone Visit q 6 weeks
Post-delivery assessment of smoking status

1.0 Background

Smoking is the most important modifiable risk factor for adverse pregnancy outcomes including preterm birth, neonatal death, and maternal complications [1-4]. Smoking rates in pregnancy remain unacceptably high with approximately 11% of American women reporting smoking during pregnancy, with higher rates in younger women, those with lower educational levels, and women residing in the Southeastern United States [5, 6]. Based on the 2010 Pregnancy Risk Assessment Monitoring System (PRAMS), in Tennessee over 34% of women reported smoking during the 3 months before pregnancy and 22% reporting smoking in the last 3 months of pregnancy [6]. The identification of a safe and effective adjuvant therapy that reduces tobacco-related adverse pregnancy events and promotes smoking cessation in pregnant women would have a powerful clinical impact on maternal-fetal health outcomes.

Smoking is a risk factor for adverse pregnancy outcomes. Up to 8% of preterm-related births, 19% of term infants with intrauterine growth restriction, and 34% of sudden infant death syndrome deaths are attributed to prenatal smoking [6]. Women who smoke have a more than 2-fold increase in the risk of preterm birth and low birth weight neonates. Just as with smoking rates, preterm birth rates in Tennessee are also above the national average (11% versus 9.6%) [7-10]. The immediate and long-term care of preterm infants requires substantial utilization of medical resources with costs associated with preterm birth in the United States estimated at $26.2 billion in 2005 [11].

Rates of smoking cessation during pregnancy are low, particularly in underserved populations. Rates of smoking cessation during pregnancy range from 25 to 75%, with risk factors for persistent smoking including lower educational status, higher cigarettes smoked per day, and coexisting psychiatric conditions [5, 6, 12]. Only 35% of pregnant smokers in Tennessee are successful at stopping smoking during their pregnancy, a proportions which is the second lowest in the United States [6]. Promoting smoking cessation in pregnancy is challenging due to the limitations of available pharmacotherapy for pregnant and breast-feeding women for whom FDA-approved smoking cessation medications are generally not recommended [13-15]. Intensive behavior therapy alone in pregnant smokers produces quit rates as high as 35% by late pregnancy, however the majority of women continue to smoke despite counselling [16].

Cigarette smoking is associated with a relative deficiency in circulating n-3 long-chain polyunsaturated fatty acid (n-3 LCPUFA) levels. We and others have found that red blood cell (RBC) phospholipid membrane concentrations of n-3 long chain polyunsaturated fatty acids (LCPUFAs) are significantly reduced in smokers compared to non-smokers [17-21]. In our pilot study, we have also found marked reductions in RBC membrane n-3 LCPUFAs (eicosapentaenoic acid [EPA] and docosahexaenoic acid [DHA]) in pregnant smokers. The mechanisms behind smoking-related n-3 LCPUFA deficiencies are unknown; however, possible reasons include 1) dietary differences; 2) lipid peroxidation; and 3) tobacco related changes in PUFA metabolism [18, 19, 22, 23].

n-3 LCPUFAs may also reduce nicotine craving and support smoking cessation. Given our findings that pregnant women have low circulating levels of n-3 LCPUFAs, correcting this nutritional deficiency may likely be an important mechanism behind the potential beneficial effect of n-3 LCPUFAs supplementation. In addition, emerging evidence supports the potential role of n-3 LCPUFAs in directly modifying smoking behavior. In animal models, n-3 LCPUFA deficiencies result in structural changes in nervous tissue which impacts dopaminergic and serotonergic systems and correction of these deficiencies can reverse these changes [24-26]. In particular, n-3 LCPUFA deficiency can result in hypo functioning of the dopamine mesocorticolimbic pathways which are related to reward and dependence [27, 28]. Specifically, nicotine administration (via smoking or other means) results in stimulation of nicotinic receptors in the ventral tegmental area which in turn raises dopamine in the nucleus accumbens, which is associated with the pleasurable sensations related to nicotine use [29, 30]. As such, it has been hypothesized that correcting the hypo functioning dopaminergic system through n-3 LCPUFA supplementation may reduce the symptoms of withdrawal associated with smoking cessation and reduce nicotine cravings [31]. In our pilot study involving pregnant smokers, we found that RBC n-3 LCPUFA levels are negatively correlated to measures of nicotine dependence.

2.0 Rationale and Specific Aims

2.1 Rationale

Smoking is the most important preventable risk factor for adverse pregnancy outcomes with almost 11% of pregnant women reporting smoking during their pregnancy [5, 6]. Smoking during pregnancy is associated with preterm birth, low birth weight neonates, intrauterine growth restriction, and sudden infant death syndrome [6-9]. The adverse effects associated with maternal smoking not only affect the health of the infant, but also place a substantial financial burden on healthcare resources.

It has been shown that smoking is correlated with lower levels of circulating n-3 LCPUFAs [21], furthermore, our group has found that both pregnant and non-pregnant smokers, have a relative deficiency in n-3 LCPUFAs [19]. Determining if n-3 LCPUFAs, which are well-tolerated when supplemented during pregnancy, lessen the adverse neonatal health consequences of smoking is of great public health relevance.

Currently there have been no clinical trials studying the effect of n-3 LCPUFA supplementation exclusively in pregnant smokers. One published study evaluated the effect of smoking status on n-3 LCPUFA supplementation and pregnancy outcomes, however, the study is limited due to its *post-hoc* analysis [23]. In addition, smoking status was not biologically validated, blood levels of n-3 LCPUFAs were not reported, and longitudinal smoking behavior was not recorded in this study. The two prior clinical trials evaluating the impact of n-3 LCPUFA supplementation on smoking status are limited due to 1) small sample size, 2) no biological assessment on n-3 LCPUFA status and smoking status, 3) selectively enrolling subjects who were not interested in quitting; and 4) no inclusion of a behavioral component.

Given the high burden of disease associated with smoking-related adverse pregnancy outcomes, the current lack of safe and efficacious pharmacotherapies to aide cessation for pregnant smokers, and the limitations of prior clinical trials, a rigorously designed and adequately powered randomized control trial is necessary to determine if supplemental n-3 LCPUFAs 1) improve neonatal outcomes in pregnant smokers and 2) enhance smoking reduction efforts.

2.2 Specific Aims

The specific aims of this study are:

- AIM 1: To determine the effect of supplemental n-3 LCPUFAs compared to placebo on gestational age at delivery and preterm labor in pregnant smokers.
    - H1: We hypothesize that pregnancy smokers who are supplemented with n-3 LCPUFAs will have a lower risk of preterm labor compared to placebo.

- AIM 2: To determine the effect of supplemental n-3 LCPUFAs compared to placebo on tobacco use in pregnant smokers
    - H2: Pregnant smokers who are supplemented with n-3 LCPUFAs will have a greater reduction in cigarettes per day at 12-weeks compared to placebo.

- AIM 3: To determine if the effect of supplemental n-3 LCPUFAs on preterm labor is mediated by changes in smoking behavior and/or increases in circulating n-3 LCPUFAs.
    - H3: Pregnant smokers allocated to n-3 LCPUFA supplements will have a lower risk of preterm birth compared to placebo which will be mediated by reduction in tobacco use.

2.3 Significance and Projected Impact

Smoking is the leading preventable cause of fetal and maternal morbidity. Despite this fact, existing smoking cessation therapies are either questionably effective or unsafe to use during pregnancy. Previous findings show evidence of low levels of circulating n-3 LCPUFAs in pregnant smokers. Correcting this nutritional deficiency could likely be an important mechanism behind the potential benefits of n-3 LCPUFA supplementation during pregnancy. Additionally, emerging evidence supports the potential role of n-3 LCPUFAs in directly modifying smoking behavior. n-3 LCPUFAs supplements are well tolerated in pregnancy but currently are not recommended as part of routine prenatal care in smokers. Even large doses of fish oil supplements are well tolerated in pregnancy, with the most common side effect reported being fishy “burps” with no differences in bleeding complications, nausea, emesis, diarrhea, or abdominal pain [32]. In our pilot study we found no increase in GI symptoms in women taking 4.2 grams/day of n-3 LCPUFAs. Although several expert panels recommend pregnant and lactating

women to consume at least 200 to 300 mg/day of n-3 LCPUFAs on average, no formal guidelines exist advocating the use of supplements [33, 34].

No prior placebo-controlled, randomized trial of n-3 LCPUFAs has recruited pregnant smokers exclusively and utilized validated biomarkers of n-3 PUFA status and smoking exposure. No prior study exists evaluating the impact of supplemental n-3 LCPUFAs in smokers who are interested in quitting and no prior study has evaluated supplemental n-3 LCPUFAs with behavioral interventions for smoking cessation. Given the high burden of disease associated with smoking-related adverse pregnancy outcomes, the current lack of safe and efficacious pharmacotherapies to aide cessation for pregnant smokers, and the limitations to prior clinical trial work, a rigorously designed and adequately powered randomized control trial is necessary to determine if supplemental n-3 LCPUFAs 1) improve neonatal outcomes in pregnant smokers and 2) enhance smoking reduction efforts.

INFANTS will be the first study to evaluate the impact on n-3 LCPUFAs on tobacco use in pregnant smokers who wish to quit, thus identifying a novel strategy to reduce tobacco use that could be relevant for all smokers. Our proposal is innovative in the choice of population targeted for our intervention; a population, which based on preliminary data, may stand to benefit the most from n-3 LCUFA supplementation. Thus, if our study demonstrates that supplemental n-3 LCPUFAs are effective at reducing the risk of tobacco-related adverse neonatal outcomes and/or reducing tobacco use during pregnancy, our results could have an immediate and major clinical impact on pregnancy care and neonatal outcomes in the United States.

3.0 Previous Human Studies

In order for us to test the feasibility of a full-scale trial we conducted a placebo-controlled pilot trial. This pilot randomized control trial (RCT) of n-3 LCPUFAs in 29 pregnant smokers found the intervention to be feasible and to result in lower cravings for nicotine based on the Fagerstrom Nicotine Tolerance Test (change from baseline score of -2.5 versus 0, p = 0.01) and a non-statistically significant reduction in urine cotinine at 4 weeks (change from baseline -472 ng/mL versus -268 ng/mL, p = 0.37) compared to placebo, laying groundwork for a full scale RCT.

In a study published in 2016, investigators measured red blood cell (RBC) phospholipid membrane concentrations of fatty acids in smokers, former smokers, and people who never smoked. A multivariable-adjusted analysis revealed that compared to former/never smokers, current smokers had higher RBC membrane fatty acid percentages of palmitic acid and steric acid and lower RBC membrane fatty acid percentages of arachidonic acid, adrenic acid, and all n=3 LUPUFAs [19].

A secondary analysis of a larger multicenter randomized control trial assessing the effects of omega-3 supplementation on pregnancy outcomes by smoking status discovered associations between supplementation and adverse pregnancy outcomes.

Investigators discovered that omega-3 supplementation was associated with a lower risk of spontaneous preterm delivery in smokers, but not in non-smokers. Low birth weight was also less frequent in omega-3 supplemented smokers compared to non-smokers. Although this was a secondary analysis and the study population was low, these results still indicate that omega-3 supplementation in pregnancy may offer a protective effect against spontaneous preterm delivery and low birth weight babies [23].

More recently, a multicenter, double-blind, randomized trial, published in The New England Journal of Medicine in 2019, concluded that n-3 LCPUFA supplementation from early pregnancy until 34 weeks of gestation did not result in a lower incidence of preterm delivery. In this study, pregnant women were instructed to take 900 mg of either n-3 LCPUFA or vegetable-oil capsules daily beginning before 20 weeks gestation continuing until 34 weeks gestation. In addition to not seeing a significant difference in the incidence of early preterm labor they also reported not seeing a higher incidence of interventions in post-term deliveries. They did however note a higher percentage of babies born to the n-3 LCPUFA group were very large for gestational age at birth. This study did not take smoking status into account in any of its analyses. The primary endpoint was early preterm delivery which is <34 weeks and did not look at deliveries between 34 weeks and 37 weeks, which are also considered preterm in nature. [35]

Support for our hypothesis comes from multiple observational studies which have reported lower levels of circulating n-3 LCPUFAs to be associated with adverse pregnancy outcomes, including preterm birth, intrauterine growth restriction, and preeclampsia, as well as higher risks for asthma in offspring [36-39]. RCT of n-3 LCPUFA supplementation in pregnant women have had mixed results on preeclampsia risk and intrauterine growth restriction; however, recent meta-analyses have suggested a beneficial effect of supplemental n-3 PUFAs on preterm labor risk [32, 40].

4.0 Inclusion and Exclusion Criteria

4.1 Inclusion Criteria

Inclusion Criteria:

- ≥ 16 or ≤ 45 years of age
- Currently reporting daily cigarette use (≥ 1 CPD; reporting 5 or more CPD prior to pregnancy)) -Daily smoker includes recent daily smokers defined as individual who smoke ≥ 1 CPD within the last 3 months and has recently cut down but is still smoking

- Between 10- and 26-weeks gestation as estimated from the last menstrual period and adjusted for the first-trimester ultrasound
- Has a cell phone or landline that can be reached directly

4.2 Exclusion Criteria

Exclusion Criteria:

- Allergy to fish including fish oil supplements or Lovaza
- Currently using fish oil supplements and unwilling to stop over the course of the study
- Active substance abuse (not including supervised buprenorphine therapy)
- Unable to give consent or obtain assent for minors
- Unstable pregnancy related medical problems such as pre-eclampsia, known fetal or placental abnormality such as previa, accrete, increate, and percreta.
- Multiple gestations
- History of:
    - Hypertension as defined as defined by two or more recorded blood pressures of 140/90 or greater
    - Seizure disorder as noted in the patient's problem list
    - Clotting disorder as noted in the patient's problem list
    - White's classification D or higher diabetes
- Planned cerclage
- Plans to move from the Middle-Tennessee area within the next 9-months
- Insufficient time to perform the complete enrollment process
- Barrier to communication (e.g., low English proficiency or hearing/speech impairment)

5.0 Study Procedures

Eligible patients will be pregnant women who are currently smoking and receiving care at Vanderbilt University Medical Center (VUMC) or Tennessee Maternal-Fetal Medicine (TMFM). We will recruit from approximately 8 obstetrical clinics across the Middle Tennessee region, approximately four sites affiliated with VUMC and four affiliated with TMFM. Recruitment will involve a review of the potential participants medical record, direct provider referral, and advertisement in clinic locations. Our clinic catchment area represents a roughly 40-mile radius around the city of Nashville.

All research related activities will be conducted through VUMC employed faculty and staff. TMFM will not be engaged in research activities and will act as a performance site for participant recruitment only.

5.1 Recruitment

Potential Participant Identification/Wavier of Consent for Chart Review

Tobacco use data is routinely collected by obstetrical nurses during clinic uptake at both VUMC and Tennessee Maternal-Fetal Medicine (TMFM) clinics. Recruitment will involve review of clinical visits through medical record review with on-site recruitment, direct provider referral, and advertisement in clinic locations.

We will identify pregnant women by querying upcoming obstetrics visits occurring within the next month as potential subjects. Chart reviews in EPIC and TMFM electronic

medical record systems will be reviewed to identify patients with upcoming appointments who have smoking listed in their social history or problem list. The date and time of the upcoming appointment will be noted, and the medical record will be reviewed to assess for preliminary eligibility.

Dissemination of Information and Advertisements

Study staff will review clinic schedules on a daily basis to identify potential participants to approach before or after clinical visits. In addition, the PI's will address providers and nurses at all clinical sites, introducing them to the study and distributing advertisement and contact information. We will attend staffing meetings to introduce the study and take questions. We have found that involving local providers and identifying a clinic "champion" helps to establish study buy-in at clinics and enhances recruitment. Study staff will physically be located at clinics to attempt to identify potential participants who are new to the healthcare system.

Fliers will be disseminated in clinical rooms, waiting rooms, and clinic bathrooms which will briefly describe the study and give appropriate contact information for potential participants to contact if they are interested in joining.

Clinics and Alternative Clinic Arrangements

Vanderbilt Obstetrics offers multiple clinical locations including Nashville (One Hundred Oaks-the site of our pilot data), Cool Springs in Franklin, Tennessee, Thompson Station Site in Thompson Station, TN, and Northcrest Medical Center in Springfield Tennessee.

Tennessee Maternal Fetal Medicine also offers multiple clinical sites from which we can recruit. This includes the Nashville location, Murfreesboro clinic (Murfreesboro TN), Mount Juliet clinic (Mount Juliet, TN) and Franklin TN locations.

Potential participants who are identified outside of a clinical visit (i.e., respond to fliers and directly contact study staff) will be asked about their next clinic visit. If the next clinical visit falls outside of the window of enrollment, then alternative arrangements may be made to conduct the first study visit. Options include use of clinical research space at VUMC. Based on our pilot data, we can complete survey administration, , blood collection, and exhaled CO measurements in approximately 30-40 minutes, thus allowing flexibility in the location that this information is collected.

Recruitment Milestones

One-fourth of the total number of proposed participants should be recruited within the first year of recruiting. This trend should continue with approximately 25% of the total number of participants to be recruited each year. In total, all participants should be recruited by the end of 2023.

5.2 Screening

Prior to clinic visit 1, potential participants will be screened by study staff to establish potential eligibility. Routine smoking status is documented in the electronic health record

(EHR) by a nurse at the participants establishment at the OB/GYN clinic as a patient producing a daily list of smokers which the study staff can access in EPIC. Patients whom appear on the smoking list with upcoming appointments in the OB/GYN clinic will be screened by study staff for preliminary eligibility. This preliminary screening will consist of obtaining information from the patient's medical record including age, smoking status, gestational age, known allergies, presence of known medical conditions, and information regarding the presence of pregnancy-related medical problems. If medical record review revels that a patient is preliminarily eligible, a text message or a phone call may be placed by study staff or a letter maybe mailed to the patient to assess study interest. The patient has the option to opt in or out of the study prior to their clinic visit 1.

Potential participants will be approached in person by study staff within the clinic before or after their normally scheduled visit. The RA will visit those patients who are preliminarily eligible based on the chart review eligibility form to verify eligibility, explain the study in detail using a written handout, and obtain informed consent. Screening for eligibility will include reviewing the patient's chart in the EHR and completion of the RA Screening Form (RASF). If a potential participant express their interest to join the study via text or call and agree to go over the study details on a call with the RA before their scheduled visit with the clinic, the RA may proceed with the call to explain the study details, answer any questions the potential participant has and may send the electronic informed consent form to the potential participant via text or email. If the patient wishes to have more time to consider study participation, she will be given the name of the RA and study phone number to call if they are interested. Data collected on participants who screen as ineligible or who choose not to join will be kept in order to have an accurate record of the rate of enrollment among those screened for participation and to be able to identify reasons why potential participants are ineligible. Patients who are deemed ineligible or choose not to join the study may be provided with information regarding smoking cessation programs sponsored through the Department of Health such as "Baby & Me".

Once study staff has determined eligibility and thoroughly explained the details of the study the potential participant will have the opportunity to ask questions and if willing, they will sign the informed consent document.

5.3 Informed Consent

Consent will be fulfilled by the subject's signature on the consent form. In the case where a minor is being consented, the minor will sign the assent form and their legal guardian will sign the parental consent. During the in-person or remote consent process, patients will be consented by a member of the key study personnel. Patient signatures can be obtained using one of several methods: typed signature, written signature – via stylus/cursor, etc.). Upon completion of the consent, patients will be provided with a copy of their version of the consent document. Potential participants will be told that their participation is completely voluntary, and that they can opt out at any time or refuse to participate in any portion of the study. During the consent process,

potential participants will be informed of all the study procedures, including possible side effects of medications used.

5.4 Enrollment

After eligibility is confirmed and consent is obtained, the patient will complete the enrollment process. Participants will be randomized into a treatment group and complete a brief baseline survey which will be administered by study staff and entered directly into REDCap study databases. Address and contact information will be confirmed. Biological specimens will be collected, and participants will be given study related material including information on other smoking cessation resources such as the Tennessee Tobacco quitline sponsored through the Department of Health

Initial Delivery of SCRIPT Behavioral Intervention for Smoking Cessation
Participants will receive the booklet "A Pregnant Woman's Guide to Quit Smoking" at study enrollment [41]. Study staff will provide an overview of the SCRIPT material to the participant and conduct a brief behavioral intervention session to introduce the participant to useful skills and techniques to assist in their quit smoking attempt. A Tobacco Treatment Specialist (TTS) will contact participants by phone approximately one week after enrollment to provide additional evidence-based care, guided by the booklet, that includes discussion of tobacco use during pregnancy, assessment of readiness to quit, and development of a plan to promote long term cessation. The TTS will reinforce the importance of utilizing support such as the free state quitline, which offers multi-session telephone counseling and 2 weeks of free mailed NRT. Study staff will offer to refer participants to the quitline via an automated referral from the EHR. A feedback report is sent from the quitline directly to the patient's chart in the EHR that includes whether or not the participant was reached, as well as use of quitline services. The TTS will have access to and use these reports to augment counseling of INFANTS participants.

5.5 Randomization

After providing informed consent at visit 1, participants will be randomized based on permuted blocks stratified by smoking amount (<10 cigarettes per day or ≥10 cigarettes per day), history of preterm labor, and supervised buprenorphine use. The randomization schema will be developed by the study statistician and maintained by the Investigational Drug Service (IDS).

5.6 Blinding

All individuals conducting the clinical study will be blinded to patient therapy assignments until patient enrollment in the study has ceased, all enrolled patients have completed the study, and final data lock has occurred. At the DSMB's request the PIs and DSMB may be unblinded to the therapy assignments for regulatory safety reporting of serious adverse events.

Patient treatment assignments will be managed through a central randomization system by IDS. Aside from regulatory safety reporting requirements, IDS will not provide the PIs with any information that could potentially un-blind the therapy assignment of any enrolled patient. IDS will also be responsible for the storage, preparation and labeling of drugs. The Clinical Research Pharmacist will devise standard operating procedures of the pharmacy to follow with regard to preparing, labeling, blinding and dispensing study drug.

The biostatistician will complete and prepare interim report data and present these results to the DSMB. This individual will receive data sets created by IDS (mock blind) and the study data manager. The biostatistician will perform specified statistical analyses.

5.7 Sample Collection

Biological specimens will be collected at all clinic visits. During enrollment at clinic visit 1, clinical phlebotomists will attempt to collect a blood sample of approximately 20 mL (a little over a tablespoon). Blood samples will be used to determine Red Blood Cell (RBC) phospholipid membrane determination, to determine blood levels of nicotine and nicotine metabolites (for NMR), and for future analysis of genetics and other potentially relevant biological markers. During clinic visit 1 the participant will also be asked to provide an exhaled CO sample. Participants will be asked to consent separately for these data to be collected for the proposed research project and for potential future research. Participants will also be asked to sign a separate consent if they choose to provide a sample for genetic analysis.

At clinic visit 2, the participant will be asked to provide a blood sample of approximately 20 mL. Blood samples will be used for RBC phospholipid membrane determination and to determine blood cotinine levels. Additional blood may be stored for future analysis. An expired CO sample will also be collected at clinic visit 2.

Blood Sample Collection Procedure
Blood samples will be collected at all clinic visits. A total of approximately 20 mL of blood will be collected at clinic visit 1 for NMR, red blood cell phospholipid membrane determination, and genetics/future studies. At clinic visit 2, a total of approximately 20 mL of blood will be collected for NMR, RBC lipid quantification, and future analysis. Red blood cell phospholipid content is not influenced by fasting status, unlike plasma levels, and as such study participants do not need to be fasting for blood draws [42]. Participants will be advised that blood may be discarded under certain circumstances (e.g., if they elect to not enroll in the study or if they withdraw from the study).

End-Exhaled CO Procedure
The Smokerlyzer ED50 CO meter (Bedfont Instruments) will be used to assess end expired carbon monoxide [43]. The results of the device will be recorded into the REDCap database. If a participant is unable to complete an in-person CO, then a

personal CO device (such as a CoVita iCO Smokerlyzer) may be mailed to the participant to complete the CO reading remotely. We have successfully used this protocol for mailed cotinine samples and CO testing in the past.

5.8 Behavioral Support for Smoking Intervention

We have made every effort to provide a rigorous standard of care for all study participants, regardless of treatment arm.

Participants will receive the SCRIPT booklet "A Pregnant Woman's Guide to Quit Smoking" at study enrollment [41]. A Tobacco Treatment Specialist (TTS) will contact participants by phone within one week after enrollment to provide evidence-based care, guided by the booklet that includes discussion of tobacco use during pregnancy, assessment of readiness to quit, and development of a plan to promote long term cessation. The TTS will offer to refer participants to the state quitline, which offers multi-session telephone counseling and 2 weeks of free mailed NRT, via an automated referral from the EHR. A feedback report is sent from the quitline directly to the patient's chart in the EHR that includes whether or not the participant was reached, as well as use of quitline services. We will also provide the participant with information regarding smoking cessation services offered through the Department of Health.

Study staff will work closely with the recruitment clinics in order to build a rapport with the staff and study participants.

5.9 Study Medication Intervention

The intervention period will have two Phases. Phase 1 will be approximately 12-weeks duration. This will begin at randomization (clinic visit 1) and end at the in-person clinic visit 2. This Phase will be used to determine impact on smoking behavior and compliance. Phase II will be the period from clinic visit 2 to 37 weeks gestation or delivery, whichever comes first. This period could range from 0 weeks to approximately 13 weeks in duration. Medication dispensing could occur on two or more occasions. The first will be after randomization at Visit 1 and includes the dispensing of a 12-week supply of study medication. The second will occur after the clinic visit 2 and will include an estimated supply to reach 37 weeks gestation. This amount will be determined after visit 2. Research staff may follow-up with a phone call to the participant to ensure receipt of the study agent.

Study medication: Fish Oil Capsules

Participants allocated to n-3 LCPUFA supplementation will be instructed to take four 1000 mg n-3 LCPUFA capsules (Metagenics™) daily. As it is unclear if EPA versus DHA might be more beneficial we have chosen to utilize a combination product for the study. Certificate of Analysis and Eurofins analysis for contaminants demonstrated <0.005 mg/kg mercury and these supplements are well below all the WHO requirements for toxic equivalents of dioxins, furans and dioxin-like PBCs. This will provide a total

daily dose of 4000 mg n-3 LCPUFAs (2840 EPA and 1160 DHA). Participants will be instructed to take study medications with meals as concomitant consumption of n-3 LCPUFA supplements with protein increased tissue bioavailability [42, 44]. Metagenics™ fish oil tablets include tocopherol anti-oxidants and are rigorously tested for over 250 contaminate. Pharmacological grade fish oil capsules have the advantage of providing high concentrations of n-3 LCPUFAs, low levels of contaminants and almost no fish odor.

Rationale for Dose of Study Medication

As described above, two prior studies have been conducted in smokers to assess the effects of supplemental n-3 LCPUFAs on nicotine cravings [45, 46]. While both studies reported a statistically significant reduction in self-reported nicotine cravings in the intervention arms, a reduction in cigarettes smoked per day was only seen in the study of high-dose n-3 LCPUFA (> 4 grams/day) as opposed to low dose n-3 LCPUFA (1 gram/day). Since large doses of fish oil supplements are well tolerated in pregnancy and the aforementioned studies suggest a dose effect of n-3 LCPUFAs on cigarettes per day we have chosen to utilize a high-dose n-3 LCPUFA intervention.

Rationale for Choice of Placebo Capsules

We will use oleic acid as our placebo. The reason for the use of oleic acid is several-fold. Oleic acid (olive oil) capsules have a similar texture, size, color, and consistency to EPA capsules. Oleic acid has been used as a placebo in several prior studies of fish oil supplementation and is well tolerated and is safe in pregnancy [36, 39, 47].

Directions for Use

The prescription and the signed consent document (for clinic visit 1 only) will be delivered via fax or other method such as secure email or study staff hand-delivering these (in the case that fax or secure email is not functional) to the Vanderbilt IDS. IDS will notify study staff of the randomization and study product will be provided on site. Should on site delivery not be available, the Vanderbilt IDS will mail the capsules directly to the participants preferred address. Participants will be instructed to take the study medication with food to decrease the risk of GI upset and to store the medication in a refrigerator. Refrigeration of the product is to reduce the risk of eructation and "fishy" taste in the mouth. They will be instructed not to freeze the capsules. To enhance tolerability subject will be given the following dose-escalation schedule.

- Day 1: Take 2 capsules
- Day 2: Take 3 capsules
- Day 3: Take 4 capsules

Participants are allowed to take all 4 capsules at one time or in divided doses. Participants will be advised to maintain the same dosing pattern over the course of the study to improve compliance. Participants will be given a pill diary as a visual memory aide at clinic visits 1. Between clinic visits 1 to 2, subjects will receive compliance telephone reminders approximately every two-weeks to encourage medication compliance using a standardized script. This message can be left either on a voice mail

or with a different household member if the participant allows. In addition, alternative message options (email communications or texting) will be allowed for medication and visit reminders if the participant indicates these as their preferred method of communication.

Contraindications
Fish oil is contraindicated in participants with a known hypersensitivity to fish.

Concomitant Medications
There are no restricted concomitant medications.

Dose Modification
Participants reporting minor GI symptoms (≤ grade 2) will be assessed to ensure they are taking the medication as suggested (with meals and refrigerated). Those who wish to remain on trial will be instructed to reduce their study medication from 4 capsules daily to 3 capsules daily. If symptoms persist or worsen over the next 3 days, they will be instructed to reduce their daily dose from 3 capsules per day to 2 capsules per day. If symptoms persist or worsen over the next 3 days, they will be instructed to reduce their daily dose from 2 capsules per day to 1 capsule per day or if necessary 1 capsule every other day. If symptoms persist or worsen over the next 3 days, they will be instructed to discontinue the study medication.

To minimize unmasking and improve tolerability participants are instructed to titrate up their dose over a period of 1 week to the study dose. Participants will be instructed to take the study medication with food to decrease the risk of GI upset and to store the medication in a refrigerator. Refrigeration of the product is to reduce the risk of eructation and “fishy” taste in the mouth. They will be instructed not to freeze the capsules. We have utilized these strategies as part of the FORTUNE pilot RCT.

Medication Adherence/Compliance
Study participants will be provided with a pill diary upon enrollment into the study. Participants will be expected to track their medication adherence everyday throughout the duration of the study. Unused medications will be collected at clinic visit 2 for pill counts. In addition to pill counts, we will ask participants to self-report medication compliance and additionally we will measure RBC membrane content of n-3 LCPUFA as a biomarker of compliance. Prior studies have confirmed that this measure is a valid method to determine compliance with fish oil supplementation and have documented increased levels of RBC membrane EPA in subjects randomized to fish oil supplements [43, 48-51].

5.10 Study Flow

Potential participants will be identified through medical record reviews, direct advertisements within the study clinics, and provider referrals. To further increase our recruitment, we will embed research staff in the clinic sites to identify potential participants during regularly scheduled clinical visits. Potential participants will be

approached in person within the clinic during their regularly scheduled visit or over the phone and the study will be described to them in detail. Once the study has been described, the potential participant will have the opportunity to ask questions and sign the informed consent document if they join the study. When the participant signs the consent form, the study flow begins with clinic visit 1. Below are details of data collected which are summarized in **Table 1**.

Clinic visit 1 will consist of the following:

- Assessment and confirmation of eligibility and obtaining informed consent
- Randomization into either intervention or placebo group
- Completion of baseline survey
- Participants receive the SCRIPT booklet "A Pregnant Woman's Guide to Quit Smoking" [41] at study enrollment along with a brief counseling session based upon the contents and other strategies to quit smoking
- Collection of exhaled CO measurements and blood samples for both red blood cell (RBC) phospholipid content quantification, NMR, and storage for genetics
- Participants will be given their study medication and a pill diary for them to keep track of their medication usage
- Participants will be reimbursed for their participation in clinic visit 1

Approximately **1 week** after enrollment:

- The participant will be contacted by a study Tobacco Treatment Specialist (TTS) who will provide evidenced based care, guided by the SCRIPT booklet [41]
- Readiness of participant to quit smoking will be assessed
- Along with the participant, the TTS will develop a plan to promote long term cessation

Approximately every **2-week interval** after initial enrollment:

- Participants will be contacted by text, email, or phone to encourage compliance with study medications
- Participant may receive reminders about upcoming appointments.

Clinic Visit 2 will occur approximately **12 weeks after Clinic Visit 1,** consisting of:

- Completion of follow up questionnaire administered by study staff
- Exhaled CO measurements and blood will be collected for red blood cell (RBC) phospholipid content quantification, cotinine level determination, and storage for further use.
- Medication will be dispensed to provide participant with enough to reach approximately 37 weeks gestation, if necessary
- Unused pills from the previous 12 weeks will be collected by study staff
- Study staff will review the medication diary with the participant
- Participants will be reimbursed for their participation in clinic visit 2

Shortly after **delivery**, study staff will obtain the following information through EHR chart review:

- Review medical records pertaining to delivery in order to obtain the following information:
    - Gestational age at delivery
    - Apgar scores (1 minute and 5 minute)
    - Birth weight
    - Maternal weight at delivery
    - Presence of any fetal/neonatal conditions or complications
    - Presence of any maternal conditions or complications present at birth
    - Method of delivery
    - Maternal smoking status

At **6 weeks post-delivery** the participant will receive a call that will consist of the following:

- Completion of smoking-related questionnaires administered by study staff
- Obtain self-reported measures of smoking status
- Reimbursement to participant for study participation

Table 1: Schedule of Study Events

	Screening: -2 weeks	**Visit 1: Baseline : 0 weeks**	**TTS Follow-up Phone Visit: BL + 1 week**	**Phone Visits: q 2 weeks**	**Visit 2: ~12 weeks from Baseline**	**Delivery:**	**Post-Delivery Follow Up: 6 weeks after Delivery**
Recruitment	X	X					
Eligibility Assessments	X	X					
Text message or Letter or Phone call to assess study interest	X						
Electronic Health Record (EHR) Review	X					X	
Informed Consent		X					
Randomization		X					
Clinic Visit 1 Baseline Survey		X					
Blood sample collection		X			X		
Exhaled CO test		X			X		
Dispense study medication		X			X		
Behavioral Intervention		X	X				
Clinic visit 2 follow-up survey					X		
Collect and Count Unused Medication					X		

Review Medication Diary for Compliance					**x**		
Medication Compliance Reminder		**x**	**x**	**x**	**x**		
6-week Post Delivery Survey							**x**
Participant Payment		**x**			**x**		**x**

5.11 Assessments

Survey Assessments (Table 2): See page 34 for full table. Survey assessments will consist of a baseline survey at clinic visit 1 and two follow up surveys approximately 12 weeks after clinic visit 1 at clinic visit 2 and approximately 6 weeks after delivery.

Baseline questionnaire:
Study RAs will administer the baseline questionnaire via REDCap (or paper, if needed) at clinic visit 1. On the baseline survey assessment, participants may be asked about sociodemographic characteristics (education and household income), perceived importance of quitting smoking, the impact of the COVID-19 pandemic on smoking thoughts and habits, social support and self-efficacy, resiliency, alcohol use, smoking-related characteristics (years of smoking; use of other tobacco products, the Fagerstrom Test for Nicotine Dependence, the Minnesota Nicotine Withdrawal Symptom Check List (MNWS), the Questionnaire of Smoking Urges-brief (QSU-brief), a medical symptom screen, and measurements of anxiety (GAD-7), depression (PHQ-8), Post-Traumatic Stress Disorder screen (PC-PTSD-5)[52], Adverse Childhood Experiences Questionnaire (CDC-Kaiser Adverse Childhood Experiences (ACEs) Questionnaire[53]), and delay discounting (Kirby Monetary Choice Questionnaire[66]; Holt and Laury Risk Preference Questionnaire[67]).

Follow up questionnaires:
Follow up assessments regarding the participants smoking habits will be conducted at approximately 12 weeks post recruitment (clinic visit 2) and at 6-weeks post-delivery. These brief follow-up assessments may include questions related to nicotine dependence and smoking urges, medical symptom assessments, and measurements of anxiety and depression, resilience, opioid use, and delay discounting similar to questions given at baseline. Participants who are unable to complete the surveys in person, will be sent a REDCap link to complete remotely via text message or email.

Self-reported smoking assessment:
Self-reported smoking amounts will be collected from self-completed survey responses at multiple time-points throughout the study period including clinic visit 1, clinic visit 2, and 6-weeks post-delivery.

Self-reported and objectively measured medication adherence

Adverse event assessment:
Fish oil is generally well tolerated and as such study participants will be asked to report any side effects related to medication use to study staff.

Medication diary assessment:
Study participants will be provided with pill diaries which will be used as an assessment of compliance. On study visit 2, participants will be asked to return all unused medication for pill counts.

Biochemical Data (Table 3)

Red Blood Cell Phospholipid Membrane Determination:
Total lipids will be extracted from 200 µl of double washed packed red blood cells using the method described by Folch et al [54]. Fatty acid values will be presented as percentage of total RBC membrane phospholipid fatty acid content. The lowest level of detection for individuals' fatty acids is less than 0.5% of the total profile. Red blood cell phospholipid content is not influenced by fasting status, unlike plasma levels, and as such study participants do not need to be fasting for blood draws [42].

End-Expired CO levels:
We will use the Smokerlyzer ED50 CO meter (Bedfont Instruments) to assess end expired carbon monoxide [43]. Participants will be advised to hold their breath for up to 20 seconds before blowing in the device or less as needed for comfort. Output is in carbon monoxide parts per million (CO ppm). Subjects self-reporting abstinence may have smoking status confirmed biochemically using end expired carbon monoxide. Biochemically validated abstinence will be defined as end expired carbon monoxide less than 4 ppm.

Nicotine, Cotinine and Nicotine Metabolites:
Nicotine and its metabolites, cotinine and trans-3'hydroxycotinine, will be measured using quantitative liquid chromatography-tandem mass spectrometry using a commercially available testing center. The assay requires a minimum of 1 mL of serum. Cotinine has an advantage over end-expired CO as it reflects a greater period of abstinence detecting nicotine exposure for up to 72 hours as opposed to 12-24 hours for expired CO.

5.12 Study Endpoints

Primary Study Endpoint: Preterm labor risk based on gestational age at delivery, which will be abstracted from medical records by study staff. Gestational age at randomization will be determined according to a previously described algorithm on the basis of the last menstrual period and earliest ultrasound examination and will not be revised after being assigned a study group [44]. Briefly, gestational age will be

estimated from the last menstrual period (LMP) and adjusted for the first-trimester ultrasound. If a self-reported LMP is greater than 7 days from the calculated ultrasound LMP, the ultrasound will be used to assign gestational age.

The primary outcome for Specific Aim 2 will be percentage change from baseline in cigarettes per day (CPD) at 12 weeks. The outcome will be determined based on participant self-report. We will confirm self-reported reduction in CPD based on biochemically confirmed reduction in end-expired CO and serum cotinine levels.

Secondary Endpoint: Secondary endpoints will include both neonatal and maternal endpoints. Neonatal endpoints include: 1) fetal death and still birth, 2) neonatal death (birth to 28 days); 3) individualized birth weight Z-score (adjusted for gestational age and maternal weight; 4) Apgar score; 5) intraventricular hemorrhage; 6) neonatal enterocolitis; 7) congenital abnormality; 8) neonatal respiratory distress

Maternal endpoints will include: 1) maternal mortality; 2) mode of delivery; 3) hypertension in pregnancy. Secondary endpoints will be collected from medical records.

The secondary outcome for Specific Aim 2 will be point prevalence abstinence from smoking at 12 weeks based on self-reported smoking cessation and biochemically confirmed by end-expired carbon monoxide and blood cotinine levels. Additional secondary endpoints will include changes in nicotine dependence based on changes in the Fagerström Test for Nicotine Dependence.

Study Medication Discontinuation: Participants may want to discontinue their medications due to possible side effects. Before medication is discontinued participants will be encouraged to adjust their medication routine. For example, taking the pills at a different time of day, taking them with or without food, or altering the dose. Those who choose to discontinue their medication will be followed and analyzed by intention to treat.

Study Withdrawal: A participant can voluntarily withdraw from the study at any point in time throughout the course of the study. The participant will not be penalized in any way for voluntarily withdrawing from the study. To withdraw, the patient will be asked to inform the research team in writing of their intent to withdraw including their reason for leaving the study. If the participant withdraws, they will no longer be eligible for compensation through the study for surveys or tests that they had not completed prior to withdrawal.

Study Termination: The study PI(s) has the right to discontinue the study at any time.

5.13 Quality Assurance

Assessment quality assurance
REDCap will be used to capture survey assessments, ensuring uniformity, completeness, and security of data. Paper assessments will be used if necessary. All

paper forms used will be stored securely in a locked cabinet that is only accessible to study staff.

Call Back Quality Assurance
Study tobacco treatment counselors/coaches will have dedicated, protocolized training in tobacco treatment. A subset of calls will be monitored by the study coordinator and/or Principal Investigator (PI) for quality assurance.

5.14 Compensation

We will offer reimbursements to participants. Participants will be compensated $225 for full participation in the study if all the procedures are completed. Participants will receive $100 for completion of clinic visit 1, $100 for completion of clinic visit 2, and $25 for completion of the 6-week post-delivery follow-up survey. This is to compensate for any financial cost due to travel and the inconvenience of blood draws. The participant compensation plan will be pro-rated based on the number of study visits completed. Participants will receive reimbursement in the form of cash or a merchandise/pre-paid gift card.

5.15 Retention

From our pilot study and exit interviews, we have identified several barriers to retention. The primary barrier involved the requirement for an independent, geographically-separate research visit. To improve retention, most research activities will occur immediately following clinical standard-of-care visits and occur at the clinical setting. If necessary, study staff may conduct a home visit to complete either the enrollment (Visit 1) or follow-up (Visit 2) process if it is not possible to have them occur within the clinical setting. Research procedures have been streamlined to complete the visit in 30-40 minutes. We will offer reimbursements to participants. Finally, based on our experienced in retaining underserved populations for cohort studies, we will include text reminders, birthday cards and study newsletters, and additional incentives.

This population is disadvantaged and thus remaining in the study could be a challenge. We will try to improve retention by 1) timing all research related activities to occur during scheduled clinic visits, 2) limiting the amount and length of a visit (estimated 30-40 minutes additional time; 3) sending periodic text reminders to our population to keep them engaged in the study and reinforce compliance with the medication and 4) offering study reimbursement. We found an improvement in lost to follow up in our pilot study when we increased subject reimbursement. Higher than anticipated rates of lost to follow up should only impact our secondary outcomes, as tobacco exposure would require an in-person visit. To address this possibility, we have powered our study to account for dropouts. Our primary outcome will be extracted from medical records and as such we anticipate more complete records. Coleman et al. published a study of

nicotine replacement therapy in pregnant smokers with smoking cessation as the primary outcome [55]. Their lost to follow up in this study was 18% with regards to follow-up measures of tobacco use. The secondary outcome was birth outcomes abstracted from medical records. For this outcome there was missing data on less than 1% of subjects.

6.0 Risks

The study will include vulnerable populations including pregnant women, neonates, and children. Protections for neonates and children will involve parental consent and assent in children 16 to 17 years of age. For neonates, we will only be conducting minimal risk procedures (medical record review). Risk will be minimized by collecting the minimal amount of blood necessary to perform study related biological assays, which fall within CFR guidelines for minimal risk. The study includes a Data Safety and Monitoring Board which will conduct interim analyses to monitor for any signs of adverse effects associated with n-3 LCPUFA supplementation.

Risks are minimized as preliminary studies have been conducted that provide safety data for n-3 LCPUFAs in pregnant women, the risk is the least possible for achieving the objectives of the research, the research holds out the prospect of benefit to the pregnant woman or fetus, consent of the pregnant woman will be obtained, each women will be fully informed regarding the reasonably foreseeable impact of the research on her and the fetus, for children who are pregnant, assent and permission will be obtained in accord with the provisions for 45CFR 46 subpart D, no inducements will be offered to terminate the pregnancy and the individuals engaged in the research will have no part in any decision as to the timing, method, or procedures used to terminate a pregnancy.

The potential risk to the participants from study participation are related 1) to the breach of data confidentiality, 2) drawing of venous blood, 3) collection of end-expiratory CO, and 4) the use of fish oil or olive oil supplements

All risks and alternative treatments as well as the rationale for the proposal will be included in the informed consent document and discussed with participants.

6.1 Breach of Data Risks

An important potential risk to participants would be a loss of confidentiality related to a disclosure of personal health information. As part of this proposal numerous confidentiality safeguards are in place including: 1) deidentifying data after entry into the analytical database; 2) limiting access to the study “key” to only study personnel; 3) containing all computerized records in a password protected secured network server; and 4) storing all paper based records in locked file cabinets that only the study PIs and research staff will have access.

The risks associated with completing questionnaires are minimal and involves predominately inconveniences of time.

6.2 Medication Risks

n-3 LCPUFAs have been part of the human diet for millennia and have uncommon and generally trivial side effects. In 1997 the Food and Drug Administration rules that an intake of up to 3 g/day of marine n-3 LCPUFAs are Generally Recognized as Safe (GRAS). This ruling specifically considered the possible effects of fish oil on bleeding time, glycemic control, and LDL cholesterol. N-3 LCPUFAs supplements have been used in several large randomized controlled studies of pregnant women. The most common side effect of fish oil is a fishy after-taste which is less of a problem with pharmaceutical grade supplements. Minor gastrointestinal symptoms occur in 5 percent of patients. Fish oil has been approved by the FDA for treatment of hypertriglyceridemia.

Olive oil is a natural substance with no known adverse effects reported related to the consumption of this product during pregnancy.

6.3 Blood Draw and End-expiratory CO Testing

Sampling of venous blood is a routine procedure that is considered standard of care in clinical medicine. The blood draw will be performed by trained personnel using universal precautions to protect both the participant and personnel. The risks to subjects are minimal, but may include pain, allergic reaction, infection, or bleeding at the needle stick site. These usually resolve without any specific medical therapy over the course of minutes to days.

End-expiratory CO analysis has no known reported risks, but some people may feel mildly short of breath.

7.0 Reporting of Adverse Events or Unanticipated Problems involving Risk to Participants or Others

7.1 Specification of Adverse Events

An **Adverse Event (AE)** is defined as any untoward or unfavorable medical occurrence or undesirable experience associated with the use of a medical product 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. AE's are graded as Mild (no limitation of usual activities), Moderate (some limitation) or Severe (inability to carry out usual activities) and attributed according to the relationship to the study drug and/or procedure as Not related, Unlikely related, possibly related, probably related, or Definitely related.

An AE can therefore be any new sign, reaction, symptom, event, disease or a worsening in frequency or severity of a preexisting condition that occurs during the course of the study. Stable chronic conditions that were present prior to study entry and do not worsen are not considered AEs. An event is considered "related" if it is likely to have resulted from participation in the research study and is considered "unanticipated" when it was unforeseeable at the time of its occurrence. An **unanticipated problem (UP) involving risk to participants or others** is "Any event that was (1) unanticipated, (2) related, and (3) places subjects or others at a greater risk of physical or psychological harm than was previously known or recognized."

A **serious adverse event** is defined as any adverse event that results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

We define an **unexpected adverse event** as any adverse event, the specificity or severity of which is not consistent with the current Investigator Brochure; or, if an Investigator Brochure is not required or available, the specificity or severity of which is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended.
"Unexpected," as used in this definition, refers to an adverse event that has not been previously observed rather than from the perspective of such experience not being anticipated from the pharmacological properties of the product and not listed in the Investigator's Brochure.

Suspected Adverse Drug Reaction – Any adverse event for which there is a reasonable possibility that the drug caused the adverse event. Reasonable possibility means there is evidence to suspect a causal relationship. It is considered unexpected if it is not consistent with the risk information described in the general investigational plan. A suspected adverse drug reaction will be defined as a recorded adverse event that is unexpected and deemed to be possibly, probably, or definitely related to the study drug.

7.2 Methods and Time for Assessing, Recording, and Analyzing Safety Parameters

- Participant symptoms will be assessed during the baseline survey to document any chronic conditions or symptoms that existed prior to the study. These will be documented on the Baseline AE log. This list will be reviewed and compared to reported events throughout the study. If the participant reports the same symptom,

occurring at the same severity, during subsequent visits, the symptom should not be recorded as an Adverse Event (AE). However, if the event was not previously reported or the severity has worsened, as determined by RA, then the AE should be reported.

- Throughout the course of the study and follow up, the participant will be asked about their health status to assess the presence of any possible side effects they may be experiencing or have experienced since the last assessment. The baseline assessment of symptoms and conditions will be reassessed at each subsequent follow up time point and reviewed and compared to baseline and previous follow ups by the study RA. Any event that meets the criteria for an AE/SAE/UP will be recorded. In the case of unresolved AEs, clinical staff will update the AE log with any follow-up information that is gathered during their investigation.
- All AEs will be assessed to determine if they meet criteria for a Serious Adverse Event (SAE). If the AE is serious, then the SAE form must be completed, and appropriate reporting measures followed (see below). Investigators will consult with the IRB or NIH, if they are uncertain how to classify an event.

Adverse Event Severity Grading Guidelines

Grade	Severity	Description
1	Mild	Mild; asymptomatic or mild symptoms; clinical or diagnostic observation only; intervention no indicated.
2	Moderate	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)[1]
3	Severe	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limited self-care ADLs [2]
4	Life-threatening	Life-threatening consequences; urgent intervention indicated
5	Fatal	Death related to AE

7.3 Procedures for Eliciting Reports of and for Recording and Reporting Adverse Events

Plan for unanticipated AE reporting
An Adverse Event Report form will be completed and returned to the IRB and DSMB within 5 working days of the reported event.

Plan for annual reporting of AEs
Annual reports will be submitted to the VUMC IRB and will contain a) the number of adverse events and an explanation of how each event was handled, b) the number of complaints and how each complaint was handled, c) the number of subject withdrawals

and an explanation of why the subject withdrew or was withdrawn, and d) the number of protocol violations and how each was handled.

Plan for safety review
Patients will be contacted by telephone every two weeks to assess for any medication side effects. Study staff will document frequency of side effects and intensity. All changes in either frequency of side effects or intensity will be discussed with the study PIs.

7.4 Advice for Participants

Individuals who report any GI symptoms ≥ grade 3, such as dyspepsia, heartburn, or diarrhea, will be instructed to discontinue the study medication immediately.

Participants reporting minor GI symptoms (≤ grade 2) will be assessed to ensure they are taking the medication as suggested (with meals and refrigerated). Those who wish to remain on trial will be instructed to reduce their study medication from 4 capsules daily to 3 capsules daily. If symptoms persist or worsen over the next 3 days, they will be instructed to reduce their daily dose from 3 capsules per day to 2 capsules per day. If symptoms persist or worsen over the next 3 days, they will be instructed to reduce their daily dose from 2 capsules per day to 1 capsule per day. If symptoms persist or worsen over the next 3 days, they will be instructed to discontinue the study medication immediately. It should be noted that in the pilot study, there were no discontinuations of study medication due to GI symptoms and the medication was well tolerated with no significant differences in reports GI side effects between fish oil and placebo.

7.5 Adverse Event Reporting

All adverse events will be reported to the IRB by the study team within the mandated time frames. Ultimately, the PI and study coordinator will review any serious adverse events and report them to the IRB and NIH.

The PI will report Unanticipated Problems and Adverse Events to the VUMC IRB in accordance with IRB policies:

- Required reporting to the IRB within 7 calendar days of the Investigator's knowledge of the problem which includes serious adverse events, injuries, side effects, deaths, or other problems occurring at VU, VUMC or other locations in which the Investigator is responsible for the conduct of the research and the VUMC IRB serves as the IRB of Record:
    - Any serious adverse event that in the Investigator's opinion was unanticipated or unexpected, involved risk to participants or others and was possibly related to the research procedures; and/or
    - Any noncompliance with the IRB-approved protocol that increased risk or affected the participant's rights, safety, or welfare.

- Any unanticipated problem listed above requires reporting to the IRB even after the participant has completed the study or after the participant has withdrawn from the study including after study closure.

- All individual AEs are maintained by the study team. Adverse events may include a participant's death as a result of a longtime illness (non-related), a breach in confidentiality or any complaint of a participant unless the risk involved is serious. In which case, the event is reported as an unanticipated problem involving risk to participants or others or as a serious adverse event at the time of occurrence.

7.6 Data Safety and Monitoring Board

Although the study proposes to administer a dietary supplement that is also FDA approved for use in treating elevated triglycerides and has been safely used in pregnancy, we will form a three-member independent Data Safety and Monitoring Board (DSMB). The members of the DSMB will not be involved with the project, will have no conflicts of interests, and will be selected so that they provide the appropriate clinical research and safety expertise. The function of the DSMB will be to provide objective, independent review of results, particularly as they relate to patient safety. The team will draft the Charter document for the DSMB which will be reviewed, edited and approved by the DSMB prior to study initiation. A brief description of the functions of the DSMB is specified below.

The DSMB is responsible for ensuring subject safety (by reviewing blinded and unblinded safety data on a regular basis and assessing the safety of study procedures) and for monitoring the overall conduct of the studies. The DSMB will monitor enrollment, follow up and adverse events.

Initially, the DSMB will meet and review the protocol and consent forms. The DSMB will approve them or recommend changes. Thereafter the committee will be provided with quarterly reports of the progress of the study. These reports will provide timely information regarding adverse events, data quality and patient recruitment. The committee will remain blinded as to whether individual patients are receiving n-3 LCPUFA supplements or placebo but will be provided with a randomization key should unbinding be deemed necessary. It is expected that unbinding would not occur without reasonable concern regarding patient safety or data validity. Interim analyses will be prepared for the DSMB the study biostatistician. The results will be blinded and presented on a coded group basis (i.e. A or B groups). The Committee will have the authority to modify the study protocol or terminate the study if they deem such actions to be warranted. The DSMB will provide summary reports to the IRB, the NIH, and the investigators.

During the conduct of the trial, any serious adverse event will be reported to the DSMB within 24 hours. Board members will be provided all of the available clinical data surrounding the clinical occurrence. During its regularly scheduled quarterly meetings,

the DSMB will also be provided with a list of non-serious adverse events organized by treatment group. The DSMB will be charged with the prompt review of this information and with providing feedback to the PIs as necessary. The DSMB will have access to the study biostatisticians to provide additional data or analysis if required.

The DSMB will meet quarterly to discuss participant accrual and study procedures. Two interim analyses have been planned for specific safety and trial futility analyses. The first interim analyses will occur after 50 participants have been enrolled in the study and completed all research-related interventions (post-delivery). The interim analysis will be conducted with patient therapy assignments blinded to the biostatistician. Members of the DSMB will receive blinded therapy assignments (mock codes assigned), clinical data listings and summaries prior to each DSMB meeting. If the DSMB recommends continuation with the trial a second interim analysis is planned after 200 participants have completed all research-related interventions. The study statistician will develop the group sequential boundaries which will be in place as study stopping rules. Additional stopping rules will be in place related to subject recruitment. Should recruitment fall below 25% of the projected accrual rate the DSMB will consider stopping the trial.

8.0 Statistical Considerations

8.1 Statistical Analysis for Aims 1-3

A detailed statistical design and power plan has been included within the Human Subjects and Clinical Trials Information Form per NIH guidelines.

If a significant n-3 LCPUFA - gestational age at birth association is observed, we will conduct an exploratory analysis what proportion of this association is mediated through smoking reduction and what proportion through an increase in circulating n-3 LCPUFA levels [50, 51]. The ability of this analysis to difference between these two possible mechanisms will depend on how strongly correlated the two are. However, provided there are a sufficient number of patients who increase their n-3 LCPUFA without reducing their cigarettes per day, this study will be in a unique position to investigate the mechanism behind n-3 LCPUFA supplementation and a higher average gestational age at delivery (lower risk of preterm birth).

8.2 Study Power

We will recruit 500 subjects to ensure at least 400 observed outcomes, allowing for a 20% loss to follow up rate. The performance of the proposed statistical methods and estimated statistical power were done through simulating batches of 20,000 datasets under various settings. To account for the potential skew in some outcomes, transformations of the Poisson distribution were used to generate outcome data with the placebo arm's mean and standard deviation calibrated to those observed in Coleman, et al [55]. Detectable effect sizes were calculated at 80% and 90% power. They are presented on the clinical scale for the primary outcomes and presented as standardized effect sizes for generalized estimates applicable to the continuous secondary outcomes.

Aim 1: A 0.55 week difference in mean gestational age at delivery is detectable at 80% power; a 0.63 week difference at 90%.
Aim 2: Assuming a common standard deviation for percent cigarettes per day reduction of 15%, the study will detect mean differences of 4.3% and 4.9% with 80% and 90% power, respectively.

Secondary outcomes: These detectable effect sizes correspond to standardized effect sizes of 0.29 and 0.33, respectively. Thus, for continuous and semi-continuous outcomes, the study is well powered to detect even small to medium effect sizes and will detect larger effect sizes with near certainty.

For dichotomous outcomes, all of which are secondary or exploratory, the detectable effect size depends on the outcome prevalence in the placebo arm. Assuming a 10% preterm delivery rate in the placebo arm, risk differences of 7% and 8% are detectable with 80% and 90% power, respectively. Similar risk differences are detectable assuming a 10% smoking cessation rate. These risk differences correspond to relative risks of 0.28 and 0.21. Thus, the study is powered to detect large effect sizes in the dichotomous outcomes.

8.3 Interpretation of Results

With regards to our primary outcome, we anticipate that supplemental n-3 LCPUFAs will result in a greater gestational age at delivery. This is an objective measure derived from standardized clinical data and the study is adequately powered to detect our estimated effect. A null finding would suggest that n-3 LCPUFA are not advantageous for smokers or has a more modest effect than reported in post-hoc analyses. We will be able to assess for compliance and thus can conduct a per-protocol secondary analysis along with our intention to treat analysis if noncompliance with the study drug is deemed to be high. A harmful effect of supplemental n-3 LCPUFA, although unexpected, would be useful and require further investigation as to the mechanism.

For our secondary outcome we will evaluate smoking behavior. A reduction in tobacco use in our intervention arm would suggest a beneficial effect on n-3 LCPUFAs in smoking cessation. This result would then identify a possible adjuvant to currently approved smoking cessation therapies which could then be investigated in nonpregnant populations. If we see changes in tobacco use, we will conduct mediation analyses to determine if changes in smoking behavior are responsible for changes in our primary outcomes. If we see no reduction in smoking behavior yet an improvement in our primary outcomes, this would suggest that correcting the relative deficiency in n-3 LCPUFAs in pregnant smokers may be beneficial regardless of smoking behavior.

9.0 Privacy/Confidentiality Issues

9.1 Data Collection

All study data will be captured electronically and stored via a secure, web-based data capture system, REDCap. Paper forms will be used during enrollment, if needed, and stored securely in a locked cabinet that only study staff will have the ability to access.

9.2 Quality Control Process

Quality control measures will include: detailed and unambiguous specifications for completion of data forms, including rules for coding skipped questions and missing data, training of study staff responsible for data collection and built-in validation rules, error checks, question skips for electronic data capture, and computer algorithms to check for out-of-range codes and internal inconsistencies. All data, regardless of capture method, will be reviewed for logic, skip patterns, response ranges, out-of-range codes, and internal inconsistencies, and converted to SAS (or equivalent statistical package) datasets for analysis.

9.3 Data Security and Confidentiality

To prevent the loss of data, all electronic information is stored within the VUMC firewall and is password-protected. If there are any hard-copy, original consent forms and other study documents including participant names and contact information will be kept in binders and locked in a filing cabinet. Electronic participant data will be placed into a password-protected, web-based database on encrypted computers and iPads by study personnel. Electronic medical records, and electronic participant tracking spreadsheets will be stored on a secure server. Only the research staff, the study PI and co-investigators will have access to this data. Data quality (including visits completed during intervention window, data missingness, and recruitment rates) will be monitored monthly by the database manager and systematic data problems will be reported to the PI.

A unique identification number will be used to protect the confidentiality of the study participants. This number will be assigned at the time of study enrollment. Only the investigator, coinvestigators and research personnel will have access to the key and information that identifies participants as being in this study. Digital data files will be coded so that the participant name or other such identifiers are not in the filename.

All procedures are in accordance with best practices for Federal Health IT as determined by the Office of the National Coordinator for Health Information Technology (ONC). Protected health information will not be exchanged outside of the approved study personnel at VUMC.

10.0 Follow-up and Record Retention

10.1 Follow up to Collect Research Data

This population is very disadvantaged and thus remaining in the study could be a challenge. We will try to improve retention by 1) timing all research related activities to

occur during scheduled clinic visits, 2) limiting the amount and length of a visit (estimated 30-40 minutes additional time; 3) sending periodic text reminders to our population to keep them engaged in the study and reinforce compliance with the medication and 4) offering study reimbursement. Higher than anticipated rates of lost to follow up should only impact our secondary outcomes, as tobacco exposure would require an in-person visit. Our primary outcome will be extracted from medical records and as such we anticipate more complete records.

10.2 Record Retention

Records will be kept indefinitely but will be destroyed if no longer needed.

11.0 Conclusion

The objective of this proposal is to determine the efficacy of supplemental n-3 long chain polyunsaturated fatty acids (LCPUFA) on preterm labor risk in pregnant smokers. We will also determine the effect of supplemental n-3 LCPUFAs on smoking behavior. This study is a randomized, double-blind, placebo controlled parallel arm study that will randomize approximately 400 pregnant smokers to either supplemental n-3 LCPUFAs or placebo. Participants will be enrolled between 10 and 24 weeks gestation and followed through delivery until 6-weeks post-delivery. We will recruit participants from the Middle-Tennessee area. We will assess smoking behavior after 12-weeks of supplementation using validated biomarkers of tobacco exposure (plasma cotinine and expired carbon monoxide). We will measure medication compliance and response to supplementation using biological markers of n-3 LCPUFA status (red blood cell phospholipid membrane fatty acid percentages). The studies primary outcome is gestational age at delivery obtained from medical records. Secondary outcomes include change in cigarettes per day from baseline at 12-weeks and smoking abstinence at 12 weeks.

Table 2: Survey and Abstracted Electronic Health Record Data

Construct	Measures	Source	Pre-recruitment Screening Form	Baseline timepoint (RASF, Baseline survey)	Clinic Visit 2	Delivery	6-week Post Delivery
Sociodemographic Factors	Age, marital status, education, race/ethnicity, income, intention to relocate	Patient	X	X			
Medical Condition/Pre-existing Conditions Checklist	**Pre-existing condition** – Chronic hypertension, seizure disorder, clotting disorder, planned cerclage, diabetes, allergy to fish or fish oil supplement	Patient /EHR	X	X			
	Pregnancy related conditions – Due date, unstable pregnancy-related conditions (pre-eclampsia, known fetal abnormalities, threatened abortion, placental abnormalities)		X	X			
	Current use of fish oil supplements			X			
Medical Conditions/ Complications related to Delivery	Gestational age at delivery, birth weight, maternal weight at delivery, Apgar scores (1 and 5 minute), Mode of delivery, presence of fetal/neonatal conditions or complications at birth, maternal conditions or complications at birth, maternal smoking status at time of delivery					X	
Tobacco Related Variables	**Nicotine dependence** Fagerstrom Test of Nicotine Dependence (FTND-modified) [56]	Patient		X	X		X
	Minnesota Nicotine Withdrawal Scale (MNWS) [57]			X	X		
	Questionnaire on Smoking Urges (QSU-Brief) [58]			X	X		
	Tobacco use			X	X		X

	Baseline: cigarettes per day, years smoked, other tobacco use, abstinence in previous 3 months Follow-up: duration of abstinence since previous clinic visit, cigarette and other tobacco product use in the past 7 days, date of last use						
	Electronic cigarette use Baseline and Follow-up: use in the past 7 days, frequency of use			X	X		X
	Secondhand smoke exposure [59]			X	X		X
	Quit attempt Definition: intentional tobacco abstinence lasting >24 hours. Baseline: past 3 months Follow-up: since last follow-up contact or clinic visit			X	X		X
	Tobacco cessation treatment Definition: FDA-approved cessation medications, behavioral support (telephone quit line, in-person counseling, internet programs), other Baseline: Prior use (in previous 3 months and/or 2 weeks) Follow-up: Use since previous clinic visit or last follow-up contact and previous 2 weeks	Patient		X	X		X
Other Substance Use	**Alcohol use** Alcohol Use Disorders Identification Test (AUDIT-C) [60]	Patient /EHR		X	X		X
	Drug use Marijuana, cocaine, opioids, stimulants, drugs by injection (Veterans Aging Cohort Study - VACS, *modified*) [61]	Patient /EHR	X	X	X		X
	Depression symptoms	Patient		X	X		X

Psychological Factors and Quality of Life	Patient Health Questionnaire (PHQ-8) [62]						
	Anxiety symptoms Generalized Anxiety Disorder (GAD-7) [63]			X	X		X
	Resiliency Connor-Davidson Resilience Scale (CD-RISC2) [64]			X	X		X
	Post-Traumatic Stress Disorder PTSD screen (PC-PTSD-5)[52]			X			
	Adverse Childhood Experiences (ACEs) CDC-Kaiser Adverse Childhood Experiences (ACEs)questionnaire [53]			X			
	Delay discounting: self-report module Kirby (1999) Moneatry Choice Questionnaire [66]			X	X		X
	Delay discounting: risk module Holt and Laury (2002) Risk Preference Questionnaire [67]			X	X		X
COVID-19 Pandemic Influence	Patient awareness of infection, changes in smoking behavior due to COVID-19, quit attempts as a result of COVID-19	Patient		X			
Program Feedback	**Services provided by the study** Helpfulness of reminder calls, counselor contact feedback, recommendation of program to others, patient's belief regarding treatment arm	Patient					X

12.0 References

1. Ellard GA, Johnstone FD, Prescott RJ, Ji-Xian W, Jian-Hua M: **Smoking during pregnancy: the dose dependence of birthweight deficits**. *British journal of obstetrics and gynaecology* 1996, **103**(8):806-813.
2. Li CQ, Windsor RA, Perkins L, Goldenberg RL, Lowe JB: **The impact on infant birth weight and gestational age of cotinine-validated smoking reduction during pregnancy**. *Jama* 1993, **269**(12):1519-1524.
3. Mitchell EA, Tuohy PG, Brunt JM, Thompson JM, Clements MS, Stewart AW, Ford RP, Taylor BJ: **Risk factors for sudden infant death syndrome following the prevention campaign in New Zealand: a prospective study**. *Pediatrics* 1997, **100**(5):835-840.
4. Pineles BL, Hsu S, Park E, Samet JM: **Systematic Review and Meta-Analyses of Perinatal Death and Maternal Exposure to Tobacco Smoke During Pregnancy**. *American journal of epidemiology* 2016, **184**(2):87-97.
5. Curtin SC, Matthews TJ: **Smoking Prevalence and Cessation Before and During Pregnancy: Data From the Birth Certificate, 2014**. *National vital statistics reports : from the Centers for Disease Control and Prevention, National Center for Health Statistics, National Vital Statistics System* 2016, **65**(1):1-14.
6. Tong VT, Dietz PM, Morrow B, D'Angelo DV, Farr SL, Rockhill KM, England LJ: **Trends in smoking before, during, and after pregnancy--Pregnancy Risk Assessment Monitoring System, United States, 40 sites, 2000-2010**. *Morbidity and mortality weekly report Surveillance summaries (Washington, DC : 2002)* 2013, **62**(6):1-19.
7. Cnattingius S, Forman MR, Berendes HW, Graubard BI, Isotalo L: **Effect of age, parity, and smoking on pregnancy outcome: a population-based study**. *American journal of obstetrics and gynecology* 1993, **168**(1 Pt 1):16-21.
8. Heffner LJ, Sherman CB, Speizer FE, Weiss ST: **Clinical and environmental predictors of preterm labor**. *Obstetrics and gynecology* 1993, **81**(5 (Pt 1)):750-757.
9. Shiono PH, Klebanoff MA, Rhoads GG: **Smoking and drinking during pregnancy. Their effects on preterm birth**. *Jama* 1986, **255**(1):82-84.
10. [https://www.marchofdimes.org/materials/premature-birth-report-card-united-states.pdf]
11. Institute of Medicine Committee on Understanding Premature B, Assuring Healthy O: **The National Academies Collection: Reports funded by National Institutes of Health**. In: *Preterm Birth: Causes, Consequences, and Prevention.* edn. Edited by Behrman RE, Butler AS. Washington (DC): National Academies Press (US) National Academy of Sciences.; 2007.
12. Alves E, Azevedo A, Correia S, Barros H: **Long-term maintenance of smoking cessation in pregnancy: an analysis of the birth cohort generation XXI**. *Nicotine & tobacco research : official journal of the Society for Research on Nicotine and Tobacco* 2013, **15**(9):1598-1607.
13. Hotham ED, Gilbert AL, Atkinson ER: **A randomised-controlled pilot study using nicotine patches with pregnant women**. *Addictive behaviors* 2006, **31**(4):641-648.
14. Myung SK, Ju W, Jung HS, Park CH, Oh SW, Seo H, Kim H: **Efficacy and safety of pharmacotherapy for smoking cessation among pregnant smokers: a meta-analysis**. *BJOG : an international journal of obstetrics and gynaecology* 2012, **119**(9):1029-1039.

15. Pollak KI, Oncken CA, Lipkus IM, Lyna P, Swamy GK, Pletsch PK, Peterson BL, Heine RP, Brouwer RJ, Fish L *et al*: **Nicotine replacement and behavioral therapy for smoking cessation in pregnancy**. *American journal of preventive medicine* 2007, **33**(4):297-305.
16. Chamberlain C, O'Mara-Eves A, Porter J, Coleman T, Perlen SM, Thomas J, McKenzie JE: **Psychosocial interventions for supporting women to stop smoking in pregnancy**. *The Cochrane database of systematic reviews* 2017, **2**:Cd001055.
17. Harris WS, Pottala JV, Lacey SM, Vasan RS, Larson MG, Robins SJ: **Clinical correlates and heritability of erythrocyte eicosapentaenoic and docosahexaenoic acid content in the Framingham Heart Study**. *Atherosclerosis* 2012, **225**(2):425-431.
18. Hibbeln JR, Makino KK, Martin CE, Dickerson F, Boronow J, Fenton WS: **Smoking, gender, and dietary influences on erythrocyte essential fatty acid composition among patients with schizophrenia or schizoaffective disorder**. *Biological psychiatry* 2003, **53**(5):431-441.
19. Murff HJ, Tindle HA, Shrubsole MJ, Cai Q, Smalley W, Milne GL, Swift LL, Ness RM, Zheng W: **Smoking and red blood cell phospholipid membrane fatty acids**. *Prostaglandins, leukotrienes, and essential fatty acids* 2016, **112**:24-31.
20. Nosova EV, Chong KC, Alley HF, Harris WS, Boscardin WJ, Conte MS, Owens CD, Grenon SM: **Clinical correlates of red blood cell omega-3 fatty acid content in male veterans with peripheral arterial disease**. *Journal of vascular surgery* 2014, **60**(5):1325-1331.
21. Simon JA, Fong J, Bernert JT, Jr., Browner WS: **Relation of smoking and alcohol consumption to serum fatty acids**. *American journal of epidemiology* 1996, **144**(4):325-334.
22. Pawlosky RJ, Hibbeln JR, Salem N, Jr.: **Compartmental analyses of plasma n-3 essential fatty acids among male and female smokers and nonsmokers**. *Journal of lipid research* 2007, **48**(4):935-943.
23. Kuper SG, Abramovici AR, Jauk VC, Harper LM, Biggio JR, Tita AT: **The effect of omega-3 supplementation on pregnancy outcomes by smoking status**. *American journal of obstetrics and gynecology* 2017, **217**(4):476.e471-476.e476.
24. Chalon S: **Omega-3 fatty acids and monoamine neurotransmission**. *Prostaglandins, leukotrienes, and essential fatty acids* 2006, **75**(4-5):259-269.
25. Chalon S, Vancassel S, Zimmer L, Guilloteau D, Durand G: **Polyunsaturated fatty acids and cerebral function: focus on monoaminergic neurotransmission**. *Lipids* 2001, **36**(9):937-944.
26. Zimmer L, Delpal S, Guilloteau D, Aioun J, Durand G, Chalon S: **Chronic n-3 polyunsaturated fatty acid deficiency alters dopamine vesicle density in the rat frontal cortex**. *Neuroscience letters* 2000, **284**(1-2):25-28.
27. Ahmad SO, Park JH, Radel JD, Levant B: **Reduced numbers of dopamine neurons in the substantia nigra pars compacta and ventral tegmental area of rats fed an n-3 polyunsaturated fatty acid-deficient diet: a stereological study**. *Neuroscience letters* 2008, **438**(3):303-307.
28. Zimmer L, Vancassel S, Cantagrel S, Breton P, Delamanche S, Guilloteau D, Durand G, Chalon S: **The dopamine mesocorticolimbic pathway is affected by deficiency in n-3 polyunsaturated fatty acids**. *The American journal of clinical nutrition* 2002, **75**(4):662-667.
29. Mansvelder HD, McGehee DS: **Cellular and synaptic mechanisms of nicotine addiction**. *Journal of neurobiology* 2002, **53**(4):606-617.

30. Zaniewska M, Przegalinski E, Filip M: **Nicotine dependence - human and animal studies, current pharmacotherapies and future perspectives**. *Pharmacological reports : PR* 2009, **61**(6):957-965.
31. Zaparoli JX, Galduroz JC: **Treatment for tobacco smoking: a new alternative?** *Medical hypotheses* 2012, **79**(6):867-868.
32. Makrides M, Duley L, Olsen SF: **Marine oil, and other prostaglandin precursor, supplementation for pregnancy uncomplicated by pre-eclampsia or intrauterine growth restriction**. *The Cochrane database of systematic reviews* 2006(3):Cd003402.
33. Koletzko B, Cetin I, Brenna JT: **Dietary fat intakes for pregnant and lactating women**. *The British journal of nutrition* 2007, **98**(5):873-877.
34. Koletzko B, Lien E, Agostoni C, Bohles H, Campoy C, Cetin I, Decsi T, Dudenhausen JW, Dupont C, Forsyth S *et al*: **The roles of long-chain polyunsaturated fatty acids in pregnancy, lactation and infancy: review of current knowledge and consensus recommendations**. *Journal of perinatal medicine* 2008, **36**(1):5-14.
35. Makrides M, Best K, Yelland L, McPhee A, Zhou S, Quinlivan J, Dodd J, Atkinson E, Safa H, van Dam J *et al*: **A Randomized Trial of Prenatal n-3 Fatty Acid Supplementation and Preterm Delivery**. *The New England journal of medicine* 2019, **381**(11):1035-1045.
36. Leventakou V, Roumeliotaki T, Martinez D, Barros H, Brantsaeter AL, Casas M, Charles MA, Cordier S, Eggesbo M, van Eijsden M *et al*: **Fish intake during pregnancy, fetal growth, and gestational length in 19 European birth cohort studies**. *The American journal of clinical nutrition* 2014, **99**(3):506-516.
37. Markhus MW, Skotheim S, Graff IE, Froyland L, Braarud HC, Stormark KM, Malde MK: **Low omega-3 index in pregnancy is a possible biological risk factor for postpartum depression**. *PloS one* 2013, **8**(7):e67617.
38. Meher A, Randhir K, Mehendale S, Wagh G, Joshi S: **Maternal Fatty Acids and Their Association with Birth Outcome: A Prospective Study**. *PloS one* 2016, **11**(1):e0147359.
39. Wadhwani N, Patil V, Joshi S: **Maternal long chain polyunsaturated fatty acid status and pregnancy complications**. *Prostaglandins, leukotrienes, and essential fatty acids* 2018, **136**:143-152.
40. Kar S, Wong M, Rogozinska E, Thangaratinam S: **Effects of omega-3 fatty acids in prevention of early preterm delivery: a systematic review and meta-analysis of randomized studies**. *European journal of obstetrics, gynecology, and reproductive biology* 2016, **198**:40-46.
41. Windsor RA: **Pregnant Woman's Guide to Quit Smoking**: Health Promotion Group; 1997.
42. Harris WS, Varvel SA, Pottala JV, Warnick GR, McConnell JP: **Comparative effects of an acute dose of fish oil on omega-3 fatty acid levels in red blood cells versus plasma: implications for clinical utility**. *Journal of clinical lipidology* 2013, **7**(5):433-440.
43. Donny EC, Denlinger RL, Tidey JW, Koopmeiners JS, Benowitz NL, Vandrey RG, al'Absi M, Carmella SG, Cinciripini PM, Dermody SS *et al*: **Randomized Trial of Reduced-Nicotine Standards for Cigarettes**. *The New England journal of medicine* 2015, **373**(14):1340-1349.
44. Velez Edwards DR, Baird DD, Hasan R, Savitz DA, Hartmann KE: **First-trimester bleeding characteristics associate with increased risk of preterm birth: data**

from a prospective pregnancy cohort. *Human reproduction (Oxford, England)* 2012, **27**(1):54-60.
45. Rabinovitz S: **Effects of omega-3 fatty acids on tobacco craving in cigarette smokers: A double-blind, randomized, placebo-controlled pilot study**. *Journal of psychopharmacology (Oxford, England)* 2014, **28**(8):804-809.
46. Zaparoli JX, Sugawara EK, de Souza AA, Tufik S, Galduroz JC: **Omega-3 Levels and Nicotine Dependence: A Cross-Sectional Study and Clinical Trial**. *European addiction research* 2016, **22**(3):153-162.
47. Schafer T, Dirschedl P, Kunz B, Ring J, Uberla K: **Maternal smoking during pregnancy and lactation increases the risk for atopic eczema in the offspring**. *Journal of the American Academy of Dermatology* 1997, **36**(4):550-556.
48. Cartwright IJ, Pockley AG, Galloway JH, Greaves M, Preston FE: **The effects of dietary omega-3 polyunsaturated fatty acids on erythrocyte membrane phospholipids, erythrocyte deformability and blood viscosity in healthy volunteers**. *Atherosclerosis* 1985, **55**(3):267-281.
49. Prisco D, Filippini M, Francalanci I, Paniccia R, Gensini GF, Abbate K, Neri Serneri GG: **Effect of n-3 polyunsaturated fatty acid intake on phospholipid fatty acid composition in plasma and erythrocytes**. *The American journal of clinical nutrition* 1996, **63**(6):925-932.
50. Steen J, Loeys T, Moerkerke B, Vansteelandt S: **Flexible Mediation Analysis With Multiple Mediators**. *American journal of epidemiology* 2017, **186**(2):184-193.
51. VanderWeele TJ, Vansteelandt S: **Mediation Analysis with Multiple Mediators**. *Epidemiologic methods* 2014, **2**(1):95-115.
52. Prins A, Bovin MJ, Smolenski DJ, Marx BP, Kimerling R, Jenkins-Guarnieri MA, Kaloupek DG, Schnurr PP, Kaiser AP, Leyva YE *et al*: **The Primary Care PTSD Screen for DSM-5 (PC-PTSD-5): Development and Evaluation Within a Veteran Primary Care Sample**. *J GEN INTERN MED* 2016, **31**(10):1206-1211.
53. Felitti VJ, Anda RF, Nordenberg D, Williamson DF, Spitz AM, Edwards V, Koss MP, Marks JS: **Relationship of childhood abuse and household dysfunction to many of the leading causes of death in adults. The Adverse Childhood Experiences (ACE) Study**. *Am J Prev Med* 1998, **14**(4):245-258.
54. Folch J, Lees M, Sloane Stanley GH: **A simple method for the isolation and purification of total lipides from animal tissues**. *The Journal of biological chemistry* 1957, **226**(1):497-509.
55. Coleman T, Cooper S, Thornton JG, Grainge MJ, Watts K, Britton J, Lewis S: **A randomized trial of nicotine-replacement therapy patches in pregnancy**. *The New England journal of medicine* 2012, **366**(9):808-818.
56. Heatherton TF, Kozlowski LT, Frecker RC, Fagerstrom KO: **The Fagerstrom Test for Nicotine Dependence: a revision of the Fagerstrom Tolerance Questionnaire**. *British journal of addiction* 1991, **86**(9):1119-1127.
57. Hughes JR, Hatsukami D: **Signs and symptoms of tobacco withdrawal**. *Archives of general psychiatry* 1986, **43**(3):289-294.
58. Cox LS, Tiffany ST, Christen AG: **Evaluation of the brief questionnaire of smoking urges (QSU-brief) in laboratory and clinical settings**. *Nicotine & tobacco research : official journal of the Society for Research on Nicotine and Tobacco* 2001, **3**(1):7-16.
59. Prochaska JJ, Grossman W, Young-Wolff KC, Benowitz NL: **Validity of self-reported adult secondhand smoke exposure**. *Tob Control* 2015, **24**(1):48-53.

60. Bush K, Kivlahan DR, McDonell MB, Fihn SD, Bradley KA: **The AUDIT alcohol consumption questions (AUDIT-C): an effective brief screening test for problem drinking. Ambulatory Care Quality Improvement Project (ACQUIP). Alcohol Use Disorders Identification Test**. *Arch Intern Med* 1998, **158**(16):1789-1795.
61. Smola S, Justice AC, Wagner J, Rabeneck L, Weissman S, Rodriguez-Barradas M: **Veterans aging cohort three-site study (VACS 3): overview and description**. *Journal of clinical epidemiology* 2001, **54 Suppl 1**:S61-76.
62. Kroenke K, Strine TW, Spitzer RL, Williams JB, Berry JT, Mokdad AH: **The PHQ-8 as a measure of current depression in the general population**. *Journal of affective disorders* 2009, **114**(1-3):163-173.
63. Spitzer RL, Kroenke K, Williams JBW, Löwe B: **A Brief Measure for Assessing Generalized Anxiety Disorder: The GAD-7**. *Archives of internal medicine* 2006, **166**(10):1092-1097.
64. Connor KM, Davidson JR: **Development of a new resilience scale: the Connor-Davidson Resilience Scale (CD-RISC)**. *Depression and anxiety* 2003, **18**(2):76-82.
65. Augenblick N, Rabin M: **An experiment on time preference and misprediction in unpleasant tasks**. *Review of Economic Studies* 2019, **86**(3):941–975.
66. Kirby KN, Petry NM, Bickel WK: **Heroin addicts have higher discount rates for delayed rewards than non-drug-using controls**. *J Exp Psychol Gen* 1999, **128**(1):78–87.
67. Holt CA, Laury SK: **Risk aversion and incentive effects**. *Am Econ Rev* 2002, **92**(5):1644–1655.