Senolytic Drugs Attenuate Osteoarthritis-Related Articular Cartilage Degeneration: A Clinic Trial

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Sponsor:
Johnny Huard, Ph.D.

Principal Investigator:
Thomas A. Evans, M.D.

Confidentiality Statement

Authorities. By accepting this document, you agree to maintain the information as confidential and to use it only for the purpose of conducting the study.

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1.0 Steadman Philippon Research Institute Representatives

This Study is an Investigator Sponsored clinical trial to be carried out by The Steadman Philippon Research Institute (SPRI) using grant support from the Office of Naval Research, US Department of Defense.

Overall responsibility for the Study will be held by:

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Independent Medical Monitoring will be provided by an appropriately certified physician responsible for overseeing the occurrence of adverse events and for providing medical advisory assistance to the Study Team.

Medical Monitor

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The Study Team

Dr. Evans and his team will lead the clinical elements of the study. In addition, the teams of Dr. Kim, Dr. Leslie Vidal, Dr. Armando Vidal, and Dr. Godin at TSC will assist in enrollment of patients into the study. Dr. Ho will read and analyze all MRI imaging. Dr. Tashman will lead a team of research associates for all duties related to functional performance, muscle strength testing, and kinematics. There will also be a team of investigators and research associates at SPRI selected by Dr. Huard to whom appropriated duties will be delegated according to their specific qualifications. Collectively Dr. Evans, Dr. Kim, Dr. L. Vidal, Dr. A. Vidal, Dr. Godin, Dr. Huard, Dr. Ho, Dr. Tashman and their designees are referred to as the "Study Team".

Primary Contact for the Study

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2.0 Protocol Signature Page

The signature below constitutes the approval of this protocol entitled, *Senolytic Drugs Attenuate Osteoarthritis-Related Articular Cartilage Degeneration: A Clinical Trial* with attachments, and provides the necessary assurances that this trial will be conducted in compliance of all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and, regulatory requirements, International Conference on Harmonization Good Clinical Practice E6 (ICH-GCP), and applicable US regulatory requirements.

Principal Investigator: _____
Print/Type

Title: _____

Signed: _____ Date**:** _____

3.0 Study Synopsis

Sponsor/ Investigator Sponsored Study	Johnny Huard, Ph.D.
Study Title:	Senolytic Drugs Attenuate Osteoarthritis-Related Articular Cartilage Degeneration: A Clinical Trial
Protocol Number:	2019-16
Study Design:	Randomized, double-blind, placebo controlled
Study Duration:	Approximately 36 months Enrollment 18 months Follow-up 18 months
Randomization:	Subjects are to be assigned to one of two treatment groups using a 1:1 (Fisetin/placebo) randomization scheme.
Study Arms/Medication	*Active Treatment:* Fisetin 100 mg capsules (~20 mg/ kg/ day) will be administered orally for two consecutive days (days 1 and 2) followed by 28 days off. A second course will be given for two consecutive days (days 31 and 32) *Placebo Control:* Appearance matched capsule p.o. dose to match the number of capsules as if subjects were receiving active treatment. Placebo capsules will be administered orally for two consecutive days (days 1 and 2) followed by 28 days off. A second course will be given for two consecutive days (days 31 and 32)
Blinding Procedure:	The block randomization chart will be securely kept by an Unblinded Study Team member and at the Vail Hospital Pharmacy (point of subject distribution). All study subjects and other Study Team members including the Principal Investigator will be blinded to treatment through 18 months follow-up. In addition, any individuals engaged by the Principal Investigator to provide and/or document assessment of study endpoints will be blinded to treatment.
Sample Size and Study Population:	A total of 100 male or female subjects 40-80 years of age will be recruited from a single clinical site, where the Principal Investigator conducts patient care.
Objectives:	Primary Objective

	To evaluate as compared to placebo: The safety of administering Fisetin in subjects with osteoarthritis (OA) of the knee. Secondary Objectives *To evaluate as compared to placebo:* 1. Reduction of pro-inflammatory and cartilage degenerating SASP markers; 2. Improvement in physical function of the Study Knee; 3. Improvement in patient reported outcomes; 4. Improvement in the quality of articular cartilage in the Study Knee with quantitative magnetic resonance imaging (MRI); 5. Reduction in time to conversion to alternative treatment.
Endpoints:	Primary Endpoint: Occurrence of adverse events. Secondary Endpoints *Statistically significant as compared to placebo:* 1. Improvement in serum measures of inflammation and cartilage degenerating senescence associated secretory phenotype (SASP) markers; 2. Improvement in lower-extremity kinematic testing with video-motion analysis; isokinetic dynamometry testing, functional performance testing, range of motion (ROM);

	3. Improvement in patient reported outcomes (PROs), including: IKDC, Lysholm, TEGNER, WOMAC and SF-12 surveys; 4. Improvement in the quality of articular cartilage in the knee joint as measured by T2 and T1rho relaxometry; 5. Days from day one of Study Medication intake to conversion to alternative therapy. Additionally, we will review and discuss any possible correlation among secondary endpoint results.
Inclusion Criteria	Subjects will be included if <u>all</u> the following criteria are met: 1. Are male or female, ages 40-80; 2. Are willing to comply with all study related procedures and assessments; 3. Are ambulatory as defined by ability to complete functional performance testing; 4. Radiographic evidence of Kellgren-Lawrence grade II-IV osteoarthritis in one or both knees; 5. Scores 4-10 on the Numerical Rating Scale (NRS) for pain; 6. Stable dose of screening/baseline medications for at least 2 months prior to the anticipated date of study drug dosing.
Exclusion Criteria:	Subjects will be excluded if any of the following criteria are met: 1. Females who are nursing, pregnant or planning to become pregnant during the duration of study drug dosing; 2. Males who do not wish to abstain from sex or use contraceptive protection during study drug dosing and for 2 weeks after the last dose; 3. Subjects who do not have the capacity to consent themselves; 4. Subjects who are unable to tolerate oral medication; 5. Subjects having previously undergone any of the following treatments in the stated time window: -Surgery on the Study Knee in the past 6 months;

	-Partial or complete joint replacement in the study knee. Partial or complete joint replacement in the contralateral knee is acceptable as long as the surgery was performed at least 6 months prior to enrollment and the operative knee is asymptomatic; -Patients who have undergone arthroscopic surgery (including microfracture and meniscectomy) on the StudyKnee in the last 2 years prior to the Screening visit or are anticipated to have arthroscopic surgery on either knee at any time during the study period; -Steroid injection, including extended-release corticosteroid (e.g., Zilretta®) within the last 5 months; -Biologic (platelet-rich plasma, bone marrow, adipose tissue/cells) or hyaluronic acid injection into the Study Knee in the past 6 months; 6. Subjects with any of the following drug/medication statuses: - Currently taking Losartan; -Currently taking Warfarin or related anticoagulants; - Opioid analgesics taken in the past 8 weeks and are not willing to discontinue these medications through the duration of the study; - Senolytic agents taken within the past 6 months and are not willing to discontinue these medications through the duration of the study, including: Fisetin, Quercetin, Luteolin, Dasatinib, Piperlongumine, or Navitoclax; -Drugs that induce significant cellular stress and are not willing to discontinue these medications through the duration of the study, including alkylating agents, anthracyclines, platins, other chemotherapy drugs; **- Subjects taking the following other drugs if they cannot be held (per the Principal Investigator) for at least 2 days before and during administration of Fisetin**: cyclosporine, tacrolimus, repaglinide, and bosentan ; 7. Subjects with any of the following disease statuses:

	-Significant liver disease (i.e. greater than or equal to 2x the upper limit of normal bilirubin levels) or as in the opinion of the Principal Investigator; -Significant renal disease (eGFR of <60 ml/min/1.73m2) or as in the opinion of the Principal Investigator; -History of other formally diagnosed joint diseases including, osteonecrosis, acromegaly, Paget's disease, Ehlers-Danlos Syndrome, Gaucher's disease, Cushing's syndrome, Stickler's syndrome, joint infection, hemophilia, hemochromatosis, or neuropathic arthropathy of any cause; -Any active systemic autoimmune disease with musculoskeletal involvement or any history of systemic inflammatory arthritis; -Patients with type 1 or 2 diabetes (HbA1c $\geq$ 6.5%) and/or taking medications that affect insulin levels, including: Metformin (within the last week), Glucocorticoids (within the last month), Acarbose (within the last week); 8. Subjects unable to safely practically undergo an MRI (BMI > 40 kg/m2) or size exceeding limits of MRI equipment, implanted metal in study knee near joint surface, incompatible implant/device, severe claustrophobia; 9. Subjects that have any medical condition, including laboratory findings and findings in the medical history or in the pre-study assessments, that in the opinion of the Investigator constitutes a risk or contraindication for participation in the study or that could interfere with the study objectives, conduct or evaluation or prevent the patient from fully participating in all aspects of the study.
Screening	**Visit 1 (Screening/Enrollment)** At the first patient visit, the following procedures will be performed: 1. Full explanation of the clinical study, execution of Informed Consent, copy of signed informed consent sent home with research subject; 2. Subject will be assigned a de-identified study number;

	3. Vital signs; 4. Medical and surgical history; 5. Demographic information; 6. Details of injury; 7. Severity of injury; 8. Medications; 9. Physical exam with range of motion (ROM) of the target limb; 10. BMI; 11. Urine pregnancy test for Women of Child Bearing Potential (WOCBP); 12. Blood analysis; 13. Completion of augmented PROs, including: NRS, IKDC, Lysholm, TEGNER, WOMAC and SF-12; 14. A radiograph of the target knee will be performed to confirm the presence of Kellgren-Lawrence grade II-IV OA; 15. The patients' medical record will be reviewed, and the subject will be interviewed to determine if inclusion/exclusion criteria are met; 16. Randomization. Patients who have met all eligibility criteria will be assigned, 1:1 via block randomization, to one of the two blinded study groups. **Visit 2 (Baseline) (Within 3 months of Visit 1)** Some procedures performed in this visit may be performed in Visit 1 provided that Informed Consent has been signed. 1. Urine pregnancy test for Women of Child Bearing Potential (WOCBP) 2. Quantitative MRI; 3. Kinematic movement; 4. Assessment of muscle strength, isokinetic dynamometry; 5. Functional performance testing.

	After randomization is performed, a Study Team member will then inform the Vail Hospital pharmacist of the subject's assigned randomization code the study medication will be obtained. The subject will take the first dose of study medication at the clinic. Subjects will be instructed to self-medicate for day 2 followed by 28 days off. They will be instructed to begin a second course of treatment for two consecutive days (days 31 and 32). Subjects will be reminded at Visit 3 in-person and over the phone 2-4 days prior to the last dosing of study medication about their upcoming dosing. If applicable, they will also be reminded to withhold current medications as instructed by the PI. A Medication Log recording medication administration will be provided to the subject with instructions to complete during the period of administration.
Subject Follow-up:	All adverse events and protocol deviations will be assessed at each follow-up visit. **Visit 3: Two Weeks after first dose of Fisetin or Placebo** (+/- 2 days) All subjects will return to the study site 2-weeks from the subject's last dose of Fisetin or placebo. The following procedures will be performed: 1. Blood Analysis **Visit 4: Two Weeks from the Subject's Last Dose of Fisetin or Placebo** (+/- 10 days) All subjects will return to the study site 2-weeks from the subject's last dose of Fisetin or placebo. The following procedures will be performed: 1. Vital signs; 2. Medical and surgical history; 3. Physical exam with ROM of target limb; 4. Blood analysis;

	5. Medications; 6. Completion of augmented PROs, including: NRS, IKDC, Lysholm, TEGNER, WOMAC and SF-12; 7. Protocol deviation assessment; 8. Adverse event assessment. **Visit 5: Six Months from the Subject's First Dose of Fisetin or Placebo** (+/- 4 weeks) 1. Vital signs; 2. Medical and surgical history; 3. Physical exam with ROM of target limb(s); 4. Blood analysis; 5. Completion of augmented PROs, including: NRS, IKDC, Lysholm, TEGNER, WOMAC and SF-12; 6. Medication; 7. Protocol deviation assessment; 8. Adverse event assessment; 9. Quantitative MRI; 10. Lower-extremity kinematic testing with video-motion analysis; 11. Isokinetic dynamometry testing; 12. Functional performance testing. **Visit 6: 12 Months following First Dose of Study Medication** (+/- 4 weeks) All procedures performed in Visit 5 will be repeated. **Additional Remote Follow-up** In addition to the in-clinic follow-up visits above, the following remote follow-up will be conducted:

	• **Once Every 3 days During FIRST 6 weeks of Study Medication Treatment then Once a Week for an ADDITIONAL 6 weeks** Subjects will complete an augmented NRS questionnaire. • **At 18-Months After First Dose of Study Medication** (+/- 4 weeks) Subjects will complete the augmented PROs electronically, including: NRS, IKDC, Lysholm, TEGNER, WOMAC and SF-12. • **Phone Calls** Subjects will be contacted by phone three times while they are taking study medication to remind them to take the next dose of drug and to capture any adverse events.
Statistical Methods	Please refer to **Section 15.0** of this document

4.0 Terms and Abbreviations

Abbreviation	Term
AE	Adverse Event
CBC	Complete Blood Count
CLIA	Clinical Laboratory Improvement Amendments
DICOM	Digital Imaging and Communications in Medicine
FIS	Fisetin
MRI	Magnetic Resonance Imaging
OA	Osteoarthritis
PRO	Patient Reported Outcome
PHI	Protected Health Information
PTOA	Post-traumatic Osteoarthritis
ROM	Range of Motion
SASP	Senescence Associated Secretory Phenotype
SCAPs	Senescent-Cell Anti-Apoptotic Pathways
SOC	Standard of Care
SPRI	Steadman Philippon Research Institute
TSC	The Steadman Clinic

5.0 Introduction

5.1 Background and Significance

The Steadman Clinic (TSC) and the Steadman Philippon Research Institute (SPRI) are proposing a Phase 1-2 prospective clinical study to collect safety and efficacy data on the use of Fisetin (FIS), which is a nutritional supplement and senolytic agent, in treating patients with Osteoarthritis (OA) of the knee. This study is entitled: *Senolytic Drugs Attenuate Osteoarthritis-Related Articular Cartilage Degeneration: A Clinical Trial.*

Recently, several senolytic compounds that selectively kill senescent cells in vitro and in vivo without affecting quiescent or proliferating cells were identified[1]. These senolytic drugs target and inhibit anti-apoptotic pathways that are upregulated in senescent cells, thereby inducing apoptotic cell death and abrogating systemic Senescence-Associated Secretory Phenotype (SASP) factors.

As further discussed in **5.3** of this protocol, we have demonstrated in pre-clinical studies that treatment with senolytic drugs including (FIS, Dasatinib (D), Quercetin (Q) and Alvespimycin) can reduce the incidence/severity of many age-related disorders, including OA in naturally aged and progeroid mice (accelerated aging). These studies have shown that senolytic treatment (D/Q and FIS) can delay articular cartilage degeneration in a murine model of natural and accelerated (progeria) aging.

Based on this promising pre-clinical data, we believe it may be possible to delay or even reverse joint degeneration, damage to ligaments, menisci, cartilage, thereby resulting in the extension of high-level function and preservation of quality of life.

In this randomized, double-blind, placebo-controlled clinical trial, we intend to measure and compare safety via the gathering of all adverse events and preliminary evidence of efficacy through recording of SASP, inflammatory biomarkers and senescent cells. In addition, magnetic resonance imaging (MRI) exams, self-reported outcomes, functional performance and other relevant clinical data will be gathered. Possible correlation among outcomes, both structural and non-structural, will be described.

Each subject is to be followed for 18 months.

5.2 Rationale

Aging is associated with the accumulation of senescent cells, which have lost their ability to proliferate and resist apoptosis. Aging cells produce a SASP consisting of potent pro-inflammatory and stress inducing factors[2-5]. OA, along with a litany of other age-related pathologies, is associated with cellular senescence, which is thought to promote aging via the chronic induction of inflammation[2-6].

OA is a debilitating and costly joint disease that affects millions of individuals each year for which there are no available disease modifying therapies[7,8]. Several studies have shown that spontaneous age-associated and post-traumatic OA (PTOA) is characterized by an increase in senescent chondrocytes within the joint capsule[9-13] that is thought to promote early inflammation and OA pathogenesis. Importantly, it was found that injection of senescent cells into the joint capsule of healthy mice could alone induce OA-like conditions in mice including severe cartilage degeneration, erosion of femoral condyles, subchondral bone structure alteration, osteophyte formation, and meniscal damage[14]. Inversely, other groups have shown that local clearance of senescent cells genetically within the intra-articular space significantly reduces development of injury-induced OA and promotes a pro-regenerative environment[15]. Thus, the link between cellular senescence and OA pathogenesis is strong, highlighting senolytic drugs as a very appealing and innovative approach to prevent or treat OA.

There are currently three Phase II clinical trials designed to investigate FIS in senescence associated diseases, including frailty, inflammation, diabetic and chronic kidney disease NCT03430037, NCT03675724, NCT03325322. To date there have been no human studies that have evaluated senolytic drugs for the treatment of knee OA.

5.3 Pre-Clinical Data

The following pre-clinical studies present an animal model for the study of OA, a proof of concept connection between cell senescence and loss of chondrogenic capacity, proteoglycan content, which may lead to OA, as well as the potential protective effect of senolytic agents.

5.3.1 Progeroid Musculoskeletal Symptoms in Z24$^{-/-}$ Mice Are Associated with Increased Senescent Cell Burden

Muscle derived stem/progenitor cells (MDSPCs) are a population of adult muscle progenitors that the SPRI laboratory uses for various regenerative medicine applications. MDSPCs isolated from skeletal muscle of 2 months old Z24$^{-/-}$ Mice with progeroid symptoms were found to have significantly elevated β-galactosidase (β-gal+) cells when compared to Wild-Type (WT) healthy control cells. β-gal is a

hydrolytic enzyme whose activity is upregulated during aging and an established marker for cellular senescence[16]. Further, β-gal expression is associated with increased expression of other senescence associated cell cycle regulator transcription factors, including p16 and p21. These transcripts are elevated in senescent cells and keep them in a non-proliferative state, a hallmark of senescence. Senescent cells were also detected in gastrocnemius muscle from progeroid $Z24^{-/-}$ mice.

It has been reported that skeletal muscle histopathology are seen with a dysfunction of MDSCs in $Z24^{-/-}$ mice[17]. Therefore, we believe these musculoskeletal pathologies observed in **$Z24^{-/-}$ mice** are associated with increased senescent cell burden.

5.3.2 Primary Chondrocytes from Progeroid $Z24^{-/-}$ Mice Exhibit Reduced Chondrogenic Capacity *in vitro* and Spontaneous Loss of Articular Cartilage Proteoglycan Content *in vivo*.

$Z24^{-/-}$ mice are deficient in the metalloprotease Zmpste24, which results in the accumulation of unprocessed Lamin A, very similar to progerin in patients with Hutchinson-Gilford progeria syndrome (HGPS). This causes blebbing of the nuclear membrane[18]. Disruption of the nuclear envelope due to Lamin A accumulation leads to destabilization of heterochromatin, DNA damage, and eventual cell cycle arrest and senescence[19]. Chondrocytes are the sole cell type in articular cartilage and are responsible for maintaining the specialized extracellular matrix proteoglycan content on joint surfaces[19]. To gauge the effects of Zmpste24 loss specifically in chondrocytes, primary costal chondrocytes from 2-month-old $Z24^{-/-}$ mice were isolated as previously described[20]. As expected, chondrocytes from $Z24^{-/-}$ mice lacked Zmpste24 expression, exhibited nuclear blebbing and were found to have reduced expression of type II collagen (Col2).

Accordingly, pellet culture of isolated chondrocytes were significantly smaller with decreased Col2 signal and toluidine blue stain intensity indicating decreased chondrogenic capacity. These data suggest that progeroid $Z24^{-/-}$ chondrocytes are sensitive to senescence and dysfunction which may initiate or potentiate OA. Indeed, safranin O staining of $Z24^{-/-}$ articular cartilage revealed obvious loss of proteoglycan content versus WT at only 5 months of age.

5.3.3 $Z24^{-/-}$ Mice Are More Sensitive to Injury-Induced OA Versus Age-Matched WT Animals.

PTOA accounts for roughly 12% of OA cases. Although associated with acute initiation, PTOA is characterized by similar cellular phenotypes to age- associated OA[21,22]. Surgical destabilization of the medial meniscus (DMM) is an established method to model post-traumatic OA that allows for the immediate initiation of OA with more severe phenotypes including overt inflammation, cartilage

degeneration, and osteophyte formation. These effects can be detected histologically and radiologically[23]. Given that $Z24^{-/-}$ animals showed diminished proteoglycan content, an early sign of OA, we hypothesized that they might be more sensitive to injury- induced OA.

With this in mind, a small cohort of $Z24^{-/-}$ and WT mice received DMM as described[23], then were sacrificed at 12 weeks. Changes in their articular cartilage was examined histologically.

As expected, DMM in WT mice resulted in decreased proteoglycan content within the articular cartilage as well as elevated MMP-13 and ADMTS-5 expression (ECM degrading enzymes up-regulated during OA). However, cartilage degeneration was significantly worse in $Z24^{-}$ mice, when compared to normal mice, demonstrating their sensitivity to injury-induced OA.

These data highlight the utility of the $Z24^{-/-}$ model to evaluate senolytic therapies in moderate progressive symptomatic OA as well as severe more aggressive injury-induced OA.

5.3.4 D/Q Protects from Spontaneous Proteoglycan Loss and OA-Associated ADMTS4 Expression in Articular Cartilage Of $Z24^{-/-}$ Mice.

As a proof of concept, a pilot study was conducted with Dasatinib + Quercetin (D/Q) (5 mg/kg; 50 mg/kg) using $Z24^{-/-}$ mice. Mice were administered D/Q via oral gavage as a single dose (D/Q_{sin}) at 4 months of age, or monthly dose starting at 2 months of age (D/Q_{mul}) prior to sacrifice at 5 months old.

Quite surprisingly, a single dose of D/Q alone was enough to mitigate proteoglycan loss in articular cartilage of Z24-/- mice. These effects were even more dramatic in mice treated with multiple doses of D/Q demonstrating a dose response to D/Q treatment throughout the short 3 months treatment window. In addition, $Z24^{-/-}$ mice in the D/Q_{mul} group were found to have decreased ADMTS-4 positive cells indicative of less ECM degradation.

While only preliminary, and from a single time point (5 months), these data strongly suggest that senolytic drug treatment may facilitate the retention of articular cartilage proteoglycan content and possibly mitigate age-related OA pathogenesis.

5.4 Safety of Administering Fisetin to Subjects with OA

Fisetin (FIS) is available as a dietary supplement. Other flavonoids, such as Quercetin (Q), have been safely used in clinical trials in amounts up to 500 mg twice daily for up to 8 weeks. To date, no reports of significant toxicity for FIS have been made, and weight of the current evidence from multiple in vitro studies demonstrates safety of this class of naturally occurring molecules[24,25]. However, FIS, along with other flavones and flavonoids, have been shown to inhibit cytochrome P450 2C9. Therefore, precaution will be taken to avoid significant drug-drug interactions, such as with warfarin[26].

More recent (unpublished) data in humans has been submitted to FDA in an Annual Report containing data gathered in three human studies of Fisetin from 3/5/2018 to 02/28/2019 under approved IND 134052. James L. Kirkland, M.D., Ph.D. of the Mayo Clinic as Sponsor-Investigator of this referenced IND has authorized FDA via separate submission (1571 SN0007) and Dr. Johnny Huard, Ph.D. the proposed Sponsor-Investigator for this IND application, to reference any and all information contained in IND 134052.

Under this referenced IND 36 subjects have been treated. Thirty-two subjects have been treated at a dose of Fisetin 20mg/kg/day, orally for 2 consecutive days. Four have been treated with Fisetin 20/mg/kg/day, orally for 2 consecutive days, for 2 consecutive months. There have been no serious adverse events reported.

However, one subject experienced a transient reduction in kidney function (creatinine increased from 2.76 to 3.68) on Day 14 after administration of the randomized intervention (either placebo or Fisetin). Interim safety labs were obtained which showed return of kidney function (creatinine 2.40) at Day 23. The change in kidney function was determined to be possibly related to the administration of Fisetin or Placebo. Given this occurrence, we have incorporated routine laboratory testing including serum creatinine at study visits 1, 3, 4, 5 and 6.

6.0 Summary of Proposed Placebo Controlled Trial

Our objective is to determine (through the performance of an adequate and well-controlled clinical trial) if the administration of Fisetin (FIS) safely reduces inflammation and pain, reduces senescent cell burden, mitigates cartilage degeneration, and improves joint function in patients with moderate to severe OA of the knee.

Patients suffering from OA of one or both knees will be recruited from the clinical practice of the Principal Investigator at TSC. Patients diagnosed with Kellgren-Lawrence grade II-IV OA of the knee via radiographic analysis, who meet all enrollment criteria will be offered the opportunity to participate in this study.

At the first visit, each potential subject will be fully informed regarding all aspects of the proposed study and will be required to review and sign a full, IRB approved, Informed Consent Form (ICF) attached as **Appendix A** in **Section 22.1.** Subjects who sign Informed Consent will be screened by a member of the Study Team. The patients' medical record will be reviewed to determine if medical history indicates that the eligibility criteria are met. If so, subjects will be screened for OA and senescent cells by T-Cell assay, C12FDG Flow assay, and by senescence biomarker assay. Further blood analyses will be performed.

Subjects who meet eligibility criteria will be assigned, 1:1 via block randomization, to one of the two blinded study groups. FIS and the inactive placebo are collectively referred to as Study Medication.

n=36: Active Treatment: FIS: 100 mg capsules (~20 mg/ kg/ day);
n=36: Placebo Control: Appearance matched capsules (dose to match the number of capsules as if subjects were receiving active treatment).

At visit two, Quantitative MRI, kinematic movement and strength testing, range of motion (ROM) and functional performance testing. Subjects will complete numerous patient reported outcomes tools, including an augmented patient reported outcomes (PROs), including numerical rating scale (NRS) for pain (as described in **Section 11.5**) of this protocol International Knee Documentation Committee (IKDC), Lysholm, Tegner, Western Ontario McMaster Universities Osteoarthritis Index (WOMAC) and short form-12 health survey (SF-12), which will provide a baseline assessment of joint pain and function.

After randomization is performed, a Study Team member will then inform the Vail Hospital pharmacist of the subject's assigned randomization code, and the medication will be obtained from the pharmacy. Subjects will take their first dose of study medication at the clinic.

Then, subjects will self-medicate on day 2 followed by 28 days off. A second course will be taken for two consecutive days (days 31 and 32). Day 1 will be defined as the first day of intake of the Study Medication. A Medication Log recording medication administration will be maintained by the subject during the period of administration.

Subjects will complete an augmented NRS pain scale. This will be completed electronically once every 3 days for 6 weeks after the first dose and then weekly thereafter for an additional 6 weeks.

Subjects will return to the clinic for a blood draw (visit 3) 2 weeks after the first dose of study medication (+/- 2 days).

Subjects will be called on the telephone three times during study medication administration to capture adverse events and to remind subjects to take the next dose of study medication.

On the fourth visit (2 weeks after the last medication dosage +/- 10 days) subjects will be asked to return to the study site. A review of study medication and concomitant medication use, vital signs, and a physical exam with ROM will be performed. The subject will be asked to complete the augmented NRS, IKDC, Lysholm, TEGNER, WOMAC and SF-12. All adverse events and protocol deviations will be documented. A blood draw will be performed to measure senescent cells by T-Cell assay and by senescence biomarker assay. Further blood analyses will be performed.

At 6-, 12- and 18-months post first dose of Study Medication, subjects will be asked to complete the augmented NRS, IKDC, Lysholm, TEGNER, WOMAC and SF-12 remotely, either electronically or on paper.

At 6- and 12-months post first dose (Visits 5 and 6) subjects will be evaluated at the study site. A review of concomitant medications, vital signs, and a physical exam with ROM will be performed. All adverse events and protocol deviations will be documented. A blood draw will be performed to measure senescent cells by T-Cell assay and by senescence biomarker assay. Further blood analyses will be performed. A quantitative MRI, lower-extremity kinematics testing with video motion analysis, isokinetic dynamometry testing, and functional performance testing will be performed.
adverse event and protocol deviation data will be collected.

6.1 Blinding

Blinding will be maintained through study follow-up until the last subject has been seen at 18- months and data has been analyzed. All study subjects and Study Team members responsible for subject evaluation, data management and analysis (including the Principal Investigator) will be blinded. In addition, any individual engaged by the Principal Investigator to provide radiographic, laboratory testing and and/or verification of data will be blinded to treatment.

Those unblinded include the Group Allocation Manager (not a member of the Study Team) that will be responsible for maintaining group assignment. In addition, the pharmacist responsible for medication distribution (including their team) will maintain a similar log and need to be unblinded to distribute study medication accurately.

6.2 Non-Prohibition of Additional/Alternative Procedures

In the event that a subject feels that he/she is unresponsive to the Study Medication (Fisetin or placebo) and/or and is having difficulty managing knee pain, he/she will be free to receive a single corticosteroid intra-articular knee injection and still remain in the study. In addition, subjects will be free to seek alternative treatment at any time; however, this may necessitate withdraw from the study as further described in **Section 14.3** of this protocol.

7.0 Study Medication

7.1 Fisetin

Fisetin (FIS) is widely available as a dietary supplement. To our knowledge, FIS has not been withdrawn from any market globally.

FIS (3,3',4',7-tetrahydroxyflavone) is a member of the flavonoid family, a family of naturally occurring polyphenolic compounds. FIS, a high Trolox-equivalent antioxidant, is present in low concentrations in many fruits and vegetables, such as apples, persimmon, grapes, onions, and cucumbers, with the highest concentration found in strawberries (160μg/g)[27-29]. Fisetin capsules supplied are to be opaque blue in color.

7.2 Placebo Control

The placebo comparator used for this study is to be manufactured using the same size #3 opaque blue capsule. Each placebo capsule is mainly composed of cellulose along with some coloring agents to approximate the appearance of the active capsule.

7.3 Supply

While Fisetin (FIS) is currently available and marketed as a dietary supplement, this study will use a product manufactured under cGMP conditions. The FIS will be supplied in 100 mg capsules to be administered orally. Both Fisetin and Placebo will be obtained from Vital Nutrients, a contracted GMP manufacturer.

Vital Nutrients
45 Kenneth Dooley Drive
Middletown, CT 06457

Vital Nutrients will ship Study Medication directly to the Vail Health Hospital Pharmacy within Vail Health Hospital. All subjects will obtain their Study Medication directly from Vail Health Hospital Pharmacy.

Methods for ordering, dispensing and tracking Study Medication shipments are outlined in specific Standard Operating Procedures maintained by the Vail Health Hospital Pharmacy.

7.4 Administration of Study Medication

7.4.1 Route and Dosage

Fisetin (FIS) 100 mg capsules (~20 mg/ kg/ day) will be administered orally for two consecutive days (days 1 and 2) followed by 28 days off. A second course will be given for two consecutive days (days 31 and 32)
Placebo (visually identical) will be taken orally using the same dosing schedule described for FIS. Number of capsules per day will be the same number as if receiving FIS.

There are no recommended dosing adjustments for kidney or liver dysfunction.

Subjects will be instructed to complete dosing 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. They will further be instructed not to increase medication dosing should a particular dose be missed.

7.4.2 Rationale for FIS Dose and Intermittent Dosing Schedule

7.4.3 Dose

We previously treated 4 male and 4 female 8-month-old C57/Bl6 mice with FIS 500 mg/kg/day, (a dose 25-fold higher than the dose to be used in this clinical trial) by oral gavage for two consecutive days and compared study endpoints to four male and four female mice treated with control. We found no evidence of substantial toxicity, as assessed by monitoring activity, food intake, and respiratory quotient in metabolic cages (Comprehensive Laboratory Animal Monitoring System, Columbus Instruments) for 48 hours after the last FIS dose.

In addition, we administered 100 mg/kg/day for 2 consecutive days by mouth (a dose five times the dose proposed in in this clinical trial) to two older Rhesus monkeys, ages 18 and 28 years. Because these animals are considered elderly for their species it would be expected that they be more vulnerable to adverse events. In over one month of close monitoring, neither monkey showed evidence of debilitation, including reduced appetite, altered activity, vomiting, or diarrhea.

Finally, others have treated mice with FIS 50 mg/kg/day for weeks without evidence of toxicity[30].

Based on this data and considering the high rate of drug metabolism in mice compared to humans and differences in physiology between non-human primates and humans, we believe that a daily dose of ~20 mg/kg/day is a safe dosage at which to complete this early stage clinical study. In addition, this smaller dose serves to minimize excessive pill burden for study subjects.

7.4.4 Intermittent Dosing Schedule

Senolytic drugs (including FIS) can effectively kill senescent cells with one to two doses given systemically[1]. We believe that allowing two week breaks between dosing will be more tolerable to patients as compared to sustained dosing, therefore minimizing protocol deviations.

We further believe that intermittent dosing may minimize the occurrence of potential drug-drug interactions and hopefully decreasing the frequency or magnitude of any potential side effects further discussed in **Section 13.1**of this protocol.

7.4.5 2 Month Duration

We have chosen a 2-month (32 days) dose duration based upon pre-clinical studies reviewed in **Section 5.3** of this protocol. Several studies have treated mice with our proposed dose and more, for over two months (equivalent of ~20 years in the human lifespan). Considering the comparative life span between the mouse model and of humans, we believe that a 2-month duration is a safe and effective length of time.

7.4.6 Human Evidence of Safe Dosing

As discussed in Section 5.4 there appears to be no serious safety barriers to administration of
Fisetin at the proposed dosage.

7.5 Study Design and Duration

7.5.1 Study Design

This is a prospective, randomized, placebo controlled, double blind study to be conducted at the Steadman Clinic and the Steadman Philippon Research. A total of no more than 100 will be randomized 1:1 to receive either Fisetin (FIS) or placebo.

7.5.2 Study Duration

When every treated subject has reached 18 months following treatment, the study will be considered complete, and the data will be summarized. It is estimated that enrollment will take approximately 18 months. Each study subject will be followed for 12 months, which will be considered the final visit. The PROs will be administered electronically at 18 months. The total duration of Study is approximately 36 months. An additional 4 months will be spent closing the study database and in data analysis.

The proposed start date is December 1, 2019, with anticipated last visit September 1, 2022.

8.0 Study Objectives and Endpoints

8.1 Objectives

8.1.1 Primary Objective

To evaluate as compared to placebo:

1. The safety of administering Fisetin (FIS) in subjects with osteoarthritis (OA).

8.1.2 Secondary Objectives

To evaluate as compared to placebo:

1. Reduction of pro-inflammatory and cartilage degenerating SASP markers;
2. Improvement in physical function of the target knee;
3. Improvement in PROs;
4. Improvement in the quality of articular cartilage in the knee joint;
5. Reduction in time to conversion to alternative treatment.

8.2 Study Endpoints

8.2.1 Primary Endpoint:

1. Occurrence of adverse events.

8.2.2 Secondary Endpoints

Statistically significant as compared to placebo:

1. Improvement in serum measures of inflammation and cartilage degenerating SASP markers;
2. Improvement in lower-extremity kinematic testing with video-motion analysis; isokinetic dynamometry testing, functional performance testing, ROM;
3. Improvement in IKDC, Lysholm, TEGNER, WOMAC and SF-12 surveys;
4. Improvement in the quality of articular cartilage in the knee joint as measured by T2 and T1rho relaxometry;
5. Days from day one of Study Medication intake to conversion to alternative therapy.

Additionally, we will review and discuss any possible correlation among secondary endpoint results.

9.0 Study Population

Male and female patients who present to the clinical practices of the Principal Investigator or Sub-Investigators will be enrolled if they meet all eligibility criteria. Subjects from the community who self-identify in response to a flyer, newspaper advertisement, or website will be enrolled if they meet all eligibility criteria.

9.1 Enrollment Criteria

9.1.1 Inclusion Criteria

Subjects will be included if <u>all</u> of the following criteria are met:

1. Are male or female, ages 40-80;
2. Are willing to comply with all study related procedures and assessments;
3. Are ambulatory as defined by ability to complete functional performance testing;
4. Radiographic evidence of Kellgren-Lawrence grade II-IV osteoarthritis in one or both knees;
5. Scores 4-10 on the Numerical Rating Scale (NRS) for pain;
6. Stable dose of screening/baseline medications for at least 2 months prior to the anticipated date of study drug dosing.

9.1.2 Exclusion Criteria

Subjects will be excluded if <u>any</u> of the following criteria are met:

1. Females who are nursing, pregnant or planning to become pregnant during the duration of study drug dosing;
2. Males who do not wish to abstain from sex or use contraceptive protection during study drug dosing and for 2 weeks after the last dose;
3. Subjects who do not have the capacity to consent themselves;
4. Subjects who are unable to tolerate oral medication;
5. Subjects having previously undergone any of the following treatments in the stated time window:
    a. Surgery on the Study Knee in the past 6 months;
    b. Partial or complete joint replacement in the study knee. Partial or complete joint replacement in the contralateral knee is acceptable as long as the surgery was performed at least 6 months prior to enrollment and the operative knee is asymptomatic;

c. Patients who have undergone arthroscopic surgery (including microfracture and meniscectomy) on the Study Knee in the last 2 years prior to the Screening visit or are anticipated to have arthroscopic surgery on either knee at any time during the study period;
d. Steroid injection, including extended-release corticosteroid (e.g., Zilretta®) within the last 5 months;
e. Biologic (platelet-rich plasma, bone marrow, adipose tissue/cells) or hyaluronic acid injection into the Study Knee in the past 6 months;

6. Subjects with any of the following drug/medication statuses:
    a. Currently taking Losartan;
    b. Opioid analgesics taken in the past 8 weeks and are not willing to discontinue these medications through the duration of the study;
    c. Senolytic agents taken within the past 6 months and are not willing to discontinue these medications through the duration of the study, including: Fisetin, Quercetin, Luteolin, Dasatinib, Piperlongumine, or Navitoclax;
    d. Drugs that induce significant cellular stress and are not willing to discontinue these medications through the duration of the study, including alkylating agents, anthracyclines, platins, other chemotherapy drugs;
    e. **Subjects taking the following other drugs if they cannot be held (per the Principal Investigator) for at least 2 days before and during administration of Fisetin**: cyclosporine, tacrolimus, repaglinide, and bosentan.;
7. Subjects with any of the following disease statuses:
    a. Significant liver disease (i.e. greater than or equal to 2x the upper limit of normal bilirubin levels) or as in the opinion of the Principal Investigator;
    b. Significant renal disease (eGFR of <60 ml/min/1.73m2) or as in the opinion of the Principal Investigator;
    c. History of other formally diagnosed joint diseases including, osteonecrosis, acromegaly, Paget's disease, Ehlers-Danlos Syndrome, Gaucher's disease, Cushing's syndrome, Stickler's syndrome, joint infection, hemophilia, hemochromatosis, or neuropathic arthropathy of any cause;
    d. Any active systemic autoimmune disease with musculoskeletal involvement or any history of systemic inflammatory arthritis;
    e. Patients with type 1 or 2 diabetes (HbA1c $\geq$ 6.5%) and/or taking medications that affect insulin levels, including: Metformin (within the last week), Glucocorticoids (within the last month), Acarbose (within the last week);

8. Subjects unable to safely practically undergo an MRI (BMI > 40 kg/m2) or size exceeding limits of MRI equipment, implanted metal in study knee near joint surface, incompatible implant/device, severe claustrophobia;
9. Subjects that have any medical condition, including laboratory findings and findings in the medical history or in the pre-study assessments, that in the opinion of the Investigator constitutes a risk or contraindication for participation in the study or that could interfere with the study objectives, conduct or evaluation or prevent the patient from fully participating in all aspects of the study.

10.0 Study Visits

A full Study Schemata is provided in **Section 23.1** of this document.

Visit 1 (Screening/Enrollment)

At the first patient visit, the following procedures will be performed:

1. Full explanation of the clinical study, execution of Informed Consent, copy of signed informed consent sent home with patient;
2. Subject will be assigned a deidentified study number;
3. Vital signs;
4. Medical and surgical history;
5. Demographic information;
6. Details of injury;
7. Severity of injury;
8. Medications;
9. Physical exam with ROM of target limb;
10. BMI;
11. Urine pregnancy test for pre-menopausal women;
12. Blood analysis;
13. A radiograph of the target knee will be performed to confirm the presence of Kellgren-Lawrence grade II-IV OA;
14. The patients' record will be reviewed, and the subject will be interviewed to determine if the eligibility criteria are met;
15. Randomization.

Patients who have met all eligibility criteria will be assigned, 1:1 via block randomization, to one of the two blinded study groups.

10.1 Visit 2 (Baseline): (Within 3 months of Visit 1)

Some procedures performed in this visit may be performed in Visit 1 provided that Informed Consent has been signed.

1. Urine pregnancy test for Women of Child Bearing Potential (WOCBP)

2. Quantitative MRI;
3. Kinematic movement;
4. Assessment of muscle strength, isokinetic dynamometry;
5. Functional performance testing.

After randomization is performed, a Study Team member will then inform the Vail Hospital pharmacist of the subject's assigned randomization code, and obtain study medication. Subjects will take their first dose of study medication at the clinic.

Subjects will be instructed to self-medicate for day 2 followed by 28 days off. A second course will be taken for two consecutive days (days 31 and 32).

A Medication Log recording medication administration will be provided to the subject with instructions to complete during the period of administration.

10.2 Visit 3: Two Weeks after first dose of Fisetin or Placebo (+/- 2 days)

All subjects will return to the study site 2-weeks from the subject's last dose of Fisetin or placebo. The following procedures will be performed:

1. Blood Analysis.

10.3 Visit 4: Two Weeks from the Subject's Last Dose of Fisetin or Placebo (+/- 10 days)

All subjects will return to the study site two-weeks from the subject's last dose of Fisetin (FIS) or placebo. The following procedures will be performed:

1. Vital signs;
2. Medical and surgical history;
3. Physical exam with ROM of target limb;
4. Blood analysis;
5. Medications;

6. Completion of augmented PROs, including: NRS, IKDC, Lysholm, TEGNER, WOMAC and SF-12;
7. Protocol deviation assessment;
8. Adverse event assessment.

10.4 Visit 5: Six Months from the Subject's First Dose of Fisetin or Placebo (+/- 4 weeks)

1. Vital signs;
2. Medical and surgical history;
3. Physical exam with ROM of target limb(s);
4. Blood analysis;
5. Completion of augmented PROs, including: NRS, IKDC, Lysholm, TEGNER, WOMAC and SF-12;
6. Medications;
7. Protocol deviation assessment;
8. Adverse event assessment;
9. Quantitative MRI;
10. Lower-extremity kinematic testing with video-motion analysis;
11. Isokinetic dynamometry testing;
12. Functional performance testing.

10.5 Visit 6: 12 Months following First Dose of Study Medication (+/- 4 weeks)

All procedures performed in Visit 5 will be repeated.

10.6 Additional Remote Follow-up

In addition to the in-clinic follow-up visits above, the following remote follow-up will be conducted:

10.6.1 Once Every 3 days During FIRST 6 weeks of Study Medication then Once a Week for an ADDITIONAL 6 weeks

Subjects will complete an augmented NRS questionnaire.

10.6.2 18-Months After First Dose of Study Medication (+/- 4 weeks)

Subjects will complete the augmented PROs electronically, including: NRS, IKDC, Lysholm, TEGNER, WOMAC and SF-12 questionnaires.

10.7 Unscheduled Visits and/or Subject Contact

In the event that additional visit(s) or subject contact is made other than those described in the protocol, all data must be documented in the appropriate study document including the occurrence or adverse events and/or protocol deviations.

10.8 Phone Calls

Subjects will be contacted by phone three times while they are taking study medication to remind them to take the next dose of drug and to capture any adverse events.

11.0 Study Tools

11.1 Vital Signs (Visits 1, 3, 4, 5, 6)

Vital signs are to include measurement of temperature, blood pressure and heart rate.

11.2 Physical Exam: (Visits 1, 4, 5, 6)

Physical exams are to include height, weight, ROM of the target limb, and general wellness check up to examine any adverse events.

11.3 Range of Motion (ROM): (Visits 1, 4, 5, 6)

Both the active and passive range of motion should be assessed. The normal knee extension is between 0 to 10 degrees. The normal knee flexion is between 130 to 150 degrees. Any pain, abnormal movement, or crepitus of the patella will be noted.

11.4 Blood Laboratory Analysis: (Visits 1, 3, 4, 5, 6)

Analyses in Column A will be performed by a CLIA-Certified Laboratory:
Vail Health Laboratory.

Analyses in Column B will be performed at the Steadman-Philippon Research Institute (SPRI)

Column A	Column B
CBC w/Diff CMP CRP ESR Creatine Kinase Uric Acid Vit D (25 hydroxy) Hb A1c	T-Cell Assay: Peripheral blood CD3+ T cell assay for P16INK4a: 30 mL blood will be collected using EDTA tubes to measure p16 INK positive lymphocyte population in the peripheral blood- a biomarker of senescence and chronological aging. T-Cells will be enriched from whole blood using a commercial kit following manufactures instructions (RosetteSep, Stem Cell Technologies #15021). P16 expression will be measured by RT-PCR using Taq-man primer-probe system from enriched T-cells. C_{12}FDG Detection of Senescent Cells in Flow: A portion of enriched T-cells collected from 30 ml blood described above will be stained with C_{12}FDG, a fluorescent marker to detect senescent cells using flow cytometry (Guava EasyCyte, Luminex). Biomarker Assessment for Senescent cells: Quantification of Growth Factor and Cytokine/Chemokine Composition: The whole blood sample will be pipetted to microcentrifuge tubes for Luminex® multiplex immunoassays (EMD Millipore Corp, Billerca, MA) that measure concentrations of growth factors, cytokines and chemokines. Specific immunoassay kits (EMD Millipore Corp, Billerca, MA) a human cytokine/chemokine magnetic bead panel, TGF-β magnetic bead panel, MMP magnetic

bead panel 1, and MMP magnetic bead panel 2. A standard manufacturer's protocol for the Luminex® 200 (Luminex Corp, Austin, TX) multiplex instrument will be utilized as previously published (64). Plate will be analyzed with the Luminex® 200 xPONENT 3.1 system (Luminex Corp, Austin, TX) using the xPonent® software (EMD Millipore Corp, Billerica, MA), which created a standard curve for each respective analyte utilizing a five-parameter logistic curve-fitting method with the median fluorescent intensity. The proteins to be assayed include: sCD40L, EGF, Eotaxin, FGF-2, Flt-3, IFN-α2, IFN-γ, IL-1α, IL-1β, IL-1ra, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p40 and p70), IL-13, IL-15, IL-17A, IP-10, MCP-1, MCP-3, MDC, MIP-1α, MIP-1β, PDGF-AA, PDGF-AB/BB, RANTES, TGF-α, TNF-α, TNF-β, VEGF, MMP (1,2,3,7,9,10,12,13), TGF-β (isoforms 1,2,3).

11.5 Patient Reported Outcomes (PROs): (Visits 1, 2, 4, 5, 6)

All PROs may be completed either on paper documents or by online questionnaire administration conducted via formsite (Vroman Systems, Inc.). Patients may complete questionnaires on a variety of electronic devices (personal computer, mobile phone, tablet).

PRO data to be collected include:
Augmented Numerical Rating Scale (NRS) (visit 2): At baseline then once every 3 days during FIRST 6 weeks of study medication treatment then once a week for an ADDITIONAL 6 weeks.

IKDC, Lysholm, TEGNER, WOMAC and SF-12 (visit 2, visits 4, 5, and 6 and remote assessments at 3- and 18-months post completion of medication).

The Numerical Rating Scale is a simple 0-10 self-reported severity of pain scale, (zero being no pain and 10 being the worst pain imaginable). This tool has been validated to provide excellent test-retest reliability in assessing knee pain associated with OA[31]. The Steadman Philippon Research Institute has added several stand-alone questions that are administered with the questionnaire. Only the validated portion, the simple NRS will be used to screen patients. In follow-up, each question will be analyzed individually. There is no composite score. A sample can be found in **Appendix C, Section 22.3** of this document.

The Lysholm knee scale is a condition-specific outcome measure that was originally designed to assess ligament injuries of the knee. It has been tested to provide excellent construct validity and overall acceptable psychometric performance for outcomes assessment of various chondral disorders of the knee. It is recommended that this tool be administered with additional psychometric measurements[32,33].

The TEGNER Activity Scale is a numerical scale ranging from 0 to 10. Each value indicates the ability to perform specific activities. An activity level of 10 corresponds to participation in the most physically demanding activities while a zero is assigned if a person is on sick leave or receiving a disability pension because of knee problems. It is simple to use, and it has been demonstrated to provide acceptable psychometric performances as outcome measures for patients with a meniscal injury of the knee. Like the Lysholm, it is recommended that it be used in conjunction with other psychometric tools such as the SF 12[32].

The Western Ontario and MacMaster Universities (WOMAC) Osteoarthritis Index is a widely used self-administered health status measure assessing pain, stiffness, and function in patients with OA of the hip or knee. It has been demonstrated to fulfill conventional criteria for face, content and construct validity, reliability, responsiveness and relative efficiency. It has been shown to be an excellent instrument for evaluative research in osteoarthritis clinical trials[34].

The SF-12 is a short form of the SF-36 which is a widely accepted tool, known to provide a validated method to assess quality of life in physical and mental domains[35].

The SF-12 has been validated against the SF-36 and has been shown to demonstrate the empirical validity of the 12-item short-form (SF-12) health survey summary measures and 8-scale profile in comparison with SF-36 summary measures and scales[36].

11.6 Imaging Assessment of OA

11.6.1 Radiographic Assessment: (Visit 1)

Subjects will receive baseline radiographs of the knee(s). If a subject has had a satisfactory radiograph within the previous 6 months of screening, no additional radiograph is required.

The knee joint is typically evaluated using the extended-knee radiograph, which is a bilateral anteroposterior image acquired while the patient is weight-bearing, with both knees in full extension. More recently, flexed-knee radiographs with varying degrees of flexion and x-ray beam angles have been employed to improve intra-articular visualization. Radiographs are used to evaluate osteophyte formation and joint space narrowing (JSN); grading schemes such as the Kellgren-Lawrence grading scheme[37] and the Osteoarthritis Research Society International classification score establish guidelines for the diagnosis of OA progression[37].

11.6.2 Quantitative Magnetic Resonance Imaging (MRI): (Visits 2, 5, 6)

Quantitative MRI will be performed at the Steadman clinic. In preparation for MRI, subjects will be asked to remove their shoes and any metal that may interfere with the scan (e.g. metallic clothing, jewelry) and will be provided with disposable, MRI-compatible clothing if needed.

Images will be acquired on a 3T Siemens Magnetom Skyra-fit scanner.

For the screening and follow-up MR scans, morphological T1 MR images will be acquired. These images provide evaluation of knee structure. It is well known that structural alteration can lead to loss of collagen organization (e.g., torn fibers and scar tissue) and changes in water content (e.g., dehydration).

In addition, quantitative T2 MR images, which provide data on tissue MR signal relaxometry values, will be acquired to provide information regarding cartilage health including the relative level of water and collagen organization within the tissue.

A Study Team member will physically collect the hard drive and will transfer MR images to the SPRI Imaging Research network drive, accessible only to specific SPRI personnel via password protected accounts. The images will also be uploaded to the secure TSC PACS (Picture Archiving And Communication System) for radiological assessment by a blinded radiologist. Objective morphological measurements and Quantitative MRI value analysis will be performed from the DICOM files using Mimics, ImageJ, 3D Slicer, and MATLAB. Specifically, the regions of interest will be segmented in either Mimics or 3D Slicer (equivalent function software) and the masks will be exported to BPM format (bitmap) and imported into MATLAB for extraction of T2 or T2 values within the regions of interest. Morphological measurements may be taken in either Mimics, ImageJ, or 3D Slicer depending upon software scheduling coordination. All of these programs perform equivalently for this purpose. Results will be output to an Excel spreadsheet for statistical analysis.

11.7 Functional Performance Testing: (Visits 2, 5, 6)

Measures of functional performance will include 6 min walk test (6MW), timed-up-and-go test (TUG), and 4-meter walk (4mW) tests[38-40]. The 6MW test measures the distance walked in 6 minutes. This test was developed and used extensively to measure endurance. The test has been validated to measure functional mobility following knee arthroplasty. The 6MW test has been demonstrated to have excellent test-retest reliability, with intra-class correlation coefficients from 0.95-0.97, and a low coefficient of variation (10.4%)[41,42]. The 6MW test is safe, easy to administer, well tolerated, and does not require extensive patient training[38-40].

The timed up and go TUG measures the time it takes a patient to rise from an arm chair (seat height of 46 cm), walk 3 m, turn and return to sitting in the same chair without physical assistance[43]. This test has excellent reliability as measured in a group of 60 functionally disabled older adults (mean age 80 years)[43]. The 4MW will be assessed at the fastest safe speed for each participant. This test assesses the capacity for performance of certain activities (e.g. crossing a street before the light changes). The time it takes for each participant to ascend and descend 9-12 stairs will be measured which will assess joint strength, stability and agility.

This measure was selected because it has been shown to predict risk of physical disability, higher health care utilization and increased mortality[44-46]. It has been established as a meaningful outcome measure in older persons with a wide range of conditions[47-49]. Additionally, it is a valid and reliable measure[50-52]; and is well tolerated by patients varying in condition and degree of health[53,54].

11.8 Lower-Extremity Kinematics, Video-Motion Analysis: (Visits 2, 5, 6)

Video-motion analysis can assess even subtle changes in musculoskeletal function due to limited joint range of motion, stiffness, pain and/or weakness. Subjects will be equipped with a full-body retro-reflective marker set (including four-marker thigh and shank clusters on each leg). Kinematics measurements will be captured with a video-motion analysis system consisting of 18 infrared, 12-megapixel motion capture cameras (Oqus 7, Qualisys AB, Gothenburg, Sweden). Ground reaction forces will be acquired simultaneously using an instrumented treadmill or force plates (Bertec, Columbus, OH). Angular kinematics and net joint moments will be determined for the trunk, pelvis, hips, knees and ankles using Visual3D software (C-Motion, Inc., Germantown, MD). Tasks will include treadmill gait (1 m/s) and a 4-step stair ascent/descent.

11.9 Assessment of Muscle Strength, Isokinetic Dynamometry: (Visits 2, 5, 6)

The isokinetic muscle test will involve the use of the HUMAC NORM Isokinetic testing system (Computer Sports Medicine Inc, Stoughton, MA)[55]. Participants will sit in the chair and press their leg against a padded bar as hard as they can while moving their leg back and forth (flexing and extending). They will do this at one speed (60°/s) with three repetitions at each speed for both the left and right leg. This will allow measurement of muscle activity surrounding the knee joint and the amount of force they can produce. Participants will be given a warm-up period (5 minutes walking or cycling), practice trials, and adequate rest time between repetitions (30 sec) and between left/right sides (5 min). As is standard practice when using the HUMAC NORM system, padded straps will be used on the participant's torso and legs in order to isolate the lower leg movements and reduce the contribution of other muscles. All measurements will be normalized to % body weight.

12.0 Study Management of Adverse Events

12.1 Definition

An adverse event (AE) is any untoward medical occurrence in a subject administered a Study Medication and which 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 whether or not related to the Study Medication (ICH E2A II/A/1, 21 CFR 312.32).

All pre-existing medical conditions will be recorded on the medical history study document. Starting with the administration of the Study Medication any new experience that was not present at baseline or worsening of an event present at baseline in intensity or frequency, is considered an adverse event.

Note: Unchanged, chronic conditions are NOT adverse events and should not be recorded.

It is recognized that subjects will exhibit (throughout follow-up) symptoms of the underlying disease process of that fluctuates in severity and duration.

Adverse events will include those occurrences, which when compared to before treatment meets any of the following criteria:

- Represent a new event or escalation of an event;
- Require a new escalation in treatment;
- Lasts longer;
- Experienced more frequently;
- More intense;
- Different in character (e.g. stabbing vs. ache);
- Experienced in a different part of the body; and/or
- Brought on by activity that previously did not cause the symptom.

12.2 Recording Adverse Events

Adverse events that occur during Study Medication administration will be recorded. Terms should be recorded consistently, using acceptable medical terms. When possible, a diagnosis should be identified as the AE (i.e., disease or syndrome) rather than the component signs and symptoms, and recorded on the case report form (e.g., record congestive heart failure rather than dyspnea, rales and cyanosis). However, signs and symptoms considered unrelated to encountered syndromes or diseases are to be recorded as individual AEs (e.g., if congestive heart failure and severe headaches are observed at the same time, each experience is to be recorded as an individual AE). The AE should not be recorded as a procedure or clinical measurement (i.e., a laboratory or vital sign) but should reflect the reason for the procedure or diagnosis.

Death is considered to be an outcome of an AE. The cause of death (rather than the term "death") should be recorded on the serious AE and death report case report forms.

Subjects should be encouraged to report AEs spontaneously or in response to general, non-directed questioning. At each required visit (or whenever reported) during the study, all AEs that have occurred since the previous visit must be recorded on the AE case report form.

All AEs, regardless of seriousness, severity, or presumed relationship to the treatment must be recorded using medical terminology.

All AEs must be followed until resolution or until a stable clinical endpoint is reached. All measures required for AE management and the ultimate outcome of the AE must be recorded in the study document. All adverse events that occur in the study population will be tabulated and summarized.

12.2.1 Date of Onset and Resolution

The date of onset is the time at which the subject reports that the incident began. This may or may not be the date on which the Study Team became aware of the event. The date of resolution is the date at which the event was no longer apparent to the subject and/or the Study Team. This may be unknown if at the end of the study the event is not resolved. In this case, the event will be indicated to be unresolved.

12.2.2 Incidence

An AE may be classified as “intermittent” or “continuous if has no periods of abatement.

12.2.3 Relationship to Study Medication

The Principal Investigator is required to assess whether there is a reasonable possibility that the Study Medication caused or contributed to an AE.

Many terms and scales are used to describe the degree of causality between a Study Medication and an event. The expression “reasonable causal relationship” is meant to convey in general that there are facts (evidence) or arguments to suggest a causal relationship (ICH E2A, III/A/1).
Determination of whether there is a reasonable possibility that a treatment caused or contributed to an AE includes assessing temporal relationships, biologic plausibility, association (or lack of association) with underlying disease, and presence (or absence) of a more likely cause.

As is recommended in FDA draft guidance document, Safety Reporting Requirements for INDs and BA/BE Studies (2010), we define four degrees of relatedness: “unrelated”, “probably not related”, “suspected adverse drug reaction” (SADR) or “adverse reaction”.

Unrelated (clearly not related to the research)
The occurrence of the AE is not reasonably related in time, OR the AE is considered unlikely to be related to the treatment (biologically implausible).

Probably not related (doubtfully related to the research)
The administration of the treatment and the AE are not considered reasonably related in time AND the AE could also be explained by causes other than the treatment (concurrent illness/underlying disease, other drugs or procedures).

Suspected Adverse Drug Reaction (SADR)

Suspected adverse reaction means any AE for which there is a reasonable possibility that the treatment caused the AE. A 'reasonable possibility' means there is evidence to suggest a causal relationship between the treatment and the AE. A SADR reaction implies a lesser degree of certainty about causality than adverse reaction, which means any AE caused by a treatment.
The Principal Investigator should consider if exposure to the treatment and the AE are reasonably related in time AND the treatment is more likely than other causes to be responsible for the AE or is the most likely cause of the AE.

Adverse Reaction
An adverse reaction means any adverse event caused by a drug. Adverse reactions are a subset of all suspected adverse reactions for which there is reason to conclude that the treatment caused the event.

If the event is felt by the Principal Investigator to be either a SADR or an adverse reaction, every effort will be made to identify the event as related to the Study Medication. Adverse events that are generally felt to be attributable to the Study Medication occur in close temporal relationship to the onset of dosing. A discussion of AEs that are common with the Study Medication is found in the **Section 13.0** of this document.
All adverse reactions and SADRs will be followed until resolution or the Investigator judges the experience to be chronic or stable.

12.2.4 Severity (Intensity) of Adverse Events

The severity of the adverse events should be assessed based on the following grading scale:

- Grade 1 (Mild): Adverse event that is noticeable to the subject and may require additional therapy;
- Grade 2 (Moderate): Adverse event that interferes with the subject's activities and requires intervention or additional therapies;
- Grade 3 (Severe): Adverse event that is intolerable, or necessitates additional therapy or places the subject at immediate risk of harm;
- Grade 4: Adverse event that is life-threatening or disabling (hospitalization);
- Grade 5: Adverse event that results in Death.

12.2.5 Expected and Unexpected Adverse Events

"Expected" AEs are those that have been previously reported as associated with the disease process. In addition, adverse events associated documented potential risk identified in Section 13.0 of this protocol will be noted as "Expected". All others are to be recorded as "Unexpected".

12.2.6 Serious Adverse Events

An AE or suspected adverse reaction is considered "serious" if, in the view of either the Principal Investigator, it results in any of the following outcomes:

- Death;
- A life-threatening adverse event;
- Hospitalization;
- Disability or permanent damage;
- Congenital anomaly/birth defect;
- Other serious events that may jeopardize the patient and may require medical or surgical intervention (treatment) to prevent one of the other outcomes.

An AE or suspected adverse reaction is considered "life-threatening" if, (in the view of the Principal Investigator) its occurrence places the patient or subject at immediate risk of death. It does not include an AE or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

Serious and/or unexpected adverse event(s) may present as either a local or systemic response, or both, which may present as an anaphylactic response associated with generalized urticaria, shortness of breath, or respiratory or circulatory arrest.

Subjects will be instructed to contact the study site immediately if a systemic reaction occurs between scheduled study visits. Subjects may be assessed initially over the phone and may be asked to return to the study site for an additional visit to assess the reaction.

12.3 Reporting of Adverse Events

12.3.1 Function of the Medical Monitor and FDA

An Independent Medical Monitor will be selected to oversee study safety. A detailed medical Monitoring plan will be submitted to FDA upon appointment of this individual and before the first subject is treated. This individual is to be an appropriately certified physician experienced in monitoring clinical trials.

The Principal Investigator will be responsible for confirming the severity of all adverse events. All serious events will be reported to the Independent Medical Monitor within 24 hours of their disclosure to the Principal Investigator. The Medical Monitor will review data or events reported by the Study Team within 72 hours of disclosure to the Medical Monitor.

During this time, appropriate care will be given to the study subject. If managing adverse events requires unblinding to the any member of the Study Team, the appropriate team member will disclose the group identity to the necessary medical provider.

The IRB will be notified of unanticipated and serious adverse events within 5 business days of identification. This reporting may come from the Study Team, the Independent Medical Monitor, or both.

Investigator Sponsor will notify FDA in an IND safety report (Form 3500A), unexpected fatal or life threatening suspected adverse reactions where there is evidence to suggest a causal relationship between the Study Medication and the adverse event will be reported as a serious suspected adverse reaction. This will be reported to the FDA 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 Medication 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.

The sponsor must identify all IND safety reports previously submitted to FDA concerning a similar suspected adverse reaction and must analyze the significance of the suspected adverse reaction in light of previous, similar reports or any other relevant information.

13.0 Potential Risks to Study Subjects and Risk Mitigation

Expected risks to subjects in this study include all those currently associated with osteoarthritis (OA) of the knee and its progression. Additional risk to subjects which might arise as the result of study participation are discussed below.

13.1 Associated with Fisetin Administration

Based upon the review of the published literature, pre-clinical data and available clinical data, we do not anticipate the occurrence of serious adverse events related to the prescribed regimen of Fisetin (FIS) in this study. However, potential side effects include the following:

- Reduced appetite,
- Altered activity,
- Nausea,
- Dry mouth,
- Diarrhea,
- Vomiting,
- Fatigue.
- There have been positive findings with Fisetin in the Ames and micronucleus assays.

In addition, FIS has been demonstrated to have inhibitory activity of cytochrome P450, and to have adverse interaction with caffeine, tobacco/nicotine consumption for which we have established exclusion criteria. These are listed in detail in **Section 9.1.2** of this protocol. In addition, a list of possible drug interactions with FIS are provided in **Section 22.4***, Appendix D* of this document.

13.2 Risk Associated with Placebo Administration

If results of this study reveal that administration of FIS improves OA symptoms and/or joint structure, subjects randomized to placebo may miss these benefit(s).

All subjects will be fully informed that they have only a 50% chance of being randomized to receive FIS. In addition, subjects will be told that the advantages of receiving FIS in the treatment of OA in humans are unknown.

13.3 Associated with MRI

The MRI scan involves exposure to loud noise and positioning in a small space. Subjects may feel claustrophobic, fatigued or nauseated especially if they are uncomfortable with tight spaces. The MRI scan does not involve the use of x-rays or injectable dyes. There are no known reports of increased cancer or birth defects associated with this procedure. The MRI scan exposes the subject to high magnetic fields, which can be dangerous for those with pacemakers and some metal implants.

All subjects will be carefully screened for potential contraindications for MRI. Subjects will be provided with hearing protection and may listen to their choice of streamed music for comfort. MRI-compatible padding and/or blankets may be used as requested for subject comfort. Subjects will be provided with a 'squeeze ball' activated microphone to allow communication with the MRI technologists so that they can notify the technologist of any problems or the desire or need to abort the scan.

13.4 Associated with PRO Questionnaires

The completion of numerous PRO questionnaires can be laborious and uncomfortable presenting a potential risk of emotional distress.

Whenever possible PRO questionnaires will be completed remotely through electronic link in order to minimize time needed to complete questionnaires. The subject will be told that he/she may discontinue questionnaire completion at any time if the questionnaire causes emotional distress.

13.5 Associated with Physical Exam

There is a potential risk of pain and/or discomfort. Palpation and low-magnitude manual force applied to the knee could result in mild to moderate discomfort, depending upon a subject's subjective perception of discomfort and knee condition.

Care will be taken to limit this discomfort to not exceed that experienced during normal daily activities such as walking, climbing stairs, putting on shoes, etc. Only experienced, trained clinicians will perform the exam, and the exam will be modified if the subject indicates

more than mild discomfort. The exam will be stopped if the subject indicates moderate or greater discomfort or requests that the exam be stopped.

13.6 Associated with Blood Draw

The potential risks of blood draw include fatigue, light-headedness, nausea, vasovagal syncope, pain at the insertion site, damage to the blood vessels, erythema, and or potential infection.

To avoid such occurrences, blood draws will be performed only by persons qualified by license and experience.

13.7 Associated with Isokinetic Muscle Strength Testing

Participants may experience mild discomfort including risk of fall, dizziness, muscle strain, stretching, or mild fatigue. Participants may also experience bruising from pushing against a padded bar, skin irritation (including reddening and itchiness) from the straps, and general joint discomfort from isolating the knee joint.

Subjects will be informed of this possibility as well as their option to take rest periods and/or discontinue isokinetic muscle strength testing at any time during the evaluation. All participants will be given a warm-up period. The bar and straps will be positioned as comfortably as possible for each participant.

13.8 Associated with Motion Capture

There is a small risk of skin irritation from the tape used to attach the reflective markers. The tasks performed during motion capture are activities of daily living and therefore would place the participants at minimal risk for additional injury.

If skin irritation occurs, they will be instructed to apply over-the-counter anti-inflammatory creams, ointment or spray of their choice to the skin. In addition, to avoid injury subjects will be coached to take rest periods if needed and/or discontinue motion at any time during the evaluation.

13.9 Associated with Functional Performance Testing

There is a small risk of general fatigue or discomfort, slight risk of fall, additional knee pain from performing the tests, muscle soreness, shortness of breath and dizziness. To mitigate this, the staff conducting the tests will assist with the procedures and apply protections.

13.10 Associated with Breach of Confidentiality

The subject's Private Health Information could be accidentally breached during the research process. Many system wide safety guards are in place to prevent this occurrence, including:
Subject de-Identification, Badge-access, locked offices; controlled access to research offices and documents, password protected work stations and password complexity enforced; state-of-the-art and promptly updated firewall to block external web traffic; end-to-end encryption on all connections, automatic email encryption system for all outbound (off TSC server) email, Electronically secure online questionnaire access.

13.11 Risk Analysis

The Study Team will perform regular review of cumulative adverse events and will modify mitigation strategy as necessary. Baseline risk analysis is shown in **Table 13.11** below. It is intended that risks with higher overall risk score will be subject to greater and more frequent scrutiny and discussion of mitigation. As the study progresses, modifications to this analysis will be fully document and retained.

Table 13.11 Risk Analysis

Risk	Likelihood of Occurrence 1= not likely 2= possible 3= very likely	Potential Impact on Subject Safety 1=little 2= moderate 3= severe	Detectability 1= high 2= moderate 3= low	Overall Risk Score (Hierarchy of assessment and follow-up) (3-9)
Associated with FIS Administration	2	3	2	7

Associated with Placebo Administration	1	1	3	5
Associated with MRI	2	1	1	4
Associated With PRO Questionnaires	2	1	1	4
Associated With Physical Exam	2	1	1	4
Associated With Blood Draw	2	2	1	5
Associated With Isokinetic Muscle Strength Testing	2	1	1	4
Associated with Functional Performance Testing	2	1	1	4
Associated With Motion Capture	2	1	1	4
Associated with Breach of Confidentiality	2	2	2	6

14.0 Stopping Rules/Termination of Study Enrollment

The triggering of stopping rules will result in prompt discontinuation of subject enrollment, subject dosing and notification of the Medical Monitor and IRBs. Although dosing and enrollment will cease, all subjects currently taking or have completed Study Medication treatment up until that point will be followed according to the protocol for the occurrence of adverse events. Efficacy follow-up will be continued only if, in the Principal Investigators judgment, efficacy follow-up would be clinically meaningful. Subject dosing and enrollment will be reconvened only when permission is granted by the IRB.

14.1 Termination of Enrollment by the Principal Investigator

The Principal Investigator may terminate study enrollment at any time for any of the following reasons.

1. Any unexpected serious or life-threatening AE that cannot be determined to be unrelated to Study Medication;
2. It is determined that the protocol precipitates multiple subject non-compliance issues beyond reasonably missed follow-up.

14.2 Termination of Enrollment by the Medical Monitor

The Medical Monitor may terminate enrollment due to the emergence of significant safety signals.

14.3 Removal of Subjects from the Study

Principal Investigators may remove a subject from the study if:

1. A subject demonstrates poor compliance with study protocol;
2. There is concurrent illness or required medical treatment that interferes with study assessments;
3. The Principal Investigator determines that the subject's health, safety or welfare is at risk.

14.4 Withdrawal of Consent

Subject may withdraw their consent to participate in the Study at any time, for any reason.

15.0 Statistical Considerations

15.1 Study Overview, Aims and Objectives

The aim of this study is to perform a single-center randomized clinical trial (RCT) to test whether the dietary supplement Fisetin (FIS) can improve symptoms, kinematic function and soft tissue quality in patients with knee osteoarthritis. The proposed therapeutic systemic mechanism of FIS in this population is by selectively killing senescent cells that have been shown to associate with aging and age-related disease, including osteoarthritis (OA).

Assessments occur over 18 months following enrollment, and include safety monitoring, biomarker assessment, kinematic testing, quantitative MRI, knee-related patient reported outcomes, and observation of conversion to alternative treatment modalities. We hypothesize that the FIS treated group will not experience adverse events at a higher rate than the placebo group. Additionally, we hypothesize that improved OA disease status will be observed among the several pain, function and tissue quality endpoints for the FIS group when compared to the placebo group.

15.2 Study Design

The study will follow a two-arm, double-blind, placebo-controlled approach. Allocation will be 1:1 between the two groups. Study subjects, treating physicians, medical assistants, and other members in the research team who conduct subject contact and/or data analysis will remain blinded throughout the study period.

15.3 Randomization

To better ensure equivalent group sizes in the event of unenrollment or loss-to-follow-up, a block randomization scheme will be followed with block size of n=8. The following steps will be adhered to when enrolling a subject into one of the two study arms:

1. Eight tokens, numbered 1-8, will be held in a jar stored in the study manager's office within TSC and SPRI.
2. Upon enrollment, a token will be drawn from the jar, and not replaced back into the jar.
3. The Block Number and Within-Block Number will be recorded by the study team on a blinded version of the Enrollment/Randomization log.
4. The Block Number and Within-Block Number will be disclosed to the Vail Health Hospital Pharmacy who will arrange the proper medication and record the subject onto an unblinded version of the Enrollment/Randomization log.

5. When final token is taken from jar, enrollment within that block is complete, and all 8 tokens will be placed back in the jar in preparation for the next subject and the new block.

Figure 15.3 Portion of the randomization/enrollment log demonstrating group allocation for subjects enrolled into block #1.

Block Number	Within-Block Number	Group (Randomization not finalized, for illustration only)		Subject ID
Number	Patient ID			
Enrollment Date				
1	1	Fisetin	1	
	2	Placebo	2	
	3	Placebo	3	
	4	Fisetin	4	
	5	Placebo	5	
	6	Fisetin	6	
	7	Fisetin	7	
	8	Placebo	8	

15.4 General Data Handling, Analysis, and Reporting Strategy

Intent-to-treat analysis will be performed.

Baseline covariates will be summarized and reported in a table, stratified by group. This is a randomized trial, thus the expected population group differences with respect to baseline covariates are zero by definition. For this reason, no statistical comparisons will be performed to test for differences in baseline covariates between groups.

Whenever applicable, assessment of endpoints that are measured at serial time points will be analyzed using methods that account for this repeated measure structure. Listwise deletion in response to missing data points and categorization/dichotomization of continuous measurements will be avoided to every extent possible.

Summary statistics will be reported as group medians with quartiles or extrema. Meanwhile estimates calculated for statistical inference will be reported with (1-α)% confidence intervals. Model fit and satisfactorily meeting model assumptions will be assessed for all multivariable regression and linear mixed-effects models using residual analysis.

The Statistical Computing Language R will be used to produce all analyses and plots.

15.5 Endpoints

15.5.1 Primary Endpoint

The safety of FIS administration is the primary endpoint of this study. All adverse events will be compared by category of description incidence, relatedness to treatment, severity, duration and expectedness. The occurrence of non-serious and serious and unexpected adverse events will be compared between treatment groups. Bivariate analysis will be performed between group and each safety variable of interest independently. When comparing the two treatment groups, dichotomous (yes/no) endpoints will be assessed for association using Fisher's exact test. Adverse events and symptoms that may occur multiple times in a single subject will be analyzed as count variables, and group differences will be assessed using simple Poisson regression or simple negative binomial regression.

This is a small Phase I/II trial in which to assess safety, thus we will report all safety data thoroughly and aim to liberally identify potential side-effects and risks that may warrant close study in future, larger trials. No adjustments will be made for multiple comparisons in this set of safety endpoints.

15.5.2 Secondary Endpoints

15.5.2.1 Imaging Assessment of OA

Quantitative MRI will be performed at baseline and at 6 months and 12 months after initiation of medication. T2 and T1rho relaxometry will be performed. Linear mixed-effects modeling with random intercepts will be used to appropriately match the repeated measures design. A group-by-time interaction will be modeled to investigate both early improvement in tissue quality and maintenance of such an effect to 12 months as well.

Secondarily, multivariable modeling techniques such as support vector machines (SVM) and/or principal components analysis (PCA) will be pursued to characterize the SASP response profile that distinguishes the FIS and placebo groups.

15.5.2.2 Kinematic, Strength and Functional Performance Testing

Measurements from the timed-up-and-go test (TUG), 4-meter walk test (4mW), 6-minute walk test (6MW), time to descend 9-12 stairs test (SCT), video motion capture of lower-extremity kinematics, and isokinetic muscle test using the HUMAC NORM system will be assessed as continuous variables. Previous testing has identified these measurements follow a relatively Gaussian distribution, so parametric comparison of means (Welch's t-test) is planned between treatment groups.

15.5.2.3 Pain and PRO Assessment

The hypothesis in this study is that FIS will induce a systemic anti-inflammatory and senolytic effect, thereby reducing pain and improving self-reported function and quality of life compared to the placebo group. Early subjective assessment (every 3 days for 6 weeks following the first dose of study medication and once weekly for the following 6 weeks) will be limited to a short augmented numerical rating scale questionnaire. Linear mixed-effect random-intercepts modeling with will be used to characterize the trend in self-reported pain over 3 months, and to test whether treatment group is a significant predictor of pain reduction.

The IKDC, WOMAC, Tegner activity scale and Lysholm PRO measures will be administered at 2 weeks after the last dose of study medication and 6, 12, and 18 months following initiation of medication. For each time point, ANCOVA will be used with the subject's baseline PROM value used as a covariate. Among the 4 postoperative time-points for assessing these validated PROMs, the Holm-Bonferroni method will be used to control the family-wise type-1 error rate to 0.05 within each PROM scale tested. No multiplicity adjustments will be made across different PROMs, which are commonly highly intercorrelated.

15.5.2.4 Conversion to Secondary Interventions

In important endpoint for subjects enrolled in this study will be conversion to alternative treatments. The protocol allows for a single intra-articular corticosteroid injection into the knee to manage knee pain without necessitating removal of the subject from the study. However, if a study subjects elects to convert to a regenerative medicine treatment (e.g. platelet rich plasma or bone marrow aspirate concentrate) or a surgical intervention (e.g. microfracture or total joint replacement), they will be removed from the study. Cox proportional hazards regression will be performed to compare the hazard rate of conversion to each of these two treatment categories between treatment groups.

15.6 Statistical Power and Sample Size

Anticipated effect sizes for the group difference with respect to MRI-assessed cartilage quality were derived from the literature, however a paucity of literature was found in the knee OA literature when comparing various grades of advanced osteoarthritis. As a simplifying assumption, we derived an anticipated effect size for clinically meaningful change in T1rho values from a recent meta-analysis (MacKay, et al, 2018, J Osteoarthritis and Cartilage) that reported the standardized mean difference (SMD) between mild OA and healthy controls.

We aim to power this study to detect an effect size between the FIS and placebo treatment groups that is equal to the meta-analyzed effect size described above (SDM=0.73). The statistical method for this unadjusted, independent groups comparison of means, and the significance level will be set at α=0.05. Assuming 2-tailed testing and requiring 80% statistical power, 30 patients per group are sufficient to detect the effect size of interest. Anticipating a 20% drop-out or incomplete follow-up rate, we aim to enroll no more than 100 total OA patients (30 per groups, and 6 per group to account for 20% drop out). Power calculations were performed using G*Power (version 3.1; Universität Düsseldorf).

15.7 Missing Data

Whenever possible, statistical methods such as linear mixed-effects modeling which naturally handle missing data will be utilized. Missing data will be investigated carefully by the biostatistician, the study manager and the clinical staff to assess its probable cause, and whether missing not at random (MNAR, missingness dependent on outcome), missing at random (MAR, missingness not dependent

on outcome but can be fully accounted for by other non-missing covariates) or missing completely at random (MCAR, missingness independent of both observed variables and unobserved parameters of interest) is most likely.

If any missing data patterns are determined to be MNAR, this observance will be reported narratively in the final report(s) and publication(s), and no imputation methods will be pursued. Little's test will be used to assess whether missing values are missing completely at random (MCAR) For methods that require complete data or listwise deletion (e.g. PCA), and where missing data is determined to be reasonably missing at random (MAR or MCAR), multiple imputation using predictive mean matching will be employed.

15.8 Data Preparation and Maintenance of Blinding

The study manager will direct the data compilation steps of various team members according to consultation with the senior biostatistician. Gathering of partial midpoint data will be performed at reporting milestones throughout the study, but no statistical inference testing will be conducted until study completion. Research scientists, engineers, clinicians and database managers will assemble their respective data spreadsheet using the patient ID number and will remain blinded to treatment group allocation throughout this process. As a final step before data analysis, group allocation will be added to the research spreadsheets, but this will be limited to "Group A" and "Group B". The full statistical analysis will be performed prior to unblinding which group received the FIS supplement.

16.0 Principal Investigator's Ethical and Regulatory Obligations

The Principal Investigator is responsible for ensuring that the trial is conducted according to the signed investigator statement, the study protocol and applicable regulations; for protecting the rights, safety, and welfare of subjects under the investigator's care; and for the control of the Study Medication. The Principal Investigator shall confirm informed consent for each subject to whom Fisetin (FIS) or placebo, is administered. A discussion of the Principal Investigator's minimal responsibilities follows.

16.1 Study Conduct: Ethics and Good Clinical Practice

Principal Investigator will administer Study Medication only to subjects under his personal supervision or under the supervision of an appropriate Study Team designee responsible to the Principal Investigator.

This study must be carried out in compliance with the protocol and in accordance with the Good Clinical Practice, ICH Guidelines and the Declaration of Helsinki, concerning medical research in humans (Recommendations Guiding Physicians in Biomedical Research Involving Human Subjects, Helsinki 1964, amended Tokyo 1975, Venice 1983, Hong Kong 1989, Somerset West 1996).

The Principal Investigator agrees, when signing the protocol, to adhere to the instructions and procedures described in it and thereby to adhere to the applicable regulatory requirements and to the principles of Good Clinical Practice to which it conforms.

16.2 Institutional Review Board

The Principal Investigator shall assure that an IRB that complies with the requirements set forth in ICH and FDA guidelines will be responsible for the initial and continuing review and approval of the proposed clinical study. The Principal Investigator shall also ensure that he or she will promptly report to the IRB all changes in the research activity and all unanticipated problems involving risk to human subjects or others. Except when the changes constitute minor administrative ones or where necessary to eliminate apparent immediate hazards to human subjects, the Principal Investigator will not make any changes in the research without IRB approval.

Before implementing this study, the protocol, the proposed informed consent form and other subject information, must be reviewed and approved by the Institutional Review Board (IRB).

Other Principal Investigator responsibilities relative to the IRB include the following:

- Submit all protocol amendments to the IRB for review;
- Report to the IRB any information about serious AEs reported in other studies associated with treatment;
- Provide the IRB with any other information it requests before or during the conduct of the study;
- Report to the IRB all adverse events as required by the approving IRB(s);
- Maintain a file of IRB/study-related information;
- Update the IRB on a minimum of a yearly basis;
- Maintain IRB approval during the duration of the study.

16.3 Informed Consent

Principal Investigators must explain to each subject the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits involved and any discomfort it may entail. Each subject must be informed that participation in the study is voluntary and that he or she may withdraw from the study at any time and that withdrawal of consent will not affect his or her subsequent medical treatment or relationship with the treating physician.

This informed consent should be given by means of a standard written statement, written in non-technical language. The subject should read and consider the statement before signing and dating it and should be given a copy of the signed document.

No subject can enter the study before his/her informed consent has been obtained.

The Informed Consent Form must be submitted by the Principal Investigator for IRB approval.

16.4 Record Keeping and Study Documents

Study documents used to record demographic, procedural, and follow-up data, as well as any adverse clinical events that may occur during the study must be retained by the Principal Investigator.

The Principal Investigator must verify personal oversight of the clinical trial and confirm that all data recorded is accurate and current.

All data entries must be made only by the Principal Investigator and/or other site trained personnel. Training will be documented and logged in the site study binder.

16.4.1 Study Medication Accountability

It is the responsibility of the Principal Investigator to ensure that all Study Medication is inventoried and strictly accounted for throughout the study. This accounting is to be recorded in appropriate documents kept at the clinical site.

16.4.2 Study Documents

The investigator/institution should maintain adequate and accurate source documents and trial records that include all pertinent observations on each of the site's trial subjects. Source data should be attributable, legible, contemporaneous, original, accurate, and complete. Changes to source data should be traceable, should not obscure the original entry, and should be explained if necessary (e.g., via an audit trail).

16.4.3 Records Retention

All correspondence related to this clinical study should be kept in appropriate study files at the clinical site. Records of subjects, study documents, product inventory, and IRB and Study Team correspondence pertaining to the study must also be kept on file.

The Principal Investigator must retain Essential Study Documents including: protocols, amendments, IRB/EC approvals, signed and dated consent forms, source documents, medical records, case report forms, device accountability records, all correspondence and all other documents pertaining to the conduct of the study.

16.4.4 Records/Reports Inspection

Investigator sites and study documentation may be subject to Quality Assurance (QA) audits during the course of the study. In addition, inspections may be conducted by regulatory bodies at their discretion, during and after study completion.

The Investigator agrees to allow inspectors from regulatory agencies to have access to all study records, including subject source documents. By participating in this study, the Investigators agree to these requirements and will assist the inspectors in their duties. The Investigator should immediately notify the Sponsor of an upcoming inspection.

Investigators shall upon request from any properly authorized officer or employee of FDA, at reasonable times, permit such officer or employee to have access to, and copy and verify any records or reports made by the Investigator pursuant to CFR 312.62. The Investigator is not required to divulge subject names unless the records of particular individuals require a more detailed study of the cases, or unless there is reason to believe that the records do not represent actual case studies, or do not represent actual results obtained.

16.4.5 Study Oversight

The investigator is responsible for supervising any individual or party to whom the Principal Investigator delegates trial-related duties and functions conducted at the trial site.

If the investigator/institution retains the services of any individual or party to perform trial-related duties and functions, the investigator/institution will ensure this individual or party is qualified to perform those trial-related duties and functions and will implement procedures to ensure the integrity of the trial-related duties and functions performed and any data generated.

16.4.6 Interim and Final Study Reports

The Principal Investigator shall furnish all progress reports required by applicable regulations the FDA, IRB and to the Medical Monitor including an analysis of data reported.

16.4.7 Publication Plan

The Principal Investigator holds the primary responsibility for publication of the results of the study. We will register with ClinicalTrials.gov within 21 days of enrollment of the first participant. We will post results to ClinicalTrials.gov within 12 months of final data collection for the primary outcome.

17.0 Monitoring of Study

The primary goal of study monitoring is to protect subject safety. The study monitoring program will apply a risked- based approach to ensure that:

1. All treatment emergent adverse events are accurately recorded;
2. The rights and well-being of the human subjects are being protected;
3. The reported data are accurate, complete, and verifiable from source documents;
4. The conduct of the study complies with the currently approved protocol and any amendments, and in accordance with all local, state and/or federal regulatory agency requirements and good clinical practice (GCP) guidelines.

During the course of the study, monitoring visits will be conducted according to a written Monitoring Plan. The Investigator must provide allocate sufficient time for the monitor to inspect subject medical records, source documents, case report forms, Study Medication accountability records, and regulatory documents.

Key trial personnel must be available to assist the monitor. Although the Principal Investigator must give monitors access to relevant hospital or clinical records, to confirm their consistency with the CRF entries, no information in these records about the identity of the subjects will leave the study site. Monitoring standards require full verification for the presence of informed consent, adherence to the inclusion/exclusion criteria, and documentation of all AEs, SAEs and the recording of efficacy and safety variables.

Checks of the consistency of the source data with the CRFs are performed according to the study specific Monitoring Plan. Monitoring Reports will include a summary of what the monitor reviewed as well as complications, significant findings, deviations and deficiencies, conclusions, and actions taken or to be taken to ensure site compliance.

A Study Monitoring Plan will be submitted to FDA prior to the first subject treated.

18.0 Quality Assurance

18.1 Subject Completion

Any subject who does not return for a scheduled follow-up evaluation will be contacted by telephone by the Principal Investigator, or his or her designee, to determine the cause for the missed appointment. Two telephone calls will be made and documented in the study binder, in addition to sending a certified letter requesting the subject to contact the office and return for his/her evaluation. If a returned letter receipt or an undeliverable response is received, this notice will be retained in the subject's study binder that will note the subject is lost to follow-up. If the study subject wishes to withdraw from the study, the reason(s) for discontinuation will be recorded in the subject's chart and on the appropriate CRF, if the subject gives a reason. If a significant symptom or adverse event is associated with discontinuation, this will be recorded on the CRF as well.

18.2 Data Management

Data is to be managed in accordance with a written Data Management Plan which is to be retained as described in **Section 16.4.3** of this protocol. A detailed data Monitoring Plan will be submitted to FDA prior to the first subject treated.

18.3 Quality of Data

Subject CRFs will be collected and reviewed for completeness and accuracy, as well as for any evidence suggesting subject risk. Data will be compared to source documents within the Investigator's site. Where any discrepancies are noted, the discrepancy will be resolved with the Investigator and/or an individual designated by the Investigator. When the data are incomplete, attempts will be made to obtain the missing data.

18.4 Data Security

Limited study personnel will have access to study data stored on the institutional formsite account, and minimum password complexity requirements will be enforced, and recaptcha and two-factor authentication provide additional layers of security. Formsite servers are co-located in a cloud-based architecture with amazon web services (aws). All data stored on formsite are encrypted using the aes-256 encryption algorithm, and high-grade sha-256 rsa encryption is used for https connections over tls. Secure network access is enforced by multi-tiered firewalls, custom system configurations and multi-zones networks. Grant Dornan, Center for Orthopaedic-Based

Outcomes, Director, manages institutional access to the TSC formsite account, and creates sub-user accounts that enable access to individual form(s) for specific research studies.

Standard of care patient questionnaire data is automatically exported from formsite.com onto The Steadman Clinic (TSC) server and into the TSC patient outcomes database. Upon successful transfer, questionnaire data is automatically deleted from formsite.com. Formsite uses secure "https" links by default for all patients that complete surveys, and no personally identifiable information (PII) is recorded through any website technologies on formsite.com.

18.5 Qualification of Study Staff

All study staff will be confirmed to be qualified as assigned to appropriately delegated study tasks. A Delegation of Authority Log will be kept at the study site in addition to documentation of applicable training and experience.

18.6 Training

Prior to initiating the study, all site personnel will undergo thorough training regarding the Protocol. Documentation of training will be retained at the study site.

18.7 Protocol Amendments

No changes in the study procedures shall be affected without mutual agreement of the Investigator and the applicable IRB. All protocol amendments must be documented in writing, including the date and justification for the change. All changes must be documented by signed protocol amendments, which will be submitted to the FDA as appropriate.

18.8 Handling of Study Medication

18.8.1 Dispensing and Labeling

Study Medication will be provided to the Vail Health Hospital Pharmacy in bulk by Vital Nutrients Compounding Pharmacy and will be stored in a secure location with limited access. Vail Health Hospital Pharmacy will fill prescriptions for each subject randomized. A prescription will be provided by the Study Team to the pharmacy indicating the appropriate randomization group and the number of pills to dispense.

The study medication label will contain the following elements: Protocol IRB #, PI Name, Name of Study Medication, Directions, and the statement, "Caution – New Drug Limited by Federal Law to Investigational Use."

18.8.2 Accountability

A medication tracking sheet will be kept by the Vail Health Hospital Pharmacy, which is to include the lot number, expiration date: date Vail Health Hospital Pharmacy received from Vital Nutrients, name of person receiving, date dispensed, name of person dispensing, randomization code, subject initials, number of capsules dispensed, date and number of returned capsules, name of person receiving, date and number discarded and by whom the capsules were discarded.

18.8.3 Disposition of Unused Study Medication

All unused, not previously dispensed Study Medication will be returned to the Study Sponsor for proper disposal or reallocation.

1. For remaining previously dispensed Study Medication, the label (including identifiable information) is taken off of the bottle and discarded in a shred bin by Vail Health Pharmacy;
2. Any remaining previously dispensed Study Medication is discarded in a secure biohazard bin by Vail Health Pharmacy;
3. Unused Study Medication that was not dispensed will be returned to the Study Sponsor to be stored or reallocated until the Study Medication expires. Upon expiration, the Study Medication will then be discarded in a secure biohazard bin.
4. Biohazard is picked up for disposal on a weekly basis.

18.9 Laboratory Qualification

All available information regarding laboratory qualification name and a copy of the medical license for laboratory directors, current normal ranges for the laboratory and proof of appropriate laboratory certification as appropriate will be maintained in the regulatory binders.

19.0 Disclosure and Confidentiality

By signing the protocol, the Principal Investigator agree to keep all study documents in strict confidentiality and to request similar confidentiality from his/her staff and the Institutional Review Board. study documents (Protocols, and other material) will be stored appropriately to ensure their confidentiality.

20.0 Declaration of Helsinki

The study will be conducted according to the guidelines established in the Declaration of Helsinki (World Medical Association 1964). Good Clinical Practices (GCPs) and local ethical and legal requirements. Subjects will be free to withdraw from the study at any stage without prejudice to their subsequent treatment.

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22.0 Appendices

22.1 Appendix A: Informed Consent

Research Information and Consent for Participation in Biomedical Research
Senolytic Drugs Attenuate Osteoarthritis-Related Articular Cartilage Degeneration: A Clinical Trial
Study #: 2019-16

I. Overview: You are being asked to take part in a research study. The information in this document should help you to decide whether you want to participate in this study. The sections in this Overview provide the basic information about the study. More detailed information is provided in the remainder of the document.

Study Staff: The Principal Investigator for this study is Dr. Thos A. Evans, MD, who is a physician at The Steadman Clinic and who will lead the study team. Other approved and appropriately qualified research staff may act on behalf of the Principal Investigator.

Study Details: This study is being conducted at The Steadman Clinic and Steadman Philippon Research Institute and is supported by The United States Department of Defense: Office of Naval Research. The purpose of the study is to test an investigational drug called Fisetin in patients with knee osteoarthritis to improve joint health and reduce pain. If you are already a patient of one of the study doctors (Dr. Evans, Dr. Kim, Dr. Godin, Dr. Leslie Vidal, or Dr. Armando Vidal), you may have already undergone a knee physical exam, X-rays and/or an MRI, and completed questionnaires that can be used for this study. All other exams, procedures, and treatments that are part of the research study will be provided at no cost to you.

If you are not currently a patient of one of the above listed study doctors, a study doctor will meet with you to evaluate your knee pain and determine if you have a condition called knee osteoarthritis. They will order a knee x-ray and do a physical exam of you knee to make this determination. This will only be done after you sign this consent form and the costs will be covered by this study.

For established patients of the study doctors and new patients, research procedures include:

- Random assignment into either the Fisetin group or placebo group. You will be provided either Fisetin (the investigational drug) or placebo tablets for 2 treatment cycles over the course of 2 months;
- Pregnancy test
- Blood draw;
- Knee X-rays;
- Magnetic resonance imaging (MRI);
- Functional performance testing;
- Physical exam;
- Muscle strength testing;
- Lower-extremity video-motion analysis;
- Knee questionnaires.

Participants: You are being asked to take part because you have been diagnosed with a painful joint condition called knee osteoarthritis. If you agree to participate in this study, your involvement will last for 18 months (1 year and 6 months).

Voluntary Participation: Your participation in this research is voluntary. You do not have to participate and may stop your participation at any time. Your decision whether or not to participate will not affect your current or future dealings with The Steadman Clinic. If you decide to participate, you are free to withdraw at any time without affecting that relationship. Alternatives to participating in the study do exist and can be discussed with the study team doctors. These strategies may or may not preclude you from participating in the study.

Benefits, Compensation, and Risks: We do not know if you will receive any benefit from your participation. If you are assigned to the placebo treatment group, you are not expected to directly benefit from participating in this research. You will receive a $25 Amazon gift card for each visit that requires you to return to the study site for research purposes. The total value is $100 if you complete the 4 follow-up research visits. The most common and most serious risk that may be related to taking part in this research is that the new treatment (Fisetin) may not be effective or as effective as current standard of care procedures to manage your knee osteoarthritis. Some of the common standard of care procedures include intra-articular steroid injection, over the counter non-steroidal anti-inflammatory drug administration (such as aspirin or ibuprofen), or autologous biologics therapies (injections of products derived from your own blood or bone marrow). You may consult your doctor for more information regarding these treatment alternatives. Another risk is that you may not be part of the treatment group and thus receive a placebo (non-active) drug. Known potential side effects of taking Fisetin or placebo, although very uncommon, include: nausea, dry

mouth, diarrhea, vomiting, and fatigue. The common drug interactions and warnings include: (1) lowering the effectiveness of other medications you may be taking, such as anti-coagulant, anti-diabetic, anti-hypertensive, and anti-seizure medications; (2) developing sleep problems; and/or (3) restlessness if you consume high amounts of caffeine or tobacco products while taking Fisetin.

Confidentiality: Even if we publish the findings from this study, we will keep your individual study information private and confidential. Anyone with the authority to look at your records must keep them confidential.

II. Conflict of Interest

If you are currently receiving care at The Steadman Clinic, your health care provider may be an investigator on this research protocol, and as an investigator, is interested in both your clinical welfare and in the conduct of this study. Before entering this study or at any time during the research, you may ask for a second opinion about your care from a clinician who is not associated with this project. You are not obligated to participate in any research project offered by your clinician. Your participation in this research study is voluntary, and you do not have to participate. The decision to not participate will not affect your clinical care now or in the future.

Neither the study doctors nor any members of the research team have any financial relationships with any manufacturer or supplier of Fisetin.

Johnny Huard, PhD is the Principal Investigator on the contract of all four of our studies funded by the Department of Defense (DOD), including this one. While Dr. Huard is the lead PhD scientist on the four DOD-funded studies, Thos Evans, MD, a physician, is the principal investigator of the present study due to its clinical nature. ProofPoint Biologics (PPB) is an operating division of the Steadman Clinic that may draw and prepare your blood for analysis.

Some of Dr. Huard's compensation is covered by PPB. Dr. Thos Evans is the medical director and part owner of PPB. Dr. Raymond Kim is a part owner of PPB. However, their financial interest in and/or part ownership of PPB will not affect the research procedures or outcomes of this study.

III. Why am I being asked to participate in this research?

You have been asked to participate in the research because you have a painful joint condition called knee osteoarthritis.

Approximately 100 subjects may be involved in this joint research project of The Steadman Clinic and the Steadman Philippon Research Institute.
Even though you agree to participate in this study and complete the informed consent, the study doctor may decide that you are not eligible to participate in this study.
You are eligible to participate in this study if you:

- Are 40-80 years of age;
- Are willing to comply with all study related procedures and assessments;
- Have been diagnosed with knee osteoarthritis.

You are not eligible to participate in this study if you:

- Are unable to take oral medications;
- Are pregnant or nursing a child;
- Women who plan on becoming pregnant during the dosing of fisetin or placebo;
- Have a medical condition or history that, in the opinion of the Investigator:
    - Might constitute a risk for participation in the study,
    - Could interfere with the study objectives, conduct, or evaluation of the study, or
    - Could prevent you from fully participating in all aspects of the study;
- Have previously undergone recent surgery or injections to your knee;
- Are currently taking any medications that may be incompatible with Fisetin use;
- Are unable to safely undergo an MRI (due to metal implants, severe claustrophobia, or body size).

- **Behavioral Modification –** You will be encouraged to reduce use caffeine use (i.e. coffee, soda, tea) by 50% prior to and during the 2-day study drug dosing periods. Due to interactions with fisetin and caffeine, your body may not clear caffeine from your system as effectively as normal.

IV. Study Procedures: What will happen during this study?

This research will be performed at The Steadman Clinic, Steadman Philippon Research Institute, and Vail Health.

Fisetin is a commercially available dietary supplement that is found in many plants, such as fruits and vegetables. The common side-effects and drug-to-drug interactions of Fisetin are generally understood by doctors and scientists. Dietary supplements do not require approval from the Food and Drug Administration (FDA); however, we have received approval from the FDA to study Fisetin as a treatment for patients with knee pain due to osteoarthritis and for this reason, Fisetin is referred to as an investigational drug in this consent form.

You will be asked to take Fisetin or placebo capsules for 2 cycles of treatment over the course of approximately 5 weeks:

- Cycle 1: 2 day on (days 1 and 2), followed by 28 days off (day 30).
- Cycle 2: 2 days on (days 31 and 32).

You will need to come to the study site 4 more times over the next 12 months, for a total of five visits in all. Each research visit will take between 1 and 4 hours to complete.

Procedure Visits:
If you are an existing patient of one of the study doctors, the physical exams, medical record review, and questionnaire collection are part of standard of care procedures at The Steadman Clinic and may be used to determine eligibility and for Visit 1 of the research study. All other research procedures and treatments for existing patients will be provided at no cost to you.

If you are not a current patient of one of the study doctors, all of the research-related procedures and treatments will be provided at no cost to you.

Visit 1 (Pre-Treatment/Enrollment)

- **Time frame**: Immediately
- **Visit length**: 2-3 hours
- A clinical member of the study team will determine if you are eligible to participate. If you're an existing patient, a clinical team member will access your medical records to collect information about you and your medical history. This will include any medications you currently take and other information in your medical records related to your condition or treatment that may be important to your participation in the study. If you are not an existing patient, a member of the clinical team will ask you about yourself and your medical history to determine your eligibility.
- A pregnancy test will be performed for all pre-menopausal female participants.

- If you do not have recent x-rays on file, you will have x-rays taken of your knees.
- You will have a 50% chance of being randomly assigned to one of the two treatment groups:
    1. Fisetin group (investigational group);
    2. Placebo group (control group).
    - "Randomization": How we decide which study group you will be in
        - This study will assign all participants to one of two different treatment groups. To decide which group you will be in, we will use a method of chance. This method, called randomization, is like flipping a coin or rolling dice.
    - "Double-Blind": You will not know which group you are in
        - You will not know which study group you are in, and neither will your study doctor. Therefore, this study is called "double-blind." Your group assignment needs to be kept secret so that the study is based on scientific results and not on peoples' opinions. However, we can obtain your group assignment if you have an emergency. **If you have an emergency, make sure you tell the emergency staff about your participation in this study.** They can contact us, and we will give them all relevant information.
    - What the placebo in this study is:
        - The placebo is a pill that looks like medicine but is not real; it will have no medical effect on you.
- You will have your blood drawn to collect 50 milliliters (10 teaspoons) of blood for lab screenings and protein testing.

<u>Visit 2 (Treatment)</u>

- **Time frame**: within 3 months of Visit 1
- **Visit length**: 3-4 hours
- Vital signs, medication information, and any adverse events that occurred since the last visit will be collected.
- A pregnancy test will be performed for all pre-menopausal female participants.
- The following procedures and tests will be performed:
    - Medication instructions;
    - Magnetic resonance imaging (MRI) scan;
    - Functional performance testing;
    - Physical exam;
    - Muscle strength testing;
    - Video-motion analysis.
- Note that the blood test and knee x-ray may be performed at this visit if it was not done at visit 1.
- Some of the screening procedures may need to be repeated for accuracy, fidelity, and/or safety.

- Vail Health Pharmacy will be responsible for dispensing the Fisetin or placebo capsules. Fisetin or placebo capsules will be given to you in a bottle with a set of instructions.
- After completing the study tests for this visit, you will be given Fisetin or placebo capsules as treatment. You will be instructed to take capsules while at the study site and the next day (10-15 capsules per day, amount based on body weight). You will be instructed to take the capsules for 2 cycles of treatment:
    - Cycle 1: 2 days on (days 1 and 2), followed by 28 days off (day 30).
    - Cycle 2: 2 days on (days 31 and 32).
    - Treatment will occur over the course of approximately 5 weeks.
- All capsules must be taken within 60 minutes after the first capsule is consumed.
- At Visit 4, please return any remaining capsules in the bottle. These extra capsules will be counted and discarded at your next on-site visit.
- You will be given a drug diary/schedule to log each dose you take during the 2 treatment cycles.
- A member of the clinical team will contact you via phone call three times while you are scheduled to take Fisetin or placebo to remind you of the schedule and record any adverse events you may experience. Depending on the severity of the adverse event, the member of the clinical team may request that you visit the study site for further evaluation.

Non-Visit, Pain Questionnaire

- **Time Frame:** Every 3 days for 6 weeks after the first dose of and once weekly for the following 6 weeks
- **Length:** 5 minutes
- You will complete an electronic pain questionnaire every 3 days for 6 weeks after the first dose of Fisetin or placebo and once weekly for the following 6 weeks. The first questionnaire will be sent to you 3 days after your first dose.
- If you are unable to fill out the questionnaire electronically, we will provide you with a paper form at Visit 2.

Visit 3 (Blood Draw)

- **Time frame**: 2 weeks (± 2 days) from your first dose of Fisetin or Placebo
- **Visit length**: 1 hour
- You will have your blood drawn to collect 50 milliliters (10 teaspoons) of blood for lab screenings and protein testing.

Visit 4 (Testing)

- **Time frame**: 2 weeks (± 10 days) from your LAST dose of Fisetin or Placebo
- **Visit length**: 1-2 hours
- You will return any extra capsules in the medication bottle at this visit.
- Vitals, medication information, a knee questionnaire, and any adverse events that occurred during the treatment course will be collected.
- You will have your blood drawn to collect 50 milliliters (10 teaspoons) of blood for lab screenings and protein testing.
- The following procedure will be performed:
    - Physical exam.

Visit 5 (Testing)

- **Time frame**: 6 months (± 4 weeks) from your FIRST dose
- **Visit length**: 3-4 hours
- Vital signs, medication information, a knee questionnaire, and any adverse events that occurred over the last 3 months will be collected.
- You will have your blood drawn to collect 50 milliliters (10 teaspoons) of blood for lab screenings and protein testing.
- The following procedures and tests will be performed:
    - MRI scan;
    - Functional performance testing;
    - Physical exam;
    - Muscle strength testing;
    - Video-motion analysis.

Visit 6 (Testing)

- **Time frame**: 12 months (± 4 weeks) from your FIRST dose
- **Visit length**: 3-4 hours
- Vital signs, medication information, a knee questionnaire, and any adverse events that occurred over the last 12 months will be collected.
- You will have your blood drawn to collect 50 milliliters (10 teaspoons) of blood for lab screenings and protein testing.
- The following procedures and tests will be performed:

- MRI scan;
- Functional performance testing;
- Physical exam;
- Muscle strength testing;
- Video-motion analysis.

Non-Visit, Pain Questionnaire

- **Time Frame:** 18 months after your FIRST dose
- **Length:** 20-30 minutes
- You will complete an electronic knee questionnaire

If you are unable to fill out the questionnaire electronically, we will provide you with a paper form.

Alternative Procedures:

If at any time you wish to seek an alternative treatment due to overt pain or functional deficiency, you may consult with your study doctor regarding treatment options. If prescribed by your doctor, you would be allowed to receive a corticosteroid intra-articular knee injection at any time during the study and remain enrolled in the study. If you wish to seek an alternative therapy other than a corticosteroid intra-articular knee injection, (e.g., a biologics-based therapy or surgery), you will be removed from the study. You or your health insurance plan would be responsible for the costs of any alternative procedures.

Research Subject Responsibilities:

As a research subject in this study, it is important that you:

- Tell your doctor or clinical team member if your condition or circumstances change during the study;
- Return to the site for scheduled study visits;
- Call the doctor's office to:
    - Report any injuries, hospitalizations, emergency room visits, abnormal symptoms (including severe nausea, diarrhea, vomiting, chills, excessive swelling), or complaints;
    - Schedule an upcoming visit or reschedule a missed research visit.

Please call 970-476-1100 and request your study doctor for any immediate assistance.

Study Procedures:

1. Pregnancy Test: You will complete a urine pregnancy test.

2. Blood Draw: A certified phlebotomist will insert a needle into your vein and will collect 50 milliliters (10 teaspoons) of blood. Your blood samples will be used to assess your health and measure proteins within your blood.
3. Medication Instructions: A member of the study team will provide you with the medication tablets at Visit 2. You will be instructed to take tablets (amount based on body weight) for 2 days and then again for 2 days 4 weeks later. You will need to store the medications at room temperature. You will be given a set of instructions and a drug diary/schedule to log the following:
    - Date;
    - Time of day tablets were taken;
    - Number of tablets taken.
4. X-ray: If you do not have recent knee x-rays on file with the clinic, you will undergo a standard, 3-view x-ray sequence of both knees.
5. Magnetic Resonance Imaging (MRI): A scan in a 3 Tesla MRI scanner that uses a strong magnetic field to produce detailed imagines of your knee joint. To set up the MRI scan, you will be first given a safety screening questionnaire to determine if you are a candidate for this procedure. If you meet the criteria, a technician will prepare you for the MRI scan. A set of headphones will be given to you and a device will be set up over your knee. Once the technician has prepared you for the MRI scan, you will be moved into the scanner. The scans will take up to half an hour each time.
6. Functional Performance: Researchers will assess the coordination of your knee joints, strength, and control by asking you to perform several different movements. Researchers will measure distance, time, or number of times you are able to complete the test. Each of the tests will be repeated three times:
    - *Stair-Climbing Test:* This is a timed test that will assess how quickly you walk up and down 12 stairs.
    - *Timed Up and Go:* This is a timed test that will assess how quickly you can stand up from an armed chair, walk 3 meters (approximately 9 feet), turn, and sit back down without physical assistance.
    - *4 Meter Fast Walk:* This is a timed test that will assess how quickly you can walk 4 meters (approximately 13 feet).
    - *6 Minute Walk:* This is a timed test that will assess the distance you can walk in 6 minutes.
7. Physical exam: A process in which a medical professional will evaluate your knee joint for any signs of injury. This procedure will take approximately 15-20 minutes to perform and includes a manual muscle test and range of motion assessment.
8. Muscle Strength: Muscle strength will be assessed for both knees using a strength testing system. You will be escorted from the Steadman Philippon Research Institute Biomotion lab to Howard Head (which is located on the same floor as the Biomotion lab) where the strength testing system is located. You will be placed in a seated position with joint stabilizing cushions. You will be asked to flex and extend your knee while contracting your muscles. The first time will be a warm-up. This test will be repeated 3 times.
9. Biomotion/Video-Motion Analysis: The movement of your joints will be measured in the Biomotion Lab of the Steadman Philippon Research Institute during two common activities:

- *Walking on a treadmill:* You will be asked to walk for 5 short periods of time (less than 1 minute each) on a treadmill. The treadmill speed will be set to 1 meter per second (2.2 miles per hour), which is slower than the average walking speed of 3 miles per hour. The treadmill has handrails for safety. You will be allowed to rest between walks if necessary.
- *Ascending and descending stairs:* You will be asked to walk up and down a short (4-step) stairway at your own pace, using handrails if needed. This activity will be performed 5 times with rests in between if necessary.

During this testing, you will wear reflective markers attached using double-sided tape (Figure 1). The motion of these markers will be tracked using an 18-camera video-motion capture system and will be used to determine the three-dimensional motion of your joints. You will be dressed in snug-fitting exercise clothes so that markers placed on clothing will not move excessively relative to the skin (the lab will provide compression clothing if you do not have your own).

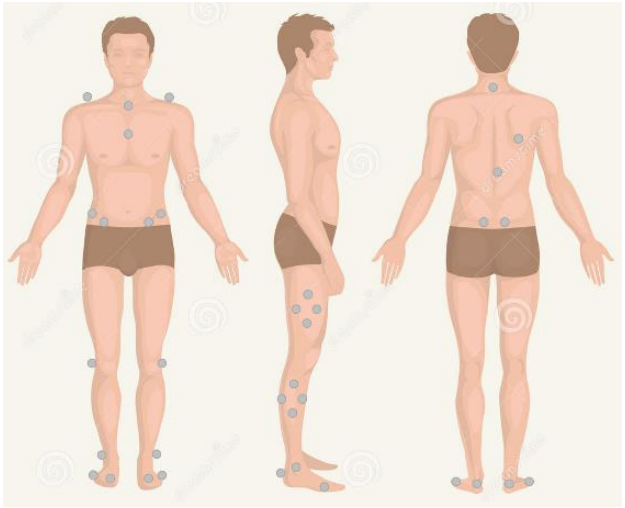

Figure 1: Approximate locations of marker placement (grey dots) for video motion analysis. *Note: You will be given compression shorts to wear throughout testing and will wear a close-fitting shirt or sports bra, if applicable. Markers will be placed on skin or clothing where necessary.*

10. Self-Reported Pain Questionnaire (Short-Form): A questionnaire that assesses your knee pain will be provided to you electronically every 3 days for 6 weeks after the first dose of and once weekly for the following 6 weeks in an e-mail or on an iPad tablet or in paper form. This procedure will take approximately 5 minutes to complete.
11. Self-Reported Knee Questionnaire (Long-Form): A questionnaire that assesses your knee's function will be provided to you electronically in an e-mail or on an iPad tablet or in paper form. This procedure will take approximately 20-30 minutes to complete.

The study team asks permission to access your medical record to collect the following health information for research purposes at the time of your evaluation:

- Gender
- Age
- Height
- Weight
- Body mass index (BMI)
- Smoking status
- Chronic diseases or conditions (not related to your current knee condition)
- Medication use such as non-steroidal anti-inflammatory drugs (e.g., aspirin, ibuprofen, etc.)

The study team also asks permission to access your medical record to collect the following injury information for research purposes from your imaging and surgical reports:

- Pre-existing injury details
- Severity of injury details
- Imaging findings (X-ray or MRI)
- Injury type
- Previous treatment details (related to your current knee condition)

V. What are the potential risks and discomforts?

There are risks associated with participation in this study. These risks are listed below, but it's possible that some risks are not yet known.

Side Effects Associated with Fisetin: A clinical member of the study team will describe the possible side effects of Fisetin and answer any questions you may have. The common side effects for Fisetin, although very uncommon, include: reduced appetite, altered activity, nausea, dry mouth, diarrhea, vomiting, and fatigue. We do not anticipate the occurrence of serious side effects with the prescribed treatment regimen in this study. However, we have identified a variety of drug interactions with Fisetin. The study team will review

your current medication regimen. If you are taking therapeutic doses of drugs that may interact with Fisetin, you may not be eligible for this study.

Your doctor does not know all the side effects that you may experience from fisetin. Like all investigational drugs, all side effects may not have been identified at this time; some may be mild or others very serious. Everyone taking part in this study will be watched closely for any side effects. If you experience any unexpected behavior changes or unpleasant symptoms, please contact your study doctor immediately. If you use tobacco or high amounts of caffeine (>1000 mg/day), a member of the study team will describe the possible complications or adverse events when taking Fisetin (such as sleep problems and/or restlessness). As a precaution, we advise that you limit the amount of caffeine and tobacco products while taking Fisetin or placebo during the 2 dosing periods (two periods of 2 days separated by 4 weeks).

Fisetin was shown to cause DNA damage to human cells in the test tube. The relevance of this result to the study in which you are participating is unknown.

Risks Associated with Placebo Group Assignment: It is unknown whether Fisetin will provide a beneficial effect for the treatment of knee osteoarthritis. Therefore, there is no known risk for NOT receiving Fisetin. Some of the common side effects of placebo treatment include: reduced appetite, altered activity, nausea, dry mouth, diarrhea, vomiting, and fatigue.

Risk of Improper Disclosure of Your Personal Information: There is a possibility that your personal information will be accidentally or inappropriately breached during this research. The Steadman Clinic and Steadman Philippon Research Institute use many physical, technologic, and administrative measures to protect your information. Locking offices and cabinets protect information on paper, while password-locked workstations and encryption protect electronic information. Whenever possible, information will be used and analyzed in a manner that does not contain any of your identifiable information. When results of this research are presented at scientific meetings and in medical journals, all information that can possibly identify you will be removed.

Risk or Discomforts Associated with the Blood Draw: The following potential risks are associated with the collection of blood: fainting, fatigue, lightheadedness, dizziness and nausea, and pain or possible infection at the blood-draw site. Clinical precautions will be in place to monitors these associated risks.

Risks associated with the Knee X-Ray: An anterior (front) view x-ray of your knees is required to determine the extent of osteoarthritis in your knee. If you have been treated for a knee disorder at The Steadman Clinic, you will probably have had an x-ray taken of your knee as part of your clinical care which can be used for this research. If you do not have a recent knee x-ray on file, an anterior knee x-

ray will be acquired as part of this research study. The radiation exposure from this x-ray is less than half of what is received from a typical intraoral dental x-ray. As part of everyday living, everyone is exposed to a small amount of background radiation from space and naturally occurring radioactive minerals. The radiation dose you will receive in this study (0.002 mSv or less) will give your body the equivalent of a few hours' worth of this natural radiation.

This radiation exposure may not be required for your medical care but is necessary to obtain the research information desired. There is no dose of ionizing radiation which is considered to be free of risk. However, there is no evidence to suggest that the small levels of radiation involved in this study have measurable adverse effects. The risks from radiation exposure to your knee are low because there are no sensitive organs exposed.

Risks or Discomforts Associated with MRI: The MRI scan involves loud noises and positioning in a small space. You may feel claustrophobic, tired, or nauseated, especially if you are uncomfortable with tight spaces. The MRI scan does not involve the use of X-rays or injectable dyes. There are no known reports of increased cancer or birth defects associated with this procedure. However, the MRI scan exposes you to high magnetic fields, which can be dangerous if you have a pacemaker or certain metal implants.

Risk of Injury and Discomfort with Functional Performance, Muscle Strength, and Video-motion Analysis Testing: This study involves completion of activities which are common during daily life. These activities are unlikely to cause additional injury at the time you will be completing them. However, there is a chance of accidental injury during these activities, such as falling, pulling a muscle, or other injury. It is also possible that these movements could cause some discomfort, pain, or fatigue consistent with physical exercise. *If at any time during data collection you do not feel that you can safely complete an activity, you must stop and tell a researcher immediately. Your safety and well-being are more important than the researchers' data.*

Risk of Skin Irritation: Motion capture markers are attached to the skin with double-sided tape, which may cause minor skin irritation.

Risks or Discomforts Associated with Self-Reported Questionnaires and Physical Exam: There is a potential risk of emotional distress associated with questionnaires and pain and/or discomfort associated with the physical exam. However, these risks should not exceed that experienced during normal activities of daily living.

VI. Are there reproductive risks to participation in this study?

If you are a woman: Participating in this research may involve risks to pregnant women and/or an unborn baby which are currently unforeseeable. To protect against possible side effects of the study drug Fisetin, you may not take part in this study if you are pregnant

or nursing a child. If you are a woman of childbearing ability, the study doctor recommends that you at least two method of birth control or to be abstinent (i.e., not have sex) throughout the study. Acceptable methods of birth control include: oral contraceptive and condom, intra-uterine device (IUD) and condom, diaphragm with spermicide and condom. If you think that you have become pregnant during the study, you must tell the doctor immediately.

If you are a man: To protect against possible side effects of the study drug to an unborn baby, you must not get a partner pregnant while taking the study drug and for 2 weeks after the last dose. The principal investigator recommends that you and your spouse abstain from sex or use contraceptive protection while taking the study drug and for 2 weeks after the last dose. Acceptable methods of birth control include: oral contraceptive and condom, intra-uterine device (IUD) and condom, diaphragm with spermicide and condom.

VII. What are the possible benefits of the study?

This study is designed for the researcher to learn more about Fisetin. This study is not designed to treat any illness or to improve your health.

VIII. Are there alternative treatments?

There may be other ways of treating your knee osteoarthritis. These other ways might include medication, injections, or surgery. You could also choose to get no treatment at all.

You should talk to your doctor about your choices. Make sure you understand all of your choices before you decide to participate in this study. You may leave this study and still have these other choices available to you.

IX. What are the costs for participating in this research?

If you are already an established patient with one of the study doctors, with the exception of the data collected at Visit 1, all physical exams and self-reported questionnaires are collected for research purposes. All other procedures and treatments are part of the research study and will be provided at no cost to you. For example, no-cost services include every treatment (Fisetin or placebo), procedure, and evaluation following the initial visit.
If you were not already a patient with one of the study doctors and heard about this study from a flyer or advertisement, all procedures and treatments are part of the research study and will be provided at no cost to you.

If you receive a bill from The Steadman Clinic or Vail Health Laboratory for any research procedures or visits that you feel is you should not have received, please contact Suzanne Page at 970-401-8770.

X. Will I be paid for my participation in this research?

You will receive a $25 Amazon gift card for each completed follow-up study visit (Visits 3, 4, 5 and 6). If you do not finish the study, you will be compensated for the visits you have completed. If you complete the study, you will receive a total of $100 in Amazon Gift cards. You will receive your payment by a member of the study team at the end of each research visit.

XI. What if I am injured as a result of my participation?

If you get ill or injured from being in the study, your study doctor will help you get medical treatment. You should let the study doctor know right away that you are ill or injured. If you believe you have become ill or injured from this study, you should contact your study doctor at (970) 476-1100. If you believe your illness or injury to be an emergency, call 911.

You should let any health care provider who treats you know that you are in a research study. If you do seek medical treatment, please take a copy of this document with you because it may help the doctors where you seek treatment to treat you. It will also provide the doctors where you seek treatment with information they may need if they want to contact the research doctors.

If you receive care for an injury, you or your health insurance plan will be billed. No money has been set aside to pay the costs of this treatment. Health insurance plans may or may not cover costs of research-related injury or illness. You should check with your insurance company before deciding to participate in this research study. The study staff will assist you in obtaining pre-authorization from your insurance company. Costs not covered by insurance could be substantial.

The Steadman Clinic and Steadman Philippon Research Institute have not set aside any money to pay you or to pay for your treatment if you get ill or injured from being in the study. There are no plans for The Steadman Clinic and Steadman Philippon Research Institute to provide other forms of compensation (such as lost wages or pain and suffering) to you for research related illnesses or injuries. The only exception to this policy is if it is proven that your injury or illness is directly caused by the negligence of a Steadman Clinic and Steadman Philippon Research Institute employee.

By signing this form, you are not giving up any legal rights to seek compensation of injury.

XII. Can I withdraw or be removed from the study?

Taking part in this study is voluntary. You have the right to choose not to take part in this study. If you decide to participate, you are free to withdraw your consent and discontinue participation at any time without affecting your future care at The Steadman Clinic. You will still receive standard of care treatment for your condition if you volunteer to withdraw.

You have the right to leave a study at any time without penalty. If leaving could affect your safety, the investigator will provide information about recommended steps for leaving the study.

The researchers also have the right to stop your participation in this study without your consent if they believe it is in your best interest.

XIII. Future use of identifiable private information

Your identifiers might be removed from your private records. Your information could be used and/or distributed to another investigator for future research studies without additional consent from you. We use your blood samples only for purposes directly related to this study. Any remaining samples will be destroyed upon study completion.

XIV. What about your privacy and confidentiality?

The people who will know that you are a research subject are members of the research team, and if appropriate, your physicians and nurses. No information about you, or provided by you, during the research, will be disclosed to others without your written permission, except if necessary to protect your rights or welfare (for example, if you are injured and need emergency care) or if required by law.

Study information which identifies you and the consent form signed by you will be looked at and/or copied for examining the research by:

- Food and Drug Administration (FDA)
- Department of Defense, Office for Human Research Protections (OHRP)
- Vail Health Institutional Review Board

A possible risk of the research is that your participation in the research or information about you and your health might become known to individuals outside the research. Your original signed consent document and relevant medical information will be added to your Medical Record at The Steadman Clinic. All other personal information that will be collected for this study will be stored separately

from the research data, with password-protected access limited to authorized personnel on the study team. The information collected for research only will be maintained for up to two years following completion of the study (or longer, if required by federal agency regulations). De-identified data and information entered into the Medical Record may be kept indefinitely.
When the results of the research are published or discussed in conferences, no information will be included that would reveal your identity.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. law. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

XV. What happens if new information becomes available about the study?

During the course of this study, we may find more information that could be important to you. This includes information that, once learned, might cause you to change your mind about being in this study. We will notify you as soon as possible if such information becomes available.

We may learn things about you from the study activities that could be important to your health or to your treatment, meaning they are "clinically significant." By participating in this study, you will receive blood tests and MRIs that are for research purposes only and are not part of your clinical care. These research tests will not be read or evaluated by a health care professional and will not be added to your medical records (they are used exclusively for research data processing). If a researcher notices an anomaly in any of these tests that might indicate a serious, life-altering condition they will notify the Principal Investigator. The Principal Investigator will evaluate this information, which may include consulting with an expert such as a radiologist or cardiologist, to determine if the finding is likely to have enough health importance to disclose it to you. If it is determined that this information should be reported to you, the Principal Investigator will communicate the findings to you and will discuss with you the provision of care by the Steadman Clinic, referral to another clinic, physician or provider, or information about alternative resources for obtaining care.

The above results will not be placed in your medical record. You may need to meet with professionals with expertise to help you learn more about your research results. The study team/study will not cover the costs of any follow-up consultations or actions. If you are not interested in receiving this information, please do not consent to participate in this study.

XVI. Who should I contact if I have questions about the research?

Contact the researchers at 970-476-1100:

- if you have any questions about this study or your part in it,
- if you feel you have had a research-related injury (or a bad reaction to the study treatment), and/or
- if you have questions, concerns, or complaints about the research.

XVII. Who should I contact if I have questions about my rights as a research subject?

If you have questions about your rights as a research subject or concerns, complaints, or to offer input you may call Mary Crumbaker, Chief Ethics and Compliance Officer at Vail Health at 970-477-5197.

XVIII. Authorization to use and disclose Protected Health Information

The purpose of this section is to give your permission to the research team to obtain and use your patient information. Your patient information will be used to do the research named above.

State and federal privacy laws protect your patient information. These laws say that, in most cases, your health care provider can release your identifiable patient information to the research team only if you give permission by signing this form.

You do not have to sign this permission form. If you do not sign it, you will not be allowed to join the research study. Your decision to not sign this permission will not affect any of your treatment or any other treatment, healthcare, enrollment in health plans, or eligibility for benefits.

What information will be obtained and used?

"Patient information" means the health information in your medical or other healthcare records. It also includes information in your records that can identify you. For example, it can include your name, address, phone number, birthdate, and medical record number.

By signing this form, you are giving permission to the following organization(s) to disclose your patient information for use in this research.

- Vail Health (includes Shaw Cancer Center, Howard Head and all Diversified Services clinic locations)
- The Steadman Clinic

- Steadman Philippon Research Institute

What information will be released for research use?

If you give your permission and sign the last page of this form, you are allowing the health care providers indicated above to release the following medical records containing your Personal Health Information to the researchers for use in this project. Your Personal Health Information includes health information in your medical records, financial records and other information that can identify you.

The specific information that will be released and used for this research is described below*:*

- Medical history / treatment
- Consultation
- Diagnostic imaging report
- Radiology images (like X-rays or CT scans or MRI's)
- Laboratory / diagnostic tests
- Operative reports (about an operation)
- Patient-reported outcomes from questionnaires
- Basic demographic information:
    - Gender
    - Age
    - Height
    - Weight
    - Body mass index (BMI)
    - Smoking status
- Medical comorbidities (presence of two chronic diseases or conditions)

How will my patient information be used?

The following groups of people may also be able to see your health information and may use that information to conduct this research:

- The research team for the research described in the Consent Form;
- Vail Health Institutional Review Board (VH IRB);
- Others who are required by law to review the quality and safety of the research, including U. S. government agencies such as The U.S. Food and Drug Administration (FDA) or the Department of Defense Office of Human Research Protections.

Your patient information will be used and/or given to others for the following reasons:

- To do the research
- To study the results, and
- To see if the research was done right

If the results of this study are made public, information that identifies you will not be used.

The researcher will use your patient information only in the ways that are described in the research consent form that you sign and as described in this HIPAA Authorization.

You can ask questions about what the research team will do with your information and how they will protect it.

The privacy laws do not always require the receiver of your information to keep your information confidential. After your information is given to an organization that is not subjected to the privacy laws, e.g., a research organization, there is a risk that it could be shared without your permission.

How long will this authorization be valid?

This permission for the researchers to obtain your patient information:

- Ends when the research is complete and any required monitoring of the study is finished.

Cancelling your permission:

You may change your mind at any time. To take back your permission, you must send your **written** request to:

Kate Wilmouth
Steadman Philippon Research Institute
181 W. Meadow Dr. Suite 1000
Vail, CO 81657

If you take back your permission, the research team may still keep and use any patient information about you that they already have. But they can't obtain more health information about you for this research unless it is required by a federal agency that is monitoring the research.

If you take back your permission, you will need to leave the research study. Changing your mind will not affect any other treatment, payment, health care, enrollment in health plans, or eligibility for benefits.

Consent to take Part in Research and Authorization for the Collection, Use, and Disclosure of Health Information

Signature of Subject

I have read (or someone has read to me) the above information. I have been given an opportunity to ask questions and my questions have been answered to my satisfaction. I agree to participate in this research and authorize the use of my health information as outlined above. I will be given a copy of this signed and dated form.

Signature of Subject	**Date**
Print Name of Subject	**Time of Consent Signing**

Statement of Person Obtaining Informed Consent and Research Authorization

I have carefully explained to the person taking part in the study what he or she can expect from their participation. I confirm that this research subject speaks the language that was used to explain this research and is receiving an informed consent form in their primary language. This research subject has provided legally effective informed consent.

Signature of Person Obtaining Consent *(must be the PI or delegated MD, NP, or PA)*	**Date (must be same as subject's)**

Printed Name of Person Obtaining Consent

22.2 Appendix B – Study Schemata

Visit Type	Screening	Baseline *(4)*	Emailed patient questionnaires	Safety Blood Draw	Phone Calls	Study Visit	Study Visit	Study Visit	Emailed patient questionnaires	Unscheduled
Clinic Visit Number	1	2		3		4	5	6		
Visit Date			Every 3 days for 6 wks after first dose of study drug and weekly for 6 wks thereafter	2 weeks after first dose of study drug	3 times after first dose of study drug	2 weeks after last dose of study drug	6 months after first dose of study drug	12 months from first dose of study drug	18 months after first dose of study drug	As needed for AEs/SAEs
Acceptable Visit Window		within 3 months of screening	± 1 day	± 2 days		± 10 days	± 4 weeks	± 4 weeks	± 4 weeks	N/A
Procedures & Assessments										
Informed Consent *(1)*	X									
Randomization (Placebo or Fisetin) *(5)*	X	X								
Medical/Surgical History & Demographics	X									
Physical Exam with Range of Motion of Study Knee	X					X	X	X		X
Vitals	X	X				X	X	X		X
BMI	X									
Record concomitant medications & supplements	X	X				X	X	X		X
Blood Draw *(6)*	X	X		X		X	X	X		X
Urine pregnancy test for WOCBP *(2)*	X	X								
PRO questionnaires			X			X	X	X	X	
Radiograph of Study Knee *(3)*	X									
Quantitative MRI		X					X	X		
Biomotion tests (functional performance, joint range of motion, muscle strength, video motion analysis)	X	X					X	X		
Dispense study drug		X								
Provide study drug diary		X								
Return study drug and diary						X				
Review and Assess AEs and SAEs				X	X	X	X	X		X

FOOTNOTES:

(1) Informed consent must be signed by the patient prior to ANY research-related procedures. The informed consent document can only be signed by the study PI and/or the treating clinician (MD/NP/PA) who is on the protocol.

(2) WOCBP = Women of Child Bearing Potential

(3) To confirm the presence of Kellgren-Lawrence grade II-IV OA.

(4) Procedures at Baseline may be performed at the Screening Visit after the Informed Consent Form (ICF) is signed.

(5) Randomization can occur at Screening or Baseline as long as the ICF has been signed and patient eligibility in the study is confirmed.

(6) If the Baseline visit occurs greater than 3 months after the Screening Visit, blood should be collected again for safety purposes.

22.3 Appendix C-Augmented Numerical Rating Scale

THE STEADMAN CLINIC
AND
STEADMAN PHILIPPON RESEARCH INSTITUTE

APPROVED

Clinical Trial #2019-16: Pain Questionnaire

Greetings from The Steadman Clinic!

This short pain questionnaire is part of the clinical trial that you are participating in at The Steadman Clinic.

We thank you very much for your time!

If you have missed an item, it will appear in RED. At the end of the , there is a submit button. Once you click the submit button, a new page with a "Thank You for Your Response" message will appear.

There is no need to print this form.

On a scale of 0 to 10 please describe your current level of knee pain:

Today: * ○ 0 - No Pain ○ 1 ○ 2 ○ 3 ○ 4 ○ 5 ○ 6 ○ 7 ○ 8 ○ 9 ○ 10 - Severe Pain

At its worst: * ○ 0 - No Pain ○ 1 ○ 2 ○ 3 ○ 4 ○ 5 ○ 6 ○ 7 ○ 8 ○ 9 ○ 10 - Severe Pain

Following activity: * ○ 0 - No Pain ○ 1 ○ 2 ○ 3 ○ 4 ○ 5 ○ 6 ○ 7 ○ 8 ○ 9 ○ 10 - Severe Pain

Please select the one response that best reflects your knee pain: *

○ None or you ignore it
○ Slight, occasional, no compromise in activity
○ Mild, no effect on average activities
○ Moderate pain, tolerable but concessions to pain are made.
○ Marked pain, serious limitations of activity
○ Totally disabled, crippled, pain in bed, bedridden

After 6 to 9 blocks (about 1 mile), please describe how you would walk: *

○ No limp ○ Slight limp ○ Moderate limp ○ Severe limp

How much trouble do you have with...

Stiffness in your knee? * ○ NONE ○ MILD ○ MODERATE ○ SEVERE ○ EXTREME

Decreased motion of your knee? * ○ NONE ○ MILD ○ MODERATE ○ SEVERE ○ EXTREME

How would you rate your current level of function? *

○ Normal ○ Nearly Normal ○ Abnormal ○ Severely Abnormal

22.4 Appendix D: Potential Drug Interactions with Fisetin

Fisetin has been demonstrated to have inhibitory activity of cytochrome P450

Fisetin has been demonstrated to have inhibitory (competitive mainly) activity of cytochrome P450 isozymes especially CYP2C9 and CYP3A4. CYP2C9 is an important cytochrome P450 isozyme with a major role in the oxidation of both xenobiotic and endogenous compounds. CYP2C9 makes up about 18% of the cytochrome P450 protein in liver microsomes (data only for antifungal). Some 100 therapeutic drugs are metabolized by CYP2C9, including drugs with a narrow therapeutic index such as warfarin and phenytoin and other routinely prescribed drugs such as acenocoumarol, tolbutamide, losartan, glipizide, and some nonsteroidal anti-inflammatory drugs. CYP3A4 is a critical hepatic "first-pass" metabolism enzyme for a litany of drugs including Fisetin which is estimated to be responsible for approximately 40-50% of Fisetin's bioavailability loss upon oral administration. As a competitive inhibitor of CYP3A4, Fisetin can thus significantly modulate the serum concentrations of other co-administered drugs that have narrow therapeutic ranges, potentially leading to adverse effects. Several classes of drugs are metabolized by cytochrome P450 isozymes. These again include anti-coagulants (i.e. warfarin or Acenocoumarol), diabetes drugs or gluco-regulatory drugs (i.e. Tolbutamide or Glucotrol), anti-seizure drugs (i.e. Phenytoin or Dilantin). To accommodate for this, we have constructed an exclusion criteria to mitigate these effects and provide a list of drugs known to interact with crucial CYPs that must be withheld for 2 days prior to Fisetin dosing and during Fisetin administration.