Vitamin E Alpha-Tocopherols to Prevent Recurrent Stroke in Patients with a Minor Neurovascular Event

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VITAMIN E ALPHA-TOCOPHEROLS TO PREVENT RECURRENT STROKE IN
PATIENTS WITH A MINOR NEUROVASCULAR EVENT

A Thesis Presented to
The Faculty of the School of Medicine
Yale University

In Candidacy for the Degree of
Master of Medical Science

April 2021

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Minor ischemic strokes and transient ischemic attacks involve impaired perfusion to corresponding brain regions leading to neurological deficits that may or may not resolve after the event. Adults suffering one of these minor neurovascular events have high odds of a recurrent stroke within the first 90 days, mitigated but not prevented by standard treatment of clopidogrel and aspirin. Vitamin E alpha-tocopherols have been shown to reduce platelet aggregation, potentiate aspirin, and reduce ischemic damage. This study will assess the efficacy of vitamin E alpha-tocopherol, in addition to the standard treatment, on stroke recurrence in adults with a minor neurovascular event compared to the standard treatment alone. In this randomized, double-blinded, placebo-controlled trial, participants will receive the standard treatment of clopidogrel and aspirin with either vitamin E alpha-tocopherol or placebo supplements. This study may help decrease the proportion of stroke recurrence and may ultimately reduce morbidity and mortality.
CHAPTER 1: INTRODUCTION

1.1 Background

Cerebrovascular accidents, or strokes, are a leading cause of disability in the United States with 800,000 people suffering from a new or recurrent stroke a year. Strokes may be due to ischemia, a subarachnoid hemorrhage, or an intracerebral hemorrhage with 87% of all strokes having an ischemic etiology. An ischemic stroke involves arterial occlusion or blockage resulting in reduced blood perfusion to corresponding brain regions leading to neurological deficits. Ischemic strokes are subcategorized into mild, moderate, and severe by the National Institute of Health Stroke Scale (NIHSS), a 42-scale scoring system that is utilized to quantify stroke severity in patients. A minor ischemic stroke is classified by a NIHSS score \( \leq 3 \). A transient ischemic attack (TIA) occurs when there is temporary occlusion of a blood vessel causing brief neurological symptoms that resolve within 24 hours before an ischemic injury occurs. TIA’s precede approximately 15% of all cerebrovascular accidents and 33% of all ischemic strokes. The ABCD\(^2\) 7-scale score predicts the stroke recurrence risk at 2-days, 7-days, and 90-days in patients who have a TIA. A patient who has a TIA that is at moderate to high-risk of a recurrent stroke is defined by an ABCD\(^2\) score \( \geq 4 \).

Unfortunately, both minor ischemic strokes and transient ischemic attacks place patients at a high-risk of a subsequent stroke with sources suggesting a recurrence risk ranging from 3-17% within the first 90 days of the primary neurovascular event. We propose a trial to reduce stroke recurrence in patients with a primary neurovascular event using vitamin E supplements. Currently, patients with a moderate to high-risk TIA or minor ischemic stroke with an identified thrombotic etiology are given antiplatelet therapy to
prevent a recurrent ischemic event. The regimen that is often prescribed is aspirin and clopidogrel (DAPT) but the regimen has its limitations.\textsuperscript{12,13}

The Platelet-Oriented Inhibition in New TIA and Minor Ischemic Stroke (POINT) study was an international, multi-center study that determined that DAPT was associated with a significantly lower risk of stroke recurrence (hemorrhagic and ischemic) compared to aspirin alone (HR, 0.74; 95\% CI, 0.58 to 0.94). Of note, there was a significant increase in the risk of major hemorrhage in the DAPT group compared to the aspirin alone group (0.9\% vs 0.4\%, HR, 2.32, 95\% CI, 1.10 to 4.87).\textsuperscript{12} In a similar China based trial, clopidogrel and aspirin were administered for 21 days followed by clopidogrel for the remainder of 90 days had a significant decrease in recurrent strokes (hemorrhagic and ischemic) compared to aspirin alone (8.2\% vs 11.7\%; HR, 0.68; 95\% CI, 0.57 to 0.81).\textsuperscript{13} DAPT has been shown to decrease stroke recurrence in TIA and minor ischemic stroke patients when used for less than a month. However, treatment surpasses 1 month, there is a significant increase in major bleeding events.\textsuperscript{19} This study aims to determine if there is a difference in the proportion of patients experiencing recurrent strokes (hemorrhagic or ischemic) in patients who receive vitamin E alpha-tocopherol supplements in addition to the standard treatment compared to patients who receive the standard treatment alone after the initial minor neurovascular event.

There are several mechanisms that vitamin E alpha-tocopherol takes part in that support its use in this trial. Several studies suggest that alpha-tocopherol inhibits protein kinase C stimulation (PKC) leading to an increase in nitric oxide (NO) and decrease in free radicals.\textsuperscript{20-24} NO then activates cyclic guanosine monophosphate (cGMP) causing inhibition of platelet aggregation.\textsuperscript{25,26} In addition, alpha-tocopherol inhibits
cyclooxygenase (COX) and as a result, when combined with aspirin, antiplatelet effects are potentiated.\textsuperscript{22,27,28} If alpha-tocopherol reduces platelet aggregation then it may play a role in reducing the incidence of secondary ischemic events, including ischemic stroke, post-minor neurovascular event.

Both animal studies and human studies support the aforementioned mechanism of vitamin E alpha-tocopherol. Animal studies have shown that both alpha-tocopherol alone and in combination with aspirin have led to less platelet aggregation and a longer time to thrombus formation compared to no alpha-tocopherol or aspirin alone, respectively.\textsuperscript{21,24,29} The studies have also suggested that vitamin E alpha-tocopherol protected cortical neurons from damage under ischemic conditions.\textsuperscript{24,28} Human in-vitro studies have further validated vitamin E alpha-tocopherol’s mechanism through a reduction in platelet aggregation both alone and in combination with aspirin.\textsuperscript{22,24,25,30,31} A human in-vivo study showed a reduction in primary ischemic strokes when used as an oral supplement alone for 6 years in male smokes compared to male smokers with placebo supplements.\textsuperscript{32} However, only one in-vivo study has investigated vitamin E alpha-tocopherol’s effects on stroke recurrence. A study from 1995 found that 400IU vitamin E alpha-tocopherol plus 325mg of aspirin for 2 years in patients with a TIA or minor ischemic stroke had a 2% recurrence risk compared to 12.5% in patient’s taking 325mg aspirin alone (\textit{P}<0.05). There was a 5.7% recurrence risk of total stroke (hemorrhagic or ischemic) in the vitamin E plus aspirin group compared to 12.5% in the group that took aspirin alone (\textit{P}<0.05). Unfortunately, the study has many limitations and the study has not been reproduced with the current DAPT.\textsuperscript{33}

Despite the preventative treatment for stroke recurrence, there remains to be a high incidence of disability among patients with a TIA or minor ischemic stroke. New treatment
regimens are needed to reduce the burden that this high-risk population faces. Although vitamin E alpha-tocopherol appears to have a promising mechanism, the literature is lacking studies that have investigated secondary prevention of stroke recurrence with short term use of alpha-tocopherol. Given the proposed mechanisms of alpha-tocopherol, this study aims to determine the association of 200IU vitamin E alpha-tocopherol oral supplements on stroke recurrence when given for 90 days in addition to the antiplatelet regimen in patients with a transient ischemic attack or minor ischemic stroke and compare it to patients taking clopidogrel and aspirin alone. By doing so, it may be possible to reduce the proportion of stroke recurrence (hemorrhagic or ischemic) in this high-risk population thus reducing mortality and morbidity.

1.2 Statement of the Problem

Strokes are a leading cause of morbidity and mortality in the United States population. Although great strides have been made in our understanding of strokes and their pathophysiologic consequences, they continue to be a daunting experience for physicians and patients alike. Specifically, there remains to be approximately an 8.2% stroke (ischemic and hemorrhagic) recurrence risk among patients with a primary transient ischemic attack or minor ischemic stroke that ultimately leads to disability, death hospital crowding, and high billing costs.\textsuperscript{13,34} Approximately one-half of patient’s that experience a recurrent stroke become disabled.\textsuperscript{34} By demonstrating the effectiveness of an alternative treatment regimen, this study may not only reduce the burden among healthcare providers and the healthcare system, but more importantly the quality of life of people who suffer from minor neurovascular events.
Literature suggests vitamin E alpha-tocopherols have antiplatelet, antioxidant, and aspirin potentiation properties. However, studies lack examining this mechanism with the current standard treatment in specifically this high-risk population. Therefore, we will perform a randomized, multi-center, placebo-controlled study to demonstrate the effectiveness of vitamin E alpha-tocopherol in the prevention of secondary ischemic strokes and compare it to the currently accepted standard of clopidogrel and aspirin.

1.3 Goals and Objectives

The randomized, multi-center, double-blinded, placebo-controlled trial aims to assess the efficacy of vitamin E alpha-tocopherol supplements, plus the standard treatment, in reducing the proportion of stroke recurrence in patients with a primary transient ischemic attack or minor ischemic stroke compared to the standard treatment alone.

1.4 Hypothesis

Patients with a transient ischemic attack (ABCD\(^2\) ≥ 4) or minor ischemic stroke (NIHSS ≤ 3) over the age of 18 years who are treated with 200IU vitamin E alpha-tocopherol in addition to the standard treatment of aspirin and clopidogrel will have a statistically significant difference in the proportion of stroke recurrence (hemorrhagic or ischemic) within 90 days of randomization compared to patients treated with aspirin and clopidogrel alone.
1.5 Definitions

**ABCD² Score**: A 7-scale score that predicts the risk of stroke recurrence of a person with a transient ischemic attack. Scoring considers age, blood pressure, clinical features, duration of symptoms, and diabetes.⁹ Scoring can be found in Appendix C.

**Alpha-Tocopherol**: Alpha-tocopherol is one of the eight components of the antioxidant, vitamin E, and the most predominant form with the chemical formula of C₂₉H₅₀O₂.³⁵

**Aspirin (Acetylsalicylic Acid/ASA)**: A synthetic, anti-inflammatory and antiplatelet agent that non-selectively and irreversibly inhibits COX-1 and COX-2 enzymes.

**Clopidogrel (Plavix)**: A synthetic, pharmaceutical, antiplatelet agent that irreversibly inhibits adenosine phosphate-induced platelet aggregation.

**Minor Ischemic Stroke**: A minor ischemic stroke is involving occlusion of the brain vessels resulting in a decrease in blood flow to the corresponding brain structures resulting in focal deficits. Specifically, a minor ischemic stroke is defined by a NIHSS score ≤ 3.

**NIH Stroke Scale (NIHSS)**: A 42-scale scoring system that is utilized to quantify stroke severity in patients, taking into consideration the patient’s level of consciousness, gaze ability, visual ability, facial paralysis, extremity motor drift, lower limb ataxia, sensation, aphasia, dysarthria, and extinction.⁴ Full scoring can be found in Appendix E.

**Transient Ischemic Attack (TIA)**: A brief, non-ischemic arterial occlusion in the brain that causes focal neurological deficits, lasting no longer than 24 hours. A patient with a TIA with moderate to high risk of stroke recurrence is defined by an ABCD² score ≥ 4.
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CHAPTER 2: REVIEW OF THE LITERATURE

2.1 Literature Search Strategy

A literature review was conducted between July 2020 and March 2021 using Ovid (Medline), Scopus, The Cochrane Library, and PubMed with the help of the Yale School of Medicine librarians. Studies were initially selected based on the title and abstract that were relevant to our proposed trial. International trials were included into the review based on the relevance of the study and its contribution to the progression of the literature. Initially, our research was confined to 2010 but was then extended to 1980 and beyond to include significant, pertinent trials that contributed to the development of this proposed trial.

Search strategy utilized the following MeSH headings and terms: TIA, transient ischemic attack, minor ischemic stroke, ischemic stroke, strokes, vitamin E, vitamin E alpha-tocopherol, alpha-tocopherol, tocopherols, recurrence, stroke recurrence, prevention, secondary prevention, treatment, therapy, clopidogrel, aspirin, DAPT, dual antiplatelet therapy, dual antiplatelet treatment, antiplatelet, platelet aggregation, thrombosis, antithrombosis, anticoagulation, nitric oxide, NO, vascular, neurovascular, antioxidant, oxidant, mechanism, mechanism of action and adults.

2.2 Review of Empirical Studies

2.2.1 Minor Neurovascular Events

We will define minor neurovascular events as a moderate to high-risk transient ischemic attack (TIA) and minor ischemic stroke. Specifically, a moderate to high-risk TIA will be classified as an ABCD² score of equal to or greater than 4. The ABCD² 7-scale
system determines a patient’s risk of recurrent stroke (Appendix C). In one study, patients with a high-risk TIA (ABCD² ≥ 6) had an 8.1% 2-day risk and a 36-43% 90-day risk of a recurrent stroke. Patients with a moderate risk TIA (ABCD² = 4 or 5) had a 4.1% 2-day risk and 19-24% 90-day recurrence risk. Patients with a low-risk TIA (ABCD² = 0 to 3) had a 1.0% risk and 0-7% 90-day recurrence risk.1,2 A study validated the scoring system by using it across different independent groups of patients.1 Given the validity of the scoring in the ability to predict increased risk of secondary stroke in patients with a moderate to high-risk TIA and evidence suggesting that a higher ABCD² score has a greater likelihood of being a true TIA (not a misdiagnosis), we will include patients with a moderate to high-risk TIA (ABCD² ≥ 4).

A minor ischemic stroke will be defined as a National Institute of Health Stroke Scale (NIHSS) score as less than or equal to 3. The NIHSS scoring is a 42-point scale accounts for the level of consciousness, gaze ability, visual ability, facial paralysis, extremity motor drift, lower limb ataxia, sensation, aphasia, dysarthria, and extinction (Appendix E).3 However, there is controversy on whether a NIHSS score of 4 or 5 should be classified as a minor stroke as well. The literature that has specifically looked at clopidogrel and aspirin included patients with a minor ischemic stroke with a NIHSS of less than or equal to 3 so we will use the same scoring to define our minor ischemic stroke population.4,5

Patients with a minor neurovascular event are at high risk of a subsequent stroke with sources demonstrating a recurrence risk ranging from 3-17% within the first 90 days of the index event.4-10 It is estimated that 50% of those who have a recurrent stroke will experience the event within weeks of the ischemic stroke with the risk decreasing and
remaining steady by 6 to 12 months after the primary event. The recurrent event often leads to disability and can lead to death. Recurrent stroke events may occur with worsening of the primary stroke through plaque formation and/or vasoconstriction causing ischemia or a new atheroembolic, cardioembolic, or hemorrhagic stroke after resolution of the primary stroke. Patients with a TIA or minor ischemic stroke with an identified thrombotic etiology are given antiplatelet therapy to prevent a recurrent ischemic event. This section will discuss the different regimens for preventing a secondary ischemic stroke.

2.2.2 Current Treatment of Minor Neurovascular Events

2.2.2.1 Aspirin + Clopidogrel

![Proposed Alpha-Tocopherol Mechanism](image)

**Figure 1:** Proposed Alpha-Tocopherol Mechanism Adapted from Gkaliagkousi et al. AA: arachidonic acid; AC: adenylyl cyclase; ATP: adenosine triphosphate; Ca²⁺: Calcium; cAMP: cyclic adenosine monophosphate; cGMP: cyclic guanosine monophosphate; COX-1: cyclooxygenase-1; DAG: 1,2-diacylglycerol; eNOS: endothelial nitric oxide synthase; IP₃: inositol 1,4,5-trisphosphate; NO: nitric oxide; O₂⁻: superoxide; ONOO⁻: peroxynitrite; P2Y12: adenosine diphosphate receptor; PKC: protein kinase c; PLA₂: phospholipase a₂; PLCβ: phospholipase cβ; ROS: reactive oxygen species; TXA₂: thromboxane 2. Solid arrows indicate activation, dashes indicate inhibition.

This section will review the accepted dual-antiplatelet therapy (DAPT) of clopidogrel and aspirin that is used to prevent recurrent strokes in patients with a moderate
to high-risk transient ischemic attack (TIA) or minor ischemic stroke. A multitude of randomized controlled trials have assessed the efficacy of DAPT versus antiplatelet monotherapy. DAPT has since become a widely accepted and recommended regimen to prevent stroke recurrence for this patient population.\textsuperscript{21} The two pharmaceutical drugs are thought to work synergistically to reduce platelet aggregation causing a reduction in stroke recurrence risk compared to antiplatelet monotherapy.\textsuperscript{22,23}

However, there is debate in the literature over the efficacy of DAPT compared to antiplatelet monotherapy. The MATCH trial was a double-blinded, multi-center, placebo-controlled study that randomized patients over 40 years old with a recent ischemic stroke or TIA and an additional vascular risk factor to receive an 18-month course of DAPT or clopidogrel alone. There was no significant difference between DAPT and clopidogrel alone (15.7\% vs 16.7\%, RR reduction 6.4\%; 95\% CI, -4.6 to 16.3). Notably, participants in the DAPT group had significantly more life-threatening bleeds than participants who received only clopidogrel (2.6\% vs 1.3\%; absolute risk increase 1.3\%, 95\% CI, 0.6 to 1.9).\textsuperscript{24} It is important to note that the antiplatelet therapy was given for a course of 18 months but time to event analysis was not performed to determine if the increased risk of bleeding could be due to prolonged antiplatelet use. The trial also had a broad inclusion criterion that allowed participants to join if they had an ischemic stroke or TIA in the past 3 months. While broad inclusion criteria tend to increase external validity and generalizability, studies have shown that the risk of recurrent stroke is highest within 90 days of the index event.\textsuperscript{25} Additionally, trials have shown that DAPT should be given within 12 to 24 hours of the event to have maximum benefit.\textsuperscript{4,5} Studies have also shown a benefit of DAPT in patients with a TIA with an ABCD\textsuperscript{2} score of greater than or equal to 4
or a minor ischemic stroke with a NIHSS score of less than or equal to 3.\textsuperscript{4,5,9} DAPT is not the recommended treatment for cardioembolic strokes, minor TIA’s, moderate and severe minor ischemic strokes.\textsuperscript{21}

The CHARISMA trial, a multi-center, double-blinded, placebo-controlled study, randomized 15,603 participants, over the age of 45 years with risk factors for atherothrombotic events, such as diabetes, smoking, coronary disease, and cerebrovascular disease, to receive DAPT of clopidogrel and aspirin or aspirin alone for a median of 28 months. There was no significant difference in severe bleeding (RR, 1.25; 95% CI, 0.97 to 1.61; P=0.09) or in intracranial hemorrhage (RR, 0.96; 95% CI, 0.56 to 1.65; P=0.89), or in the composite of myocardial infarctions, strokes, or mortality from cardiovascular causes (RR, 0.93; 95% CI, 0.83 to 1.05; P= 0.22) between the two groups. Notably, participants in the DAPT group had a significantly greater risk of developing moderate bleeding (RR, 1.62; 95% CI, 1.27 to 2.08; P<0.001) but a lower risk of having a non-fatal stroke (RR, 0.03; 95% CI, 0.64 to 0.98; P=0.03) or hospitalization from unstable angina, TIA, or revascularization (RR, 0.90; 95% CI, 0.82 to 0.98; P=0.02) compared to participants taking aspirin alone. Only 37\% of participants in each group had a history of a stroke or TIA. No subgroup analysis was performed for these participants.\textsuperscript{26} Neither the MATCH nor the CHARISMA trial fully captured our target population. For the purpose of this study, we will focus specifically on the literature that investigated DAPT in patients with minor ischemic strokes and TIA’s as it has shown to be more efficacious in reducing stroke recurrence compared to antiplatelet monotherapy.

The POINT study was a multi-center, randomized, double-blinded, placebo-controlled international trial that randomized 4,881 patients over 18 years old with a minor
ischemic stroke (NIHSS ≤ 3) or moderate to high-risk TIA (ABCD2 ≥ 4) within 12 hours of symptom onset to receive either a 90-day course of DAPT of clopidogrel and aspirin or aspirin alone with placebo tablets. The study found that patients who received DAPT had a significantly lower risk of having major ischemic events (ischemic stroke, myocardial infarction, or death from an ischemic vascular event) at 90 days compared to patients taking aspirin alone (5% vs 6.5%; HR, 0.75; 95% CI, 0.59 to 0.95; P=0.02). Most ischemic events occurred during the first week after the index event. Patients in the DAPT group had a significantly greater risk of developing a major hemorrhage compared to patients who received aspirin alone (0.9% vs 0.4%; HR, 2.32; 95% CI, 1.10 to 4.87; P=0.02). However, there was no significant difference in death from hemorrhagic etiology in either group (0.1% vs 0.1%). The study had a large sample size, randomized the participants, and blinded both participants and researchers. This increases the internal validity and thus strengthens the findings between DAPT and aspirin alone. The trial also increased the external validity of the trial by including multiple international centers, thus enhancing the generalizability of the trial.

The CHANCE trial, similarly, sought to determine the efficacy of short-term DAPT of clopidogrel and aspirin followed by clopidogrel alone to prevent secondary ischemic strokes in patients in China with a moderate to high-risk TIA or minor ischemic stroke. Participants that met the eligibility criteria within 24 hours of their index event were randomized to either receive a 21-day course of DAPT followed by clopidogrel alone from day 22 to day 90 or aspirin plus placebo tablets for the full 90 days. 8.2% of participants that received DAPT had a stroke (ischemic or hemorrhagic) by day 90 while 11.7% of participants in the aspirin alone group had a stroke (ischemic or hemorrhagic) by day 90.
More specifically, ischemic stroke occurred in 7.9% in the DAPT group vs 11.4% in the aspirin alone group (HR, 0.67; 95% CI, 0.56 to 0.81; p < 0.001). In addition, hemorrhagic stroke occurred in 0.3% of participants in each group (P=0.93) and moderate or severe hemorrhaging, as defined by the GUSTO criteria (Table 4) occurred in 0.3% of participants in each group P = 0.73). There was a trend in overall bleeding in the DAPT group, however this was not significant when compared to the aspirin alone group, unlike the finding in the POINT trial.\textsuperscript{4,5} It is important to note that although this trial was large, involving 5,170 patients from 114 centers, it was specific towards patients in China, decreasing external validity.\textsuperscript{5} However, similar trials investigating DAPT, including the POINT trial included the US, the CARESS trial done in Europe, and the CLAIR trial done in Asia, all support the regimen’s greater efficacy in reducing stroke recurrence, ischemic in particular, compared to aspirin alone.\textsuperscript{4,27,28} Given the large sample size in the CHANCE trial and equal risk of developing moderate to severe hemorrhage and hemorrhagic stroke between groups (0.3% vs 0.3%, p=0.73), it was accepted into the guidelines for high-risk TIA/minor ischemic stroke treatment guidelines by the American Heart Association.\textsuperscript{5,21}

A meta-analysis that included five randomized controlled trials, including the POINT, CHANCE, and CARESS trial recently described, found that short term DAPT of aspirin and clopidogrel for 1 month or less significantly reduced the risk of recurrent ischemic stroke (RR, 0.53; 95% CI, 0.27-0.78) and major cardiovascular events (RR, 0.72; 05%CI, 0.58-0.90) and did not increase the risk of major bleeding (RR, 1.82; 95% CI, 1.36-2.56) compared to aspirin alone. Similarly, DAPT given for 1 to 3 months (intermediate term) significantly reduced the risk of recurrent ischemic stroke (RR, 0.72; 95% CI, 0.58-
0.90) and cardiovascular events (RR, 0.76; 95% CI, 0.61-0.94). However, participants given DAPT for the intermediate term had an increased risk of major bleeding events compared to participants who received DAPT for the short term (RR, 2.58; 95% CI, 1.19-5.60). Participants who took DAPT for the long-term (over 3 months) had no significant reduction in the risk of recurrent ischemic stroke (RR, 0.81; 95% CI, 0.63-1.04) or in adverse cardiovascular events (RR, 0.87; 95% CI, 0.71-1.07) and showed a significantly increased risk of major bleeding compared to participants who received DAPT for the short term (RR, 1.82; 95% CI, 0.91-3.62).29 The majority of the participants that were included in this study had a minor ischemic stroke and TIA patients. A couple of studies included ischemic stroke patients were specific to lacunar strokes.30,31 The studies also varied on the dosing of both clopidogrel and aspirin, which could have altered the meta-analysis results.

When looking specifically at moderate to high-risk TIA and minor ischemic stroke patients, however, a multitude of studies have found a benefit to using DAPT, specifically clopidogrel and aspirin, to reduce the risk of developing a recurrent ischemic stroke.4,5,27,29,31 However, studies have varied in their length of onset to treatment, duration of the DAPT and dosing. This variability could explain the argument regarding dual antiplatelet therapy versus antiplatelet monotherapy when balancing ischemic stroke recurrence and hemorrhaging.

2.2.2.2 Other Treatments

Aspirin + Ticagrelor

Trials have been exploring new secondary prevention strategies to reduce stroke recurrence in patients with a moderate to high-risk transient ischemic attack (TIA) or minor ischemic stroke compared to other treatments. In an international double-blinded
controlled trial, patients with a moderate to high-risk TIA ($\text{ABCD}^2 \geq 4$) or mild to moderate ischemic stroke ($\text{NIHSS} \leq 5$) were randomized within 24 hours of the index event to receive a 90-day course of either ticagrelor, an antiplatelet agent, or aspirin. There was no significant difference in stroke occurrence, myocardial infarction, or death within 90 days in patients who took ticagrelor plus aspirin versus aspirin alone ($6.7\%$ vs $7.5\%$, HR, 0.89, 95% CI, 0.78 to 1.01, $P = 0.07$). Secondary analysis found no significant difference in ischemic stroke between the two groups ($5.8\%$ vs $6.7\%$, HR, 0.87, 95% CI, 0.76 to 1.00). A similar international trial, double-blinded trial, patients with a high-risk TIA ($\text{ABCD}^2 \geq 6$) or minor to moderate ischemic stroke ($\text{NIHSS} \leq 5$) were randomized within 24 hours of the index event to receive a 30-day regimen of either ticagrelor plus aspirin or aspirin plus placebo. There was a $5.5\%$ chance of stroke or death within 30 days and $6.6\%$ chance in the aspirin alone group (HR, 0.83; 95% CI, 0.71 to 0.96; $P = 0.02$). Specifically, ischemic stroke occurred in $5.0\%$ in the ticagrelor-aspirin group and $6.3\%$ in the aspirin group (HR, 0.79; 95% CI, 0.68 to 0.93; $P = 0.004$). Significantly more participants experienced severe bleeding in the ticagrelor plus aspirin group than the participants in the aspirin alone group ($0.5\%$ vs $0.1\%$, $P=0.001$). Although, there may be a decrease in stroke recurrence in the ticagrelor-aspirin group within 30 days of the primary event, the trial did not fully capture the high risk of recurrent strokes that exist within the full 90 days. Further investigation is needed in the efficacy of ticagrelor and its potential role in dual antiplatelet therapy for the full 90 days and should be compared to the current standard of clopidogrel and aspirin.

Aspirin + Dipyridamole

Another potential regimen to prevent stroke recurrence is aspirin plus dipyridamole, an antiplatelet agent. In one study, participants with an ischemic stroke were
randomized to receive either aspirin plus dipyridamole or clopidogrel alone. There was a lower risk of mortality and subsequent stroke or myocardial infarction events within 90 days in the aspirin plus dipyridamole group (HR, 0.62, 0.43 to 0.91; HR, 0.70, 0.52 to 0.94). However, a different study found that patients taking aspirin plus dipyridamole had similar rates of recurrent strokes, compared to patients taking clopidogrel alone (9.0% vs 8.8%, respectively, HR, 1.01, 95% CI, 0.92 to 1.11). The international, double-blinded, randomized trial involved 20,333 participants who had an ischemic stroke, defined by focal neurological symptoms lasting over 24 hours or evidence of an infarction on imaging, that were prescribed either 25mg aspirin plus 200mg extended-release dipyridamole twice daily or 75mg clopidogrel daily for 2.5 years. Neither study mentioned represented patients with a transient ischemic attack and did not specify the severity of the ischemic stroke patients, decreasing the external validity of the trial. Despite the regimen’s possible role in stroke recurrence, multiple studies have shown that almost half of patients on dipyridamole had a headache that resulted in a significant number of patients to discontinue the drug.

2.2.3 Vitamin E Alpha-Tocopherol as a Potential Treatment

Vitamin E is a collection of fat-soluble molecules known as tocopherols and tocotrienols. Alpha-tocopherol, in particular, is the most widely available and studied component of vitamin E. This section will discuss the different proposed mechanisms of vitamin E alpha-tocopherol including inhibition of platelet aggregation, potentiation of aspirin, and reduction in oxidative stress. The mechanisms suggest a possible role in reducing the incidence of stroke recurrence in patients with a high-risk transient ischemic attack (TIA) or minor ischemic stroke.
2.2.3.1 Antiplatelet Properties

Vitamin E alpha-tocopherol (AT) has been shown to inhibit protein kinase C (PKC), a platelet aggregate regulator, leading to an increase in nitric oxide (NO), decrease in superoxide, and decrease in lipid peroxidation.\textsuperscript{18,39–43} An increase in platelet NO leads to an increase in cyclic guanosine monophosphate (cGMP). cGMP activates cGMP-dependent protein kinase (PKG) ultimately leading to inhibition of platelet aggregation.\textsuperscript{44,45} The decrease in superoxide and lipid peroxidation contribute to AT’s antioxidant properties discussed later in this section.

Mice fed a high vitamin E diet for a mean of 15 days had significantly less platelet aggregation and a longer time to thrombus formation compared to mice fed a regular diet (P<0.05).\textsuperscript{46} Similarly, Li et al. found a significantly lower platelet count in mice that received 8-weeks of either low dose vitamin E supplements (P<0.01) or high dose vitamin E supplements (P<0.01) compared to mice who received placebo. There was a positive dose effect relationship in the vitamin E alpha-tocopherol dose and platelet count.\textsuperscript{47}

In a study, human platelets were incubated with different alpha-tocopherol and found a significant decrease in adenosine diphosphate (ADP)-induced platelet aggregation compared to the control (P<0.05). In addition, there was a significant decrease in lipid peroxidation products an increase in NO compared to the control.\textsuperscript{47} ADP is a platelet aggregation activator that is also inhibited by clopidogrel as seen in Figure 1.\textsuperscript{48} In another study, it was found that peripheral blood from healthy volunteers incubated with vitamin E had significantly less platelet aggregation when induced with Phorbol 12-myristate 13-acetate (PMA), a PKC activator, compared to control peripheral blood that was induced with PMA (P<0.05).\textsuperscript{49} Another study isolated platelet’s from human’s plasma and
significant reduction in platelet aggregation when infused with alpha-tocopherol compared to the vehicle of 5% ethanol (r = -0.78; P < 0.02). To determine the mechanism of the alpha-tocopherol, the study utilized PKC-dependent and independent platelet agonists. Alpha-tocopherol treated platelets inhibited PMA-induced phosphorylation of 47-kD proteins, substrates of the PKC. In the same study, patients were randomized to alpha-tocopherol supplements of a dose ranging from 400, 800, or 1200IU/d for 14 days. Blood samples were then obtained by the participants. PKC-dependent agonist phorbol 12-myristate 13-acetate (PMA) and alpha-tocopherol supplementation at all doses significantly has less platelet aggregation compared to pre-supplementation baseline (r = .67; P<0.01). While the findings are significant, it is notable that the study only included 15 participants, decreasing the internal validity of the trial.40

Similar findings have been seen in other trials. Participants who were supplemented with 400IU alpha-tocopherol daily for 14 days had a significant increase in NO production (P<0.05) and less ROS (P<0.05) compared to the control. The study also found that through immunoprecipitation, platelets incubated with alpha-tocopherol did not have PKC-dependent eNOS phosphorylation, suggesting that alpha-tocopherol increases NO release through a PKC dependent mechanism.18 This trial also had a very small sample size of 5 participants, decreasing the internal validity of the results. However, given the results of the aforementioned studies, it may be proposed that alpha-tocopherol inhibits platelet aggregation through the PKC pathway, as demonstrated in Figure 1.

The antiplatelet mechanism of alpha-tocopherol has been supported clinically as well. Notably in the ATBC study, healthy men who were given 50mg (approximately 75IU) of alpha-tocopherol had a significantly lower risk of cerebral infarctions and no increased risk
of subarachnoid hemorrhages compared to those taking placebo (P=0.035; P=0.07).\textsuperscript{50} Glynn et al. randomized participants over the age of 45 years old to receive 600IU of vitamin E or placebo for a median of 10.2 years. People in the vitamin E group had a significantly lower risk of developing a venous thromboembolism (VTE) (relative hazard, 0.73; 95% CI, 0.66 to 0.94; P=0.010). The risk is lowered in those who had a history of a VTE (relative hazard, 0.56; 95% CI, 0.31 to 1.00; P=0.048), no history of a VTE (relative hazard, 0.82; 95% CI, 0.68 to 0.99; P=0.048), and a history of a factor V Leiden or prothrombin mutation (relative hazard, 0.51; 95% CI, 0.30 to 0.87; P=0.014).\textsuperscript{51} These results further support the antiplatelet properties of vitamin E.

2.2.3.2 Aspirin Potentiation

In addition to the nitric oxide (NO) increase, research also suggests that alpha-tocopherol increases prostacyclin synthesis through inhibition of phospholipase A2 and cyclooxygenase (COX) by a similar mechanism as aspirin as seen in Figure 1. As a result, when vitamin E alpha-tocopherol is used in combination with aspirin, the antiplatelet effects are potentiated resulting in a significant decrease in platelet aggregation.\textsuperscript{13,14,17,52} Gonzalez-Corrales et al. found that rats treated with vitamin E alone had significantly less platelet aggregation than saline treated rats (P<0.001). There was significantly less platelet aggregation in the rats treated with aspirin and alpha-tocopherol compared to the control/nondiabetic rats, the saline treated diabetic rats, the aspirin treated diabetic rats, and the alpha-tocopherol treated diabetic rats (P<0.05; P<0.0001; P=0.015; P=0.002, respectively). The combination of aspirin and alpha-tocopherol had greater inhibition of thromboxane production compared to alpha-tocopherol alone (P=0.038). The combination
also potentiated prostacyclin production compared to alpha-tocopherol alone (P<0.0001) and aspirin alone (P<0.0001). Aspirin plus alpha-tocopherol also potentiated NO compared to alpha-tocopherol alone and aspirin alone (P=0.020, P=0.011, respectively). The study has limited external validity given a population of strictly type 1 diabetic rats. Of note, saline treated diabetic rats had significantly more platelet aggregation than the control nondiabetic rats (P<0.05). However, the aspirin and vitamin E alpha-tocopherol group did show significantly less platelet aggregation than the control non-diabetic rats (P<0.05). The study also supports the mechanism of alpha-tocopherol’s inhibition of thromboxane, increase in prostacyclin, increase in NO, and overall inhibition of platelet aggregation. These findings suggest vitamin E antiplatelet properties that synergistically work with aspirin and potentiate platelet aggregation inhibition.\(^\text{17}\)

Whole blood from healthy men was mixed with either aspirin alone or aspirin and alpha-tocopherol. NO was significantly higher in whole blood treated with both aspirin and alpha-tocopherol compared to aspirin alone resulting in less platelet aggregation (P<0.05). Vitamin E spared (at low doses) and significantly increased prostacyclin levels (at high doses) while aspirin significantly decreased prostacyclin synthesis (P<0.05) compared to the control. When alpha-tocopherol and aspirin were used together, there was significantly less prostacyclin inhibition compared to aspirin alone (IC\(_{50}\) 12.92 ± 1.10uM vs IC\(_{50}\) 1.81 ± 0.15uM; P<0.05).\(^\text{14}\) In another study, human platelets were incubated with vitamin E and both the low dose, 50uM, (P<0.001) and high dose, 100uM, (P<0.001) inhibited platelet adhesion compared to platelets treated with aspirin alone. Because platelet activation was inhibited using collagen, the component that is exposed during endothelial damage thus
leading to recruitment of platelets, vitamin E has again shown to potentiate aspirin’s inhibition of platelet aggregation.\textsuperscript{52}

In one double-blinded, placebo-controlled trial 100 patients, over 18 years old, with a minor stroke, reversible ischemic neurologic deficit, retinal ischemic event, or TIA within 8 weeks from enrollment were randomized to a 2-year course of either aspirin plus alpha-tocopherol or aspirin alone. Patients who received aspirin plus alpha-tocopherol had a significantly lower risk of recurrent stroke (hemorrhagic or ischemic) compared to patient’s who took aspirin alone (5.7\% vs 12.5\%, P<0.05). There was no significant difference in hemorrhagic strokes between the two groups. Unfortunately, the trial has many issues. First, the study was not completed at the time of publication. At the time only 33 patients had reached a termination point and 16 patients had completed the 2-year study. Therefore, the analysis on recurrent stroke was only performed on about half of the total sample size of 100 patients. No follow-up study has been found for the complete study. So, while the preliminary results suggest a significant difference in stroke recurrence and platelet adhesion, the trial’s limitations must be considered.\textsuperscript{53} Notably, studies have not investigated short-term administration of vitamin E alpha-tocopherol nor in combination with antiplatelet drugs. Given the gap in the literature, this study will investigate further on the efficacy of alpha-tocopherol plus antiplatelet drugs on stroke recurrence in patients with high-risk TIA or minor ischemic stroke. This gap and study’s limitations further emphasize the need for this trial.
2.2.3.3 Antioxidant Properties

Reactive oxygen species (ROS) are free radicals that damage cell membranes through lipid peroxidation. Lipid peroxidation is the process in which free radicals remove electrons from lipids that construct cell membranes. Removal of electrons cause disrupted, unstable cellular membranes that lead to cell death.\(^{54}\) Under standard conditions, ROS is produced through aerobic metabolism but is scavenged by endogenous antioxidants such as glutathione peroxidase and vitamin E thus preventing cell damage. Specifically, alpha-tocopherol is a fat-soluble antioxidant that donates a hydrogen to inactivate free radicals, reducing lipid peroxidation and thus maintaining cellular membrane integrity.\(^{15,20}\) During an ischemic stroke, there is excessive metabolic production of ROS that are not scavenged causing neuronal cell death and possibly disability.\(^ {55}\) Given alpha-tocopherol’s ability to bind to free radicals, supplements may have a potential role in reducing cell death and ultimately, reducing function deficit post-infarction.\(^ {17,56}\)

In an animal trial, transient focal cerebral ischemia for 90 minutes was induced in rats. Rats who were pre-treated with alpha-tocopherol had significantly less brain edema \((P < 0.001, 80.1 \pm 0.32\% \text{ vs } 83.8 \pm 0.11)\) and less disruption of the blood brain barrier (as seen by less Evans Blue leakage) \((P < 0.001, 6.66 \pm 0.87 \mu \text{g/g vs } 14.58 \pm 1.29)\) compared to the control group. In addition, the alpha-tocopherol group had a significantly reduced concentration of malondialdehyde, an oxidative stress marker and marker of lipid peroxidation, \((P < 0.001, 26.84 \pm 4.79 \text{ nmol/mg vs } 63.57 \pm 5.42 \text{ nmol/mg})\) and elevated concentration of glutathione, an endogenous antioxidant, \((P < 0.01, 10.17 \pm 0.83 \text{ mmol/mg vs } 5.86 \pm 0.31 \text{ mmol/mg})\) compared to the control group post-ischemia. The alpha-tocopherol group also had significantly diminished cerebral lesion volumes \((P < 0.01, 241\)
± 29 mm³ vs 422 ± 41 mm³) and less severe motor disabilities (as measured by the neurological deficit score) (P < 0.05, 1.75 ± 0.25 vs 4 ± 0.5) compared to the control group. These findings imply that pre-treatment with alpha-tocopherol may protect the brain from edema and disruption of the blood brain barrier through an antioxidant mechanism by reducing oxidative stress and increasing endogenous antioxidants leading to less cerebral death resulting in reduced disability severity compared to no pre-treatment.57

Numakawa et al. found that 24 hour pretreated cultured cortical neurons from rats either alpha-tocopherol (P<0.001), alpha-tocotrienol (P<0.001), gamma-tocopherol (P<0.01), and gamma-tocotrienol (P<0.001) had significantly less neuronal death than the vehicle-treated neurons after H2O2 administration.55 Notably, both animal studies mentioned have limited external validity as they are specific to rats. However, the study may improve our understanding of mechanism of vitamin E and its potential role in reducing oxidative damage from ischemic stress. Research has shown that pre-treatment with vitamin E may be protective against cell damage and death if the animal undergoes an ischemic event.55,57 Therefore, vitamin E may not only help reduce the incidence of stroke but if a stroke occurs, it may reduce ischemic damage thus reducing the degree of disability if a recurrent ischemic stroke were to occur.

Unfortunately, human trials investigating alpha-tocopherol’s antioxidant mechanism post-cerebral ischemia is limited. In one trial patients with an acute ischemic stroke were randomized to receive either 727 mg (800IU) alpha-tocopherol and 500mg of vitamin C or no treatment for 14 days. The study reproduced similar findings to the aforementioned animal studies, showing a reduction in the change in malondialdehyde concentration from day 0 to day 7 in patients who received vitamin E and C compared to
the control group (P<0.01, median -0.13μmol/l vs 0.03, respectively). Patients treated with vitamin E and C also had a significantly greater median change from day 0 to day 7 of total antioxidant capacity compared to the control group (P<0.05, median 0.09mmol/l vs 0.01, respectively). The study had a small sample size of 48 participants and was performed at a single hospital in England thus limiting the external validity of the trial’s findings. The trial also combined vitamin E and C supplements and did not compare the effects of the drugs separately reducing the internal validity. \(^5\) Despite the study design of the aforementioned study, other human trials investigating other disorders have demonstrated that vitamin E alpha-tocopherol supplements at doses as low as 100IU is associated with a significantly lower malondialdehyde concentration and increased glutathione concentration, further supporting the vitamin E alpha-tocopherol antioxidant mechanism in humans. \(^59-61\)

The literature discussed has shown that alpha-tocopherol scavenges free radicals, increases endogenous antioxidants, reduces brain edema, protects the blood brain barrier, and ultimately, reduces disability severity. To further investigate vitamin E alpha-tocopherols potential antioxidant effects, we will measure degree of disability using the modified Rankin Scale (mRS) at baseline and at day 90 and compare the mean change in mRS between groups. We will also compare mean mRS scores between groups in patients who had a recurrent stroke (ischemic or hemorrhagic) to investigate the relationship between disability in those were pre-treated with and without alpha-tocopherol.

2.3 Identifying Potential Confounding Variables

There are several potential confounding variables that may influence stroke recurrence that need to be accounted for in this trial. Previous randomized controlled trials have investigated potential confounding variables and their potential effect on stroke
recurrence. A 2017 study performed a subgroup analysis of the CHANCE trial to determine potential risk factors associated with recurrent stroke in patients with dual antiplatelet therapy (DAPT). The study found that patients with a history of hypertension (P<0.009), history of smoking (P<0.02), higher body mass index (P<0.02), less likely to have a lipid lowering agent (P<0.01), and a baseline NIHSS score of 2 (HR 2.12; 95% CI, 1.07 to 4.21) or 3 (HR 4.11; 2.05 to 8.22) compared to those who did not have a stroke of DAPT. We will also control for diabetes mellitus and co-existing cardiovascular diseases has they have been shown to place patients with a transient ischemic attack (TIA) or minor ischemic stroke at an increased risk for a recurrent stroke.\(^{10,63,64}\)

In addition, age and race have been associated with an increased risk of stroke recurrence. We will account for age given research has demonstrated that those over the age of 60 years who have had a minor neurovascular event are at an increased risk of developing a recurrent stroke compared to those younger than 60 years old.\(^1,2\) In addition, a study suggests that Black individuals at higher risk of early stroke recurrence compared to White individuals (HR, 1.6; 95% CI, 1.1-2.3).\(^65\)

Although there is no difference in recurrent stroke in patients with a TIA or minor stroke between men and women as demonstrated by multiple studies, women score worse on the modified-Rankin Scale (mRS) 1 to 6 months after a TIA or ischemic stroke, implying women may be more disabled after the event.\(^66\) Therefore, we will account for gender given its potential influence on disability, one of our secondary outcomes. In addition, time from initial presentation to randomization, and number of TIA and minor ischemic stroke patients. To control for potential confounders, we will first randomize the patients and then perform a secondary analysis utilizing multiple logistic regression.
2.4 Review of Relevant Methods

2.4.1 Study Design

The trial will be a prospective, multi-center, randomized, double-blinded, placebo-controlled trial performed to test the efficacy of vitamin E alpha-tocopherol in addition to clopidogrel and aspirin versus clopidogrel and aspirin alone in patients who have had a minor ischemic stroke or transient ischemic attack (TIA) who are at risk for a recurrent stroke. Similar to the trials that have investigated new treatment regimens for minor ischemic stroke and TIA patients, such as the CHANCE and POINT trial, this trial will mimic their study’s design.\(^4,5\) We will compare our intervention to the standard treatment to determine the efficacy of the new regimen.

Multiple stroke centers will be selected using the NIH Stroke Net to increase the generalizability and reduce selection bias.\(^6,7\) Participants will undergo the typical neurological work-up according to the stroke center’s protocol. Once the patient meets the eligibility criteria and is enrolled into the study, they must obtain a magnetic resonance imaging (MRI) or computed tomography (CT), if not done already, within 24 hours of the index event with no evidence of hemorrhage or nonischemic cause of a neurological deficit as done in previous trials.\(^4,5\) Similar to the POINT trial, we will use an interactive, computer-based system, WebDCU, that will randomize patients in a 1:1 ratio to receive either DAPT plus vitamin E alpha-tocopherol supplements or DAPT plus placebo.\(^4\) Given current guidelines and results from the CHANCE trial, both groups will receive a 21-day course of both clopidogrel and aspirin followed by clopidogrel alone for the remainder of the 90 days to reduce the incidence of serious bleeding.\(^5,21\) Patients will receive either vitamin E alpha-tocopherol or placebo tablets for the full 90 days. Both participants and
researchers will be blinded to the intervention with the use of the assignment of a randomized identification number and the use of placebo tablets that will be identical to the alpha-tocopherol supplements.

2.4.2 Control and Interventional Groups

Participants will be randomized to either the control group, which will be dual antiplatelet therapy (DAPT) of clopidogrel and aspirin plus placebo tablets, or the interventional group, which will be DAPT plus 200IU vitamin E alpha-tocopherol supplements. Several studies have shown that DAPT is superior to other treatment regimens in reducing stroke recurrence in patients with a history of a high-risk transient ischemic attack (TIA) or minor ischemic stroke.\textsuperscript{4,5} We will mimic the procedures of the CHANCE trial and administer loading doses of 300mg oral clopidogrel and open label 72mg to 300mg oral aspirin within 12 hours of the index event to both groups. Both groups will then receive 75mg aspirin per day from day 2 to day 21 and 75mg clopidogrel per day from day 2 to day 90.\textsuperscript{5} In addition, the control group will receive a 90-day course of placebo supplements that resemble and taste like the vitamin E alpha-tocopherol supplements.

The interventional group will receive 200IU of vitamin E alpha-tocopherol from day 1 to day 90 in addition to the DAPT. It has not escaped us that 400IU is the most widely studied dose in vitamin E alpha-tocopherol trials. Controversy remains with studies suggesting no benefit of low dose 200IU alpha-tocopherol and no benefit of alpha-tocopherol at higher doses platelets and oxidative stress.\textsuperscript{68,69} Studies have shown that vitamin E alpha-tocopherol increases nitric oxide and reduces platelet aggregation at doses
from 75IU/day to 200IU/day.\textsuperscript{70-73} Given the controversial research, risk of bleeding, and new antiplatelet regimen, we will utilize 200IU of alpha-tocopherol for 90 days. We hypothesize that this dose will be sufficient to potentiate aspirin in the first 30 days as well as provide antiplatelet and antioxidant characteristics in combination with clopidogrel to reduce the incidence of stroke recurrence within 90 days of the index event.

2.4.3 Inclusion and Exclusion Criteria

To determine the inclusion and exclusion criteria, we analyzed literature that studied the use of clopidogrel, aspirin, and alpha-tocopherol. By basing our inclusion and exclusion criteria on studies of all three drugs, we will encapsulate the full and adequate population that capture the population that may benefit from the intervention while minimizing adverse events. Specifically, we will be modeling our inclusion and exclusion criteria off of the CHANCE trial, POINT trial, and Leppalla et al study.\textsuperscript{4,5,50} Of note, we will exclude patients with a creatinine of greater than 2mg/dl or with hepatic insufficiency given hepatic metabolism and partial renal excretion of vitamin E, aspirin, and clopidogrel.\textsuperscript{74-76} The full inclusion and exclusion criteria can be found in Table 1.

2.3.4 Primary Outcome

The primary outcome that we will be measuring is stroke recurrence (ischemic and hemorrhagic) within 90 days of the index event. This outcome has been the main measure when trialing new therapies for a primary neurovascular event and will determine the effectiveness of the treatment regimen.\textsuperscript{5,27,30,77,78} Similar to the CHANCE trial, ischemic stroke will be defined as a sudden onset of new focal neurological deficit, evidence on imaging of an infarction for 24 hours or more, quick worsening of an existing focal
neurological symptom or sign, and not due to a nonischemic cause. Hemorrhagic stroke will be defined as blood in the parenchyma or subarachnoid space as confirmed on imaging with associated neurological signs or symptoms. Further analysis will be performed to analyze the incidence of ischemic stroke recurrence and hemorrhagic occurrence separately between the two groups. Though studies have measured this outcome as a time to recurrence, we will first measure it as a proportion. Secondary analysis will be performed to measure time to first recurrence using hazards ratio. This is further explained in section 3.10.

2.3.5 Secondary Outcomes

We will measure myocardial infarction (MI), all-cause mortality, degree of disability, and day 90 serum alpha-tocopherol levels as secondary outcomes. Previous studies have measured myocardial infarction as a primary or secondary outcome. A systemic review that analyzed 39 studies, including 65,996 patients, found that the annual risk for MI’s was 2.2%. In addition, a subgroup analysis of the CHANCE trial and found that 1.7% of the participants who were prescribed DAPT had a MI within 90 days of the initial event. The international POINT trial found only 0.4% of patients on DAPT had a MI within 90 days of the index event. Given the low percentage but its ischemic etiology and vitamin E’s potential antiplatelet role, MI will be measured as a secondary outcome. In addition, similar other studies, this trial will include 90-day all-cause mortality as a secondary outcome.

Other secondary outcomes include degree of disability (mRS) and serum alpha-tocopherol levels. Degree of disability at 90 days will be measured using the modified
Rankin, a 6-leveled scale that evaluates recovery from a stroke and the patient’s level of
disability, as seen in similar trials.\textsuperscript{5,81,82} A literature review analyzed 224 articles and found
evidence to suggest that the mRS is reliable with strong test-re-test reliability (K=0.81 to
0.95) and is valid when comparing the mRS score with the stroke type, lesion size,
perfusion and impairment.\textsuperscript{83} We will assess the relationship between the degree of
disability and the administration of vitamin E supplements in addition to DAPT. Multiple
studies have shown that DAPT improves functional outcome, as measured by degree of
disability, at 90 days when compared to aspirin.\textsuperscript{4,5,81} Lastly, we will measure a fasting
serum alpha-tocopherol level at the end of the 90 days and the mean serum alpha-
tocopherol level will be compared between groups. By doing so, we will determine if there
is a significant difference in serum alpha-tocopherol and if a significantly higher or lower
serum level can be associated with the primary or secondary outcomes as seen in other
studies.\textsuperscript{53,84}

2.4.6 Safety Outcome

A meta-analysis, including 8 primary and secondary prevention trials, states that
vitamin E increases the risk of hemorrhagic stroke (pooled relative risk 1.22; 95% CI, 1.00
to 1.48; P=0.045) and reduces ischemic stroke risk by 10% (pooled relative risk 0.90; 95%
CI, 0.82 to 0.99; P=0.02). The study suggests a significant relationship between
hemorrhagic stroke and vitamin E; however, the confidence interval of the study includes
1.00 and therefore, there may in fact be no relationship.\textsuperscript{85} A 2020 meta-analysis including
18 randomized controlled trials, investigating primary and secondary prevention of stroke
with the use of vitamin E supplements, demonstrated a significant risk reduction in
ischemic stroke (RR=0.92, 95% CI 0.85–0.99, p=0.04) but not in hemorrhagic stroke (RR=1.17, 95% CI 0.98–1.39, p=0.08). There was no significant difference in total stroke (RR=0.98, 95% CI 0.92–1.04, p=0.57). Stroke was not the primary outcome in multiple of the studies and the trial’s included also had varying populations with different comorbidities. Only half of the studies tested secondary prevention strategies for cardiovascular disease and many did not examine bleeding risk. As a precaution given the novel treatment and antiplatelet use, we will include hemorrhagic stroke in our primary outcome (in addition to ischemic stroke) and measure bleeding as a safety outcome. In addition, a data safety and review board will incrementally look at outcome data in order to identify if there is a statistical safety concern and determine if the trial should be halted.

There was an increased risk in major bleeding in patients who had a minor ischemic stroke or a high-risk transient ischemic attack (TIA) and received aspirin and clopidogrel (DAPT) for 90 days compared to antiplatelet monotherapy of aspirin (0.9% vs 0.4%). However, when a 21-day course of DAPT is prescribed followed by clopidogrel alone for the remainder of the 90 days, there was no significant increase in moderate or severe bleeding events compared to aspirin alone (0.3% vs 0.3%). As a result, the latter trial had a higher risk of secondary stroke of 8.2% compared to the first trial with a risk of 6.5%. We propose that patients take a 21-day course of clopidogrel and aspirin followed by clopidogrel from day 22 to day 90. In addition, 200IU vitamin E alpha-tocopherol supplements, a mild antiplatelet, for the entirety of the 90 days as described in Section 2.3.2. We will utilize the GUSTO criteria to define severe, moderate, and mild bleeding as used by the CHANCE trial and as described in Section 3.8.

2.4.7 Sample Size Calculation
Our sample size was calculated using randomized controlled trials addressing stroke recurrence (hemorrhagic and ischemic) within 90 days of randomization. In the CHANCE trial, the recurrence risk for secondary stroke after a high-risk transient ischemic attack (TIA) (ABCD2 ≥ 4) or minor ischemic stroke (NIHSS ≤ 3) was 8.2% associated with a 90-day course of clopidogrel and 21-day course of aspirin compared to 11.7% recurrence risk with aspirin alone (HR 0.68, 95% CI, 0.57 to 0.81, p<0.001). In the study’s interventional group, clopidogrel was given as a loading dose of 300mg on day 1 followed by 75mg daily from day 2 to day 90. Aspirin was given as a loading dose with an open label of 72mg to 300mg on day 1 followed by 75mg daily from day 2 to day 21. The limitation to this study is that the population was confined to China, limiting the generalizability of the study. Also, 6.4% of the sample size discontinued from the trial. However, the study was a large sample of over 5,000 participants and accounted for a 5% drop-out in its sample size. Because the study population and the interventional group in the CHANCE trial closely resembles this proposed trial’s control group, the 8.2% recurrent risk was used when calculating this study’s sample size.5

To estimate the rate difference we were limited, due to the lack of research, to a small study that observed the relationship between the administration of aspirin and alpha-tocopherol plus aspirin had a recurrent stroke (hemorrhagic or ischemic) versus aspirin alone. 5.7% of patients who were given a 2-year regimen of 400IU vitamin E alpha-tocopherol plus aspirin had a recurrent stroke (hemorrhagic or ischemic) versus 12.5% in patients taking aspirin alone (p<0.05). A major limitation to this study is its analysis of only 50% of its small sample size of 100 participants because the trial was still ongoing and has not followed up with another publication with the full data.
In addition, it prescribed 375mg of Aspirin for 2 years instead of the new standard of 90-day course of 75mg clopidogrel with 21-day course of 75mg of aspirin. Given the aforementioned study’s population and intervention, the addition of clopidogrel, and the POINT and CHANCE trial suggesting a 1.5-3.5% rate difference, the incidence of 5%, to create a 3.2% rate difference, was used when calculating the study’s sample size for this proposed trial.\textsuperscript{4,5,53} When calculating our sample size, we used a two-tailed alpha of 0.05 and a power of 80% to detect a rate difference of 3.2%. We also took into account an 8% withdrawal rate. Please see Chapter 3 for the calculation.

2.5 Conclusion

The literature previously described support the need for this randomized controlled trial. The proposed mechanism of vitamin E alpha-tocopherol and studies on its effects on platelet aggregation, aspirin potentiation, and antioxidant properties support vitamin E alpha-tocopherol’s potential role in preventing stroke recurrence in patients with a minor neurovascular event. However, the literature is limited in addressing this intervention in combination with clopidogrel and aspirin in this high-risk population. This study aims to fill this gap and improve clinical outcomes for patients with a moderate to high-risk TIA or minor ischemic stroke.
2.6 References

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CHAPTER 3: METHODS

3.1 Study Design

The study will involve multiple stroke centers including multiple emergency departments and neurology units that are responsible for discharging patients with a minor neurovascular event and giving prescriptions for outpatient medications. We will evaluate the effect of a 90-day regimen of 200IU vitamin E alpha-tocopherol supplements, in addition to the standard treatment of clopidogrel and aspirin, on stroke recurrence among patients who have had a moderate to high-risk TIA or a minor ischemic stroke. When a patient arrives to a study trial center and are determined to have a minor neurovascular event, they will be blindly randomized to the intervention or control group.

The primary null hypothesis of this randomized, double-blinded, multi-center, placebo-controlled trial is that patients with a moderate to high-risk TIA (ABCD² ≥ 4) or minor ischemic stroke (NIHSS ≤ 3) who receive a 90-day regimen of 200IU vitamin E alpha-tocopherol in addition to the treatment of clopidogrel and aspirin will not have a statistically significant difference in the proportion of stroke recurrence (hemorrhagic or ischemic) within 90 days from the primary neurovascular event.

3.2 Study Sites, Population, & Sampling

The study population will include patients over the age of 18 years with a primary moderate to high-risk transient ischemic attack (TIA) (ABCD2 ≥ 4) or minor ischemic stroke (NIHSS ≤ 3) who present within 12 hours to an emergency stroke center located within a tertiary medical center. The stroke center must be certified by the Joint Commission to contain the necessary training and resources for stroke patients. In
collaboration with the NIH Stroke Net, we will select stroke centers across the United States to participate in this interventional trial. We plan to include at least 10 stroke centers to recruit at least 2,002 participants over the course of a year. Each study site will have a physician investigator and research assistant who will remain in contact with the site’s emergency medicine physicians and neurologists who are primarily responsible for diagnosing and treating stroke patients to ensure enrollment of eligible patients. A clinical research coordinator (CRC) will also be put into place to oversee, direct and coordinate the clinical trial, ensuring that the protocols are met at each center. The CRC will also be responsible for ensuring resources are transported from the hub (Yale New Haven Hospital) to the satellite medical centers. The full research proposal will be submitted to the central Institutional Review Board (IRB) that is responsible for multi-center trials. Once the central IRB has accepted our proposal, we will begin recruiting eligible participants.

Participants must be at least 18 years old and have either a TIA with an ABCD² score of 4 or greater or a minor, nondisabling, ischemic stroke with a NIHSS score of 3 or less at the time of randomization. Patients who experience a TIA must have resolution of neurological symptoms at the time of randomization. A head computed tomography (CT) scan, CT angiography, magnetic resonance imaging (MRI), or MR angiography scan must be obtained and demonstrate that the neurological symptoms are NOT due to a hemorrhage or another brain pathology such as a tumor or vascular malformation as recommended by American Heart Association guidelines.¹ If the inclusion and exclusion criteria are met, all participants must sign and date a consent form (Appendix A) in order to be included and randomized to the trial. Please see Table 1 for the extensive exclusion criteria and a review of the inclusion criteria.
### Table 1. Inclusion and Exclusion Criteria

<table>
<thead>
<tr>
<th>Inclusion Criteria</th>
<th>Exclusion Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Age ≥ 18 years old</td>
<td>1. Age ≤ 18 years old</td>
</tr>
<tr>
<td>2. Acute, minor, nondisabling stroke with a NIHSS ≤ 3 at time of randomization</td>
<td>2. TIA symptoms limited to isolated numbness, visual changes, or dizziness/vertigo</td>
</tr>
<tr>
<td>3. TIA with resolution of symptoms by the time of randomization and a moderate-high risk of stroke recurrence as classified by an ABCD2 score ≥ 4</td>
<td>3. Candidate for thrombolysis, endarterectomy, or endovascular intervention as determined by providing physician (unless patient refuses the intervention by time of randomization)</td>
</tr>
<tr>
<td>4. Head CT or MRI showing no evidence of a hemorrhage or other brain pathology that could contraindicate treatment or explain neurological deficits (i.e., tumor, vascular malformation, abscess, multiple sclerosis)</td>
<td>4. Received intravenous or intra-arterial thrombolysis within 1 week of primary event</td>
</tr>
<tr>
<td>5. Must be randomized within 12 hours of symptom onset</td>
<td>5. Major surgery within 3 months prior to the primary event</td>
</tr>
<tr>
<td>6. Signed and dated informed consent</td>
<td>6. GI bleed within 3 months prior to primary event</td>
</tr>
<tr>
<td></td>
<td>7. Clear indication for anticoagulation therapy or anticipation to treat during study period</td>
</tr>
<tr>
<td></td>
<td>8. Ischemic event due to angiography or surgery</td>
</tr>
<tr>
<td></td>
<td>9. Non-cardiovascular comorbidity with life expectancy of ≤ 3 months</td>
</tr>
<tr>
<td></td>
<td>10. Score of more than 2 on the modified Rankin scale immediately before occurrence of the primary ischemic stroke or TIA</td>
</tr>
<tr>
<td></td>
<td>11. Contraindication to clopidogrel or aspirin (known allergy, serum Cr &gt;2 mg/dL, hepatic insufficiency, severe cardiac failure, hemostatic disorder, systemic bleeding, history of thrombocytopenia or neutropenia, current thrombocytopenia platelet count &lt;100x10^9, current neutropenia &lt;1x10^9/l, history of drug-induced hematologic or hepatic abnormalities)</td>
</tr>
<tr>
<td></td>
<td>12. Anticipated requirement for long-term non-study antiplatelets or NSAIDs affecting function of platelets</td>
</tr>
<tr>
<td></td>
<td>13. Currently receiving an investigational therapy or treatment within the last 7 days</td>
</tr>
<tr>
<td></td>
<td>14. Other neurological complications that complicate assessment of outcomes</td>
</tr>
<tr>
<td></td>
<td>15. Premenopausal or postmenopausal women at risk for pregnancy within 12 months of last menses without a negative pregnancy test or without reliable contraception</td>
</tr>
<tr>
<td></td>
<td>16. Unwilling or unable to discontinue concomitant medications</td>
</tr>
<tr>
<td></td>
<td>17. Unavailable for follow-up</td>
</tr>
<tr>
<td></td>
<td>18. Missing signed and dated informed consent</td>
</tr>
<tr>
<td></td>
<td>19. No use of individual supplements of vitamin A, E or beta carotene for more than once a week</td>
</tr>
</tbody>
</table>

### 3.3 Participant Confidentiality

This multi-center trial must be reviewed and approved by the Central Institutional Review Board (central IRB) prior to study initiation. In addition, all research members must be certified through the Health Insurance Portability and Accountability Act (HIPAA) before recruitment begins. For patient privacy, patient records and data will be secured through device encryption and password protected data. The information will only be accessible to members of the research team. In addition, we will de-identify all participants and utilize a computerized program that will randomize participants to identification.
numbers. After we complete data analyses, all participant data and information will be properly disposed.

After the participant has met the inclusion and exclusion criteria, then he or she will be required to complete written, informed consent (Appendix A). A research assistant at each clinical site will explain the consent form and ensure that each participant has signed and dated the form prior to randomization. The consent form will include a detailed description of the trial, potential risks and benefits, economic considerations, and confidentiality. The form will also state that participants have the ability to withdraw from the trial at any point. Once written, informed consent is obtained, the participant will be randomized into the trial.

3.4 Study Variables

The independent variable will be 200IU vitamin E alpha-tocopherol supplements that will be delivered in oral capsules. The control variable will be oral placebo tablets that will have the same packaging and be identical to the taste and appearance of the alpha-tocopherol supplements. The primary outcome will be stroke recurrence (hemorrhagic or ischemic), including fatal and non-fatal events, within 90 days of randomization. An ischemic stroke is defined as an acute, arterial block reducing or halting blood flow to the brain resulting in focal symptoms. Hemorrhagic stroke is defined as extravasation of blood into the parenchymal or subarachnoid space of the brain. The safety outcome will be moderate and major bleeding as defined by the GUSTO criteria described in Table 4.

The secondary dependent variables that will be measured are degree of disability, serum vitamin E alpha-tocopherol, proportion of myocardial infarction (MI), and
proportion of all-cause mortality. Degree of disability will be measured in all participants using the modified Rankin Scale (mRS) at the time of randomization and at the end of 90 days. Vitamin E alpha-tocopherol level is defined as the concentration of alpha-tocopherol within the patient’s serum. The reference range for alpha-tocopherol is defined as 5.5-17.0 mg/L in people 18 years or older. MI, with or without coronary revascularization, will be defined as myocardial necrosis with myocardial ischemia and we will use the Universal Definition of Myocardial Infarction algorithm that accounts for cardiac biomarkers, EKG abnormalities, imaging, pathology, and clinical setting. All-cause mortality will be defined as all participant deaths that occur within 90 days of randomization.

Table 2. Baseline Characteristics Description and Analysis

<table>
<thead>
<tr>
<th>Baseline Characteristics</th>
<th>Description</th>
<th>Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Measured in years</td>
<td>ANOVA</td>
</tr>
<tr>
<td>Female Sex</td>
<td>No./total No. (%)</td>
<td>Chi-Square</td>
</tr>
<tr>
<td>Body Mass Index</td>
<td>Median (Range)</td>
<td>ANOVA</td>
</tr>
<tr>
<td>Race</td>
<td>White, Black, Asian, Hispanic, Other (No./total No. (%))</td>
<td>Chi-Square</td>
</tr>
<tr>
<td>Ischemic Heart Disease</td>
<td>No./total No. (%)</td>
<td>Chi-Square</td>
</tr>
<tr>
<td>Hypertension</td>
<td>No./total No. (%)</td>
<td>Chi-Square</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>No./total No. (%)</td>
<td>Chi-Square</td>
</tr>
<tr>
<td>Smoking Pack Years</td>
<td>(# cigarettes per day) x (years smoked): Mean Pack Years ± SD</td>
<td>ANOVA</td>
</tr>
<tr>
<td>Clopidogrel Use at Presentation</td>
<td>No./total No. (%)</td>
<td>Chi-Square</td>
</tr>
<tr>
<td>Aspirin Use at Presentation</td>
<td>No./total No. (%)</td>
<td>Chi-Square</td>
</tr>
<tr>
<td>Time from Initial Presentation to Randomization</td>
<td>Mean time (hrs) ± SD</td>
<td>ANOVA</td>
</tr>
<tr>
<td>ABCD² Score</td>
<td>Median (Range)</td>
<td>ANOVA</td>
</tr>
<tr>
<td>NIHSS Score</td>
<td>Median (Range)</td>
<td>ANOVA</td>
</tr>
<tr>
<td>TIA Subjects</td>
<td>No./total No. (%)</td>
<td>Chi-Square</td>
</tr>
<tr>
<td>Minor Ischemic Stroke Patients</td>
<td>No./total No. (%)</td>
<td>Chi-Square</td>
</tr>
</tbody>
</table>

Stroke recurrence within 90 days of the neurovascular event can be attributed to many baseline characteristics and have the potential to impact the study groups. We will control for many factors including age, gender, body mass index, race, known risk factors (ischemic heart disease, hypertension, diabetes mellitus, smoking history), medication use at presentation (aspirin, clopidogrel, multivitamin use), time from initial presentation to randomization, neurovascular event (TIA vs minor ischemic stroke), and median qualifying neurologic score (ABCD², NIHSS). A baseline evaluation will occur at the time
of randomization after the patient meets the inclusion criteria. Statistically significant
differences in baseline characteristics found between study groups will be controlled for
using the multiple logistic analysis approach. Please refer to Table 2 for description and
analysis of the baseline characteristics.

3.5 Methodology

3.5.1 Group Randomization & Blinding

Participants who enter the stroke centers who meet the inclusion and exclusion
criteria will be randomized in a 1:1 ratio to either the interventional (vitamin E alpha-
tocopherol plus standard treatment) or control (standard treatment alone) group. This
randomization will be performed using a computerized program. Trained users will use the
computerized program to complete an eligibility checklist. If the program finds that the
patient is eligible then the patient will be enrolled and assigned to a randomization number.
Participants, research assistants, and physician investigator at every study site will be
blinded to the group assignment. All participants in both groups will be given the same
instructions on the use, benefits, and risks of the medications. Research assistants and
physician investigators will also be blinded to the primary, secondary, and safety outcome.
The clinical coordinator center located at the hub of the trial will be responsible for creating
and distributing study packets with either the placebo or the vitamin E tablet. The pharmacy
at each study site will assist in this process by ensuring that the placebo pills are identical
to the vitamin E alpha-tocopherol pills in appearance and taste. This method will allow for
standardization of delivery as well as ensuring that the participants, research assistants and
physician investigator are blinded to the intervention the participant receives at each study
center. If any complications arise and the participant or provider needs to be un-blinded, the clinical research coordinator may be contacted to identify what the participant received.

3.5.2 Intervention Design

Participants in the interventional group will receive clopidogrel and aspirin plus vitamin E alpha-tocopherol. The initial loading dose on day 1 will include 3 oral tablets: 300mg clopidogrel, open label 72mg-300mg aspirin, and 200IU alpha-tocopherol. The aspirin loading dose will be determined by the primary physician. All three medications must be given within 12 hours of the primary event. The participants will receive a 20-day supply of 75mg clopidogrel, 20-day supply of 200IU alpha-tocopherol, and 20-day supply of 75mg aspirin. Patient’s will take one oral tablet of clopidogrel, alpha-tocopherol, and aspirin by mouth once daily in the evening after eating a meal from day 2 to day 21. From day 21 to day 90 study participants will discontinue the aspirin and continue protocol for alpha-tocopherol and clopidogrel. Patient’s will receive a refill for clopidogrel and alpha-tocopherol at the follow-up appointments to encourage adherence.

3.5.3 Control Design

Participants in the control group will receive the standard treatment of clopidogrel and aspirin plus placebo. The initial loading dose on day 1 will include 3 oral tablets: 300mg clopidogrel, open label 72mg-300mg aspirin, and a placebo tablet identical to that of alpha-tocopherol. The aspirin loading dose will be determined by the primary physician. All three medications must be given within 12 hours of the primary event. The participants will receive a 20-day supply of 75mg clopidogrel, 20-day supply of placebo, and 20-day supply of 75mg aspirin. Patient’s will take one oral tablet of clopidogrel, placebo, and
aspirin by mouth once daily in the evening after eating a meal from day 2 to day 21. From
day 21 to day 90 study participants will discontinue the aspirin and continue the same
protocol for the placebo and clopidogrel. Patient’s will receive a refill for clopidogrel and
placebo at the follow-up appointments to encourage adherence.

3.5.4 Other Considerations

If the patient has yet to take the loading dose of 300mg clopidogrel and open label
72mg-300mg aspirin, then they will take it in conjunction with the alpha-tocopherol or
placebo supplement. If the patient has already received the clopidogrel, aspirin, or both, at
the time of randomization then they will take the alpha-tocopherol or placebo tablet
alongside the missing medication or alone. Patients should receive the oral vitamin E
supplements within 12 hours of the primary event. Participants who are admitted to the
hospital will be provided the daily oral vitamin E alpha-tocopherol supplements alongside
the clopidogrel or aspirin by their providing physician. When participants are discharged,
they will be instructed on the use of vitamin E alpha-tocopherol supplements. Instructions
will remain consistent among both the interventional and control groups.

3.6 Data Collection

The baseline characteristics described in Table 2 will be recorded at the time of
randomization by blinded researchers using the form found in Appendix B. Time will be
allocated to training research assistants on how to survey, utilize stroke scales, document
patient data, interpret laboratory values, and interpret radiologist neuroimaging results.

Stroke recurrence (hemorrhagic or ischemic) is the primary outcome and will be
measured utilizing a stroke free questionnaire (Appendix F) imaging. The patient will
undergo either a head computerized tomography (CT) scan or a magnetic resonance image (MRI) scan that will be read by a neuroradiologist who will determine if there are signs of an ischemic or hemorrhagic stroke. Imaging will be performed when symptoms arise within 90 days of randomization or at the end of the trial at day 90. Patients will have imaging done at the time of randomization to determine patient’s current stroke status and a baseline image to compare future CT/MRI results.

The secondary outcome of change in disability will be measured at time of randomization and at the end of the 90 days. Disability will be measured using the six leveled modified Rankin Scale (mRS).³ Research assistants will be trained and given Appendix D to determine the disability of each participant in both arms. Once the study is complete, the mean change of disability within each group will be determined. The mean change of disability between groups will then be analyzed.

In order to determine serum vitamin E alpha-tocopherol levels, we will collect whole blood via peripheral blood draws at the end of the 3 months of each participant. For accurate results, all participants must be fasting for 12-hours prior to the blood draw. Serum alpha-tocopherol levels will be determined by using high-performance liquid chromatography. The blood specimen will be collected in a red top tube and will be placed in an amber vial to protect from light. If the specimen is unable to be analyzed within 7-days then the specimen must be refrigerated or frozen and analyzed within 44 days. If the blood specimen is grossly hemolyzed or has gross lipemia then it will be rejected. The mean day 90 serum alpha-tocopherol levels will be compared between groups.

Myocardial infarction (MI), including fatal and non-fatal events, will be determined by the providing physician who will use the Universal Definition of Myocardial Infarction
algorithm that takes into account cardiac biomarkers, EKG abnormalities, imaging, pathology, and clinical setting. When a patient presents with signs or symptoms of a MI or there is a clinical suspicion by the provider, patients will undergo the standard testing.

Blinded research assistants will follow up with study participants at day 7 from randomization to document verbal compliance and address any issues or concerns with medication use or effects. The study participants will then have an appointment with a blinded clinician at day 21 to ensure withdrawal of aspirin, continuation of vitamin E alpha-tocopherol and clopidogrel. At day 90 the study participants will have an in-person appointment where trained blinded research assistants will measure the modified Rankin Scale (mRS) score and the National Institute of Health Stroke Scale score as described in Appendix D and E, respectively. At each visit, the participants will be questioned on stroke recurrence since randomization and will be assessed for any stroke symptoms or signs using the Questionnaire for Verifying Stroke-Free Status (QVSFS) detailed in Appendix F. We will also ask about myocardial infarction events within the past 90 days. We will also use patient medical and death records to support the measurement of our outcomes, including all-cause mortality.

3.7 Adherence

Participants will be closely monitored throughout the trial. At day 7, 21 and 60, the participants will have a follow up appointment with the clinicians. At the time of follow-up, they will receive a refill of the clopidogrel, aspirin, and alpha-tocopherol (or placebo) supplements. At the appointment, participants will be asked if they have been adherent to the medication using the Morisky scale. Patients will be considered adherent if they report
at least moderate compliance. The inability to adhere to the medication will disqualify the patient from participating in the trial.

**Table 3: Proposed Trial Design Influenced by CHANCE and POINT trial**

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Baseline/Screening/Randomization Day 1</th>
<th>Phone f/u Day 7</th>
<th>Phone f/u Day 21</th>
<th>Phone f/u Day 60</th>
<th>In-person f/u Day 90</th>
<th>Event Visit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eligibility</td>
<td>X</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Signed Consent Form</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Randomization</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Baseline Characteristics/Medical History</td>
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<td></td>
<td></td>
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<tr>
<td>ABCD² Score (for TIA)</td>
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<td></td>
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<tr>
<td>Modified Rankin Scale (mRS)</td>
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<td>X</td>
<td></td>
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<td>NIHSS Score</td>
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<td>X</td>
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<td>Vital Signs</td>
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<td>Basic Labs (CBC, CMP)</td>
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<td></td>
<td>X</td>
<td>X</td>
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<tr>
<td>Serum Alpha-Tocopherol Level</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
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</tr>
<tr>
<td>Head CT/MRI</td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>EKG</td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
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<tr>
<td>Current Medications</td>
<td>X</td>
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<td>X</td>
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<tr>
<td>Stroke Free Questionnaire</td>
<td></td>
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<td>X</td>
<td>X</td>
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<tr>
<td>Medication Adherence Questionnaire</td>
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<td>Medication Refill</td>
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<td></td>
<td></td>
<td>X</td>
</tr>
</tbody>
</table>

3.8 Monitoring for Adverse Events

An adverse event is defined as an unexpected medical occurrence that happens during pharmaceutical treatment. We will submit a data and safety monitoring plan that will go into detail about collecting adverse events that will be performed by the clinical research coordinator (CRC). A data monitoring center will be utilized to monitor all such events throughout the trial. Participants will be closely monitored for any adverse events throughout the entirety of the trial. All events will be documented, reported and included in a final clinical study report. As a safety outcome, severe or moderate bleeding will be
monitored for. If an adverse event occurs, the CRC may be contacted to unblind the participant, research investigator, and primary physician to the intervention. Serious adverse events will be reported to the IRB immediately. If bleeding occurs, then patients will be instructed to stop the alpha-tocopherol tablets. In cases of serious active bleeding and prolonged prothrombin time, vitamin K supplementation may be considered by the primary physician.7

Table 4: GUSTO Criteria8

<table>
<thead>
<tr>
<th>Severe Bleeding</th>
<th>Intracranial hemorrhage or bleeding causing hemodynamic compromise requiring blood or fluids, inotropic support to maintain blood pressure, surgical intervention (other than bleeding site repair), ventricular assist devices or CPR to maintain cardiac output.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moderate Bleeding</td>
<td>Bleeding requiring blood transfusions but does not cause hemodynamic compromise.</td>
</tr>
<tr>
<td>Mild Bleeding</td>
<td>Bleeding that does not require transfusions nor cause hemodynamic compromise.</td>
</tr>
</tbody>
</table>

3.9 Sample Size Calculation

Our study aims to determine the difference in stroke recurrence among patients who have a moderate to high-risk transient ischemic attack or minor ischemic stroke who receive 200IU vitamin E alpha-tocopherol in addition to the standard treatment versus those who receive the standard treatment alone. Taking into consideration the literature discussed in the sample size literature review and using the Power and Precision software (Appendix G), we determined that this trial will require at least 2,002 participants, with 1,001 participants per arm, in order to provide 80% power to detect a rate difference of 3.2% in the vitamin E alpha-tocopherol group, with a two-sided type I error of 0.05 and an 6% withdrawal rate, assuming an 8.2% event rate in the group of aspirin and clopidogrel alone.
3.10 Analysis

The statistical data will be analyzed using the intention-to-treat protocol by blinded researchers. The statistical significance will be defined by a p-value less than 0.05. Our dichotomous, primary outcome of stroke recurrence within 90 days will be measured as a proportion in the control and intervention group. Therefore, the primary outcome will be compared between groups using a chi-squared test. Because baseline characteristics and other confounding variables may be associated with the dependent variable, multiple logistic regression will be performed after the initial data analysis. To determine baseline characteristic variation between the study arms, we will compare each variable using the appropriate statistical analysis as suggested in Table 2.

Secondary outcomes include mean change in disability, mean serum vitamin E alpha-tocopherol, proportion of myocardial infarction (MI), and proportion of all-cause mortality. Mean change in disability, as measured by the mRS scale, from baseline to day 90 will be compared between groups using student’s t-test. Mean serum vitamin E alpha-tocopherol at day 90 will be compared between groups using student’s t-test. Proportion of MI and all-cause mortality will both be compared between groups using chi-square analysis.

We will analyze our data as a time to stroke recurrence using the Kaplan-Meier survival analysis as a secondary analysis to investigate risk of stroke recurrence at different time points. We will then use Cox proportional hazards regression to account for confounding variables. We are confident in utilizing this analysis as a secondary approach after calculating that only a few hundred participants are needed for this survival analysis. This participant count is less than our proposed 2,002 participants.
3.11 Timeline

The estimated total duration of the recruitment and data collection will be **32 months**. The pre-enrollment phase will take approximately **6 months** and will include attaining central IRB approval, preparing and distributing the vitamin E alpha-tocopherol to all study sites, and training research assistants at each study site how to survey, utilize stroke scales, document patient data, interpret laboratory values, and interpret neuroimaging results. We will allocate **20 months** towards continuous enrollment and follow-up. After halting recruitment, an additional **3 months** will be given towards follow-up completion. Final data analysis will take approximately **6 months**.

**Table 5. Proposed Trial Timeline**

<table>
<thead>
<tr>
<th>Pre-enrollment Phase (IRB approval, Training)</th>
<th>Recruitment and Follow-up</th>
<th>Completion of Follow-Up</th>
<th>Data Analysis &amp; Publication</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 MONTHS</td>
<td>20 MONTHS</td>
<td>3 MONTHS</td>
<td>6 MONTHS</td>
</tr>
</tbody>
</table>

**Total Duration: 32 months**
3.11 References


CHAPTER 4: CONCLUSION

4.1 Strengths

We anticipate this trial to possess many strengths. First, the trial is novel in investigating alpha-tocopherol’s proposed mechanism, in addition to dual antiplatelet therapy (DAPT) of clopidogrel and aspirin, and the regimen’s potential efficacy in reducing the proportion of stroke recurrence in patients with a transient ischemic attack (TIA) or minor ischemic stroke compared to DAPT alone. The prospective study design allows for the collection of specific data in real time unlike retrospective trials that are more prone to biases and confounders. To increase the internal validity of the trial, we will implement a strict randomization process, blind the participants and researchers, and have a placebo group. Randomizing patients to either the control or interventional group utilizing a computer-based program will aid in reducing selection bias and producing comparable groups with respect to baseline characteristics. In addition, this trial will be double-blinded, with the use of a randomized identification number and identical placebo supplement, to reduce observer and confirmation bias. By increasing the validity of this trial, we will be able to establish a relationship between vitamin E alpha-tocopherol and stroke recurrence. We will also increase the external validity by having a multi-center trial design. Utilizing the NIH Stroke Net, we will randomly select multiple stroke centers throughout the United States to increase the generalizability of the trial.

4.2 Limitations

We anticipate our randomized, multi-center, double-blinded trial to have many strengths, but also have a few limitations. First, we will be including only stroke centers
within tertiary medical centers. While these hospitals treat the majority of stroke patients, the study may not be generalizable to patients in more rural settings who lack the access to tertiary medical centers and are treated primarily at rural hospitals. However, because the majority of patients are treated at stroke centers that have the appropriate training and resources, we feel that the inclusion of only these certified centers is the appropriate step in determining a new treatment regimen to reduce the risk of stroke recurrence in patients who experience a minor neurovascular event.

Another potential issue is the trial’s inability to enroll around the clock leading to a possible convenient sample causing a potential selection bias and limiting the generalizability of the study. Selection bias may also occur through the loss to follow up. The trial is also at risk of information bias; however, we will attempt to reduce this with the use of standard questionnaires, measurements and imaging results. We also will have an extensive exclusion criterion that will exclude patients with a cardioembolic or hemorrhagic stroke, mild TIA, moderate or severe ischemic stroke, hepatic insufficiency, renal disease, and cardiac failure. Therefore, the sample size may not fully encompass all stroke patients who are risk for a recurrent stroke. In addition, patients may have varying vitamin E diet’s that may contribute to stroke recurrence and alter the levels of their serum alpha-tocopherol levels. However, to accurately measure serum alpha-tocopherol levels, the participant must be fasting for 12 hours prior to the blood draw. However, after our primary analysis, we will perform secondary analysis of both multiple logistic regression and cox-proportional hazards regression to control for confounding variables.

Given the trial’s novelty in delivering vitamin E alpha-tocopherol supplements in addition to DAPT to stroke patients, we will administer a low dose of 200IU of alpha-
tocopherol to reduce the risk of adverse effects, such as major bleeding, that may be seen with higher dosing of 400IU, despite its positive results in the literature. If the addition of 200IU alpha-tocopherol does not result in an increased risk of moderate or major bleeding then future directions could include an adaptive study design that could examine the effects of different doses of vitamin E alpha-tocopherol with the use of a larger sample size.

4.3 Clinical Significance

Reducing the incidence of recurrent strokes would lessen the burden that exists for healthcare professionals, including physician assistants, and reduce resource usage in emergency departments and neurology units. According to the American Heart Association statistics, stroke has a large healthcare burden with 800,000 strokes yearly, costing approximately $34 billion a year.¹ About 25% of these yearly strokes are recurrent, suggesting an estimated cost of over $8.5 billion.¹ Not only does stroke recurrence take a toll on the healthcare system, but, more importantly, on the individual. According to a study, a patient’s healthcare bill totals to $34,639 following discharge of a recurrent, non-cardioembolic ischemic stroke. This is significantly higher than the $25,036 that is spent for a new stroke occurrence.² If we reduce the incidence of recurrent ischemic strokes in this vulnerable population then their quality of life can be improved with a decrease in mortality, morbidity and healthcare expenses.
4.4 References

APPENDICES

Appendix A: Consent Form

Adapted from Consent for Participation in a Research Project, 200 FR.1 (2016-2)
YALE UNIVERSITY SCHOOL OF MEDICINE

Study Title: VITAMIN E ALPHA-TOCOPHEROLS TO PREVENT RECURRENT STROKE IN PATIENTS WITH A MINOR NEUROVASCULAR EVENT

Principal Investigator: Charles Wira, MD

Invitation to Participate and Description of Project

You are invited to participate in a research study that is designed to look at the treatment options for patients who have a transient ischemic attack (TIA) or minor ischemic stroke. This study will specifically look at the addition of vitamin E supplements to the current treatment regimen. You have been asked to participate in this study because you have recently been diagnosed with a moderate to high-risk TIA or a minor stroke and are at high risk for stroke recurrence.

In order to decide whether or not you wish to be a part of this research study, you should know enough about the study’s risks and benefits to make an informed decision. This consent form gives you detailed information about the research study, which a member of the research team will discuss with you. This discussion should go over all aspects of this research: the purpose, the procedures that will be performed, any risks of the procedures, possible benefits and possible alternative treatments. Once you understand the study, you will be asked if you wish to participate; if so, you will be asked to sign this form.

Description of Procedures

If you agree to participate in this study, you will be asked various questions regarding your baseline characteristics including your age, gender, race, and past medical history. If you have not had one already as part of your workup, you will undergo a computed tomography (CT) scan or magnetic resonance imaging (MRI) prior to initiation to determine any ischemic damage or presence of bleeding. A research assistant will then utilize a computer system that will assign you to receive either vitamin E alpha-tocopherol supplements or placebo pills (a pill causing no therapeutic benefit or harm) for a total of 90 days. In addition, you will be assigned a unique identification number. Randomization through this computer-based program ensures an equal chance for all participants to be assigned to either group. The supplements will be taken as an adjunct to your dual antiplatelet treatment of clopidogrel and aspirin.
Once you are determined to have a minor ischemic stroke or a transient ischemic attack, you will receive a loading dose of 300mg clopidogrel, 72mg-300mg aspirin (as determined by the provider), and either 200IU vitamin E alpha-tocopherol or a placebo pill. You will then be prescribed a 20-day course of 75mg clopidogrel and 75mg aspirin taken once daily from day 2 to day 21. From day 22 to day 90, you will continue to take clopidogrel once daily. From day 2 to day 90, you will be prescribed either 200IU vitamin E alpha-tocopherol or placebo supplements to be taken once daily. All medications should be taken in the evening, together, with a meal. The treatments will be given as a monthly supply and will be administered at the time of randomization. Your medication will be refilled at your follow-up appointments.

- **DAY 1** you will receive:
  - 300mg clopidogrel
  - 72mg-300mg aspirin (dose determined by provider)
  - 200IU vitamin E alpha-tocopherol OR placebo

- **DAY 2 to DAY 21**
  - 75mg clopidogrel
  - 75mg aspirin
  - 200IU vitamin E alpha-tocopherol OR placebo

- **DAY 22 to DAY 90**
  - 75mg clopidogrel
  - 200IU vitamin E alpha-tocopherol OR placebo

You will be contacted via telephone for follow-up on days 7, 21, and 60 from time of randomization. The follow-up appointments will include a questionnaire to assess for signs and symptoms of a stroke and receive your medication refill. The study will conclude on day 90 after your in-person appointment. At this appointment, you will undergo an MRI and a questionnaire to assess for stroke recurrence. After the study is complete, we recommend follow-up with your neurologist to determine next steps in your treatment plan.

A description of this study will be available on [http://www.ClinicalTrials.gov](http://www.ClinicalTrials.gov), as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of this results. You can search this Web site at any time.

You will be told of any significant findings that are developed during the course of your participation in this study that may affect your willingness to continue to participate. Research results will not be returned to your doctor. If research results are published, your name and other personal information will not be given.

**Risks & Inconveniences**

Participation in this trial may place you at an increased risk of bleeding. Antiplatelet drugs, including clopidogrel, aspirin, and vitamin E, are blood thinners that prevent blood cells from clumping together to form a clot. Therefore, the addition of vitamin E to the standard regimen may increase your risk of bleeding. Patient’s more commonly experience minor
bleeding such as bruising easily or prolonged bleeding from minor cuts. However, though rare, bleeding may occur in the brain, gastrointestinal tract, nosebleeds, eyes, etc. Bleeding may require hospitalization, blood transfusions and cause fatality. If signs and/or symptoms arise, you should go to the nearest hospital to be assessed and undergo diagnostic measures. If bleeding occurs, this will be considered an adverse event and you may be withdrawn from the study.

You may also experience other side effects related to the three medications. These side effects include weakness, dizziness, nausea, abdominal pain, diarrhea, constipation and other possible side effects listed on the label of each medication.

You will also receive exposure to radiation through computed tomography (CT) and/or magnetic resonance imaging (MRI) as standard diagnostic measures for a stroke or transient ischemic attack. A CT scan or MRI is required prior to randomization. Imaging will aid in your diagnosis and in ruling out other possible causes of your symptoms and/or signs such as brain bleeding, tumors, etc. At the end of the study, you will undergo an MRI to aid in the diagnosis of a recurrent stroke.

Benefits

The results of this study may help the medical community in determining the most effective treatment regimen for secondary prevention of recurrent strokes. A potential benefit of taking vitamin E alpha-tocopherol supplements in addition to the clopidogrel and aspirin is reducing the risk of recurrent strokes within 90 days of having a minor ischemic stroke or transient ischemic attack. Reducing the risk of recurrent stroke may increase quality of life and reduce disability severity.

Economic Considerations

Participation in this study is voluntary. No direct compensation will be given for participation in this trial. You will be reimbursed for the vitamin E supplements, placebo supplements, serum alpha-tocopherol blood draws, and other non-standard of care procedures. If you are injured or become ill while on the study, seek immediate treatment and contact the study doctor. If your illness is due to the study drug, vitamin E, you will not be financially responsible. You will receive a parking voucher for your day 90 in-person appointment at the end of the trial.

Treatment Alternatives

The alternative is to deny participation in this study. If you decline participation into this study then your primary medical provider will provide you your treatment plan for the transient ischemic attack or minor ischemic stroke.
Confidentiality

Information about your study participation will be entered into your Electronic Medical Record (EMR). Once placed in your EMR, these results are accessible to all of your providers who are appropriate to have access to your EMR (e.g., health insurance company, disability provider.) The protected information that will be collected in this study includes demographics, medical history, physical examinations, routine lab tests, current medications, vital signs, CT/MRI results, and information recorded in study questionnaires.

Information about you and your health which might identify you may be used or given to:

- The U.S. Department of Health and Human Services (DHHS) agencies
- Representatives from Yale University and the Human Investigation Committee (the committee or Institutional Review Board that reviews, approves, and monitors research on human subjects), who are responsible for ensuring research compliance. These individuals are required to keep all information confidential
- Your providers who are participants in the Electronic Medical Record (EMR) system
- Those individuals at Yale who are responsible for the financial oversight of research including billings and payments
- The study doctor, Dr. Charles Wira, and the Yale study team
- The U.S. Food and Drug Administration (FDA). This is done so that the FDA can review information about the new drug product involved in this research. The information may also be used to meet the reporting requirements of drug regulatory agencies.
- The study sponsor or manufacturer of study drug and/ or their representatives
- Health care providers who provide services to you in connection with this study.
- Laboratories and other individuals and organizations that analyze your health information in connection with this study, according to the study plan.
- Data and Safety Monitoring Boards and others authorized to monitor the conduct of the Study

Any identifiable information that is obtained in connection with the study will remain confidential and will be disclosed only with your permission or as required by the U.S. or State law. Examples of information that we are legally required to disclose include abuse of a child or elderly person, or certain reportable diseases. Study data will be kept confidential through the use of encrypted servers and locked cabinets. When the results are of the research are published or discussed in conferences, no information will be included that would reveal your identity unless your specific consent for this activity is obtained.

All healthcare providers subject to HIPAA (Health Insurance Portability and Accountability Act) are required to protect the privacy of your information. The research staff at the Yale School of Medicine are required to comply with HIPAA and to ensure the confidentiality of your information. Some of the individuals or agencies listed above may not be subject to HIPAA and therefore may not be required to provide the same type of confidentiality protection. They could use or disclose your information in ways not
mentioned in this form. However, to better protect your health information, agreements are in place with these individuals and/or companies that require that they keep your information confidential.

By signing this form, you authorize the use and/or disclosure of the information described above for this research study. The purpose of the uses and disclosures you are authorizing is to ensure that the information relating to this research is available to all parties who may need it for research purposes.

**Voluntary Participation & Withdrawal**

Participating in this study is voluntary. You are free to choose deny participation in this study. Refusing to participate will involve no penalty or loss of benefits to which you are otherwise entitled (such as your health care outside this study, the payment for your health care, and your health care benefits). However, you will not be able to enroll in this research study and will not receive study procedures as a study participant if you do not allow use of your information as part of this study.

If you do become a subject, you are free to stop and withdraw from this study at any time during its course. To withdraw from this study, you can call a member of the research team at any time and tell them you no longer want to take part. This will cancel any future appointments. The researchers may withdraw you from participating in the research if necessary. You may be withdrawn if necessary. You may be withdrawn if you have a serious side-effect related to vitamin E alpha-tocopherol such as moderate or major bleeding events. You may also be withdrawn from the study if you do not adhere to the treatment.

Withdrawing from the study at any time will involve no penalty or loss of benefits to which you are entitled. Withdrawing will also not harm your relationship with your providers or interfere with receiving standard treatment from your providers, if requested. When you are withdrawn from the study, no new health information identifying you will be gathered after that date. Information that has already been gathered may still be used and given to others until the end of the research study, as necessary to ensure the integrity of the study and/or study oversight.

**Questions**

We have used some technical terms in this form. Please feel free to ask about anything you don’t understand and to consider this research and the permission from carefully – as long as you feel it necessary – before you make a decision.
**Authorization**

_I have read (or someone has read to me) this form and have decided to participate in the project described above. In general purposes, the particulars of my involvement and possible hazards and inconveniences have been explained to my satisfaction. My signature also indicates that I have received a copy of this consent form._

________________________________________

Name of Subject

________________________________________

Signature                                      Date

________________________________________

Signature of Person Obtaining Consent          Date

If you have any further questions about this project or if you have a research related problem, you may contact the co-investigator Veronica Guirguis at veronica.guirguis@yale.edu.

If, after you have signed this form you have any questions about your privacy rights, please contact the Yale Privacy Officer at (203) 432-5919. If you would like to talk with someone other than the researchers to discuss problems, concerns and questions you may have concerning this research, or to discuss your rights as a research subject, you may contact the Yale Human Investigation Committee at (203) 785-4688.
Appendix B: Baseline Characteristics Survey

Participant Number: _______________  Research Assistant: _______________

Research Center: _______________  Date: ____________

Study Setting: _______________

Demographics:

Age: __________

Gender: __________

Race: __________

Please Check Below if Participant Has the Following:

Hypertension _____

Ischemic Heart Disease _____

Diabetes Mellitus _____

Smoking History
  If yes:  Current? Former?
  How many cigarettes a day?
  How many years?

Clopidogrel Use
  If yes: What is the dose?
  For how long?

Aspirin Use
  If yes: What is the dose?
  For how long?

ABCD² Score (if TIA): __________

NIHSS Score (if ischemic stroke): __________

Modified Rankin Scale (mRS) Score: __________
Baseline Laboratory Values:

<table>
<thead>
<tr>
<th>Laboratory Test</th>
<th>Laboratory Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT</td>
<td></td>
</tr>
<tr>
<td>PTT</td>
<td></td>
</tr>
<tr>
<td>INR</td>
<td></td>
</tr>
<tr>
<td>Alpha-tocopherol</td>
<td></td>
</tr>
<tr>
<td>Total Cholesterol (mg/dL)</td>
<td></td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td></td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td></td>
</tr>
<tr>
<td>Chol/HDL Ratio (calculated)</td>
<td></td>
</tr>
<tr>
<td>Non-HDL Cholesterol (mg/dL, calculated)</td>
<td></td>
</tr>
<tr>
<td>WBC (K/uL)</td>
<td></td>
</tr>
<tr>
<td>RBC (M/uL)</td>
<td></td>
</tr>
<tr>
<td>Hgb (gm/dL)</td>
<td></td>
</tr>
<tr>
<td>Hct (%vol)</td>
<td></td>
</tr>
<tr>
<td>MCV (fl)</td>
<td></td>
</tr>
<tr>
<td>Platelets (k/uL)</td>
<td></td>
</tr>
<tr>
<td>MCH (pg)</td>
<td></td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td></td>
</tr>
<tr>
<td>RDW (%)</td>
<td></td>
</tr>
<tr>
<td>MPV (fl)</td>
<td></td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td></td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td></td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td></td>
</tr>
<tr>
<td>Basophils (%)</td>
<td></td>
</tr>
<tr>
<td>Neutrophils (K/uL)</td>
<td></td>
</tr>
<tr>
<td>Lymphocytes (K/uL)</td>
<td></td>
</tr>
<tr>
<td>Monocytes (K/uL)</td>
<td></td>
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<tr>
<td>Eosinophils (K/uL)</td>
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<tr>
<td>Serum Creatinine (mg/dL)</td>
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<tr>
<td>BUN (mg/dL)</td>
<td></td>
</tr>
<tr>
<td>Estimated GFR (mg/dL)</td>
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</tr>
<tr>
<td>BUN/Cr Ratio</td>
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</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td></td>
</tr>
<tr>
<td>Sodium (mmol/L)</td>
<td></td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td></td>
</tr>
<tr>
<td>Chloride (mmol/L)</td>
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</tr>
<tr>
<td>Carbon Dioxide (mmol/L)</td>
<td></td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
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</tr>
<tr>
<td>Alkaline Phosphatase (U/L)</td>
<td></td>
</tr>
<tr>
<td>Aspartate Aminotransferase (U/L)</td>
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<tr>
<td>Alanine Aminotransferase (U/L)</td>
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</tr>
<tr>
<td>Bilirubin, Total (mg/dL)</td>
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</tr>
<tr>
<td>Protein, Total (g/dL)</td>
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</tr>
<tr>
<td>Albumin (g/dL)</td>
<td></td>
</tr>
<tr>
<td>Globulin (g/dL)</td>
<td></td>
</tr>
<tr>
<td>A/G Ratio</td>
<td></td>
</tr>
<tr>
<td>Anion Gap</td>
<td></td>
</tr>
</tbody>
</table>
Appendix C: ABCD² Score¹

FOR TIA PATIENTS (SCORE MUST BE ≥ 4 TO QUALIFY)

ABCD2 Score: ____/7

<table>
<thead>
<tr>
<th></th>
<th>No (0 points)</th>
<th>Yes (1 point)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age ≥ 60 years</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>BP ≥140/90 (initial BP. Either</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>SBP ≥ 140 or DBP ≥ 90</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Clinical Features of the TIA</strong></td>
<td>Other Symptoms (0 points)</td>
<td>Speech Disturbance without Weakness (1 point)</td>
</tr>
<tr>
<td><strong>Duration of Symptoms</strong></td>
<td>&lt;10 minutes (0 points)</td>
<td>10-59 minutes (1 point)</td>
</tr>
<tr>
<td><strong>History of Diabetes</strong></td>
<td>No (0 points)</td>
<td>Yes (1 point)</td>
</tr>
</tbody>
</table>
Appendix D: Modified Rankin Scale (mRS)²

FOR ALL PATIENTS

Modified Rankin Scale (mRS) Score:

<table>
<thead>
<tr>
<th>Level</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No symptoms</td>
</tr>
<tr>
<td>1</td>
<td>No significant disability; able to perform usual activities</td>
</tr>
<tr>
<td>2</td>
<td>Slight disability: able to carry out own affairs but unable to perform previous activities without assistance</td>
</tr>
<tr>
<td>3</td>
<td>Moderate disability: require some assistance but is able to walk without help</td>
</tr>
<tr>
<td>4</td>
<td>Moderately-Severe disability; unable to walk or attend to bodily needs without assistance</td>
</tr>
<tr>
<td>5</td>
<td>Severe disability: bedridden, incontinent, requires nursing care</td>
</tr>
<tr>
<td>6</td>
<td>Death</td>
</tr>
</tbody>
</table>
Appendix E: National Institute of Health Stroke Scale (NIHSS)\(^3\)

Minor ischemic stroke patients at time of randomization (score must be \(\leq 3\) to qualify)

For all patients on day 90

<table>
<thead>
<tr>
<th>NIHSS Score: ___/42</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>+0</th>
<th>+1 POINT</th>
<th>+2 POINTS</th>
<th>+3 POINTS</th>
<th>+4 POINTS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Consciousness</strong></td>
<td>Alert, keenly responsive</td>
<td>Aroused to minor stimulation</td>
<td>Requires repeated stimulation to arouse OR Movements to pain</td>
<td>Postures or Unresponsive</td>
</tr>
<tr>
<td><strong>Ask Month &amp; Age</strong></td>
<td>Both questions right</td>
<td>1 question right OR Dysarthric/intubated/traumatic language barrier</td>
<td>0 questions right OR Aphasic</td>
<td></td>
</tr>
<tr>
<td><strong>'Blink eyes' 'Squeeze hands'</strong></td>
<td>Perform both tasks</td>
<td>Performs 1 task</td>
<td>Perform 0 tasks</td>
<td></td>
</tr>
<tr>
<td><strong>Horizontal Extraocular Movements</strong></td>
<td>Normal</td>
<td>Partial gaze palsy: can be overcome OR Partial gaze palsy corrects with oculocephalic reflex</td>
<td>Forced gaze palsy: cannot be overcome</td>
<td></td>
</tr>
<tr>
<td><strong>Visual Fields</strong></td>
<td>No visual loss</td>
<td>Partial hemianopia</td>
<td>Complete hemianopia</td>
<td>Bilaterally blind OR Bilateral hemianopia</td>
</tr>
<tr>
<td><strong>Facial Palsy</strong></td>
<td>Normal symmetry</td>
<td>Minor paralysis (flat nasolabial fold, smile asymmetry)</td>
<td>Partial paralysis (lower face)</td>
<td>Unilateral complete paralysis (upper/lower face) OR Bilateral complete paralysis (upper/lower face)</td>
</tr>
<tr>
<td><strong>Left Arm Motor Drift</strong></td>
<td>No drift for 10 sec OR Amputation/joint fusion</td>
<td>Drift, but doesn’t hit bed</td>
<td>Drift, hits bed OR Some effort against gravity</td>
<td>No effort against gravity No movement</td>
</tr>
<tr>
<td><strong>Right Arm Motor Drift</strong></td>
<td>No drift for 10 sec OR Amputation/joint fusion</td>
<td>Drift, but doesn’t hit bed</td>
<td>Drift, hits bed OR Some effort against gravity</td>
<td>No effort against gravity No movement</td>
</tr>
<tr>
<td><strong>Left Leg Motor Drift</strong></td>
<td>No drift for 5 sec OR Amputation/joint fusion</td>
<td>Drift, but doesn’t hit bed</td>
<td>Drift, hits bed OR Some effort against gravity</td>
<td>No effort against gravity No movement</td>
</tr>
<tr>
<td><strong>Right Leg Motor Drift</strong></td>
<td>No drift for 5 sec OR Amputation/joint fusion</td>
<td>Drift, but doesn’t hit bed</td>
<td>Drift, hits bed OR Some effort against gravity</td>
<td>No effort against gravity No movement</td>
</tr>
<tr>
<td><strong>Limb Ataxia</strong></td>
<td>No ataxia OR Amputation/joint fusion OR Doesn’t understand OR Paralyzed</td>
<td>Ataxia in 1 limb</td>
<td>Ataxia in 2 limbs</td>
<td></td>
</tr>
<tr>
<td><strong>Sensation</strong></td>
<td>Normal/no sensory loss</td>
<td>Mild-moderate loss: less sharp/duller OR Mild-moderate loss: can sense being touched</td>
<td>Complete loss: can’t sense being touched at all OR No response &amp; quadriplegic OR Coma/unresponsive</td>
<td></td>
</tr>
<tr>
<td><strong>Language/Aphasia</strong></td>
<td>Normal/no aphasia</td>
<td>Mild-moderate aphasia: some obvious changes, without significant limitation</td>
<td>Severe aphasia: fragmentary expression, inference needed, can’t identify materials</td>
<td>Mute/global aphasia: no usable speech/auditory comprehension OR Coma/unresponsive</td>
</tr>
<tr>
<td><strong>Dysarthria</strong></td>
<td>Normal OR Intubated/Unable to rest</td>
<td>Mild-moderate dysarthria: slurring but can be understood</td>
<td>Severe dysarthria: unintelligible slurring or out of proportion to dysphasia OR Mute/anarthric</td>
<td></td>
</tr>
<tr>
<td><strong>Extinction/inattention</strong></td>
<td>No abnormality</td>
<td>Visual/tactile/auditory/spatial/personal inattention OR Extinction to bilateral simultaneous stimulation</td>
<td>Profound hemi-inattention (ex: does not recognize own hand) OR Extinction to &gt;1 modality</td>
<td></td>
</tr>
</tbody>
</table>
Appendix F: Patient Follow-Up

Participant Identifying Number: ___________
Stroke Center Site: ___________
Name of Researcher: ___________
Today’s Date: ___________

Known Recurrent Stroke? ___________

If yes, how many days after your index stroke? ___________

Known Myocardial Infarction?

If yes, how many days after your index stroke? ___________

Medication Compliance:

Morisky Scale Score: ___________

Disability

Modified Rankin Scale Score: ___________
NIHSS Score (only if index event was minor ischemic stroke): ___________

Labs and Imaging (for day 90 only)

Serum alpha-tocopherol level: ___________
Notable Labs (out of normal range):

________________________________________________________________________

________________________________________________________________________

________________________________________________________________________

MRI Findings (evidence of new infarction): ________________
Questionnaire for Verifying Stroke-Free Status (QVSFS)

1. Were you ever told by a physician that you had a stroke? (since last visit)
   ___ Yes    ___ No    ___ Unknown

2. Were you ever told by a physician that you had a TIA, ministroke, or transient ischemic attack? (since last visit)
   ___ Yes    ___ No    ___ Unknown

3. Have you had sudden painless weakness on one side of your body?
   ___ Yes    ___ No    ___ Unknown

4. Have you had sudden numbness or a dead feeling on one side of your body? (since last visit)
   ___ Yes    ___ No    ___ Unknown

5. Have you had sudden painless loss of vision in one or both eyes? (since last visit)
   ___ Yes    ___ No    ___ Unknown

6. Have you suddenly lost one half of your vision? (since last visit)
   ___ Yes    ___ No    ___ Unknown

7. Have you suddenly lost the ability to understand what people are saying? (since last visit)
   ___ Yes    ___ No    ___ Unknown

8. Have you ever suddenly lost the ability to express yourself verbally or in writing? (since last visit)
   ___ Yes    ___ No    ___ Unknown
Using the Power and Precision 4, we determined that the trial will require at least 2,002 participants, with 1,001 participants per arm, in order to provide 80% power to detect a rate difference of 3.2% in the vitamin E alpha-tocopherol group, with a two-sided type I error of 0.05 and an 6% withdrawal rate, assuming an 8.2% event rate in the group of aspirin and clopidogrel alone.
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