MENOGENIX, INC.

CLINICAL STUDY PROTOCOL

Study Title:	A Randomized, Double-Blind, Placebo-Controlled Phase 1b Study to Assess the Safety and Effect of Repeated Administration of Granulocyte Colony-Stimulating Factor (G-CSF; filgrastim) on Hot Flashes and Other Vasomotor Symptoms of Menopause in Postmenopausal Women
Study Drug:	Granulocyte Colony-Stimulating Factor (G-CSF, filgrastim Neupogen®) or placebo (sterile physiological saline)
Sponsor:	MenoGeniX, Inc. Bioscience Park Center 12635 East Montview Blvd. Suite 100 Aurora, CO 80045
Protocol ID:	MNGX-102
Sponsor Contact:	[redacted]
Medical Contact:	[redacted]
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Protocol Version 3:	29 May 2018 (Amendment 2)
Protocol Version 4:	12 October 2020 (Amendment 3)

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Protocol MNGX-102

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Final, Version 4, Amendment 3

12 October 2020

PROTOCOL AUTHORS:

Signature: _____

[redacted] Date

Signature: _____

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PROTOCOL APPROVED BY:

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STUDY ACKNOWLEDGMENT

A Randomized, Double-Blind, Placebo-Controlled Phase 1b Study to Assess the Safety and Effect of Repeated Administration of Granulocyte Colony-Stimulating Factor (G-CSF; filgrastim) on Hot Flashes and Other Vasomotor Symptoms of Menopause in Postmenopausal Women

Protocol MNGX-102

Version 4, Amendment 3

12 October 2020

This protocol has been approved by MenoGeniX, Inc.

INVESTIGATOR STATEMENT

I have read the protocol, including all appendices, and I agree that it contains all necessary details for me and my staff to conduct this study as described. I will conduct this study in compliance with all applicable regulations and guidelines as stated in the protocol and other information supplied to me. I will make a reasonable effort to complete the study within the time designated.

I will provide all study personnel under my supervision with copies of the protocol and access to all information provided by MenoGeniX, Inc. I will discuss this material with them to ensure that they are fully informed about the drug(s) and the study.

Signature of Principal Investigator	Date
Print Name	Site Number

PROTOCOL SYNOPSIS

MenoGeniX, Inc.

Protocol MNGX-102

Title of Study:	A Randomized, Double-Blind, Placebo-Controlled Phase 1b Study to Assess the Safety and Effect of Repeated Administration of Granulocyte Colony-Stimulating Factor (G-CSF; filgrastim) on Hot Flashes and Other Vasomotor Symptoms of Menopause in Postmenopausal Women.
Objectives:	The primary objective of this Phase 1b study is to assess the safety and pharmacodynamic (PD) effect of repeated subcutaneous injection of G-CSF in healthy postmenopausal women. The secondary objective of this proposed study is to assess the efficacy of repeated administration of G-CSF in reducing the frequency and severity of hot flashes in postmenopausal women. The tertiary objectives are to assess additional measures of hot flash burden and to test the association between G-CSF administration, changes in hot flash frequency and severity, and markers of inflammation and reproductive hormones.
Study Design:	This is a 12-week, multicenter, randomized, double-blind, placebo-controlled study. Eligible subjects will be stratified by natural or surgical menopause and randomized (1:1) to receive 3 single injections, 28-days apart, of either 300 mcg G-CSF or placebo.
Study Phase:	Phase 1b
Number of Subjects Planned:	A total of 65 subjects will be enrolled in this study.
Target Population:	Healthy female subjects aged 49 to 65 who are naturally postmenopausal or aged 40 to 65 who are surgically postmenopausal, and experiencing at least 7 moderate to severe hot flashes per day on average, or at least 49 moderate to severe hot flashes per week.
Study Duration:	Subjects will be followed for 12 weeks to evaluate safety, PD, and efficacy.
Main Eligibility Criteria:	To be eligible for the study, the following eligibility criteria must be met: <u>Inclusion:</u> • Female, aged 49 to 65 for natural postmenopausal or aged 40 to 65 for surgical postmenopausal • Body Mass Index (BMI) 18 to 35 • At least 7 moderate to severe hot flashes per day on average (or at least 49 moderate to severe hot flashes per week) • Naturally postmenopausal or surgically postmenopausal women: o Naturally postmenopausal is defined as having no menstrual periods for at least 12 months prior to study

entry; with a biochemical criteria of menopause (FSH ≥ the reference range for menopause for the local laboratory used for screening)
    - Surgically postmenopausal is defined as at least 3 months after documented bilateral salpingo oophorectomy
- Normal pelvic exam within 2 years
- Normal pap smear (if cervix /uterus were present) within 2 years
- Signed informed consent

Exclusion:

- Radiation or chemotherapy-induced (including GnRH agonist) menopause
- Prior chemotherapy or radiation therapy for cancer
- Prior diagnosis of hematologic malignancy
- Type 1 diabetics or Type 2 diabetics with HbA1c > 7.0%
- Use of hormone replacement therapy or oral contraceptives within the past three months
- Use of alternative or complementary medicines or herbs for menopausal symptoms within 30 days (refer to Appendix 2)
- Use of any SSRI or SNRI within 30 days
- Use of selective estrogen receptor modulators within 30 days
- Use of gabapentin within 30 days
- Use of clonidine within 30 days
- Use of megestrol acetate (Megace) within 30 days
- Use of, prescription corticosteroids within 30 days (nasal or other inhaled corticosteroids and OTC topical corticosteroids excepted)
- Current use of lithium therapy (related to possible risk of G-CSF)
- History (in the past year) or presence of drug or alcohol use which, in the opinion of the Investigator, might compromise the study or confound the study results
- History of use of any anti-inflammatory biologics
- History of or current splenomegaly (related to possible risk of G-CSF)
- History of sickle cell disease (related to possible risk of G-CSF)
- High risk for medical complications that might affect the subject's ability to complete the trial without a serious co-morbid event, based on medical history, physical examination and laboratory screening evaluation in the opinion of the Investigator
- Presence of an acute or chronic condition (such as a hematological, rheumatologic auto-immune disease, chronic inflammatory disorder, chronic lung disease or osteoporosis)

	based on history, clinical, or laboratory evaluation, which, in the opinion of the Investigator, might compromise the study, confound the study results or place the subject at risk • Follicle stimulating hormone (FSH below the reference range for menopause for the local laboratory used for screening • Thyroid stimulating hormone (TSH) outside normal limits at study entry • Absolute neutrophil count (ANC) $\leq 1.0 \times 10^9$/L • Total white blood cell count (WBC) $\leq 3.0 \times 10^9$/L • Platelet count (PLT) $\leq 150 \times 10^9$/L • Hemoglobin count (HGB) consistent with anemia • Positive urine pregnancy test at Baseline visit • Allergy or hypersensitivity to *E coli*-derived proteins, G-CSF, or any component of the product • Mentally or legally incapacitated such that informed consent cannot be obtained • Inability or unwillingness to complete daily hot flash diary and study questionnaires appropriately • Participation in another investigational trial within the past 30 days
Study Procedures/ Frequency:	Subjects must complete the Screening, plus 14-day run-in period and daily diary entry of hot flash frequency and severity, to confirm eligibility prior to Baseline. Site staff will call subject on day 15 after 14-day run-in period to review and record hot flash frequency and severity and to confirm eligibility before scheduling the Baseline visit. Eligible subjects will be randomized to receive a single injection of G-CSF or placebo at Baseline and Days 28 and 56. **Laboratory Assessments:** • Blood samples for clinical chemistry, non-fasting (including sodium, potassium, calcium, ALT, AST, bilirubin, creatinine, BUN, alkaline phosphatase, and albumin), will be obtained at Screening, Baseline and Days 1, 21, 28, 29, 49, 56, 57, and 84. If >14 days between Screening and Baseline visit, labs will be repeated and reviewed prior to Randomization. • A blood sample for non-fasting HbA1c will be obtained at Screening. • Blood samples for CBC with differential (including RBC, WBC, platelets, hemoglobin, hematocrit, RDW, MCV, MCH, MCHC, neutrophils, lymphocytes, monocytes, eosinophils, basophils, neutrophils [absolute], lymphocytes [absolute], monocytes [absolute], eosinophils [absolute], basophils [absolute]) will be obtained at Screening, Baseline, Days 1, 21, 28, 29, 49, 56, 57, and 84. **The lab results from the CBC with differential obtained at Days 1, 21, 28, 29, 49, 56, 57, and 84 will not be returned to the Investigator in order to avoid treatment unblinding and will be reviewed by the Medical/Safety Monitor**.

	• Serum samples for circulating hormone levels (including FSH, LH, TSH, DHEA, DHEAS, testosterone, and estradiol) will be obtained at Baseline and at Days 1, 21, 28, 29, 49, 56, 57, and 84. • A blood sample for FSH and TSH will be obtained at Screening for verification of inclusion criteria. • Serum samples for circulating cytokines (including but not limited to IL-1, IL-6, IL-8, and TNF-alpha) will be obtained at Screening, Baseline, and at Days 1, 21, 28, 29, 49, 56, 57, and 84. Negative urine pregnancy test will be confirmed prior to administration of G-CSF or placebo at Baseline and at Days 28 and 56 and also at the end of the study (Day 84). **Study Assessments:** Assessments include collection of demographics, medical history, smoking and alcohol history, hormone use, reproductive and hot flash history, concomitant medications and any dietary supplements, and physical exam (including vital signs, temperature, weight, and height) completed at Screening. A medical history and concomitant medication update will be collected at Baseline. Concomitant medications and adverse events will be collected at every visit. Vital signs, temperature, and weight also collected at Baseline, Days 28, 56 and 84. Collection of daily menopausal symptoms using a hot flash frequency and severity diary will be completed by subject twice daily (AM and PM) for the duration of study. **Other Assessments/Questionnaires:** Questionnaires will include Hot Flash Related Daily Interference Scale (HFRDIS), Insomnia Severity Index (ISI), Menopause Specific Quality of Life Questionnaire (MENQOL), Fatigue Severity Scale (FSS), and Arthritis Impact Measurement Scale (AIMS) completed at Baseline and Days 28, 56, and 84. Coordinator-to-Subject phone calls will occur every week that the subjects are not in clinic (calls performed Weeks 1, 2, 5, 6, 9, 10, and 11) to inquire if any adverse events have occurred, remind subject about diary completion, and will be documented. Follow-up Coordinator-to-Subject phone call will occur approximately 28 days following last clinic visit to inquire if any adverse events occurred (will be documented), and to inquire about subject status. Subjects will be requested to review their hot flash diary at each visit. Additional visits and / or tests may be performed if clinically indicated.
Test Product, Dose, and Mode of Administration:	G-CSF will be provided as Neupogen® packaged in individual 1.0 mL vials containing 300 mcg filgrastim. Individual vials of sterile physiological, preservative free, isotonic saline will be used for placebo. Three single subcutaneous injections (repeated 28-days apart), in the outer area of either upper arm, of G-CSF (300 mcg)) or placebo (sterile physiological saline) in a total volume of 1 mL will be given at Baseline visit and Days 28 and 56.
Safety Evaluation:	Safety will be assessed by adverse events, clinical laboratory tests (clinical chemistry and CBC with differential) and vital signs. The

	study will have an independent Data Safety Reviewer assigned by the NIH to review safety data at regular intervals.
Evaluation of Effect:	**Primary Endpoint** Safety and PD are the primary endpoints of this study. Pharmacodynamic Endpoints: Assessment of changes from baseline in circulating white blood cells, hormone and inflammatory cytokine concentrations. **Secondary Endpoints** Clinical endpoints The effect of 3 repeated doses of G-CSF in the following indicators of vasomotor symptoms in women with naturally occurring or surgically induced menopause at weeks 2, 4, 6, 8, 10 and 12 weeks post-administration: Hot flashes – change from baseline in: • Frequency of total daily hot flashes (mild + moderate + severe hot flashes) • Daily frequency of moderate + severe hot flashes • Daily composite hot flash severity score (1 x mild + 2 x moderate + 3 x severe hot flashes) • Hot flash severity score ((1 x mild + 2 x moderate + 3 x severe hot flashes)/total hot flashes) Quality of life questionnaires - change from baseline in: • Hot Flash Related Daily Interference Scale (HFRDIS) • Insomnia Severity Index (ISI) • Menopause-specific Quality of Life Questionnaire (MENQOL) • Fatigue Severity Scale (FSS) • Arthritis Impact Measurement Scale (AIMS)
Statistical Methods:	As this is a Phase 1b study intended to evaluate the preliminary safety, PD and efficacy of G-CSF in postmenopausal women, no *a priori* assumptions are made regarding estimated treatment effects or statistical power. Exploratory analyses will be performed to determine the effects of G-CSF administration on circulating hormone levels (FSH, LH, TSH, DHEA, DHEAS, testosterone, and estradiol) and circulating pro-inflammatory cytokine levels (including but not limited to IL-1, IL-6, IL-8, and TNF-alpha).
Protocol Date:	**12 October 2020**

GLOSSARY OF ABBREVIATIONS

AE	adverse event
AIMS	Arthritis Impact Measurement Scale
ANC	absolute neutrophil count
ANOVA	analysis of variance
ARDS	acute respiratory distress syndrome
ALT	alanine aminotransferase (SGPT)
AST	aspartate aminotransferase (SGOT)
BID	bis in die (twice daily)
BMI	body mass index
BMT	bone marrow transplant
BRCA1	breast or cervical cancer gene
BRCA2	breast or cervical cancer gene
BSA	body serface area
BUN	blood urea nitrogen
CBC	complete blood count
C	celsius
CRA	Clinical Research Associate
CRF	case report form
CTCAE	Common Terminology Criteria for Adverse Events
DHEA	dehydroepiandrosterone
DHEAS	dehydroepiandrosterone sulfate
DSMB	Data Safety Monitoring Board
dL	decilitre
ECG	electrocardiogram
ET	estrogen
EPT	estrogen plus progesterone
F	farhrenheit
FDA	Food and Drug Administration
FSH	follicle stimulating hormone
FSS	Fatigue Severity Scale
g	grams
GCP	Good Clinical Practice
G-CSF	granulocyte colony-stimulating factor
GM-CSF	granulocyte/macrophage colony-stimulating factor
GnRH	gonadotropin-releasing hormone
HbA1c	hemoglobin A1c
HF	hot flash
HFRDIS	Hot Flash Related Daily Interference Scale
HGB	hemoglobin
HT	hormone therapy
ICF	informed consent form
ICH	International Conference on Harmonization
IL-1	interleukin 1
IL-6	interleukin 6
IL-8	interleukin 8
IRB	Institutional Review Board
ISI	Insomnia Severity Index
IV	intravenous
kg	kilogram
L	litre
LH	lutenizing hormone
mcg	microgram
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MCV	mean corpuscular volume
MENQOL	MENopause specific Quality of Life Questionnaire
mIU	milli international units
mL	millilitre
MNGX	MenoGeniX, Inc.
NCI	National Cancer Institute
OTC	over-the-counter
PBPC	peripheral blood progenitor cell
PD	pharmacodynamic
PI	principal investigator
PK	pharmacokinetic
PLT	platelet
PSQI	Pittsburgh Sleep Quality Index
QD	quaque die (once daily)
RBC	red blood cell
RDW	red blood cell distribution width
SAE	serious adverse event
SERM	selective estrogen receptor modulator
SNRI	serotonin norepinephrine reuptake inhibitor
SSRI	selective serotonin reuptake inhibitor
SC	subcutaneous
TNF-alpha	tumor necrosis factor-alpha
TSH	thyroid stimulating hormone
U	units
US	United States
WBC	white blood cell
WHI	Women's Health Initiative

1 INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

Prior to trial initiation, the Investigator at each site must provide to MenoGeniX, Inc. (MenoGeniX), a protocol signature page, a Financial Disclosure Form, FDA Form 1572 and documentation of Institutional Review Board (IRB) approval.

The trial will be administered and monitored by employees or representatives of MenoGeniX. The Clinical Research Associates (CRAs) will monitor each site on a periodic basis and perform verification of source documentation for each subject. MenoGeniX will be responsible for ensuring timely reporting of expedited Serious Adverse Event (SAE) reports to regulatory agencies, IRB, and Investigators, as applicable by local regulations.

2 INTRODUCTION

A comprehensive review of filgrastim (recombinant methionyl human granulocyte colony-stimulating factor [r-metHuG-CSF], marketed by Amgen under the trademark Neupogen® and generic name filgrastim) is contained in the Investigator's Brochure. This document should be reviewed prior to initiating the study.

2.1 Background Therapeutic Information

G-CSF is being developed for the treatment of hot flashes and other vasomotor symptoms associated with natural, surgical or chemically-induced menopause. Up to 86% of all naturally menopausal women consult a doctor during their menopausal transition to discuss symptom management [1]. The primary symptom that triggers a woman to seek treatment during her menopausal transition is the hot flash. Symptoms appear to be more prevalent and severe in women who have undergone hysterectomy with bilateral oophorectomy (surgical menopause), have premature menopause [2] or are on Tamoxifen or aromatase inhibitors for the treatment of breast cancer. A similar proportion of men who undergo androgen ablation therapy for prostate cancer will also report hot flashes [3]. Although hot flashes subside over time for the majority of women who are affected, 16% have been reported to remain symptomatic 4-5 years past their final menstrual period [4] and ~2% of untreated women in their 70's report experiencing hot flashes or night sweats [5].

Hormone therapies (HTs) consisting of estrogens or estrogens plus progesterone are the only approved medications to treat hot flashes. However, as described in **Section 2.2**, there is much confusion and concern about the use of HT to safely treat these symptoms [6,7]. An effective, non-hormonal treatment for hot flashes and related vasomotor symptoms would be a welcome, novel and useful treatment modality.

Evidence exists that a single, 300 mcg (~5 mcg/kg), subcutaneous (SC) injection of G-CSF (as Neupogen®) administered to assess cyclic neutropenia in a menopausal woman resulted in a significant and long-term (approximately 3-month) reduction in hot flash frequency and severity. A second single 300 mcg administration of G-CSF (as Neupogen®) to the same individual ~3 years later resulted in a similar 3-month diminution in hot flash frequency and severity. This anecdotal observation led to a confirmatory, 12-week, randomized, placebo-controlled, phase 1 clinical trial in 30 women with naturally-occurring and surgically-induced menopause which showed that a single non-weight-adjusted, 275 mcg dose of G-CSF (mean dose = 3.9 mcg/kg; range = 3.1 – 5.3 mcg/kg) resulted in a statistically significant reduction in hot flash frequency and severity through three weeks post administration with 37% of the

G-CSF-treated women reporting greater than 50% reduction in hot flashes by 2 weeks and 70-85% reduction at 12 weeks post-administration.

2.2 Background and Significance

Hot flashes occur in over 75% of women who are traversing menopause and they persist in up to 16% of women more than a decade after menopause [4,8]. Hormone therapy is the only FDA-approved therapy for the treatment of moderate to severe menopausal symptoms. Hormone therapy is highly effective and was approved based on its reported efficacy in reducing hot flash frequency in post-menopausal women by 70 – 90% [2,4]. However, results from the Women's Health Initiative (WHI) study published in 2002, resulted in significant "boxed" warnings about HT use including an increased risk of developing cancer, heart disease, stroke, and dementia [6,7]. In addition, HT is contraindicated for women with breast cancer and for survivors of breast cancer undergoing estrogen suppression, and may not be appropriate for those women who undergo bilateral oophorectomy because of a genetic susceptibility to develop breast or cervical cancer (*BRCA1* and/or *BRCA2* positive). Finally, HT is an inappropriate treatment for hot flashes due to other causes (e.g., in men with prostate cancer undergoing androgen ablation).

The current indications for HT are a relationship between menopause and hot flashes (hot flashes/night sweats), sleep disturbances, and mood disorders [8,9]. Both the physiological mechanisms underlying a hot flash and the exact mechanism of action of HT in alleviating menopausal symptoms remain unknown.

Due to the published serious health risks and boxed warnings associated with HT [6,7], several non-hormonal alternatives are often prescribed for off-label use by healthcare providers. The current non-hormonal alternative to HT that is approved is Brisdelle™. In 2013, the FDA approved Brisdelle™, a low dose version of the anti-depressant paroxetine, for the treatment of moderate to severe vasomotor symptoms associated with menopause. Additionally, other non-hormonal alternatives to HT and paroxetine that are not approved for this use but which have shown a limited degree of efficacy in recent clinical studies include antidepressants such as the selective serotonin reuptake inhibitor (SSRI) and serotonin norepinephrine reuptake inhibitor (SNRI) classes of drugs (e.g., venlafaxine, paroxetine, fluoxetine, citralopram, escitalopram) and the anti-epilepsy pain medication gabapentin [10]. The former drug class can cause significant side effects, including addiction and severe withdrawal symptoms while gabapentin is limited in daytime use by its chief side effect, drowsiness. Anti-depressants and gabapentin have less than half the effectiveness of HT [11]. Treatment with nutraceuticals has also been investigated and found to be non-effective. In

summary, there are no alternative non-hormonal treatments currently under development that match HT's demonstrated efficacy in reducing the frequency and/or severity of hot flashes.

2.3 G-CSF

Human granulocyte colony-stimulating factor (G-CSF) is currently indicated to stimulate the proliferation and differentiation of granulocytes. G-CSF is available in the US as Neupogen®, TevaGrastim® and Zarxio®. Neupogen® is the Amgen Inc. trade name for filgrastim, recombinant methionyl human granulocyte colony-stimulating factor (r-metHuG-CSF), synthesized in bacteria. TevaGrastim® is the Teva Pharmaceuticals trade name for filgrastim. Zarxio® is the Sandoz, a Novartis company, trade name for filgrastim. Both TevaGrastim® and Zarxio® are approved as Neupogen® biosimilars. Since filgrastim is produced in bacteria, it differs slightly from that of the naturally produced human G-CSF.

G-CSF is given daily to cancer patients during the course of chemotherapy and life-long to those with chronic severe neutropenia [12]. G-CSF, normally produced in healthy individuals in response to infections, stimulates the production of a subset of white blood cells (WBC). This subset of cells, including neutrophils, basophils, and eosinophils, is collectively referred to as granulocytes and is involved in fighting infections caused by bacteria, fungi and parasites. Although all WBCs are derived from precursor cells in the bone marrow, granulocytes, in particular, have a very short life span and are regenerated constantly. Certain chemotherapy regimens damage these precursor cells in the bone marrow resulting in low WBC counts, a condition referred to as neutropenia. Chronic neutropenia is also observed in rare individuals that are born with the disease (congenital) or who develop the disease for unknown reasons (idiopathic), the latter of which can also be cyclic. When neutrophil cell counts drop to a very low level, patients become susceptible to severe, life-threatening bacterial infections.

A factor that stimulated neutrophil growth was first described in 1967 by Drs. Metcalf, Robinson, and Bradley at the Walter and Elisa Hall Institute in Melbourne [13]. The mouse protein was purified in 1983 by Metcalf and colleagues and the human gene was cloned in 1986 by two additional groups of researchers, eventually resulting in the FDA-approved version of recombinant human G-CSF. G-CSF, which increases peripheral granulocytes and precursors in humans over 5 to 7 days, is used for the treatment of oncology patients to accelerate recovery from chemotherapy-induced neutropenia allowing for higher-intensity treatment regimens in breast cancer in particular. G-CSF, marketed under the trade name Neupogen®, was first approved in 1991 to decrease the incidence of bacterial infection, as manifested by febrile neutropenia, in patients with non-myeloid malignancies receiving

myelosuppressive anti-cancer drugs. Additional market approvals were later granted for the reduction of incidence and sequalae of low WBC counts in symptomatic children and adults with congenital, cyclic, or idiopathic severe neutropenia.

G-CSF has been used successfully both chronically in patients with idiopathic neutropenia over several years and as a burst therapy (daily use for up to 14 days) in patients with chemotherapy-induced neutropenia [14]. To date, over 9 million patients have received G-CSF therapy [13]. Meta-analysis of studies shows a 46% decrease in febrile neutropenia and 40% decrease in early mortality. The demonstration of such robust efficacy has led to physician recommendations of its use as a prophylactic measure when risk of neutropenia is >20% [13]. Cost is the major limitation to increased widespread use [13]. The most common side effect with repeated injections is bone pain due to expansion of bone marrow cell populations [15]. Bone pain was reported more frequently in patients treated with high intravenous (IV) doses (20 to 100 mcg/kg/day), and less frequently in patients treated with lower SC doses of Neupogen® (3 to 10 mcg/kg/day) [16].

Due to the low toxicity of G-CSF that has been observed in animal models and in extensive clinical experience with neutropenic patients, additional clinical studies in non-neutropenic subjects have been undertaken and have shown G-CSF treatment to be safe, beneficial and well tolerated for the prevention and/or treatment of infections in patients at high risk of infection undergoing surgery, those with HIV, and with diabetic foot ulcers [17,18]. G-CSF has also been used to aid expansion of bone marrow precursor cells after marrow transplantation and to expand peripheral blood stem cells in healthy human donors [19].

Beyond its obvious effects on bone marrow, G-CSF also acts as a growth stimulating and survival factor for neurons. The G-CSF receptor is expressed by neurons in the brain and spinal cord which, upon activation, induces growth of nerve cells and increases neuroplasticity. Of interest, estrogen has also been reported to have neurotrophic effects. Basic studies have examined the effects of G-CSF on neuroprotection and neural repair and survival. Current clinical investigations are exploring the role of G-CSF in ischemic stroke and in spinal cord injury [20].

The G-CSF receptor is also expressed widely in endothelium cells and throughout the reproductive system. In this regard, G-CSF has shown some potential in human clinical trials to induce beneficial angiogenesis in cardiovascular disease and to prevent recurrent miscarriages [21]. These studies are on-going and no version of G-CSF is currently approved for any indication other than to prevent febrile disease in chemotherapy-induced, congenital

or idiopathic severe neutropenia. Nonetheless, these studies suggest that G-CSF may have effects beyond stimulation of granulocyte precursors.

As the mechanisms underlying hot flashes are poorly understood, it is difficult to postulate a potential mechanism of how administration of G-CSF would alleviate them. Additional clinical investigation of G-CSF may lead to identification and characterization of this mechanism, and eventually to an effective alternative to HT for the treatment of subjects suffering these symptoms.

2.4 Safety Experience with G-CSF

The current study proposes to subject thirty healthy postmenopausal women to three single, subcutaneous injections of G-CSF at 28 day intervals (300 mcg).

As described in the Investigator's Brochure and in the Neupogen® package insert, filgrastim was administered to monkeys, dogs, hamsters, rats and mice as part of a preclinical toxicology program which included single-dose acute, repeated-dose subacute, subchronic, and chronic studies. Single-dose administration of filgrastim by the oral, IV, SC or IP routes resulted in no significant toxicity in mice, rats, hamsters, or monkeys at doses as high as 3450 mcg/kg in mice, rats and monkeys.

2.4.1 Previous G-CSF Experience in Phase 1 Single-Dose Healthy Volunteer Studies

Since its approval by the FDA in 1991, G-CSF has been administered chronically to more than 9 million individuals with chemotherapy-induced, congenital, and idiopathic severe neutropenia [13]. The common side effects and AEs described for G-CSF are associated with chronic daily use in the setting of chemotherapy-induced, congenital, and idiopathic severe neutropenia at daily dosage levels that often exceed the single SC dose to be administered in this proposed clinical trial (300 mcg per subject, given once). The following Phase 1 study summaries are included to give background clinical experience from studies investigating single injection doses of G-CSF in healthy volunteers.

2.4.1.1 *Amgen Neupogen® Phase 1 (Healthy volunteer; Single dose)*

The following Phase 1 study was the only single dose, healthy volunteer study conducted by Amgen prior to advancing filgrastim directly into their target chemotherapy induced neutropenic population.

A Phase 1 single-dose, placebo-controlled study in 21 normal healthy volunteer males was conducted to determine the safety, pharmacodynamic (PD) effects, and pharmacokinetic (PK) profile of filgrastim [22].

Intravenously administered filgrastim resulted in a dose-dependent and rapidly reversible increase in absolute neutrophil counts (ANC) when administered as single doses in a range of 0.575 – 3.45 mcg/kg. Filgrastim was well tolerated with no clinically important AEs recorded. Filgrastim showed first order pharmacokinetics with an elimination half-life of 160 minutes.

The effect of filgrastim on increasing ANC was highly specific. Minor increases seen in monocyte, eosinophil, basophil, and WBC precursor counts were clinically insignificant. The most frequently reported AE was bone pain, which was generally mild to moderate in severity.

2.4.1.2 *Biosimilar AVI-014 and Neupogen® Phase 1 (Healthy volunteer; Single dose)*

AVI-014 is a chicken egg white-derived, recombinant, human (G-CSF). This healthy volunteer study was the first human investigation of AVI-014 [23].

Twenty-four male and female subjects received a single subcutaneous injection of AVI-014 at 4 or 8 mcg/kg. Sixteen control subjects received 4 or 8 mcg/kg of Neupogen® in a partially blinded, parallel fashion. All subjects completed the trial. The age range of subjects was 21-

50. There were no serious adverse events (SAEs) reported during the study. A total of 47.5% of the enrolled subjects experienced at least one AE. Of a total of 42 AEs, thirty-four events were assessed as possibly related to study drug. The most common adverse events were headache, myalgia, back pain, and bone pain. Fifty percent (12 of 24) of subjects treated with AVI-014 and 43.8% (7 of 16) of those treated with Neupogen® experienced an AE. Musculoskeletal complaints, which included bone pain and muscle aches, occurred in 37.5% (9 of 24) subjects receiving AVI-014 and 18.8% (3 of 16) of subjects receiving Neupogen®.

Headache occurred in 20.8% (5 of 24) subjects receiving AVI-014 and 18.8% (3 of 16) of subjects receiving filgrastim.

2.4.1.3 *Biosimilar Nivestim™ and Neupogen® Phase 1 (Healthy Volunteer; Single Dose)*

This was a Phase 1, single-center, open-label, randomized trial designed to evaluate and demonstrate equivalence between the PK characteristics of Hospira manufactured filgrastim (Nivestim™) and Amgen manufactured filgrastim [24].

Forty-eight healthy volunteers (male and female, aged 18 – 45 years) were randomized to receive IV or SC dosing and then randomized to order of treatment. Volunteers in each of the two dosing groups received a single 10 mcg/kg dose of Hospira filgrastim or Amgen filgrastim, with subsequent crossover to the opposite arm. Forty-six volunteers completed the study.

No significant differences in AE profiles were observed between Hospira filgrastim and Amgen filgrastim. The overall incidence of AEs was lower in volunteers who received IV Hospira filgrastim compared with those who received IV Amgen filgrastim; however, similar incidences of AEs were reported between volunteers in both arms who received study drugs via the subcutaneous route. The most commonly reported AEs included headache, back pain, and nausea. All AEs were mild or moderate in intensity and no SAEs were reported. No clinically significant changes in heart rate, 12-lead ECG, hematology, biochemistry, urinalysis, or changes in physical examination were reported.

2.4.1.4 *Biosimilar Zarzio® and Neupogen® Phase 1 (Healthy Volunteer; Single and Multiple Doses)*

Zarzio®, a recombinant G-CSF (filgrastim), has been evaluated in healthy volunteers and neutropenic patients in Phase 1 and 3 studies [25]. Healthy volunteers (male and female) in randomized, two-period crossover studies received single- and multiple-dose SC injections of 1 mcg/kg (n = 24), 2.5 mcg/kg (n = 28), 5 mcg/kg (n = 28), or 10 mcg/kg (n = 40), as well as

single-dose IV infusions of 5 mcg/kg (n = 26), of Zarzio® or the reference product (Neupogen®). Filgrastim serum levels were monitored; PD parameters included absolute neutrophil count (all studies) and $CD34^+$ cells (multiple dose studies). Safety assessments in the Phase 1 studies consisted of monitoring and recording all AEs and SAEs, assessments of physical condition, vital signs, electrocardiogram, and monitoring of laboratory values. Local tolerance was evaluated by self-assessment of the subjects using a visual analog scale and by the investigators using the injection site reaction score.

In the Phase 1 crossover studies, 146 healthy volunteers (81 males and 65 females, ages 21-54 years) were treated with Zarzio® and Neupogen®. Study drug-related AEs frequently observed in healthy volunteers under Zarzio® or Neupogen® treatment were as expected (mild to moderate musculoskeletal pain, leukocytosis, thrombocytopenia, and headaches). There were no clinically-relevant differences between Zarzio® and Neupogen® in the frequency or type of AEs by system organ class and severity (all generally mild or moderate). No SAEs were observed and no deaths occurred during any of these studies. Results from the laboratory tests, vital signs measurements, and physical examinations confirmed the absence of marked changes in the subjects' state of health.

In summary, the combined results of these healthy volunteer studies are reflective of the already established mild toxicity of Neupogen®, especially as relevant to a single SC injection, and as characterized in multiple large randomized trials as well as a large post-marketing safety database.

2.4.2 Previous G-CSF Experience in Chemotherapy-induced, Congenital and Idiopathic Severe Neutropenia

As described previously, as well as in the Neupogen® (filgrastim) package insert and in the Investigator's Brochure, G-CSF has been chronically administered to millions of individuals subcutaneously (4 – 8 mcg/kg/day) as well as intravenously (5 – 10 mcg/kg/day). Clinical use in these settings has shown the drug to be safe, well tolerated, and uniformly efficacious in raising circulating granulocyte cell counts. The most common adverse events (AEs) that were consistently reported in phase 2 and phase 3 clinical trials of Neupogen® for neutropenic conditions included mild to moderate bone pain (24 – 33%) and headache (<10%); both were readily controlled by non-narcotic analgesics [16]. Bone pain was reported more frequently in patients treated with higher doses (20 to 100 mcg/kg/day) administered IV, and less frequently in patients treated with lower SC doses of Neupogen® (3 to 10 mcg/kg/day) [16]. Allergic-type reactions occurring on initial or subsequent treatment have been reported in <1 in 4000 patients treated with Neupogen®. Reactions tended to occur within the first

30 minutes after administration and appeared to happen more frequently in patients administered IV Neupogen®. Rapid resolution of symptoms occurred in most cases following administration of antihistamines, steroids, bronchodilators, and/or epinephrine.

The following adverse reactions were identified during post-approval of Neupogen®: splenic rupture; acute respiratory distress syndrome (ARDS); alveolar hemorrhage and hemoptysis, sickle cell crisis; cutaneous vasculitis; and Sweet's syndrome (acute febrile neutrophilic dermatosis). Because these reactions are reported voluntarily from a population of large, but uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to drug exposure.

2.5 Background and Rationale for the Current Study

2.5.1 Clinical Study MNGX-101: A Randomized, Double-Blind, Placebo-Controlled Pilot Phase 1b Study to Assess the Safety and Effect of a Single Subcutaneous Dose of Granulocyte Colony-Stimulating Factor (G-CSF; filgrastim) to Treat Hot Flashes in Postmenopausal Women

MXGX-101 was a 12-week multicenter, double-blind, placebo-controlled, study to evaluate the safety and effect of a single, non-weight adjusted, 300 mcg, SC injection of G-CSF (mean dose = 3.9 mcg/kg; range = 3.1 – 5.3 mcg/kg) in women who were experiencing postmenopausal hot flashes. Subjects were stratified by natural or surgical menopause and randomized (2:1) to receive either G-CSF or placebo. The study enrolled female subjects aged 50 to 65 who were postmenopausal and experiencing at least 7 moderate to severe hot flashes per day on average, or at least 49 moderate to severe hot flashes per week. G-CSF was provided as Neupogen® packaged in individual 1.0 mL vials containing 300 mcg filgrastim. A single subcutaneous injection, in the outer area of either upper arm, of G-CSF (300 mcg) in a total volume of 1 mL was given at Baseline to subjects randomized to receive G-CSF. The women kept daily diaries recording the number of mild, moderate and severe hot flashes that they experienced for the subsequent 84 days post administration. A total of 30 subjects were enrolled in the study, 11 in the placebo group and 19 in the G-CSF treatment group. All subjects completed the study and were included in the Intent-to-Treat (ITT) and Safety populations.

The primary objective of this pilot study was to describe the effect of a single subcutaneous injection of G-CSF on the frequency and severity of hot flashes in postmenopausal women. The secondary objectives of this pilot study were to assess additional measures of hot flash burden, circulating hormone and inflammatory cytokine concentrations, and safety.

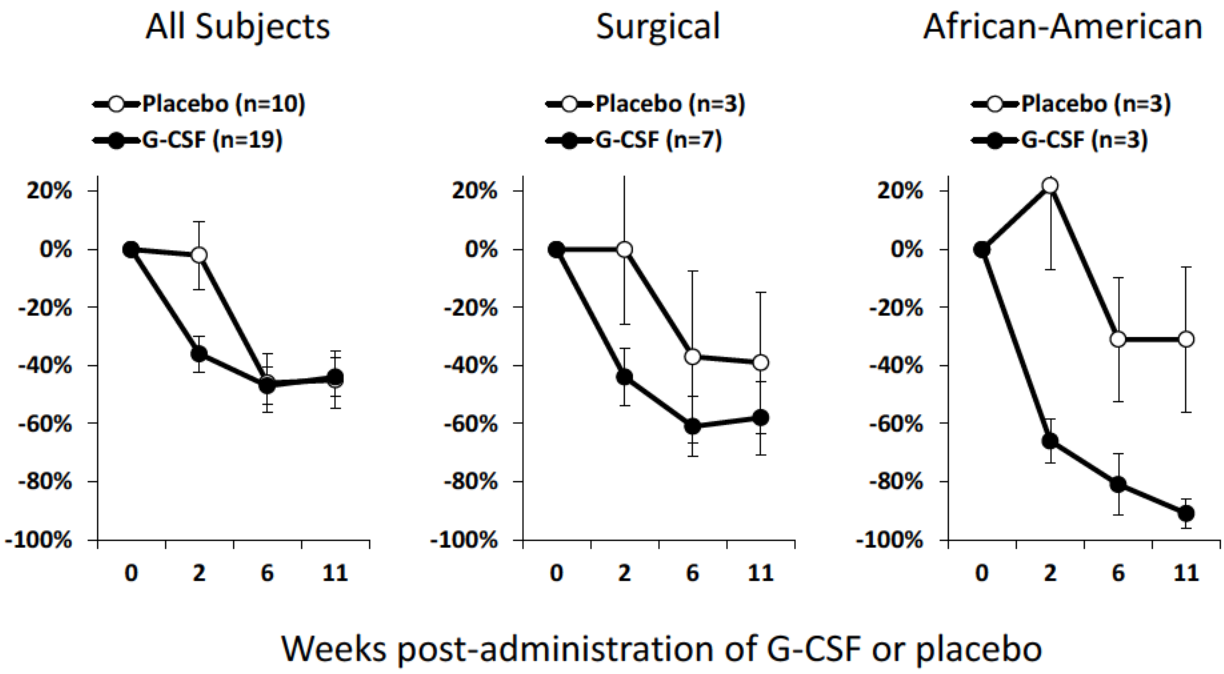
In patients with frequent hot flashes at baseline (>7 moderate + severe per day), a single injection of G-CSF at time 0 resulted in a statistically significant efficacy signal at week two post-administration compared to placebo. Specifically, a statistically significant reduction from baseline was observed at two weeks in:

1) Mean daily total hot flashes (mild + moderate + severe) (35% reduction in the G-CSF group vs. 5% reduction in the placebo; p=0.016);
2) Mean daily moderate plus severe hot flashes (36% reduction in the G-CSF group vs. 5% reduction in the placebo; p=0.013) (see Figure 3);
3) Mean daily severe hot flashes (46% reduction in the G-CSF group vs. 27% increase in the placebo; p=0.019);
4) Mean composite daily hot flash score ((1 x mild)+(2 x moderate)+(3 x severe) (39% reduction in the G-CSF group vs. 1% reduction in the placebo; p=0.015); and
5) Mean daily hot flash severity score (0.23 unit lessening of severity in the G-CSF group vs. 0.08 unit worsening in the placebo group; p=0.002) (see Figure 4)

This strong effect was highly significant when the modest number of subjects (n=29) is considered.

A leveling-off of response was observed in the treated group after week 3 which is consistent with the known pharmacological effects of G-CSF in the setting of neutropenia clearance and effect on circulating neutrophil cell counts); however, we also observed an unexpected reduction in hot flash frequency but not severity in the placebo group starting at week 4.

Figure 3: Percent change in moderate + severe hot flashes reported at the indicated week post-administration relative to baseline where baseline moderate + severe hot flashes = 0%



Quality of life questionnaires including net change in the Hot Flash Related Daily Interference Scale (HFRDIS) (see Figure 5), net change in the Insomnia Severity Index (ISI), net change in the Pittsburgh Sleep Quality Index (PSQI), and net change in the Fatigue Severity Scale (FSS), showed a general trend towards greater improvement in the G-CSF group vs. placebo at the two, four and twelve week time points when they were assessed but did not achieve statistical significance.

Figure 4: **Net change in hot flash severity score ((1 x mild) + (2 x moderate) + (3 x severe)/Total hot flashes) at the indicated week post-administration relative to baseline where baseline HFSS = 0.00**

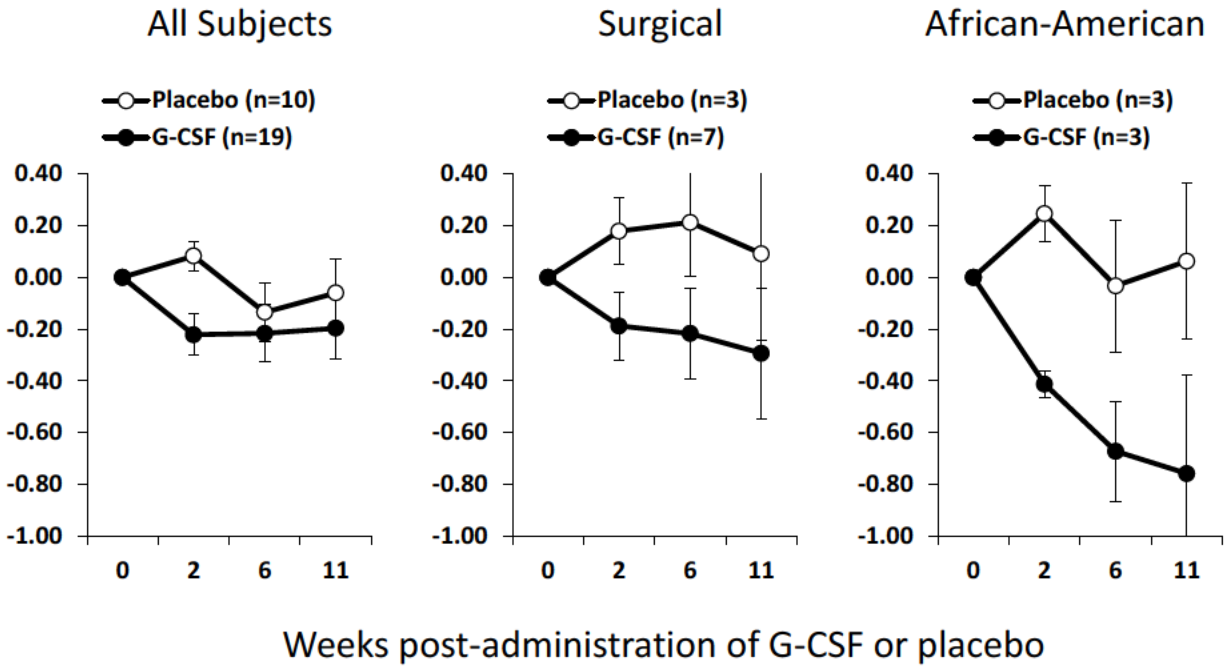
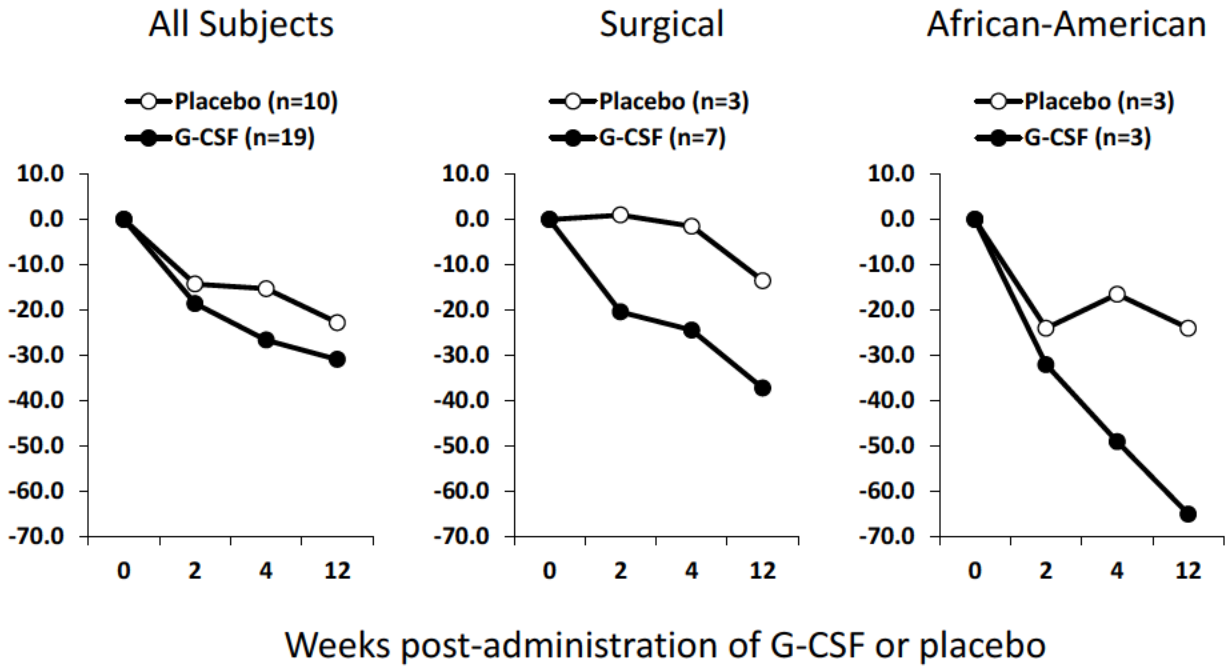


Figure 5: **Net change in Hot Flash Related Daily Interference Score at the indicated week post-administration relative to baseline where baseline = 0.0**



Retrospective analysis of the data, focusing on the 2 week time point revealed that 37% (7 out of 19) of the treated subjects vs. none (0 out of 10) of the placebo subjects reported a greater than 50% reduction in total hot flash frequency and a greater than 55% reduction in moderate + severe hot flashes and composite daily score (p=0.032) (see Figure 6). Weight-adjusted dose did not predict "high" responders and this "potential threshold effect" will require further research. This finding suggests the possibility that there may be a threshold dose which is required to elicit a response and implies that a higher dose or more frequent dosing may be effective in a higher percentage of subjects. It should be further noted that the reduction in hot flash frequency and severity reported by the treated subjects at 2 weeks provided a reasonable prediction of their response at 12 weeks in terms of improvement in hot flashes and QOL whereas the placebo response at 2 weeks was not predictive.

Summary of Adverse Events in MNGX-101

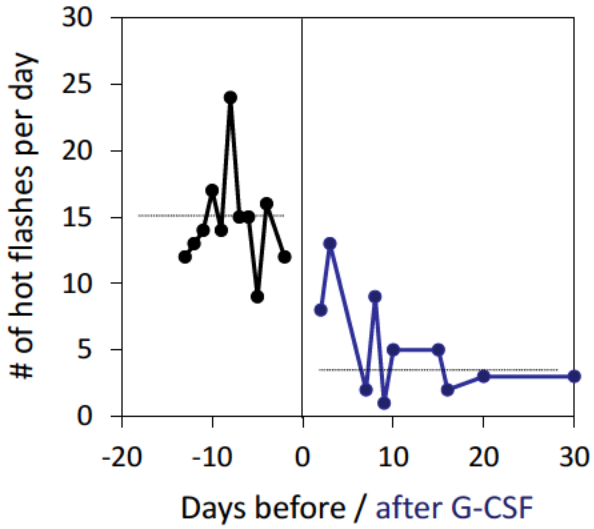
Adverse Event	**Placebo** (n=11)		**MNGX-100** (n=19)		**All** (n=30)	
	n	%	n	%	n	%
Common Gastrointestinal Distress	1	9	2	11	3	10
Ulcerative colitis	0	0	1	5	1	3
Headache	4	36	7	37	11	37
Musculoskeletal pain	3	27	4	21	7	23
Clenching Jaws	0	0	1	5	1	3
Common Respiratory Ailments	4	36	8	42	12	40
Left chest pain	1	9	0	0	1	3
Nosebleed	0	0	1	5	1	3
Menstrual cramps and spotting	1	9	1	5	1	3
Fatigue	1	9	1	5	2	7
Elective surgery	0	0	2	11	2	7
Dizziness	1	9	0	0	1	3
Pruritus	0	0	1	5	1	3
Bone pain	0	0	0	0	0	0

2.5.2 Original Case Study

A 52-year old female experienced onset of menopause with severe, frequent hot flashes. Due to a family history of breast cancer and personal preferences, the subject declined hormonal therapy. The hot flashes occurred both day and night, and were disruptive to the subject's sleep and daily activities at home and at work. She was otherwise healthy. In her annual visit to her physician she was noted to have persistently low WBC (range 2.9 – 3.5 x10^9/L) and ANC (1.3 – 1.5 x 10^9/L) counts (normal reference ranges are 4.0 – 11.1 x 10^9/L and 1.8 – 7.8 x 10^9/L respectively). She was evaluated by a hematologist at the University of Colorado for cyclic neutropenia and, after exclusion of other etiologies by her physician, was given a single injection of Neupogen® (300 mcg/SC). Within four days her WBC and ANC counts increased into the normal reference range (4.1 x 10^9/L; 2.4 x 10^9/L) and returned to pre-treatment levels by 30 days (3.1 x 10^9/L; 1.4 x 10^9/L). The only AE experienced after the injection was a transient mild tingling sensation, of short duration. Five to seven days after the injection she noted a dramatic decline in the frequency and severity of her hot flashes. This alleviation of her symptoms persisted for approximately 3 months, despite the absence of other changes in weight, dietary supplements, or medications. Because the effect was so dramatic, the literature was reviewed, and no obvious explanation was found. Approximately three years later, she received another single injection of Neupogen® (300 mcg/SC).

Before receiving the second injection, she monitored her daily hot flash frequency and severity. As previously noted, beginning at five to seven days following the injection, the frequency of her hot flashes again dramatically decreased by approximately 75%. A daily diary was kept for 30 days (see Figure 1) and for approximately the next 2 months, she noted that her hot flashes were much less frequent and much less bothersome. The hot flashes gradually returned to the pre-treatment level approximately 3 months after the injection. She also noted that her hot flashes were much less bothersome, and her joint and muscle pain had improved. Again, the only AE experienced after the injection was a transient mild tingling sensation, of short duration.

Figure 1: Recorded frequency of hot flashes in case study patient before and after administration of G-CSF (15 days before injection; and 30 days after injection)



In its FDA-approved form, G-CSF administration is known to be safe as a daily injection, often given over a period of many years, with a well-established and understood safety profile. Based on these anecdotal observations, it was initially hypothesized that G-CSF could be administered as a single injection on an as-needed basis to control hot flashes.

Clinical Study MNGX-102 is designed to further evaluate the potential use of G-CSF as an effective, non-hormonal intervention to reduce hot flashes in postmenopausal women. This study will provide the preliminary data to justify larger, controlled, proof of concept studies.

2.5.3 G-CSF Compared to Hormone Therapy and Paroxetine

The current study is designed to assess the effect of repeated injection of G-CSF vs. placebo on hot flash frequency and severity in healthy post-menopausal women. The safety of repeat administration will also be assessed in this - study. The long-term goal is to seek FDA approval for G-CSF to treat hot flashes and perhaps other vasomotor symptoms in individuals in need of such therapy. G-CSF could provide a safer alternative to HT and paroxetine for the ~70 million postmenopausal women world-wide that currently seek therapy.

2.5.2.1 Relative Safety of HT / Paroxetine vs. G-CSF

Approximately 5 million post-menopausal women are treated each year with hormone therapies despite the well-known and well publicized health risks associated with HT. HT and paroxetine are the only FDA-approved therapies for postmenopausal symptoms.

Brisdelle® and other neuroactive anti-psychotic, anti-depression and pain drugs that are often prescribed off-label carry their own significant health risks and they appear to be prescribed mainly because of the perceived risks of HT. The following information summarizes the boxed warnings, warnings and precautions that are described in the prescribing information for the HT Premarin®.

Boxed Warnings for HT: Risk of development of endometrial cancer; cardiovascular disease; breast cancer; and dementia.

Warnings for HT: See boxed warnings plus: cardiovascular disorders including stroke, coronary heart disease and venous thromboembolism; malignant neoplasms including endometrial, breast and ovarian cancers; probable dementia; gallbladder disease; hypercalcemia; visual abnormalities; anaphylactic reactions and angioedema; and exacerbation of hereditary angioedema.

Precautions for HT: Elevated blood pressure; hyperglyceridemia; hepatic impairment; hypothyroidism; fluid retention; hypocalcemia; exacerbation of endometriosis; and exacerbation of asthma, diabetes, epilepsy, migraine, porphyria, SLE, and hepatic hemangiomas.

Since publication of the Women's Health Initiative in 2002, use of HT has declined greatly due to the published health risks.

The following information summarizes the boxed warnings, warnings and precautions that are described in the prescribing information for the HT Brisdelle™.

Brisdelle™, is a low dose version of the anti-depressant paroxetine, approved for the treatment of moderate to severe vasomotor symptoms associated with menopause (REF). Brisdelle™ was approved despite a 10-4 vote against approval by the Advisory Committee due to safety concerns and lack of efficacy. It showed less than half the efficacy of HT after 24 weeks of daily dosing in terms of reduction of hot flash frequency and almost no reduction in hot flash severity (REF). The following information summarizes the boxed warnings, warnings and precautions that are described in the prescribing information for Brisdelle™ (REF).

Boxed Warnings for Brisdelle™: Potential for increased risk of suicidal thinking and behavior. Monitor for worsening or emergence of suicidal thoughts and behaviors.

Warnings for Brisdelle™: See boxed warnings plus: development of serotonin syndrome alone but particularly with concomitant use of serotonergic drugs; potential impact on

tamoxifen efficacy; abnormal bleeding; hyponatremia; bone fracture; requirement to screen patients for bipolar disorder and monitoring for mania/hypomania; seizures; akathisia; acute angle closure glaucoma and potential for cognitive and motor impairment.

Although millions of oncology and other severely neutropenic patients have received treatment with filgrastim since Neupogen was approved in 1991, there have been no clinical studies to date specifically investigating filgrastim in women who are experiencing postmenopausal hot flashes. Therefore, available data from clinical trials where filgrastim was administered to healthy volunteers provides the most relevant clinical experience data for the current patient population. Safety data obtained from trials in cancer patients and other patient populations are also included for reference information.

Based on data available from Phase 1 through Phase 3 clinical trials, including head-to-head comparison studies in healthy volunteers, and from a post-marketing safety database of over 9 million neutropenic patients, G-CSF administered as a single dose to postmenopausal women is expected to be very well tolerated, with no significant adverse reactions. On the basis of currently available data, G-CSF appears to have reproducible biologic activity and an acceptable short-term safety profile in normal subjects. The common adverse events consist mainly of bone pain, headache, fatigue and nausea. Anxiety, noncardiac chest pain, myalgias, insomnia, night sweats, skin rashes, anorexia, dizziness, weight gain, local reactions at the injection site, and vomiting have occasionally been reported as well. Of interest, G-CSF-related fever has been rarely reported. Clinically relevant drug interactions between G-CSF and other drugs have not been reported.

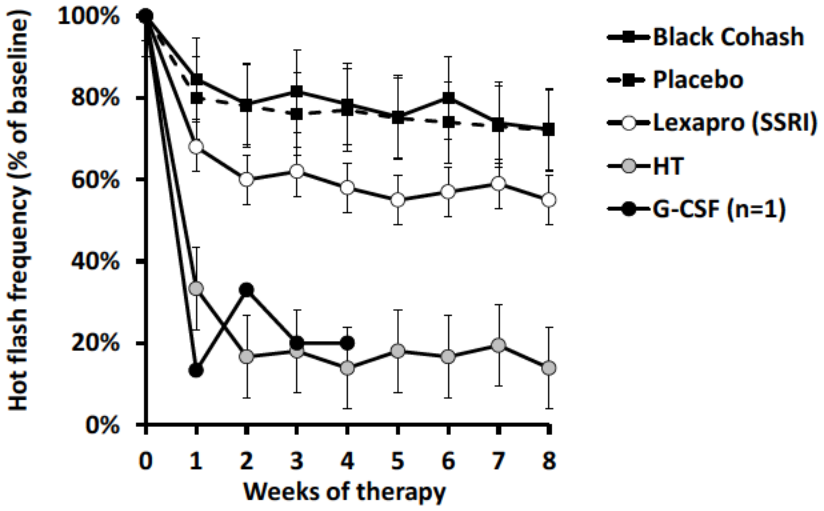
The warnings and precautions regarding the clinical use of G-CSF apply to the setting of chemotherapy-induced, congenital and idiopathic severe neutropenia and involve multiple daily injections over extended periods of time. Based on current available healthy volunteer data with a single SC injection, where serious safety events were not observed, most of the warnings/precautions observed with chronic administration are much more serious than what is expected in a healthy postmenopausal woman following a single injection of G-CSF.

Potentially relevant side effects with a single SC injection of G-CSF include sensitivity at the injection site, bone pain, muscle aches, and headaches. Unlike HT and paroxetine, G-CSF does not have a black box warning. The safety profile of three, 4-week repeated injections of G-CSF in post-menopausal women compares reasonably well with the safety profile of short-term HT use in this same population.

2.5.2.2 Relative Efficacy of HT vs. G-CSF

The relative efficacy of HT vs. G-CSF and other forms of experimental therapies, to ameliorate hot flashes is shown in Figure 2. These data have been adapted from Freeman et al. [26] and from Newton et al. [27] and are representative of other studies that have been published. In brief, HT was approved based on evidence that it reduces hot flash frequency and severity by 70 – 90%. As shown in Figure 2, the positive effect of HT is readily apparent compared to the placebo effect (20 – 30% reduction). Nutraceuticals have uniformly been found to be no different than placebo. The example that is shown here is for the Chinese herb black cohosh. As described above, other drugs that are prescribed include the SSRI and SNRI anti-depressants. The example shown below, in Figure 2, is for the SSRI Lexapro® (escitalopram). As has been reported in other studies, anti-depressants show approximately half the efficacy of HT (40 – 50% reduction).

Figure 2: Comparison of treatments for hot flash frequency (% of baseline) over weeks of therapy (Note: 30 day G-CSF data is based on the single case study involving a single administration of G-CSF on day 1)



In summary, the anecdotal results obtained with a single dose of G-CSF in reducing hot flash frequency in a postmenopausal woman compare favorably with those seen with HT and are clearly discriminated from the reported effect of placebos as well as anti-depressants.

2.5.4 Dose Selection

The recommended starting dose of Neupogen® in cancer patients receiving myelosuppressive chemotherapy is 5 mcg/kg/day, administered as a single daily injection by SC bolus injection, short IV infusion (15 to 30 minutes), continuous SC infusion, or continuous IV infusion.

Cancer patients receiving bone marrow transplant (BMT) have a recommended dose of 10 mcg/kg/day given as an IV infusion of 4 or 24 hours, or as a continuous 24-hour SC infusion.

When used for peripheral blood progenitor cell collection (PBPC) and therapy in cancer patients, the recommended dose is 10 mcg/kg/day SC, either as a bolus or a continuous infusion.

The recommended daily starting dose for congenital neutropenia is 6 mcg/kg BID SC while the recommended daily starting dose for idiopathic or cyclic neutropenia is 5 mcg/kg as a single injection SC QD (once daily).

The current study proposes to subject thirty healthy postmenopausal women to three single, subcutaneous injections of 300 mcg G-CSF at 28 day intervals.

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3 STUDY OBJECTIVES

The primary objective of this Phase 1b study is to assess the safety and pharmacodynamic (PD) effects of three single subcutaneous injection(s) of 300 mcg G-CSF administered at 28 day intervals in healthy menopausal women.

The secondary objectives of this proposed study are to assess the efficacy of three single subcutaneous injection(s) of 300 mcg G-CSF administered at 28 day intervals in reducing the frequency and severity of hot flashes in postmenopausal women and to assess additional measures of hot flash burden.

4 STUDY DESIGN AND PLAN

This is a 12-week, multicenter, double-blind, placebo-controlled study. Eligible subjects will be stratified by natural or surgical menopause and randomized (1:1) to receive three injections, 28-days apart, of either 300 mcg G-CSF or placebo.

Subjects enrolled will be given three single 1.0 mL SC injections (repeated 28-days apart), in the outer area of either upper arm, of either G-CSF or placebo (sterile physiological saline) at Baseline, Day 28 and Day 56. Subjects will be followed for 12 weeks and will complete hot flash diary entries every day for the duration of treatment. Safety will be assessed by adverse events, clinical laboratory tests (clinical chemistry and CBC with differential) and vital signs. The study will have an independent Data Safety Reviewer assigned by the NIH to review safety data at regular intervals.

4.1 Treatment Plan and Regimen

4.1.1 Treatment Plan

In total, 65 subjects will be enrolled in this study.

4.1.2 Dose, Time of Administration and Duration of Treatment

Subjects randomized to receive the active treatment dose of G-CSF will be given one 1.0 mL SC injection at Baseline, Day 28 and Day 56, prepared from an individual 1.0 mL vial containing 300 mcg G-CSF. Subjects randomized to receive placebo will be given one 1.0 mL SC injection at Baseline, Day 28 and Day 56 prepared from an individual vial of sterile, physiological, preservative free, isotonic saline (placebo), at the clinical investigational site.

Treatment with G-CSF or placebo will be given three times, 28-days apart, as a single SC injection in the outer area of either upper arm at Baseline, Day 28 and Day 56.

The study duration is 12 weeks and subjects will continue unless they meet any of the criteria for discontinuation (see **Section 7.3**).

4.2 End of Study

The end of the study will be deemed as the date of the last visit of the last subject participating in the clinical trial.

4.3 NIH-assigned Data Safety Reviewer Board

The NIH assigned Data Safety Reviewer will be responsible for reviewing safety data at regular intervals.

5 SUBJECT POPULATION

Questions regarding eligibility criteria should be addressed with MenoGeniX PRIOR to randomization of the potential subject. Eligibility criteria are standards required to ensure that subjects who enter this study are medically appropriate candidates for treatment with G-CSF.

5.1 Inclusion Criteria

Subjects must meet *all* of the following inclusion criteria to be eligible for participation in this study.

1. Female, aged 49 to 65 for naturally postmenopausal and aged 40 to 65 for surgically postmenopausal;
2. Body Mass Index (BMI) 18 to 35;
3. At least 7 moderate to severe hot flashes per day on average (or 49 moderate to severe hot flashes per week);
4. Naturally postmenopausal or surgically postmenopausal:
    a. Naturally postmenopausal is defined as having no menstrual periods for at least 12 months prior to study entry; with a biochemical criteria of menopause (FSH ≥ the reference range for menopause for the local laboratory used for screening);
    b. Surgically postmenopausal is defined as at least 3 months after documented bilateral salpingo oophorectomy;
5. Normal pelvic exam within 2 years;
6. Normal pap smear within 2 years (if uterus/cervix were present); and
7. Signed informed consent.

5.2 Exclusion Criteria

Subjects who meet *any* of the following exclusion criteria are not to be enrolled in this study.

1. Radiation or chemotherapy-induced (including GnRH agonist) menopause;
2. Prior chemotherapy or radiation therapy for cancer;
3. Prior diagnosis of hematologic malignancy;
4. Type 1 diabetics or Type 2 diabetics with HbA1c > 7.0%;
5. Use of hormone replacement therapy or oral contraceptives within the past three months;

6. Use of alternative or complementary medicines or herbs for menopausal symptoms within 30 days (refer to Appendix 2 for list of exclusionary drugs/supplements);
7. Use of any SSRI or SNRI within 30 days;
8. Use of selective estrogen modulators within 30 days;
9. Use of gabapentin within 30 days;
10. Use of clonidine within 30 days;
11. Use of megestrol acetate (Megace) within 30 days;
12. Use of prescription corticosteroids within 30 days (nasal or other inhaled corticosteroids and OTC topical corticosteroids excepted);
13. Current use of lithium therapy (related to possible risk of G-CSF);
14. History (in the past year) or presence of drug or alcohol use which, in the opinion of the Investigator, might compromise the study or confound the study results;
15. History of use of any anti-inflammatory biologics ;
16. History of or current splenomegaly (related to possible risk of G-CSF);
17. History of sickle cell disease (related to possible risk of G-CSF);
18. High risk for medical complications that might affect the subject's ability to complete the trial without a serious co-morbid event, based on medical history, physical examination and laboratory screening evaluation in the opinion of the Investigator;
19. Presence of an acute or chronic condition (such as a hematological, rheumatologic auto-immune disease, chronic inflammatory disorder, chronic lung disease or osteoporosis) based on history, clinical, or laboratory evaluation, which, in the opinion of the Investigator, might compromise the study, confound the study results or place the subject at risk;
20. Follicle stimulating hormone (FSH) below the reference range for menopause for the local laboratory used for screening
21. Thyroid stimulating hormone (TSH) outside normal limits at study entry;
22. Absolute neutrophil count (ANC) $\leq 1.0 \times 10^9$/L;
23. Total white blood cell count (WBC) $\leq 3.0 \times 10^9$/L;
24. Platelet count (PLT) $\leq 150 \times 10^9$/L;
25. Hemoglobin count (HGB) consistent with anemia;
26. Positive urine pregnancy test at Baseline visit;

27. Allergy or hypersensitivity to *E coli*-derived proteins, G-CSF, or any component of the product;
28. Mentally or legally incapacitated such that informed consent cannot be obtained;
29. Inability or unwillingness to complete daily hot flash diary and study questionnaires appropriately; and
30. Participation in another investigational trial within the past 30 days.

6 STUDY DRUG(S) AND CONCOMITANT MEDICATIONS

6.1 Description and Handling of Study Drug

The term study drug refers to G-CSF and placebo.

6.1.1 Study Drug

G-CSF will be obtained as Neupogen® (filgrastim) and is a sterile, clear, colorless, preservative-free liquid for parenteral administration.

Clear, sterile physiological, preservative free, isotonic saline will be used for the placebo.

Additional information regarding G-CSF can be found in the Investigator's Brochure.

6.1.2 Packaging and Labeling

G-CSF will be used as packaged in individual 1.0 mL vials containing 300 mcg of G-CSF (labelled as Neupogen® (filgrastim) [16]. Sterile physiological, preservative free, isotonic saline will be used from individual vials for placebo.

6.1.3 Storage and Handling

Vials of study drug should be stored in a refrigerator at a temperature between 2° and 8°C (36° to 46°F). Avoid shaking. Prior to injection, study drug may be allowed to reach room temperature for a maximum of 24 hours. Any vial or prefilled syringe left at room temperature for greater than 24 hours should not be used. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit; if particulates or discoloration are observed, the container should not be used [16].

Study drug should be stored in a secure, limited-access storage area accessible only to designated study personnel.

Saline should be stored in a refrigerator at a temperature between 2° and 8°C (36° to 46°F) in order to follow the exact same process for storage and handling of G-CSF.

6.1.4 Procedure

Additional details regarding the procedures to prepare syringes will be provided in a separate Pharmacy study manual.

Subjects will be assigned to double-blind treatment by the randomization number in sequential order.

Subjects, investigators, site staff with contact with subjects or subject records will remain blinded to study treatment during the study.

6.1.5 Breaking the Blind

An unblinded designee will be responsible for maintaining the blind throughout the study. If a subject becomes seriously ill or pregnant during the study, the blind will be broken only if knowledge of the administered study drug will affect treatment options available to the subject. Every attempt will be made to maintain the blind throughout the study and the blind can only be broken with the written consent of the MenoGeniX medical/safety director. Instructions for breaking the blind in the event of an emergency can be found in the study manual provided to each site.

6.2 Preparation and Administration of Study Drug

Study drug will be administered in a double-blind fashion for the study as a SC injection on an outpatient basis.

All unused study materials are to be returned to MenoGeniX or its designee after the clinical phase of the study has been completed.

6.2.1 Adverse Events Associated with Drug Administration

G-CSF is an FDA-approved drug with widespread human experience to date. See Table 1 for a summary of AEs associated with G-CSF (filgrastim) administration. For additional description of anticipated safety in single dose healthy volunteer studies, see **Section 2.4**.

Additional safety data are summarized in the Investigator's Brochure. The warnings and precautions regarding the clinical use of G-CSF apply to the setting of chemotherapy-induced, congenital or idiopathic severe neutropenia and involve multiple daily injections over extended periods of time. These events are much more serious than what is expected with a single SC dose repeated 28-days apart in healthy postmenopausal women which should be comparable to published healthy volunteer data, where serious safety events were not observed and where adverse events were generally mild [22,23,24,25], as also observed in the pilot study MNGX-101.

Table 1: Common Side Effects Associated with Drug Administration [16]

Common Side Effects Included in the Neupogen® Package Insert
• Sensitivity at injection site
• Risk of mild to moderate bone pain (normally seen with repeated dosing)

6.3 Drug Accountability

MenoGeniX requires that drug accountability logs be maintained. These logs must record quantities of study drug and quantity dispensed to subjects, including lot/batch number, date and time dispensed, subject identifier number, protocol number, dose, balance forward, expiry date, the initials of the person preparing the study drug syringes, and the person giving the injection should be recorded. Used drug vials will be maintained on-site until end of study closeout, and will be labeled with subject initials, subject number and date and time dispensed.

6.4 Concomitant Medications/Dietary Supplements

All concomitant medications and any dietary supplements will be recorded on the CRF.

6.4.1 Prohibited Concomitant Medications and Potential for Drug Interactions

Drug interactions between G-CSF and other drugs have not been fully evaluated. Drugs which may potentiate the release of neutrophils, such as lithium and corticosteroids, should not be used while on study.

Hormone replacement therapy or oral contraceptives, alternative or complementary medicines or herbs for menopausal symptoms, any SSRI, SNRI, or SERM, gabapentin, clonidine, megestrol acetate (Megace), and any anti-inflammatory biologics are prohibited while on study (see **Appendix 2** for full list).

6.4.1.1 *Investigational Drug Therapies*

Subjects must not receive any other investigational drugs, devices, or procedures during the study period.

7 STUDY PROCEDURES

The study procedures to be conducted for each subject enrolled in the study are presented in Appendix 1 and detailed in the text that follows.

7.1 Subject Enrollment and Study Drug Randomization

Before recruitment of subjects into the study, written IRB approval of the protocol, informed consent, advertising materials, and any additional subject information must be obtained. A screening log will be maintained to record all subjects who sign the informed consent form (ICF).

The principal investigator is responsible for verifying that the subject is eligible before study drug treatment. If any of the inclusion criteria are not met or any of the exclusion criteria are met, the subject should not be enrolled to receive study drug, however if the principal investigator would like to request a deviation for a subject, this must be approved by MenoGeniX. A site sequential screening number will be assigned to all subjects who sign an informed consent form. At Baseline, a unique subject number will be assigned to each enrolled subject. Once a subject is enrolled in the study, she will only be identified by her initials and the assigned subject number.

Randomization will be performed by a MenoGeniX unblinded designee. Randomization lists will be provided to each study site (electronically or paper). Subjects who have been confirmed for all eligibility criteria at Baseline will be randomized to active treatment with 300 mcg G-CSF or placebo in a 1:1 ratio.

Subjects must complete 14 days of run-in, then be treated at the Baseline Visit within 1 day after the run-in period. If there are more than 21 days between Screening and Baseline visits, labs will be repeated and reviewed prior to Randomization.

7.2 Study Procedures and Assessments

The Study Procedures Table is presented in Appendix 1.

Participants who meet screening eligibility requirements will participate in the study over approximately 12 weeks. The Screening visit will occur after the subject has been telephone screened by the research site staff. Some items during the screening visit may be performed via Telehealth/Zoom when appropriate. After the subjects complete the first 7 days of the 14-day run-in period of hot flash diary collection, the site staff will call the subject to review and record the daily hot flash frequency and severity. If a subject appears to qualify with at least 7 moderate to severe hot flashes per day on average, (equivalent to 49 moderate to severe hot

flashes per week), the subject will be scheduled for the Baseline visit, which should be scheduled after the completion of the 14-day run-in period, and the subject will be instructed to continue recording their hot flashes on the diary. Study drug administration will occur at the Baseline visit. The final study visit will occur at the end of 12 weeks.

Subjects must complete the Screening 14-day run-in period and daily diary entry of hot flash frequency and severity, to confirm eligibility prior to Baseline.

Procedures/assessments must be completed in the order as described below:

Visit 1 (Screening visit):

- Sign informed consent (may be performed via Telehealth/Zoom when appropriate);
- Screening number assigned (site sequential);
- Demographics, medical and medication/dietary supplement history (including smoking and alcohol history) and medical record documentation of a pelvic exam and pap smear within 2 years is required (may be performed via Telehealth/Zoom when appropriate);
- General physical exam (including vitals, temperature, weight, and height);
- Hormone status/reproductive & hot flash history (may be performed via Telehealth/Zoom when appropriate);
- Blood and serum samples will be collected for non-fasting clinical chemistry, HbA1c, CBC with differential, TSH, FSH, and cytokines (time recorded); and
- Subjects are instructed on hot flash diary completion. Subject instructed to complete diary twice a day at same time (AM and PM) and given hot flash diary to complete for the next week(s).

Coordinator-to-Subject phone call (on day 7 of the 14-day run-in period [day -8]):

- Subject is called to review and record the daily hot flash frequency and severity for the last 7 days. Subject must have at least 7 moderate to severe hot flashes per day on average, or 49 moderate to severe hot flashes per week to continue. If the minimum number of hot flashes is confirmed, the Baseline visit will be scheduled to occur after the 14-day run-in.
- Subject will be instructed to continue recording daily hot flashes on diary.

Visit 2 (Baseline visit – Day 0):

- Hot flash diary collected, reviewed and confirmed for eligibility requirements (must have minimum of 14 full days recorded). This part of the visit may take place via Telehealth/Zoom when appropriate;
- The HFRDIS, ISI, MENQOL, FSS, and AIMS will be completed by subject using RedCap prior to the in-person segment of this visit (paper questionnaires will be available to subjects as back up);
- Vitals, temperature, and weight will be obtained;
- A urine sample will be collected, and pregnancy test performed to confirm negative pregnancy test prior to study drug injection;
- Concomitant medications and medical history update is reviewed/documented (may be performed via Telehealth/Zoom when appropriate);
- Blood and serum samples will be obtained for CBC with differential, hormones and cytokines (time recorded). Lab results are not required prior to study drug injection;
- Subject number assigned;
- **After all eligibility requirements are confirmed**, subject will be given injection of study drug (G-CSF or placebo) per randomization list;
- Subject instructed to complete diary twice a day at same time (AM and PM) and given hot flash diary to complete for the next three weeks; and
- Next visit is scheduled.

Visit 3 (Day 1):

- Hot flash diary reviewed for completion;
- AEs and concomitant medications/dietary supplements are reviewed and documented and physical exam as necessary based on history (may be performed via Telehealth/Zoom when appropriate, with the exception of the physical exam);
- Blood and serum samples will be obtained for non-fasting clinical chemistry, CBC with differential, hormones and cytokines (time recorded);
- Subject instructed to continue completing diary twice a day at same time (AM and PM); and
- Next visit is scheduled.

Coordinator-to-Subject phone call (end of weeks 1 and 2):

- Subject is called as follow-up between clinic visits to inquire if any adverse events have occurred (will be documented), and as reminder to complete hot flash diary at same time twice a day (AM and PM).

Visit 4 (Day 21):

- Hot flash diary collected and reviewed for completion;
- AEs and concomitant medications/dietary supplements are reviewed and documented and physical exam as necessary based on history (may be performed via Telehealth/Zoom when appropriate, with the exception of the physical exam);
- Blood and serum samples will be obtained for non-fasting clinical chemistry, CBC with differential, hormones and cytokines (time recorded);
- Subject instructed to complete diary twice a day at same time (AM and PM) and given hot flash diary to complete for the next week; and
- Next visit is scheduled.

Visit 5 (Day 28):

- The HFRDIS, ISI, MENQOL, AIMS, and FSS are completed by subject, using RedCap, prior to the in-person segment of this visit (paper questionnaires will be available to subjects as back up);
- Vitals, temperature, and weight will be obtained;
- A urine sample will be collected and pregnancy test performed to confirm negative pregnancy test prior to study drug injection;
- Hot flash diary collected and reviewed for completion;
- AEs and concomitant medications/dietary supplements are reviewed and documented and physical exam as necessary based on history (may be performed via Telehealth/Zoom when appropriate, with the exception of the physical exam);
- Blood and serum samples will be obtained for non-fasting clinical chemistry, CBC with differential, hormones and cytokines (time recorded);
- Subject will be given injection of study drug (G-CSF or placebo) per randomization list;

- Subject instructed to complete diary twice a day at same time (AM and PM) and given hot flash diary to complete for the next three weeks; and
- Next visit is scheduled.

Visit 6 (Day 29):

- Hot flash diary reviewed for completion;
- AEs and concomitant medications/dietary supplements are reviewed and documented and physical exam as necessary based on history (may be performed via Telehealth/Zoom when appropriate, with the exception of the physical exam);
- Blood and serum samples will be obtained for non-fasting clinical chemistry, CBC with differential, hormones and cytokines (time recorded);
- Subject instructed to continue completing diary twice a day at same time (AM and PM); and
- Next visit is scheduled.

Coordinator-to-Subject phone call: (end of weeks 5, and 6):

- Subject is called as follow-up between clinic visits to inquire if any adverse events have occurred (will be documented), and as reminder to complete hot flash diary at same time twice a day (AM and PM).

Visit 7 (Day 49):

- Hot flash diary collected and reviewed for completion;
- AEs and concomitant medications/dietary supplements are reviewed and documented and physical exam as necessary based on history (may be performed via Telehealth/Zoom when appropriate, with the exception of the physical exam);
- Blood and serum samples will be obtained for non-fasting clinical chemistry, CBC with differential, hormones and cytokines (time recorded);
- Subject instructed to complete diary twice a day at same time (AM and PM) and given hot flash diary to complete for the next week; and
- Next visit is scheduled.

Visit 8 (Day 56):

- The HFRDIS, ISI, MENQOL, AIMS, and FSS are completed by subject, using RedCap, prior to the in-person segment of this visit (paper questionnaires will be available to subjects as back up);
- Vitals, temperature, and weight will be obtained;
- A urine sample will be collected, and pregnancy test performed to confirm negative pregnancy test prior to study drug injection;
- Hot flash diary collected and reviewed for completion;
- AEs and concomitant medications/dietary supplements are reviewed and documented and physical exam as necessary based on history (may be performed via Telehealth/Zoom when appropriate, with the exception of the physical exam);
- Blood and serum samples will be obtained for CBC with differential, hormones and cytokines (time recorded);
- Subject will be given injection of study drug (G-CSF or placebo) per randomization list;
- Subject instructed to complete diary twice a day at same time (AM and PM) and given hot flash diary to complete for the next four weeks; and
- Next visit is scheduled.

Visit 9 (Day 57):

- Hot flash diary reviewed for completion;
- AEs and concomitant medications/dietary supplements are reviewed and documented and physical exam as necessary based on history (may be performed via Telehealth/Zoom when appropriate, with the exception of the physical exam);
- Blood and serum samples will be obtained for non-fasting clinical chemistry, CBC with differential, hormones and cytokines (time recorded);
- Subject instructed to continue completing diary twice a day at same time (AM and PM); and
- Next visit is scheduled.

Coordinator-to-Subject phone call: (end of weeks 9, 10 and 11):

- Subject is called as follow-up between clinic visits to inquire if any adverse events have occurred (will be documented), and as reminder to complete hot flash diary at same time twice a day (AM and PM).

Visit 10 (Final – Day 84):

- The HFRDIS, ISI, MENQOL, AIMS, and FSS are completed by subject, using RedCap, prior to the in-person segment of this visit (paper questionnaires will be available to subjects as back up);
- General physical exam (including vitals, temperature, and weight);
- A urine sample will be collected, and pregnancy test performed to confirm negative pregnancy test;
- Hot flash diary collected and reviewed for completion;
- Adverse events and concomitant medications/dietary supplements are reviewed and documented and physical exam as necessary based on history (may be performed via Telehealth/Zoom when appropriate, with the exception of the physical exam); and
- Blood and serum samples will be obtained for chemistry, CBC with differential, hormones and cytokines.

Follow-up Coordinator-to-Subject phone call: (30-days post Final Visit):

- Subject is contacted approximately 30-days following last clinic visit to inquire if any adverse events occurred (will be documented), and to inquire about subject status.

7.2.1 Clinical Laboratory Tests

Additional laboratory handling instructions can be found in the Laboratory procedures study manual provided to each site. Date and time will be recorded for all laboratory collections.

Local laboratory results (chemistry, HbA1c, and TSH, BUT NOT CBC with differential) should be reviewed promptly by the investigator and will be transcribed into the electronic Case Report Forms (eCRFs) for data collection.

CBC with differential results at Days 1, 21, 28, 29, 49, 56, 57, and 84 will not be returned to the Investigator in order to avoid treatment unblinding and will be reviewed by the Medical/Safety Monitor. If of significant clinical concern, the results will be discussed with the Investigator.

A copy of the local laboratory normal reference ranges and laboratory certifications should be provided to MenoGeniX prior to the initiation of the study. MenoGeniX should be notified of any subsequent revisions to the normal ranges.

Chemistry (including sodium, potassium, calcium, ALT, AST, bilirubin, creatinine, BUN, alkaline phosphatase, and albumin will be performed at Screening in order to assess eligibility for the study and repeated at every visit. TSH, FSH, and HbA1c will be performed at Screening to assess for eligibility.

CBC with differential (including RBC, WBC, platelets, hemoglobin, hematocrit, RDW, MCV, MCH, MCHC, neutrophils, lymphocytes, monocytes, eosinophils, basophils, neutrophils [absolute], lymphocytes [absolute], monocytes [absolute], eosinophils [absolute], basophils [absolute]) will be performed at Screening in order to assess eligibility for the study and repeated at every study visit.

Hormone and cytokine levels will be collected at Baseline (additional baseline cytokine collection at the Screening visit) and repeated at every visit. The circulating hormone levels to be assessed will include FSH, LH, TSH, DHEA, DHEAS, testosterone, and estradiol. The circulating cytokine levels to be assessed will include (but will not be limited to) IL-1, IL-6, IL-8, and TNF-alpha. The samples for both the hormones and cytokines will be collected, processed, aliquoted, and frozen at -70 degrees Celsius or lower and will be sent to MenoGeniX at the end of the study for batch analysis in 2 separate shipments. If a -70 degrees or lower Celsius freezer is not available at the site, samples will be couriered the same day to a facility with a -70 degrees Celsius freezer is available. Please refer to the Laboratory procedures study manual for additional details.

7.2.2 Daily Hot Flash Diary

Subjects will be instructed to complete the hot flash diary twice-a-day at the same time for the duration of the study. A time in the morning and evening should be scheduled with the subject to record hot flash frequency and severity. A new diary will be given at each visit and collected at the following visit. If, upon review of the hot flash diary, it is noted the subject has not been compliant with completing the diary, this should be discussed with the subject during the visit. If the subject is noncompliant for at least a total of two weeks, consideration should be made regarding discontinuing the subject from the study following discussion between the Investigator and MenoGeniX.

The coordinator will discuss with the subject if an additional reminder should be set-up (such as daily text or email).

Data from the hot flash diary will be transcribed on the CRFs for data collection and the diary filed as a source document.

7.2.3 Questionnaires

Hot Flash Related Daily Interference Scale (HFRDIS) (Appendix 3), Insomnia Severity Index (ISI) (Appendix 4), Menopause-specific Quality of Life Questionnaire (MENQOL) (Appendix 5), Fatigue Severity Scale (FSS) (Appendix 6) and Arthritis Impact Measurement Scale (AIMS) (Appendix 7) will be completed at Baseline and repeated at Visit 5 (Day 28), Visit 8 (Day 56) and Visit 10 (Day 84/Final Visit).

Questionnaires should be completed by the subject at the beginning of the visit before collection of the hot flash diary and before any other procedure or assessment is completed.

7.2.4 Coordinator-to-Subject Phone Calls

Coordinator-to-Subject phone call will occur at the end of every week the subject is not seen in clinic (Weeks 1, 2, 5, 6, 9, 10, and 11) and will be documented. A time should be scheduled for the coordinator to call the subject. The subject will be asked how they are doing and asked about compliance in completing the hot flash diary at same time twice-a-day (AM and PM). If any adverse events are relayed by the subject, these should be recorded by the coordinator.

If the subject cannot be reached at the scheduled time, 2 additional documented attempts should be made by the coordinator to reach the subject each week.

7.2.5 Final Coordinator-to-Subject Phone Call

Subject is contacted approximately 30 days following last clinic visit to inquire if any adverse events occurred (will be documented), and to inquire about subject status.

7.3 Criteria for Study Discontinuation

Criteria for study discontinuation will include:

- Pregnancy, medical or ethical reasons;
- Total of at least two weeks of noncompliance with hot flash diary entry, following discussion between the Investigator and MenoGeniX;
- Subject request (excluding adverse events);
- Regulatory reasons; and/or
- Administrative reasons

8 ADVERSE EVENTS

8.1 Safety Assessment

Assessments will consist of monitoring and recording physical exams, AEs/SAEs, clinical chemistry, and CBC. The study-specific procedures and assessments are described in **Section 7** and summarized in the Study Procedures Table (Appendix 1). All subjects who have received an injection of study drug will be evaluated for safety of the study drug.

8.1.1 Clinical Laboratory Abnormalities

It is the responsibility of the Investigator to assess the clinical significance of all abnormal values as defined by the list of normal values from the local laboratory. All clinically significant laboratory values should be recorded as AEs if they meet the definition in **Section 8.2**.

CBC with differential results Days 1, 21, 28, 29, 49, 56, 57, and 84, will be reviewed by the Medical/Safety Monitor and results that may be clinically significant will be discussed with the Investigator.

8.2 Definition of Adverse Event

An adverse event (AE) is any untoward medical occurrence, including the exacerbation of a pre-existing condition, in a subject or clinical investigation subject administered a pharmaceutical product. This does not necessarily have a causal relationship with this treatment.

8.3 Definition of Serious Adverse Event

A serious adverse event (SAE) is any experience that suggests a significant hazard, contraindication, side effect, or precaution. This includes any experience that:

- Results in death;
- Is acutely life-threatening;
- Requires inpatient hospitalization or prolongs the existing hospitalization;
- Results in persistent or significant incapacity or substantial disruption of ability to conduct normal life functions;
- Is a congenital anomaly/birth defect; or
- Requires medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or

convulsions that do not result in in-subject hospitalization, or the development of drug dependency or drug abuse. This definition includes intercurrent illness or injuries, exacerbation of pre-existing conditions and adverse events occurring as a result of drug withdrawal, abuse or overdose.

If a SAE is reported from the study, the causal relationship must be provided by the investigator for all potential trial drugs, i.e., G-CSF or placebo according to the study design. If the SAE meets the regulatory criteria of being related to the drug, and is not listed in the Investigator's Brochure, then it must be reported to the manufacturer of the drug under the appropriate regulatory statutes of post-marketing safety reporting.

8.4 Adverse Event Reporting Period

Any AE (i.e., a new event or an exacerbation of a pre-existing condition) that occurs after the subject is randomized and receives study drug treatment must be recorded as an AE on the appropriate page(s) of the CRF. Any SAE that occurs more than 30 days after the last study visit should be reported if considered related to study drug. The evaluation of an AE should continue until the adverse event resolves, or until the Investigator or Sponsor determines the subject's condition is stable.

8.5 Adverse Event Assessment and Documentation

A consistent methodology for eliciting AEs should be adopted. Examples of nondirective questions include: "How have you felt since your last clinical visit?" or "Have you had any new or changed health problems since you were last here?" Clinically significant laboratory abnormalities should be reported as AEs.

All AEs will be assessed by the Investigator and recorded on the appropriate CRF page, including the dates of onset and resolution, severity, relationship to study drug, seriousness, and the action taken with the study drug. Any medication necessary for treatment of the serious adverse event must be recorded on the concomitant medication section of the subject's CRF and, if applicable, on the Serious Adverse Event Report Form.

Correct medical terminology/concepts should be used when recording AE terms. Abbreviations should be avoided. A diagnosis is preferred rather than individual signs and symptoms (e.g., record pneumonia rather than fever, cough, pulmonary infiltrate). An adverse event that meets serious criteria should be recorded **both** on the Adverse Event CRF and on the Serious Adverse Event Report Form. The Serious Adverse Event Report Form must be submitted to MenoGeniX within 24 hours of when the investigator becomes aware of the SAE (see **Section 8.6**).

The adjectives "severe" and "serious" are not synonymous. Serious is a regulatory definition (see **Section 8.3**), while severity describes the intensity of the adverse event. Severity should be recorded and graded according to the CTCAE, V5.0 (refer to the following website for the CTCAE manual or the CTCAE document):

https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

The relationship to study drug therapy should be assessed using the following definitions:

- **Not Related:** Evidence exists that the adverse event has an etiology other than the study drug (e.g., pre-existing condition, underlying disease, intercurrent illness, or concomitant medication). This includes events that are considered remotely or unlikely related to study drug.
- **Related:** A temporal relationship exists between the event onset and administration of the study drug. It cannot be readily explained by the subject's clinical state, intercurrent illness, or concomitant therapies. In case of cessation or reduction of the dose, the event abates or resolves and reappears upon re-challenge. It should be emphasized that ineffective study drug treatment should not be considered as causally related in the context of adverse event reporting. This includes events that are considered possibly, probably, or definitely related to study drug.

These criteria, in addition to good clinical judgment, should be used as a guide for determining the causal assessment.

8.5.1 Definition of Severity of Adverse Events to Study Drug

All adverse events will be examined to determine severity. All AEs should be graded according to the Common Toxicity Criteria V5.0 (dated November 2017). In the case where an AE does not have a criterion, the categories are as follows:

Mild:	Awareness of sign, symptom, or event, but easily tolerated
Moderate:	Discomfort enough to cause interference with usual activity and may warrant intervention
Severe:	Incapacitating with inability to do usual activities or significantly affects clinical status, and warrants intervention
Life-threatening:	Immediate risk of death

8.6 Serious Adverse Event Reporting Requirements

MenoGeniX is required to evaluate and expedite reporting of SAEs to regulatory authorities; therefore, the appropriate parties, as specified in this section, must be notified immediately regarding the occurrence of any serious adverse event.

All SAEs, regardless of relationship to study drug, must be reported to the Sponsor within 24 hours of the Investigator's knowledge. This should be done by faxing the completed SAE Form to the Sponsor at the number provided on the SAE Form.

The procedure for reporting serious adverse events, regardless of causal relationship, is as follows:

- Complete and sign the SAE Report Form. The form must contain sufficient information to enable medical assessment by MenoGeniX.
- If it is felt that a SAE is not related to study drug therapy, then an alternative explanation should be provided on the SAE Report Form.
- Fax the completed SAE Form to the Sponsor at the number provided on the SAE Form.
- Copies of medical records including radiology reports, laboratory results, and autopsy reports may also be attached to the serious adverse event report as applicable. Please omit the subject's name and medical record number from the documents to ensure anonymity.

MenoGeniX may request additional information from the Investigator to ensure the timely completion of accurate safety reports.

8.7 Serious Adverse Event Report Distribution

A SAE may qualify for reporting by MenoGeniX to regulatory authorities according to the statutes of post-marketing safety reporting and all Investigators actively participating in a clinical study of G-CSF if the SAE is attributable to the study drug and is unexpected/unlisted based upon safety information in the current Investigator's Brochure. In this case, a formal notification describing the SAE and similar reports will be communicated by MenoGeniX.

MenoGeniX will notify the Investigator and the Investigator must notify the IRB of SAEs in writing and in accordance with regulations and local institutional policy.

8.8 Expected Adverse Events

The MenoGeniX Investigator's Brochure contains a complete description of the safety information for G-CSF. An expected adverse event is one that is described in the Investigator's Brochure and is not different in severity.

8.9 Pregnancy

While not expected to occur in this study's population, pregnancies are not considered to be AEs or SAEs; however, pregnancies must be reported to MenoGeniX following the SAE timelines. Pregnancies will be followed through to outcome.

Available data from published studies, including several observational studies of pregnancy outcomes in women exposed to filgrastim products and those who were unexposed, have not established an association with Neupogen® use during pregnancy and major birth defects, miscarriage, or adverse maternal or fetal outcomes. Additional information is described in the Investigator's Brochure and in the Neupogen® package insert.

9 STATISTICAL METHODS

The primary objective of this pilot study is to describe the effects of three single (repeated) subcutaneous injection of G-CSF on the frequency and severity of hot flashes in postmenopausal women.

The secondary objectives of this pilot study are to assess additional measures of hot flash burden, circulating hormone and inflammatory cytokine concentrations, and safety.

9.1 Sample Size

In total, 65 subjects will be enrolled in this study. Subjects will be stratified by natural menopause or surgical menopause groups, and randomized 1:1 to either 300 mcg G-CSF or placebo.

The sample size of 65 subjects is based on clinical feasibility. This is a phase 1b study to determine feasibility of the treatment and the ability to conduct a larger powered study.

9.2 Study Endpoints

9.2.1 Safety

Safety of three single subcutaneous injections of G-CSF at 28-day intervals in a postmenopausal healthy subpopulation;

9.2.2 Evaluation of Effect

Changes from baseline throughout the entire 12 weeks will be evaluated in the:

Primary Endpoints:

- Safety and pharmacodynamics.
    - Pharmacodynamic Endpoints: Assessment of changes from baseline in circulating white blood cells, hormone and inflammatory cytokine concentrations.

Secondary Endpoints:

- Clinical endpoints

  The effect of repeated administration of G-CSF in the following indicators of vasomotor symptoms in women with naturally occurring or surgically induced menopause at weeks 2, 4, 6, 8, 10 and 12 weeks post-administration:
- Hot flashes – change from baseline in:

- Frequency of total daily hot flashes (mild + moderate + severe hot flashes)
- Daily frequency of moderate + severe hot flashes
- Daily composite hot flash severity score (1 x mild + 2 x moderate + 3 x severe hot flashes)
- Hot flash severity score ((1 x mild + 2 x moderate + 3 x severe hot flashes)/total hot flashes)

- Quality of life questionnaires - change from baseline in:
  - Hot Flash Related Daily Interference Scale (HFRDIS)
  - Fatigue Severity Scale (FSS)
  - Insomnia Severity Index (ISI)
  - Menopause-specific Quality of Life Questionnaire (MENQOL)
  - Arthritis Impact Measurement Scale (AIMS)
  - Frequency and severity of hot flashes.

9.2.3 Exploratory Analyses

Changes from baseline throughout the entire 12 weeks and will be evaluated in the:

Secondary Endpoints:

- Circulating hormone levels as a result of three injections of G-CSF or placebo; and
- Circulating pharmacodynamics and inflammatory cytokine levels as a result of three injections of G-CSF or placebo.

9.3 Data Analysis

All analyses will be looked at as per protocol population and intent to treat population, as applicable, and will employ descriptive statistics (means, medians, etc.) and post-hoc hypothesis testing.

This is an exploratory, pilot study intended to gather descriptive information on safety and effect, and no *a priori* assumptions are made regarding estimated treatment effects or statistical power.

9.3.1 Evaluation of Effect

The frequency of hot flashes per day will be calculated for each week, providing that within a week at least 4 days' worth of information were gathered. Missing data will be averaged from available weekly data. Descriptive statistics will be used to describe changes from baseline in average frequency and severity of hot flashes.

Descriptive statistical analyses will be performed to compare pre-treatment to all post-treatment points using analysis of variance (ANOVA), with appropriate *post hoc* testing, to determine statistical significance of the observed effects.

Primary Endpoints:

Descriptive statistics will be used to analyze changes from baseline throughout the entire 12 weeks in frequency and severity of hot flashes. The frequency and severity of hot flashes will be examined separately by comparing the Baseline hot flash frequency and severity per Baseline week to the frequency throughout the entire 12 weeks.

Secondary Endpoints:

Descriptive statistics will be used to analyze changes from baseline throughout the entire 12 weeks in Hot Flash Related Daily Interference Scale (HFRDIS); Insomnia Severity Index (ISI); Menopause Specific Quality of Life Questionnaire (MENQOL); Fatigue Severity Scale (FSS), and Arthritis Impact Measurement Scale (AIMS). The HFRDIS, ISI, MENQOL, FSS, and AIMS will also be scored at all relevant study weeks (Baseline, Days 28, 56, and 84).

9.3.2 Exploratory Analysis

9.3.2.1 *Circulating Hormone and Proinflammatory Cytokine Levels*

Descriptive exploratory analyses will be performed to determine whether or not G-CSF or placebo administration affects endogenous circulating hormone levels (FSH, LH, TSH, DHEA, DHEAS, testosterone, and estradiol) and proinflammatory cytokine effects (including but not limited to IL-1, IL-6, IL-8, and TNF-alpha) at every visit. ANOVA will be performed as described above.

9.3.3 *Safety*

Safety will be analyzed as intent to treat population. All subjects who receive the injection of G-CSF or placebo will be considered evaluable for all safety analyses.

Descriptive statistics will be used to summarize safety data, and summary tables for all AEs will be generated. Additional summary tables may be generated for the following population subsets: subjects with related AEs, subjects with SAEs, subjects with related SAEs, subject deaths, and subjects who discontinue due to AEs.

9.3.3.1 *Adverse Events*

All incidences of AEs will be looked at including seriousness, deaths, and discontinuation due to adverse events. Severity, Investigator-attributed relationship to study drug, duration,

and outcome of the events will also be recorded. These AEs will be coded by body system. The number and percent of each event will be calculated and summarized by body system.

9.3.3.2 *Laboratory Data*

Descriptive statistics will be used to summarize lab data. Chemistry and CBC data will be displayed in tabular form, with values outside of normal ranges noted.

10 STUDY CONDUCT

10.1 Adherence to the Protocol

The Investigator is responsible for ensuring the study is conducted in accordance with the procedures and evaluations described in this protocol.

Deviations from the protocol should be minimized. In the case of significant deviations from the protocol, the sponsor must be notified immediately. In the case of major deviation, the sponsor and the investigator will determine whether the IRBs and regulatory authorities, should be notified in accordance with local requirements.

Changes to the protocol may be made only when a written protocol amendment provided by the Sponsor has been signed by the Investigator and approved by the IRB and applicable regulatory agencies in accordance with local requirements.

10.2 Recording and Collecting of Data

10.2.1 Case Report Forms

MenoGeniX will provide CRFs, either in a paper or electronic format, for the recording and collecting of data. All CRFs, including any corrections, will be completed by site staff in a manner appropriate to the CRF format. The investigator or site designee must complete CRFs within a reasonable time period after data collection.

The Investigator will sign and date all CRF signature modules to indicate that, to his/her knowledge, the data contained in the CRF are complete and accurate.

Once the CRFs have been collected, queries or site notifications will be generated and submitted to the sites for completion. The Investigator will be responsible for assuring that all corrections have been addressed and have been acknowledged by the Investigator's or site designee's signature and date on each query or site notification form.

10.2.2 Study Files and Subject Source Documents

The Investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. These documents include Investigators' Study Files and original subject clinical source documents generated at the study site. The term "original" means the first recording of the data.

The Investigator will ensure the Study Files are maintained, including the CRFs and query forms, protocol/amendments, IRB and regulatory approvals with associated correspondence,

informed consents, study drug records, staff curriculum vitae, all correspondence, and other appropriate documents.

Subject clinical source documents may include, but are not limited to, subject hospital/clinic records, physicians' and nurses' notes, appointment books, diaries, questionnaires, laboratory reports. The Investigator must ensure that all original source documents are available to support monitoring activities.

10.3 Monitoring

The Sponsor of this study is responsible for ensuring the proper conduct of the study with regard to protocol adherence and validity of data recorded in the CRFs. The Sponsor will oversee monitoring of the study by a CRA. The CRA is responsible for reviewing the CRFs at regular intervals throughout the study, verifying adherence to the protocol, ensuring completeness, consistency, and accuracy of the data, and reviewing study files and drug accountability (see **Section 10.3.1**). The original medical records and laboratory results (with the exception of the blinded CBC results) will be reviewed as part of source document verification to ensure validity of the data. The Investigator's responsibility is to ensure that any issues detected in the course of a monitoring visit are resolved. When the CRF pages are completed and signed by the Investigator, they will be collected by the CRAs as applicable.

10.3.1 Drug Accountability

As outlined in **Section 6.1**, the Investigator is responsible for ensuring adequate accountability of all used and unused study drug. All drug supplies and associated documentation will be reviewed and verified by the CRA. Unused material cannot be disposed of until approval is obtained from the CRA. The study site is responsible for the disposal and/or destruction of all unused study drug supplies, according to the site's standard operating procedures. If the site cannot dispose of these materials, arrangements should be made between the site and the Sponsor or its representative for destruction or return of the unused study drug supplies.

10.4 Retention of Records

All clinical study documents must be retained by the Investigator until at least 2 years after the last approval of a marketing application in an ICH region (i.e., US, Europe, or Japan) and until there are no pending or contemplated marketing applications in an ICH region; or, if no application is filed or if the application is not approved for such indication, until 2 years after the investigation is discontinued and regulatory authorities have been notified. Investigators

may need to retain documents longer if required by applicable regulatory requirements or if requested by the Sponsor.

Should the Investigator wish to assign the study records to another party or move them to another location, the Sponsor must be notified in advance.

If the Investigator cannot guarantee this archiving requirement at the study site for any or all of the documents, special arrangements must be made between the Investigator and the Sponsor to store these documents in sealed containers in an off-site storage location so they can be retrieved by the Investigator in case of a regulatory inspection. Where source documents are required for the continued care of the subject, appropriate copies should be made for off-site storage.

10.5 Inspection

Good Clinical Practice (GCP) regulations require independent inspection of clinical program activities. Such inspections may be performed at any time before, during, and/or after the study. The Investigator and study staff are responsible for maintaining a comprehensive and accurate filing system of all study-related documentation that will be suitable for inspection at any time by the Sponsor, its designees, and/or regulatory agencies. In signing this protocol, the Investigator understands and agrees to give access to the necessary documentation and files.

10.6 Legal and Ethical Requirements

10.6.1 Good Clinical Practice

The Investigator will ensure that this study is conducted in full compliance with GCP, which includes ICH GCP guidelines, applicable GCP regulations, and with any other applicable laws and regulations of the country in which the research is conducted, whichever affords the greater protection to the study subject.

10.6.2 Institutional Review Board Approval

The Investigator must submit this protocol, the informed consent form(s), and any accompanying material that will be provided to the subject (such as advertisements, subject information sheets, or descriptions of the study used to obtain informed consent) to the IRB, if unable to use the central IRB. Approval from the board/committee must be obtained **before** starting the study and documented in writing to the Investigator specifying the protocol number, protocol version, documents reviewed, and date on which the committee met and granted the approval. Written evidence of the approval must be made available to the

Sponsor. Any modifications made to the protocol after receipt of IRB approval must also be submitted to the board/committee for approval prior to implementation.

Appropriate reports on the progress of the study will be made to the IRB in accordance with applicable regulations, institutional policy, and in agreement with policies established by the Sponsor.

10.6.3 Informed Consent

The Investigator will submit the informed consent to the Sponsor for approval prior to submitting to the IRB. The Investigator is responsible for obtaining written, informed consent(s) from each subject interested in participating in this study prior to conducting any study-related procedures. Written informed consent should be obtained after adequate, thorough, and clear explanation of the aims, methods, objectives, potential risks, and benefits of the study, as well as any use of the subject's genetic information from the study. The Investigator must use the most current IRB-approved consent form for documenting written informed consent. Each informed consent will be appropriately signed and dated by the subject and the person obtaining consent. The investigational site must retain the original signed consent and provide a copy to the subject. Documentation of the consent process should be documented in the subject's medical record.

Significant new safety information received by the Investigator should be provided to current and future study subjects at the first available opportunity.

10.6.4 Study Termination

The Sponsor, the Investigator, and/or the regulatory authorities reserve the right to terminate the study at any time. Should termination be necessary, all parties will formulate and coordinate termination procedures. In terminating the study, the Sponsor and the Investigator will ensure that subjects' safety and rights are carefully protected.

10.6.5 Regulatory Approval

The Sponsor will determine the appropriate local, national, and/or regional regulatory approval(s) that need to be obtained in order to conduct this study.

10.7 Compensation, Insurance, and Indemnity

The Sponsor will compensate the study center for providing care for the acute treatment of injury to a subject, which, in the judgment of the Investigator and the Sponsor, occurs directly as a result of the proper use of the study drug or procedures performed in accordance with the protocol, so long as (i) the injury was not a foreseeable side effect or attributable to

the negligence or willful misconduct of the Investigator or the study center or its personnel or attributable to the pre-existing abnormal medical condition or underlying disease, or (ii) the expense is not covered by the subject's insurance. The subject's right at law to claim compensation for injury where negligence can be proven is not affected.

The Sponsor and the study center shall both maintain professional liability and other insurance reasonably necessary to insure themselves against any losses connected directly or indirectly with the study. The Sponsor will provide evidence of such coverage to the study center upon request. Issues of indemnity may be the subject of a separate document.

10.8 Disclosure of Information

Information concerning G-CSF, the protocol, the study information, patent applications, and processes, scientific data, or other pertinent information is confidential and remains the property of MenoGeniX. The Investigator may use this information for the purpose of the study only.

MenoGeniX will use information developed in this clinical study in connection with the development of G-CSF and, therefore, may disclose it as required to other clinical Investigators participating in this study and to regulatory agencies. In order to allow the use of the information derived from this clinical study, the Investigator understands that he/she has an obligation to provide all data produced during this study to MenoGeniX.

MenoGeniX considers that clinical data (complete or incomplete) constitute sensitive information and MenoGeniX must fulfill its board directed obligations. Consequently, MenoGeniX requires that verbal or written discussion of results prior to study completion and full reporting should only be undertaken with MenoGeniX's written consent.

10.9 Confidentiality and Data Protection

Individual subject medical information obtained as a result of this study is considered confidential. The Investigator and the study center will adhere to all applicable laws relating to the protection of subject information. To assure that subject confidentiality is maintained, subject data will be identified by a study-assigned number and initials only.

All MenoGeniX personnel will handle subject data in a confidential manner in accordance with applicable regulations governing clinical research. Subject records will be inspected only in connection with this research project. Information generated as a result of a subject's participation in this study may be disclosed to third parties for research and regulatory purposes in any country as determined by MenoGeniX. However, subjects will not be

individually identified but will be referred to by the study-assigned number and the subject's initials.

10.10 Study Report and Publications

The Coordinating Investigator will have the responsibility to review the final study results to confirm to the best of his/her knowledge it accurately describes the conduct and results of the study. A Coordinating Investigator will be selected from the participating Investigators by MenoGeniX prior to database lock.

After conclusion of the study, investigators in this study may make oral presentations of study results or publish such results in scientific journals or other scholarly media without prior written approval from MenoGeniX, only after the following conditions have been met:

- The results of the study in their entirety have been publicly disclosed by or with the consent of MenoGeniX in an abstract, manuscript, or presentation forum;
- The investigator has complied with all requests from MenoGeniX to delete any references to its confidential information (other than study results); and
- The study has been completed at all study sites for at least 2 years.

The investigator may submit to MenoGeniX any proposed publication or presentation along with information about the scientific journal or presentation forum at least 90 days prior to submission of the publication or presentation (6 weeks for abstracts). The investigator will comply with requests from MenoGeniX to delete references to its confidential information (other than the study results) in any paper or presentation and agrees to withhold publication or presentation for an additional 120 days in order to obtain patent protection if deemed necessary.

No such communication, presentation, or publication will include MenoGeniX's confidential information.

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12 APPENDICES

Appendix 1: Study Procedures Table

Activity	Screening 14-day Run-In Visit 1 (-21 to -14d)	Day 0 Baseline [ab] Visit 2 (+8 to +14 d)	Day 1 Visit 3 (24 hr ± 1 hr)	Day 21 Visit 4 (± 1 d)	Day 28 Visit 5 (± 1 d)	Day 29 Visit 6 (24 hr ± 1 hr)	Day 49 Visit 7 (± 1 d)	Day 56 Visit 8 (± 1 d)	Day 57 Visit 9 (24 hr ± 1 hr)	Day 84 Visit 10 / Final (± 3 d)	30 day Follow-up (± 2 d)
Informed Consent - Prior to any assessments	X										
Demographics	X										
Medical History [c]	X	X[c]									
General Physical Exam [d]	X	X[d]			X[d]			X[d]		X	
Hormone Status/Reproductive & Hot Flash History	X										
Clinical Chemistry and HbA1c [e]	X[e]	X	X	X	X	X	X	X	X	X	
Urine Pregnancy Test [f]		X			X			X		X	
CBC with Differential [g]	X[g]	X[g]	X[g]	X[g]	X[g]	X[g]	X[g]	X[g]	X[g]	X[g]	
Hormones [h]	X	X	X	X	X	X	X	X	X	X	
Cytokines [i]	X	X	X	X	X	X	X	X	X	X	
Hot Flash Diary Review/Collection [j]	Diary provided	X	X	X	X	X	X	X	X	X	
Questionnaires (HFRDIS, ISI, MENQOL, FSS, AIMS) [k]		X			X			X		X	
Randomization [l]		X									
Study Drug Injection (G-CSF or Placebo)		X			X			X			
Adverse Events (AE)			X	X	X	X	X	X	X	X	
Concomitant Medications/Dietary Supplements	X	X	X	X	X	X	X	X	X	X	
Coordinator-to-Subject Phone Calls [m]	X (at day 7 of the 14-day run-in period)		Weeks 1 and 2			Weeks 5 and 6			Weeks 9, 10, and 11		Phone call

[a] Hot flash frequency must be determined after the 14-day Run-In period to meet eligibility criteria before study drug injection.
[b] Baseline, Days 28 and 56 visits must be performed on a Monday, Tuesday, Wednesday or Thursday, in order for 24 hour follow-up visit/lab collection to occur.
[c] Smoking and alcohol history collected at Screening. Documented history of normal pelvic/pap smear within 2 years. Medical history update only at Baseline.
[d] Physical exam includes vital signs, temperature, weight and height. Physical exam may be performed at subsequent visits, as necessary based on history. Vital signs, temperature, and weight will also be obtained at Baseline, Days 28 and 56.

[e] Clinical chemistry includes (non-fasting): electrolytes, calcium, liver function tests (transaminase), bilirubin, creatinine, BUN, alkaline phosphatase, total protein, and albumin. HbA1c will only be checked at the Screening visit.
[f] Negative urine pregnancy test must be confirmed prior to study drug injections.
[g] CBC with differential includes: RBC, WBC, platelets, hemoglobin, hematocrit, RDW, MCV, MCH, MCHC, neutrophils, lymphocytes, monocytes, eosinophils, basophils, neutrophils [absolute], lymphocytes [absolute], monocytes [absolute], eosinophils [absolute], basophils [absolute]). CBC with differential results at Days 1, 21, 28, 29, 49, 56, 57, and 84, will not be returned to the Investigator in order to avoid treatment unblinding and will be reviewed by the Medical/Safety Monitor. If of significant clinical concern, the results will be discussed with the Investigator.
[h] Hormones will include FSH, LH, TSH, DHEA, DHEAS, testosterone, and estradiol. Sample for FSH and TSH only at screening to confirm eligibility, remaining visit sample collections will be stored frozen (-70 degrees C) and batch shipped at end of study.
[i] Cytokines will include (but will not be limited to) IL-1, IL-6, IL-8, and TNF-alpha. Two baseline serum samples will be collected, one at Screening and one at Baseline. Samples from all visits will be stored frozen (-70 degrees C) and batch shipped in two separate shipments at end of study. If a -70 degrees Celsius freezer is not available at the site, samples will be couriered the same day to a facility with a -70 degrees Celsius freezer (additional details provided in the Laboratory procedures study manual).
[j] Collection of completed hot flash diary from subject (review for compliance) and new diary provided to subject. Diary records daily menopausal symptoms; hot flash frequency and severity; completed by subject twice daily (AM and PM) for duration of study.
[k] Questionnaires completed by subject at clinic during indicated visit.
[l] Study drug administration and randomization should occur at the completion of screening, after all eligibility is confirmed at the Baseline visit.
[m] Coordinator-to-Subject phone calls will occur every week, except the weeks subjects are in clinic (Day 7 of the 14-day Run-in period, Weeks 1, 2, 5, 6, 9, 10, and 11) and will be documented. Subject is called as reminder to complete hot flash diary at same time twice-a-day (AM and PM) and document AEs. 30-day follow-up contact to inquire about subject status and record any AEs.

Appendix 2: Prohibited Drugs and Supplements

- Drugs which may potentiate the release of neutrophils, such as lithium and corticosteroids (exception of nasal or other inhaled corticosteroids and OTC topical corticosteroids)
- Any hormone therapy including oral contraceptives (natural or synthetic)
- Selective serotonin reuptake inhibitors (SSRIs):
    - Citalopram (Celexa, Cipramil, Cipram, Dalsan, Recital, Emocal, Sepram, Seropram, Citox, Cital)
    - Dapoxetine (Priligy)
    - Escitalopram (Lexapro, Cipralex, Seroplex, Esertia)
    - Fluoxetine (Depex, Prozac, Fontex, Seromex, Seronil, Sarafem, Ladose, Motivest, Flutop)
    - Fluoxamine (Luvox, Fevarin, Faverin, Dumyrox, Favoxil, Movox, Floxyfral)
    - Indalpine (Upstene)
    - Paroxetine (Paxil, Seroxat, Sereupin, Aropax, Deroat, Divarius, Rexetin, Xetanor, Paroxat, Loxamine, Deparoc)
    - Sertraline (Zoloft, Lustral, Serlain, Asentra)
    - Zimelidine (Zelmid, Normud)
- Serotonin-norepinephrine reuptake inhibitors (SNRIs):
    - Venlafaxine (Effexor)
    - Desvenlafaxine (Pristiq)
    - Duloxetine (Cymbalta, Yentreve)
    - Milnacipran (Dalcipran, Ixel, Savella)
    - Sibutramine (Meridia, Reductil)
- Gabapentin (Neurontin, Gralise)
- Clonidine (Catapres, Jenloga, Kapvay)
- Selective estrogen-receptor modulator (SERMs)
    - Clomifene
    - Femarelle
    - Ormeloxifene
    - Raloxifene
    - Tamoxifen
    - Toremifene
    - Lasofoxifene
- Megestrol acetate (Megace)
- Medroxyprogersterone acetate (Provera)
- Methyldopa (Aldomet)
- Pregabalin (Lyrica)
- Anti-inflammatory biologics (such as the following):
    - Actemra

- Cimzia
- Enbrel
- Humira
- Kineret
- Orencia
- Remicade
- Rituxan
- Simponi

- Any supplements specifically used for hot flashes (such as the following):
  - Dong Quai
  - Epa (Eicosapentaenoic Acid)
  - Evening Primrose Oil
  - Flaxseed
  - Kudzu
  - Progesterone
  - Pycnogenol
  - Red Clover
  - Sage
  - Wild Yam
  - Black Cohosh
  - Magnesium
  - Soy products including proestrogens
  - Vitamin E

Appendix 3: Hot Flash Related Daily Interference Scale

Appendix 4: Insomnia Severity Index

Appendix 5: Menopause Specific Quality of Life Questionnaire

Appendix 6: Fatigue Severity Scale

Appendix 7: Arthritis Impact Measurement Scale