Phase 2 Clinical Trial of FIsetin to Treat CArpal Tunnel Syndrome (FITCATS)

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LIST OF ABBREVIATIONS

AE	Adverse Event/Adverse Experience
BCTQ	Boston Carpal Tunnel Syndrome Questionnaire
CBC	Complete Blood Count
CFR	Code of Federal Regulations
CRF	Case Report Form
CTS	Carpal Tunnel Syndrome
DSMB	Data and Safety Monitoring Board
DSMP	Data and Safety Monitoring Plan
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
HUVECs	Human Umbilical Vein Endothelial Cells
IB	Investigator's Brochure
ICL	Immunochemical Core Laboratory
IND	Investigational New Drug Application
IRB	Institutional Review Board
PHI	Protected Health Information
PI	Principal Investigator
SAE	Serious Adverse Event/Serious Adverse Experience
SABG	Senescence-Associated β-Galactosidase
SASP	Senescence-Associated Secretory Phenotype
SOP	Standard Operating Procedure
SSCT	Subsynovial Connective Tissue
SSS	Symptom Severity Score
UPIRTSO	Unanticipated Problem Involving Risk to Subjects or Others
WCBP	Women of Child Bearing Potential

Study Summary

Title	**Phase 2 Clinical Trial of FIsetin to Treat CArpal Tunnel Syndrome (FITCATS)**
Running Title	FITCATS
Protocol Number	21-010406
Phase	Phase II
Methodology	Interventional
Overall Study Duration	2 years
Subject Participation Duration	180 days
Single or Multi-Site	Single Site Study
Objectives	To evaluate the effectiveness of treatment of Fisetin to treat mild-moderate Carpal Tunnel Syndrome (CTS)
Number of Subjects	100 screened, 40 enrolled
Diagnosis and Main Inclusion Criteria	Adult men and women between age 21 and 80 who have a clinical diagnosis of Carpal Tunnel Syndrome (CTS)
Study Product, Dose, Route, Regimen	This study will involve a regimen with Fisetin,100 mg capsules/(~20 mg/kg body weight/day)
Duration of Administration	2 consecutive days and, after 1 month, another 2 consecutive days
Statistical Methodology	This is a phase 2 trial to determine if Fisetin has any effect on CTS, powered based on the results of known effective treatments of CTS. The primary hypothesis is that the Boston CTS questionnaire score will improve by 1 point from pre-treatment to 60 days post-treatment. The secondary hypothesis is that clinical improvement will be proportional to the effect in reducing blood markers of cellular senescence

1 Introduction

This document is a protocol for a human research study. This study will be carried out in accordance with the applicable United States government regulations and Mayo Clinic research policies and procedures.

1.1 Background

Carpal tunnel syndrome (CTS), a compression neuropathy of the median nerve at the wrist, causes numbness and weakness in the hand, resulting in loss of hand dexterity, sensation, and strength. CTS is the most commonly diagnosed disorder of the upper extremity, with a lifetime risk of 25% to 30%. It is more common in women than in men, and typically develops in people aged 45 and older. The etiology of CTS is unknown in most cases, but it is associated with other disorders of aging as well as disorders known to be associated with cellular senescence, such as diabetes and obesity. One of the most characteristic findings in CTS is progressive non-inflammatory fibrosis of the subsynovial connective tissue (SSCT) within the carpal tunnel. Current treatment of CTS is empiric, with activity modification and steroid injections for mild to moderate cases, and surgical decompression for more severe disease. Currently, roughly 50% of all patients with CTS require surgery. A better, mechanistically based, non-surgical treatment for CTS is urgently needed.

We have identified an increased burden of senescent cells in the SSCT as a potential mediator of CTS, as evidenced by increased expression of $p16^{INK4A}$, CTGF/CCN, and TGF-ß and dysregulation of multiple transcriptional markers of senescence. Further, we have preliminary data showing that senolytics, including Fisetin, can clear senescent SSCT cells *in vitro*. Fisetin has already been approved by the FDA to be used in clinical trials and is currently being used at Mayo in several Phase 2 trials to treat: senescence associated fragility in older women (AFFIRM, ClinicalTrials.gov Identifier: NCT03675724); CoV infection (NCT04476953); diabetic and chronic kidney disease (NCT03325322), and to alleviate age-related osteoporosis (NCT04313634).

Like the AFFIRM trial, our proposed FITCATS trial will enroll approximately 40 subjects with mild-moderate CTS and evidence of senescence from blood markers into a short term (180 days) prospective phase 2 study of Fisetin therapy, using as outcome measures a well-accepted patient reported outcome questionnaire (Boston CTS questionnaire, BCTQ) as well as reduction from baseline in senescence markers in the blood, including SASP factors and inflammatory markers. Based on the known performance of the BCTQ, we estimate that a sample size of 40 will be sufficient to show a clinically important difference in outcome, if it is present. A placebo control is inappropriate, because other, effective (but more invasive-so blinding is also not possible) treatments are available, and our own experience in a previous CTS trial (DUCATS; NCT02219555) has shown that patients will not agree to enroll in a trial with randomization to a placebo arm, even for periods as short as one month. Mayo sees over 1,000 patients with CTS each year, so completing this study with 40 subjects within a 2-year time frame is feasible. Each subject will be enrolled for a six-month period. Subjects may withdraw at any time, for any reason.

If this short-term Phase 2 study shows that Fisetin reduces symptoms and senescence markers and increases function in subjects with mild-moderate CTS, we will then proceed to a larger Phase 3 study comparing Fisetin to a known effective non-surgical treatment, steroid injection.

1.2 Investigational Agent

See Investigator's Brochure

1.3 Preclinical Data

See Investigator's Brochure

1.4 Clinical Data to Date

The senolytic drug used in this study is Fisetin. This proposal outlines use of the dietary supplement Fisetin dosed twice for a 2-day period one month apart.

To our knowledge, there are no published studies utilizing Fisetin for CTS. Several studies involve use of Fisetin for its anti-oxidative and anti-apoptotic effects in animal models. Fisetin may reduce oxidative stress, alleviate hyperglycemia, and improve kidney function[3,4-9]. Based on our preclinical experience with 46 agents/drugs examined and recent studies in Fisetin, these senolytics induce apoptosis of senescent cells *in vitro* with minimal effects on non-senescent or healthy cells[1,2]. Removal of these senescent cells has broad potential implications including improvements in CTS symptoms and more general metabolic parameters. Preliminary data have shown that markers of cellular senescence are elevated in the carpal tunnel synovium of patients with CTS. We hypothesize that Fisetin will reduce these markers, and thereby improve the symptoms of CTS in these patients.

1.5 Dose Rationale

See Investigator's Brochure

1.6 Risks and Benefits

In this study, we will examine the effect of Fisetin as a senolytic agent in humans, which may have immense potential as a treatment for CTS in addition to other clinical benefits. As an off-target effect, removal of senescent cells may allow for functional improvement of neighboring or distant cells and tissues, allowing enhancement in overall function.

Based upon the review of the published literature and our pre-clinical data, we do not anticipate the occurrence of serious adverse events with the prescribed regimen in this study. We have identified a variety of drug-drug interactions and behavior activities (caffeine, tobacco/nicotine consumption) for which we established exclusion criteria or modification plans to minimize associated risks. We have also followed the Clinical Trials Facilitation and Coordination Group's protocol recommendations for including women of childbearing potential. Therefore, the risks of using this drug are likely minimal compared to the anticipated benefits and the knowledge that may be gained from these clinical investigations.

2 Study Objectives

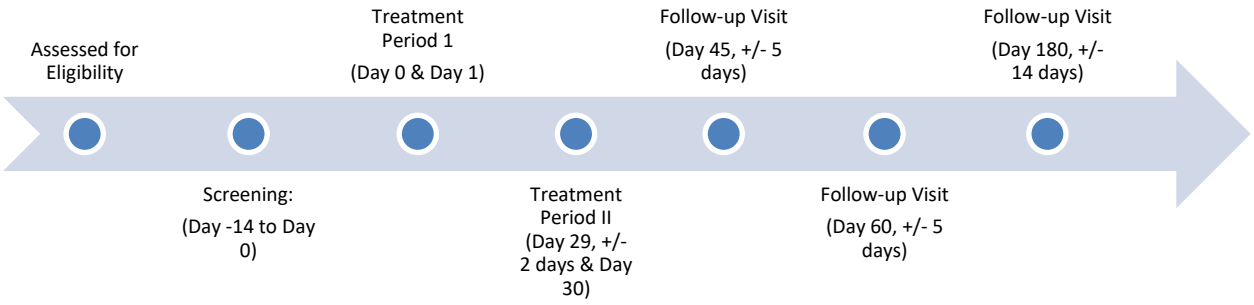
Primary Objective

To test the efficacy of the senolytic drug (Fisetin) in reducing the symptoms of CTS, as measured by the Boston CTS questionnaire, a validated measure of CTS outcome, in 20 men and 20 women with Carpal Tunnel Syndrome (CTS).

Secondary Objective

To study the effect of Fisetin on circulating and urine biomarkers of senescence in subjects with CTS, and to identify any composite clinical and biomarker measures that may improve the ability to predict outcome after treatment for CTS.

3 Study Design



3.1 General Description

This study is a phase II study evaluating the efficacy of the senolytic drug Fisetin in reducing symptoms (primary outcome) and senescent cell abundance by markers of senescence and inflammation (secondary outcomes) in men and women with CTS.

Subjects will be screened and interested qualified subjects will be consented and offered participation in this trial.

Once consent has been obtained, subjects will be screened, baseline values will be obtained, and subjects will begin treatment and follow-up for the next 180 days. The overall study duration is 2 years.

3.2 Number of Subjects

A total of 20 adult men and 20 adult women between age of 21 and 80 who have a clinical diagnosis of Carpal Tunnel Syndrome.

3.3 Duration of Participation

Participants in this study will remain in this study for the duration of 180 days (6 months), but we will request that they allow us to retain their blood and urine specimens indefinitely for possible future analysis, depending on the outcome of the study.

3.4 Primary Study Endpoints

In this pilot study, our primary endpoint will be a decrease in CTS symptoms at 60 days (2 months) compared to baseline, as measured by the Boston CTS questionnaire.

3.5 Secondary Study Endpoints

Our secondary endpoints will be the change in circulating and urine biomarkers of senescence from enrollment to the 60-day end point, and the long-term symptom outcome at 180 days.

3.6 Primary Safety Endpoints

Safety endpoints are undertaken with the measurement of safety laboratory testing, adverse event monitoring, and subject questionnaires.

3.7 Identification of Source Data

The following source data will be directly recorded on the Case Report Form (CRF) or electronic Case Report Form (eCRF) in REDCap

- Subject Eligibility Form
- Interactions & Contraceptive Measures
- Katz Stirrat Hand Diagram
- Subject Drug Diaries
- Adverse Event/Serious Adverse Event Data
- Protocol Deviations
- Patient Questionnaires and Assessments
- Hand Examination Data

The following source data will not be directly collected in the Case Report Form (CRF) but will be captured in the electronic medical record (EMR).

- Laboratory results of safety labs
- Pregnancy Test results and Screening and Safety Lab results
- Concomitant Medications, Vital Signs, and Medical History
- Investigational Product administration
- Investigational Product tracking

The following source data will be received from the testing laboratory and stored in the electronic subject files

- Peripheral Blood and Urine results

4 Subject Selection, Enrollment, and Withdrawal

4.1 Inclusion Criteria

Subjects must meet all of the following inclusion criteria to be eligible for enrollment:

- Males and females between age 21 and 80 years of age.
- Symptoms of numbness or tingling for at least 4 weeks in at least two digits on one hand that include thumb, index, long, or radial border of ring finger.
- Classic or probable carpal tunnel syndrome on Katz-Stirrat hand diagram.
- A clinical diagnosis of carpal tunnel syndrome. Patients with bilateral CTS will have the more severe hand enrolled.
- Able to complete English-language questionnaires and clinical evaluations.
- Willingness to avoid pregnancy.
    - Female participants of childbearing potential must have a negative pregnancy test at screening (serum) and before the first dose on Day 1 (urine), before the third dose on Day 29 (urine), and 60 days after the final dose on day 60 (urine).
    - Sexually active female participants of childbearing potential must agree to take appropriate precautions to avoid pregnancy from screening until 30 days after the last dose of study drug (day 60). Permitted methods in preventing pregnancy (see Appendix A) will be communicated to the participants and their compliance confirmed.
    - All female participants of childbearing potential will refrain from donating oocytes from screening-day 60 of the study.
    - Women without child bearing potential (ie., surgically sterile with hysterectomy and/or bilateral oophorectomy and/or bilateral salpingectomy OR ≥ 12 months of amenorrhea and at least 50 years of age) are eligible to participate without the above precautions.
- Willing and able to comply with study procedures and requirements and attend all study visits as defined in this protocol.

4.2 Exclusion Criteria

Subjects with any of the following exclusion criteria will not be eligible for enrollment:

4.2.1 General Exclusion Criteria:

- Unable or unwilling to give informed consent.
- Pregnant or breast feeding
- Previous carpal tunnel release on the study hand
- History of steroid injection into carpal tunnel or surgery on the affected wrist within the past 6 months.

- Prisoners, institutionalized individuals, or others who may be considered vulnerable populations, such as individuals with dementia.

4.2.2 Laboratory Exclusion Criteria:

The following laboratory tests as indicated or as *per* clinical judgement:

- Bilirubin > 2.0; serum aspartate transaminase (AST) > 4 X upper limit of normal, or alanine aminotransferase (ALT) > 4 X upper limit of normal as a marker of liver disease
- Hemoglobin < 7g/dL; white blood cell count ≤2,000/mm3 (≤2.0 x 109/L) or ≥20,000/mm3 (≥20 x 109/L); platelet count ≤40,000/μL (≤40 x 109/L); absolute neutrophil count ≤1 x 109/L; lymphocyte count <0.3 x 109/L at screening as a marker of poor nutrition
- Plasma and/or serum fasting glucose > 300 or HbA1c > 9 as a marker of poor diabetic control
- Creatinine >2.5, cystatin c >3 or eGFR< 25 ml/min/1.73 m^2 as a marker of advanced kidney disease,
- CRP > 10 or ESR >25 as a marker of systemic inflammation
- Unstable (as per clinical judgement) major cardiovascular, renal, endocrine, immunological, or hepatic disorder

4.2.3 Clinical History Exclusion Criteria

- History of diverticulitis or diverticulosis with GI bleeding, as *per* clinical judgement
- Any of the following clinical diagnosis or conditions: cervical radiculopathy, renal failure (see laboratory exclusion criteria), liver disease (see laboratory exclusion criteria), peripheral nerve disease, uncontrolled diabetes (see laboratory exclusion criteria), or other metabolic disorder; as *per* clinical judgement
- Human immunodeficiency virus infection
- Known active hepatitis B or C infection
- Invasive fungal infection
- Uncontrolled (as per clinical judgement) pleural/pericardial effusions or ascites
- New/active invasive cancer except non-melanoma skin cancers

4.2.4 Medication Exclusion Criteria

- Known hypersensitivity or allergy to Fisetin or other flavonoids
- Currently participating in another study using Fisetin or currently taking any flavonoid as a nutritional supplement. Note that consuming foods rich in flavonoids is NOT an exclusion, both because flavonoids are present in almost all fruits and vegetables, making such an exclusion impossible as a practical matter, and, from a scientific perspective, the amount of flavonoids in general, and Fisetin in particular, present in foods is far lower than the dose that will be administered in the trial.

- Patients currently taking medications listed in the Investigator Brochure are excluded unless medication can be safely held following the guidelines in the Investigator Brochure .
- If the patient is required to initiate any medication listed in the Investigator Brochure during the study, the PI will reassess their continued participation in the study.

4.2.5 Behavioral Modifications - Participants will be educated about the risk of excessive caffeine usage and nicotine usage. Namely,

- CAFFEINE: limit daily intake to no more than the equivalent of 4 cups of coffee per day or 400 milligrams of caffeine per day.
- TOBACCO/NICOTINE: limit daily intake to no more than a pack of day (20 cigarettes) or 20 mg of nicotine per day.

Alternatively, if a subject routinely uses more than these amounts they can be considered for enrollment if a behavior modification program is in place that reduces the daily usage by half.

4.3 Subject Recruitment, Enrollment, and Screening

We will recruit patients from our Hand Clinic and other clinical areas at Mayo Clinic. We will also recruit via Mayo Clinic's social media outreach, facilitated by Research Recruitment Services of the Office of Clinical Trials. All subjects will be screened before they receive the first dose of study drug (i.e., screening on Days -14 to Day -1 and study drug administration on Days 0,1,29, 30). Adult patients who meet the inclusion and exclusion criteria will be offered enrollment into the study. We will make every effort to recruit minorities. The minority population in our local community is roughly 10%. Monthly meetings of the PI and study coordinator will assure steady progress towards accomplishing the recruitment goals.

4.4 Early Withdrawal of Subjects

4.4.1 When and How to Withdraw Subjects

All subjects will be assessed during the 2 days of medication administration, subsequent days of medication administration, and all study visits. If a severe adverse event (SAE) occurs at any time during administration of the 2-day drug regimen, a formal review will occur, and subsequent patients will be enrolled one at a time using the same regimen. If three or more events accrue, the pilot study will be held and either a potential dosing regimen revisited, or discontinuation of the study protocol will occur. Other interventions will be at the direction of the Food and Drug Administration and Mayo Institutional Review Board.

Patients may also wish to withdraw if they perceive that their symptoms of carpal tunnel syndrome are not improving, and they wish additional treatment such as surgery or a steroid injection. If they are tolerating the Fisetin otherwise, we will request that they continue the Fisetin in addition to the other treatments, and such patients will continue to be followed, but will be excluded from the final analysis. We aim to continue enrollment until 40 patients complete the 60-day trial time point without intervening treatment for their CTS.

Study Completion:
For each subject in the study, the end of study will be reached when the 60-day treatment **and** post-treatment safety follow-up period at 180 days since enrollment have been completed.

Subject withdrawal:
A subject may be withdrawn from the study prior to that subject completing all of the study related procedures. Some reasons may include:

- Subject safety issues
- Failure of subject to adhere to protocol requirements
- Disease progression
- Subject decision to withdraw from the study (withdrawal of consent)

Withdrawn subjects may not reenter the study.

Premature withdrawal from study:
Subjects may voluntarily withdraw from the study for any reason at any time. Subjects are considered withdrawn if they state an intention to withdraw further participation in all components of the study, die, or are lost to follow-up for any other reason. The investigator may withdraw a subject from the study (without regard to the subject's consent) if they believe that continued participation in the study would be contrary to the best interests of the patient.

Subjects are considered as lost to follow-up if all reasonable attempts by the investigator to communicate with the individual fail. The investigator will take preventive measures to avoid a subject being lost to follow-up *(e.g*., document different ways of contact such as telephone number, home address, e-mail address, person to be contacted in case the subject cannot be reached). If the subject cannot be reached, the investigator will make a reasonable effort to contact the subject, document all attempts and enter the loss of follow-up information into the Case Report Form (CRF). The following methods will be used: at least two telephone calls will be placed to the last available telephone number (each call on different days) and one registered letter will be sent by post to the last available home address. If the subject is still unreachable after all contact attempts listed above, he/she will be lost to follow-up.

If premature withdrawal occurs for any reason, the reason for premature withdrawal from the study, along with who made the decision (subject, investigator) will be recorded in the CRF.

Subject replacement:
Subjects withdrawn from the study will be replaced by newly recruited subjects meeting inclusion criteria matching similar baseline characteristics (age, gender, race, , and/or blood or urine assay senescence marker positivity).

4.4.2 Data Collection and Follow-up for Withdrawn Subjects

For safety monitoring, telephone contact of withdrawn subjects receiving Fisetin will be attempted. Research data will not be collected on subjects after they are withdrawn from the study, any additional evaluation will be for subject safety only.

5 Study Drug

5.1 Description

While Fisetin is currently available and marketed as a dietary supplement, this study will use a product manufactured under cGMP conditions. The Fisetin will be supplied in 100 mg capsules

to be administered orally. The investigational supplies will be obtained from Vital Nutrients or Sharp Clinical Services, contracted GMP manufacturers. Fisetin capsules manufactured by Vital Nutrients are 00 opaque blue in color, Fisetin capsules manufactured by Sharp Clinical Services are size 00 green in color.

5.2 Treatment Regimen

The treatment dosing regimen is based on the preclinical information available about Fisetin along with information about similar clinical drug trials using the alternative senolytic compounds Dasatinib and Quercetin. Participants will be administered Fisetin over a two-day course of treatment twice during the study duration. Each day subjects will receive a target dose of 20 mg/kg of body weight. Therefore, for an adult with a body weight of 75 kg, each day the participant will take 15 of the 100 mg capsules. Dosing should be completed in as short of a time as possible, with a goal of 10 minutes and a maximum time of 60 minutes to complete ingestion of the capsules. Subjects can empty the capsules into room temperature or cold drinks or puddings, such as milk, yoghourt, or apple sauce, if they are unable or unwilling to ingest multiple capsules. The drink or pudding will be consumed within 10 to 60 minutes. By mass spectrometry, we verified that Fisetin is stable under these conditions. Dosing will occur at days 0 and 1 and repeated at days 29 and 30.

5.3 Preparation and Administration of Study Drug

The request for the study drug will be sent to the research pharmacy and individually prepared for each subject. The number of capsules will be calculated for each study subject based on body weight to meet the target dose each day of approximately 20 mg/kg of body weight. Study subjects will be instructed to ingest all of the capsules from one bottle each day.

5.4 Subject Compliance Monitoring

Patient adherence to study treatment will be monitored by requiring the subjects to attend in-person visits (Days 0 and 29), where they will be provided the study drug.
The CTRU nurse will document drug administration on day 0 and 29 in the subject's chart as well as in the Subject Drug Diary. The Subject Drug Diary will document compliance for day 1 and 30 of drug administration at the subject's home.

5.5 Prior and Concomitant Therapy

Subjects may continue all treatments except for two related to CTS: steroid injection or surgery, during the first 60 days of the study. If a study participant is required to initiate any medication listed in Appendix 1 of the Investigator Brochure during the study, the PI will reassess their continued participation in the study.

5.6 Packaging

The bottles will be prepared and dispensed by the Research Pharmacy with appropriate labeling to include a statement that these products are *for investigational use only.*

5.7 Receiving, Storage, Dispensing, and Return

5.7.1 Receipt of Drug Supplies

The investigational products for this study will be delivered to and managed by the Research Pharmacy according to their established standard procedures.

5.7.2 Storage

Investigational products should be stored 2-8°C.

5.7.3 Dispensing of Study Drug

The study drug is to be used exclusively in the clinical study according to the instructions of this protocol and directions for use. The CRTU nurse is responsible for providing subjects with the study drug and instructions for dosing and proper storage of the study drug. They will record the amount of study drug dispensed, date of dispensing, as well as the amount of drug returned and drug remaining.

5.7.4 Return or Destruction of Study Drug

At the completion of the study, there will be a final reconciliation of drug shipped, drug dispensed, drug returns, and drug remaining. This reconciliation will be logged on the drug reconciliation form, signed, and dated. Any discrepancies noted will be documented and investigated, prior to return or destruction of unused study drug. Drug destroyed on site will be documented in the study files.

6 Study Procedures

A tabulated summary of all visits and assessments described in the following sections is provided in Table 1, Schedule of Events. To the extent possible, subjects will be expected to adhere to the established visit schedule.

After discussing the study with the investigator/appropriate study staff and after agreeing to study participation by signing the informed consent, subjects will be assigned a subject number. For any subject, it is the responsibility of the investigator or study team member to obtain written informed consent (subject's signature) prior to performing protocol-mandated assessments.

However, assessments performed as part of the routine care of the subject may be used to assess eligibility. The subject number will identify the subject throughout the duration of the study.

Table 1: Schedule of Events

	Screening	Treatment Period				Post-Treatment Follow-up		Post Study Follow-up
Time (Days)	Day -14 to Day 0	Day 0	Day 1	Day 29	Day 30	Day 45	Day 60 (Month 2)	Day 180 (Month 6, remote visit)
Visit	1	2A	2B	3A	3B	4	5	6
Visit window				±2 days		± 5 days	± 5 days	± 14 days
Informed Consent	x							
Inclusion/Exclusion Criteria	x	x		x				
Demographic Information	x							
Medical History	x	x		x			x	x
Weight & Height	x							
Vital signs		x		x			x	
Concomitant medications		x		x			x	x
Katz-Stirrat Diagram	x							
Boston CTS (BCTQ) Questionnaire		x		x			x	x
Hand Examination		x		x			x	
Hand/Wrist Pain & Interference VAS		x		x			x	x
Screening Labs[1]	x							
Safety Labs[4]				X[7]		x	x	
Pregnancy test, if applicable	X[5]	X[6]		X[6]			X[6]	
Urine[2]		x		x			x	
Peripheral blood[3]		x		x			x	
Study agent dosing		x	x	x	x			
AE Assessment		x		x			x	x

[1]Complete Blood Count (CBC) with differential (hemoglobin, hematocrit, erythrocytes, mean corpuscular volume (MCV), red blood cell (RBC) distribution width, leukocytes, neutrophils, lymphocytes, monocytes, eosinophils, basophils, platelet count), Comprehensive Metabolic Panel (CMP) (potassium, sodium, chloride, bicarbonate, anion gap, blood urea nitrogen (BUN), creatinine, eGFR, calcium, glucose (plasma or serum), total protein, albumin, aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT), and total bilirubin), cystatin C, Hemoglobin A1c, C-Reactive Protein (CRP) and ESR.
[2] Urine for Inflammation and Senescence Markers (to be stored- no testing)
[3] Peripheral blood for Inflammation and Senescence Markers (done in Kirkland lab)
[4] Complete Blood Count (CBC) with differential (hemoglobin, hematocrit, erythrocytes, mean corpuscular volume (MCV), red blood cell (RBC) distribution width, leukocytes, neutrophils, lymphocytes, monocytes, eosinophils, basophils, platelet count), Comprehensive Metabolic Panel (CMP) (potassium, sodium, chloride, bicarbonate, anion gap, blood urea nitrogen (BUN), creatinine, eGFR, calcium, glucose (plasma or serum), total protein, albumin, aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT), and total bilirubin), cystatin C, Hemoglobin A1c, C-Reactive Protein (CRP) and ESR.
[5]Serum pregnancy test (if applicable)
[6]Urine pregnancy test (if applicable), POC
[7] Safety labs can be performed at any Mayo Clinic site, up to 5 days before the 3A appt

6.1 Visit 1, Screening

Subjects will be invited to participate, review, and sign the consent form. This visit may be performed remotely, using video or phone visit options and the consent form may be signed using remote consent technology consistent with institutional standards.
Eligibility data will include screening blood tests, pregnancy test if applicable, review of medications and medical history, demographic information, Katz-Stirrat hand diagram, and weight and height. Subjects will be informed of the contraceptive protocol and potential interactions, and we will record their compliance with these restrictions. Blood for screening and safety tests will be collected either in Rochester or at another Mayo Clinic site more convenient to the potential subject's home. Subjects will be notified via phone, email, or patient portal contact if they pass the eligibility protocol and will schedule their 2A visit.

6.2 Visit 2A, Baseline, Day 0

Subjects will have their vital signs measured and review their medical history and concomitant medications with the CTRU nurse to ensure they continue to meet inclusion/exclusion criteria. Women of child bearing potential will confirm their pregnancy status via a point of care pregnancy test. The study team will administer the hand examination including measurement of pinch and grip strength and record the information into REDCap. The subject will fill out hand/wrist pain VAS and hand/wrist interference VAS assessments and the Boston CTS (BCTQ) questionnaire in REDCap. The subject will provide a blood and urine sample for inflammation and senescence markers. Finally, the CTRU nurse will administer the first IP dose and provide the subject with IP for the following day (20 mg/kg/day).

6.3 Visit 2B, Day 1

The 2nd IP administration will occur on Day 1 (12 -36 hours after the 1st IP administration). This dosing will occur at the subject's home. The subject will be instructed at the baseline visit to complete the Drug Diary and return it and the bottle along with any unused study drug.

6.4 Visit 3A, Day 29 (±2 days)

Blood for safety tests will be collected either in Rochester or at another Mayo Clinic site more convenient to the research subject's home within 5 days prior to the scheduled appt on day 29. These results will be reviewed prior to the third IP dose, to confirm there are no clinically significant changes. On day 29, subjects will return to Mayo Clinic's Clinical Trial Research Unit (CTRU). Subjects will have their vital signs measured and review changes to their medical history and concomitant medications with the CTRU nurse to ensure they continue to meet inclusion/exclusion criteria. Women of child bearing potential will confirm their pregnancy status via a point of care pregnancy test. The study team will administer the hand examination including measurement of pinch and grip strength and record the information into REDCap, record adverse events, and collect the Subject Drug Diary. The subject will fill out hand/wrist pain VAS and hand/wrist interference VAS assessments and the Boston CTS (BCTQ) questionnaire in REDCap. The subject will provide a blood and urine sample for inflammation and senescence markers. Finally, the CTRU nurse will administer the third IP dose and provide the subject with IP for the following day (20 mg/kg/day).

6.5 Visit 3B, Day 30 (±2 days)

The 4th IP administration will occur on Day 30 (12 -36 hours after the 3rd IP administration). This dosing will occur at the subject's home. The subject will be instructed at the baseline visit to complete a Drug Diary and return it and the bottle along with any unused study drug.

6.6 Visit 4, Day 45 (±5 days)

At the 45-day visit, blood for safety tests will be collected either in Rochester or at another Mayo Clinic site more convenient to the research subject's home. At this point the study intervention is completed, and subject will enter the post-treatment follow-up phase.

6.7 Visit 5, Day 60 (±5 days)

At the 60-day visit, subjects will return to Mayo Clinic's Clinical Trial Research Unit (CTRU). Subjects will have their vital signs measured and review changes to their medical history and concomitant medications with the CTRU nurse. Women of childbearing potential will confirm their pregnancy status via a point of care pregnancy test. The subject will provide a blood and urine sample for inflammation and senescence markers as well as safety labs. The study team will administer the hand examination and record the information into REDCAP.The subject will fill out hand/wrist pain VAS and hand/wrist interference VAS assessments and the Boston CTS (BCTQ) questionnaire in REDCap. The study team will record adverse events and collect the Subject Drug Diary.

6.8 Visit 6, Day 180 (remote visit) (±14 days)

At day 180, subjects will be contacted by phone and email. The study coordinator will assess the subject for Adverse Events, and record answers to the hand/wrist pain VAS, hand/wrist interference VAS, the BCTQ questionnaire, changes to medical history, and concomitant drugs into REDCap. Alternately, the subject can answer the hand/wrist pain VAS, hand/wrist interference VAS, the BCTQ questionnaire directly through the links sent via email.

6.9 Assessments/Procedures

6.9.1 Demographic Information

The following elements of demographic information will be collected at Day 0: date of birth, age, race, ethnicity, biological sex, phone number, email address, and physical address.

6.9.2 Medical History/Prior and Concomitant Medications

Medical history will be obtained preliminarily from subject medical records with incomplete entries to be completed during the screening visit by subject recall. At subsequent visits, the CTRU nurse will record medical history and concomitant medications. Medication history will include current medications only. The subject will also be questioned on their tobacco and caffeine usage.

6.9.3 Vital Signs

Weight and height will be recorded at Screening; Vital signs will be collected at all subsequent in person visits. These measurements include heart rate, respiratory rate, temperature, blood pressure, and body weight.

6.9.4 Hand Examination

The study coordinator will measure pinch and grip strength of each hand, and record hand dominance.

6.9.5 Subject Questionnaires, Assessments, and Diagrams

The subject will complete a Katz-Stirrat Hand Diagram during screening. The subject will complete VAS hand pain and VAS pain interference assessments and the Boston CTS Questionnaire (BCTQ) at Day 0, 30, 60, and 180. The context is symptoms of the study hand at the time of test administration.

6.9.6 Screening Labs

Screening labs will be collected within 14 days prior to Day 0: Complete Blood Count (CBC) with differential (hemoglobin, hematocrit, erythrocytes, mean corpuscular volume (MCV), red blood cell (RBC) distribution width, leukocytes, neutrophils, lymphocytes, monocytes, eosinophils, basophils, platelet count), Comprehensive Metabolic Panel (CMP) (potassium, sodium, chloride, bicarbonate, anion gap, blood urea nitrogen (BUN), creatinine, eGFR, calcium, glucose (plasma or serum), total protein, albumin, aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT), and total bilirubin), cystatin C, Hemoglobin A1c, C-Reactive Protein (CRP) and ESR.

6.9.7 Safety Labs

Safety labs will be collected on days 29, 45, and 60: Complete Blood Count (CBC) with differential (hemoglobin, hematocrit, erythrocytes, mean corpuscular volume (MCV), red blood cell (RBC) distribution width, leukocytes, neutrophils, lymphocytes, monocytes, eosinophils, basophils, platelet count), Comprehensive Metabolic Panel (CMP) (potassium, sodium, chloride, bicarbonate, anion gap, blood urea nitrogen (BUN), creatinine, eGFR, calcium, glucose (plasma or serum), total protein, albumin, aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT), total bilirubin), cystatin C, Hemoglobin A1c, C-Reactive Protein (CRP) and ESR.

6.9.8 Labs for Research (Urine and Peripheral Blood)

At days 0, 29, and 60, 40 ml of blood and 40 ml of urine will be collected to measure blood for inflammation and senescence markers.

6.9.9 Pregnancy Testing

Pregnancy testing will be required for female participants of childbearing potential (see Appendix A) and all premenarchal female participants during this study. A serum pregnancy test will be obtained at screening. Urine pregnancy tests (POC) will be performed at Day 1 and Day 29 prior to administration of study drug, and at day 60.

If the investigator is notified of a pregnancy (confirmed by a serum pregnancy test), see Section 8.4 for reporting requirements both during the study and 30 days (female participants of childbearing potential) after the last dose of study drug.

6.9.10 Contraceptive Compliance

All female participants of childbearing potential will be required to record their compliance with the contraceptive measures listed in Appendix 1, in accordance with the Clinical Trials Facilitation

and Coordination Group recommendations[10]. Men, women not of childbearing potential, and women who are not sexually active with opposite sex partners will not be required to record contraceptive measures.

6.9.11 Interaction Compliance

All participants will be questioned regarding their alcohol and caffeine usage and will record their compliance to reduce their consumption, if needed, based on the study protocol.

7 Statistical Plan

7.1 Sample Size Determination

Using data from a previous study from a similar study population (Schrier *et al*, Ultrasound in Medicine and Biology, 46:2236-44, 2020), the variability (SD) of a 3-month change of the Boston Carpal Tunnel Questionnaire (BCTQ) subscores (symptom severity score SSS, functional severity score FSS) was approximately 0.7. With 40 subjects, the minimum detectable change in the mean value is 0.3/0.4 with 80%/90% power and a significance level of 0.05 based on a paired t-test. Given that the desired detectable difference is 1.0, there should be sufficient power to detect that difference. For the secondary goal of detecting differences between males and females, assuming 20 subjects per group the minimum detectable difference is 0.6/0.7 with 80%/90% power. Thus, the study will have sufficient power to detect large group differences.

7.2 Statistical Methods

Descriptive Statistics

The data will be summarized using standard statistics including the mean, median, and standard deviation at each visit. Graphical displays of the data (scatterplots, boxplots) will also be used.

Handling of Missing Data

Basic summaries of subject demographics and baseline BCTQ will be run for subjects who drop out prior to completion of the study *vs*. those who completed the 60-day portion of the study.

Primary Hypothesis:

The primary hypothesis is that the BCTQ score will improve by 1 point from pre-treatment to 60 days post-treatment. Confidence intervals of the change in score, along with paired t-tests will be run, however the focus will be on the confidence intervals. Mixed effects models including all BCTQ measurements will be used as a secondary analysis. Additional exploration comparing the BCTQ changes between males and females will be made using a two-sample t-test. Similar analyses will be run using the SASP markers.

Secondary Hypotheses:

There are two secondary hypotheses. The first secondary hypothesis is that clinical improvement will be proportional to the effect in reducing blood markers of cellular senescence. Analysis will

include correlations between baseline SASP markers and the BCTQ score and between the change in SASP markers and the change in BCTQ. The second secondary hypothesis is that the symptomatic changes noted on the BCTQ at 2 months will be maintained at 6 months.

Interim Analysis

No interim analysis is planned.

7.3 Subject Population(s) for Analysis

As a pilot study, all subjects who complete the 60-day study visit (60 ± 5 days) will be the main population of interest, though summaries will be made of those who do not reach that point.

8 Safety and Adverse Events

Safety evaluations will include adverse event (AE) and serious AE (SAE) reporting. Adverse event reporting will be conducted throughout the study for all subjects. The reporting period begins at administration of IP (Day 0) and continues through the post treatment follow-up period, –Day 180). Adverse events will be assessed at Day 0, 29,, 60, and 180. Subjects will be encouraged to contact the Primary Investigator or study coordinator immediately if they experience any AE or SAE's during the study.

8.1 Definitions

Unanticipated Problems Involving Risk to Subjects or Others (UPIRTSO)
Any unanticipated problem or adverse event that meets the following three criteria:

- Serious: Serious problems or events that results in significant harm (which may be physical, psychological, financial, social, economic, or legal) or increased risk for the subject or others (including individuals who are not research subjects). These include: (1) death; (2) life threatening adverse experience; (3) hospitalization - inpatient, new, or prolonged; (4) disability/incapacity - persistent or significant; (5) birth defect/anomaly; (6) breach of confidentiality; and (7) other problems, events, or new information (*i.e.* publications, DSMB reports, interim findings, product labeling change) that in the opinion of the local investigator may adversely affect the rights, safety, or welfare of the subjects or others, or substantially compromise the research data, **AND**
- Unanticipated: (*i.e.*, unexpected) problems or events are those that are not already described as potential risks in the protocol, consent document, not listed in the Investigator's Brochure, or not part of an underlying disease. A problem or event is "unanticipated" when it was unforeseeable at the time of its occurrence. A problem or event is "unanticipated" when it occurs at an increased frequency or at an increased severity than expected, AND
- Related: A problem or event is "related" if it is possibly related to the research procedures.

Adverse Event
An untoward or undesirable experience associated with the use of a medical product (*i.e.*, drug, device, biologic) in a patient or research subject.

Serious Adverse Event
Adverse events are classified as serious or non-serious. Serious problems/events can be well defined and include:

- An outcome of death
- An immediate life-threatening adverse experience
- Inpatient hospitalization or prolongation of existing hospitalization *Emergency room visits that do not result in admission to the should be evaluated for one of the other serious outcomes (*e.g.*, life-threatening; required intervention to prevent permanent impairment or damage; other serious medically important event).
- Persistent or significant disability or incapacity; an event that results in significant, persistent, or permanent change, impairment, damage, or disruption of the subject's body function/structure, physical activities, and/or quality of life.
- Substantial disruption of the ability to conduct normal life functions
- Exposure to a medical product prior to conception of during pregnancy resulting in a birth defect/congenital anomaly and/or according to the protocol may be problems/events that in the opinion of the sponsor-investigator may have adversely affected the rights, safety, or welfare of the subjects or others, or substantially compromised the research data.

All adverse events that do not meet any of the criteria for serious, should be regarded as **non-serious adverse events**. For this study, such non-serious events would include changes in clinical status that would cause a subject to meet our exclusion criteria at some point after enrollment.

Adverse Event Reporting Period
For this study, the treatment period is defined as 30 (±2) days. The adverse event reporting period ends when the subject completes the study.

Preexisting Condition
A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings
At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

Post-study Adverse Event
All unresolved adverse events should be followed by the sponsor-investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the sponsor-investigator should instruct each subject to report, to the sponsor-investigator, any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study.

Abnormal Laboratory Values

A clinical laboratory abnormality will be documented as an adverse event if it changes from a value within the normal range (or as *per* clinical judgement) before treatment to one outside and worse than the normal range after treatments. Such changes will prompt a repeat test, telephone call to check the subject's status, a repeat visit, and/or referral to the subjects' primary care physician.

Hospitalization, Prolonged Hospitalization, or Surgery
Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for an adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:
The following hospitalizations will not be considered SAE for this study:

- a visit to the emergency department or other hospital department <24 hours, that does not result in admission (unless considered an important medical or life-threatening event). However, the reason for the ER visit should be considered/recorded as an adverse event
- elective surgery planned prior to signing consent
- admissions according to the protocol for a planned medical/surgical procedure
- routine health assessment requiring admission for baseline/trending of health status (*e.g.*, routine mammogram)
- medical or surgical admission other than to remedy ill health and planned prior to entry into the study. Appropriate documentation will be obtained in these cases

8.2 Recording of Adverse Events

At each contact with the subject, the study team must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event section of the case report form (CRF) or in a separate adverse event worksheet. All clearly related signs, symptoms, and abnormal diagnostic, laboratory, or procedure results should be recorded in the source document.

All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been ultimately determined that the study treatment or participation is not the probable cause. Serious adverse events that are still ongoing at the end of the study period must be followed up, to determine the final outcome. Any serious adverse event that occurs during the Adverse Event Reporting Period and is considered to be at least possibly related to the study treatment or study participation should be recorded and reported immediately.

8.3 Reporting of Serious Adverse Events and Unanticipated Problems

When an adverse event has been identified, the study team will take appropriate action necessary to protect the study participant and then complete the Study Adverse Event Worksheet and log. The sponsor-investigator will evaluate the event and determine the necessary follow-up and reporting required.

8.3.1 Sponsor-Investigator Reporting: Notifying the Mayo IRB

The sponsor-investigator will report to the Mayo IRB any UPIRTSOs and Non-UPIRTSOs according to the Mayo IRB Policy and Procedures.

Information collected on the adverse event worksheet (*and entered in the research database)*:

- Subject's name
- The date the adverse event occurred
- Description of the adverse event
- Relationship of the adverse event to the research drug: Not Related, Possibly Related, Probably Related, or Related
- If the adverse event was expected
- The severity of the adverse event: (Mild, symptoms such as GI upset, nausea, or headache; Moderate, symptoms such as vomiting, vertigo, chest pain; Severe, any problem requiring more than one day of OTC medication, or requiring a doctor visit, such as chest pain, vertigo, GI bleeding, *etc*)
- If any intervention was necessary/action taken
- Resolution: was the incident resolved spontaneously or after discontinuing treatment
- Date of Resolution

The Investigator will review all adverse event reports to determine if specific reports need to be made to the IRB and FDA. The sponsor-investigator will sign and date the adverse event report when it is reviewed. For this protocol, only directly related SAEs/UPIRTSOs will be reported to the IRB. Non-UPIRTSOs/non-serious AEs will be reported at the time of annual continuing review, according to the IRB policy.

8.3.2 Sponsor-Investigator Reporting: Notifying the FDA

The sponsor-investigator will report to the FDA all unexpected, serious suspected adverse reactions according to the required IND Safety Reporting timelines, formats, and requirements.

Unexpected fatal or life threatening suspected adverse reactions, where there is evidence to suggest a causal relationship between the study drug and the adverse event, will be reported as a serious suspected adverse reaction. This will be reported to the FDA on FDA Form 3500A, **no later than 7 calendar days** after the sponsor-investigator's initial receipt of the information about the event.

Other unexpected serious suspected adverse reactions, where there is evidence to suggest a causal relationship between the study drug and the adverse event, will be reported as a serious suspected adverse reaction. This will be reported to the FDA on FDA Form 3500A, no later than 15 calendar days after the sponsor-investigator's initial receipt of the information about the event.

Any clinically important increase in the rate of serious suspected adverse reactions over those listed in the protocol or product insert will be reported as a serious suspected adverse reaction. This will be reported to the FDA on FDA Form 3500A **no later than 15 calendar days** after the sponsor-investigator's initial receipt of the information about the event.

The sponsor-investigator must also notify the FDA (and sponsors must notify all participating investigators) in an IND safety report of potential serious risks, from clinical trials or any other source, as soon as possible, but in **no case later than 15 calendar days** after the sponsor determines that the information qualifies for reporting under § 312.32(c)(1)(i)-(iv).

Findings from other studies in human or animals that suggest a significant risk in humans exposed to the drug will be reported. This will be reported to the FDA on FDA Form 3500A, **no later than 15 calendar days** after the sponsor-investigator's initial receipt of the information about the event.

8.4 Pregnancy

Pregnancy, in and of itself, is not regarded as an AE unless there is suspicion that the study drug may have interfered with the effectiveness of a contraceptive medication or method. When a pregnancy has been confirmed in a participant during maternal exposure to study drug, the following procedures should be followed in order to ensure safety:

- The study drug must be discontinued immediately.
- The investigator must complete and submit a Clinical Trial Pregnancy Form to the sponsor or its designee with 24 hours of learning of the pregnancy.

Pregnancy should be recorded on a Clinical Trial Pregnancy Form and reported by the investigator to the sponsor or its designee. Pregnancy follow-up information should be recorded on the same form and should include an assessment of the possible causal relationship to the sponsor's study drug to any pregnancy outcome, as well as follow-up to the first well-baby visit or the duration specified in local regulations, whichever is later.

Any SAE occurring during pregnancy of a study participant must be recorded on the Serious Adverse Event Report Form and submitted to the sponsor or its designee.

Abnormal pregnancy outcomes (eg, spontaneous abortion, fetal death, stillbirth, congenital anomalies, or ectopic pregnancy) are considered SAEs and must be reported.

8.5 Stopping Rules

All subjects will be assessed during the 2 cycles each lasting for 2 days of Fisetin administration (after Visits 1 and 2; total 4 days) and during each visit following administration of the first cycle of drugs. If a severe adverse event occurs at any time during administration of the 2-day drug regimen, the study will pause, and a formal review will occur. Subsequent subjects will only be enrolled if the SAE is determined to be unrelated to the study drug. Other interventions will be as according to the direction of the FDA and Mayo IRB.

Individual Subject Stopping Rules
Any subject who experiences a SAE of any sort, or who is found to meet, after enrollment, any exclusion criterion, will no longer receive the study drug.

Toxicity stopping rule
The study will be terminated if the number of **treatment related** deaths exceeds 0 or if SAE's in the subjects already registered and treated in the trial exceeds 3.

8.6 Medical Monitoring

It is the responsibility of the Principal Investigator to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan (see section 10 "Study Monitoring, Auditing, and Inspecting"). Medical monitoring will include a regular assessment of the number and type of serious adverse events.

8.6.1 Internal Data and Safety Monitoring Board

There will not be a formal DSMB. However, we will have a DSMP and the PI will monitor the progress of the study and all adverse events.

9 Data Handling and Record Keeping

9.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (long term survival status that the subject is alive) at the end of their scheduled study period.

9.2 Source Documents

Source data are all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

9.3 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. Case reports in the form of completed checklists will be kept assuring inclusion/exclusion criteria and review of adverse events/toxicity. All data requested on the CRF will be recorded; data will be entered directly into REDCap or the subject's CRF. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". All entries will be printed legibly. If any entry error has been made, to correct such an error, a single straight

line will be drawn through the incorrect entry and the correct data will be entered above it. All such changes will be initialed and dated. Errors will not be erased and "white-out" will not be used to correct errors. For clarification of illegible or uncertain entries, the clarification will be printed above the item, initialed, and dated. If the reason for the correction is not clear or needs additional explanation, details will be added related to the justification for the correction.

Data Management
The data will be housed in hard copy CRFs, and/or eCRFs (electronic CRFs) in REDCap, and/or directly in the participant's medical record in EPIC.

Data Security and Confidentiality
Source documents and CRFs and original consents will be stored in secured locations. All data will be entered into a password protected, limited access database. Individually identifiable subject history and medical record information will be stored in a database under coded accession numbers. Clinical laboratory values will be stored in the electronic medical record system, requiring protected password access. These data are monitored regularly for access and a formal policy regarding protection of personal privacy is in place. The key to identification of subjects will be maintained in a secure office environment under the direction of the principal investigators.

Data Quality Assurance
Manual and computerized quality checks will occur during data collection and analyses and any discrepancies will require Case Report Form (CRF) review and validation of correct data.

Data Clarification Process
The Research Clinical Coordinator will be responsible for resolving queries in a timely manner.

9.4 Records Retention

The sponsor-investigator will maintain records and essential documents related to the conduct of the study. These will include subject case histories and regulatory documents.
The sponsor-investigator will retain the specified records and reports for:

1. Up to 2 years after the marketing application is approved for the drug; or, if a marketing application is not submitted or approved for the drug, until 2 years after shipment and delivery of the drug for investigational use is discontinued and the FDA has been so notified OR as outlined in the Mayo Clinic Research Policy Manual – "Retention of and Access to Research Data Policy" [redacted] whichever is longer.

10 Study Monitoring, Auditing, and Inspecting

10.1 Study Monitoring Plan

The Investigator will allocate adequate time for such monitoring activities. The investigator will also ensure the monitor or other compliance, or quality assurance reviewer has access to all study-related documents and study related facilities (*e.g.*, pharmacy, diagnostic laboratory, *etc.*), and has adequate space to conduct the monitoring visit.

As a service to the sponsor-investigator, this study may be monitored during the conduct of the trial by staff from the Mayo Clinic Office of Research Regulatory Support. Clinical trial monitoring may include review of the study documents and data generated throughout the duration of the study to help ensure the validity and integrity of the data along with the protection of human research subjects. Written reports will be provided to the sponsor, investigator, and Lead Study Coordinator. This will aid in complying with Food and Drug Administration regulations.

10.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB, the sponsor, and government regulatory agencies, of all study related documents (*e.g.*, source documents, regulatory documents, data collection instruments, study data, *etc*.). The investigator will ensure the capability for inspections of applicable study-related facilities (*e.g.*, pharmacy, diagnostic laboratory, *etc*.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable compliance offices.

11 Ethical Considerations

This study is to be conducted according to United States government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted local Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study. The decision of the IRB concerning the conduct of the study will be made in writing to the sponsor-investigator before commencement of this study.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the approved IRB consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or the subject's legally authorized representative and the individual obtaining the informed consent.

12 Study Finances

12.1 Funding Source

Institutional funding will be used to support this study.

12.2 Conflict of Interest

Any study team member who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, *etc*.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a

Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor-investigator prior to participation in this study.

12.3 Subject Payments

Remuneration will consist of up to $270.00 if the subject completes the entire study. Subjects will receive remuneration for each visit as follows:

- Visit 1, Screening: $50.00
- Visit 2A, Day 0: $50.00
- Visit 3A, Day 29: $50.00
- Visit 4, Day 45: $50.00
- Visit 5, Day 60: $50.00
- Visit 6, Day 180: $20.00

Completion of a study visit means that the subject followed all study-related procedures for each day as described in the consent form. In addition, subjects will receive parking passes for the time involved with completing the study visits or travels to the research center for study visits. Subjects will be directed where to park in order to receive the parking passes.

13 Publication Plan

The principal investigators, Drs. Amadio and Kirkland, hold the primary responsibility for publication of the results of this study.

We will register with ClinicalTrials.gov prior to subject recruitment and enrollment. We will post results to ClinicalTrials.gov within 12 months of final data collection for the primary outcome.

14 References

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10 Clinical Trials Facilitation and Coordination Group. Recommendations related to contraception and pregnancy testing in clinical trials. 2020.

APPENDIX A. INFORMATION REGARDING EFFECTIVENESS OF CONTRACEPTIVE METHODS AND DEFINITIONS

Definitions:
For female participants of childbearing potential

- A female who is considered fertile following menarche and until becoming postmenopausal unless permanently sterile (see below).
- Females in the following categories are not considered female participants of childbearing potential:
    - Premenarchal
    - Premenopausal with 1 of the following:
        - Documented hysterectomy
        - Documented bilateral salpingectomy
        - Documented bilateral oophorectomy
    - Postmenopausal
        - A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.

For female participants who are of childbearing potential

- The following methods during the Protocol-defined timeframe that can achieve a failure rate of less than 1% per year when used consistently and correctly are considered as highly effective birth control methods:
    - Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation
        - Oral, Intravaginal, Transdermal
    - Progestogen-only hormonal contraception associated with inhibition of ovulation
        - Oral, Injectable, Implantable
    - Intrauterine device
    - Intrauterine hormone-releasing system
    - Bilateral tubal occlusion
    - Vasectomized partner
    - Sexual abstinence from penile-vaginal intercourse

Source: Clinical Trials Facilitation and Coordination Group 2020.