Transdermal Nitroglycerin Therapy for Menopausal Hot Flashes

(Public title: Flushing Reduction Associated with Nitrates-- FRAN)

Protocol Version 1.6

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SUMMARY OF CHANGES FOR VERSION 1.6

Section E.3 Exclusion Criteria

- Exclusion of women unable or unwilling to use remote platforms (i.e., video, telephone, and/or mail-in platforms) to the above if necessary to promote social distancing

Section G.1.3

- Modification to include if visits are conducted by video with participants at home, trained coordinators will guide participants step-by-step and in real time in obtaining the above measurements using automated monitors distributed to participants in advance of the visit.

Section H

- Addition video-based visits and procedure options to promote social distancing.
- Section H.1.3.B Screening Clinic Visits
    - Modified ECG procedure to candidates who have cardiovascular risk factors such as hypertension, hyperlipidemia, or smoking will undergo a screening resting 12-lead electrocardiogram (ECG) administered by a trained clinical coordinator.

SUMMARY OF CHANGES FOR VERSION 1.5

Section H.1.3:

- Clarification that the screening electrocardiogram (ECG) will take place during the Screening Clinic Visit

Section I.1.2:

- Acknowledgement that the screening ECG is designed to provide additional protection against risks of hypotension/lightheadedness and chest pain.

Section F.1.7

- Addition of a more detailed description of the weaning process for each of the final dosages of study medication.

Section G.1.3

- Acknowledgement that participants will be instructed to contact study staff between study visits to report any chest pain suggestive of ischemia, syncope, or other severe anticipated side effects.

Section J.2

- Addition of a more detailed description of the weaning process for each of the final dosages of study medication.

SUMMARY OF CHANGES FOR VERSION 1.4

Section E.3:

- Change in washout periods for exclusion for prior use of estrogen or progestin therapy
- Exclusion of women using riociguat, a soluble guanylate cyclase stimulator medication
- Exclusion of women with evidence of prior myocardial infarction on screening electrocardiogram (ECG)
- Exclusion of women with uncontrolled tachyarrhythmias or second or third-degree AV block on ECG
- Exclusion of women unwilling to take measures to prevent pregnancy (if not yet postmenopausal)
- Exclusion of women with normal resting blood pressure but evidence of orthostatic hypotension
- Exclusion of women currently or recently breastfeeding, or planning to breastfeed during the study

Section F.2.2:

- Clarification that use of riociguat during the treatment period will be considered a protocol violation

Section G.1.3:

- Addition of orthostatic measurements of blood pressure and heart rate to safety procedures

Section H.1.3:

- Addition of orthostatic blood pressure and heart rate measurements to screening procedures
- Addition of protections against accidental pregnancy for women who are not yet postmenopausal

Section H.2.5:

- Addition of orthostatic blood pressure and heart rate measurements to follow-up visits
- Addition of protections against accident pregnancy for women who are not yet postmenopausal
- 3-week telephone call added for participants who were started on 0.6 mg/hr dose at 2 weeks

Section H.2.8:

- 3-week telephone call added for participants who were started on 0.6 mg/hr dose at 2 weeks

Section I.1.1:

- Acknowledgement of potential for skin irritation with screening ECG electrodes

Section I.1.2:

- Clarification of the threshold for frequency of baseline headache that will exclude women from the study
- Clarification that women reporting current or recent chest pain suggesting ischemia or recent syncope, or those having measured severe hypertension at follow-up visits, will be referred for emergency care.
- Clarification that women with normal resting blood pressure but orthostatic hypotension will be excluded
- Clarification that women with ECG evidence of uncontrolled tachyarrhythmias or second or third-degree atrioventricular node block will be excluded
- Description of measures to protect against skin irritation from ECG electrodes

Section J.1:

- Clarification that participants who develop syncope attributable to NTG, resting hypotension or symptomatic orthostatic hypotension that does not respond to dose decrease, persistent severe hypertension, or pregnancy will be discontinued from the study

TABLE OF CONTENTS

A. PRÉCIS 6
B. STUDY OBJECTIVES 7
B.1 Primary Objective 7
B.2 Secondary Objectives 7
C. BACKGROUND & RATIONALE 7
C.1 Background 7
C.2 Rationale 8
C.3 Preliminary Data 8
D. STUDY DESIGN 9
E. SELECTION AND ENROLLMENT OF PARTICIPANTS 9
E.1 Overview 9
E.2 Inclusion Criteria 9
E.4 Recruitment and Consent Procedures 11
F. INTERVENTIONS, RANDOMIZATION, AND BLINDING 12
F.1 Study Drug and Placebo 12
F.2 Concomitant Interventions 13
F.3 Adherence Assessment 14
G. STUDY MEASUREMENTS AND OUTCOMES 14
G.1 Study Measurements 14
G.2 Primary and Secondary Outcomes 17
H. STUDY PROCEDURES 17
H.1 Screening and Baseline Evaluations 17
H.1.2 Consenting procedure 17
H.1.3 Screening and baseline procedures 18
I. SAFETY ASSESSMENTS 24
I.1 Potential Risks and Protective Measures 24
I.2 Methods and Timing of Safety Assessments 28
I.3 Adverse Events and Serious Adverse Events 28
I.4 Independent Safety Monitoring 30
J. INTERVENTION DISCONTINUATION 31
J.1 Discontinuation Decisions 31
K. STATISTICAL CONSIDERATIONS 32
K.1 General Design Issues 32
K.2 Data Analyses 33
K.3 Sample Size Determination 33
K.4 Additional Statistical Considerations 34
L. DATA COLLECTION AND QUALITY ASSURANCE 34
L.1 Data Collection Forms 34

L.2 Data Management 35
L.3 Quality Assurance 35
M. PARTICIPANT RIGHTS AND CONFIDENTIALITY 36
M.1 Institutional Review Board (IRB) Review 36
M.2 Informed Consent Forms 36
M.3 Participant Confidentiality 37
N. ETHICAL CONSIDERATIONS 37
O. STEERING COMMITTEE 37
P. PUBLICATION OF RESEARCH FINDINGS 37
APPENDIX A: Summary of Measures and Procedures at Study Visits 38
REFERENCES CITED 39

A. PRÉCIS

Study title

Transdermal Nitroglycerin Therapy for Menopausal Hot Flashes
(a.k.a. Flushing Reduction Associated with Nitrates, or FRAN)

Objectives

To evaluate the efficacy, safety, and tolerability of uninterrupted transdermal NTG therapy for menopausal hot flashes.

Design

Randomized, double-blinded, parallel-group, placebo-controlled trial of uninterrupted transdermal NTG therapy in peri- or postmenopausal women with hot flashes.

Interventions

Random assignment to uninterrupted daily use of transdermal NTG patches (participant-directed dose escalation from 0.2 to 0.6 mg/hr) for 12 weeks, versus uninterrupted daily use of identical-appearing placebo patches for 12 weeks.

Population

Women aged 40 to 62 years who are postmenopausal or in the late menopausal transition, document an average of at least 7 hot flashes per day, do not have coronary disease or multiple risk factors for coronary disease, and do not have contraindications to transdermal NTG therapy, recruited from the San Francisco Bay Area.

Outcomes

The primary outcome is change in the average frequency of any hot flashes from baseline to 4 and 12 weeks as documented by symptom diaries. Secondary efficacy outcomes include changes in frequency of moderate-to-severity hot flashes and total hot flash severity score from baseline to 4 and 12 weeks of treatment, also documented by symptom diaries. Additional secondary efficacy outcomes include changes in sleep quality, anxiety and depression symptoms, and menopause-related quality of life from baseline to 4 and 12 weeks of treatment, assessed using validated questionnaires. Additional safety outcomes include rates of severe headache, chest pain, and syncope, as well as other adverse events with a Common Terminology for Adverse Event Criteria severity grade of 3 or higher.

B. STUDY OBJECTIVES

B.1 Primary Objective

To determine the efficacy of uninterrupted transdermal NTG therapy in reducing the frequency of hot flashes in peri- and postmenopausal women.

We hypothesize that, compared to placebo, uninterrupted use of NTG will result in at least a 20% greater decrease in frequency of hot flashes (assessed by validated 7-day symptom diaries) over 12 weeks.

B.2 Secondary Objectives

A. To evaluate the efficacy of uninterrupted transdermal NTG therapy in reducing the severity of hot flashes in peri- and postmenopausal women.

We hypothesize that, compared to placebo, uninterrupted use of NTG will result in significant improvements in frequency of moderate-to-severe hot flashes and in cumulative hot flash severity score (assessed by validated 7-day symptom diaries) over 12 weeks.

B. To evaluate the efficacy of uninterrupted transdermal NTG therapy in improving other symptom- and quality-of-life outcomes associated with hot flashes in peri- and postmenopausal women.

We hypothesize that, compared to placebo, uninterrupted use of NTG will result in significant improvements in sleep quality, anxiety and depression symptoms, and menopause-related quality of life over 12 weeks.

C. To examine the tolerability and safety of uninterrupted transdermal NTG therapy in peri- and postmenopausal women with hot flashes.

We hypothesize that, compared to placebo, uninterrupted use of NTG will be associated with higher rates of mild headache, but no significant differences in rates of chest pain, syncope, or severe adverse events defined by Common Terminology for Adverse Event Criteria severity grade 3 or higher.

C. BACKGROUND & RATIONALE

C.1 Background

Hot flashes (a.k.a. vasomotor symptoms) are the most common symptomatic complaint of menopausal women in the United States, with over two thirds reporting hot flashes at some point during the menopausal transition, and one in five reporting symptoms severe enough to require treatment (Gold, Sternfeld et al. 2000, Huang, Grady et al. 2008, Avis, Crawford et al. 2015). Although estrogen therapy is effective in suppressing hot flashes, it is associated with an increased risk of endometrial cancer, and when combined with a progestin to prevent endometrial hyperplasia, its long-term use can increase risk of coronary disease, thromboembolic events, and breast cancer (Rossouw, Anderson et al. 2002, Shumaker, Legault et al. 2003, Manson, Chlebowski et al. 2013).

To date, most efforts to identify alternate treatments for hot flashes have focused on central nervous system (CNS) mechanisms hypothesized to play a role in triggering these symptoms. However, pharmacologic agents that have targeted CNS mechanisms for hot flashes (antidepressants, gabapentin, clonidine) have so far had only modest effects on vasomotor symptoms, and are associated with multiple side effects that limit their acceptability (Stearns, Beebe et al. 2003, Grady, Cohen et al. 2007, Butt, Lock et al. 2008). Furthermore, while hot flashes may be precipitated by abnormalities in the CNS, they manifest physically in the periphery in the form of vasodilation accompanied by flushing, sweating, and intense sensations of heat over the chest, head, and arms. As a result, treatment strategies that specifically target mechanisms that mediate peripheral

vasodilation during hot flashes may offer a more direct and effective way to suppress these symptoms, while also potentially incurring fewer side effects.

C.2 Rationale

Clinical studies have shown that nitric oxide (NO) plays an important role in mediating peripheral vasodilation during hot flashes, with local cutaneous blockade of NO synthase suppressing hot flash-related vasodilation (Low, Davis et al. 2008, Hubing, Wingo et al. 2010, Low, Hubing et al. 2011). One pharmacologic agent with direct and potent effects on NO-mediated vasodilation is nitroglycerin (NTG), an organic nitrate that is widely used to treat chest pain in patients with coronary disease. Intermittent use of NTG triggers release of NO, promotes vascular smooth muscle relaxation, and triggers vasodilation. However, *continuous* use of NTG rapidly leads to tolerance to the drug's vasodilatory effects, and also induces cross-tolerance to endogenous nitrates as a result of enhanced NO degradation (Munzel, Sayegh et al. 1995, Laursen, Boesgaard et al. 1996, Laursen, Mulsch et al. 1996, Chen, Foster et al. 2005, Daiber, Mulsch et al. 2005, Thomas, DiFabio et al. 2007).

This phenomenon, widely known as nitrate tolerance, limits the use of NTG for chest pain, in that patients can only use NTG for up to 12 hours at a time before it ceases to provide therapeutic effects on chest pain, even at high doses. However, women who develop nitrate tolerance should also experience a marked reduction in vasomotor symptoms due to suppression of NO-mediated peripheral vasodilation. As a result, uninterrupted use of NTG using a method of administration that maintains steady-state levels (i.e., transdermal NTG patches) may offer a new and innovative approach to treating hot flashes.

C.3 Preliminary Data

To assess the preliminary effects of this treatment approach, our investigative team conducted a pilot single-arm trial in which 19 peri- and postmenopausal women with at least 7 hot flashes per day were recruited from the San Francisco area (Huang, Cummings et al. 2015). Women using other clinical treatments for hot flashes and those with a history of coronary disease or multiple risk factors for coronary disease were excluded, as were women with a history of chronic headache, sensitivity to adhesives, or hypotension. All eligible women were started on a generic 0.1 mg/hr NTG patch (no placebo group) applied daily without any patch-free periods. Participants who tolerated the 0.1 mg/hr patch and reported persistent bothersome hot flashes on this dose underwent dose escalation weekly to 0.2, 0.4, or 0.6 mg/hr as tolerated, and then discontinued NTG for the final week of the study.

At baseline, mean (±SD) age was 51.4 (±4.3) years, 42% of participants were racial/ethnic minorities. The mean frequency of hot flashes at baseline was 10.6 (±2.1) per day, and the mean frequency of moderate-to-severe hot flashes was 7.2 (±3.7) per day. Over 4 weeks of escalating dose treatment, the average daily frequency of hot flashes decreased by 54% to 5.0 (±2.5), and the average daily frequency of moderate-to-severe hot flashes decreased by 69% to 2.3 (±2.6) ($P<0.01$ for both). Women also demonstrated improvements in scores of 60% on the Hot Flash Daily Interference Scale, 46% on the Beck Depression Inventory, 46% on the Insomnia Severity Index, and 33% on the Menopausal Quality of Life vasomotor domain from baseline to maximum dose therapy ($P<.05$ for all). During a one-week period after discontinuation of NTG, frequency of any hot flashes increased by 23%, and frequency of moderate-to-severe hot flashes increased by 96% ($P<0.01$ for both).

Two women developed headache on the initial 0.1 mg/hr patch, leading them to discontinue treatment before the first outcomes assessment. Among women who tolerated the initial dose of TNG, however, none subsequently experienced any serious adverse events or discontinued treatment for side effects. Eleven women opted to escalate to 0.6 mg/hr dose therapy, while three preferred to remain at 0.4 mg/hr and three on 0.2 mg/hr therapy. Average improvement in hot flash frequency from baseline associated with each NTG dose was 28% for 0.1 mg/hr, 34% for 0.2 mg/hr, 39% for 0.4 mg/hr, and 53% for 0.6 mg/hr. Average blood pressure

was 127/79 mmHg, 129 /78 mmHg, 130/81 mmHg, and 125/77 mmHg on 0.1 mg, 0.2 mg/hr, 0.4 mg/hr, and 0.6 mg/hr dose NTG, respectively, and 130/81 mm Hg after discontinuation of NTG.

D. STUDY DESIGN

We will conduct a randomized, double-blinded, placebo-controlled trial to evaluate the efficacy and safety of continuous transdermal NTG for treatment of hot flashes. Peri- or postmenopausal women who report frequent hot flashes, are not using other clinical hot flash treatments, have no known history of coronary disease or multiple risk factors for coronary disease, and do not have contraindications to NTG therapy will be recruited from the San Francisco Bay area. Prior to randomization, women will be asked to record all hot flashes on a validated symptom diary over a 7-day period. They will then complete a brief, 3-day run-in period in which they will wear a 0.1 mg/hr transdermal NTG patch daily to confirm willingness to apply the patch and eliminate those with sensitivities to even low-dose NTG. Women who demonstrate an average of 7 or more hot flashes per day by their screening diaries, are successful in applying a 0.1 mg/hr patch daily during the run-in period, and meet all other eligibility criteria will then be randomized in equal ratios to daily use of transdermal NTG or placebo for 12 weeks.

Randomized participants will be started on a 0.2 mg/hr NTG (or identical-appearing placebo) patch daily, as the minimum dose previously found to produce at least a one-third decrease in hot flashes in the investigators' pilot study. Women will apply a new patch each night before going to bed, immediately after removing the old patch, and avoid patch-free periods that might interfere with development of tolerance. Women will return for follow-up at 1 week, and those who show no signs of hypotension or severe hypertension and are not already completely satisfied with the change in their symptoms will be increased to a 0.4 mg/hr NTG patch (or identical-appearing placebo) daily. Based on reassessment at 2 weeks, women may increase their NTG (or placebo) patch to 0.6 mg/hr for the remaining treatment period.

To achieve the primary efficacy objective, changes in the frequency and severity of hot flashes will be assessed over 4 and 12 weeks using a validated 7-day symptom diary. To achieve the secondary efficacy objective, changes in other hot flash-related symptoms and quality-of-life outcomes will be assessed using validated questionnaires. To achieve the secondary safety objective, specific potentially severe side effects of NTG will be systematically assessed, and other reported unanticipated adverse events will also be evaluated using standardized forms at all follow-up visits.

This design is designed to provide the first rigorous evidence to evaluate the efficacy and tolerability of uninterrupted NTG for treatment of menopausal hot flashes, while making efficient use of research resources.

E. SELECTION AND ENROLLMENT OF PARTICIPANTS

E.1 Overview

Eligibility criteria are designed to identify peri- or postmenopausal women with frequent and bothersome hot flashes, who are not using other clinical hot flash treatments, have no history of coronary disease or multiple risk factors for coronary disease, and do not have clear contraindications to transdermal NTG therapy. To maximize generalizability, women from multiple menopausal stages will be enrolled, provided they meet all hot flash-related and clinical criteria and complete measurements that can clarify menopausal/hormonal status.

E.2 Inclusion Criteria

1) Women aged 40 to 62 years (consistent with the recent MSFlash hot flash network trials, and likely to maximize enrollment of highly symptomatic women)

2) Postmenopausal or in the late menopausal transition. For this study, postmenopausal status will be defined by: 1) self-reported history of bilateral oophorectomy, 2) follicle stimulating hormone (FSH) levels > 20 mU/mL for those with a self-reported history of hysterectomy without bilateral oophorectomy, or 3) no self-reported history of hysterectomy or oophorectomy, but absence of menses in the past 12 months. Late menopausal transition will be defined by amenorrhea for at least 60 days in the past 12 months within the specified age group.
3) Documentation of an average of 7 or more hot flashes per 24 hours as well as 4 or more moderate-to-severe hot flashes per 24 hours as recorded on a validated 7-day screening symptom diary
4) Willing to refrain from initiating other treatments that are known to affect the frequency of severity hot flashes during the trial period
5) Report having a current primary health care provider (such as a general practitioner, family medicine, internal medicine, or nurse practitioner providing primary care or specializing in women's health)

E.3 Exclusion Criteria

1) Current or recent use of NTG or other nitrate-containing medications (i.e., use within 1 month of screening), or intention to use nitrate-containing medication during the interventional period
2) Current or recent use of medications already known to reduce the frequency or severity of hot flashes (e.g., vaginal or transdermal estrogens in the past 4 weeks; oral estrogens or progestins in the past 8 weeks; intrauterine progestin therapy in the past 8 weeks; progestin implants or estrogen alone injectable therapy in the past 3 months; estrogen pellet therapy or progestin injectable therapy in the past 6 months; clonidine, methyldopa, gabapentin, pregabalin and selective serotonin or norepinephrine reuptake inhibitors (SSRIs/SNRIs) in the past 1 month); or intention to use these medications during the interventional period
3) Current or recent use of phosphodiesterase inhibitor medications (within 1 month of screening), or intention to use these medications during the interventional period
4) Current or recent use of riociguat, a soluble guanylate cyclase stimulator medication, within 1 month of screening, or intention to use riociguat during the interventional period
5) Self-reported history of hypertrophic obstructive cardiomyopathy, aortic valve stenosis, or mitral valve stenosis (since symptoms of these conditions may be aggravated by NTG therapy)
6) Self-reported history of coronary disease (since patients with coronary disease may need NTG therapy for chest pain or may be at increased risk of new coronary events in the setting of nitrate tolerance), or evidence of prior myocardial infarction on screening electrocardiogram (ECG)
7) Self-reported history of diabetes or 2 or more major risk factors for coronary disease (i.e., smoking, hypertension, or hyperlipidemia with physician-recommended pharmacologic treatment)
8) Evidence of tachyarrhythmias such as atrial fibrillation or flutter without adequate rate control (>110 beats/minute) on screening ECG
9) Evidence of second or third-degree atrioventricular block on screening ECG
10) Hypotension based on measured resting blood pressure <90/60 at baseline; or normal resting blood pressure but evidence of orthostatic hypotension with change from supine to standing position)
11) Uncontrolled hypertension based on measured resting blood pressure > 180/110 at baseline
12) Self-reported headaches interfering with activities of daily activities more than twice a month, or use of prescription medication to prevent or treat headache in the past month (since these may be

worsened by NTG therapy)

13) Known allergy to nitroglycerin or other nitrate-based medications.
14) Known skin sensitivity to adhesives (which may generalize to the NTG patches)
15) Unable to complete or tolerate a brief 3-day run-in period involving the lowest available dose of NTG (0.1 mg/hr)
16) Currently pregnant, gave birth within the past 3 months, planning pregnancy during the study period, or unwilling to use regular barrier contraception or abstain from sexual activity to prevent pregnancy if not yet postmenopausal
17) Currently breastfeeding, breastfeeding within the past 3 months, or planning to breastfeed during the study period
18) Self-report heavy alcohol use (>3 drinks in a given day or >7 drinks per week) and uncomfortable or unwilling to decrease their alcohol intake during the study period.
19) Unable or unwilling to provide informed consent, fill out questionnaires, or complete study visits in English; unable or unwilling to use remote platforms (i.e., video, telephone, and/or mail-in platforms) to the above if necessary to promote social distancing
20) Report other conditions that, in the judgment of the investigators, render potential participants highly unlikely to follow the protocol, including plans to move, substance abuse, significant psychiatric problems, or severe dementia.

E.4 Recruitment and Consent Procedures

E.4.1 Recruitment strategies

Participants will be recruited from the greater San Francisco Bay area by trained research coordinators working under the supervision of the principal investigator at the UCSF Women's Health Clinical Research Center. The study team will use a multi-component IRB-approved approach that has been successfully used by the study team to recruit diverse menopausal participants in past clinical trials of experimental treatments for hot flashes. Recruitment techniques will include community-based media efforts (e.g., newspaper advertisements, mass mailings to households with female residents, internet-based and social media-based recruitment, talks at community centers), mailings to female patients at UCSF Medical Center with diagnostic codes indicating menopausal symptoms or increased risk of menopausal symptoms, recruitment from a database of past participants who have given permission to be contacted for future studies, and direct recruitment from physician offices (e.g., primary care, gynecology, breast care).

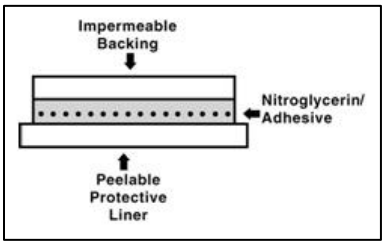
E.4.2 Informed consent & documentation

Before entering the study, all study procedures, time requirements, risks and potential benefits will be explained to each potential study participant using the information in the UCSF IRB-approved informed consent form. The potential study participant will be given adequate time to read the informed consent document and ask questions about the study procedure before providing consent. Potential participants will also be invited to see and handle a run-in NTG patch before deciding whether to enroll in the study. Eligible participants who choose to enter the study will sign the inform consent form and Health Insurance Portability and Accountability Act (HIPAA) form (which may involve electronic signature) prior to beginning study treatment. Each participant will be given a copy of the signed documents and the original will be a part of the research record.

F. INTERVENTIONS, RANDOMIZATION, AND BLINDING

F.1 Study Drug and Placebo

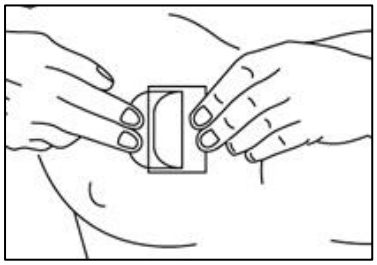
F.1.1. Transdermal nitroglycerin: 1,2,3-propanetriol, trinitrate is an organic nitrate administered in the form of a patch that provides continuous, controlled release of NTG through intact skin. For this study, the study team will purchase generic NTG patches manufactured by Mylan Pharmaceuticals in multiple dosages (0.1, 0.2, and 0.4 mg//hr), the same brand of NTG patch used in the investigators' pilot study. For each NTG patch, the rate of release of NTG is linearly dependent upon the area of the applied system. Each patch consists of 2 layers: 1) a transparent impermeable outer backing composed of a composite plastic film, and 2) nitroglycerin in acrylic-based polymer adhesive with a cross-linking agent. Prior to use, a protective peelable liner is removed from the adhesive surface. In healthy volunteers, steady-state plasma levels of NTG are reached by ~2 hours after application of a patch, and are maintained for the duration of use of the patch, with only ~6% of the total content of NTG being delivered in the first 12 hours. Upon removal of the patch, plasma concentrations decline with a half-life of approximately one hour.



F.1.2.Placebo Patch Product Specifications:

Placebo patches matching the Mylan Pharmaceuticals' NTG patches in size, shape, and color have been created by Clinical Trial Services specifically for this study. The placebo patch occlusive backing is made of a polymer of ethylene and vinyl acetate, typically used for membranes or backing films. The adhesive material used in the placebo patch is a medical grade double coated tape and transfer adhesive. The material used in the placebo patch liner is a fluoropolymer coated polyester film which provides premium release from skin contact adhesives.

F.1.3. Medication dispensing and tracking: Transdermal NTG medication will be purchased by the UCSF Department of Clinical Pharmacy and then re-packaged by Clinical Trial Services, a company specializing in medication and placebo packaging for clinical research studies. Study medication (active and placebo) will be individually packaged in foil packets, labeled, and then grouped by treatment assignment into trial master packs. Standardized study medication accountability forms and logs will be used to maintain accurate records of receipt, dispensing, return, and disposal of study medication. During the study, participants receiving study medication will be identified on the drug accountability form by study number and acrostic. In addition, the date, dosage, and quantity dispensed will be recorded each time study medication is dispensed.



F.1.4. Active and run-in patch dosing: Because data from our pilot study suggest that doses of 0.2 mg/hr or higher are more likely to result in meaningful improvement in hot flashes (see Preliminary Data section), we will use only 0.2, 0.4, and 0.6 mg/hr dosages of NTG during the active phase of the trial. For logistical reasons, the 0.6 mg/hr dosage will be obtained through simultaneous use of a 0.2 and 0.4 mg/hr patch, rather than a 0.6 mg/hr patch. Additionally, a 0.1 mg/hr patch will be used during a brief 3-day run-in period prior to randomization, to eliminate women who are unable to self-administer and tolerate even the lowest dose of transdermal NTG.

F.1.5. Schedule of administration during run-in: During the brief run-in period, women will apply a 0.1 mg/hr NTG patch to their chest or any clean, dry, hairless area of skin on the body except the extremities below the knee or elbow. Women will self-administer this patch at home daily (removing the old patch each night and replacing it with a new patch) for 3 days. Women will also be advised to rotate the location of the patch on a nightly basis to minimize risk of skin reaction or breakdown. Those who report persistent or significant

headache, develop skin reaction, hypotension, or severe hypertension at the baseline visit, or are otherwise unable to apply patches daily during run-in, will not continue to randomization.

F.1.6. Schedule of administration during treatment phase: At the baseline visit, women who successfully complete the run-in with 0.1 mg/hr NTG and meet all eligibility criteria will be assigned in a 1:1 ratio to start using a 0.2 mg/hr NTG or identical-appearing placebo patch for the treatment phase of the study. Women will again apply a new patch each night, directly after removing the old one, and avoiding patch-free episodes that could interfere with maintenance of nitrate tolerance. At a 1-week follow-up visit, those who report persistent hot flashes, do not have blood pressure <90/60 or >180/110, and pose no other major safety or tolerability issue will have their NTG (or placebo) dose increased to 0.4 mg/hr. Based on the 2-week follow-up visit, women with persistent hot flashes and no significant blood pressure, safety, or tolerability issue will have their dose increased to 0.6 mg/hr and remain at this for the remainder of the study. Women who report being completely satisfied on a dose lower than 0.6 mg/hr or who have major safety or tolerability issues will be permitted to remain on a lower dose.

F.1.7. Discontinuation of NTG/placebo after treatment phase: At the 12-week visit, participants will be asked to wean off NTG (or placebo) over the next 1 to 3 days, by decreasing their patch dose on a nightly basis before stopping NTG/placebo completely. Specifically, participants who are being weaned from the 0.6 mg dose of study medication will be instructed to switch to 0.4 mg for one day, then to 0.2 mg for one day, then to stop study drug altogether. Participants who are being weaned from the 0.4 mg dose will switch to 0.2 mg for one day before stopping medication altogether. Women who are taking the 0.2 mg (the lowest dose hypothesized to have a therapeutic effect) will simply stop study medication without any change in dose. Women will be given a last supply of NTG or placebo medication (one patch of each dose strength needed to complete the weaning). Participant and staff blinding will be maintained throughout the weaning process; for participants taking placebo, weaning will use placebo rather than active NTG patches. In the event that a participant develops a potentially severe adverse event such as chest pain during weaning, she will be directed to seek immediate emergency attention, just as she would during any other time point of the study.

F.2 Concomitant Interventions

F.2.1 Allowed interventions

The following interventions will be permitted during the study treatment period, despite their potential effects on hot flashes or frequent use by menopausal women to alleviate hot flashes:

- Aromatase inhibitors and selective estrogen receptor modular (SERM) medications: Although these medications can precipitate or worsen hot flashes, concomitant use will be permitted provided that women have been on stable doses for at least 3 months and do not intend to change doses during the study period.
- Isoflavones, black cohosh, phytoestrogens, and other herbal products: Use of these complementary products will be allowed as their efficacy in suppressing hot flashes has not been scientifically demonstrated.
- Paced respiration, mind-body therapies, and other complementary behavioral therapies: Use of these techniques is allowed as their efficacy in suppressing hot flashes has not been scientifically demonstrated.

F.2.2 Prohibited interventions

Participants will be instructed to avoid the following medications during the study treatment period due to their potential to improve hot flashes or secondary symptom or quality-of-life outcomes, overlap with study treatment, and/or interact adversely with study treatment.:

- Estrogen or progestogen medications (any injectable, oral, transdermal, or vaginal preparations), including combined estrogen and selective estrogen receptor modulator preparations
- Other medications known to improve hot flashes or menopause-related quality of life, including clonidine, methyldopa, selective serotonin or norepinephrine reuptake inhibitors (SSRIs/SNRIs), gabapentin, or pregabalin
- Outside NTG therapy (transdermal, oral, intravenous, or sublingual) or other nitrate-containing medications. Documented use of any outside nitrates from the start of the screening period to the 12-week visit will be considered a protocol violation.
- Phosphodiesterase inhibitor medications, due to risk of pharmacodynamic reaction with NTG that can result in severe hypotension. Documented use of any phosphodiesterase inhibitors from the start of the screening period to the 12-week visit will be considered a protocol violation.
- Riociguat, a soluble guanylate cyclase stimulator, due to risk of pharmacodynamic reaction with NTG that can result in severe hypotension. Documented use of riociguat from the start of the screening period to the 12-week visit will be considered a protocol violation.

F.3 Adherence Assessment

Study coordinators will review adherence at each scheduled follow-up visit or telephone call and address barriers to adherence in the event that participants appear to have missed days of patch use. Adherence to study medication will be assessed primarily in two ways: 1) daily study medication diaries in which participants will record their patch use and also affix their used patches to the diary, and 2) tabulation of unused medication patches at each study visit and at the study end.

F.3.1 Medication diaries

All participants who are started on study medication will be instructed to complete standardized medication diaries, in which they will record the date and time of each patch administration. Participants will also be instructed directly affix their used NTG (or placebo) patches to the appropriate section of each diary page after removing them to place a new one. Diary pages will be collected and reviewed by study coordinators at all in-person or video-based follow-up visits to promote assessment of adherence.

F.3.2 Tabulation of unused medication

Participants will be asked to return any unused medication after each in-person or video-based visit following administration of study medication; unused patches will be tabulated by study coordinators to assess adherence.

F.3.3 Adherence thresholds

The study team will monitor the proportion of participants who are using ≥ 75%, 50%-74%, and <50% of recommended study patches over any given time interval. This will correspond roughly to participants applying/changing their patches on at least 6 days, 4-5 days, or less than 4 days per week on average.

G. STUDY MEASUREMENTS AND OUTCOMES

G.1 Study Measurements

G.1.1 Hot flash frequency and severity (before the baseline, 5-week, and 12-week clinic visits)

Change in frequency of hot flashes will be measured using a validated 7-day diary that has been widely used in prior trials of hot flash treatments, is the FDA-recommended outcome measure for studies of hormonal therapies for hot flashes, has good test-retest reliability, and is sensitive to change (Sloan, Loprinzi et al. 2001,

Food and Drug Administration 2003, Grady, Sawaya et al. 2009). Participants will be asked to record each hot flash they experience on this diary and rate it as: 1) mild (sensation of heat without sweating), 2) moderate (sensation of heat with sweating, not preventing the participant from continuing with activity), or 3) severe (sensation of heat with sweating, causing cessation of activity); severity definitions will be provided with the diary.

For this study in which the average frequency of hot flashes at baseline will be high (i.e., at least 7 hot flashes per 24-hour period), the study team believes that this type of real-time diary will lead to more accurate assessment of hot flash frequency than alternate diary formats in which subjects only record their hot flashes once or twice a day by retrospectively recalling their hot flashes over the past 12 or 24 hours. To avoid diary fatigue, participants will be asked to complete the diary for a 7-day period before the baseline, 5-week, and 12-week visits only, rather than recording their hot flashes continuously throughout the trial. To facilitate real-time recording, the diary has been designed to be small and portable, so that women can carry it with them throughout the day and keep it at their bedside at night.

In addition to assessing change in frequency of any hot flashes, diary data will be used to assess change in frequency of moderate-to-severe hot flashes as an important secondary outcome. A total hot flash severity score will also be calculated by taking the sum of severity ratings for all hot flashes recorded during each 7-day period.

G.1.2 Other symptom and quality-of-life outcomes (at the screening, 5-week, and 12-week visits)

Additional symptom and quality-of-life outcomes will be assessed using several validated, self-administered, structured-item questionnaires, including:

A. The Hot Flash Related Daily Interference Scale (HFRDIS)

The HFRDIS is a 10-item self-administered questionnaire that assesses the degree to which hot flashes have interfered with participants' activities over the prior week. Interference with each activity is rated on a 10-point scale, and an overall score is calculated as the average of the items. The HFRDIS has been shown to have good internal consistency reliability, construct validity, and sensitivity to change (Carpenter 2001)

B. The Pittsburgh Sleep Quality Index (PSQI) and Pittsburgh Sleep Diary

Sleep quality, sleep latency, sleep efficiency, and sleep problems will be assessed using an 18-item validated questionnaire (Buysse, Reynolds et al. 1989, Buysse, Reynolds et al. 1991). A global sleep quality score ranging from 0 to 21 can be calculated, with higher scores reflecting worse sleep quality. Additionally, participants will be asked to complete a 7-day Pittsburgh Sleep Diary on the same days that they complete their hot flash diaries (Monk, Reynolds et al. 1994); in this diary, participants will record in their diaries in the morning just after waking up and at night before going to bed.

C. The Menopause Quality of Life (MENQOL)

Condition-related quality of life will be measured using the this 30-item questionnaire that has been widely used in both observational and interventional studies of women with hot flashes and has been shown to have good responsiveness and discriminative characteristics (Hilditch, Lewis et al. 1996, Lewis, Hilditch et al. 2005). For this study, analyses will focus on the vasomotor MENQOL domain, designed to be specific to impact of hot flashes.

D. The Generalized Anxiety Disorder-7 (GAD-7)

Anxiety symptoms will be assessing using the GAD-7, a 7-item structured questionnaire designed to assess the severity of anxiety symptoms over a 2-week period in primary care populations (Spitzer, Kroenke et al. 2006). Total scores range from 0 to 21, with higher scores indicating more severe

anxiety. The GAD-7 has been administered in previous hot flash trials and has been reported to modify the effects of hot flash interventions (Freeman, Guthrie et al. 2011).

E. The Center for Epidemiologic Studies Depression (CES-D) scale

Depressive symptoms will be assessed by the CES-D, a 20-item measure that has been widely used in clinical trials, including hot flashes intervention trials, and has been shown to be sensitive to change (WW, C et al. 2004). Total scores range from 0 to 60, with higher scores indicating more severe depressive symptoms.

G.1.3 Safety and tolerability measures (assessed at all follow-up assessments after initiation of therapy)

A. Blood pressure monitoring:

Blood pressure will be closely monitored before and during NTG (or placebo) therapy (also see "Safety Assessments" section below. At in-person screening and baseline visits and at each in-person follow-up visit thereafter, participants will undergo two resting blood pressure measurements by trained coordinators using calibrated monitors. Additionally, orthostatic measurements of blood pressure and heart rate will be obtained. If visits are conducted by video with participants at home, trained coordinators will guide participants step-by-step and in real time in obtaining the above measurements using automated monitors distributed to participants in advance of the visit.

In the event that participants report blood pressure readings <90/60 or >180/110 in outside contexts despite normal readings in the study visit, or participants report symptoms suggesting hypo- or severe hypertension despite normal blood pressure readings in the study visit, they will be invited to undergo additional home or ambulatory blood pressure monitoring for more intensive assessment of blood pressure.

B. Severe anticipated side effects of NTG therapy

At each follow-up visit or phone call after initiation of NTG or placebo, blinded study coordinators will systematically assess for the following potentially severe side effects of NTG therapy: 1) headache severe enough to interfere with instrumental activities of daily living; 2) chest tightness or pain interfering with instrumental activities of daily living, and 3) syncope (fainting or loss of consciousness). If reported, these symptoms will be recorded on adverse event forms that will indicate date of onset, date of resolution, likelihood of relationship to treatment, actions taken in response to the event, and severity according to the Common Terminology Criteria for Adverse Events (CTCAE) scale from the National Cancer Institute (National Cancer Institute 2009) (see "Safety Assessments" section below). Participants will also be given the phone number for the study site and will be instructed to contact study staff in between study visits to report any chest pain suggestive of cardiac ischemia, syncope, or other severe anticipated side effects such as headache interfering with daily activities.

C. Unanticipated adverse events

In addition to systematically eliciting selected anticipated adverse events as noted above, blinded study coordinators will assess for other unanticipated adverse events at each follow-up contact using the standardized question, "Have there been any changes in your health since your last visit?" Participants will also be given a telephone number that can be used to contact study staff to report any adverse events experienced in between scheduled study visits. Negative changes will be recorded as adverse events on standardized adverse event forms, with documentation of date of onset, date of resolution, likelihood of relationship to treatment, and actions taken in response to the event, and severity according to the CTCAE scale (National Cancer Institute 2009) (see "Safety Assessments" section below).

G.1.4 Demographic and clinical covariates

Structured questionnaires will collect data on the following variables in order to assess eligibility, characterize the study cohort, assess whether groups are comparable at baseline, and guide statistical adjustments in the event that significant differences between groups are detected at baseline:

A. Sociodemographic history (date of birth, race/ethnicity, education, employment, marital/relationship status);

B. Medical history (diagnosed medical conditions including coronary disease, diabetes mellitus, hypertension, hypercholesterolemia; use of prescription and OTC drugs);

C. Reproductive/menopausal history (date of last menstrual period, number of periods in the prior 12 months, hysterectomy, oophorectomy, parity, age of onset of hot flashes, current and past use of hot flash treatments)

D. Blood and urine testing: Follicle stimulating hormone levels will be measured in women under the age of 60 years with a history of hysterectomy without oophorectomy. Urine pregnancy testing will be performed in women who may still be of child-bearing potential.

G.2 Primary and Secondary Outcomes

- The primary outcome of this trial is *change in the average frequency of any hot flashes* from baseline to 4 and 12 weeks, as documented by symptom diaries.
- Secondary outcomes include changes in frequency of moderate-to-severity hot flashes and total hot flash severity score from baseline to 4 and 12 weeks of treatment, also documented by symptom diaries.
- Additional secondary efficacy outcomes include changes in sleep quality, anxiety and depression symptoms, and menopause-related quality of life from baseline to 4 and 12 weeks of treatment, assessed using validated questionnaires.
- Additional safety outcomes include rates of severe headache, chest pain, and syncope, as well as all adverse events with a Common Terminology for Adverse Event Criteria severity grade of 3 or higher.

H. STUDY PROCEDURES

H.1 Screening and Baseline Evaluations

H.1.1 Schedule of screening and baseline evaluations

Screening evaluations will be conducted by study coordinators based at outpatient facilities on the Mt. Zion campus of UCSF or in rented facilities in surrounding counties, or through video-based assessments using UCSF's secure Zoom on-line video platform. Screening procedures will include a brief preliminary Screening Telephone Interview, followed by an in-person or video-based Screening Clinic Visit, completion of a 7-day hot flash diary at home, completion of a 3-day run-in period with 0.1 mg/hr NTG, and review of the diary and run-in medication tolerance at an in-person or video-based Baseline Clinic Visit.

All screening procedures must be completed within a 90-day period in order for participants to be eligible for randomization. If screening procedures cannot be completed within 90 days, initial screening procedures must be repeated to ensure that participant eligibility has not changed prior to randomization.

H.1.2 Consenting procedure

Prior to administration of any data collection instruments, signed consent will be obtained by a research coordinator at the beginning of the Screening Clinic Visit. A single informed consent process will be used that will cover both the screening and post-randomization procedures. All participants must be able to read and

understand the consent form in English and must provide written informed consent before enrolling in the study.

Following the UCSF IRB-approved template, the consent form will describe the purpose of the study, the procedures involved in recruiting, randomizing, and monitoring participants, and the potential risks and benefits associated with participation. A study coordinator will first explain the study procedures to the potential participant, referring as necessary to the detailed IRB-approved consent form. The coordinator will then give a paper copy or electronic copy of the consent form to the potential participant to read. After the potential participant has read the consent form, she will be asked if she has any questions or concerns about the study. Once these questions/concerns have been addressed, the participant will be asked to sign or electronically sign the consent form, and will be given a copy of the consent form to take home for future reference.

Coordinators will also have each participant read and sign a Health Information Portability and Accountability Act (HIPAA) authorization form granting study staff permission to access protected health information if needed. Pursuant to California Health & Safety Code 24172, each participant will also receive a copy of the Experimental Subject's Bill of Rights. A copy of the signed consent form and HIPAA authorization form will be stored in the participants' chart in locked research offices or in a secure electronic folder.

H.1.3 Screening and baseline procedures

A. Telephone Screening Interview

- Women who call in response to recruitment advertisements or mailings or who have previously given permission to be contacted about opportunities to participate in women's health research at UCSF will be provided with a brief overview of the study goals, design, and procedures by a clinical coordinator over the telephone.
- If interested, candidates will complete a brief telephone survey to assess preliminary eligibility (including age, gender, menopausal history, duration/frequency of hot flashes, exclusionary conditions, and medications,). Potentially eligible respondents will be invited to attend a clinic screening visit (either in-person or video-based) and will be asked to bring all medications to the visit.

B. Screening Clinic Visit

- At this visit, a clinical coordinator will explain the requirements of the study while referring to the detailed informed consent form, and candidates will provide written or electronic informed consent if still interested in proceeding with the study.
- Candidates will complete questionnaires about their demographic and medical history to determine if they meet criteria related to age, gender, menopausal history, duration/frequency of hot flashes, and exclusionary conditions.
- Over-the-counter and prescription medications will be reviewed to determine if candidates are taking any exclusionary medications.
- Women will be queried about alcohol use, and those reporting >3 drinks in a given day or >7 drinks per week will be asked if they are comfortable decreasing their alcohol intake during the study period to decrease their risk of side effects of NTG therapy.
- Candidates will be asked to complete structured-item self-report questionnaires about sleep quality, anxiety and depression symptoms, and condition-specific quality of life.
- Height, weight, and resting and orthostatic blood pressure and heart rate measurements will be obtained; candidates may be excluded for either high or low blood pressure.

- A urine sample will be collected to rule out pregnancy (for candidates who may still be of child-bearing potential).
- Candidates under the age of 60 who have a history of hysterectomy without oophorectomy will be sent for FSH hormone testing.
- Candidates who have cardiovascular risk factors such as hypertension, hyperlipidemia, or smoking will undergo a screening resting 12-lead electrocardiogram (ECG) administered by a trained clinical coordinator.
- Potentially eligible women will be instructed on completing a 7-day hot flash diary at home to document the frequency and severity of their hot flashes
- Potentially eligible women will also be given a supply of 0.1 mg/hr NTG patches and instructions on using patches during a 3-day run-in period following completion of their 7-day hot flash diary; of note, participants will be specifically instructed to wait until they have completed their 7-day diary before starting to use the run-in NTG medication.
- Women who are not yet postmenopausal will be counseled to refrain from sexual activity or use barrier contraceptives during the study period.
- A Baseline Clinic Visit will be scheduled at least 10 days after the Screening Clinic Visit to review the completed hot flash diary, assess tolerance to the run-in medication, and determine final eligibility.
- Women will be given a list of prohibited medications that they should avoid for the duration of their participation in this study.

C. Baseline Clinic Visit

- Participants will return the completed 7-day hot flash diary, and results will be reviewed by a clinical coordinator to determine if they have sufficient frequency and severity of hot flashes to be eligible for the study.
- Potentially severe side effects of NTG will be systematically assessed and recorded, other unanticipated adverse events that are reported will also be documented according to standard protocols, and participants' overall success in using the run-in NTG patches will be assessed.
- Additional resting and orthostatic measurements of blood pressure and heart rate will be obtained to assess whether blood pressure readings are within acceptable parameters for randomization. If the screening diary reveals sufficient frequency and severity of hot flashes, average blood pressure at both the screening and baseline visits is within acceptable parameters, the participant was successful in applying and changing the 0.1 mg/hr patch during run-in without developing a significant safety or tolerability issue, and she meets all other eligibility criteria, then she will be eligible for randomization to NTG or placebo.
- Women who are not yet postmenopausal will be reminded to refrain from sexual activity or use barrier contraceptives; those who report unprotected sexual activity will be asked to provide a urine sample for pregnancy testing.

H.1.4 Enrollment, randomization, and blinding

A. Enrollment

Enrollment in the main part of the study will be defined by randomization to either the NTG or placebo interventions. Informed consent will not be formally reassessed upon enrollment, as the original informed consent process will encompass both screening and post-randomization procedures. However, if a participant declines to proceed with randomization at the Baseline Clinic Visit, then she

will not be formally enrolled in the main part of the study.

B. Randomization

Following run-in, eligible women will be randomized in equal ratios to the NTG or placebo interventions using a computer algorithm with randomly permuted blocks of size 2 and 4. To ensure similar distribution of women of different menopausal stages in each group, randomization will be stratified by menopausal stage (i.e., postmenopausal vs late menopausal transition), although there will be no pre-set sample size threshold for any specific stratum. Within each category, randomization numbers identifying the supply of medication for each participant will be generated and then assigned sequentially in the order participants are randomized.

To avoid manipulation of randomization, standard allocation concealment procedures will be followed (Schulz and Grimes 2002). Randomization numbers will be pre-printed on medication foil packets and master packs, prepared by Clinical Trials Services. At baseline, after screening data have been reviewed and the principal investigator has confirmed eligibility, a coordinator will enter the date and participant ID into the randomization log next to the number that has not yet been assigned, and dispense a supply of the drug identified by the randomization number. To provide a check on validity, an analyst will check that the randomization dates and times follow the order of the sequence numbers.

Based upon randomization, participants will be given a supply of either 0.2 mg/hr NTG or placebo patches and will be instructed to apply their first patch that night and change their patches on a nightly basis, without any patch-free periods, until the 1-week visit.

C. Blinding

Consistent with a double-blinded design, participants, investigators, and any study staff who have contact with participants or role in assessing outcomes will be blinded to treatment assignment. Only the statistician responsible for generating the randomization scheme and the staff of Clinical Trials Services contracted to package the active and placebo patches will have access to treatment assignment. Unless stipulated by the Data & Safety Monitoring Board or required for management of a participant with a serious adverse event, assignment codes will not be broken until all participants have completed the study, the trial data are cleaned, and the dataset is locked.

H.2.5 Follow-up visits

Follow-up visits will consist of a 1-week clinic visit, 2-week clinic visit, 5-week clinic visit, 8-week telephone call, 12-week clinic visit, and 4-week post-discontinuation telephone call. These visits will be conducted by coordinators based at outpatient research facilities on the Mt. Zion campus of UCSF or in rented facilities in surrounding counties, or through secure video-based platforms.

A. 1-Week Clinic Visit

- Approximately 1 week after randomization, women will return for an in-person or video-based visit, bringing their study medication diaries and any unused medication.
- Potentially severe side effects of NTG will be systematically assessed and recorded, and other unanticipated adverse events that are reported will also be documented according to standard protocols.
- Study coordinators will review medication diaries and tabulate unused patches to assess adherence and trouble-shoot barriers to adherence.
- Two resting blood pressure and heart rate measurements and orthostatic measurements will be obtained by a trained study coordinator.
- Women who report no symptoms of hypotension or other safety issues, have blood pressures

within acceptable parameters, and continue to report bothersome hot flashes will be asked to increase to the 0.4 mg/hr NTG patch (or identical placebo) at this visit. Those who are not eligible or who decline to increase to 0.4 mg/hr will be permitted to remain on 0.2 mg/hr.

- Women who are not yet postmenopausal will be reminded to refrain from sexual activity or use barrier contraceptives; those who report unprotected sexual activity will be asked to provide a urine sample for pregnancy testing.
- An appropriate supply of study medication (or placebo) will be dispensed, and participants will be asked to apply NTG or placebo patches on a nightly basis leading up to the 2-Week Clinic Visit.

B. 2-Week Clinic Visit

- Approximately 2 weeks after randomization, women will return for an in-person or video-based visit, bringing their study medication logs and any unused medication.
- Potentially severe side effects of NTG will be systematically assessed and recorded, and other unanticipated adverse events that are reported will also be documented according to standard protocols.
- Study coordinators will review medication diaries and tabulate unused patches to assess adherence and trouble-shoot barriers to adherence.
- Two resting blood pressure and heart rate measurements and orthostatic measurements will be obtained by a trained study coordinator during an in-person visit, or participants will be guided by the coordinator in obtaining these measurements at home during a video visit.
- Women who report no symptoms of hypotension or other safety issues, have blood pressures within acceptable parameters, and continue to report bothersome hot flashes will be asked to increase to the next higher dose patch (0.6 mg/hr for those previously on 0.4 mg/hr, or 0.4 for those previously on 0.2 mg/hr) or corresponding placebo. Those who are not eligible or who decline to increase their dose will be permitted to remain on a lower dose.
- Women who are not yet postmenopausal will be reminded to refrain from sexual activity or use barrier contraceptives; those who report unprotected sexual activity will be asked to provide a urine sample for pregnancy testing.
- Participants will be given a second hot flash diary and asked to record all hot flashes during the 7 days prior to their 5-Week Clinic Visit.
- An appropriate supply of study medication (or placebo) will be dispensed and participants will be asked to apply NTG or placebo patches on a nightly basis leading up to the 5-Week Clinic Visit

C. 3-Week Telephone Call (for women taking 0.6 mg/hr NTG (or placebo)

- If a participant was started on 0.6 mg/hr NTG (or placebo) at the 2-Week Clinic Visit, the study coordinator will call the participant 1 week later to discuss adherence, assess adverse events, and address questions.
- If a safety concern is raised by this call, the participant will be asked to come in immediately for an in-person or video-based visit (or directed to seek emergency medical attention, as appropriate)
- Participants will be reminded of when to start their next hot flash diary at least 7 days before the 5-Week Clinic Visit.

D. 5-Week Clinic Visit

- Approximately 5 weeks after randomization, women will have a follow-up visit, returning their study medication logs and any unused medication, as well as their second completed hot flash diary.
- Potentially severe side effects of NTG will be systematically assessed and recorded, and other unanticipated adverse events that are reported will also be documented according to standard protocols.
- Study coordinators will review medication diaries and tabulate unused patches to assess adherence and trouble-shoot barriers to adherence.
- Two resting blood pressure and heart rate measurements and orthostatic measurements will be obtained by a trained study coordinator during an in-person visit, or participants will be guided by the coordinator in obtaining these measurements at home during a video visit.
- Data from the second completed hot flash diary will be reviewed and abstracted by a blinded coordinator.
- Participants will complete the same questionnaires about sleep quality, anxiety and depression symptoms, and menopausal quality of life that they completed at baseline.
- Participants will be given a third hot flash diary and asked to record all hot flashes during the 7 days prior to their 12-Week Clinic Visit.
- Women who are not yet postmenopausal will be reminded to refrain from sexual activity or use barrier contraceptives; those who report unprotected sexual activity will be asked to provide a urine sample for pregnancy testing.
- An appropriate supply of study medication (or placebo) will be dispensed or mailed, and participants will be asked to apply NTG or placebo patches on a nightly basis leading up to the 12-Week Clinic Visit.

E. 8-Week Telephone Call

- Approximately 8 weeks after randomization, the study coordinator will call each participant to discuss adherence, assess adverse events, and address questions.
- At the participant's request, or if there is a safety concern, this telephone call can be converted to an in-person visit.
- Participants will be reminded to start their third hot flash diary one week before the12-Week Clinic Visit.

F. 12-Week Clinic Visit

- Approximately 12 weeks after randomization, women will return for an in-person or video-based visit, bringing their study medication logs and any unused medication, as well as their third completed hot flash diary.
- Potentially severe side effects of NTG will be systematically assessed and recorded, and other reported adverse events will also be documented according to standard protocols.
- Study coordinators will review medication diaries and tabulate unused patches to assess adherence and trouble-shoot barriers to adherence.
- Participants will undergo repeat measurements of height and weight.
- Two resting blood pressure and heart rate measurements and orthostatic measurements will be obtained by a trained study coordinator.

- Data from the third completed hot flash diary will be reviewed and abstracted by a blinded coordinator.
- Participants will complete the same questionnaires about sleep quality, anxiety, and depression symptoms, and menopausal quality of life that they completed at baseline.
- Participants will be asked to wean off NTG (or placebo) over the next 1 to 3 days, by decreasing their patch dose on a nightly basis before stopping NTG/placebo completely. Women will be given a last supply of NTG or placebo medication to complete the weaning.
- Once weaning is complete, participants will be instructed to avoid using any NTG or nitrate products until after the post-discontinuation telephone call.
- A participant satisfaction questionnaire will be administered.

G. 4-Week Post-Discontinuation Telephone Call

- At least 28 days after the 12-Week Clinic Visit or after the participant discontinues study medication, study coordinators will contact participants by telephone.
- Potentially severe side effects of NTG will be systematically assessed and recorded by phone, and other reported adverse events will also be documented according to standard protocols.
- At the participant's request, or if there is a safety concern, this telephone call can be converted to an in-person visit.

H.2.6 Early termination

If a randomized participant opts to terminate the study before the 12-Week Clinic Visit, or if the principal investigator in consultation with the Data and Safety Monitoring Board determines that early termination is necessary to protect the safety of a participant or the integrity of the study, the study coordinator will encourage the participant to complete an early termination visit. This visit will include as many of the procedures originally scheduled to take place at the 12-Week Clinic Visit as possible, including: completion of a final hot flash diary; assessment of adverse events; re-administration of questionnaires about sleep quality, anxiety and depression symptoms, and condition-specific quality of life; review of current medications; re-assessment of weight, blood pressure, and heart rate; and administration of a satisfaction questionnaire. Study staff will provide instructions for weaning off NTG (or placebo) medication, and provide additional patches needed to complete the weaning. The participant's reasons for terminating the study early will be explored and documented in her study file.

H.2.7 Adherence to procedures and visits

Every effort will be made to assure that participants complete follow-up procedures and visits according to the study protocol. Participant contact information including address, phone number(s), fax number(s), and email address will be obtained at the Screening Clinic Visit. Study coordinators will also request contact information for two family members or friends who will be able to locate the participant. The study team will encourage retention by educating participants about the importance of the study, maintaining a friendly and efficient study clinic environment, and encouraging excellent staff-participant rapport. Participants who miss a visit will be contacted by the study coordinator to reschedule the visit and to provide assistance in completing the visit. Participants will also be offered gift cards with the return of each completed hot flash diary ($25 at the Baseline Clinic Visit and 5-Week Clinic Visit, and $50 at the 12-Week Clinic Visit).

H.2.8 Follow-up visit time windows

Suggested time windows for completion each of the post-randomization follow-up evaluations will be as follows:

Follow-Up Assessment	Time Window

1-week clinic visit	7 (± 3) days after the baseline clinic visit (i.e., 4 to 10 days after the baseline visit)
2-week clinic visit	14 (± 3) days after the baseline clinic visit (i.e., 11 to 17 days after the baseline visit)
3-week telephone call	21 (± 3) days after the baseline clinic visit (i.e., 18 to 24 days after the baseline visit)
5-week clinic visit	5 weeks (± 6 days) after the baseline clinic visit (i.e., 29 to 41 days after the baseline visit)
8-week telephone call	8 weeks (± 6 days) after the baseline clinic visit (i.e., 49 to 63 days after the baseline visit)
12-week clinic visit	12 weeks (± 6 days) after the baseline clinic visit (i.e., 78 to 90 days after the baseline clinic visit)
4-week post-discontinuation telephone call	28 to 42 days after the 12-week clinic visit (or discontinuation of study medication)

I. SAFETY ASSESSMENTS

I.1 Potential Risks and Protective Measures

I.1.1 Potential risks of study procedures

A. Potential risks associated with the therapeutic intervention (transdermal NTG) include:

Headache: Potential side effects of NTG therapy are related to the medication's vasodilatory action. The most commonly reported side effect is headache, occurring most frequently in the first 24 hours of administration (Tfelt-Hansen and Tfelt-Hansen 2009). In previous placebo-controlled trials of intermittent therapy with transdermal NTG (in which the NTG patch was removed at bedtime and replaced the next morning) at 0.2 to 0.8 mg/hr, approximately 60% of participants taking transdermal NTG reported headache, compared to 18% of participants taking placebo (Rossouw, Prentice et al. 2007). Among persons using continuous transdermal nitrate (immediately replacing the old patch with a new patch), development of tolerance to the vasodilatory effects of transdermal nitrate is expected within 12 to 24 hours, which should diminish the frequency and severity of headache. In the study team's prior pilot trial of continuous NTG for treatment of hot flashes in peri- and postmenopausal women, 37% of participants reported headache at some point during the 6-week study but the average duration of headache was less than 24 hours.

Lightheadedness/hypotension: Lightheadedness has been reported with initiation of NTG therapy, presumably due to lowering of blood pressure during the first 12 to 24 hours of administration. In prior placebo-controlled trials of intermittent NTG therapy for other indications, 6% of participants taking transdermal NTG reported transient lightheadedness thought to be associated with lowering of blood pressure, compared to 4% of participants taking placebo (Rossouw, Prentice et al. 2007). It is possible but uncommon for patients using transdermal NTG patches to develop hypotension severe enough to cause syncope. In the case of hypotension, removal of the transdermal patch should result in immediate (within seconds to minutes) normalization of blood pressure. Any lightheadedness or lowering of blood pressure associated with transdermal NTG therapy should

also diminish within 12 to 24 hours of continuous administration as tolerance to the vasodilatory effect of NTG therapy develops. In the study team's prior pilot trial of NTG for treatment of hot flashes, no participants developed lightheadedness or hypotension requiring discontinuation of treatment; average blood pressure remained in the normal range on all NTG doses: 127/79 (±14/9) mmHg, 129 /78 (±17/10) mmHg, 130/81 (±16/10) mmHg, and 125/77 (±13/11) mmHg on 0.1 mg, 0.2 mg/hr, 0.4 mg/hr, and 0.6 mg/hr nitroglycerin, respectively.

Chest pain or tightness: Among individuals with pre-existing coronary artery disease, continuous administration of transdermal nitrate beyond 12 to 24 hours could theoretically increase the risk of chest pain and/or de novo coronary events. This is because these individuals, having developed nitrate tolerance, may have decreased physiologic capacity to dilate their coronary arteries in response to exogenous or endogenous nitrates. Additionally, animal-based studies have pointed to the possibility of increased oxidative stress and/or endothelial dysfunction associated with chronic nitrate therapy, although there are currently no longitudinal data to indicate whether this may translate into adverse cardiovascular outcomes in humans. To minimize any safety concerns with the use of NTG (which at this point are largely theoretical), only individuals at very low risk of cardiovascular disease will be enrolled. In the study team's prior pilot trial of NTG for treatment of menopausal hot flashes, no chest pain was reported during four weeks of treatment or during the week after discontinuation of therapy.

Hypertension: With uninterrupted NTG therapy, there is also a theoretical risk of increased blood pressure associated with cross-tolerance to endogenous nitrates and decreased NO-mediated vasodilation, although limited studies of chronic use of nitrate medications in human subjects have not demonstrated significant increases in hypertension (Stokes, Bune et al. 2005). In the investigators' prior pilot trial of NTG for treatment of hot flashes, in which women with hypertension were permitted to enroll provided that they did not also have other major cardiovascular risk factors, no participants developed new-onset hypertension during the treatment period, and average blood pressure remained in the normal range on all NTG doses (i.e., 127/79 (±14/9) mmHg, 129 /78 (±17/10) mmHg, 130/81 (±16/10) mmHg, and 125/77 (±13/11) mmHg on 0.1 mg, 0.2 mg/hr, 0.4 mg/hr, and 0.6 mg/hr nitroglycerin, respectively) and after discontinuation of NTG (130/81 (±20/12).

Skin irritation: The nitroglycerin patch may also result in irritation or redness of the skin at the site where it is administered, due to local reaction to the patch adhesive.

B. Potential risks associated with other study interventions and data collection procedures include:

Placebo patch: Similar to the NTG patch, the placebo patch may result in irritation or redness of the skin at the site where it is administered, due to local reaction to the patch adhesive.

Study diaries and questionnaires: Although the information collected by diary and questionnaire will be kept confidential, some participants may feel embarrassed at having to answer questions about their hot flashes, their mood, or their quality of life. There will be slight inconvenience in time and effort to complete diaries and questionnaires.

Physical exam measurements: There are no direct risks associated with undergoing measurement of height, weight, blood pressure, and heart rate, which are routine physical measurements obtained in clinical practice. Nevertheless, some participants may experience these measurements as inconvenient or unpleasant.

Blood draws for sex hormone testing: Participants may develop pain and/or bruising at the site of the blood draw. There is also a possibility that participants will develop infection at the site, although this risk is minimized by use of sterile technique. Participants may experience the blood draw as inconvenient or unpleasant.

Urine pregnancy testing: There are no direct risks associated with urine pregnancy testing, although participants may experience this as inconvenient or unpleasant.

Electrocardiography (ECG) testing: It is possible for participants to develop a mild rash where the ECG electrodes are attached, but this rash usually resolves without treatment.

I.1.2 Protections against risks

A. Protections against potential risks of uninterrupted transdermal NTG therapy:

Headache: To reduce headache, women reporting a history of more than two headaches per month that interfere with their daily activities, or taking prescription medication to prevent or treat headache in the past month, will be excluded from participating in this study. Women reporting heavy alcohol use that could increase their risk of headache and who are uncomfortable decreasing their alcohol intake during the study will also be excluded. Women who develop significant headache during the brief run-in period with the 0.1 mg/hr NTG patch will also be excluded before randomization. Prior to administration of either nitroglycerin or placebo, women will be given handouts describing simple over-the-counter headache remedies, including acetaminophen (Tylenol) and ibuprofen (Motrin), as tolerated. At follow-up visits, transdermal NTG therapy may be decreased or stopped in women reporting headache associated with therapy that interferes with daily activities. Study staff will instruct participants who experience persistent headache that is not attributable to NTG therapy to seek further evaluation and treatment from their regular source of health care.

Lightheadedness/hypotension: To address potential lightheadedness/hypotension with initiation of dose-escalation of NTG, all potential and enrolled participants will undergo systematic blood pressure monitoring before, during, and after discontinuation of treatment. First, all women expressing interest in this study will undergo two seated blood pressure measurement at the screening and baseline visits. Women found to have resting blood pressures less than 90/60 mm Hg (both measurements) at either visit will be ineligible to enroll in the trial. Women will also undergo orthostatic blood pressure and heart rate measurements, and those who have normal resting blood pressure but show orthostatic changes with change in position will also be ineligible. Participants who have even one risk factor for coronary artery disease will undergo a screening ECG, and we will also exclude participants with uncontrolled tachyarrhythmias or second or third degree heart block on screening electrocardioigram (ECG), as being at greater risk of negative consequences from blood pressure fluctuations.

Women reporting heavy alcohol use that could increase risk of lightheadedness or hypotension and who are uncomfortable limiting alcohol intake during the study will also be excluded. Women with ECG evidence of uncontrolled tachyarrhythmias or second or third atrioventricular block will also be excluded due to potential increased risk of blood pressure fluctuations in these women. To minimize symptoms of hypotension associated with initiation of therapy, women will start their first transdermal nitrate patch at night shortly before going to bed, so that lightheadedness will be minimized by the fact that women will be lying supine overnight. Women will also be cautioned to maintain adequate fluid intake (at least 6 glasses of non-caffeinated, non-alcoholic fluid per day) to avoid low blood pressure associated with hypotension, and to consider limiting their intake of alcoholic beverages that may exert an additive vasodilatory effect with NTG.

At all on-treatment interim follow-up visits (i.e., 1-week, 2-week, 5-week), transdermal NTG will be decreased or stopped in women with blood pressures <90/60 mm Hg (both measurements), or in those with blood pressures <100/60 mm Hg and symptoms such as dizziness that are suggestive of hypotension. Study medication will also be decreased or stopped in women with symptoms such as dizziness who have a resting blood pressure in the normal range but demonstrate orthostatic changes in blood pressure or heart rate when rising from the supine to standing position. These

women will also be given a standardized letter documenting their blood pressure reading to take to their regular health care provider to promote further monitoring and/or evaluation. In the event that there is a concern about the reliability of clinic measurements, or women report symptoms suggestive of hypotension despite having blood pressure measurements in the normal range, home-based or ambulatory blood pressure measurements will be obtained, to detect episodes of hypotension that may be have been missed by clinic measurements. In the (hopefully unlikely) event that a participant reports a recent episode of syncope or loss of consciousness, study staff will also urge the participant to seek emergency medical attention.

Chest pain or tightness: Women reporting a history of coronary artery disease, diabetes, or two or more other major risk factors for coronary artery disease (smoking, hypertension, or hyperlipidemia with physician-recommended pharmacologic treatment) will be excluded from the study, to avoid the theoretical risk of increased chest pain or coronary events that could be associated with sustained transdermal nitrate therapy. Participants who have even one risk factor for coronary artery disease will undergo a screening ECG, and we will also exclude participants with evidence of myocardial infarction on screening ECG. Occurrence of chest pain severe enough to interfere with instrumental activities of daily living will be carefully monitored during the treatment period as part of the safety aim of the trial. Although the study team does not anticipate that many participants will develop severe chest pain interfering with daily activities, if any participant reports current or recent chest pain suggestive of ischemia at a study visit, she will be instructed to seek emergency medical attention. At the end of the treatment period, participants will be weaned off study medication rather than discontinuing medication abruptly, to minimize any risk of rebound chest pain.

Hypertension: Potential participants will undergo measurement of blood pressure at both the screening and baseline visits, and those with blood pressure >180/110 at baseline will be excluded from the study due to the theoretical risk of increased blood pressure with induction of nitrate cross-tolerance. (Women may still seek further anti-hypertension therapy from their regular sources of health care and re-present for screening if desired). At all on-treatment interim follow-up visits (i.e., 1-week, 2-week, 5-week), blood pressure will be reassessed, and transdermal NTG will be decreased or stopped in women with blood pressures >180/110 mm Hg. Women with blood pressure >180/110 at any study visit will also be urged to seek emergency medical attention. Additionally, women with blood pressures >160/100 will be given a standardized letter to take to their regular health care provider to promote further evaluation and/or treatment. In the event that there is a concern about the reliability of clinic measurements, or women report high blood pressure measurements obtained elsewhere despite having blood pressure measurements in the normal range at their study visits, home-based or ambulatory blood pressure measurements may be obtained, to detect uncontrolled hypertension that may be have been missed by in the study clinic. At the end of the treatment period, participants will be weaned off study medication rather than discontinuing medication abruptly, to minimize any risk of rebound hypertension.

Skin irritation: Women with known allergy to adhesives will be excluded, as these women may have a more significant reaction to the adhesive associated with the transdermal NTG patch. Women will also be advised to rotate the location of the patch on a nightly basis to minimize risk of skin reaction or breakdown. During the brief run-in period with the 0.1 mg/hr NTG patch, women who are unable to tolerate the patch will be excluded. If women subsequently develop significant skin irritation in response to using the transdermal nitrate (or placebo) patch, their participation in the study will be terminated early.

B. Protections against potential risks associated with other study data collection procedures:

Placebo patch: Women with known skin sensitivity to adhesives will be excluded, as these women may have a more significant reaction to the adhesive associated with the placebo patch. Women will also be advised to rotate the location of the patch on a nightly basis to minimize risk of skin

reaction or breakdown. During the brief run-in period with the 0.1 mg/hr NTG patch, women who are unable to tolerate the patch adhesive will be excluded. If women subsequently develop significant skin irritation in response to using the patch, their participation in the study will be terminated early.

Study diaries and questionnaires: Any paper-based source forms will be stored in a locked file cabinet in a locked room in the research clinic. All electronic data will be stored on password-secured servers and accessed by secure computers based at the UCSF Women's Health Clinical Research Center. All study staff will be required to be fully trained in good clinical practice, HIPAA procedures, and data security. At weekly study team meetings led by the principal investigator, the importance of participant confidentiality will be emphasized.

Physical exam measurements: Clinical coordinators will be trained to be courteous and discrete when obtaining physical exam measurements. They will also be trained to follow-up with referrals to appropriate medical personnel when measurements are outside allowable ranges. Every clinical staff member will complete a dedicated in-person training session and will be certified by the principal investigator or project director before being permitted to obtain height, weight, blood pressure, and heart rate measurements as part of the study.

Blood draws for sex hormone testing: Participants will be sent to the Quest Diagnostics Laboratory for blood draws by experienced phlebotomists, who will use standard sterile technique and are trained to minimize patient discomfort.

Urine pregnancy testing: Clinical coordinators will be trained to collect urine samples in a sensitive and professional manner. This test can also be performed by the participant at home, under the guidance of a coordinator during a video visit, using a home pregnancy testing kit provided to the participant.

Electrocardiography (ECG) testing: Women with known skin sensitivity to adhesives will be excluded.

I.2 Methods and Timing of Safety Assessments

To monitor participant safety, a clinical coordinator will assess for adverse events at each in-person or follow-up contact following initiation of study medication, starting with the Baseline Clinic visit. As noted in section I.3 below, coordinators will systematically and proactively assess for a pre-established set of severe side effects that are potentially attributable to NTG therapy. They will also encourage participants to volunteer information about other unanticipated adverse events by asking the standardized, open-ended question, "Have there been any changes in your health since your last visit?" Negative changes in health will be recorded as adverse events or serious adverse events on standardized forms as appropriate (see section I.3.1 below for definitions and documentation). Additionally, if participants contact clinical coordinators in between scheduled study visits and calls and report negative changes in their health, these will also be recorded as adverse events or serious adverse events.

I.3 Adverse Events and Serious Adverse Events

I.3.1 AE/SAE definitions

An Adverse Event (AE) is any untoward or unfavorable medical occurrence in a study participant, including any abnormal sign (e.g. abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the participants' involvement in the research, whether or not considered related to participation in the research. Medical conditions or diseases present before starting study interventions will only be considered adverse events if they worsen after starting the intervention.

A Serious Adverse Event (SAE) is any AE that results in death, Is life threatening, or places the participant at immediate risk of death from the event as it occurred, requires or prolongs hospitalization, causes persistent or significant disability or incapacity, results in congenital anomalies or birth defects, or any other important event judged by the investigators to jeopardize the safety of a participant based upon appropriate medical judgment.

I.3.2 AE/SAE documentation

At each follow-up visit or phone call after initiation of NTG or placebo, blinded study coordinators will systematically assess for the following severe side effects that could be attributable to NTG therapy: 1) headache severe enough to interfere with instrumental activities of daily living; 2) chest tightness or pain interfering with instrumental activities of daily living, and 3) syncope (fainting or loss of consciousness). If reported, these symptoms will be recorded on standardized SAE or AE forms, depending on whether they meet the threshold for being an SAE or a non-serious AE. These standardized SAE and AE forms will indicate date of onset, date of resolution, likelihood of relationship to treatment, and actions taken in response to the event.

To collect information about other or unanticipated adverse events, participants will also be asked about any other negative changes in their health at each telephone and in-person follow-up contact after initiation of therapy, using the standardized question, “Have there been any changes in your health since your last visit or call?” Participants will also be given telephone numbers to call study staff in between scheduled study visits or calls if they wish to report any health changes in between scheduled study visits or calls. Any negative changes in health that are reported will again be recorded as AEs or SAEs using standardized forms, as appropriate.

Additionally, using the Common Terminology Criteria for Adverse Events (CTCAE) system, all adverse events will be graded in severity using the 5-point CTCAE scale. According to this scale, AEs are considered grade 1 if they represent only mild symptoms or asymptomatic laboratory findings; grade 2 if they cause moderate symptoms that limit instrumental activities of daily living (shopping, transportation, household tasks); grade 3 if they are serious or disabling events or result in hospitalization or prolongation of hospitalization without being directly life-threatening; grade 4 if they are life-threatening events in which the participant is at risk of death at the time of the event if immediate intervention is not undertaken; or grade 5 if they are fatal adverse events.

I.3.3 AE/SAE reporting procedures

A. SAE reporting

Study personnel will notify the principal investigator immediately when a SAE is discovered. The principal investigator will report any SAE deaths to the NIA Program Officer within 24 hours of awareness of the event. Other SAEs that are potentially related to the intervention will also be reported to the NIA Program Officer within 24 hours of awareness of the event. The NIA Program Officer will then notify the Data and Safety Monitoring Board (DSMB) Chairperson, who also serves as the Safety Officer for this study, within 24 hours of notification by the principal investigator.

Other SAEs that are unlikely to be related to the intervention may be handled in a less urgent manner but should also be reported to the NIA Program Officer within 5 working days of awareness of the event; the NIA Program Officer will then forward the report to the DSMB Chairperson. Additionally, all SAEs will be reported to the UCSF Institutional Review Board (IRB) within 5 working days of awareness of the event, consistent with UCSF IRB guidelines.

If a clinical coordinator discovers an AE that does not meet the definition of a SAE but: 1) is unexpected in terms of nature, severity, or frequency; 2) is potentially associated with study interventions or procedures, and 3) may still pose a substantial risk of harm to a participant, then he/she will report this unexpected problem to the principal investigator within 1 working day, in addition to filling out a standardized AE form. The principal investigator will in turn report the event to the UCSF IRB and the NIA Program Officer within 2 weeks of awareness of the event, along with

a proposed corrective plan and measures to prevent reoccurrence. The NIA Program Officer will forward the report and the proposed plan to the DSMB Chair.

Unblinding of treatment assignment may be performed if the principal investigator in cooperation with co-investigators determines that knowledge of treatment assignment is necessary to assist with the provision of care to a participant suffering an SAE. Unblinding decisions will be made on a case-by-case basis during the course of the study, with input from participants' health providers as appropriate. Similarly, the study investigators may decide to terminate participants' involvement in the trial prior to their expected termination date, if early termination is thought to be necessary to safeguard the health of participants with SAEs.

B. Non-serious AE reporting

Adverse events that are not SAEs and are not unexpected problems that are potentially associated with the study therapy will not necessarily be reported to the IRB, the DSMB, or the NIA on an individual basis, but cumulative trends in non-serious AEs will be reviewed by the principal investigator and the DSMB members at scheduled DSMB meetings, and the summary report of DSMB will be forwarded to the IRB after each meeting (see "Data and safety monitoring board" section below). More frequent review of non-serious adverse events may be performed if recommended by the DSMB or desired by the principal investigator.

I.4 Independent Safety Monitoring

The conduct of the study and safety of participants will be evaluated by an independent Data and Safety Monitor Board, composed of three members who collectively provide experience in clinical trials, research ethics, and statistics (see Data and Safety Monitoring Plan). The DSMB members will periodically review the conduct and outcomes of the study and provide feedback to the NIA sponsor and the investigators, with particular attention to protecting the safety of study participants. The DSMB members will be independent of the institution and the investigators as well as the sponsor and have no financial ties to the outcome of the study.

Prior to initiation of the trial, the DSMB will review and approve the study design and plans for recruitment, adherence, interventions, data quality, and safety monitoring. At periodic intervals during the course of the trial, the DSMB will evaluate the adequacy and timeliness of participant recruitment, evaluate the ability of the trial to reach stated goals, review adherence to visits and protocols, assess data quality and timeliness, evaluate the safety of participants, provide a report to the investigators and the IRB on the scientific progress of the trial and the safety of participants, make recommendations to the investigators on continuation, termination, or other modifications of the trial, and consider factors external to the study (i.e., new scientific or therapeutic developments) when relevant to the safety of the participants or the ethical conduct of the trial.

Prior to the start of participant recruitment, the DSMB will review and approval the study protocol, Data and Safety Monitoring Plan, and plans for recruitment and follow-up. After recruitment beings, the DSMB will periodically review aggregate and unblinded trial data after 20, 50, 80, and 110 participants complete the 5-week visit. In the event that a year has passed since the last DSMB review but the usual milestone for DSMB review has not yet been reached, an annual DSMB review meeting will still be held. An emergency meeting may also be called at any time should questions of participant safety arise. Each review will include an assessment of the adequacy and timeliness of participant recruitment, adherence to the visit and intervention protocols, data quality and timeliness, adverse effects, and participant safety.

Given that the study is of short duration, no assessment of interim efficacy will be done, and the study will not be stopped or altered for unexpected efficacy or futility. Interim reports for the DSMB will be prepared by an unblinded biostatistician at the Women's Health Clinical Research Center, and sent to the DSMB at least 7

days prior to a pre-scheduled meeting or conference call. A copy of the interim reports will be retained in a locked, confidential file by the DSMB chair.

After each interim review, the DSMB chair will provide a signed statement that indicates whether the study should continue, terminate, or be altered based on ability to meet study recruitment and data quality goals and participant safety. The DSMB will include any recommendations for changes to the protocol if necessary to enhance participant safety or potentiate the ability of the trial to answer the research hypotheses. This statement will be forwarded to the principal investigator and will be sent to the UCSF IRB. All materials, discussions, and proceedings of the DSM process will be completely confidential.

J. INTERVENTION DISCONTINUATION

J.1 Discontinuation Decisions

Study medication may be discontinued if deemed necessary to protect the safety of a participant or preserve the integrity of the study. The decision to discontinue study medication will be made on a case-by-case by the principal investigator, with input from the steering committee and the DSMB as needed. In most cases, discontinuation of study medication will prompt early termination of study participation, although there may be select cases where study medications are discontinued only temporarily, and the participant is subsequently restarted on study medication and then followed for the full duration of the study.

Specific reasons for discontinuation of study medication and/or early termination of the study include: 1) syncopal episode attributable to NTG-induced hypotension or vasodilation; 2) development of persistent blood pressure <90/60, blood pressure <100/60 with symptoms of dizziness, or orthostatic blood pressure with symptoms of dizziness, which do not resolve with reduction in study medication dose; 3) development of persistent severe hypertension defined by blood pressure >180/110; 4) development of pregnancy or decision to try to become pregnant (among women who are not yet postmenopausal); 5) development of another clinically significant adverse event limiting a participant's ability to safety continue to use the study drug; 6) unusually disruptive behavior exhibited by a participant during study visits that endangers the safety or well-being of the participant or study staff; or 7) decision to terminate the study by the IRB, the NIA, or other regulatory bodies.

Participants may also withdraw voluntarily from participation in the study at any time and for any reason. If a participant decides to discontinue the study drug or withdraw from the study, the study coordinator will try to elicit the reason for this decision. The coordinator may also try to trouble-shoot participant concerns or discuss strategies for overcoming barriers to continued participation in the study in order to try to retain the participant, provided that the decision to terminate is not driven by a safety issue.

J.2 Discontinuation/Termination Procedures

In the event that study medication is discontinued early, study coordinators will attempt to schedule an early termination visit to collect outcomes data, provided that the participant is willing, and provided that the principal investigator (with input from the steering committee and DSMB as appropriate) judges that it is safe and feasible to do so.

This visit will include as many of the procedures originally scheduled to take place at the 12-Week Clinic Visit as possible, such as: completion of a final hot flash diary; assessment of adverse events; re-administration of other symptom and quality-of-life questionnaires; review of current medications; re-assessment of weight, blood pressure, and heart rate; and administration of a satisfaction questionnaire.

Regardless of whether the participant is able to complete an early termination visit, the study coordinator will document the reason for early termination in the participant's file, except in cases where a participant is lost to follow-up or declines to provide a reason for deciding to discontinuing participant early.

Upon study termination, participants will be asked to wean off NTG (or placebo) over the next 1 to 3 days, by decreasing their patch dose on a nightly basis before stopping NTG/placebo completely. Specifically, participants who are being weaned from the 0.6 mg dose of study medication will be instructed to switch to 0.4 mg for one day, then to 0.2 mg for one day, then to stop study drug altogether. Participants who are being weaned from the 0.4 mg dose will switch to 0.2 mg for one day before stopping medication altogether. Women who are taking the 0.2 mg (the lowest dose hypothesized to have a therapeutic effect) will simply stop study medication without any change in dose. Participant and staff blinding will be maintained throughout the weaning process; for participants taking placebo, weaning will use placebo rather than active NTG patches. If necessary, women will be given a last supply of NTG or placebo medication (one patch of each dose strength needed to complete the weaning).

In the event that a participant develops a potentially severe adverse event such as chest pain during weaning, she will be directed to seek immediate emergency attention, just as she would during any other time point of the study. Although all study medication will be stopped while the participant receives emergency care, it should be possible to manage any side effects safely in the emergency care setting regardless of medication discontinuation.

Study coordinators will also try to schedule and complete a post-discontinuation telephone call that will occur at least 28 days after discontinuation of study medication, to promote appropriate monitoring of adverse events following discontinuation of study drug.

K. STATISTICAL CONSIDERATIONS

K.1 General Design Issues

A randomized, parallel-group, placebo-controlled trial design has been selected to examine the following aims and test the following hypotheses related to the efficacy, safety, and tolerability of transdermal NTG for treatment of menopausal hot flashes:

Objective 1: Determine the efficacy of uninterrupted transdermal NTG therapy in reducing the frequency of hot flashes in peri- and postmenopausal women.

Hypothesis 1: Compared to placebo, uninterrupted use of NTG will result in at least a 20% greater decrease in the frequency of hot flashes (measured by validated symptom diaries) over 12 weeks.

Objective 2: To evaluate the efficacy of uninterrupted transdermal NTG therapy in reducing the severity of hot flashes in peri- and postmenopausal women.

Hypothesis 2: Compared to placebo, uninterrupted use of NTG will result in significant improvements in frequency of moderate-to-severe hot flashes and in cumulative hot flash severity score (assessed by validated symptom diaries) over 12 weeks.

Objective 3: Evaluate the efficacy of uninterrupted transdermal NTG therapy in improving other symptom- and quality-of-life outcome associated with hot flashes in peri- and postmenopausal women.

Hypothesis 3: Compared to placebo, uninterrupted use of NTG will result in significant improvements in hot flash-related sleep quality, anxiety and depression, and menopause-related quality of life over 12 weeks.

Objective 4: Examine the tolerability and safety of uninterrupted transdermal NTG therapy in peri- and postmenopausal women with hot flashes.

Hypothesis 4: Compared to placebo, uninterrupted use of NTG will be associated with higher rates of mild

headache during the first 24 hours of therapy, but no significant differences in rates of chest pain, syncope, or severe adverse events defined by CTCAE severity grade 3 or higher.

K.2 Data Analyses

K.2.1 Analyses for Objective 1

Analyses to address aim 1 will examine change in average daily hot flash frequency from baseline to 4 and 12 weeks of treatment, measured over 7 days by validated hot flash diaries (Sloan, Loprinzi et al. 2001, Food and Drug Administration 2003). The effect of NTG therapy will be estimated using linear mixed models (LMMs) for the repeated changes, adjusting for baseline values, under the assumption of an approximately constant treatment effects at 4 and 12 weeks; in supplementary analysis we will assess evidence for differences in the treatment effects at these two time points. The distribution of the LMM residuals will be assessed using Q-Q plots, and outcomes will be transformed if necessary to achieve approximate normality. Analyses will be by intention to treat, according to treatment assignment, and without regard to adherence.

K.2.2 Analyses for Objective 2

For additional confirmation of treatment effects on frequency of any hot flashes, we will also assess treatment effects on moderate-to-severe hot flashes and on total hot flash severity scores as secondary outcomes, also assessed by diary, using the same methods. The distribution of the LMM residuals will again be assessed using Q-Q plots, and outcomes will be transformed if necessary to achieve approximate normality. Analyses will again be by intention to treat, according to treatment assignment, and without regard to adherence.

K.2.3 Analyses for Objective 3

Analyses to address aim 2 will examine changes in sleep quality and duration, anxiety and depressive symptoms, and menopausal quality-of-life from baseline to 4 and 12 weeks. These outcomes will be captured by scores on validated self-administered questionnaires, with supplementation by sleep diary in the case of sleep duration. Intervention effects on these outcomes will be estimated using LMMs, adjusted for baseline scores. Approximate normality of the residuals will be checked using Q-Q plots, and outcomes will be transformed if necessary. Analyses will once again be by intention to treat and without regard to adherence.

K.2.4 Analyses for Objective 4

To assess tolerability and safety of NTG, we will compare group-specific rates of: 1) severe symptoms that are potentially attributable to NTG therapy (i.e., headache limiting activities of daily living, chest pain or tightness limiting activities of daily living, or syncope/fainting); and 2) any adverse events with a severity rating of 3 or higher on the CTCAE severity scale (National Cancer Institute 2009). We will also examine group-specific rates of all adverse events, regardless of severity and of anticipated relationship to NTG therapy. Inferences for rare outcomes will be obtained using Fisher's exact test. Because intention-to-treat analysis is conservative, any suggestive differences in safety outcome rates will be assessed accounting for adherence, final dose, and their interactions with treatment.

K.3 Sample Size Determination

Sample size calculations are based on parameter estimates from prior pharmacologic hot flash trials conducted by the investigators such as the FAST(Grady, Cohen et al. 2007) and CHIMES trials (Grady, Sawaya et al. 2009), which had similar eligibility criteria with respect to age, hot flashes, and treatment history. We assume the study sample will be characterized by: a) mean baseline hot flash frequency of >9 hot flashes/day; b) mean reduction in hot flash frequency in the control group of <3 hot flashes/day (i.e., <33%); c) standard deviation of change in hot flash frequency of ~4.5 hot flashes per day; and d) correlation between baseline and follow-up values of ~0.55.

Under these assumptions, a sample size of 140 (70 per group) should provide an estimated 87% power in 2-sided tests with type-I error of 5% to detect an 20% greater reduction in hot flash frequency (or 1.8 hot flashes per day) in the active treatment arm relative to the control arm. This will correspond to a 50-55% decrease in hot flash frequency from baseline in the active treatment group, which has previously been described as a clinically important effect in prior hot flash research (Butt, Deng et al. 2007). Estimates account for correlation of the repeated measures and loss to follow-up of up to 15%, and indicate that power will remain ≥80% even if actual effect size or other parameters are less favorable than expected. This sample of 70 per group will also provide >80% power in 2-sided tests with type-I error of 5% to detect a between-group difference in mean frequency of moderate-to-severe hot flashes of ~1.5 hot flashes per day, as well as ~3.3 points in hot flash severity scores.

K.4 Additional Statistical Considerations

K.4.1 Drop-out rates

Based on past experience, participant dropout levels are expected to remain under 15% over 12 weeks. The assumption of non-informative dropout will be examined by comparing the baseline characteristics and early post-randomization outcomes of participants who are lost to follow-up versus retained in the study. If drop-out rates differ substantially between groups, analyses will be adjusted for baseline covariates associated with retention. In sensitivity analyses, we will also use multiple imputation of missing outcomes under plausible informative missingness assumptions, along with standard methods for calculating summary estimates and confidence intervals, to assess the potential impact of loss to follow-up on treatment effect estimates (Schafer 1999).

K.4.2 Dose effects

In the event that an overall treatment benefit is detected over 12 weeks, we will conduct exploratory analyses to probe for differences in the magnitude of treatment effects by highest-achieved NTG dose, by testing for interaction between treatment assignment and highest-achieved NTG dose in a linear mixed model for change in hot flash frequency. This analysis will control for the main effect of highest achieved dose to control the placebo effect of this self-selected variable, as well as baseline factors that may confound it, such as age, body mass index, and baseline hot flash frequency or severity.

K.4.5 Subgroup analyses

After assessing overall treatment effects, we will explore differences in treatment effects within select subgroups defined by key demographic and clinicopathologic factors in multivariate regression analysis. For example, we will explore whether age, menopausal stage, body mass index, and use of estrogen receptor modulators influence treatment effects on primary and secondary outcomes. Analyses will necessarily be exploratory, as the study will be primarily powered to detect overall treatment effects rather than subgroup effects. Subgroup estimates will be considered only if the interaction between treatment and subgroup is significant at $p<0.05$

L. DATA COLLECTION AND QUALITY ASSURANCE

L.1 Data Collection Forms

Study data will be obtained from participant-completed symptom diaries and medication diaries, participant- or interviewer-administered questionnaires, physical examination measures, and blood and urine sampling/testing. Forms for recording or abstracting data will be developed specifically for this study, including:

- Hot flash diary abstraction form: After participants complete their diary at home, they will return the completed diary to the study clinic, and diary data will be abstracted by trained study coordinators using

a paper-based Hot Flash Diary Abstraction Form. Data from abstraction forms will then be entered into the electronic database by a blinded staff member.

- Questionnaires assessing sleep quality, menopause-related quality of life, mood, and participant satisfaction. Participants will complete these self-report questionnaires during clinic- or video-based study visits. In most cases, participants will enter directly enter their responses into an electronic tablet or laptop computer or use an electronic questionnaire link; however, if a participant prefers to record her responses on a paper-based questionnaire form, a blinded staff member will then enter the data into the electronic database after the visit.
- Questionnaires assessing demographic history, general medical history, medication use, and tobacco and alcohol use: These questionnaires will be administered by a clinical coordinator during telephone and clinic- or video-based study visits; data from questionnaires will be directly entered by the coordinator into the electronic database.
- Physical examination findings forms: Clinical coordinators will enter data obtained from height and weight measurements and blood pressure and heart rate measurements directly into the electronic database.
- Blood and urine test result forms: Clinical coordinators will directly enter the results of blood and urine tests on into the electronic database.

L.2 Data Management

Data will be entered, managed, and edited using Medrio web-based Electronic Data Capture (EDC) software for Clinical Research, which is available for use without charge for investigator-initiated, university- or government-sponsored research. Medrio software meets the requirements for electronic records and signatures (21 CFR Part 11) and HIPAA. Data entered via Medrio can be accessed from machines on any network, can accommodate multiple users, allows for users at different sites to be issued differed password-protected logins, and includes tools to allow users to load data that collected offline.

L.3 Quality Assurance

L.3.1 Staff Training

All investigators, project directors, and clinical coordinators involved in the study will complete training in Human Subjects Research offered by the Collaborative Institutional Training Initiative (CITI), which includes specific training modules on assessing risk to subjects, avoiding group harms, conflicts of interest, cultural competence, FDA-regulated research, HIPAA-regulated research, informed consent, IRB member responsibilities, IRB chair responsibilities, records-based research, research with vulnerable subjects, and unanticipated problems and reporting. All investigators, project directors, and coordinators will maintain active CITI certification for the duration of the study.

Coordinators and analysts involved in data collection will also attend a study-specific training meeting led by the principal investigator and the project directors prior to the start of participant recruitment. At a minimum, the training meeting will include: (1) an introduction to the goals, design, and procedures of the FRAN study; (2) an overview of goals, structure, and procedures for administering and abstracting data from the hot flash diary and other study-specific outcome measures; (3) a review of definitions and procedures for assessing, documenting, and reporting adverse events.

Coordinators and analysts will also receive training and undergo supervised practice in reviewing and abstracting data from the hot flash symptom diary as the primary efficacy measure in the trial. They will also receive training and undergo supervised practice performing measurements of blood pressure and heart rate. Following the training, clinical staff will be certified for performing these functions.

L.3.2 Data Quality Assessment

Data collection forms will be reviewed on an ongoing basis for data completeness and accuracy as well as protocol compliance. A subset of hot flash diaries used to collect data on the primary outcome will be verified against paper-based source forms by a staff member who was not involved in initial abstraction of the data.

Quality of data entry will be periodically assessed by the principal investigator and project managers using measures such as number of missing forms, number of missing queries, and proportion of all study variables queried. To promote formal and independent data quality monitoring, these data quality metrics will be incorporated into study progress reports to the DSMB and reviewed at scheduled DSMB meetings.

L.3.3 Protocol Deviations

Exceptions to the protocol are expected to occur rarely or not at all and, where possible, will be approved in advance by the principal investigator. Protocol exceptions may occur for the following reasons:

- exceptions necessary to protect the safety or well-being of a participant (in this case, the protocol exception should apply to that participant only)
- exceptions due to oversight or error on the part of study staff, which are subsequently detected by the investigators, project managers, clinical coordinators, or data analysts.

For each protocol exception, study staff will document the exception on a Protocol Exceptions Log (located in the Regulatory Binder). Entries in the Protocol Exceptions Log should include the participant ID, date of the exception, date exception is being recorded, description of exception, and action taken in response to the exception, if any. The principal investigator will document approval for each exception determined in advance, or acknowledgement for each exception detected after the fact.

For this study, missed medication doses will not be considered a protocol deviation, although study staff will make every effort to promote adherence to medication use.

L.3.4 External Auditing

If desired by the NIA, the study team will undergo on-site auditing by an auditor who is independent of both the sponsor and UCSF. Site monitoring visits may include review of participant records, informed consent forms, source data collection forms, and the electronic study database. The schedule of site monitoring will be agreed upon in advance by the NIA, the principal investigator, and the independent monitoring organization. The principal investigator, project manager, and study coordinators will be available to meet in person with the independent monitor and provide access to all study-specific forms, materials, and databases.

M. PARTICIPANT RIGHTS AND CONFIDENTIALITY

M.1 Institutional Review Board (IRB) Review

The study protocol, informed consent document, data and safety monitoring plan, and data collection forms will be reviewed and approved by the UCSF IRB prior to implementation of study procedures. Any subsequent modifications to these documents will also be reviewed and approved by the UCSF IRB prior to administration in the study.

M.2 Informed Consent Forms

A signed consent form will be obtained from each participant at the Screening Visit before in-person or video-based data collection procedures are initiated (see section 6.2.2. for description of informed consent procedures). A copy of the consent form will be given to each participant, and documentation of signed consent will be filed in the participant's study file.

M.3 Participant Confidentiality

Study data will be protected to preserve participant confidentiality both during and after the study. Each participant will be assigned a unique numerical study identifier which will be used on study forms instead of names or other identifying information. The document linking study ID to participant identifiers (name, address, contact names and addresses) will be maintained in a password-protected file stored on a secure server protected by firewalls. Only the clinical coordinators or study investigators who need to get access to participant identifiers to contact participants will have access to the password to this file.

Any paper source forms will be stored in a locked cabinet in a locked office at the UCSF Women's Health Clinical Research Center, and only research staff who need to access these forms for data collection, data editing, or quality monitoring purposes will have the key to this cabinet. Information that could identify individual participants will not be released without written permission of the participant, except as necessary for monitoring by the IRB, NIA, the OHRP, or other government agencies responsible for protecting participant safety. Paper-based source forms will be securely destroyed 3 years after the end of the study.

N. ETHICAL CONSIDERATIONS

This research will be conducted in accordance with principles outlined in, "The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects in Research" (National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research, April 1979), which include respect for persons, beneficence, and justice.

O. STEERING COMMITTEE

The conduct of this research will be overseen by a steering committee composed of the principal and co-investigators—Alison Huang, MD; Deborah Grady, MD, MPH; Steven Cummings, MD; Peter Ganz, MD; and Eric Vittinghoff, PhD.

P. PUBLICATION OF RESEARCH FINDINGS

Any publications resulting from this research will be made available to the public consistent with the NIH public access policy. At the request of the NIA program officer, the principal investigator will also provide a copy of any abstracts or manuscripts resulting from this work to the program officer prior to submission.

APPENDIX A: Summary of Measures and Procedures at Study Visits											
Measures	Telephone Screening	Screening Clinic Visit	Run-in period	Baseline Clinic Visit	1-Week Clinic Visit	2-Week Clinic Visit	3-Week Phone Call (for 0.6 mg)	5-week Clinic visit	8- Week Phone Call	12-Week clinic Visit	4-Week Post-Discontinuation Phone Call
Brief telephone eligibility survey	X										
Informed consent documentation		X									
Sociodemographics questionnaire		X									
Medical/reproductive history questionnaire		X									
Blood and Urine Testing		X									
Electrocardiography		X									
Current medications inventory		X									
Alcohol and tobacco use questionnaire		X									
Height and weight measurement		X								X	
Resing blood pressure & heart rate		X		X	X	X		X		X	
Orthostatic blood pressure and heart rate		X		X	X	X		X		X	
0.1 mg/hr NTG patch run-in period			X								
Hot flash symptom diary return				X				X		X	
Pittsburgh Sleep Diary		X		X				X		X	
Randomization to NTG or placebo				X							
Review of adherence to patches				X	X	X	X	X	X	X	
Patch dose escalation					X	X					
Weaning of NTG or placebo patches										X	
Hot Flash Related Daily Interference Scale		X						X		X	
Pittsburgh Sleep Quality Index		X						X		X	
Menopause-specific Quality of Life		X						X		X	
Generalized Anxiety Disorder – 7		X						X		X	
Center for Epidemiologic Studies Depression		X						X		X	
Adverse events assessment				X	X	X	X	X	X	X	X

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