Official Title:	Colchicine for the Treatment of Osteoarthritis of the Knee (CLOAK) A Phase II, Randomized, Double-Blind, Placebo-Controlled, Single-Center Study of the Effects of Colchicine on Pain and Inflammation in Subjects with Knee Osteoarthritis.
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Colchicine for the Treatment of Osteoarthritis of the Knee (CLOAK)

A Phase II, Randomized, Double-Blind, Placebo-Controlled, Single-Center Study of the Effects of Colchicine on Pain and Inflammation in Subjects with Knee Osteoarthritis.

Regulatory Sponsor:	NYU Langone Health Division of Rheumatology, Department of Medicine 333 East 38th Street New York, NY 10016 646-501-7400
Funding Sponsor:	Rheumatology Research Foundation 2200 Lake Blvd., NE Atlanta, GA 30319 404-633-3777 Hikma Pharmaceuticals USA Inc. 246 Industrial Way West Eatontown, NJ 07724 Future manufacturing site of the drug product: Hikma Pharmaceuticals 1809 Wilson Road Columbus, OH 43228
Study Product:	Colchicine
Protocol Number:	s16-01796
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List of Abbreviations

AE:	Adverse Event
BMI:	Body Mass Index
CMC:	Center for Musculoskeletal Care
CPP:	Calcium Pyrophosphate
CPPD:	Calcium Pyrophosphate Deposition Disease
CRF:	Case Report Form
CV:	Cardiovascular
DMOADs:	Disease Modifying Osteoarthritis Drugs
DSMC:	Data Safety Monitoring Committee
eCRF:	Electronic Case Report Form
eGFR:	Estimated Glomerular Filtration Rate
FDA:	Food and Drug Administration
FMF:	Familial Mediterranean Fever
hsCRP:	Highly Sensitive C-Reactive Protein
IL-1Ra:	IL-1 Receptor Antagonist
IMD:	Intermargin Distance
IND:	Investigational New Drug Application
iNOS:	Nitric Oxide Synthetase
JNS:	Joint Space Narrowing
JSW:	Joint Space Width
KL:	Kellgren and Lawrence Scoring System
KOOS:	Knee injury and Osteoarthritis Outcome Score
MDRD Equation:	Modification of Diet in Renal Disease
MTP:	Medial Tibial Plateau
MSK-US:	Musculoskeletal Ultrasound
MSU:	Monosodium Urate
NIH:	National Institutes of Health
NO:	Nitric Oxide
NYC:	New York City
NSAIDs:	Non-Steroidal Anti-Inflammatory Drugs
OA:	Osteoarthritis
OARSI:	Osteoarthritis Research Society International
PA:	Posterior/Anterior
PBL:	Peripheral Blood Leukocyte
PI:	Principal Investigator
SAE:	Serious Adverse Event
SF36:	Short Form Health Survey
SKOA:	Symptomatic Knee Osteoarthritis
US:	United States
USA:	United States of America
VAS:	Visual Analog Scale
WOMAC:	Western Ontario and McMaster Universities Arthritis Index

Study Summary

Title	**Colchicine for the Treatment of Osteoarthritis of the Knee: A Phase II, Randomized, Double-Blind, Placebo-Controlled, Single-Center Study of the Effects of Colchicine on Pain and Inflammation in Subjects with Knee Osteoarthritis.**
Short Title	CLOAK
Protocol Number	s16-01796
Phase	Phase 2
Methodology	Double blind, randomized, placebo controlled
Study Duration	Estimated duration for the main protocol (e.g. from start of screening to last subject processed and finishing the study) – 36 months
Study Center(s)	Single-center
Objectives	To investigate whether colchicine has utility in the treatment of knee OA pain, and in the modulation of both blood-based and imaging biomarkers that may associate with symptomatic and structural progression of knee OA.
Number of Subjects	120
Diagnosis and Main Inclusion Criteria	Subjects must have symptomatic and radiographic knee OA
Study Product, Dose, Route, Regimen	Colchicine 0.8 mg or 0.6 mg orally once daily
Duration of administration	90 days
Reference therapy	Placebo in capsule identical to study drug
Statistical Methodology	The following will be used where appropriate: descriptive statistics, tests of normality of continuous outcome measurements, T-tests or Wilcoxon nonparametric counterpart, chi-square (Fisher exact) test to compare proportions of outcomes between groups, multivariate linear (logistic) regression methods with adjustment of potential confounding variables. When multiple time points are considered such as at baseline, 1, 2 and 3 months, we will model linear trajectories of outcome scores across time using mixed-effects regression models. The models will be specified with random intercepts and slopes such that a linear trajectory representing outcome scores will be compared between treatment groups.

1 Introduction

This document is a protocol for a human research study. This study is to be conducted in accordance with US government research regulations, and applicable international standards of Good Clinical Practice, and institutional research policies and procedures.

Our project seeks to investigate whether colchicine, an already FDA-approved treatment for gout, has utility in osteoarthritis (OA). OA is the most common arthritis, with more than 30 million people affected in the United States. OA can be painful, debilitating and may even shorten life expectancy. Unfortunately, approved drug treatments are only analgesic, and for many patients work poorly and/or are poorly tolerated (indeed, recent data suggests that acetaminophen, the first line therapy for OA, is essentially ineffective). There are no drugs approved in the US to actually slow down the progression of OA. OA processes are beginning to be better understood, and it is now appreciated that local inflammation is likely to play a role in OA joint pain, impairment of joint function, and the health of joint cells including cartilage cells (chondrocytes) and synovial cells, whose proper function is essential to maintaining joint health. We and others have reported that local OA inflammation can actually be detectible systemically through biomarkers in the blood of some OA patients, and that in those patients OA may progress faster. In this project we propose to test an anti-inflammatory drug known as colchicine, which has been in use for more than 2,000 years for gout and other inflammatory disorders. At low doses colchicine is well tolerated, and preliminary evidence suggests that colchicine 1) targets some of the processes now appreciated to play a role in OA, and 2) may have a positive impact on OA pain. We will enroll patients with knee OA, already accessible to us from a previous study (Leukocyte Gene Expression in Osteoarthritis, LGEO), along with information collected from that study, and treat them with colchicine or placebo daily. In addition to measuring impact on pain and function, we will measure colchicine's impact on markers of inflammation, in both the blood and the joint fluid (including uric acid levels as a novel biomarker of serum and synovial fluid). We will use imaging to assess the impact of colchicine on inflammatory synovitis within the joints. If successful, our studies could identify a new (old!) drug as a potential treatment for OA, and perhaps as a potential agent than can actually alter OA biology. If colchicine is effective on some but not all outcomes, that will still be a potential major treatment advance. Even if colchicine does not improve OA status, the intensive assessment that is intrinsic to the trial will extend our prior, NIH-funded study of OA natural history, and provide important insights into OA biomarkers and progression.

1.1 Background

Osteoarthritis (OA): Scope of the Problem: OA affects more than 30 million adults in the USA (1, 2), yet disease modifying OA drugs (DMOADs) are currently lacking. Few randomized controlled trials have assessed the effect of medications on both symptom and structure modification, based upon proposed OA pathologic mechanisms, and none have shown unequivocal success.

OA as an Inflammatory Disease: We and others have shown that OA is locally, and perhaps systemically inflammatory (3-7). Synovitis is present (7,8), and cytokines including IL-1β and TNF-α can be detected in the synovial fluid and peripheral blood of patients with OA joint degeneration (4,9). Furthermore, our analyses of peripheral blood leukocyte (PBL) gene expression profiles have identified a subset of symptomatic knee OA (SKOA) patients, whose increased expression of mRNA for IL-1β, TNF-α, and COX-2 is associated with increased pain and risk for radiographic progression at 24 months (**Tables 1 and 2**)(4). We have also shown in multiple cohorts that the pro-inflammatory lipids PGE2 and 15-HETE

Table 1. Symptomatic knee OA patients with elevated PBL IL-1β expression (OAIL-1) exhibit elevated baseline WOMAC and visual analog scale (VAS) pain scores compared to patients with normal PBL IL-1β expression (OAnl). IL-1β expression was dichotomized based on median levels to define two groups of subjects (e.g., with biomarker above vs. at or below the threshold); mean pain score values were compared in the two subject groups using a two-sample t-test. * P values significant at 5% alpha level are shown in bold type.

Measure		Mean (standard deviation) OA^{IL-1} (n=78)	OA^{nl} (n=101)	*p value*
WOMAC at baseline	Sum pain	40.46 (24.98)	33.09 (21.32)	**0.0359***
	Total score	127.99 (72.25)	104.47 (61.92)	**0.0209***
VAS Pain	Baseline	48.45 (28.47)	39.58 (28.09)	**0.0396***

are detectable in the plasma of ~40% of SKOA patients, and are similarly associated with OA disease severity (10). Most recently, we found that plasma levels of IL-1Ra (IL-1 receptor antagonist) are independently associated with SKOA severity and progression (11). Taken together, these findings suggest that there is chronic low-grade inflammation in SKOA, and that at least some soluble and cellular markers of OA joint inflammation can be detected in peripheral blood. It is not surprising, then, that certain anti-inflammatory strategies (e.g., steroid injections, NSAIDs) have some capacity for ameliorating OA symptoms, although these agents do not appear to affect OA structural outcomes (12). Given the prevalence, pain and disability of OA, and the current lack of adequate treatments, the possibility that other anti-inflammatory strategies might prove equally or ideally more effective for ameliorating OA symptoms, and certainly, the possibility that the "right" anti-inflammatory strategies might have disease modifying activities, deserves serious consideration.

	IL-1β (PBL mRNA)			TNFα (PBL mRNA)			COX-2 (PBL mRNA)		
	OA^{IL-1} (n=54)	OA^{nl} (n=55)	*p* value	$OA^{TNF\alpha}$ (n=54)	OA^{nl} (n=55)	*p* value	OA^{COX-2} (n=54)	OA^{nl} (n=55)	*p* value
Baseline Medial knee JSW (mm)	3.85 (1.39)	3.46 (1.29)	0.131	3.60 (1.28)	3.70 (1.45)	0.706	3.76 (1.43)	3.55 (1.27)	0.418
24 month Medial knee JSW (mm)	3.14 (1.58)	3.12 (1.49)	0.933	2.85 (1.58)	3.41 (1.43)	0.057	3.05 (1.57)	3.21 (1.49)	0.571
JSN (mm)	0.71 (0.93)	0.34 (0.82)	**0.032***	0.75 (1.03)	0.30 (0.67)	**0.007***	0.71 (0.90)	0.34 (0.86)	**0.027***

Table 2. Association of PBL transcriptome IL-1β, TNFα and COX-2 (relative gene expression levels were dichotomized by median), with joint space narrowing (JSN) at 24 months in 109 patients with symptomatic knee osteoarthritis in the medial compartment for whom gene expression data were available. Biomarkers were dichotomized based on median levels to define two groups of subjects (e.g., with biomarker above vs. at or below the threshold); mean JSN values were compared in the two subject groups using a two-sample t-test. *p-values significant at 5% alpha level are shown in bold.

A Role for Crystals in OA? Clinicians have long assumed an association between crystal arthropathies and OA presence, but investigations assessing a clinical, or indeed a pathogenic relationship between crystals and OA are few and far between. Crystal-induced mechanical cartilage damage could theoretically stimulate OA progression; in this regard it is relevant that OA and gout or chondrocalcinosis frequently co-occur (13). Muehlemann *et al* investigated the prevalence of monosodium urate (MSU) and calcium pyrophosphate (CPP) crystals in 7855 cadaveric tali. The presence of crystals was strongly associated with cartilage lesions that seemed to be biomechanically induced. Strikingly, however, only 34% of crystals detected were CPP; the remaining 66% composed of MSU, suggesting a role for MSU crystals even in the absence of clinical gout (14). Indeed, in ultrasound studies from our group and others, MSU crystals on cartilage can be detected in a significant percentage of patients with asymptomatic hyperuricemia, and even occasionally in patients with neither hyperuricemia nor gout (15). In addition to biomechanically-induced cartilage damage leading to OA, MSU and other crystals might act biochemically, biophysically or via receptor (e.g., Toll-like Receptor 4) interactions to induce chondrocyte and/or synoviocyte activation, leading to OA. In gout and pseudogout, the concept of MSU crystals mediating joint inflammation is well established. In particular, MSU (and CPP) crystals promote the release of IL-1β and IL-18 via activation of the NLRP3 inflammasome (16). Despite the role of IL-1β in OA, it remains uncertain whether the IL-1β /inflammasome pathway contributes to the development of OA in joints affected by MSU-triggered inflammation. Supporting such a model, synovial fluid urate concentrations have recently been shown to correlate with synovial fluid IL-1β and IL-18, and with radiographic severity of knee OA (9), leading the investigators to propose that chondrocyte necrosis in early OA results in the release of urate as a "danger signal," promoting IL-1β generation, additional inflammation, and further chondrocyte death. Other mechanisms could also play a role in the contribution of MSU deposition to OA. For example, Liu *et al* have demonstrated that MSU crystals can directly (i.e., independent of IL-1β) up-regulate inducible nitric oxide synthetase (iNOS), nitric oxide (NO) release and MMP-3 secretion, all mediators with potential roles in OA pathogenesis (17,18).

Our group has conducted a prospective pilot study to compare the prevalence of knee OA between gout, asymptomatic hyperuricemic and control subjects (19). Using standardized radiographs to assess for knee OA, and musculoskeletal ultrasound to assess for occult MSU deposition, we found a markedly increased prevalence and severity of knee OA in gout patients vs. non-hyperuricemic controls, and an intermediate prevalence and severity of knee OA in asymptomatic hyperuricemia patients (**Figure 2**). Moreover, patients with MSU cartilage deposition on ultrasound were more likely to have knee OA than those without, regardless of gout versus hyperuricemic status. Interestingly, MSU deposition conveyed risk for knee OA even when the deposition was in OA-unaffected joints, suggesting the possibility of a metabolic or systemic inflammatory effect.

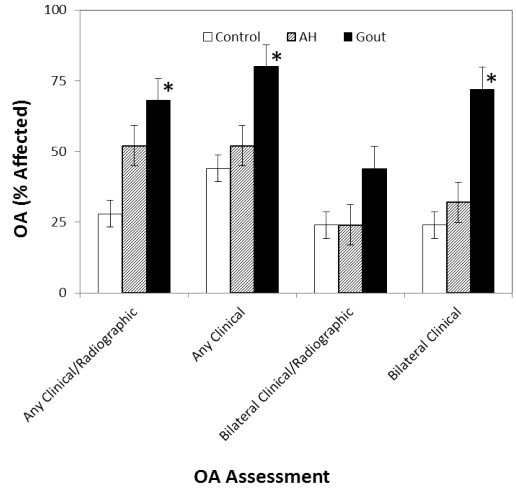


Figure 2. Prevalence of knee OA, and bilateral knee OA, among control, AH and gout groups. Subjects were assessed for presence of knee OA and/or bilateral knee OA using either ACR Clinical/Radiographic or ACR Clinical OA criteria, as indicated (*P<0.05 vs control group).

More recently**,** we have taken the opposite approach, reviewing a group of patients with known medial knee OA for whom we had available standing knee X-rays at baseline and after two years. Using banked serum, we assessed patient baseline urate values and compared them with the degree of progression. Consistent with an interaction between urate and OA, we observed a direct correlation between baseline urate values and the degree of joint space narrowing (JSN) over the observation period (**Figure 3**).

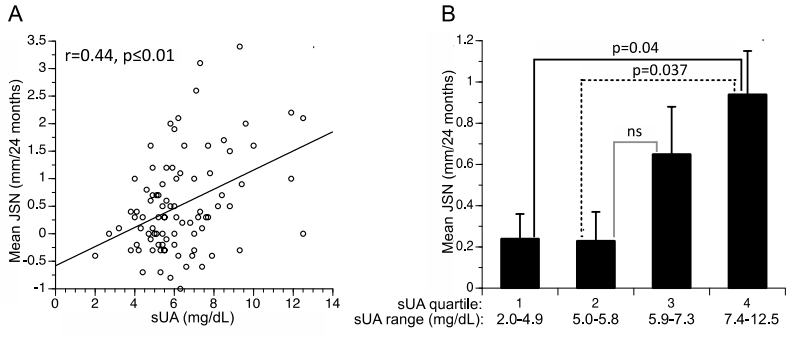
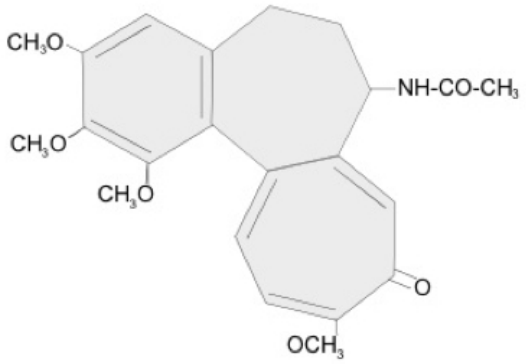


Figure 3. Serum urate correlates with 24 month joint space narrowing (JSN) in patients with established OA. **A**, Scatter plot. **B**, Serum urate arranged according to quartiles.

1.2 Investigational Agent

Colchicine, most commonly used to treat gout and calcium crystal-induced inflammation, is a generally safe and well-tolerated drug, with more than two thousand years of physician experience (20). Colchicine is a tricyclic, lipid-soluble alkaloid with a long terminal half-life (20 to 40 hours) and bioavailability ranging from 24% to 88% (**Figure 4**). Within the bloodstream, ~40% of colchicine binds to albumin. Although peak plasma concentrations occur 1 hour after administration, maximal anti-inflammatory effects develop over 24 to 48 hours, based on leukocyte accumulation. Colchicine reaches much higher concentrations in leukocytes than in plasma, and persists there for several days after ingestion, with concentrations ranging from 4 to 64 ng/10^9. Colchicine uptake into other cells has not been as rigorously defined, but evidence suggests that the drug also affects monocyte/macrophages and synovial fibroblasts. Whether colchicine directly affects chondrocytes has not been determined.

Figure 4 *Molecular Structure of Colchicine*



For this study we will be employing a non-standard dose of Colchicine, specifically 0.81 mg daily, compared to the FDA approved 0.6 mg dose. The doses are between the minimum and maximum recommended daily dose of Colchicine (0.6-1.2mg). For this study, Hikma Pharmaceuticals is formulating the 0.81 mg dose with the same capsule used for the standard daily dose 0.6 mg. The formulation of that capsule can be found in Table 3.

Table 3 Dose and Exposure

– Formulation and dose:

Ingredient	**Mg/cap**
Colchicine, USP	0.6 mg or 0.81mg[1]
Microcrystalline Cellulose, NF (Avicel pH 101)	5.65mg
Anhydrous lactose, NF (Tablet Grade)	101.77mg[2]
Sodium Starch Glycolate, NF (Primojel)	3.4mg
Colloidal Silicon Dioxide, NF (Cab-o-sil)	0.56mg
Magnesium Stearate, NF	1.13mg
#4 Med Orange/Lt. Blue Hard Gelatin Capsules printed "Hikma Pharmaceuticals119" in white ink	(40mg)
Total Weight (Target Filled Capsule)	153.32mg

[1]Equivalent to 0.808mg/cap (1% excess based on Anhydrous, Solvent Free Basis, adjusted for assay, moisture content, and ethyl acetate.
[2]Adjusted to make Total 113.32mg/cap

– Route of Administration: Oral
– Planned exposure (90 days)

1.3 Preclinical Data

Colchicine inhibits microtubules and adhesion molecules in inflammatory cells, abrogating multiple processes that contribute to the production of catabolic mediators in the joint (21, 22). Colchicine inhibits neutrophil chemotaxis and lysosomal enzyme release (23). Colchicine modulates E- and L-selectin distribution and expression, impeding leukocyte-endothelial cell interactions (22). More recently it has been shown that colchicine also inhibits caspase-1 in the monocyte/macrophage NLRP3 inflammasome, thus blocking the conversion of pro-IL-1β to active IL-1β, mitigating the release of IL-1β and, secondarily, of TNFα and other inflammatory mediators that are also implicated in OA (16) (**Figure 5**).

Colchicine is most commonly used to treat acute gouty flares, and as prophylaxis for gouty flares as well as for prophylaxis and acute treatment of calcium pyrophosphate crystal arthritis (i.e., pseudogout). It also

used in familial Mediterranean fever (FMF), some types of vasculitides and neutrophilic dermatoses, pericarditis, and more recently has been studied in coronary artery disease and atrial fibrillation.

The apparent association between crystals and OA lends biologic plausibility to the possibility that colchicine, by diminishing inflammation, could be an effective treatment for OA. **Figure 5** displays how colchicine's mechanisms of action target several processes implicated in OA. Importantly, colchicine warrants study even if crystals are not a driving factor in OA pathogenesis, given its ability to inhibit the production of cytokines and inflammatory markers that are known to be associated with OA joint pain and degeneration. Critical to this study, our group has had significant research expertise in studying inflammation and colchicine for many years. For example, in patients with gout, we are currently studying whether chronic colchicine use is associated with reduced rates of acute cardiovascular (CV) syndromes (24). We are also collaborating with our interventional cardiology colleagues to test whether cardiac patients who are given colchicine prior to percutaneous CV intervention experience reduced rates of post-procedure inflammation (a marker of poorer long-term outcomes), compared with patients who receive placebo.

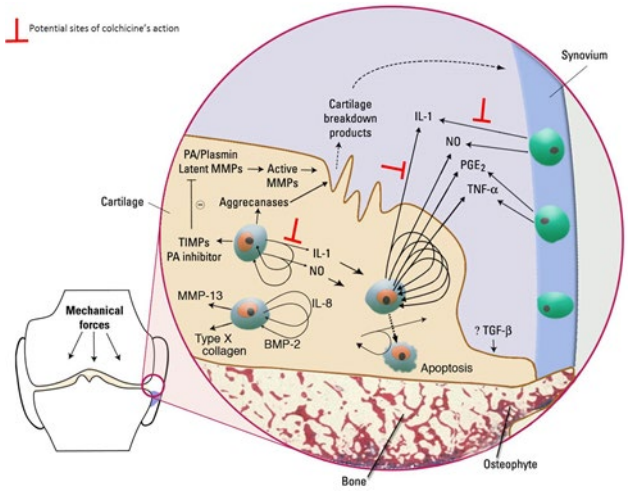


Figure 5. Inflammatory processes that contribute to OA pathogenesis and colchicine's potential therapeutic targets therein.

1.4 Clinical Data to Date

Colchicine is FDA-approved, in doses of 0.6 mgs once or twice daily, for long-term use in gout prophylaxis. In familial Mediterranean fever, colchicine is used at doses up to 1.8 mgs daily in divided doses, essentially for a lifetime. To date, three small, randomized controlled trials have assessed the potential for a symptom-modifying effect of colchicine in knee OA (25,26,27). Aran et al examined physician and patient global assessment endpoints, using non-validated 15-point VAS scales, in 61 female patients, and found that patients receiving colchicine had improved assessment scores and approximately a 50% decrease in the use of breakthrough pain medication (acetaminophen) compared to placebo controls (25). Das et al studied the addition of colchicine to nimesulide (an NSAID) in 36 patients, and found significantly improved total WOMAC and VAS pain scores in the colchicine vs placebo groups (26). Das also examined the addition of colchicine to intra-articular steroids and piroxicam in 39 patients with clinical evidence of "inflammation" of the knee, of whom 38% had radiographic chondrocalcinosis and 74% had CPPD crystals on polarizing microscopy (27). They found a greater reduction in 15-point VAS pain and greater improvement in a modified WOMAC scale, in the group who received colchicine vs placebo.

Limitations in the designs of the aforementioned studies, as well as the brief time frames of their study periods, underline the need for investigations incorporating rigorous methodology and validated outcome measures. Moreover, these prior studies did not address the cellular, inflammatory or structural impact of colchicine treatment. The possibility that a well-established, relatively safe, and FDA-approved drug might improve the management of a currently untreatable disease lends further urgency to the question. We therefore propose a double-blind, randomized controlled trial, to determine whether colchicine treatment can:

- improve pain, function, and quality of life in SKOA patients
- improve inflammatory PBL, plasma, and synovial fluid markers in SKOA patients
- reduce OA synovitis and/or effusion, determined by musculoskeletal ultrasound (MSK-US); we will also use MSK-US to correlate the presence of crystals observed on cartilage both with biomarker inflammation and OA response to colchicine

To accomplish this, we will employ a well-established SKOA cohort, whom we have been following in natural history studies since 2005, and for whom we already possess baseline clinical, radiographic, ultrasound, genetic, PBL genomic and plasma/serum biomarker data.

1.5 Dose Rationale

Subjects will be randomized to take colchicine 0.6mg, 0.8mg, or placebo daily. In gout practice, an option to use colchicine 0.6mg (U.S.) or 0.5mg (internationally) once or twice daily is recommended for prophylaxis of gout flares. For acute flares, a dosing regimen of 1.2 mg followed by 0.6mg 1 hour later showed no greater gastrointestinal adverse events than placebo (Terkeltaub A&R 2010). For our study, a new, once-daily dose of colchicine 0.8mg is being formulated. This dose was chosen because we expect it to be 1) almost universally tolerated, 2) likely to have a higher compliance rate than twice-daily dosing, 3) less likely than twice daily dosing to require dose adjustment for co-morbidities or concomitant medications and 4) potentially more efficacious than a once-daily 0.6mg dose. However, because recent studies suggest that a dose of 0.6mg may be sufficient for chronic inflammatory suppression and may reduce the already low risk of toxicity of 0.8mg, for the remainder (second half) of the study we will switch from the 0.8mg to a 0.6mg dose to allow comparison of the two doses (36).

1.6 Research Risks & Benefits

1.6.1 Risks of Study Drug

As a bioactive substance, colchicine is generally well tolerated within the dose range being studied. However, some patients may experience upset stomach, nausea/vomiting and/or loose stools. Colchicine can induce bone marrow suppression and a neuromyopathy, mostly after overdose; such occurrences would be extremely rare at the doses and duration being studied, particularly since we will exclude individuals whose metabolism of colchicine may be altered by liver disease or medication use. *See Appendices I (PI – Package Insert) and II (MSDS-Material Data Safety Sheets).*

1.6.2 Other Risks of Study Participation

Blood Drawing
Risks of venipuncture include bruising, pain at site of phlebotomy, fainting, and rarely, infection.

Ultrasound
The ultrasound assessment does not pose any adverse risks, dangers or radiation exposure. It is a standard procedure performed by trained MDs frequently employed during arthritis care to assess for signs of inflammation and structural abnormalities in and around the knee. The ultrasound probe is placed onto the skin and mild pressure is applied using a lubricating gel in between to improve the image quality on the screen. The pressure might be uncomfortable, but it should not cause any bruising, bleeding, or injury.

For evaluating the knees by ultrasound, there is no gold standard method in the literature.
Thus we will capture 5 semiquantitative scores on a scale of 0-3 (see below) for osteoarthritis, and 3 yes/no questions about crystal disease.

1) Size of effusion in the anterior suprapatellar space in the longitudinal view, anechoic and compressible, on a scale of 0-3 (by millimeter cutoffs)
2) Degree of synovial hypertrophy (by gray scale) in the anterior suprapatellar space in the longitudinal view, hypoechoic and NOT compressible, on a scale of 0-3
3) Degree of synovitis by Doppler signal in the anterior suprapatellar space in the longitudinal view, on a scale of 0-3 (none, scattered, less than 50%, greater than 50% of the area)
4) Size of osteophytes in the medial or lateral joint lines (whichever more severe) in the longitudinal view, hyperechoic, on a scale of 0-3

5) Degree of femoral articular cartilage (FAC) wear in the suprapatellar transverse view with knee flexed, on a scale of 0-3
6) FAC double contour sign (hyperechoic line across top of anechoic FAC) in the suprapatellar transverse view with knee flexed: yes/no
7) FAC evidence of CPPD (hyperechoic deposits in the middle of the anechoic FAC) in the suprapatellar transverse view with knee flexed: yes/no
8) meniscal evidence of CPPD (hyperechoic deposits in the triangular meniscus edges) in the medial and/or latera joint line: yes/no

Knee Radiographs

As an entry criterion, bilateral standardized knee radiographs showing the presence of a Kellgren-Lawrence grade 2 or 3 osteoarthritis in at least one knee within the past year will be required. The knee radiograph will need to be in the NYU Langone system. Any patient who is being considered for entry into the study and has not had X-rays within the past year will be expected to undergo standardized X-ray studies for confirmation during the screening visit. The X-ray will need to be read by the study radiologist. If the patient already has an existing knee radiograph that was conducted at NYU Langone, then this X-ray will need to be read by the study radiologist. There is minimum radiation exposure associated with this procedure.

Knee Arthrocentesis for Synovial Fluid Sample

Arthrocentesis involves the use of a needle to remove a quantity of fluid from the joint, in this case the knee, if swollen. It is a standard procedure performed by trained MDs frequently employed during arthritis care to assess inflammation, reduce pain and improve function. The most likely risks are temporary local pain during the procedure, and occasional bruising. To mitigate pain to the greatest extent possible we will employ local anesthesia (anesthetic topical spray followed by a soft-tissue lidocaine injection), and perform the procedure under ultrasound guidance, which typically shortens the procedure time. Patients with larger effusions may derive pain relief from the volume decompression of the procedure. The most concerning risk of arthrocentesis is the potential for introducing an infection from the skin into the joint. In general practice this risk appears to be extremely low based on available data, between 1 in 1,000 and 1 in 10,000 procedures. We will minimize this risk by 1) using meticulous aseptic technique; 2) only attempting to aspirate synovial fluid from patients with ultrasound confirmation of an effusion; 3) using ultrasound guidance for the procedure to reduce the need for blind exploration with the needle to reach the effusion pocket. Knee Arthrocentesis will be performed at the screening visit and three month visit for the subset that qualifies and consents to this additional study procedure. This procedure may be performed in one or both knees depending on ultrasound findings.

Bilateral Physical Knee Exam

This assessment does not pose any adverse risks or dangers. It is a standard exam performed by trained MDs frequently employed during arthritis care to assess inflammation, pain, tenderness, and function. It will be performed by a study physician. Participants will be asked to stand during this assessment and to bend their knees. This study procedure will be performed at the screening visit and the three month visit.

Risk to Privacy and Confidentiality

As with all research studies, the risk of participating in this study involves a loss of privacy and confidentiality. However, the study team and study doctors will assign each subject with a four-digit screening number in sequential order of the screening visit date. All participants who are eligible for this study after the screening visit will be assigned a five digit subject ID number at baseline in sequential order of the baseline visit date. These numbers will be put on the subject's questionnaires and samples so their names and identifying information will not be disclosed. Only the PI, Dr. Michael Pillinger, and his study team will have the information that links the name and study number. They will keep that information in a secured location accessible only to select research staff. All digital data collected in the study will be maintained in a secure REDCap file, which is password–protected.

1.6.3 Potential benefits

Subjects participating in this research may potentially benefit from improved pain, function and quality of life as a result of the study medication. However, it is unknown whether or not the study drug will help subjects with osteoarthritis. Indirectly, information from subjects in this study may eventually help other avoid the consequences of OA and its co-morbidities in the future.

2 Study Objectives

Purpose: OA is a common, painful, and debilitating form of arthritis for which no disease modifying therapy currently exists. Studies from our group and others indicate that OA is accompanied by occult inflammation, suggesting that inflammation may contribute to disease symptoms/progression. Colchicine is an anti-inflammatory agent used commonly for gout. Colchicine mechanisms of action target several processes implicated in OA, and small preliminary studies suggest that colchicine may ameliorate OA knee pain. We therefore propose a double-blind, randomized, placebo-controlled trial to test whether colchicine treatment of symptomatic knee OA (SKOA) patients will lower pain scores and improve physical function. We hypothesize that clinical improvement will be associated with reduction of inflammatory candidate genomic, plasma and synovial fluid biomarkers, and with improved imaging outcomes. We will recruit subjects from a well-established SKOA cohort, Leukocyte Gene Expression in Osteoarthritis (LGEO), whom we followed in a prospective natural history study beginning in 2005 (NIH R01 AR052873, PI Abramson), and who have completed that observational study. To the fullest extent possible, our interventional study will employ as entry criteria assessments and outcome measures used in the parent observational study, including clinical, radiographic, ultrasound, peripheral blood genomic, and plasma/serum biomarker results for these subjects.

Objective 1: To test whether daily colchicine treatment for three months improves clinical outcomes in SKOA. Patients will be assessed for clinical outcomes (i.e., pain, physical function) using VAS (Visual Analog Scale) for pain and KOOS (Knee Injury and Osteoarthritis Outcome Score) instruments. Pain medication use will be evaluated at each assessment time.

The ***primary clinical outcome*** will be the difference in mean changes of VAS pain scores between treatment groups at 3 months. The VAS scale asks patients to rate the pain that they've had in each knee during the past 30 days on a scale of 0-10, where *"0" means "No pain" and "10" means "Pain as bad as you can imagine."*

Secondary clinical outcomes will include:

- mean changes and absolute differences in the Knee Injury and Osteoarthritis Outcome Score (KOOS) for pain between and within the groups at 3 months.
- mean changes and absolute differences of KOOS stiffness, physical function and total KOOS scores between and within the groups at 3 months.
- mean absolute doses and changes in dosage of acetaminophen or other medications used for pain between and within the groups at baseline and 3 months.

Objective 2: To test whether colchicine suppresses peripheral blood leukocyte (PBL), plasma and synovial fluid OA biomarkers of inflammation (Abramson Lab).

PBL Genomic Studies. PBL will be isolated from all subjects at baseline (or end of parent study if within 3 months) and 3 months. qPCR will be performed on PBL RNA (buffy coat PBMC).The qPCR panel will be based on our preliminary findings, likely including IL-1β, TNF-α, IL-1Ra and COX-2. qPCR data will be pre-processed for quality control, including correction of off-scale measurement and normalization. To obtain comparable results across assays, we will use the comparative C_T method ($2^{-\Delta\Delta Ct}$ method) as our normalization strategy. Logarithmic transformation will be performed on gene expression data if not normally distributed. We will use the qPCR library (http://www.dr-spiess.de/qpcR.html) within Bioconductor 2.6.

Plasma protein/lipid biomarker specimens will be collected from all subjects at baseline and 3 months, including IL-1Ra, PGE_2, 15-HETE, highly sensitive C-reactive protein (hsCRP), MMP-1, MMP-3, MMP-9 and urate. MMP-1, 3 and 9 levels will be assayed via multiplex ELISA (#K15034C-1), and hsCRP using #K151STD-1, (Meso Scale Diagnostics). IL-1Ra will be evaluated using #DRA00B from R & D systems. PGE_2 and 15-HETE will be analyzed using the ACE competitive EIA kit (Cat no:514010; Cayman Chemical, MI). IL-1β and TNF-α soluble protein levels will not be assessed, as we have previously shown that these levels do not accurately reflect knee OA severity owing to low levels and measurement limitations.

Synovial Fluid (SF) Studies (Optional). Subjects will undergo arthrocentesis during their screening and three month visits if effusion is detected on ultrasound and they consent to participate in these substudies. Thus, we expect to collect SF from a potentially more "inflammatory" subset of SKOA subjects. SF markers, measured by ELISA in the laboratory of our collaborator Dr. Mukundan Attur, will include IL-1β, TNF-α, IL-1Ra, IL-18, MMP-3, MCP-1 and urate. The SF studies are optional and will be exploratory to comprise only part of a sub-aim of the study.
Only coded samples will be sent to Dr. Attur. He will not have access to the key which links the subject identity to the subject code for the sample. Dr. Attur's Immunology laboratory will do the synovial fluid, blood, and plasma studies.

Objective 2a: *We will compare absolute levels and mean changes in PBL gene expression levels for IL-1β, TNF-α, IL-1Ra, and COX-2* between baseline and 3 months in colchicine and placebo groups; we will also examine whether levels of individual biomarkers significantly change between baseline and 3 months within each group.

Objective 2*b*: *We will compare absolute levels and mean changes in plasma levels of IL-1Ra, PGE_2, 15-HETE, MMP-1, MMP-3, MMP-9 and CRP* between baseline and 3 months in colchicine and placebo groups; we will also examine whether levels of individual biomarkers significantly change between baseline and 3 months within each group.

Objective 2c: *We will compare absolute levels and mean changes in SF IL-1β, TNF-α, IL-1Ra, IL-18, MCP-1, MMP-3, and urate* between baseline and 3 months, in colchicine and placebo groups; we will also examine whether levels of individual biomarkers significantly change between baseline and 3 months within each group. We will additionally examine associations of SF biomarkers with their peripheral blood correlates (e.g. SF IL-1β with PBL IL-1β gene expression) at each time point measured

Aim 3: To determine the impact of three months of daily colchicine on musculoskeletal ultrasound (MSK-US) signs of synovitis and effusion:

MSK-US: We will assess for urate (as double-contour sign) and calcium pyrophosphate crystal (CPP; as chondrocalcinosis) deposition as well as degenerative change in the articular cartilage, CPPD in the menisci, effusions, osteophytes and synovitis, using standardized ultrasound methodology as well as specific approaches for synovitis. Jonathan Samuels, MD, or team physicians trained by Dr. Samuels, will scan the longitudinal suprapatellar, flexed transverse suprapatellar, medial and lateral views of the knee, using a frequency of 18 MHz (MyLab25 Gold, Biosound Esaote, Indianapolis Indiana). Subjects will undergo arthrocentesis during their screening and three month visits if effusion is detected on ultrasound and they consent to participate in these substudies.

Objective 3a*. We will compare changes in synovitis and effusion between colchicine and placebo groups*. Mean changes in MSK-US-measured synovitis and effusion at 3 months will be compared between the two groups, as well as within each group. We hypothesize that, consistent with our prior observations, OA patients will frequently demonstrate synovitis; and that synovitis will improve after colchicine but not placebo treatment.

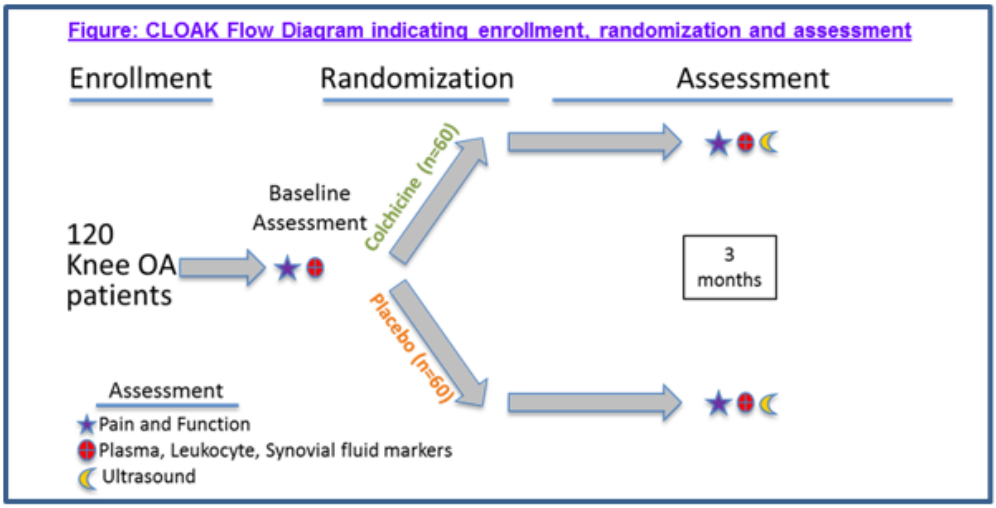
Objective 3b*. We will compare rates of crystal deposition* (urate, calcium) on MSK-US between colchicine and placebo groups over three months, and ***assess the relationship between crystal deposition, OA severity, levels of inflammation and response to colchicine***. We will examine the

association of crystal deposition and OA severity by KL grade and OARSI atlas measures. We will also examine whether crystal deposition associates with PBL, plasma, and especially SF inflammatory mediators. We hypothesize that some OA patients will have previously unappreciated crystal deposition, that may correlate with indices of pain and inflammation. We will determine whether colchicine treatment alters any associations found

3 Study Design

3.1 General Design

This Phase 2, prospective, double-blind, placebo-controlled, randomized trial will enroll 120 SKOA subjects through the Peter D. Seligman Center for Advanced Therapeutics which is located the at the NYU Langone Orthopedic Center (333 East 38th Street, Fourth Floor), New York, New York 10016. Patients meeting entry criteria will be randomized 1:1 to treatment with colchicine or placebo daily for three months. Subjects will have detailed evaluation of standardized clinical pain outcomes, candidate peripheral blood biomarkers, baseline knee radiographs as well as MSK-US, and a subset will undergo evaluation of their synovial fluid.



3.2 Primary Study Endpoints

Outcomes Measured: Clinical assessments for symptoms and medication use will be made for each study visit (according to Table of Study Visits/Procedures. Biomarker blood samples will be obtained at baseline and 3 months. MSK-US and synovial fluid will be measured on a subset of the cohort (described below) at screening and 3 months.

The ***primary outcome*** will be the difference in mean changes of VAS knee pain scores (as average daily pain) between colchicine treatment (both doses) and placebo groups at 3 months.

3.3 Secondary Study Endpoints

Secondary outcomes will include:

- Mean changes and absolute differences of KOOS pain scores between and within the groups at 3 months.
- Mean changes and absolute differences of KOOS stiffness, physical function and total KOOS scores between and within the groups at three months. We will also collect pain/stiffness data in a subject diary [Appendix I] on a daily basis.

- Mean absolute doses and changes in dosage of acetaminophen or other medications used for pain between and within the groups at baseline and three months (using a subject diary).
- Mean changes and absolute differences of peripheral blood leukocyte (e.g. IL-1β, TNF-α, IL-1Ra and COX-2), plasma (e.g. IL-1Ra, PGE2, 15-HETE, highly sensitive C-reactive protein (hsCRP), MMP-1, MMP-3, MMP-9 and uric acid) and synovial fluid (e.g.lL-1β, TNF-α, IL-1Ra, IL-18, MCP-1, MMP-3, and urate) OA biomarkers of inflammation between and within the groups at 3 months.
- Mean changes in MSK-US-measured synovitis and effusion at 3 months will be compared between the two groups, as well as within each group.
- Assessment of rates of crystal deposition (urate, calcium) on MSK-US between colchicine and placebo groups over 3 months, and assessment of the relationship between crystal deposition, OA severity, levels of inflammation and response to colchicine.
- Differences in all the above outcomes between colchicine users in the 0.8mg vs 0.6mg groups.

3.4 Primary Safety Endpoints

Primary safety endpoints will include assessment, as clinically indicated, for the most common adverse reactions known to occur with colchicine, including the following symptoms and signs:
Abdominal cramping, abdominal pain, diarrhea, nausea, vomiting

Although not expected, we will also assess for secondary safety endpoints, including:

- Neurological: weakness or numbness
- Dermatological: hair loss or rash
- Musculoskeletal: muscle weakness or pain

4 Subject Selection and Withdrawal

4.1 Inclusion Criteria

1. Capable of giving informed consent in English.
2. 40 years of age or older.
3. Have a clinical diagnosis of knee OA as per ACR criteria.
4. Have frequent knee symptoms defined as pain, aching, or stiffness in or around one or both knees on most days (i.e. more than half) for at least one month in the past 12 months.
5. VAS pain greater than or equal to 30mm in at least one knee in the month prior to the screening visit.
6. Have KL grade of 2 or 3 demonstrated on knee radiograph, in at least one painful knee.
7. Have an estimated glomerular filtration rate (eGFR) > 30 ml/min (MDRD equation) and liver transaminases < 2x the upper limit of normal.
8. Have a BMI ≤ 35kg/m^2 at the time of enrollment.
9. Agree to refrain from taking oral or topical non-steroidal anti-inflammatory medications (NSAIDs) and intra-articular therapies to the knees for the duration of the study. Low doses of aspirin (≤325mg) that do not act in an NSAID capacity, such as aspirin taken for cardiovascular conditions, are acceptable to continue taking during the study.
10. Agree to refrain from starting any new pain medications (with the exception of acetaminophen), including supplements, acupuncture, physical therapy or other non-pharmacologic modalities for the duration of the study.

11. Agree to refrain from changing the dose or frequency of any pain medication regimen (with the exception of acetaminophen), acupuncture, physical therapy or other nonpharmacologic modalities you are currently taking or participating in for the duration of the study.
12. Be able to take colchicine or placebo daily for three months.
13. Females of childbearing potential must use effective contraceptive protection (e.g., birth control pills, intrauterine device, barrier method) during the treatment period and 30 days after the last dose of study drug.
14. Male study participants who have female partners of child-bearing potential are required to be on effective contraceptive protection (e.g., birth control pills, intrauterine device, barrier method) with their female partners during treatment period and for 90 days after the last dose of the study drug.

4.2 Exclusion Criteria

1. Have received intra-articular therapies (hyaluronic acid,corticosteroid steroid injection, Platelet Rich Plasma, stem cell injections, etc.) within the past three months prior to the screening visit.
2. Have a known diagnosis of gout/pseudogout (acute calcium pyrophosphate arthropathy) or other inflammatory arthritis (rheumatoid arthritis, psoriatic arthritis, reactive arthritis, spondyloarthropathy etc). Patients with radiographic chondrocalcinosis without clinical pseudogout will not be excluded; based on our knowledge of this cohort's radiographic and MSK-US findings, we anticipate chondrocalcinosis prevalence of <10%.
3. Have any clinical disorder that required the use of corticosteroids within one week of screening visit
4. Have an estimated glomerular filtration rate (eGFR) < 30 ml/min (MDRD equation) and liver transaminases > 2x the upper limit of normal on most recent measurement
5. Have a BMI > 35kg/m2 at the time of enrollment
6. Have KL score of 0, 1, or 4.
7. Have a diagnosis of diabetes mellitus, chronic infectious disease, congestive heart failure, non-cutaneous cancer within the past 5 years or any other comorbidity that in the investigator's opinion would disqualify the subject
8. Scheduled or undergoing total knee replacement or other major surgery during the study period
9. Currently pregnant or plan to become pregnant during the study period
10. Unwilling to refrain from any medication or herbal supplements that is a strong CYP3A4 inhibitor whose metabolism may interact with colchicine (e.g., certain protease inhibitors, certain azole antifungal agents, clarithromycin) within 14 days or 5 half-lives, whichever is longer, of the first administration of the study intervention in this study. *See Appendix II.*
11. Unwilling to refrain from any medication or herbal supplements that when taken with colchicine can lead to high levels of colchicine in the body (e.g. some antifungal drugs, HIV drugs, antibiotics, and antidepressants) within 14 days or 5 half-lives, whichever is longer, of the first administration of the study intervention in this study
12. Unwilling to refrain from consuming grapefruit or grapefruit juice (CYP3A4 inhibitor) during the study period.
13. Unwilling to refrain from the use of more than 1 glass daily of red wine, or from consumption of Seville oranges, pomelos, exotic citrus fruits, or grapefruit hybrids from 7 days before the start of the study intervention
14. Unwilling to refrain from taking oral or topical NSAIDs during the study period. Low doses of aspirin (≤325mg) that do not act in an NSAID capacity, such as aspirin taken for cardiovascular conditions, are acceptable to continue taking during the study.
15. Unwilling to refrain from receiving intra-articular therapies to the knees during the study period.
16. Unwilling to refrain from starting new pain medications (with the exception, including supplements, or acupuncture, physical therapy or other pain modalities for the duration of the study
17. Unwilling to be randomized to take colchicine or placebo daily for 3 months
18. Subjects participating in other clinical studies or taking other investigational drugs

19. Nursing mothers

4.3 Subject Recruitment and Screening

Subjects will be primarily recruited from the LGEO (Leukocyte Gene Expression and Genetic Biomarkers of OA Incidence and Progression: s12-03682) natural history study described in detail below. Subjects will initially be recruited from the pool of patients whose most recent knee radiograph (LGEO exit x-ray) indicates KL grade 2 or 3. The LGEO study was a previous observational OA study. Previous sample collected included blood, synovial fluid, ultrasound, x-ray, and/or MRI. These subjects consented to be contacted for future research studies. The investigators also reserve the option to offer participation to subjects who have already participated in other prior knee OA studies at the NYU Langone Orthopedic Center. If necessary, the investigators may also seek appropriate subjects in their own clinics and practices, and will communicate through word of mouth to colleagues in the NYU/NYC rheumatology community. Alerts in the form of letters or e-mails to colleague practitioners may be employed, as necessary. The investigators may also post the flyer and colleague letter to their personal social media pages (Facebook and Twitter) to alert outside physicians and potential candidates about the study. The social media interaction will be limited to 1-way communication and people who are interested will be asked to call the number listed in the post and not respond via social media. Personal social networking sites cannot be used for communication with the study team as privacy cannot be ensured, because we are instructing those on social media not to respond on the social media platform but to call the coordinator, privacy will be maintained in the same manner as all other recruitment methods. In addition, it may become necessary to use DataCore as part of our recruitment plan. DataCore can provide a list of potential subjects, based on selected criteria, from various EMR sources, such as EPIC. Our research team will review that list to identify potential candidates. Once identified, potential subjects may be contacted, after a chart review. For patients who have not previously signed a release to be contacted for research purposes, we will first reach out to their primary provider and ask them to obtain patient permission to be contacted. In addition to DataCore, i2b2, Slicer Dicer, and MyChart will also be utilized to recruit subjects for the study. Moreover, the iConnect Volunteer Registry may be utilized to recruit potential subjects. The registry is available for whomever is interested in participating in clinical studies at NYU Langone Health. Volunteers who agree to be apart of the registry will be asked to provide standard information, including demographics (age, race, gender, etc) and medical history. Our research team may use the registry to identify a list of potential volunteers by filtering out medical conditions for knee osteoarthritis. Once identified, potential volunteers may be contacted to discuss the study, inquire interest, and complete a phone screen. Alternatively, the research team may receive referrals for potential volunteers interested in the study and request to be contacted. If potential volunteers fit the baseline criteria to be screened, the research team will obtain consent prior to initiating any study procedures.

Finally, we may consider recruitment through our arthritis clinics and primary care clinics at our affiliated hospitals, Bellevue Hospitals Center, the VA New York Harbor Healthcare System, and The Hospital for Joint Diseases. Should those sites be used, we will first secure local IRB approval.

Flyers will be distributed electronically or in print versions to rheumatologists, orthopedists and other musculoskeletal practitioners at the NYU Langone Orthopedic Center, and to primary care physicians within the NYU faculty practice network. Flyers will be displayed by the above practitioners in their offices at their own discretion.

Screening and Consent: SKOA (s14-00290) cohort subjects who meet entry criteria will be invited to participate in our prospective, double-blind, placebo-controlled, randomized trial. Subjects will be approached for written informed consent in accordance with the policies of the NYU School of Medicine Institutional Review Board and GCP and ICH Guidelines.

Subjects may also be screened over the phone using the Telephone Screening Interview form.

Signal Knee Selection

Signal knee is the knee that will be assessed for the duration of the study. For the purposes of identifying the signal knee, pain proceeds KL severity. If pain and KL severity are equal in both knees, then it will be up to the discretion of the physician as to which knee to follow for the purpose of the study.

a. Number of Subjects
Approximately 120 knee OA subjects will be enrolled in all arms of the study.

b. Gender of Subjects
Subjects will not be restricted by gender, although we anticipate that a majority of subjects will be female as the disease is more common in women, and the parent study pool is 63% female.

c. Age of Subjects
Subjects will be 40 years or older by the start of the study period.

d. Racial and Ethnic Origin
Subjects will not be selected or restricted according to racial or ethnic origin. Rather, the breakdown of these demographics in our study is expected to reflect the population of the parent pool the patients are being recruited from.

e. Vulnerable Subjects
Certain vulnerable subject groups, as specified by Federal regulations (e.g., pregnant women, neonates, children, and terminally ill patients) will either be ineligible to participate or will be specifically excluded (or will have already been excluded from the LGEO study). OA is a disease seen predominantly in aging; thus pregnant women would not likely be diagnosed with OA. Moreover, although colchicine use in clinical practice is often continued in pregnant women with familial Mediterranean fever, we will exclude all pregnant women from this study as a risk-benefit consideration. Neonates and children are under age 40, making them ineligible for the study. Terminally ill patients will be excluded, as they may not be able to complete the duration of the study based on life expectation and/or functional status. Subjects who lack capacity to consent for themselves, e.g. cognitively impaired persons, will be excluded. Members of minorities, economically and/or educationally disadvantaged persons, and students and employees will be eligible to participate if they meet inclusion and exclusion criteria. However, we will consent only in English for this study.

It is possible that employees of NYU Langone Health System may express interest in participating in the study, creating the need to define employees as a potential vulnerable population due to their employability at the institution. No employees will be coerced into participating, nor will their disinterest in participating be held against them in any way. In sum, their employability will not be impacted based on whether or not they choose to participate in this study and it will be fully explained to them that their potential involvement in the study will not impact their job position, future job standing, or employee evaluation.

Further it is possible that students of NYU may wish to participate in this study. No students will be coerced into participating, nor will their disinterest in participating be held against them in any way. It will be fully explained to them that their decision to participate, or not to participate, in this study will not influence or impact their status, position, standing, or grades at NYU. No study personnel are involved in the grading and evaluation of the students that are to be potentially recruited. Should employees or students choose to participate; the study team will treat their privacy and confidentiality with the highest regard and make every effort to ensure that the knowledge of their involvement is limited to the study staff and the participating individual.

4.4 Early Withdrawal

Subjects may voluntarily withdraw from study participation at any time without having to provide a reason. Alternatively, subjects may be withdrawn at the investigator's discretion if it is in the subject's best interest. Subjects who become pregnant during the study will be withdrawn from the study.

Every effort will be made for withdrawn subjects to undergo end of study assessments, regardless of the reason for withdrawal. If a subject refuses end of study procedures, the reason for refusal will be fully documented in the subject's source document and recorded in the study specific eCRF in REDCap. It is the subject's right to withdraw from the trial without providing a reason. In this case, the source documents and the eCRF should document the reason for discontinuation as "withdrawal of consent". Withdrawn subjects will not be replaced.

Whilst the effect of withdrawals will be investigated, subjects withdrawing for any reason will be counted as non-remitters in the primary analysis and therefore no adjustment in sample size for withdrawals is necessary.

Discontinuation of Study Therapy

Subjects may discontinue taking study medication at any time. In the event subjects permanently discontinue study medication, they will be asked to return for remaining study visits. All subjects, whether completing the study or withdrawn prematurely, will be followed up through the last visit on the schedule of assessment. The study team will collect any new adverse events (AEs) and concomitant medications at every visit.

If any subject is discontinued from study therapy, an explanation of this clinical decision will be documented. If the reason for discontinuing study therapy is an AE or an abnormal laboratory test result, the specific event or test will be recorded in the eCRF. Subjects who are discontinued from study therapy will undergo all study assessments up to and including the early termination visit, if possible.

Discontinuation of Study Drug Due to an AE or SAE

Subjects may be permanently discontinued from study drug because of an AE or SAE. It is vital to obtain follow-up data on any subject withdrawn because of an AE or SAE. In any case, every effort will be made to evaluate protocol-specified safety follow-up procedures (i.e., Reporting Procedure for AEs, SAEs and Pregnancy). If a subject is withdrawn due to an AE or SAE, the event will be followed by the Investigator through contact with the subject until resolution or stabilization has occurred. All AEs should be followed until resolution, stabilization or the subject is lost to follow-up and cannot be contacted.

4.4.1 When and How to Withdraw Subjects

Participants will be informed that they are free to withdraw from the study at any time. Subjects will otherwise be withdrawn if they experience serious adverse events to the study intervention, or non-serious adverse events that fail to resolve with ongoing treatment and/or temporary dose withholding.

4.4.2 Data Collection and Follow-up for Withdrawn Subjects

If patients choose to withdraw from the study, as delineated in the consent form, they can write a letter to the study team formalizing this request. We will include in the consent language that states that if a patient withdraws, the study will maintain her/his data as well as samples up to the point of withdrawal, for intention-to-treat analysis. Subjects who wish to withdraw their information and/or biosamples from the study will be required to submit an explicit written request, and their request for withdrawal will be kept on file by the study team as part of documentation for the study records. Every attempt will be made to contact the patient at what would otherwise be the end of the treatment period to collect data on the primary outcome, including phone calls to the subject weekly.

5 Study Drug

5.1 Description

The study drug (colchicine) will be 0.6 mg or 0.8 mg capsules. An identical placebo capsule will be provided. The study drug and placebo capsules will be provided by Hikma Pharmaceuticals, which is FDA approved to produce colchicine at a dose of 0.6 mg. The IND has been submitted for the 0.8 mg dose and will also include the 0.6 mg dose.

5.2 Treatment Regimen

Subjects will be randomized to take oral colchicine (0.6mg or 0.8mg), or oral identical placebo once daily (in the morning) for 90 days. Subjects will be instructed to take the study drug in the morning around the same time daily.

5.3 Method for Assigning Subjects to Treatment Groups

A randomization code will be generated by the statistician using random block sizes and held by the research pharmacist.

5.4 Preparation, Storage and Administration of Study Drug

Hikma Pharmaceuticals will provide matching study drug and placebo capsules. The study medication will be maintained and stored by the NYU Langone Orthopedic Center Pharmacy located at 333 East 38th Street, Fourth Floor, New York, New York 10016.

The study medication will be maintained and dispensed by the Pharmacy after randomization at the baseline visit.
The study medication will be dispensed as 90 tablets.

At both the Pharmacy, and at home by the subjects, study medication will be stored at a temperature between 20° and 25° C (66°-77° F), and protected from light and moisture. Instructions for storage will be labeled both on the shipments from Hikma, and on the bottles individually dispensed to the subjects.

5.5 Subject Compliance Monitoring

Subject Diary:

Each subject will be given a subject diary for the duration of the study. The subject diary will include data on medication compliance, pain (10-point Likert scale), other pain medication use, potential side effects and opportunity for additional comments. Subjects will be asked complete a daily entry and will be asked to do so around the same time every night for the duration of the study. *See Subject Diary in Appendix I*

Questionnaires:

Medication use will be reviewed and recorded at each visit to confirm medication adherence. Study drug pill counts will be performed at each study visit to assess study compliance. *See Medication Compliance Form in Appendix III.*

5.6 Prior and Concomitant Therapy

At screening, baseline, and all follow up visits, all concomitant medications will be recorded. Patients will be allowed to take acetaminophen (as first line therapy), topical analgesics (except topical diclofenac) and other pain medications at FDA-approved doses that they were using prior to the study, except NSAIDs. Subjects will record any analgesic use in their subject diaries. Initiation of new analgesic medications or changes to pain medication regimen will not be allowed for the duration of the study. Intra-articular therapies (hyaluronic acid,corticosteroid steroid injection, Platelet Rich Plasma, or any stem cell injections will not be allowed during or within 3 months prior to the start of the study.

5.7 General Method of Preparation and packaging

- General description of how drug is manufactured/prepared

  Blending:

  The Colchicine, USP and Microcrystalline Cellulose, NF (Avicel pH 101) are passed through an 8 mesh screen and then charged into two #4 Ball Mill Jars with approximately 9kg of grinding media per jar. The jars are sealed and then rolled for eight hours. The material is discharged through a screen to remove the grinding media from the mixed material.

  Concurrent with the ball milling, the Anhydrous Lactose, NF is processed in a fitzmill with Hammers Forward at High Speed using a #0 screen (1532-0027).

  The Colchicine/Avicel mix, Milled Lactose, Sodium Starch Glycolate, NF, and Colloidal Silicon Dioxide, NF are passed through a 20 mesh screen, loaded in a 7 ½ cubic foot tumbler, and mixed for 22.5 minutes.

  The Magnesium Stearate, NF is passed through a 30 mesh screen and then charged to the tumbler in 5 equal parts, each of which is followed by a one minute mixing time.

  After the blended material is sampled, it is discharged into drums as Colchicine – Final Blend, and held until in-process testing is complete and the material is ready for encapsulation.

  Encapsulation:

  A GKF 1500 Capsule Filler with a 7.5mm Dosing Disc and a CP-350 Capsule Polisher are used for the encapsulation process. A KKE 1500 is used to check weigh the filled capsules and a MET30+ metal detector inspects the capsules prior to depositing them into a bulk capsule container.

  The Colchicine – Final Blend is loaded into the powder hopper of the GKF 1500 Capsule Filler. No.4 med Orange/Lt Blue Opaque Hard Gelatin Empty Capsules are loaded into the empty capsule hopper of the GKF 1500. The capsule filling machine is adjusted to achieve the desired target fill weight, including adjustment of the tamping settings on the filler.

- Acceptable limits and analytical methods used to assure the identity, strength, quality, and purity of the drug substance:

Physical & Chemical Test Requirements	Specification	Methodology
Description	Pale yellow to pale greenish yellow crystalline powder	Visual (SOP-196)
Identification (IR)	Exhibits comparable maxima at same wavelengths as standard	USP <197K>
Specific Rotation	-240° to -250°	USP <781S>

Water	NMT 2.0%	USP <921> Method I
EP F Colchicine	NMT 0.15%	HPLC (TM-1775)
Limit of Ethyl Acetate	NMT 6.0%	GC (TM-1855)
Heavy Metals	NMT 20 ppm	USP <231> Method II
Residue on Ignition	NMT 0.2%	USP <281>
Assay (on anhydrous, solvent free basis)	94.0% - 101.0%	HPLC (TM-1756)
Residual Solvents: • Chloroform • Ethanol • Methanol • Methyl Chloride	 NMT 100 ppm NMT 5000 ppm NMT 3000 ppm NMT 600 ppm	GC (TM-1710)
Impurities • EP D: Colchicoside • EP E: 3-O-demethylcolchicine • EP B: Conformational Isomer • EP A: N-deactyl-N-formylcolchicine • *y*-lumicolchicine • EP C: β-lumicolchicine • Highest Unspecified Impurity • Total Specified and Unspecified Impurities	 NMT 0.15% NMT 0.15% NMT 2.0% NMT 0.15% NMT 0.06% NMT 0.06% NMT 0.10% NMT 3.0%	HPLC (TM-1756)
Particle Size D90 D50 D10	 NMT 50µ NMT 20µ NMT 10µ	HELOS (SOP-585)
XRD	Conforms to current USP RS	USP <941>
Bulk Density	0.10 – 0.40 g/mL	(SOP-107)

- Information sufficient to support stability of the drug substance during proposed human testing
  Finished product manufactured with Colchicine drug substance has been placed on accelerated and room temperature stability. Six month data is available for both conditions in bottles of 15's, 100's, and 1000's.
- **NOTE**: Reference to the current edition of the United States Pharmacopoeia – National Formulary may satisfy relevant requirements in this section.-THIS IS NOT A USP PRODUCT!

5.8 Drug Components and Drug Product

– **Ingredient**	**Mg/cap**	**Function**
Colchicine, USP	0.6 mg or 0.81mg[1]	Active
Microcrystalline Cellulose, NF (Avicel pH 101)	5.65mg	Diluent
Anhydrous Lactose, NF (Tablet Grade)	101.77mg[2]	Diluent
Sodium Starch Glycolate, NF (Primojel)	3.4mg	Disintegrant
Colloidal Silicon Dioxide, NF (Cab-o-sil)	0.56mg	Glidant
Magnesium Stearate, NF	1.13mg	Lubricant

#4 Med Orange/Lt. Blue Hard Gelatin Capsules printed “Hikma Pharmaceuticals119” in white ink	(40mg)	Encapsulation
Total Weight (Target Filled Capsule)	153.32mg	---

[1]Equivalent to 0.808mg/cap (1% excess based on Anhydrous, Solvent Free Basis, adjusted for assay, moisture content, and ethyl acetate.
[2]Adjusted to make Total 113.32mg/cap

Microcrystalline cellulose (Avicel pH 101) was selected as a diluent and carrier of the colchicine drug. Microcrystalline cellulose has a particle size of 50 microns which is very close to the particle size of colchicine. These two ingredients are Ball Milled together to mill any larger colchicine particles and to prepare a triturate of colchicine in order to obtain a proper content uniformity in the final product. Anhydrous lactose, DT grade was selected to obtain a free flowing blend which can aid in the weight uniformity during the encapsulation process. Sodium starch glycolate (Primojel) was used for faster dissolution. Colloidal silicon dioxide (Cab-O-Sil) was used as a glidant. Magnesium stearate, NF Hyqual grade, with a particle size limit of 50th percentile between 10.5-16.5 microns, was selected as a lubricant.

The following components used in the manufacture of Colcichine Capsules, 0.8mg are compendial: Microcrystaline Cellulose, Anhydrous Lactose, Sodium Starch Glycolate, Colloidal Silicon Dioxide and Magnesium Stearate.

The only non-compendial component is the empty hard gelatin capsule, which is commonly used in the manufacture of pharmaceutical capsule products.

The gelatin capsules are provided by Suheung Co., Ltd, Seoul, Korea. Capsules are manufactured from cow or pig sources and are certified BSE and TSE free.

Blend Specifications

Test	Test Specifications	Test Results
Uniformity of Blend (Content Uniformity) “C” sample		
$\bar{X}$ %RSD	90.0 – 110.0% NMT 5.0%	98.6% 1.2%
Bulk Density (g/mL)	Tentative 035 – 0.75 g/mL	0.56 g/mL
Particle Size (Sieve Analysis)		
40 mesh 100 mesh Pan	Tentative NMT 10% NMT 45% NLT 40%	 0% 8% 91%

Finished Product Specifications

Test	Test Specifications		
Average Capsule Weight: Range	(Target: 153 mg) 142 mg – 164 mg	155 mg	
Average Capsule Content: Range	(Target: 113 mg) 102 mg – 124 mg	113 mg	
Dissolution: Colchicine	NLT 80% (Q) in 20 minutes (Tentative)	X	100%
		Min	97%
		Max	104%
		Stage	1
Uniformity of Dosage:			

Content Uniformity Acceptance Value (AV) X = SD	 AV NMT 15.0 Report Result Report Result	 5.6 100.1% 2.35
Water Content	NMT 2.0%	1.0%
Assay	90.0% - 110.0%	99.3%
Impurities: • EP D: Colchicoside • EP E: 3-O-demethylcolchicine • EP B: Conformational Isomer • EP A: N-deacetyl-N-formylcolchicine • γ -lumicolchicine • EP C: β-lumicolchicine • Highest Unspecified Impurity • Total Specified and Unspecified Impurities	 NMT 0.15% NMT 0.25% NMT 2.0% NMT 0.25% NMT 0.6% NMT 0.6% NMT 0.25% NMT 3.0%	 0.06% ND 0.5% ND ND ND < 0.05% 0.6%

– Packaging procedures as appropriate for the product.

Packaging:

The Colchicine Capsules, 0.8mg are loaded into the hopper of a slat filler. Empty HDPE bottles are loaded into the bottle unscramble hopper, caps are loaded into the capper hopper, and rayon coil is fed into the cottoner (if required). When the line is started, the empty bottles are oriented and blown to clear any residual debris. The capsules are loaded into the bottle followed by a piece of rayon. The cap is applied and tightened to a torque and then run through an induction sealer and retorqued.

5.9 Blinding of Study Drug

Drug/placebo and a unique subject identifier linked to group assignment will be allocated by the research pharmacist, who will not release group assignment to the investigative team until after all subjects have completed the protocol and the database has been locked.

5.10 Receiving, Storage, Dispensing and Return

5.10.1 Receipt of Drug Supplies

Upon receipt of the of the study treatment supplies, an inventory must be performed and a drug receipt log filled out and signed by the person accepting the shipment. It is important that the designated study staff counts and verifies that the shipment contains all the items noted in the shipment inventory. Any damaged or unusable study drug in a given shipment (active drug or comparator) will be documented in the study files. The investigator must notify company providing the study drug and placebo of any damaged or unusable study treatments that were supplied to the investigator's site.

5.10.2 Storage

Colchicine must be stored at a temperature between 20° and 25° C (66°-77° F), and must be protected from light and moisture.

5.10.3 Dispensing of Study Drug

Colchicine will be supplied to the NYU Langone Orthopedic Center Investigational Pharmacy in bottles of 1000. These will contain the appropriate number of pills according to the treatment group to which the patient is randomized. Patients will receive detailed dosing instructions during screening and baseline visits. Bottles of 90 capsules will be dispensed at the baseline visit.

5.10.4 Return or Destruction of Study Drug

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

6 Study Procedures

Screening (21-day window to Baseline Visit):

- Patients may be initially screened over the phone;
- Patients will be provided detailed information about the study and will be asked to sign an informed consent form prior to undergoing any study-specific procedures;
- Patients will be assessed for inclusion/exclusion criteria,
- Height and weight will be obtained
- Patients will undergo a complete metabolic profile to assess eGFR and liver function, along with a complete blood count panel with differential to determine study eligibility. Results from standard of care tests within the last 3 months may be used in place of new screening labs to determine eligibility, unless the investigator deems it necessary to repeat them.
- Concomitant medications will be reviewed and recorded.
- Medical history will be reviewed and recorded.
- Demographics will also be obtained.
- Patients will complete VAS questionnaires for both knees to help determine the signal knee;
- Standardized bilateral, weight-bearing knee x-ray will be performed if one was not performed in the past 12 months at NYU Langone;
- Standardized knee evaluation will be performed bilaterally
- Ultrasound of the knees will be performed bilaterally, using a standardized protocol;
- Synovial fluid sample will be obtained from either knee if the patient has knee effusion detectable on musculoskeletal ultrasound and subject to the patient's agreement to this separate optional procedure;
- Serum Pregnancy test will be performed (as appropriate if the patient is female and within childbearing age).
- Review and documentation of any adverse events or serious adverse events.

6.1 Baseline (window)

- Review of inclusion and exclusion criteria for eligibility
- Subject will be randomized if eligible
- Medical history will be reviewed for any changes.
- Concomitant medications will be reviewed for any changes.
- Patients will complete VAS for both knees, KOOS for the signal knee, and pain in other joints questionnaires;
- 20 mL of blood will be obtained for research laboratory tests; Subject diary, with instructions, will be dispensed. Daily diary will include prompts to report the average knee pain over the past 24 hours, on a 0-10 scale, separately for each knee.
- Study medication will be dispensed. .
- Height and weight will be recorded.
- Review and documentation of any adverse events or serious adverse events.

6.2 Visit 1 (Week 6/ Day 42)

- Patients will complete VAS for both knees, KOOS for the signal knee, and pain in other joints questionnaires;
- Concomitant medications will be reviewed and recorded.
- Medical history will be reviewed for any changes.
- Medication pill count will be performed.
- Subject diary will be reviewed for compliance.
- Serum Pregnancy test will be performed (as appropriate)
- Height and weight will be recorded.
- Review and documentation of any adverse events or serious adverse events.
- If a patient is unable to return onsite for visit 1, this visit may be completed remotely via telephone.

6.3 Visit 2 (Week 12/ Day 84)

- Patients will complete VAS for both knees, KOOS for the signal knee, and pain in other joints questionnaires;
- Concomitant medications will be reviewed and recorded.
- Patients will undergo a complete metabolic profile to assess eGFR and liver function, along with a complete blood count panel with differential. Results from standard of care tests within 3 weeks prior to the visit may be used, unless the investigator deems it necessary to repeat them.
- Medical history will be reviewed for any changes.
- Medication pill count will be performed
- Standardized knee evaluation, will be performed bilaterally;
- 20 mL of blood will be obtained for research laboratory tests.
- Ultrasound of the knees will be performed, using a standardized protocol;
- Synovial fluid sample will be obtained from either knee if the patient has knee effusion detectable on musculoskeletal ultrasound and subject to the patient's agreement to this separate optional procedure;
- Serum Pregnancy test will be performed (as appropriate)
- Height and weight will be recorded.
- Review and documentation of any adverse events or serious adverse events.
- Patients will be informed of all abnormal lab results if they are significantly elevated as compared to previous results.

6.4 Visit 3 (Week 16/Day 112)

- Serum Pregnancy test will be performed (as appropriate)

Procedures/ Assessments	**Screening**	**Baseline**	**Visit 1/Week 6 / Day 42-[1]**	**Visit 2/Week 12/ Day 84 [1] / Early Termination / Termination Visit**	**Visit 3/Week 16/Day 112 [1] 28 days post dosing visit**

Windows		(within 21 days from Screening)	±7 days	±3 days	
Informed consent	X				
Inclusion/exclusion assessment	X	X			
Demographics	X				
Medical history and review	X	X	X	X	
Randomization		X			
Height and Weight	X	X	X	X	
Bilateral Physical Knee Exam	X			X	
Dispensation by Pharmacy		X			
Pill Count			X	X	
Bilateral VAS pain Questionnaire	X	X	X	X	
KOOS Questionnaire for Signal Knee		X	X	X	
Review of Subject Diary		X	X	X	
Pain in other joints (questionnaire)		X		X	
Bilateral Knee ultrasonography (see Appendix V)	X			X	
Bilateral Knee x-rays	X				
Blood sample (complete metabolic profile - NYU Lab)	X			X	
Blood sample (complete blood count with diff -NYU Lab)	X			X	
Serum Pregnancy (as appropriate)	X		X	X	X
Blood Samples		X		X	

(LOH Research Lab)					
Synovial fluid sample	X			X	
Concomitant Meds	X	X	X	X	
Adverse Events	X	X	X	X	

[**]Knee x-ray will be performed if subject has not had one within the past 12 months at NYU Langone Health.
[1]Telephone call prior to every visit will be made to remind subject of appointment date and time, to bring in subject diary and study medication (bottle).

7 Statistical Plan

7.1 Statistical Methods

Descriptive statistics will be used to compare the distributions of baseline subject characteristics. Frequency distributions and chi-square tests will be used to screen for major imbalances in the two randomization groups for qualitative variables; graphical displays including and box plots and summary statistics (e.g., mean, median, standard deviation, ranges, etc.) will be used for quantitative variables with t-tests (after appropriate transformations if necessary to meet the assumptions of the methods). For example, we will conduct the Kolmogorov-Smirnov or the Shapiro-Wilk test for the continuous outcomes such as VAS, pain scores from Aim 1, PBL gene expression, and plasma levels in Aim 2, to confirm normality of measurements prior to performing statistical analyses. Detection of outliers will be done using histograms, box-plots, normal plots and summary statistics. Where appropriate, logarithmic transformation will be further done.

In order to assess for significant differences in mean changes of continuous outcomes between drug/placebo groups, we will perform un-paired T-tests or Wilcoxon nonparametric counterpart where deviated from normality. When outcomes are dichotomized (e.g. progressed), we will use the chi-square (Fisher exact) test to compare proportions of outcomes between groups. These univariate analyses will be followed by multivariate linear (logistic) regression methods for an adjustment of all other potential confounding variables. Baseline factors associated with different groups will be also included in a multivariable model.

When multiple time points are considered such as at baseline and 3 months, we will model linear trajectories of outcome scores across time using mixed-effects (linear) regression models. The models will be specified with random intercepts and slopes such that a linear trajectory representing outcome scores will be compared between treatment groups. For example, longitudinal changes in all available continuous outcome scores over time of follow-ups will be displayed using locally weighted smoothing scatter (lowess) plots, identifying nonparametric mean trajectories over time. Mean trajectories will be then modeled with mixed-effects linear regression models, and if necessary using segment-linear models to approximate non-linear mean trajectories for ease of interpretation. Treatment group-specific (as well as a individual-level) intercepts and slopes over the period of follow-up will be included as random effects, while other covariates will be modeled as fixed effects. All models will be adjusted for other clinical or demographic confounders. Residual-based diagnostics will be used to evaluate validity of model assumptions. Two sided p-values <0.05 will be considered to be statistically significant. All statistical procedures will be performed using R statistical package (www.R-project.org).

7.2 Sample Size Determination

The patients will be randomized in a 1:1 ratio to each main arm of the study, for a total of 120 knee OA subjects (half colchicine and half placebo).

Assuming normality, we calculated the statistical power for various detectable effect sizes in a two-sided hypothesis test with a significance level of 0.05, where a 1:1 ratio of sample size is assumed between cases (treatment) vs. matched controls (placebo). For outcomes that are proportions, we calculated power based on rate differences. The table below shows that our sample size will have adequate power to detect the listed effect sizes. For example, we will achieve 86% power to detect a standardized mean difference of 0.50 between two groups of a size of 60 for each, which corresponds to a relative reduction by 50% in continuous outcomes. For dichotomous outcomes such as rates of MSK-US-measured synovitis and effusion, N=60 will have sufficient power to detect a difference of rate of 0.27 between two treatment groups. Note that for each domain score and the overall score, the power is the same for each effect size since they are standardized, but the mean change corresponding to a given effect size differs.

Sample size (N)	Unpaired t-test		Chi-square test	
	Effect size [1]	Power (%)	Effect size [2]	Power (%)
20	0.8	80	0.42	80.6
30	0.7	84.9	0.36	80.9
60	**0.5**	**85.9**	**0.27**	**83**
70	0.45	84.2	0.25	83
80	0.4	80.9	0.23	81.3
90	0.4	84.8	0.22	83.4

[1] standardized mean difference of 2 groups
[2] absolute proportion difference of 2 groups

7.3 Subject Population(s) for Analysis

Because the trial is short, and the anticipated rate of adverse events is low, we anticipate that most of the patients initiating the study will complete it. Our primary analysis will be intention-to-treat, and will include all treated patients who received at least one dose of study drug /placebo. We will also complete secondary analyses on only those patients who completed the study protocol to the end of 90 days, and exploratory analyses on subjects who were compliant with the study medications.

8 Safety and Adverse Events

8.1 Definitions

Unanticipated Problems Involving Risk to Subjects or Others
Any incident, experience, or outcome that meets <u>all</u> of the following criteria:

- <u>Unexpected in nature, severity, or frequency</u> (i.e. not described in study-related documents such as the IRB-approved protocol or consent form, the investigators brochure, etc)
- <u>Related or possibly related to participation in the research</u> (i.e. possibly related means there is a reasonable possibility that the incident experience, or outcome may have been caused by the procedures involved in the research)
- <u>Suggests that the research places subjects or others at greater risk of harm</u> (including physical, psychological, economic, or social harm).

Adverse Event
An ***adverse event*** (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests

- is considered by the investigator to be of clinical significance

Serious Adverse Event
Adverse events are classified as serious or non-serious. A ***serious adverse event*** is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

Subjects will be instructed to record the time of occurrence, duration and outcomes of any AEs in their subject diary.

Important medical events are those that may not be immediately life threatening but are clearly of major clinical significance. They may jeopardize the subject and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious should be regarded as ***non-serious adverse events***.

Adverse Event Reporting Period
The study period during which adverse events must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up. For this study, the study treatment follow-up is defined as 30 days following the last administration of study treatment.

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

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

Post-study Adverse Event
All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The investigator should notify the study sponsor of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study. The sponsor should also be notified if the investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study.

Abnormal Laboratory Values
A clinical laboratory abnormality should be documented as an adverse event if <u>any one of the following</u> conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity

- The abnormality is of a degree that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.

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

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should ***not*** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

Enrolled subjects will be asked to sign a medical release form so that the investigative site can easily retrieve any medical records resulting from a hospitalization or other medical encounter related to this study.

8.2 Recording of Adverse Events

At each contact with the subject, the investigator or designee must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should recorded in the source document, though should be grouped under one diagnosis.

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

8.3 Reporting of Serious Adverse Events and Unanticipated Problems

Investigators and the protocol sponsor must conform to the adverse event reporting timelines, formats and requirements of the various entities to which they are responsible, but at a minimum those events that must be reported are those that are:

- related to study participation,
- unexpected, and
- serious or involve risks to subjects or others
  (see definitions, section 8.1).

For Narrative Reports of Safety Events
If the report is supplied as a narrative, the minimum necessary information to be provided at the time of the initial report includes:

- Study identifier
- Study Center
- Subject number
- A description of the event
- Date of onset
- Current status
- Whether study treatment was discontinued
- The reason why the event is classified as serious
- Investigator assessment of the association between the event and study treatment

8.3.1 Investigator reporting: notifying the study sponsor

The following describes events that must be reported to the study sponsor in an expedited fashion.

Initial Report: within 24 hours:
The following events must be reported to the study sponsor by telephone within 24 hours of awareness of the event:

- Unanticipated problems related to study participation,
- Serious adverse events, regardless of whether they are unexpected.

Additionally, an FDA Form 3500A (MEDWATCH Form; see Attachment VIII) must be completed by the investigator and faxed to the study sponsor within 24 hours. The investigator shall maintain a copy of the MEDWATCH Form on file at the study site.

NYU Langone Health
Division of Rheumatology, Department of Medicine
NYU Langone Orthopedic Center
333 East 38th Street, Fourth Floor
New York, NY 10016
646-501-7194Telephone
646-501-7334 Facsimile

Follow-up report: within 48 hours:
As a follow-up to the initial report, within the following 48 hours of awareness of the event, the investigator shall provide further information, as applicable, on the unanticipated device event or the unanticipated problem in the form of a written narrative. This should include a copy of the completed Unanticipated Problem form, and any other diagnostic information that will assist the understanding of the event. Significant new information on ongoing unanticipated adverse device effects shall be provided promptly to the study sponsor.

Other Reportable events:

- **Deviations from the study protocol**
  Deviations from the protocol must receive both Sponsor and the investigator's IRB approval before they are initiated. Any protocol deviations initiated without Sponsor and the investigator's IRB approval that may affect the scientific soundness of the study, or affect the rights, safety, or welfare of study subjects, must be reported to the Sponsor and to the investigator's IRB as soon as a possible, but ***no later than 5 working days*** of the protocol deviation awareness.

- ***Withdrawal of IRB approval***
  An investigator shall report to the sponsor a withdrawal of approval by the investigator's reviewing IRB as soon as a possible, but ***no later than 5 working days*** of the IRB notification of withdrawal of approval.

8.3.2 Investigator reporting: notifying the IRB

Federal regulations require timely reporting by investigators to their local IRB of unanticipated problems posing risks to subjects or others. The following describes the NYULMC IRB reporting requirements, though Investigators at participating sites are responsible for meeting the specific requirements of their IRB of record.

Report Promptly, but no later than 5 working days:
Researchers are required to submit reports of the following problems promptly but no later than 5 working days from the time the investigator becomes aware of the event:

- ***Unanticipated problems including adverse events* that are unexpected and related**
  - *Unexpected: An event is "unexpected" when its specificity and severity are not accurately reflected in the protocol-related documents, such as the IRB-approved research protocol, any applicable investigator brochure, and the current IRB-approved informed consent document and other relevant sources of information, such as product labeling and package inserts.*
  - *Related to the research procedures: An event is related to the research procedures if in the opinion of the principal investigator or sponsor, the event was more likely than not to be caused by the research procedures.*
  - *Harmful: either caused harm to subjects or others, or placed them at increased risk*

Other Reportable events:
The following events also require prompt reporting to the IRB, though ***no later than 5 working days***:

- ***Complaint of a research subject*** when the complaint indicates unexpected risks or the complaint cannot be resolved by the research team.
- ***Protocol deviations or violations*** (includes intentional and accidental/unintentional deviations from the IRB approved protocol) for any of the following situations:
  - *one or more participants were placed at increased risk of harm*
  - *the event has the potential to occur again*
  - *the deviation was necessary to protect a subject from immediate harm*
- ***Breach of confidentiality***
- ***Incarceration of a participant*** when the research was not previously approved under Subpart C and the investigator believes it is in the best interest of the subject to remain on the study.
- ***New Information indicating a change to the risks or potential benefits*** of the research, in terms of severity or frequency. (e.g. analysis indicates lower-than-expected response rate or a more severe or frequent side effect; Other research finds arm of study has no therapeutic value; FDA labeling change or withdrawal from market)

Reporting Process
The reportable events noted above will be reported to the IRB using the form: "Reportable Event Form" or as a written report of the event (including a description of the event with information regarding its fulfillment of the above criteria, follow-up/resolution and need for revision to consent form and/or other study documentation).

Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator's study file.

8.3.3 Sponsor reporting: *Notifying the FDA*

The study sponsor is required to report certain study events in an expedited fashion to the FDA. These written notifications of adverse events are referred to as IND safety reports. The following describes the safety reporting requirements by timeline for reporting and associated type of event:

- ***Within 7 calendar days*** *(via telephone or facsimile report)*
  Any study event that is:
  - associated with the use of the study drug
  - unexpected,
  - fatal or life-threatening

- ***Within 15 calendar days*** *(via written report)*
  Any study event that is:
  - associated with the use of the study drug,
  - unexpected, and
  - serious, but not fatal or life-threatening

    -or-
  - a previous adverse event that was not initially deemed reportable but is later found to fit the criteria for reporting (reporting within 15 calendar days from when event was deemed reportable).

  Any finding from tests in laboratory animals that:
  - suggest a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity.

Additional reporting requirements
Sponsors are also required to identify in IND safety reports all previous reports concerning similar adverse events and to analyze the significance of the current event in light of the previous reports.

Reporting Process
Adverse events may be submitted on FDA Form 3500A (MEDWATCH Form (see Appendix VIII), or in a narrative format. If supplied as in a narrative format, the minimum information to be supplied is noted above at the beginning of section 8.3. The contact information for submitting IND safety reports is noted below:

Diana L. Walker, Ph.D., RAC
Sr. Regulatory Health Project Manager
FDA/CDER/ODE II/DAAAP
Tel: 301-796-4029
Email: Diana.Walker@fda.hhs.gov

8.4 Unblinding Procedures

Unblinding will be used in the case of any SAE which may be associated with the study drug.

8.5 Stopping Rules

Because the study drug is already used clinically in patients at higher risk than the subjects in this study, the study is only 3 months long, and because the anticipated rate of adverse events is low, we do not anticipate the need to interrupt or stop the study. Nevertheless, a Data Monitoring Committee will be created and can determine the need to interrupt or stop the study if necessary.

Subjects are free to discontinue the study at any time.

8.6 Medical Monitoring

It is the responsibility of the Principal Investigator to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan (see section 9 Auditing, Monitoring and Inspecting). Medical monitoring will include a regular assessment of the number and type of serious adverse events. In addition, the principal investigator will designate an

independent safety monitor to make determinations about the nature of any adverse events. This individual will conduct his or her review every 6 months and the information will be shared with the PI and the study team.

8.6.1 Data Monitoring Committee

There is no need for a Data Monitoring Committee. However, monitoring the study is a critical part of its success. Therefore, an internal clinical practitioners (to be determined) will be monitoring the study on a biannual basis. This monitor will work with our clinical and regulatory study teams to ensure that all procedures and assessments are being conducted in compliance with the protocol and the informed consent.

9 Sample Storage, Data Handling, and Record Keeping

9.1 Confidentiality

Primary research data will be stored at the NYU Langone Orthropedic Center (LOC). The sample collection includes: synovial fluid, plasma, and serum samples. Subjects will mark in the consent form whether or not they agree to provide a synovial fluid sample. These samples will be stored at the Immunology Lab at the NYU Langone Orthopedic Hospital located at 301 East 17th Street, Rm 1600, New York, NY 10003). The Immunology Lab is managed by Dr. Mukundan Attur. The samples will be stored for a maximum of over 20 years unless the patient requests, in writing, for the samples to be destroyed. If patients choose to withdraw from the study, as delineated in the consent form, they can write a letter to the study team formalizing this request. Samples will immediately be destroyed for patients who wish to withdraw their information from the study, and their request for withdrawal will be kept on file by the study team as part of documentation for the study records.

“True” genetic testing will not be done.

It is believed the maximum allowance for time to hold samples will allow for significant analysis and improvement in understanding surrounding rheumatic disease. Source documents will be stored in study binders, which will remain in a locked room/cabinet at the study facility. All subjects will be assigned a unique four-digit study number and all subsequent questionnaires and samples will only be encoded with this numeric code and the date of collection. A link between patient name, contact information, and identifier will be retained by Dr. Pillinger and the study team. These will be retained in a password-protected database with original CRFs retained in a locked cabinet in case report binders at LOC. The clinical database will be kept in a de-identified, password-protected secure online REDCap database, created for this study. Only the principal investigators, co-investigators, and study coordinators will have access to this data. Information with subject names will not be used in any publications resulting from this investigation.

For patients who are fully eligible, do not withdraw from the study or request that their samples be withdrawn, sample storage for future research is not optional and is a mandatory part of this research. While the study team plans to measure certain blood markers soon after each subject is enrolled in the study, stored samples would allow for further analysis as the understanding of osteoarthritis changes in the future.

When patient blood and questionnaires are obtained, they will be labeled with the appropriate unique numeric code and date of collection. All subsequent data analysis, specimen recording and samples will only be encoded with this numeric code and the date of collection. There will be no link between the informed consent forms and the subjects' samples.

Chart reviews will require patient names. However, when the data is transferred from patient charts and medical questionnaires to the database, only the appropriate numeric code will be used as an identifier.

Serum/plasma will be collected from the study subjects. These samples will be stored in sterile plastic tubes in storage containers in locked –80^{o}C freezers in a locked room, and will be accessible only to

authorized study personnel. All stored samples will be identified by unique numeric codes and dates of collection only, and stored at the Immunology Lab on the 16th Floor at the NYU Langone Orthopedic Hospital located at 301 East17th Street, Suite 1600, New York, NY 10003. There is no PHI associated with any of the biospecimens for this study. Samples will be stored for future research for up to 20 years. Subjects may also decline to participate in having samples banked for future research.

9.2 Confidentiality and HIPAA

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

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

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

9.3 Source Documents

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

9.4 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. DO NOT ERASE OR WHITE OUT ERRORS. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it.

9.5 Records Retention

It is the investigator's responsibility to retain study essential documents for at least 2 years after the last approval of a marketing application in their country and until there are no pending or contemplated marketing applications in their country or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period if required by an agreement with the sponsor. In such an instance, it is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

10 Study Monitoring, Auditing, and Inspecting

10.1 Study Monitoring Plan

This study will be monitored according to the monitoring plan in Appendix VI. The investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the above noted study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

10.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB/EC, the sponsor, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

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

11 Ethical Considerations

This study will be conducted accordance with applicable US government regulations and international standards of Good Clinical Practice (GCP), and applicable institutional research policies and procedures. In addition to standard trainings, the PI (Michael Pillinger, MD) will participate in PINDAR, NYULMC's GCP training for PIs.

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

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the IRB-approved consent form, will be obtained before that subject undergoes any study procedure. The consent form will be signed by the subject and the investigator-designated research professional obtaining the consent.

Informed consent will be obtained by the Principal Investigator or a designee such as a sub-investigator, research coordinator or research nurse. Any future participants in the consent process will be trained by the PIs and the Institution and will be placed on the protocol with IRB approval. The informed consent form will be discussed in depth by the individual obtaining consent, with initialing of the subject at each juncture to confirm understanding. Complete consent forms will be stored in each subject's individual binder on site at CMC, maintained in a secure cabinet/room.

Before the study starts, one of the investigators or research physician/personnel shall explain the full details of the protocol and study procedures as well as risks involved to patients prior to their enrollment in the study. Participants will be informed that they are free to withdraw from the study at any time. All participants will sign an IRB-approved consent form. Only patients who can consent for themselves and have the capacity to do so will be eligible for enrollment in this study.

12 Study Finances

12.1 Funding Source

Subjects will incur no undue costs as a result of participation in the study. The study procedures, study drug and placebo are financed by Hikma Pharmaceuticals.

12.2 Conflict of Interest

At the present time no investigator has a conflict of interest with this study. If, in the future, any investigator develops a conflict or potential conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.), they will submit the conflict for review by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor prior to participation in this study. All NYULMC investigators will follow the applicable University conflict of interest policies.

12.3 Subject Stipends or Payments

Subjects will receive $50.00 at each of the visits, for a total stipend of $200.00 as compensation for their time participating in the study. Subject who are required to come in for the final serum pregnancy visit will receive an additional $50.00 for a total of $250.00. The funds for each visit will be sent to the subject via ClinCard direct deposit.

13 Publication Plan

Neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided by the sponsor for the purposes of performing the study, will be published or passed on to any third party without the consent of the study sponsor and company/funding source (Hikma Pharmaceuticals). Any investigator involved with this study is obligated to provide Hikma Pharmaceuticals with complete test results and all data derived from the study.

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15 APPENDICIES

APPENDIX I: Subject Diary

CLOAK Study Diary Subject ID_____

Day	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
Date (MM/DD/YY)	/ /	/ /	/ /	/ /	/ /	/ /	/ /
Study Medication (please record time taken)	❒am	❒am	❒am	❒am	❒am	❒am	❒am
Knee Pain (0-10) Average daily pain over the prior 24 hours; please use whole numbers (0-no pain; 10 worst pain)	Right ___ Left ___	Right ___ Left ___	Right ___ Left ___	Right ___ Left ___	Right ___ Left ___	Right ___ Left ___	Right ___ Left ___
Side Effects or Adverse Reaction							
Time/Duration of side effect (include any treatment used or if side effect resolved on its own)							
Rescue Knee Pain Medication (please specify name of medication, dose and time taken – include topical medications)	Name Time Dose Name Time Dose	Name Time Dose Name Time Dose	Name Time Dose Name Time Dose	Name Time Dose Name Time Dose	Name Time Dose Name Time Dose	Name Time Dose Name Time Dose	Name Time Dose Name Time Dose
Comments							

Week of _____Reviewed by _____Date Reviewed_____

NYU Langone Health

APPENDIX II: Medications That May Interact with Colchicine*

Strong CYP3A4 Inhibitors	Moderate CYP3A4 Inhibitors	P-gp Inhibitors
atazanivir	amiodarone	cyclosporine
boceprevir	amlodipine	ranolazine
clarithromycin	amprenavir	omeprazole
danazol	aprepitant	lansoprazole
gemfibrozil	atorvastatin	pantoprazole
indinavir	azithromycin	
itraconazole	diltiazem	
ketoconazole	erythromycin	
nefazodone	fluconazole	
nelfinavir	fosamprenavir	
ritonavir	grapefruit juice	
saquinavir	nicotinic acid	
telaprevir	simvastatin	

telithromycin	verapamil	
*Note that some compounds inhibit both CyP3A4 and P-gp. For simplicity these are listed among the CYP3A4 inhibitors and not repeated in the P-gp column.		

APPENDIX III: Medication Compliance Form (to be provided by Pharmacy)

APPENDIX IV: Drug Reconciliation Form (to be provided by Pharmacy)

APPENDIX V: Ultrasonography Form

For evaluating the knees by ultrasound, there is no gold standard method in the literature.
Thus we will capture 5 semiquantitative scores on a scale of 0-3 (see below) for osteoarthritis, and 3 yes/no questions about crystal disease.

1) Size of effusion in the anterior suprapatellar space in the longitudinal view, anechoic and compressible, on a scale of 0-3 (by millimeter cutoffs)
2) Degree of synovial hypertrophy (by gray scale) in the anterior suprapatellar space in the longitudinal view, hypoechoic and NOT compressible, on a scale of 0-3
3) Degree of synovitis by Doppler signal in the anterior suprapatellar space in the longitudinal view, on a scale of 0-3 (none, scattered, less than 50%, greater than 50% of the area)
4) Size of osteophytes in the medial or lateral joint lines (whichever more severe) in the longitudinal view, hyperechoic, on a scale of 0-3
5) Degree of femoral articular cartilage (FAC) wear in the suprapatellar transverse view with knee flexed, on a scale of 0-3
6) FAC double contour sign (hyperechoic line across top of anechoic FAC) in the suprapatellar transverse view with knee flexed: yes/no
7) FAC evidence of CPPD (hyperechoic deposits in the middle of the anechoic FAC) in the suprapatellar transverse view with knee flexed: yes/no
8) meniscal evidence of CPPD (hyperechoic deposits in the triangular meniscus edges) in the medial and/or latera joint line: yes/no

APPENDIX VI: Data Safety Monitoring Plan

1. **Types of Data or Events:**
   All data collected under the study and schedule of events will be monitored and reviewed by PI and Hikma Pharmaceuticals (funding source).

   Events that would be specifically reviewed by the DSMP would be those adverse events that are known to be associated with colchicine use at the dose ranged tested.
   Adverse events include:
   - GI upset
   - Soft stools/Diarrhea
   - Occassional nausea/vomiting

   In most cases at the dose being tested, these symptoms would be mild. Events that occur with an overdose of colchicine could include neuropathy, myopathy, rhabdomyolysis, renal and bone marrow failure. However, this is unexpected at the doses tested in this study with the exclusion criteria. The occurrence of these at the doses and in the manner we are using the agent would be very rare as too be reportable. They are not a concern, but they would be reviewed by the DSMP if they did occur. The occurrence of any other unexpected events will also be noted.

2. **Responsibilities and roles for gathering, evaluating and monitoring the data:**
   - The PI and study staff will collect the required data and record and monitor all adverse events in a timely manner after each participant visit. Any unanticipated problems, SAEs or events meeting reportable IRB criteria will be submitted as per IRB and sponsor guidelines.
   - The roles of the investigators, research coordinators at the site will be documented on the delegation list for the study and signed by the PI.
   - Data accuracy will be monitored by the PI and/or primary data and safety monitor or designee upon first subject enrolled and every 3 months as needed. The safety monitor will conduct a review of adverse events biannually.
   - The primary data and safety monitor or designee will verify compliance with the protocol by comparing source documents to research case report forms for accuracy. Monitoring will occur upon first subject enrolled and biannually throughout the study..

3. **Information about the monitoring entity:**
   The PI will serve as the monitoring entity and is responsible for the data safety monitoring of the overall study, which is deemed appropriate due to:
   - the study is conducted at one site only
   - the narrow range of possible study events that could occur, supported by the FDA approval of the drug

4. **Reporting adverse events and unanticipated problems to the monitoring entity:**
   - All adverse events will be recorded and followed by study staff/PI. They will be reported if they meet IRB and sponsor guidelines for reporting within specified time frame.
   - Specific forms or electronic submission will be used to report the events if they meet reporting criteria.
   - The coordinator will prepare and submit the form signed by PI.

5. **Assessments:**

The PI will be monitoring the safety and efficacy variables throughout the study, monthly, on all 120 subjects. Monitoring will include source data matches entered data and protocol compliance. Events meeting reporting criteria as "unanticipated problems involving risks to participants or others" (i.e., as to whether they are unexpected, related and harmful) will be reported to IRB and sponsor.

6. **Criteria for action:**
   - The PI will determine if there is additional action required during study for postponing treatment or withdrawal from study. This would be related to infections or new diagnosis or treatment in which study treatment would cause harm.
   - Stopping rules will be determined by PI in cases of infection, hospitalizations, voluntary or PI withdrawal of subject.

7. **Procedures for Communicating – dissemination of safety information**
   As appropriate: Electronically
   - Outcomes of monitoring entity reviews if needed will be provided electronically for communication to the IRB and/or research sponsor as needed.

APPENDIX VII: Schedule of Events

Table of Study Visits/Procedures (Appendix VII)					
Procedures/ Assessments	**Screening**	**Baseline**	**Visit 1/Week 6 / Day 42-[1]**	**Visit 2/Week 12/ Day 84 [1] / Early Termination / Termination Visit**	**Visit 3/Week 16/Day 112 [1] 28 days post dosing visit**
Windows		**(within 21 days from Screening)**	**±7 days**	**±3 days**	
Informed consent	X				
Inclusion/exclusion assessment	X	X			
Demographics	X				
Medical history and review	X	X	X	X	
Randomization		X			
Height and Weight	X	X	X	X	
Bilateral Physical Knee Exam	X			X	
Dispensation by Pharmacy		X			
Pill Count			X	X	
Bilateral VAS pain Questionnaire	X	X	X	X	
KOOS Questionnaire for Signal Knee		X	X	X	
Review of Subject		X	X	X	

Diary					
Pain in other joints (questionnaire)		X		X	
Bilateral Knee ultrasonography (see Appendix V)	X			X	
Bilateral Knee x-rays	X				
Blood sample (complete metabolic profile - NYU Lab)	X			X	
Blood sample (complete blood count with diff -NYU Lab)	X			X	
Serum Pregnancy (as appropriate)	X		X	X	X
Blood Samples (LOH Research Lab)		X		X	
Synovial fluid sample	X			X	
Concomitant Meds	X	X	X	X	
Adverse Events	X	X	X	X	

****Knee x-ray will be performed if subject has not had one within the past 12 months at NYU Langone Health.**

APPENDIX VIII: FDA MEDWATCH FORM 3500A

Reset Form

U.S. Department of Health and Human Services
Food and Drug Administration

MEDWATCH

FORM FDA 3500A (10/15)

For use by user-facilities, importers, distributors and manufacturers for MANDATORY reporting

Page 1 of 3

Form Approved: OMB No. 0910-0291, Expires: 9/30/2018
See PRA statement on reverse.

Mfr Report #
UF/Importer Report #
FDA Use Only

PLEASE TYPE OR USE BLACK INK

Note: For date prompts of "dd-mmm-yyyy" please use 2-digit day, 3-letter month abbreviation, and 4-digit year; for example, 01-Jul-2015.

A. PATIENT INFORMATION

1. Patient Identifier	2. Age	3. Sex	4. Weight
In Confidence	☐ Year(s) ☐ Month(s) ☐ Week(s) ☐ Days(s)	☐ Female ☐ Male	☐ lb ☐ kg
	or Date of Birth *(e.g., 08 Feb 1925)* __ __ – _ _ _ – _ _ _ _		

5.a. **Ethnicity** *(Check single best answer)*
☐ Hispanic/Latino
☐ Not Hispanic/Latino

5.b. **Race** *(Check all that apply)*
☐ Asian ☐ American Indian or Alaskan Native
☐ Black or African American ☐ White
☐ Native Hawaiian or Other Pacific Islander

B. ADVERSE EVENT OR PRODUCT PROBLEM

1. ☐ **Adverse Event** and/or ☐ **Product Problem** *(e.g., defects/malfunctions)*

2. **Outcome Attributed to Adverse Event** *(Check all that apply)*
☐ Death *Include date (dd-mmm-yyyy):* __ __ – _ _ _ – _ _ _ _
☐ Life-threatening
☐ Disability or Permanent Damage
☐ Hospitalization – initial or prolonged
☐ Congenital Anomaly/Birth Defects
☐ Other Serious (Important Medical Events)
☐ Required Intervention to Prevent Permanent Impairment/Damage (Devices)

3. Date of Event *(dd-mmm-yyyy)*	4. Date of this Report *(dd-mmm-yyyy)*
__ __ – _ _ _ – _ _ _ _	__ __ – _ _ _ – _ _ _ _

5. **Describe Event or Problem**

(Continue on page 3)

6. **Relevant Tests/Laboratory Data, Including Dates**

(Continue on page 3)

7. **Other Relevant History, Including Preexisting Medical Conditions** *(e.g., allergies, pregnancy, smoking and alcohol use, liver/kidney problems, etc.)*

(Continue on page 3)

C. SUSPECT PRODUCT(S)

1. **Name, Manufacturer/Compounder, Strength**

#1 – Name and Strength	#1 – NDC # or Unique ID
#1 – Manufacturer/Compounder	#1 – Lot #
#2 – Name and Strength	#2 – NDC # or Unique ID
#2 – Manufacturer/Compounder	#2 – Lot #

2. **Concomitant Medical Products and Therapy Dates** *(Exclude treatment of event)*

(Continue on page 3)

Submission of a report does not constitute an admission that medical personnel, user facility, importer, distributor, manufacturer or product caused or contributed to the event.

3. Dose	Frequency	Route Used
#1		
#2		

4. **Therapy Dates** *(If unknown, give duration)* from/to *(or best estimate))* *(dd-mmm-yyyy)*
#1
#2

5. **Diagnosis for Use** *(Indication)*
#1
#2

9. **Event Abated After Use Stopped or Dose Reduced?**
#1 ☐ Yes ☐ No ☐ Doesn't apply
#2 ☐ Yes ☐ No ☐ Doesn't apply

10. **Event Reappeared After Reintroduction?**
#1 ☐ Yes ☐ No ☐ Doesn't apply
#2 ☐ Yes ☐ No ☐ Doesn't apply

6. Is the Product Compounded?	7. Is the Product Over-the-Counter?
#1 ☐ Yes ☐ No	#1 ☐ Yes ☐ No
#2 ☐ Yes ☐ No	#2 ☐ Yes ☐ No

8. **Expiration Date** *(dd-mmm-yyyy)*
#1 __ __ – _ _ _ – _ _ _ _ #2 __ __ – _ _ _ – _ _ _ _

D. SUSPECT MEDICAL DEVICE

1. **Brand Name**

2. Common Device Name	2b. Procode

3. **Manufacturer Name, City and State**

4. Model #	Lot #	5. Operator of Device
Catalog #	Expiration Date *(dd-mmm-yyyy)* __ __ – _ _ _ – _ _ _ _	☐ Health Professional ☐ Lay User/Patient ☐ Other
Serial #	Unique Identifier (UDI) #	

6. If Implanted. Give Date *(dd-mmm-yyyy)*	7. If Explanted. Give Date *(dd-mmm-yyyy)*
– –	– –

8. **Is this a single-use device that was reprocessed and reused on a patient?** ☐ Yes ☐ No

9. **If Yes to Item 8, Enter Name and Address of Reprocessor**

10. **Device Available for Evaluation?** *(Do not send to FDA)*
☐ Yes ☐ No ☐ Returned to Manufacturer on: __ __ – _ _ _ – _ _ _ _

11. **Concomitant Medical Products and Therapy Dates** *(Exclude treatment of event)*

(Continue on page 3)

E. INITIAL REPORTER

1. **Name and Address**

Last Name:	First Name:
Address:	
City:	State/Province/Region:
Country:	ZIP/Postal Code:
Phone #:	Email:

2. Health Professional?	3. Occupation *(Select from list)*	4. Initial Reporter Also Sent Report to FDA
☐ Yes ☐ No		☐ Yes ☐ No ☐ Unk

Reset Form

MEDWATCH

FORM FDA 3500A (10/15) *(continued)*

FDA USE ONLY

F. FOR USE BY USER FACILITY/IMPORTER *(Devices Only)*

1. **Check One** ☐ User Facility ☐ Importer
2. **UF/Importer Report Number**
3. **User Facility or Importer Name/Address**
4. **Contact Person**
5. **Phone Number**
6. **Date User Facility or Importer Became Aware of Event** *(dd-mmm-yyyy)* __ – ___ – ____
7. **Type of Report** ☐ Initial ☐ Follow-up # _____
8. **Date of This Report** *(dd-mmm-yyyy)* __ – ___ – ____
9. **Approximate Age of Device**
10. **Event Problem Codes** *(Refer to coding manual)* Patient Code ___ – ___ – ___ Device Code ___ – ___ – ___
11. **Report Sent to FDA?** *(If Yes, enter date (dd-mmm-yyyy))* ☐ Yes __ – ___ – ____ ☐ No
12. **Location Where Event Occurred** ☐ Hospital ☐ Home ☐ Nursing Home ☐ Outpatient Treatment Facility ☐ Outpatient Diagnostic Facility ☐ Ambulatory Surgical Facility ☐ Other: _____ *(Specify)*
13. **Report Sent to Manufacturer?** *(If Yes, enter date (dd-mmm-yyyy))* ☐ Yes __ – ___ – ____ ☐ No
14. **Manufacturer Name/Address**

G. ALL MANUFACTURERS

1. **Contact Office (and Manufacturing Site for Devices)** Name / Address / Email Address
2. **Phone Number**
3. **Report Source** *(Check all that apply)* ☐ Foreign ☐ Study ☐ Literature ☐ Consumer ☐ Health Professional ☐ User Facility ☐ Company Representative ☐ Distributor ☐ Other: _____
Compounding Outsourcing Facility 503B? ☐ Yes
4. **Date Received by Manufacturer** *(dd-mmm-yyyy)* __ – ___ – ____
5. NDA # _____ ANDA # _____ IND # _____ BLA # _____ PMA/510(k) # _____ Combination Product ☐ Yes Pre-1938 ☐ Yes OTC ☐ Yes
6. **If IND, Give Protocol #**
7. **Type of Report** *(Check all that apply)* ☐ 5-day ☐ 30-day ☐ 7-day ☐ Periodic ☐ 10-day ☐ Initial ☐ 15-day ☐ Follow-up # ____
8. **Adverse Event Term(s)**
9. **Manufacturer Report Number**

H. DEVICE MANUFACTURERS ONLY

1. **Type of Reportable Event** ☐ Death ☐ Serious Injury ☐ Malfunction
2. **If Follow-up, What Type?** ☐ Correction ☐ Additional Information ☐ Response to FDA Request ☐ Device Evaluation
3. **Device Evaluated by Manufacturer?** ☐ Not Returned to Manufacturer ☐ Yes ☐ Evaluation Summary Attached ☐ No *(Attach page to explain why not)* or provide code: _____
4. **Device Manufacture Date** *(dd-mmm-yyyy)* __ – ___ – ____
5. **Labeled for Single Use?** ☐ Yes ☐ No
6. **Event Problem and Evaluation Codes** *(Refer to coding manual)* Patient Code ___ – ___ – ___ Device Code ___ – ___ – ___ Method ___ – ___ – ___ – ___ Results ___ – ___ – ___ – ___ Conclusions ___ – ___ – ___ – ___
7. **If Remedial Action Initiated, Check Type** ☐ Recall ☐ Repair ☐ Replace ☐ Relabeling ☐ Notification ☐ Inspection ☐ Patient Monitoring ☐ Modification/Adjustment ☐ Other: _____
8. **Usage of Device** ☐ Initial Use of Device ☐ Reuse ☐ Unknown
9. **If action reported to FDA under 21 USC 360i(f), list correction/removal reporting number:**
10. ☐ **Additional Manufacturer Narrative** and / or 11. ☐ **Corrected Data**

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Reset Form

(CONTINUATION PAGE)
For use by user-facilities,
importers, distributors, and manufacturers
for MANDATORY reporting

MEDWATCH
FORM FDA 3500A (10/15) *(continued)*

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B.5. **Describe Event or Problem** *(continued)*

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B.6. **Relevant Tests/Laboratory Data, Including Dates** *(continued)*

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B.7. **Other Relevant History, Including Preexisting Medical Conditions** *(e.g., allergies, pregnancy, smoking and alcohol use, hepatic/renal dysfunction, etc.)* *(continued)*

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Concomitant Medical Products and Therapy Dates *(Exclude treatment of event)* *(For continuation of C.2 and/or D.11; please distinguish)*

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Other Remarks