Protocol J2D-MC-CVAB (a)

A Randomized, Double-Blind, Placebo-Controlled Study to Evaluate the Effects of Oral Single-Doses of LY3526318 on Cinnamaldehyde-Induced Dermal Blood Flow in Healthy Females

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Confidential Information

LY3526318

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Clinical Protocol Electronically Signed and Approved by Lilly on date provided below.

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1. Protocol Synopsis

Title of Study:
A randomized, double-blind, placebo-controlled study to evaluate the effects of oral single-doses of LY3526318 on cinnamaldehyde-induced dermal blood flow in healthy females.

Rationale:
LY3526318 is a small molecule that inhibits transient receptor protein ankyrin 1 (TRPA1), a calcium permeable nonselective cation channel. There is strong clinical and preclinical evidence for TRPA1 antagonism in the alleviation of chronic pain. In humans, exogenous TRPA1 agonist administration induces pain and nerve hyper-excitability. TRPA1 antagonists have been safe and well tolerated in healthy participants (ODM-108; NCT02432664) and in patients with diabetic neuropathy (GRC-17536; EU Clinical Trial Register 2012-002320-33). GRC-17536 has been efficacious for pain relief in a Phase 2a trial in a subset of patients. LY3526318 has been demonstrated to be safe and well tolerated in Study CVAA. The effect of LY3526318 on cinnamaldehyde (CA)-induced dermal blood flow (DBF) will be compared to placebo.

Objectives/Endpoints:

Objectives	Endpoints
Primary	
To assess target engagement of TRPA1 after a single dose of LY3526318	Decrease in CA-induced dermal blood flow (DBF) relative to placebo measured by laser Doppler Imaging (LDI) at 3 hours post-dose
Secondary	
To evaluate the safety and tolerability in healthy females following single oral doses of LY3526318	• Treatment-emergent adverse events • Serious adverse events
To assess target engagement of TRPA1 after a single dose of LY3526318 using an alternate imaging method	Decrease in CA-induced DBF relative to placebo measured by Laser Speckle Contrast Imaging (LSCI) at 3 hours post-dose
To assess the pharmacokinetics of LY3526318 following single oral doses of LY3526318	Summary of LY3526318 plasma concentrations by dose and time point

Summary of Study Design, Treatment Arms and Planned Duration for an Individual Participant:

Study CVAB is a randomized, double-blind, placebo-controlled, 4-way crossover study of LY3526318 in healthy female participants.

All participants will have 2 screening visits with the first to evaluate the health of the participant and the second visit to undergo a laser Doppler imaging (LDI) assessment to ensure a response of at least 100% increase in DBF between pre- and post-CA challenge. Following a screening period of up to 28 days, eligible participants will be confined to the clinical research unit (CRU) from Day -1 until all assessments are completed on Day 2 of each dosing period.

Each participant will be randomly assigned to receive 4 unique doses of study drug in a ratio of 3 LY3526318: 1 placebo, in a crossover manner with at least 6 days of washout between doses. The planned dose levels of LY3526318 will be between 10 and 100 mg.

The LDI and laser speckle contrast imaging (LSCI) assessment will be performed at approximately 3 hours and 24 hours following study drug administration. The change in DBF before and after CA application will be compared between LY3526318 and placebo.

All participants will attend scheduled visits at the CRU for follow-up safety assessments.

Number of Participants:
Sufficient number of participants will be screened to achieve 16 randomized participants.

Statistical Analysis:

For the primary endpoint, summary statistics for each cohort will be provided by treatment.

All participants who receive at least one dose of study drug and for whom the data are considered sufficient and interpretable will be included in the analyses. Additional exploratory analyses will be conducted as deemed appropriate. For continuous variables, summary statistics will include number of participants, mean, median, standard deviation, minimum, and maximum. Categorical endpoints will be summarized using number of participants, frequency, and percentages.

Plasma concentration data will be summarized and may be combined with pharmacodynamic (PD) data and pharmacokinetic (PK) data from Study CVAA, to perform population PK/PD analyses in order to inform the design of future studies.

2. Schedule of Activities

	S		Treatment Period 1				Treatment Period 2				Treatment Period 3				Treatment Period 4			FU	ED[d]
Study Day	**≤28**		**-1[b]**	**1**	**2**	**8**	**14[b]**	**15**	**16**	**22**	**28[b]**	**29**	**30**	**36**	**42[b]**	**43**	**44**	**50**	**-**
Visit Window (days)	**-**		**-**	**-**	**-**	**±1**	**-**	**-**	**-**	**±1**	**-**	**-**	**-**	**±1**	**-**	**-**	**-**	**±2**	**-**
Visit	**1[a]**	**2[a]**	**3[c]**			**4**	**5[c]**			**6**	**7[c]**			**8**	**9[c]**			**10**	
Admit to CRU			X				X				X				X				
Discharge from CRU					X				X				X				X		
CRU visit	X	X				X				X				X				X	X
Informed consent	X																		
Medical history	X																		
Height	X																		
Weight	X		X															X	X
Physical examination	C		D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	C	C
Urine drug screen and ethanol test	X		X				X				X				X			X	X
Hematology and clinical chemistry	X			P	24h			P	24h			P	24h			P	24h	X	X
Urinalysis[e]	X			P														X	X
β-hCG pregnancy test	X		X			X	X			X	X			X	X			X	X
C-SSRS	X		X				X				X				X			X	X
Vital signs including temperature	X			P and 3h	24h			P and 3h	24h			P and 3h	24h			P and 3h	24h	X	X
ECG[f]	X			P and 3h	24h			P and 3h	24h			P and 3h	24h			P and 3h	24h	X	X
Randomization				X															
Study drug administration[g]				X				X				X				X			
PK blood sample[h]				P and 3h	24h	X		P and 3h	24h	X		P and 3h	24h	X		P and 3h	24h	X	X
DBF by LDI[a,i]		X																	
DBF by LDI and LSCI[i]				P and 3h	24h			P and 3h	24h			P and 3h	24h			P and 3h	24h		
AE / Con Med	← X →																		

Abbreviations: AE = adverse events; C = compete physical examination; Con Med = concomitant medications CRU = clinical research unit; C-SSRS= Columbia-Suicide Severity Rating Scale; D = directed physical examination; DBF = dermal blood flow; ECG = electrocardiogram; ED = early discontinuation; FU = safety follow-up; h = hour; LSCI = laser speckle contrast imaging; LDI = laser Doppler imaging; P = pre-dose assessment; PK = pharmacokinetic; S = Screening.

Note: In the event that assessments are planned for the same time, assessments should be conducted in following order: LSCI DBF assessment, LDF DBF assessment, PK sample, and ECG/vital signs. The PK sample should be collected as soon as the LDI/LSCI DBF assessment is completed. The ECG and vital signs measurement should be obtained at least 30 minutes after the PK sample is drawn.

a Screening visits will be performed within 28 days before the administration of first dose. All participants will have 2 screening visits with the first to evaluate the health of the participant and the second visit to undergo the LDI DBF assessment.
b Can be performed on a day prior to dosing or pre-dose on day of dosing.
c All participants will remain in the CRU until completion of all procedures that occur the day following each dose.
d Participants who discontinue the study prior to study completion will complete the ED visit procedures.
e A standard urine dipstick may be used.
f A single 12 lead ECGs will be obtained in the supine position after at least 10 minutes rest.
g Study drug will be administered in a fasting state (8 hours pre-dose until 4 hours post-dose). The exact time study drug is administered will be recorded.
h The timing of PK sample collections may be adjusted based on clinical needs. The exact sample collection dates and times must be recorded.
i All participants will have an LDI and LSCI procedure with cinnamaldehyde challenge to measure DBF at stipulated time points. The pre-dose measurement will include at least 30 minutes of acclimatization, followed by a dermal blood flow measurement, without cinnamaldehyde application. Each post-dose measurements will include at least 30 minutes of acclimatization, followed by a pre-cinnamaldehyde dermal blood flow measurement (about 10 minutes before cinnamaldehyde application). Next, cinnamaldehyde will be applied and a post-cinnamaldehyde measurement at 20 minutes following the cinnamaldehyde application will be performed. The LDI assessment will be performed following completion of LSCI assessment.

3. Introduction

3.1. Study Rationale

LY3526318 is a small molecule designed to inhibit transient receptor protein ankyrin 1 (TRPA1), a calcium-permeable nonselective cation channel, expressed by peripheral pain-sensing nociceptors, which play a pivotal role in generation of pain (Maatuf et al. 2019). A nonclinical efficacy pharmacology study using a formalin-induced flinch model in rats showed an analgesic effect after a single dose of LY3526318. The potential of LY3526318 to produce unwanted pharmacological effects associated with central nervous system (CNS), cardiovascular, or respiratory functioning was determined in rats, monkeys, and *in vitro* systems (see Section 3.2).

As on September 2019, LY3526318 or matching placebo has been administered to 40 healthy participants in the single ascending dose (SAD) portion of the first-in-human (FIH) study J2D-MC-CVAA [CVAA]. In CVAA, single LY3526318 doses between 10 mg and 300 mg have been found to be safe and well tolerated. This CVAB study is intended to demonstrate target engagement of TRPA1 measured by dermal blood flow (DBF) response to cinnamaldehyde (CA) with LDI.

3.2. Background

Chronic pain is a major health issue affecting the quality of life of millions of patients. Nonsteroidal anti-inflammatory drugs are the primary choice of drugs for chronic pain treatment; however, efficacy is limited in many patients. Opioids are potent but cause sedation and have addictive potential, relegating them to third- and fourth-line treatment options in chronic pain (Ko et al. 2019).

The role of TRPA1 in pain and inflammation and its localization in sensory neurons has been characterized (Bodkin and Brain 2011; Ückert et al. 2017). TRPA1 agonists evoke pain and an adverse response when administered exogenously (Berta et al. 2017; Demartini et al. 2017; Maatuf et al. 2019; Wang et al. 2019). Additionally, there is genetic evidence for the contribution of TRPA1 to chronic pain (Kremeyer et al. 2010).

TRPA1 antagonists are undergoing evaluation in clinical trials, where there is evidence of efficacy and safety (ODM-108, NCT02432664; GRC 17536, EU Clinical Trial Register 2012-002320-33).

The results of the general toxicology studies, the behavioral and cardiorespiratory safety pharmacology studies, the *in vitro* human ether-à-go-go channel assay, and the *in vitro* and *in vivo* genotoxicity/mutagenicity battery justified proceeding to human clinical studies.

A CNS safety pharmacology study in the rat and a cardiovascular respiratory study (Study # 00926046) in monkeys showed no adverse findings up to the highest dose (300 mg/kg) of LY3526318 tested. No test article-related toxicity was seen at any dose in the 28 day repeat-dosing study in monkeys (Study # 00926044; highest dose was 1000 mg/kg/day). CCI [redacted]

CCI [redacted]

Ongoing review of the blinded data from the CVAA study indicated that single oral doses of LY3526318 or placebo at studied dose levels (10, 50, 100, 200 and 300 mg) were safe and well tolerated. All reported adverse events (AEs) were nonserious and mild in severity. No clinically significant observations were reported in clinical laboratory, vital signs, and electrocardiogram (ECG) data. Single dose administration of LY3526318 in the 10 to 300 mg dose range resulted in measurable LY3526318 concentrations.

3.3. Benefit/Risk Assessment

CCI [redacted]

No clinically significant safety or tolerability concerns have been identified in female participants in Study CVAA up to the highest single dose administered (300 mg).

This study is being conducted prior to non-clinical evaluations assessing the risk of developmental and reproductive toxicity by LY3526318 and its active metabolite, M6. Women of child-bearing potential (WOCBP) could become pregnant during the study, and thus expose themselves and their conceptus to LY3526318 and its metabolite M6 at levels where effects are unknown. However, these risks are mitigated by non-clinical evaluations and clinical study design. Studies in two animal species that each were 28-day repeat dose designs achieving higher concentrations than planned for this clinical study, identified no adverse effects on female organs. The risk of harm to a conceptus is minimized during the early stages of pregnancy based on *in vitro* tests for genotoxicity and mutagenicity that showed no effects by LY3526318 and M6. Clinically, WOCBP must adhere to highly effective contraception and will be monitored to exclude pregnancy including documentation of the participants last menstrual period and a negative pregnancy test as per Schedule of Activities (Section 2).

More information about the known and expected benefits, risks, serious adverse events (SAEs) and reasonably anticipated AEs of LY3526318, along with safety data from CVAA study, is provided in the Investigator's Brochure (Lilly 2019).

Additional information on scientific rationale for the study design and justification for planned LY3526318 doses are provided in Sections 5.4 and 5.5, respectively.

4. Objectives and Endpoints

Table 4-1 shows the objectives and endpoints of the study.

Table 4-1 Objectives and Endpoints

Objectives	Endpoints
Primary	
To assess target engagement of TRPA1 after a single dose of LY3526318	Decrease in CA-induced dermal blood flow (DBF) relative to placebo measured by laser Doppler imaging (LDI) at 3 hours post-dose
Secondary	
To evaluate the safety and tolerability in healthy females following a single oral dose of LY3526318	• Treatment-emergent adverse event • Serious adverse event
To assess target engagement of TRPA1 after a single dose of LY3526318 using an alternate method	Decrease in CA-induced DBF relative to placebo measured by laser speckle contrast imaging (LSCI) at 3 hours post-dose
To assess the pharmacokinetics of LY3526318 following single oral doses of LY3526318	Summary of LY3526318 plasma concentrations by dose and time points
Exploratory	
To assess target engagement of TRPA1 after a single dose of LY3526318	• Decrease in CA-induced DBF relative to placebo measured by LDI at 24 hours post-dose • Decrease in CA-induced DBF relative to placebo measured by LSCI at 24 hours post-dose
To explore the relationship between LY3526318 plasma concentration and DBF in healthy females following a single oral LY3526318 dose	• LY3526318 plasma concentrations • DBF

5. Study Design

5.1. Overall Design

This is a Phase 1 randomized, double-blind, placebo-controlled, 4-way crossover study of LY3526318 in healthy female participants.

All participants will have 2 screening visits with the first to evaluate the health of the participant and the second visit to undergo a laser Doppler imaging (LDI) assessment to ensure a response of at least 100% increase in DBF between pre- and post-CA challenge. Following a screening period of up to 28 days, eligible participants will be confined to the clinical research unit (CRU) from Day 1 until all assessments are completed on Day 2 of each dosing period.

As summarized in Table 5-1, each participant will be randomly assigned to 1 of 4 treatment sequences, with a ratio of 3 LY3526318: 1 placebo. Each participant will receive 3 doses of LY3526318 and 1 dose of placebo in a crossover manner, with at least 6 days of washout between doses. The planned dose levels of LY3526318 are anticipated not to exceed the maximum administered dose in the SAD portion of Study CVAA.

Table 5-1 Treatment Sequence

Sequence	Period 1	Period 2	Period 3	Period 4
P132 (n=4)	P	D1	D3	D2
12P3 (n=4)	D1	D2	P	D3
231P (n=4)	D2	D3	D1	P
3P21 (n=4)	D3	P	D2	D1

Abbreviations: D= LY3526318 dose; n = number of participants; P = placebo.

The LDI and laser speckle contrast imaging (LSCI) assessments will be performed at timepoints provided in Schedule of Activities (Section 2). The change in DBF before and after CA application will be compared between LY3526318 and placebo (refer to Section 9.6.1 for further details on DBF assessment).

All assessments will be performed as defined in Schedule of Activities (Section 2).

All participants will attend a safety follow-up visit about 7 days following the last dose of study drug.

Study governance considerations are described in detail in Appendix 3.

5.2. Number of Participants

Sufficient number of participants will be screened to achieve 16 randomized participants.

For purposes of this study, a participant completes the study when all scheduled procedures shown in the Schedule of Activities (Section 2) have been finished.

5.3. End of Study Definition

End of the study is the date of the last visit or last scheduled procedure shown in the Schedule of Activities (Section 2) for the last participant.

5.4. Scientific Rationale for Study Design

LY3526318 is a small molecule TRPA1 inhibitor. TRPA1 mediates neurogenic vasodilation and both TRPA1 channels and neurogenic vasodilation are activated by topical CA application. This study will assess the pharmacodynamic effects of LY3526318 following administration of single LY3526318 doses. In response to TRPA1 inhibition by LY3526318, the DBF increase induced by topical application of CA and measured by LDI and LSCI is anticipated to be reduced. Non-clinical pharmacology studies have demonstrated target engagement of other TRPA1 antagonists through changes in DBF for TRPA1 activation and antagonism (Aubdool et al. 2016). The use of two methods to measure reactive blood flow in the skin provides complementary information (Millet et al. 2011) improving the ability to translate preclinical LDI and pain reduction data to clinical data.

No SAEs are expected because doses of LY3526318 in this study were previously shown to be safe and tolerable in Study CVAA. In addition, topical application of CA has been shown to be safe and well tolerated in prior clinical studies (Buntinx et al. 2017).

5.5. Justification for Dose

The doses administered in this study will not exceed the maximum dose administered in the SAD portion of Study CVAA; which was found to be safe and well tolerated in healthy participants.

The proposed doses for this study are anticipated to be in the target engagement dose range, while providing the maximum achievable safe LY3526318 exposure, to address the study objectives.

6. Study Population

Eligibility of participants for the study will be based on the results of screening medical history, physical examination, vital signs, clinical laboratory tests, and ECG.

The nature of any conditions present at the time of the physical examination and any preexisting conditions will be documented.

Screening may occur up to 28 days prior to enrollment. If the time of screening and randomization is prolonged to greater than 28 days, the extent of rescreening will be discussed between the investigator and sponsor.

Prospective approval of protocol deviations to recruitment and enrollment criteria, also known as protocol waivers or exemptions, are not permitted.

6.1. Inclusion Criteria

Participants are eligible for inclusion in the study only if they meet all of the following criteria at screening. The timeframes included in the following criteria are relative to screening unless otherwise noted.

1. Healthy female participants as determined by medical history and physical examination.
    a. Female participants must be nonpregnant and not lactating, or of nonchildbearing potential (either surgically sterilized [e.g. tubal occlusion, hysterectomy, bilateral salpingectomy] or physiologically incapable of becoming pregnant, or postmenopausal with amenorrhea for at least 12 consecutive months)
    b. Nonpregnancy will be confirmed for all female participants through a serum pregnancy test at screening, at (each) admission to the CRU and, at other time points specified in the Schedule of Activities (Section 2). Follicle-stimulating hormone will be tested at screening for all female participants.
    c. Healthy female participants of child-bearing potential who have a fertile male sexual partner must be willing and able to practice effective contraception from screening visit ≤ 28 days to 30 days after the final visit.
    d. Sexually active participants must use a combination of 2 of the following methods of contraception, including so called 'barrier' methods:
        i. Hormonal contraceptive associated with inhibition of ovulation (oral, transdermal patches, vaginal or injectable)
        ii. Intrauterine device with or without hormones
        iii. Condom, diaphragm or cervical cap ('barrier' methods)

Contraceptive requirements do not apply for participants who are sexually abstinent or exclusively in same-sex relationships.

2. Have given written informed consent, approved by the sponsor and the Institutional Review Board (IRB) governing the site, prior to any study-specific procedures
3. Aged 18 to 45 years, inclusive.
4. Have a body-mass index of 18 to 30 kg/m^2, inclusive.
5. Are reliable and willing to make themselves available for the duration of the study.
6. Are willing to follow CRU specific study procedures including,
    a. no drugs (except study drug, hormonal contraceptives) 7 days prior to each DBF assessment and until discharge from CRU;
    b. no chocolate, alcohol, or caffeine containing products 24 hours prior to initiation of each DBF assessment and until discharge from CRU;
    c. a complete 4 hour fast (water is allowed) prior to initiation of each DBF assessment.
7. Have suitable skin characteristics for the dermal CA challenge (as determined by CRU-staff judgment) and have demonstrated at least a 100% increase in dermal flow following CA challenge as part of the screening procedures and measured through LDI.
8. Have clinical laboratory test results within normal reference range for the population or CRU, or results with acceptable deviations that are judged not clinically significant by the investigator.

6.2. Exclusion Criteria

Participants will be excluded from study enrollment if they meet any of the following criteria at screening:

1. Are male.
2. Are currently enrolled in or discontinued within the last 30 days or 5 half-lives of the study drug (whichever is longer), from a clinical study involving an investigational product or any other type of medical research judged not to be scientifically or medically compatible with this study.
3. Have received treatment with biological agents (such as monoclonal antibodies, including marketed drugs) within 3 months or 5 half-lives (whichever is longer) prior to dosing.
4. Have previously completed or withdrawn from this study or any other study investigating this study drug.
5. Have a history or presence of medical illness including, but not limited to, any cardiovascular, hepatic, respiratory, hematological, endocrine, psychiatric or neurological disease, convulsions, or any clinically significant laboratory abnormality that, in the judgment of the investigator, indicate a medical problem that would preclude study participation.
6. Have an abnormality in the 12-lead ECG that, in the opinion of the investigator, increases the risks associated with participating in the study. In addition, participants with the following findings will be excluded:

a. confirmed Frederica's corrected QT interval >470 msec One repeat ECG may be performed if required.

7. Have a history of clinically significant multiple or severe drug allergies or severe post-treatment hypersensitivity reactions, which in the opinion of the investigator may hamper participation in the study.
8. Are investigator or CRU personnel directly affiliated with this study or are immediate family members of investigator or CRU personnel. Immediate family is defined as a spouse, parent, child or sibling, whether biological or legally adopted.
9. Are Eli Lilly and Company (Lilly) employees or contractors or an immediate family member of employees or contractors.
10. Show evidence of human immunodeficiency virus (HIV) and/or positive human HIV antibodies, hepatitis C and/or positive hepatitis C antibody, or hepatitis B and/or positive hepatitis B surface antigen.
11. Have donated blood of more than 500 mL within the previous month.
12. Have an abnormal blood pressure (supine) defined as a diastolic blood pressure >90 or <50 mmHg and/or a systolic blood pressure >140 or <90 mmHg.
13. A history of drug abuse which, in the opinion of the investigator, is clinically significant or who test positive for drugs of abuse at screening or admission.
14. Cannot avoid excessive tanning (any exposure to sunlight or a tanning bed which would cause a sunburn reaction) throughout the study and cannot cover forearm for 24 hours prior to each treatment period.
15. Use lotions, oils, depilatory preparations, makeup, or other topical treatments on the arms on a regular basis, which cannot be discontinued for the duration of the study. Participants who have used any topical treatments within 7 days prior to CA application will not be enrolled in the study.
16. Are a habitual and heavy consumer of coffee or caffeinated beverages (more than approximately 4 cups of tea, coffee, or cola drinks/day). Participants who have reduced their consumption to a maximum of 4 cups of coffee/day at least 1 week prior to enrollment may participate. Participants who cannot refrain from caffeinated beverages 24 hours prior to CA application will not be enrolled in the study.
17. Are unable to refrain from drinking alcohol 24 hours prior to each DBF assessment.
18. Cannot refrain from being around second-hand smoke 24 hours prior to CA application or use nicotine-containing products. Ex-smokers should have ceased smoking at least 6 months prior to screening.

6.3. Lifestyle and/or Dietary Requirements

Throughout the study, participants may undergo medical assessments and review of compliance with requirements before continuing in the study.

6.3.1. Meals and Dietary Restrictions

The participants need to undertake fasting 8 hours pre-dose until 4 hours post-dose (water is allowed). In addition, they also need to undertake fasting 4 hours (water is allowed) before each DBF assessment.

Standard meals, according to the CRU standard operating procedures, will be provided during the stay at the CRU. Unless otherwise instructed by CRU personnel, participants will maintain their own dietary habit throughout the ambulatory phases of the study.

6.3.2. Caffeine, Alcohol, and Tobacco

Participants will need to abstain from chocolate, caffeine, and alcohol from 24 hours prior to each DB assessment until discharge from the CRU. At all other times, the participants will be instructed to abstain from drinking more than 2 alcoholic beverages per day. Caffeinated products are limited to no more than 4 consumptions per day.

Participants will need to refrain from being around second-hand smoke 24 hours prior to CA application. Ex-smokers must have ceased smoking at least 6 months prior to screening. Smoking is not allowed during the study.

6.3.3. Activity

Participants must refrain from new strenuous exercise routines 5 days prior to each CRU confinement period and throughout their CRU stay.

6.4. Screen Failures

At screening and throughout the study, procedure/lab re-tests may be conducted at the discretion of the investigator and do not constitute a re-screen. Individuals who do not meet the criteria for participation in this study (screen failure) may be re-screened once at the discretion of the investigator with sponsor's approval. If a participant is re-screened, they would be assigned a new participant number and would need to sign a new informed consent form (ICF).

7. Treatment

7.1. Treatment Administered

Treatment drugs will be administered orally in a fasted state (8 hours pre-dose until 4 hours post-dose; water is allowed).

Based on assigned treatment sequence, each participant will receive 3 doses of LY3526318 (between 10 mg and 100 mg) and 1 dose of placebo. Each dose will be separated by at least 6 days of washout period.

All 4 doses of study drug will be administered by trained CRU personnel.

The LY3526318 drug product is supplied as a hard gelatin capsule in dosage strengths of capsule in dosage strengths of 10 to 100 mg. Placebo capsules will be identical in size and shape.

The investigator or designee is responsible for

- explaining the correct use of the investigational product(s) to the participant
- verifying that the instructions are followed properly
- maintaining accurate records of investigational product dispensing and collection, and
- returning all unused medications to Lilly or its designee at the end of the study.

Note: In some cases, CRU may destroy the material if, during the CRU selection, the evaluator has verified and documented that the CRU has appropriate facilities and written procedures to dispose of clinical materials.

7.1.1. Packaging and Labeling

The drug product will be manufactured, tested, packaged, and labeled in accordance with all applicable good manufacturing practice requirements and country's regulatory requirements. A certificate of analysis will be supplied confirming that the materials are released for human use in clinical trials. LY3526318 drug products are for investigational use only and are to be used only within the context of this study.

7.2. Method of Treatment Assignment

Eligible participants will be randomly assigned to a cohort and dosing sequence (Table 5-1). Each dosing sequence will determine the administration order of 3 doses of LY3526318 and 1 dose of placebo.

A randomization table will be created by a computer software. The randomization list will be provided to the designated unblinded CRU personnel for participant randomization and dispensing purposes and kept in a secure location, accessible to the designated unblinded CRU personnel only.

7.2.1. Selection and Timing of Doses

The doses will be administered at approximately the same times on each day. The actual time of all dose administrations will be recorded in the participant's case report form (CRF).

A trained CRU personnel will administer all doses of the study drug at CRU.

7.3. Blinding

Participants, investigators, and CRU personnel performing trial-related activities or with the ability to influence study outcomes will be blinded with regards to LY3526318 and placebo treatment. To preserve the blinding of the sponsor, only a minimum number of Lilly personnel may have access to the randomization table and codes before the study is complete. Clinical research unit personnel who are responsible for participant-specific study drug preparation will not be blinded; laboratory personnel, including bioanalytical laboratory personnel, will also not be blinded. Drug product and placebo will be identical in appearance.

One set of sealed envelopes containing the randomization code will be provided to the investigator at the start of the trial. A code envelope, which reveals the treatment for a specific study participant, may be opened during the study only if the participant's well-being requires knowledge of the participant's treatment assignment.

Unblinding: In case of an emergency, the investigator, or authorized designee, has the sole responsibility when unblinding of an individual participant occurs. If the investigator, or authorized designee, determines unblinding of a participant's treatment assignment is warranted for medical management of the event, documentation must include the actual study product, dose, date of unblinding, and signature of the investigator or authorized designee. The participant's safety must always be the first consideration in making such a determination. If the investigator decides that unblinding is warranted, it is the responsibility of the investigator to promptly document the decision and rationale and notify Lilly as soon as possible.

Unless the investigator obtains Lilly medical monitor approval stating otherwise, a participant whose study treatment assignment is unblinded must be discontinued from the study. During the study, emergency unblinding should occur only by accessing the study participant's emergency code.

At the end of the study, unopened envelopes will be returned to Lilly or its designee or destroyed according to CRU procedures.

7.4. Dose Modification

Dose modification is not allowed.

7.5. Preparation/Handling/Storage/Accountability

The investigator or designee must confirm that appropriate temperature conditions have been maintained, as communicated by the sponsor, during transit for all investigational products received and any discrepancies are reported and resolved before use of the study drug. Detailed records of the amounts of study drug received, dispensed and remaining at the end of the study will be maintained.

The LY3526318 drug products are used in accordance with the protocol and will be stored in a secure and locked area with strictly limited access and monitored for temperature (manual or

automated) in accordance with the labeled storage conditions. LY3526318 drug products are allocated and dispensed by appropriately trained personnel.

Unblinded pharmacy personnel will be responsible for providing either LY3526318 or placebo to the blinded study personnel for administration based on the randomization schedule. Unblinded pharmacy personnel will follow detailed pharmacy instructions for the preparation and handling of LY3526318 drug product.

LY3526318 capsules are stable when stored refrigerated between 2°C and 8°C.

7.6. Treatment Compliance

The study drug will be administered at the CRU, and documentation of study drug administration will occur at the CRU.

A qualified designee will be responsible for monitoring the administration of the timed oral doses. A mouth check will be performed by the qualified designee to ensure that the participants have swallowed the study drug.

Participants' hands will also be verified to ensure that the study drug was ingested. Dose administration will be documented in the CRF.

7.7. Concomitant Therapy

No drugs will be allowed 7 days prior to each DBF assessment until discharge from CRU. Acetaminophen cannot be administered within 24 hours before each study drug administration.

If the need for concomitant medication arises, inclusion or continuation of the participant may be at the discretion of the investigator after consultation with the sponsor. Any medication used during the course of the study must be documented.

7.8. Treatment after the End of the Study

Not applicable.

8. Discontinuation Criteria

Participants may be withdrawn from the study if any of the following criteria are observed:

- Enrolment in any other clinical study involving an investigational product or other medical research judged not to be scientifically or medically compatible with this study.
- **Investigator's decision**: the investigator may decide that the participant should be discontinued from the study for the following reasons:

    - If, in the investigator's opinion, continuation in the study would be detrimental to the participant's well-being or study conduct, in particular in case of an AE
    - Lost to follow up after 3 attempts to contact the participant through phone, text message, or mail.

- If the investigator withdraws a participant for a study drug-related reason (according to the judgment of the investigator), he/she is considered a dropout. Dropouts may be replaced, where the sponsor deems this necessary.
- Participant's Decision: the participant requests to be discontinued from the study.
- Sponsor's Decision: Lilly stops the study or stops the participant's participation in the study for medical, safety, regulatory, or other reasons consistent with applicable laws, regulations, and good clinical practice (GCP).
- Discontinuation due to a hepatic event of liver test abnormality: Participants who are discontinued from using study drug due to a hepatic event or liver test abnormality should have additional hepatic safety data collected. Discontinuation of the study drug for abnormal liver tests should be considered by the investigator when a participant meets 1 of the following conditions after consultation with the Lilly-designated medical monitor:

    - Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) >5 × upper limit of normal (ULN)
    - ALT or AST >3 × ULN, sustained for more than 2 weeks or
    - ALT or AST >3 × ULN and total bilirubin level (TBL) >2 × ULN or prolonged prothrombin time with international normalized ratio >1.5 or
    - ALT or AST >3 × ULN with the appearance of fatigue, nausea, vomiting, right upper-quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5%)
    - Alkaline phosphatase (ALP) >3 × ULN
    - ALP >2.5 × ULN and TBL >2 × ULN
    - ALP >2.5 × ULN with the appearance of fatigue, nausea, vomiting, right quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5%).

- Pregnancy: If the pregnancy test (planned or unplanned) of female participant of child-bearing potential is positive at any time during the stay at CRU, the female participant will be discontinued from the study.
- Requirement of prohibited concomitant medication.
- Participant's failure to comply with protocol requirements or study-related procedures.
- Termination of the study by the sponsor or regulatory authorities.

Participants discontinuing from the study prematurely for any reason should complete AE and other follow-up procedures (Early Discontinuation Visit) per Section 2 (Schedule of Activities) of this protocol.

Every effort must be made to contact participants who do not return for a planned visit and the reason for withdrawal should be documented in the CRF. The participant can only be declared as 'lost to follow-up' if the investigator has had no success in contacting the participant. Participants who withdraw from the study before completion of all study activities may be replaced at the discretion of the sponsor.

9. Study Assessments and Procedures

Section 2 lists the Schedule of Activities, detailing the study procedures and their timing (including tolerance limits for timing).

Appendix 2 lists the laboratory tests that will be performed for this study.

Appendix 5 provides a summary of the maximum number and volume of invasive samples, for all sampling, during the study.

Unless otherwise stated in subsections below, all samples collected for specified laboratory tests will be destroyed within 60 days of receipt of confirmed test results. Certain samples may be retained for a longer period, if necessary, to comply with applicable laws, regulations, or laboratory certification standards.

9.1. Efficacy Assessments

Not applicable.

9.2. Adverse Events

A clinical trial AE is any untoward medical event associated with the use of a drug or drug delivery system in humans, whether or not it is considered related to that drug or drug delivery system.

Investigators are responsible for monitoring the safety of participants who have entered this study and for alerting Lilly or its designee to any event that seems unusual, even if this event may be considered an unanticipated benefit to the participant.

The investigator is responsible for the appropriate medical care of participants during the study.

Investigators must document their review of each laboratory safety report.

The investigator remains responsible for following, through an appropriate healthcare option, AEs that are serious or otherwise medically important, considered related to the study drug or a study-related procedure, or that caused the participant to discontinue the study drug before completing the study. The participant should be followed up until the event resolves, stabilizes with appropriate diagnostic evaluation, or is reasonably explained. The frequency of follow-up evaluations of the AE is left to the discretion of the investigator.

After the ICF is signed, the CRU personnel will record, via CRF, the occurrence and nature of each participant's preexisting conditions. Additionally, CRU personnel will record any change in the condition(s) and the occurrence and nature of any AEs.

Planned surgeries should not be reported as AEs unless the underlying medical condition has worsened during the course of the study.

If a participant's study drug is discontinued because of an AE, CRU personnel must report this to Lilly or its designee via CRF.

9.2.1. Serious Adverse Events

An SAE is any AE from this study that results in 1 of the following:

- death
- initial or prolonged inpatient hospitalization
- a life-threatening experience (i.e. immediate risk of dying)
- persistent or significant disability/incapacity
- congenital anomaly/birth defect
- important medical events that may not be immediately life threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent 1 of the other outcomes listed in the definition above.

The CRU personnel must alert the Lilly medical monitor, or its designee, of any SAE as soon as practically possible.

Additionally, CRU personnel must alert Lilly Global Patient Safety, or its designee, of any SAE within 24 hours of investigator awareness of the event via a sponsor-approved method. If alerts are issued via telephone, they are to be immediately followed up with official notification on study-specific SAE forms. This 24-hour notification requirement refers to the initial SAE information and all follow-up SAE information.

Although all AEs are recorded in the CRF after signing informed consent, SAE reporting to the sponsor begins after the participant has signed informed consent and has received the study drug. However, if an SAE occurs after signing informed consent, but prior to receiving the study drug, AND is considered reasonably possibly related to a study procedure then it MUST be reported.

Investigators are not obligated to actively seek AEs or SAEs in participants once they have discontinued from and/or completed the study. Serious adverse events will be collected for 30 days after the last dose of study drug. However, if the investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and he/she considers the event reasonably possibly related to the study drug or study participation, the investigator must promptly notify Lilly Global Patient Safety within 24 hours.

Pregnancy (maternal or paternal exposure to the study drug) does not meet the definition of an AE. However, to fulfill regulatory requirements any pregnancy should be reported following the SAE process to collect data on the outcome for both mother and fetus.

The designated medical monitor of the sponsor will monitor safety data throughout the course of the study. The sponsor and/or its designee will review SAEs within appropriate timeframes to meet reporting obligations imposed by regulatory authorities. All serious and unexpected AEs for this study will be reported to regulatory authorities in accordance with local laws, directives, and regulations.

9.2.1.1. Suspected Unexpected Serious Adverse Reactions

Suspected unexpected serious adverse reactions (SUSARs) are serious events that are not listed in the investigator's brochure and that the investigator reports as related to investigational product or procedure. Lilly has procedures that will be followed for the recording and expedited

reporting of SUSARs that are consistent with global regulations and the associated detailed guidance.

9.2.2. Complaint Handling

Lilly collects product complaints on investigational products and drug delivery systems used in clinical trials in order to ensure the safety of study participants, monitor quality, and to facilitate process and product improvements.

Participants should be instructed to contact the investigator as soon as possible if he or she has a complaint or problem with the study drug so that the situation can be assessed.

9.3. Treatment of Overdose

An overdose is not anticipated in the study, as all study drugs will be administered by a trained CRU member.

In case of overdose, use supportive therapy. There is no known antidote to LY3526318 therapy.

9.4. Safety

9.4.1. Laboratory Tests

For each participant, laboratory tests detailed in Appendix 2 should be conducted according to the Schedule of Activities (Section 2).

9.4.2. Vital Signs

For each participant, vital signs measurements should be conducted according to the Schedule of Activities (Section 2).

It is suggested that systolic and diastolic blood pressure and heart rate should be measured in supine position just after the ECG (if the ECG is recorded at the same time point) and before any other procedures according to the Schedule of Activities (Section 2).

Unscheduled orthostatic vital signs should be assessed, if possible, during any AE of dizziness or posture-induced symptoms. Additional vital signs may be measured during each study period if warranted.

9.4.3. Electrocardiograms

For each participant, ECGs should be collected according to the Schedule of Activities (Section 2).

Participant must be supine for approximately 10 minutes before ECG collection and remain supine but awake during ECG collection. Electrocardiograms may be obtained at additional times, when deemed clinically necessary.

In the event the assessments are planned for the same time, assessments should be conducted in following order: LSCI DBF assessment, LDI DBF assessment, PK sample, and ECG/vital signs. The PK sample should be collected as soon as the LDI/LSCI DBF assessment is completed. The

ECG and vital sign measurement should be obtained at least 30 minutes after the PK sample is drawn. The exact time of each procedure will be recorded.

All ECGs recorded should be stored at the CRU.

Any clinically significant findings from ECGs that result in a diagnosis and that occur after the participant receives the first dose of the investigational product, should be reported to Lilly, or its designee, as an AE via EDC.

Electrocardiograms will be interpreted by a qualified physician (the investigator or qualified designee) at the CRU as soon after the time of ECG collection as possible, and ideally while the participant is still present, to determine whether the participant meets entry criteria at the relevant visit(s) and for immediate participant management, should any clinically relevant findings be identified.

If a clinically significant quantitative or qualitative change from baseline is identified after enrollment, the investigator will assess the participant for symptoms (e.g. palpitations, near syncope, syncope) to determine whether the participant can continue in the study. The investigator or qualified designee is responsible for determining if any change in participant management is needed and must document his/her review of the ECG printed at the time of evaluation from each time point.

9.4.4. Physical Examination

A complete or directed physical examination will be conducted according to the Schedule of Activities (Section 2).

9.4.5. Safety Monitoring

The sponsor will monitor safety data throughout the course of the study periodically will review evolving aggregate safety data within the study through appropriate methods..

9.4.5.1. Hepatic Safety

If a study participant experiences elevated ALT ≥3X ULN, ALP ≥2X ULN, or elevated total bilirubin ≥2X ULN, liver tests (Appendix 4) should be repeated within 3 to 5 days including ALT, AST, ALP, TBL, direct bilirubin, gamma-glutamyl transferase (GGT), and creatinine kinase to confirm the abnormality and to determine if it is increasing or decreasing. If the abnormality persists or worsens, clinical and laboratory monitoring should be initiated by the investigator based on consultation with the Lilly medical monitor. Monitoring should continue until levels normalize and/or are returning to approximate baseline levels.

Additional safety data should be collected if 1 or more of the following conditions occur:

- elevation of serum ALT to ≥5X ULN on two or more consecutive blood tests
- elevated serum TBL to ≥2X ULN (except for cases of known Gilbert's syndrome)
- elevation of serum ALP to ≥2X ULN on 2 or more consecutive blood tests

- participant discontinued from treatment due to a hepatic event or abnormality of liver tests
- hepatic event considered to be a SAE.

9.5. Pharmacokinetics

For all participants, blood samples of up to 3 mL each for the determination of the concentrations LY3526318 and metabolite(s) of interest will be collected at time points specified in the Schedule of Activities (Section 2). Instructions for the collection and handling of blood samples will be provided by the sponsor.

Sampling times for PK evaluation are provided as a guidance to be adhered to as closely as possible. The actual date and time (24-hour clock time) of each sample collection must be recorded. Pre-dose samples should be obtained between waking up and dosing.

A maximum of 3 additional PK samples may be drawn at other time points during the study, if warranted and agreed upon by both the investigator and the sponsor. A PK sample should be obtained at the early termination visit, if applicable.

Plasma sample analyses of LY3526318 and possible metabolite(s) of interest will be performed using validated procedures and methods. Placebo samples are not planned to be analyzed.

Bioanalytical samples collected to measure study drug concentrations will be retained for a maximum of 1 year following last participant visit for the study.

9.6. Pharmacodynamics

9.6.1. Dermal Blood Flow (DBF) Measurements by Laser Doppler Imaging (LDI) and Laser Speckle Contrast Imaging (LSCI)

Dermal blood flow will be measured at visits and time points as specified in the Schedule of Activities (Section 2). All measurements for assessment should be performed while the participants are resting in a supine position on a comfortable bed in a quiet, ambient temperature-controlled room after at least 30 minutes acclimatization period.

Measurements of DBF will be taken with or without the application of a reactive agent to the skin. Based on the presumptive mechanism of action for LY3526318, CA will be used as the reactive agent to induce DBF.

Two methods will be used to measure DBF at each time point: DBF measurements from LDI (PeriScan PIM 3 system, Perimed, Stockholm, Sweden) and LSCI (PeriCam PSI System, Perimed, Stockholm, Sweden). The LDI assessment will be performed following LSCI assessment. The screening LDI measurements will be used to determine study eligibility.

Given that an upper extremity (forearm) requires approximately 5-days to recover from a reactive DBF measurement, the schedule between the screening and first study drug administration should account for this frequency limitation. During the first period, the extremity used for the first post-dose DBF measurements is chosen and the contralateral forearm

will be used for the 24-hour post-dose time point. Subsequent study periods should use the same schedule of DBF based on forearm and time point.

During each visit (screening and study periods), each patient will undergo at least 30 minutes acclimatization and placement of 3 rubber O-rings on the volar surface of the forearm. Prior to study drug administration, a pre-drug DBF will be collected without CA. For each time point following study drug administration, pre-CA measurements will be performed for the areas defined by the rings within 10 minutes before CA application. After the pre-CA measurements, a 20 µL topical dose of 10% CA will be applied in the proximal O-ring of the participant's forearm. Using the same procedure, a 20 µL topical dose of 3% CA will be applied in the medial ring, followed by a 20 µL topical dose of the vehicle solution to the distal ring. LDI/LSCI measurements will again be performed approximately 20 minutes after CA and vehicle applications. Specific details of the procedure will be agreed upon and documented between the sponsor and investigator and research staff operating the equipment.

9.7. Genetics

Not applicable.

9.8. Biomarkers

Not applicable.

9.9. Health Economics

Not applicable.

10. Statistical Considerations and Data Analysis

10.1. Sample Size Determination

The sample size is justified based on the statistical power to meet the primary endpoint of DBF measurements from LDI assay at t_{max}. The power calculation used 10% CA-induced absolute DBF in comparison between LY3526318 and placebo arms. For a 20% reduction in DBF (assuming mean observed placebo DBF of 444.3 arbitrary perfusion units (PU) with standard deviation of 55.1 PU) and a significance level of 0.05, a paired t-test with 16 patients results in 98% power to reject the null hypothesis. Incorporating a Bonferroni adjustment to account for multiplicity (changing the significance level to 0.05/3) lowers the power slightly to 96%.

10.2. Populations for Analyses

10.2.1. Study Participant Disposition

All participants who discontinue from the study will be identified, and the extent of their participation in the study will be summarized by treatment. If known, a reason for their discontinuation will be given.

10.2.2. Study Participant Characteristics

Participant demographics (age, race, ethnicity, height, and weight) will be summarized by treatment.

10.2.3. Concomitant Medications

Concomitant medications will be listed.

10.3. Statistical Analyses

Statistical analysis of this study will be the responsibility of Lilly or its designee.

Additional exploratory analyses of the data will be conducted as deemed appropriate. Study results may be pooled with the results of other studies for population PK and PK/PD analysis purposes.

For continuous variables, summary statistics will include number of participants, mean, median, standard deviation, minimum, and maximum. Categorical endpoints will be summarized using number of participants, frequency, and percentages. Additional analyses of the data will be conducted as deemed appropriate and may be fully detailed in a statistical analysis plan.

10.3.1. Safety Analyses

All study drug and protocol procedure AEs will be listed, and if the frequency of events allows, safety data will be summarized using descriptive methodology. Summary statistics for AE and SAE will be provided by dose level and for all placebo participants combined.

Safety assessments include laboratory tests, vital signs, ECGs, and physical examination. The parameters will be listed and summarized using standard descriptive statistics. Additional analysis will be performed as required.

10.3.2. Pharmacokinetic Analyses

Pharmacokinetic data will be presented as LY3526318 and M6 plasma concentration summaries.

10.3.3. Pharmacodynamic Analyses

The primary endpoint is the percent change from pre-CA to CA-induced DBF as measured by LDI, compared to placebo at 3 hours post-dose. Summary statistics for each cohort will be provided by treatment. Mixed-effect model for repeated measure (MMRM) will be used to evaluate the primary endpoint including covariates such as treatment, period, carry-over, and sequence. Similar analyses will also be conducted for LDI at 24 hours post dose as well as DBF outcomes as measured by LSCI (laser speckle contrast imaging) at 3 hours and 24 hours.

10.3.4. Pharmacokinetic/Pharmacodynamic Analyses

Pharmacokinetic and PD data may be combined with PK data from previous studies, to perform population PK/PD analyses in order to inform the design of future studies.

10.3.5. Interim Analyses

No interim analyses are planned for this study. If an unplanned interim analysis is deemed necessary, the Lilly medical monitor or designee will consult with the appropriate medical director or designee to determine if it is necessary to amend the protocol.

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12. Appendices

Appendix 1. Abbreviations and Definitions

Term	Definition
AE	adverse event: Any untoward medical occurrence in a patient or clinical investigation participant administered a pharmaceutical product that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
blinding	A procedure in which one or more parties to the study are kept unaware of the treatment assignment(s). Unless otherwise specified, blinding will remain in effect until final database lock. A single-blind study is one in which the investigator and/or his staff are aware of the treatment but the participant is not, or vice versa, or when the sponsor is aware of the treatment but the investigator and/his staff and the participant are not. A double-blind study is one in which neither the participant nor any of the investigator or sponsor staff who are involved in the treatment or clinical evaluation of the participants are aware of the treatment received
CA	cinnamaldehyde
CNS	central nervous system
CRU	clinical research unit
C-SSRS	Columbia-Suicide Severity Rating Scale
DBF	dermal blood flow
enroll	The act of assigning a participant to a treatment. Participants who are enrolled in the study are those who have been assigned to a treatment.
enter	Participants entered into a study are those who sign the informed consent form directly or through their legally acceptable representatives.
ECG	electrocardiogram
ED	early discontinuation
ERB	ethical review board
FIH	first-in-human
FU	safety follow-up
GCP	good clinical practice
HIV	human immunodeficiency virus

IB	Investigator's Brochure
ICF	informed consent form
ICH	International Council for Harmonization
IND	Investigational New Drug: An application to the FDA to allow testing of a new drug in humans.
informed consent	A process by which a participant voluntarily confirms his or her willingness to participate in a particular study, after having been informed of all aspects of the study that are relevant to the participant's decision to participate. Informed consent is documented by means of a written, signed and dated informed consent form.
IRB	institutional review board
investigator	A person responsible for the conduct of the clinical study at a study site. If a study is conducted by a team of individuals at a study site, the investigator is the responsible leader of the team and may be called the principal investigator.
LSCI	laser speckle contrast imaging
LDI	laser Doppler imaging
randomize	the process of assigning participant to an experimental group on a random basis
PK/PD	pharmacokinetic/pharmacodynamic
SAD	single ascending dose
SAE	serious adverse event
screen	The act of determining if an individual meets minimum requirements to become part of a pool of potential candidates for participation in a clinical study.
SUSARs	suspected unexpected serious adverse reactions
TEAE	treatment-emergent adverse event: Any untoward medical occurrence that emerges during a defined treatment period, having been absent pretreatment, or worsens relative to the pretreatment state, and does not necessarily have to have a causal relationship with this treatment
TBL	total bilirubin
TRPA1	Transient receptor protein ankyrin 1
ULN	upper limit of normal
WOCBP	woman of child-bearing potential

Appendix 2. Clinical Laboratory Tests

Safety Laboratory Tests

Hematology	**Clinical Chemistry**
Hematocrit	Sodium
Hemoglobin	Potassium
Erythrocyte count (RBC)	Glucose (fasting)
Mean cell volume	Blood urea nitrogen (BUN)
Mean cell hemoglobin	Total cholesterol
Mean cell hemoglobin concentration	Total protein
Leukocytes (WBC)	Albumin
Platelets	Total bilirubin
Differential WBC (Absolute counts and %) of:	Alkaline phosphatase (ALP)
Neutrophils	Aspartate aminotransferase (AST)
Lymphocytes	Alanine aminotransferase (ALT)
Monocytes	Creatinine
Eosinophils	Gamma-glutamyl transferase (GGT)
Basophils	
	Alcohol breath test (ethanol) [c]
Urinalysis Dipstick	**Urine Drug Screen**[a]
Specific gravity	Nicotine metabolites
pH	Amphetamine (including XTC)
Protein	Barbiturates
Glucose	Benzodiazepine
Ketones	Cannabinoids
Bilirubin	Cocaine
Urobilinogen	Methadone
Blood	Opiates
Nitrite	
RBC and WBC counts	Hepatitis B surface antigen
	Hepatitis C antibody
	HIV[b]
	FSH
	Pregnancy Test (Serum, Quantitative β-hCG)

Abbreviations: β-hCG = beta subunit of human chorionic gonadotropin; FSH = follicle-stimulating hormone; HIV = human immunodeficiency virus; RBC = red blood cells; WBC = white blood cells.

[a] Urine drug screen may be repeated prior to admission to the clinical research unit and at other times indicated in the Schedule of Activities.

[b] Performed at screening only

[c] Breath sample for ethanol test may be repeated prior to admission to the clinical research unit and at other times indicated in the Schedule of Activities.

Appendix 3. Study Governance, Regulatory and Ethical Considerations

Informed Consent

The investigator is responsible for

- ensuring that the participant understands the nature of the study, the potential risks and benefits of participating in the study, and that his/her participation is voluntary.
- ensuring that informed consent is given by each participant or legal representative. This includes obtaining the appropriate signatures and dates on the ICF prior to the performance of any protocol procedures and prior to the administration of investigational product.
- answering any questions the participant may have throughout the study and sharing in a timely manner any new information that may be relevant to the participant's willingness to continue his or her participation in the study.
- providing a copy of the ICF to the participant or the participant's legal representative and retaining a copy on file.

Recruitment

Lilly or its designee is responsible for the central recruitment strategy for participants. Individual investigators may have additional local requirements or processes. Study-specific recruitment materials should be approved by Lilly.

Ethical Review

The investigator must give assurance that the ethical review board (ERB) was properly constituted and convened as required by the ICH guidelines and other applicable laws and regulations.

Documentation of ERB approval of the protocol and the ICF must be provided to Lilly before the study may begin at the CRU. Lilly or its representatives must approve the ICF before it is used at the CRU. All ICFs must be compliant with the ICH guideline on GCP.

The study site's ERB(s) should be provided with the following:

- study protocol and any amendments during the course of the study
- the current IB and updates during the course of the study
- ICF
- relevant site personnel curricula vitae

Regulatory Considerations

This study will be conducted in accordance with the protocol and with

1. consensus ethics principles derived from international ethics guidelines, including the Declaration of Helsinki and Council for International Organizations of Medical Sciences International Ethical Guidelines
2. applicable ICH GCP Guidelines
3. applicable laws and regulations

Some of the obligations of the sponsor will be assigned to a third-party organization.

Protocol Signatures

The sponsor's medical officer responsible will approve the protocol, confirming that, to the best of his or her knowledge, the protocol accurately describes the planned design and conduct of the study.

Final Report Signature

The sponsor's medical officer responsible and statistician will sign/approve the final clinical study report for this study, confirming that, to the best of his or her knowledge, the report accurately describes the conduct and results of the study.

Data Quality Assurance

To ensure accurate, complete, and reliable data, Lilly or its representatives will do the following:

- provide instructional materials to the study sites, as appropriate.
- provide training to instruct the investigators and study coordinators. This training will give instruction on the protocol, the completion of the CRFs, and study procedures.
- make periodic visits to the study site.
- be available for consultation and stay in contact with the study site personnel through mail, telephone, and/or fax.
- review and evaluate CRF data and/or use standard computer edits to detect errors in data collection.
- conduct a quality review of the database.

In addition, Lilly or its representatives will periodically check a sample of the participant data recorded against source documents at the study site. The study may be audited by Lilly and/or regulatory agencies at any time. Investigators will be given notice before an audit occurs.

The investigator will keep records of all original source data. This might include laboratory tests, medical records, and clinical notes. If requested, the investigator will provide the sponsor, applicable regulatory agencies, and applicable ERBs with direct access to the original source documents.

Data Collection Tools/Source Data

An EDC system will be used in this study. The site must define and retain all source records and must maintain a record of any data where source data are directly entered into the data capture system.

Data Protection

Data systems used for the study will have controls and requirements in accordance with local data protection law.

The purpose and use of participant personal information collected will be provided in a written document to the participant by the sponsor.

Study and Site Closure

Discontinuation of Study Sites

Study site participation may be discontinued if Lilly or its designee, the investigator, or the ERB of the study site judges it necessary for medical, safety, regulatory, or other reasons consistent with applicable laws, regulations, and GCP.

Discontinuation of the Study

The study will be discontinued if Lilly or its designee judges it necessary for medical, safety, regulatory, or other reasons consistent with applicable laws, regulations, and GCP.

Appendix 4. Hepatic Monitoring Tests for Treatment-Emergent Abnormality

Selected tests may be obtained in the event of a treatment-emergent hepatic abnormality and may be required in follow-up with patients in consultation with Lilly medical monitor.

Hepatic Monitoring Tests

Hepatic Hematology[a]	**Haptoglobin[a]**
Hemoglobin	
Hematocrit	**Hepatic Coagulation[a]**
RBC	Prothrombin Time
WBC	Prothrombin Time, INR
Neutrophils	
Lymphocytes	**Hepatic Serologies[a,b]**
Monocytes	Hepatitis A antibody, total
Eosinophils	Hepatitis A antibody, IgM
Basophils	Hepatitis B surface antigen
Platelets	Hepatitis B surface antibody
	Hepatitis B Core antibody
Hepatic Chemistry[a]	Hepatitis C antibody
Total bilirubin	Hepatitis E antibody, IgG
Conjugated bilirubin	Hepatitis E antibody, IgM
Alkaline phosphatase	
ALT	**Anti-nuclear antibody[a]**
AST	**Alkaline Phosphatase Isoenzymes[a]**
GGT	**Anti-smooth muscle antibody (or anti-actin antibody)[a]**
CPK	

Abbreviations: ALT = alanine aminotransferase; AST = aspartate aminotransferase; CPK = creatinine phosphokinase; GGT = gamma-glutamyl transferase; Ig = immunoglobulin; INR = international normalized ratio; RBC = red blood cells; WBC = white blood cells.

[a] Assayed by Lilly-designated or local laboratory.

[b] Reflex/confirmation dependent on regulatory requirements and/or testing availability

Appendix 5. Blood Sampling Summary

This table summarizes the approximate number of venipunctures and blood volumes for all blood sampling (screening, safety laboratories, and bioanalytical assays) during the study.

Protocol J2D-MC- CVAB Sampling Summary

Purpose	Blood Volume per Sample (mL)	Number of Blood Samples	Total Volume (mL)
Screening tests[a]	11.5	1	11.5
Clinical laboratory tests[a]	6.5	10	65
Pharmacokinetics	3	16	48
Total (rounded-up)			125

[a] Additional samples may be drawn if needed for safety purposes.

Appendix 6. Protocol Amendment J2D-MC-CVAB(a) Summary

Protocol J2D-MC-CVAB has been amended in response to queries raised by Federal Agency for Medicines and Health Products (FAMHP), Belgium. The new protocol is indicated by Amendment (a), dated 15 Nov 2019, and will be used to conduct the study in place of any preceding version of the protocol.

The overall changes and rationale for the changes made to this protocol are as follows:

Section # and Name	Description of Change	Brief Rationale
6.1. Inclusion Criteria	Contraception language changed (Criterion 1.d.i) per 'Recommendations related to contraception and pregnancy testing in clinical trials of the Clinical Trial Facilitation Group (CTFG)'	Criterion updated in response to FAMHP query.

Revised Protocol Sections

Note: Deletions have been identified by ~~strikethroughs~~ or grayed out for figures. Additions have been identified by the use of underscore.

6.1. Inclusion Criteria

1. Healthy female participants as determined by medical history and physical examination.
    a. Female participants must be nonpregnant and not lactating, or of nonchildbearing potential (either surgically sterilized [e.g. tubal occlusion, hysterectomy, bilateral salpingectomy] or physiologically incapable of becoming pregnant, or postmenopausal with amenorrhea for at least 12 consecutive months)
    b. Nonpregnancy will be confirmed for all female participants through a serum pregnancy test at screening, at (each) admission to the CRU and, at other time points specified in the Schedule of Activities (Section 2). Follicle-stimulating hormone will be tested at screening for all female participants.
    c. Healthy female participants of child-bearing potential who have a fertile male sexual partner must be willing and able to practice effective contraception from screening visit ≤ 28 days to 30 days after the final visit.

d. Sexually active participants must use a combination of 2 of the following methods of contraception, including so called 'barrier' methods:

    i. Hormonal contraceptive associated with inhibition of ovulation (oral, transdermal patches, vaginal or injectable)

    ii. Intrauterine device with or without hormones

    iii. Condom, diaphragm or cervical cap ('barrier' methods)

Contraceptive requirements do not apply for participants who are sexually abstinent or exclusively in same-sex relationships.

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