A RANDOMIZED, CONTROLLED, DOUBLE-BLIND STUDY TO EVALUATE THE EFFICACY AND SAFETY OF AN INTRA-ARTICULAR INJECTION OF AMPION™ IN ADULTS WITH PAIN DUE TO SEVERE OSTEOARTHRITIS OF THE KNEE

STUDY PROTOCOL

STUDY NUMBER: AP-013

NCT 03988023

7 JUNE 2019

CLINICAL STUDY PROTOCOL

A RANDOMIZED, CONTROLLED, DOUBLE-BLIND STUDY TO EVALUATE THE EFFICACY AND SAFETY OF AN INTRA-ARTICULAR INJECTION OF AMPION™ IN ADULTS WITH PAIN DUE TO SEVERE OSTEOARTHRITIS OF THE KNEE

STUDY NUMBER: AP-013

Drug Development Phase:	Phase 3
Investigational Product:	Ampion™
Indication:	Osteoarthritis of the knee
Sponsor:	Ampio Pharmaceuticals, Inc. 373 Inverness Parkway, Suite 200 Englewood, CO 80112
Lead Investigators:	John Schwappach, MD
Date:	Version 1.0 7 June 2019

Conduct: In accordance with the ethical principles that originate from the Declaration of Helsinki and that are consistent with International Conference on Harmonisation (ICH) guidelines on Good Clinical Practice (GCP) and regulatory requirements as applicable.

PROTOCOL SIGNATURE PAGE

I have read and understand the contents of this clinical protocol for Study AP-013 dated 7 June 2019 and agree to meet all obligations of Ampio Pharmaceuticals, Inc. as detailed in all applicable regulations and guidelines.

Signed By:

<Study personnel signature> **<Enter date>**

PROTOCOL SYNOPSIS

Sponsor:	**Investigational Product:**	**Developmental Phase:**
Ampio Pharmaceuticals, Inc.	Ampion™	Phase 3

Title of Study:

A Randomized, Controlled, Double-Blind Study to Evaluate the Efficacy and Safety of an Intra-Articular Injection of Ampion in Adults with Pain Due to Severe Osteoarthritis of the Knee

Protocol Number:

AP-013

Study Center(s):

Approximately 20 sites

Lead Investigators:

John Schwappach, MD

Indication:

Pain of severe osteoarthritis of the knee (OAK)

Number of patients:

This trial is designed for 1,034 patients, 517 per study arm, randomized 1:1 for Ampion and saline intra-articular (IA) injection. There will be an interim analysis at 724 patients, 362 per study arm, randomized 1:1, Ampion and saline. A promising zone design is used with the possibility to increase the sample size by 1.5 times the original sample size up to 1,552 patients at the interim analysis.

Objectives:

The co-primary trial objectives are to evaluate the greater efficacy for pain improvement and function improvement of 4 mL Ampion versus saline intra-articular (IA) injection, when applied to patients suffering from severe osteoarthritis of the knee (OAK). Efficacy will primarily be assessed with WOMAC A pain and WOMAC C function scores.

The secondary trial objectives include the following evaluations of an intra-articular injection of Ampion, as compared to saline, when administered to patients suffering from severe OAK: assessment of safety; improvement in the Patient Global Assessment; and the amount of rescue analgesia.

Sponsor:	**Investigational Product:**	**Developmental Phase:**
Ampio Pharmaceuticals, Inc.	Ampion™	Phase 3

Exploratory objectives are to analyze the effect, if any, of the efficacy of Ampion, as compared to saline, on stiffness; Outcome Measures in Rheumatology Clinical Trials and Osteoarthritis Research Society International (OMERACT-OARSI) responder status; proportion of treatment failures; change in pain and function as a cumulative distribution function (CDF) plot; and a Bayesian meta-analysis for WOMAC A pain sub score including KL 4 patients from studies AP-003-A, AP-003-B, AP-003-C, AP-004, and AP-013.

Methods:

Study AP-013 is a randomized, controlled, double-blind study with a 28-day screening period for each patient followed by a 24-week participation period. This trial will use an adaptive design with promising zone method for sample size re-estimation.

This trial is designed for 1,034 patients, 517 per study arm, randomized 1:1 for Ampion and saline IA injection. There will be an interim analysis to allow for sample size re-estimation up to 1.5 times the original trial size for a maximum of 1,552 patients, 776 per study arm, randomized 1:1 for Ampion and saline IA injection.

The clinical effects of treatment on severe OAK will be evaluated at 2, 4, 6, 8, 10, 12, and 24 weeks, using the Western Ontario and McMaster Universities Arthritis Index (WOMAC®) osteoarthritis Index 3.1, and the Patient's Global Assessment of disease severity (PGA). Average daily WOMAC A pain scoring will be collected for seven days using a daily diary beginning one week prior to clinic visits at Week 0 (baseline) and Week 12, in order to determine a WOMAC A weekly pain score for Baseline and Week 12.

Safety will be assessed by recording adverse events (through 24 hours post-dose and at all follow up contacts) and physical examination and vitals (Baseline, Weeks 6, 12, and 24).

Diagnosis and Main Criteria for Inclusion:

1. Able to provide written informed consent to participate in the study
2. Willing and able to comply with all study requirements and instructions of the site study staff
3. Male or female, 40 years to 85 years old (inclusive)
4. Must be ambulatory
5. Index knee must be symptomatic for greater than 6 months with a clinical diagnosis of OAK and supported by radiological evidence (Kellgren-Lawrence Grade 4) that is not older than 6 months prior to the date of screening
6. Moderate to moderately-severe OA pain in the index knee (rating of at least 1.5 on the WOMAC Index 3.1 as measured by 5-point Likert Pain Subscale) assessed at screening
7. Ability to temporarily discontinue nonsteroidal anti-inflammatory drug (NSAID) for 48 hours prior to scheduled clinical efficacy evaluations
8. No analgesia (including acetaminophen [paracetamol]) taken 12 hours prior to an efficacy measure

Sponsor:	Investigational Product:	Developmental Phase:
Ampio Pharmaceuticals, Inc.	Ampion™	Phase 3

9. No known clinically significant liver abnormality (e.g. cirrhosis, transplant, etc.).

Main Criteria for Exclusion:

1. As a result of medical review and screening investigation, the Principal Investigator considers the patient unfit for the study
2. A history of allergic reactions to human albumin (reaction to non-human albumin such as egg albumin is not an exclusion criterion)
3. A history of allergic reactions to excipients in 5% human albumin (N-acetyltryptophan, sodium caprylate)
4. Presence of tense effusions
5. Inflammatory or crystal arthropathies, acute fractures, history of aseptic necrosis or joint replacement in the affected knee, as assessed locally by the Principal Investigator
6. Isolated patella femoral syndrome, also known as chondromalacia
7. Any other disease or condition interfering with the free use and evaluation of the index knee for the duration of the trial (e.g. cancer, congenital defects, spine osteoarthritis)
8. Major injury to the index knee within the 12 months prior to screening
9. Severe hip osteoarthritis ipsilateral to the index knee
10. Any pain that could interfere with the assessment of index knee pain (e.g. pain in any other part of the lower extremities, pain radiating to the knee)
11. Any pharmacological or non-pharmacological treatment targeting OA started or changed during the 4 weeks prior to randomization or likely to be changed during the duration of the study
12. Use of the following medications:
    a. No IA injected pain medications in the study knee during the study
    b. No analgesics containing opioids. NSAIDs may be continued at levels preceding the study, however may not be used 48 hours prior to efficacy evaluations, and acetaminophen is available as a rescue medication during the study from the provided supply
    c. No topical treatment on osteoarthritis index knee during the study
    d. No significant anticoagulant therapy (e.g. Heparin or Lovenox) during the study (treatment such as Aspirin and Plavix are allowed)
    e. No systemic treatments that may interfere with safety or efficacy assessments during the study
    f. No immunosuppressants
    g. No use of corticosteroids > 10 mg prednisolone equivalent per day
    h. If corticosteroid use is ≤ 10 mg prednisolone equivalent per day, and if clinically indicated, subjects should be allowed to decrease their corticosteroid use. Additionally, some subjects may need to increase their steroid dose to treat worsened symptoms in the treated knee, and subjects who increase their corticosteroid dose above their starting dose of corticosteroid during the study will be treated as "treatment failures" for efficacy analysis
13. No human albumin treatment in the 3 months before randomization or throughout the duration of the study

Sponsor:	**Investigational Product:**	**Developmental Phase:**
Ampio Pharmaceuticals, Inc.	Ampion™	Phase 3

Test Product, Dose and Mode of Administration:

Ampion, 4 mL, single intra-articular injection in the knee

Reference Therapy, Dose and Mode of Administration:

Saline, 4 mL, single intra-articular injection in the knee

Study Duration:

24 weeks

Criteria for Evaluation:

Safety:
Adverse events, physical examination, and vitals

Efficacy:
WOMAC osteoarthritis Index 3.1 and PGA

Statistical Methods:

The co-primary efficacy endpoints are the change from Baseline to Week 12 in the WOMAC A pain score and the WOMAC C function score. The primary efficacy analysis will use the intent-to-treat (ITT) analysis population with the per-protocol (PP) analysis population as supportive. WOMAC A pain and WOMAC C function scores will be analyzed using mixed-effects models with repeated measures (MMRM) with treatment assignment, week, and baseline WOMAC A pain scores and WOMAC C function scores, respectively, as covariates, with comparisons between Ampion and saline.

All treatment emergent adverse events (TEAEs) will be reported by randomization assignment. There will be no statistical tests for incidence and severity of TEAEs.

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LIST OF IN-TEXT TABLES

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviation	Definition
°C	degrees Celsius
°F	degrees Fahrenheit
µg	microgram
AE	adverse event
BP	Blood pressure
CFR	Code of Federal Regulations
CI	confidence interval
CRF	case report form
CRO	Clinical Research Organization
Da	dalton
DA-DKP	aspartyl-alanyl diketopiperazine
DBP	diastolic blood pressure
DMP	Data Management Plan
eCRF	electronic case report form
eDC	electronic data capture
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HSA	human serum albumin
IA	intra-articular
ICF	informed consent form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IND	Investigational New Drug
IRB	Institutional Review Board
ITT	intent-to-treat
kDa	kilodalton
mg	milligram
mL	milliliter
MMRM	Mixed-effects model with repeated measures
NA	not applicable
NSAID	non-steroidal anti-inflammatory drug
OA	osteoarthritis
OAK	osteoarthritis of the knee
OMERACT-OARSI	outcome measures in rheumatology clinical trials and osteoarthritis research society international
PGA	patient's global assessment of disease severity
PP	per protocol population
REB	Research Ethics Board
SAE	serious adverse event
SAP	Statistical Analysis Plan
SBP	systolic blood pressure
SD	standard deviation
SEM	standard error of the mean
SMC	Safety Monitoring Committee
SOP	standard operation procedure

Abbreviation	Definition
TEAE	treatment-emergent adverse event
WBC	white blood cell
WOMAC	Western Ontario and McMaster Universities Arthritis Index

1 INTRODUCTION

The sole starting material of Ampion, human serum albumin (HSA), has a long history of clinical use as a colloid replacement therapy, dating back over 60 years. Currently, HSA is approved for intravenous administration for the indications of hypovolemia, hypoalbuminemia, prevention of central volume depletion after paracentesis due to cirrhotic ascites, ovarian hyperstimulation, adult respiratory distress syndrome (ARDS), acute nephrosis, hemolytic disease of the newborn and burns. In addition to its effects on oncotic pressure, HSA has pharmacological effects including decreased inflammation (Quinlan 2005), decreased vascular permeability (Evans 2002) and has no adverse effect on cardiac safety (Vincent JL 2004). The low molecular weight (< 5 kilodalton (kDa)) ultrafiltrate of 5% HSA, Ampion, is being developed as an intra-articular (IA) injection to provide relief for the pain due to severe osteoarthritis of the knee (OAK).

There are no currently FDA-approved drugs for the indication of pain from severe (Kellgren Lawrence [KL] Grade 4) OAK, for which Ampion is indicated. The active components in Ampion, aspartyl-alanyl diketopiperazine (DA-DKP), N-acetyl tryptophan (NAT), and caprylic acid (Caprylate), play a role in Ampion's biological activity and have been shown to reduce pro-inflammatory cytokine tumor necrosis factor-alpha (TNFα). DA-DKP is naturally present in human serum and is a cyclized dipeptide derived from the two N-terminal amino acids of albumin, aspartic acid and alanine. These amino acids are cleaved by a dipeptidase, and the resulting dipeptide is cyclized to form a diketopiperazine (aspartyl-alanyl diketopiperazine or DA-DKP). The formation of DA-DKP occurs during the HSA pasteurization process (Bar-Or 2013). The two other active components, NAT and Caprylate, are added during the manufacture of pharmaceutical HSA as stabilizers to protect albumin from heat denaturation and oxidation.

In vitro nonclinical studies, which use cellular models designed for the study of osteoarthritis, evaluate the role of Ampion in regulating inflammatory responses with cytokine tumor necrosis factor-alpha (TNFα), and other inflammatory markers, in immune cells (peripheral blood mononuclear cells, monocytes, macrophages), and support the clinical application of Ampion. A high-level summary of these studies is shown in Table 1.1.

Table 1.1 Summary of Ampion nonclinical studies

Study Objective	Summary of Findings
Anti-inflammatory effects of HSA and DA-DKP (TNFα, IFNγ)	HSA inhibited the production of pro-inflammatory cytokines (IFNγ, TNFα) in activated immune cells; however, when the low-molecular weight ultrafiltrate was removed from HSA, no anti-inflammatory effects were observed. DA-DKP was detected in HSA from multiple vendors, and different concentrations of synthetic DA-DKP (25-500 μM) were shown to inhibit TNFα release in a dose-dependent manner. This study identified early anti-inflammatory properties of Ampion (< 5 kDa ultrafiltrate of HSA) and of the active component DA-DKP. These results were published in the journal of *Critical Care Medicine* (Bar-Or 2006).

Study Objective	Summary of Findings
DA-DKP activity on cytokine production (TNFα, IFNγ, IL-8)	DA-DKP decreased production of pro-inflammatory cytokines (IFNγ, TNFα) in T-lymphocytes. DA-DKP also increased phosphorylation of the GTPase Rap-1, which in turn, decreased phosphorylated transcription factors involved in the regulation of TNFα and IFNγ gene transcription. Release of pro-inflammatory cytokine (IL-8) was not impacted by DA-DKP, suggesting that DA-DKP induction of active Rap-1 impacted the phosphorylation of only selected transcription factor pathways and was not a global effect. These results were published in the *Journal of Trauma, Injury, Infection, and Critical Care* (Shimonkevitz 2008)
Ampion anti-inflammatory activity (TNFα inhibition)	Ampion inhibited pro-inflammatory cytokine TNFα in activated immune cells by an average of 39%. Individual components contribute to activity: DA-DKP (100 µM) 17% TNFα inhibition, NAT (3.0 mM) 17% TNFα inhibition, and Caprylate (0.6 mM) 19% TNFα inhibition and is published in the *Journal of Immunoassay and Immunochemistry* (Thomas 2016).
Dose responses of Ampion and individual components (TNFα inhibition).	Ampion and its components have been shown to inhibit TNFα in a dose-dependent manner in activated immune cells (IND 15345).

This *in vitro* data translates to *in vivo* use in humans, as it has been reported that synovial levels of TNFα correlate directly with Western Ontario and McMaster Universities Arthritis Index (WOMAC) measures of pain, stiffness, and functional disability, while other studies have reported that TNFα levels are associated with pain while standing (Stannus 2013).

1.1 Study Drug

Ampion is the < 5 kDa ultrafiltrate of 5% HSA.

1.2 Background to the Disease

Osteoarthritis (OA) remains a leading contributor to global disability, with both hip and knee OA accounting for over 17 million years lived with disability (Cross 2014). OA is the most common form of arthritis and affects up to 38 million adults in the US alone. OA is caused by inflammation of the soft tissue and bony structures of the joint which worsens over time and leads to progressive thinning of articular cartilage, narrowing of the joint space, synovial membrane thickening, osteophyte formation and increased density of subchondral bone. These changes eventually result in chronic pain and disability and deterioration of the joint and may require eventual surgery for total joint replacement. OA of the knee is defined in stages as noted by Kellgren-Lawrence (KL) grading: Grade 0 (normal knee; no osteophytes or joint space narrowing), Grade 1 (possible osteophytic lipping and doubtful narrowing of joint space), Grade 2 (definite osteophytes and possible narrowing of joint space), Grade 3 (moderate multiple osteophytes, definite narrowing of joint space and some sclerosis, and possible deformity of the bone ends) and Grade 4 (large osteophytes, marked narrowing of joint space, severe sclerosis, and definite deformity of bone ends).

The pain associated with OA is now thought to be multifactorial. The pathogenesis of pain from OA is comprised of both nociceptive and neuropathic pain pathways resulting from severely damaged cartilage, exposed subchondral bone, joint innervation, and less defined pathways including the synovium. The balance between nociceptive and neuropathic pain shifts towards

neuropathic pain as the severity of the condition increases (Akinci 2016). The multifactorial nature of the pain of OA may make pain control more difficult, particularly in patients with severe OAK (KL 4). Patients with severe OAK have few treatment options for pain management. Difficult-to-treat pain conditions, such as severe OAK, are a challenge for both medical doctors and patients. FDA has acknowledged an unmet medical need for patients with severe OAK.

The presence of synovitis in KL 4 patients indicates active 'inflammatory' disease process. These findings were supported with a linear observation of an increasing trend correlated with the severity of synovitis related to advancing KL grades. Additionally, KL 4 patients were shown to have lesions that fluctuate with pain (i.e., fluctuating bone marrow lesions and synovitis) related to a high grade of cartilage loss in KL 4 patients (Guermazi 2015). These findings were further supported using a unique *in vivo* imaging technique that detected activated and not resting macrophages (Kraus 2016). In a patient cohort that included patients with severe (KL 4) OAK, the quantity of knee-related activated macrophages was statistically associated with more severe knee pain and radiographic severity of OAK.

These data demonstrate that greater macrophage mediated inflammation is a cause of more severe pain in KL 4 OAK and provides a rationale whereby Ampion, which has demonstrated *in vitro* anti-inflammatory and anti-macrophage activity, may mediate symptomatic relief in this patient group. These data further demonstrate the need to examine patients by disease severity and supply treatment options for severely diseased patients (Guermazi 2015). Ampion has shown anti-inflammatory activity *in vitro* against macrophages, monocytes, and immune cells (Bar-Or 2015, Frederick 2016, Thomas 2016, Thomas 2016b).

1.3 Previous Human Experience

In addition to the extensive HSA clinical experience, the sole starting material of Ampion, the Company has completed numerous clinical studies to evaluate the effect of Ampion in adults with OAK. These studies are summarized in Section 1.5.

1.4 Preclinical Data

1.4.1 Pharmacology Studies

In vitro pharmacology studies demonstrated that the low molecular weight ultrafiltrate of pharmaceutical preparations of HSA, and the cyclized dipeptide, DA-DKP, contained in that filtrate have a range of anti-inflammatory properties, which may be expected to ameliorate the symptoms of inflammation, including pain, in humans (Bar-Or 2006). Further, *in vitro* studies support anti-inflammatory properties for the < 5 kDa ultrafiltrate of HSA (Ampion) and its components (DA-DKP, NAT, Caprylate) with the reduction of pro-inflammatory cytokines (Shimonkevitz 2008, Thomas 2016, IND 15345). No pharmacokinetics studies were performed as the plasma levels of the constituents of the < 5 kDa preparation, including the active components (DA-DKP, NAT, Caprylate), after intra-articular (IA) injection of a therapeutic dose in humans or in laboratory animals would be below the limits of detection. For more information, consult the Investigator Brochure.

1.4.2 Toxicity and Safety Studies

HSA and other components of serum albumin preparations are species specific and may be expected to be immunogenic if repeatedly injected into non-human species, even at very low concentrations. For this reason, it was not possible to perform formal toxicological studies in animals.

1.5 Clinical Experience

Ampio has conducted multiple clinical studies to evaluate the treatment effect and safety of Ampion in subjects with OAK. Ampion has been administered across six clinical studies as a single IA injection or as three IA injections administered every two weeks. Four single-dose pivotal Phase 3 studies, two multi-dose studies, and one multiphase single-dose Phase 1b study have been completed. An overview of the efficacy studies is provided in the Table 1.2.

For the clinical development program, all patients were required to have x-ray findings demonstrating KL 2, 3, or 4 disease severity. All studies enrolled patients with KL 4 disease. In addition, eligible subjects had at least moderate pain at Baseline (defined as a score of at least 1.5 on the WOMAC Index 3.1, as measured by the 5-point Likert pain subscale at screening) in the study knee, which was to have been symptomatic for greater than six months with a clinical diagnosis of OA confirmed by radiological evidence. The clinical effects of treatment on OA pain were evaluated during clinic visits, with phone call follow-ups according to the study visit schedule.

Table 1.2 Summary of the Studies Conducted with Ampion Administered as an Intra Articular Injection to the Knee

Study	Phase	N	N: KL 2, 3, 4	Route	Primary outcome	Primary endpoint	Additional outcomes	Test product
Single-Injection Phase 3 Studies in Support of Efficacy & Safety								
AP-003-A	3	329	115 / 139 / 75	Single IA injection	WOMAC A pain, 5- point Likert scale	Week 12	WOMAC B WOMAC C PGA OMERACT -OARSI Rescue analgesia	Ampion, 4 mL and 10mL
AP-004	3	538	133 / 195/ 210	Single IA injection	WOMAC A pain, 5- point Likert scale	Week 12	WOMAC C PGA WOMAC A pain over 12 weeks	Ampion, 4 mL

Study	Phase	N	N: KL 2, 3, 4	Route	Primary outcome	Primary endpoint	Additional outcomes	Test product
AP-003-C	3	168	0 / 0 / 168	Single IA injection	OMERACT-OARSI responder	Week 12	WOMAC A, B, C and PGA	Ampion, 4 mL
AP-003- B	3	480	101 / 247 / 132	Single IA injection	WOMAC A pain, 5- point Likert scale	Week 12	WOMAC B WOMAC C PGA Rescue analgesia WOMAC A pain with movement, pain at rest	Ampion, 4 mL
Additional Clinical Studies in Support of Safety								
AP-007	1 / 2	47	2 / 23/ 15	Multiple IA injection; 3 injections, every 2 weeks	WOMAC A pain, 5- point Likert scale	Week 20	WOMAC B WOMAC C PGA Physical activity Radiologic changes	Ampion, 4 mL
AP-008	3	342	0 / 114 / 228	Multiple IA injection; 3 injections, every 2 weeks	WOMAC A pain, 5- point Likert scale	Week 20	WOMAC B WOMAC C PGA Physical activity WOMAC A pain with movement	Ampion, 4 mL
AIK	1 / 2	103	33 / 69 / 1	Single IA injection	Pain, 10- point NRS	Day 3, 8, 30 and 84	WOMAC total, parts A, B, C Range in motion Rescue analgesia	Ampion 10 mL, alone or in solution with betamethasone and/or lidocaine and/or saline

In all four well-controlled, single-injection studies, Ampion demonstrated a larger improvement in pain in KL 4 patients compared to saline, indicating a reproducible and consistent response. Analysis of the combined data demonstrate a statistically significant result in this underserved patient population.

In addition to the efficacy analysis described in this section, Ampion has also demonstrated a robust safety profile across the four well-controlled, single-injection studies, as well as the multiple-injection and early phase trials (Table 1.3). No drug-related Serious Adverse Events (SAEs) have been reported in 2,007 patients treated during the clinical development program. The Adverse Event (AE) profile is similar for

Ampion and saline, with a majority of the AEs of minor or moderate severity and unrelated to treatment.

Table 1.3 Summary of Adverse Events

Adverse event (AE)	Overall population		KL 4 population	
	Ampion N=1,076	Saline N=931	Ampion N=475	Saline N=361
One or more AE	381 (35.4%)	386 (41.5%)	150 (30.1%)	140 (38.8%)
One or more related AE	11 (1.0%)	19 (2.0%)	3 (0.6%)	6 (1.7%)
AE by severity*				
Mild	264 (24.5%)	253 (27.2%)	100 (21.2%)	86 (23.8%)
Moderate	162 (15.1%)	190 (20.4%)	58 (12.3%)	74 (20.5%)
Severe	35 (3.3%)	32 (3.4%)	17 (3.6%)	14 (3.9%)
Serious AE (SAE)	15 (1.4%)	20 (2.1%)	5 (1.1%)	7 (1.9%)
AE leading to withdrawal	0 (0%)	0 (0%)	0 (0%)	0 (0%)
AE leading to death	0 (0%)	0 (0%)	0 (0%)	0 (0%)

**Subjects could have AEs in more than one severity category*

CONCLUSIONS

Clinical efficacy observed in previous single-injection studies suggest that Ampion safely relieves pain due to severe (KL 4) osteoarthritis of the knee, the most severe form of OAK, for which there is no FDA approved treatment. Currently, when KL 4 patients are treated for OAK, they are receiving treatment using IA therapy (such as viscosupplementation) that were not studied, nor required in their device approvals to demonstrate efficacy in the KL 4 population (Rutjes 2012). Historical treatments for OAK pain also involve opioid-based treatments or surgical intervention. Ampion treatment offers clinically meaningful pain relief for an unmet medical need in severely diseased KL 4 patients with a single IA injection.

2 RATIONALE FOR THE STUDY

This is a Phase 3 randomized study designed to confirm the efficacy of an IA injection of Ampion and is designed to support the completed single injection Ampion studies.

2.1 Rationale for the Doses and the Dosing Regimen

This trial will use the 4 mL volume of Ampion used in the single-injection studies (AP-003-A, AP-004, AP-003-B and AP-003-C).

3 STUDY DESIGN

3.1 Study Design Overview

This is a randomized, saline-controlled, double-blind study with a 28-day screening period for each patient followed by a 24-week participation period. This trial will use an adaptive design with promising zone method for sample size re-estimation at the interim analysis. Daily assessments of WOMAC A pain using a subject diary will be assessed for seven days beginning one week prior to the clinic visits at Week 0 (baseline) and Week 12 to allow for calculation of a WOMAC A weekly pain score, which will be used for conducting the primary efficacy analysis.

This trial is designed for 1,034 patients, 517 per study arm, randomized 1:1 for Ampion and saline IA injection. There will be an interim analysis at 724 patients, 362 per study arm, randomized 1:1, Ampion and saline. After the interim analysis, enrollment will either a) continue up to a total of 1,034 patients, 517 patients per study arm, randomized 1:1 for Ampion and saline IA injection; b) allow for sample size re-estimation using the promising zone method (Pocock, 2011) up to 1.5 times the original trial size for a total of 1,552 patients, 776 per study arm, randomized 1:1 for Ampion and saline IA injection.

The clinical effects of treatment on severe OAK will be evaluated at baseline 2, 4, 6, 8, 10, 12, and 24 weeks, using the Western Ontario and McMaster Universities Arthritis Index (WOMAC®) osteoarthritis Index 3.1, and the Patient's Global Assessment of disease severity (PGA).

The WOMAC® and PGA are validated scoring systems and sets the standard for the patient response. In order not to bias the collection of data, only questions from the validated WOMAC Index 3.1 and the PGA will be asked of patients.

The trial has co-primary hypotheses and requires success on both for a successful trial. First, pain reduction must be demonstrated for Ampion vs. saline control. Next, a function improvement must be shown on Ampion vs. saline control.

The value for clinical benefit is derived from the minimal clinically important improvement (MCII), which is defined as the smallest change that signifies an important improvement to the patient (Tubach 2005). The Initiative on Methods, Measurement, and Pain Assessment in Clinical Trials (IMMPACT) advocates a provisional benchmark of 10-20% for minimally important improvement (Dworkin 2008). In addition, two studies have reported results of a survey of key opinion leaders and clinicians to determine value was best for MCII, suggesting a 20% MCII may be useful in patients with OAK (Tubach 2005), including severe OAK (Salottolo 2018). Clinical benefit will be determined as observed 20% improvements from baseline in pain and function at Week 12 to determine improvements are clinically important and not just statistically significant.

Clinical meaningfulness will be determined by the end results of this trial, specifically by the apparent clinical benefit versus any adverse events or any increased apparent risk. Safety will be assessed by recording adverse events (through 24 hours post-dose and at all follow up contacts) and physical examination and vitals (Baseline, Weeks 6, 12, and 24).

3.2 Study Objectives

3.2.1 Primary Objective

The co-primary trial objectives are to evaluate the greater efficacy for pain improvement and function improvement of 4 mL Ampion versus saline IA injection when applied to patients suffering from severe OAK. Efficacy will primarily be assessed with WOMAC A pain and WOMAC C function scores.

Mean change in the WOMAC A weekly pain scores from Baseline to Week 12 will be compared between Ampion and saline control. Mean change in WOMAC C function score will be compared between Ampion and saline control. This will ensure that Ampion is superior to saline in improving pain and function.

3.2.2 Secondary Objectives

The secondary trial objectives include the following evaluations of an intra-articular injection of Ampion, as compared to saline, when administered to patients suffering from severe OAK: improvement in pain utilizing a 100 mm Visual Analogue Scale (VAS) scale; assessment of safety; improvement in the Patient Global Assessment; and the amount of rescue analgesia.

3.2.3 Exploratory Objectives

Exploratory objectives include analyzing the effect, if any, of the efficacy of Ampion, as compared to saline, on stiffness; Outcome Measures in Rheumatology Clinical Trials and Osteoarthritis Research Society International (OMERACT-OARSI) responder status; proportion of treatment failures; change in pain and function as a cumulative distribution function (CDF) plot.

Analysis with biomarkers, such as those for analgesic use and chemokines and cytokines associated with inflammation and cartilage regeneration, may be analyzed to compare differences between treatment groups using urine samples collected at Week 12.

Additionally, a Bayesian meta-analysis will be performed for WOMAC A Likert (LK) pain sub score using all KL 4 patients in studies AP-003A, AP-003-B, AP-003-C, AP-004, and AP-013.

3.3 Safety Monitoring Committee

A Safety Monitoring Committee (SMC) will be established to review the safety of Ampion as the study progresses. The SMC will consist of independent clinicians and statisticians not involved in the conduct of the clinical trial. The Medical Monitor will attend open sessions of the SMC meetings. The SMC will be primarily responsible for reviewing any serious Adverse Event (SAE) and other clinically important safety findings (e.g., discontinuations due to AEs) that may occur during the study. Prior to unblinding any treatment assignments, a charter will be developed to detail the SMC review responsibilities, the frequency of meetings, and data evaluation plans.

A promising zone analysis will be run after outcomes are available for 724 patients (70% of final sample size). The promising zone analysis will be run for WOMAC C since function will be the predominant determinant of sample size.

The SMC, with the exclusion of the Medical Monitor, will also be tasked with evaluating whether sample size increase criteria has been met for the interim efficacy analysis. The SMC committee will review the interim analyses and report the next steps to the Sponsor, which will include one of the following prespecified actions: (1) continue to the a total of 1,034 patients, 517 patients per study arm, randomized 1:1 for Ampion and saline IA injection; (2) sample size re-estimation up to 1.5 times the original trial size for a total of 1,552 patients, 776 per study arm, randomized 1:1 for Ampion and saline IA injection, has occurred using the promising zone and the new sample size will be reported.

3.4 Stopping Rules

There are no stopping rules for early success stopping. The trial will enroll 1,034 patients, or if the promising zone triggers an expansion, up to 1,552 patients.

The Safety Monitoring Committee may recommend trial termination if there is a safety concern.

3.5 Study Endpoints

3.5.1 Co-Primary Endpoint

The trial will test whether Ampion can produce a superior improvement in pain and function vs. saline control. This goal is achieved using two co-primary endpoints:

Change in the WOMAC A pain sub score between Baseline and Week 12
Change in the WOMAC C physical function sub score between Baseline and Week 12

Mean 12-week change in the WOMAC A weekly pain scores determined at Baseline and Week 12 will be compared between Ampion and saline control. Mean 12-week change in WOMAC C physical function sub score will be compared between Ampion and saline control.

3.5.2 Secondary Endpoints

- Change in PGA between Baseline and Week 12
- Change in the 100 mm VAS Pain scale between Baseline and Week 12
- Use of rescue analgesia (amount of acetaminophen used between Baseline and Week 12)
- Incidence and severity of treatment-emergent adverse events (TEAEs)

3.5.3 Exploratory Endpoints

- Change in WOMAC B stiffness sub score between Baseline and Weeks 2, 4, 6, 8, 10, 12, and 24

- Response status based on the OMERACT-OARSI criteria at Weeks 2, 4, 6, 8, 10, 12 and 24
- Proportion of treatment failures at Week 12
- Change in pain and function analysis as a cumulative distribution function (CDF) plot
- A Bayesian meta-analysis for LK WOMAC A pain sub score including KL 4 patients from studies AP-003-A, AP-003-B, AP-003-C, AP-004, and AP-013
- Change in WOMAC A pain sub score between Baseline and Weeks 2, 4, 6, 8, 10, and 24
- Change in WOMAC C function sub score between Baseline and Weeks 2, 4, 6, 8, 10, and 24
- Change in WOMAC C function sub score between Baseline and Weeks 2 through 12 and Weeks 2 through 24
- Change in PGA between Baseline and Weeks 2, 4, 6, 8, 10, and 24
- Change in WOMAC C physical function sub score between Baseline and Weeks 2, 4, 6, 8, and 10
- If collected, difference in urine analysis between treatment groups at Week 12

3.6 Blinding and Randomization

Patients will be assigned to treatment by a randomization schedule developed and maintained by an independent statistician. Patients, the investigator, Sponsor, and all study staff having a role in the day-to-day conduct of the study will remain blinded to the treatment assignment.

Ampion and saline will be provided as blinded study vials labeled with the appropriate information. To maintain study blinding, vial labels will be designed to adequately cover vial contents and prevent discrimination between active and placebo. Syringes, needles, and rescue medication (acetaminophen) will be provided to the sites.

A comprehensive presentation of the Data Management Plan (DMP) and Statistical Analysis Plan (SAP) will be approved by Ampio Pharmaceuticals, Inc. prior to unblinding of study data. The SMC will be unblinded to perform the interim analysis.

3.7 Code-breaking

Where required, safety personnel and/or investigator may be unblinded to a particular patient's treatment assignment to meet reporting requirements to Regulators. Unblinding may occur by contacting Ampio Pharmaceuticals, Inc.

4 SELECTION OF PATIENTS

4.1 Number of Patients

This trial will use an adaptive design with promising zone method for sample size re-estimation.

This trial is designed for 1,034 patients, 517 per study arm, randomized 1:1 for Ampion and saline IA injection. There will be an interim analysis at 724 patients, 362 per study arm, randomized 1:1, Ampion and saline.

Following the interim analysis, enrollment will either a) continue up to a total of 1,034 patients, 517 patients per study arm, randomized 1:1 for Ampion and saline IA injection; b) allow for sample size re-estimation up to 1.5 times the original trial size for a total of 1,552 patients, 776 per study arm, randomized 1:1 for Ampion and saline IA injection.

The Ampion effect was estimated as the average change in pain from Baseline to Week 12 for KL 4 subjects receiving Ampion from all prior single injection studies randomized 1:1 with saline control. Saline effect was estimated as the average change in pain from Baseline to Week 12 for KL 4 subjects receiving saline from all prior single injection studies randomized 1:1 for Ampion and saline. The sample size for assessing the WOMAC A Pain score alone would be 690 total patients for 90% power.

The Ampion effect was estimated as the average change in function from Baseline to Week 12 for KL 4 subjects receiving Ampion from all prior single injection studies randomized 1:1 with saline control. Saline effect was estimated as the average change in function from Baseline to Week 12 for KL 4 subjects receiving saline from all prior single injection studies randomized 1:1 for Ampion and saline. The sample size for assessing the WOMAC C Function score alone would be 984 patients for 90% power.

The total sample size is chosen as the larger of the two values. To be conservative sample sizes were estimated with a 5% dropout so 1,034 patients will be enrolled to achieve 984 total patients. All ITT patients will be included in the statistical analysis. At the assumed effect size there is a 90% probability of success at 1,034 patients.

The study design of 1,034 total patients achieves 97% power assuming a WOMAC A pain score with mean pain decreases of -0.83 for Ampion and -0.62 for saline and a standard deviation of 0.85.

As described in Section 10, a Promising Zone method will be employed for a sample size re-estimation and the trial may increase to 1,552 patients.

4.2 Recruitment Methods

Patients will be recruited from the population being seen by Investigators at the clinical sites participating in the study. In addition, notifications about the opportunity for patients to participate in a clinical trial will be sent to referring physicians. A description of the clinical trial will also be posted at ClinicalTrials.gov, and advertisements and/or other notices may be produced to advise potential study patients on how they may obtain information about study

participation. All such materials will be reviewed and approved by the Institutional Review Board (IRB) prior to their publication or dissemination.

4.3 Inclusion Criteria

It is recommended that patients should have a WOMAC A pain sub score of < 1.5 as measured by the 5-point Likert Pain Subscale in the contralateral knee, which is assessed at screening. Patients should fulfill the following inclusion criteria:

1. Able to provide written informed consent to participate in the study
2. Willing and able to comply with all study requirements and instructions of the site study staff
3. Male or female, 40 years to 85 years old (inclusive)
4. Must be ambulatory
5. Index knee must be symptomatic for greater than six months with a clinical diagnosis of OAK and supported by radiological evidence (Kellgren-Lawrence Grade 4) that is not older than six months prior to the date of screening
6. Moderate to moderately-severe OA pain in the index knee (rating of at least 1.5 on the WOMAC Index 3.1 as measured by the 5-point Likert Pain Subscale,) assessed at screening
7. Ability to temporarily discontinue NSAID for 48 hours prior to scheduled clinical efficacy evaluations
8. No analgesia (including acetaminophen [paracetamol]) taken 12 hours prior to an efficacy measure
9. No known clinically significant liver abnormality (e.g. cirrhosis, transplant, etc.)

4.4 Exclusion Criteria

Patients fulfilling one or more of the following criteria may not be enrolled in the study:

1. As a result of medical review and screening investigation, the Principal Investigator considers the patient unfit for the study
2. A history of allergic reactions to human albumin (reaction to non-human albumin such as egg albumin is not an exclusion criterion)
3. A history of allergic reactions to excipients in 5% human albumin (N-acetyltryptophan, sodium caprylate)
4. Presence of tense effusions
5. Inflammatory or crystal arthropathies, acute fractures, history of aseptic necrosis or joint replacement in the affected knee, as assessed locally by the Principal Investigator
6. Isolated patella femoral syndrome, also known as chondromalacia
7. Any other disease or condition interfering with the free use and evaluation of the index knee for the duration of the trial (e.g. cancer, congenital defects, spine OA)
8. Major injury to the index knee within the 12 months prior to screening
9. Severe hip OA ipsilateral to the index knee
10. Any pain that could interfere with the assessment of index knee pain (e.g. pain in any other part of the lower extremities, pain radiating to the knee)
11. Any pharmacological or non-pharmacological treatment targeting OA started or changed during the four weeks prior to randomization or likely to be changed during the duration of the study

12. Use of the following medications:
    a. No IA injected pain medications in the study knee during the study
    b. No analgesics containing opioids. NSAIDs may be continued at levels preceding the study, however may not be used 48 hours prior to efficacy evaluations, and acetaminophen is available as a rescue medication during the study from the provided supply
    c. No topical treatment on osteoarthritis index knee during the study
    d. No significant anticoagulant therapy (e.g. Heparin or Lovenox) during the study (treatment such as Aspirin and Plavix are allowed)
    e. No systemic treatments that may interfere with safety or efficacy assessments during the study
    f. No immunosuppressants
    g. No use of corticosteroids > 10 mg prednisolone equivalent per day
    h. If corticosteroid use is ≤ 10 mg prednisolone equivalent per day, and if clinically indicated, subjects should be allowed to decrease their corticosteroid use. Additionally, some subjects may need to increase their steroid dose to treat worsened symptoms in the treated knee, and subjects who increase their corticosteroid dose above their starting dose of corticosteroid during the study will be treated as "treatment failures" for efficacy analysis
13. Any human albumin treatment in the three months before randomization or throughout the duration of the study.

4.5 Inclusion of Patients Incapable of Giving Informed Consent

No patient incapable of giving informed consent may be enrolled in the study.

5 STUDY PLAN AND PROCEDURES

Patients will be screened for inclusion into the clinical study, which will include the ability to record pain on a daily basis for seven days prior to randomization. Patients will be randomized into a study arm and receive an IA injection of Ampion or saline. Telephone contact will be made with the patient 24 hours after the IA injection. The patient will be followed for 24 weeks and the clinical effects of treatment on OAK pain will be evaluated during clinic visits at 6, 12, and 24 weeks, and during telephone contacts at 2, 4, 8 and 10 weeks, using the WOMAC osteoarthritis Index 3.1 (pain sub score, stiffness sub score and function sub score) and an overall global severity assessment (PGA).

Safety will be assessed by recording adverse events (through 24 hours post-dose and at all follow-up in-clinic visits and telephone contacts), vital signs (Baseline, and Weeks 6 and 12), recording prior and concomitant medications including start/stop dates, indication, dose and frequency (through 24 hours post-dose and at all follow-up in-clinic visits and telephone contacts) and physical examination (Baseline and Weeks 6, 12, and 24). The assessments and procedures performed at each patient visit or contact are described in Section 5 and in Table 6.1.

5.1 Description of Study Visits

5.1.1 Visit -1 (Day -28 through Day 0, in-clinic): Screening

The following procedures will be performed:

- Obtain written informed consent before the start of any study specific procedure.
- Review medical history including all previous treatments for OA
- Record prior and concomitant medications including start/stop dates, indication, dose and frequency
- Record demographic data including year of birth, age, gender and race
- Measure and record height and weight
- Perform and record physical examination
- Record KL Grade from radiological assessment that is not older than 6 months prior to screening. Patient may have an x-ray at screening in order to satisfy this requirement
- Record pain in the index knee with the WOMAC Index 3.1 using both the 5-point Likert scale and the 100mm VAS scale
- Record pain in the contralateral knee with the WOMAC Index 3.1 pain subsection using both the 5-point Likert scale and the 100mm VAS scale
- Record vital signs. (Measure body temperature, systolic blood pressure [SBP], diastolic blood pressure [DBP] and pulse rate)
- Evaluate all inclusion and exclusion criteria to ensure that patients meet all inclusion criteria and none of the exclusion criteria
- Review collection instructions of daily diary pain scores with subject

5.1.2 Visit 0 (Day -6, telephone contact): Pre-Baseline Daily Assessments

The following procedures will be performed:

- Review requirements for daily collection of WOMAC A pain evaluation using both the 5-point Likert scale and the 100mm VAS scale
- Confirm Baseline visit date
- It is possible, depending on the Baseline visit date and successful completion of Screening criteria defined above, that the first Pre-Baseline Daily Assessment may occur during the Screening visit.

5.1.3 Visit 1 (Day 0, in-clinic): Baseline/Randomization/Treatment

The following procedures will be performed:

- Confirm eligibility (review inclusion/exclusion criteria)
- Record concomitant medications
- Conduct pregnancy test, as applicable
- Perform and record physical examination
- Record vital signs (Measure body temperature, SBP, DBP, and pulse rate) pre- and post-injection
- Perform and/or review the final Pre-Baseline WOMAC A pain evaluation using both the 5-point Likert scale and the 100mm VAS scale
- Perform WOMAC B and C prior to treatment
- Perform PGA evaluations prior to treatment
- Randomize patient to study arm
- Perform intra-articular injection of blinded study treatment
- Record post-injection AEs if observed
- Issue rescue medication (acetaminophen [paracetamol])

5.1.4 Visit 2 (Day 1, telephone contact)

The following procedures will be performed:

- Record AEs
- Record concomitant medications
- Review rescue medication use

5.1.5 Visit 3 (Week 2 ± 7 days, telephone contact)

The following procedures will be performed:

- Perform WOMAC and PGA evaluations of the study knee
- Record AEs
- Record concomitant medications
- Review rescue medication use

5.1.6 Visit 4 (Week 4 ± 7 days, telephone contact)

The following procedures will be performed:

- Perform WOMAC and PGA evaluations of the study knee
- Record AEs
- Record concomitant medications
- Review rescue medication use

5.1.7 Visit 5 (Week 6 ± 7 days, in-clinic)

The following procedures will be performed:

- Perform and record physical examination
- Record vital signs. (Measure body temperature, SBP, DBP and pulse rate)
- Perform WOMAC and PGA evaluations of the study knee
- Record AEs
- Record concomitant medications
- Review rescue medication use

5.1.8 Visit 6 (Week 8 ± 7 days, telephone contact)

The following procedures will be performed:

- Perform WOMAC and PGA evaluations of the study knee
- Record AEs
- Record concomitant medications
- Review rescue medication use

5.1.9 Visit 7 (Week 10 ± 7 days, telephone contact)

The following procedures will be performed:

- Perform WOMAC and PGA evaluations of the study knee
- Record AEs
- Record concomitant medications
- Review rescue medication use

5.1.10 Visit 8 (Week 11 ± 7 days, telephone contact): Pre-Week 12 Daily Assessments

The following procedures will be performed:

- Review requirements for daily collection of WOMAC A pain scores using both the 5-point Likert scale and the 100mm VAS scale
- Confirm Week 12 visit date

5.1.11 Visit 9 (Week 12 ± 7 days, in-clinic)

The following procedures will be performed:

- Collect a urine sample for testing
- Perform and record physical examination
- Record vital signs. (Measure body temperature, SBP, DBP and pulse rate)
- Perform and/or review the final WOMAC A pain evaluation using both the 5-point Likert scale and the 100mm VAS scale
- Perform WOMAC B and C evaluations
- Perform PGA evaluations of the study knee
- Record AEs
- Record concomitant medications
- Review rescue medication use

5.1.12 Visit 10 (Week 24 ± 7 days, in-clinic)

The following procedures will be performed:

- Perform and record physical examination
- Record vital signs (Measure body temperature, SBP, DBP and pulse rate)
- Perform WOMAC and PGA evaluations of the study knee
- Record AEs
- Record concomitant medications
- Review rescue medication use

5.1.13 Early Termination Visit

The following procedures will be performed at Early Termination Visit:

- Perform and record physical examination
- Record vital signs. (Measure body temperature, SBP, DBP and pulse rate)
- Perform WOMAC and PGA evaluations of the study knee using both the 5-point Likert scale and the 100mm VAS scale
- Record AEs
- Record concomitant medications
- Review rescue medication use

5.1.14 Unscheduled Visits

Additional visits may be scheduled at the discretion of the Investigator, for example as part of follow-up of AEs. Unscheduled Visits will follow the visit structure described in Section 5.1.7 for the Week 6 visit.

5.1.15 Missed Visits

Patients unable to complete a study visit as scheduled should be re-scheduled for a replacement visit as soon as possible. If a subject misses any scheduled follow-up visit and cannot be seen within the +/- 7-day visit window, as described in Table 6.1, the visit is considered missed.

6 METHODS OF ASSESSMENT

Demographic Data

At Visit 1(Screening), patient demographic data will be collected. These include year of birth, age, gender and race.

Medical History

At Visit 1(Screening) a complete medical history, including prior interventions to the study knee, will be obtained from each patient.

Concomitant Medications

Detailed history of medications will be documented for each patient at Screening and Baseline. Concomitant medications (especially any changes in medication) will be documented for each patient at each scheduled visit.

Physical Examination and Vital signs

Height in feet and inches will be measured at Screening.

Body weight in pounds (lb) will be measured at Screening.

Body temperature (deg F) will be measured at each visit.

SBP and DBP and pulse rate will be measured with the patient in a seated position.

The full physical examination will consist of examining the following body systems: cardiovascular, respiratory, abdominal, skin and musculoskeletal other than the knee. The physical examination of the target knee will consist of evaluating the knee joint for effusion and tenderness on palpation. Table 6.1 below lists the various study activities and timing.

Biomarkers

At Week 12 urine samples will be collected and may be analyzed to compare differences between treatment groups for biomarkers, such as those for analgesic use and chemokines and cytokines associated with inflammation and cartilage regeneration.

6.1 Efficacy Assessments

Note: Efficacy questionnaire questions will be asked “with reference to study knee” i.e. to obtain scores specific for the treated knee.

6.1.1 WOMAC® Osteoarthritis Index (Bellamy 1988)

The clinical effects of treatment on OA pain and function will be evaluated during clinic visits at 6, 12, and 24 weeks, and telephone contacts at 2, 4, 8 and 10 weeks, using the WOMAC Index

3.1, and the PGA. All patients are required to take at least five minutes to complete the questionnaire.

Patients are asked about their pain, stiffness, and function in the knee (study joint) due to arthritis during the last 24 hours.

In addition to WOMAC scoring during clinic visits, daily WOMAC A pain sub scores will be collected for seven days up to and including Baseline and Week 12 to allow for calculation of WOMAC A weekly pain scores, which will be used for conducting the primary efficacy analysis.

Patients respond to each subscale by using a 5-point adjectival Likert score (0 = None, 1 = Mild, 2 = Moderate, 3 = Severe, 4 = Extreme) and 100mm VAS.

The WOMAC B and WOMAC C sub scores will be collected at Weeks 0 (Baseline), 2, 4, 6, 8, 10, 12, and 24 using the 5-point Likert scale. Patients respond to each subscale by using a 5-point adjectival Likert score (0 = None, 1 = Mild, 2 = Moderate, 3 = Severe, 4 = Extreme).

6.1.2 Patient's Global Assessment of Disease Severity (PGA)

The PGA should be completed by patients at Screening, pre-dose Day 1 (prior to injection), and at Weeks 2, 4, 6, 8, 10,12 and 24.

Patients are asked the following question: "Considering all the ways in which your arthritis affects you, please indicate how you are doing."

Patients respond by using a 5-point adjectival Likert score (0 = Very Well, 1 = Well, 2 = Fair, 3 = Poor, 4 = Very Poor).

6.2 Safety Parameters

Based on results of a Phase 1b and a Phase 2 randomized, controlled, repeat-blind study of a single IA injection of Ampion < 5 kDa ultrafiltrate of 5% HSA in adults with OAK (AIK study), in which no clinically significant differences between active and placebo were found in either electrocardiogram or blood parameters, safety will be assessed by recording adverse events, vital signs, results of physical examination, and by recording prior and concomitant medications.

6.2.1 Vital Signs

Vital signs (radial pulse rate, SBP, DBP, and body temperature) should be recorded at Screening, pre- and post–treatment Day 1, and then on visits at Weeks 6,12 and 24.

Vital signs should be taken after the patient has rested in a seated position for at least five minutes.

Table 6.1 Schedule of Assessments and Procedures

	Screening	Pre-Baseline (phone)	Baseline	24-hours (phone)	Week 2 (phone)	Week 4 (phone)	Week 6	Week 8 (phone)	Week 10 (phone)	Pre-Week 12 (phone)	Week 12	Week 24	Early Term.
Assessments & Procedures	**Visit -1 Day-28 to 0**	**Visit 0 Day -6 to 0**	**Visit 1 Day 0**	**Visit 2 Day 1**	**Visit 3 Day 14 ± 7**	**Visit 4 Day 28 ± 7**	**Visit 5 Day 42 ± 7**	**Visit 6 Day 56 ± 7**	**Visit 7 Day 70 ±7**	**Visit 8 Day 77 ±7**	**Visit 9 Day 84 ± 7**	**Visit 10 Day 174 ± 7**	
Informed Consent	X												
Inclusion/ exclusion criteria	X		X										
Medical history/prior medications	X		X										
Concomitant medications	X		X	X	X	X	X	X	X		X	X	X
Urine sample			X**								X		
Physical examination	X		X				X				X	X	X
Vital Signs	X		X				X				X	X	X
Randomization			X										
Review Diary Completion Instructions	X								X				
Phone Contact Daily Collection Reminder		X								X			
WOMAC A (Likert)	X	X*	X***		X	X	X	X	X	X*	X***	X	X
WOMAC A (VAS)	X	X*	X***							X*	X***		X
WOMAC B and C (Likert)	X		X		X	X	X	X	X		X	X	X
PGA	X		X		X	X	X	X	X		X	X	X
X-ray[1]	X												
Study treatment			X										
Dispense Rescue medication			X										
Review Rescue medication				X	X	X	X	X	X		X	X	X
Adverse Events			X	X	X	X	X	X	X		X	X	X

Visits are in clinic except for Day 1 and Weeks 2, 4, 8 and 10 when patients will be contacted by telephone

[1]X-ray may be acquired at Screening to satisfy inclusion criteria, "Index knee must be symptomatic for greater than six months with a clinical diagnosis of OA and supported by radiological evidence (KL 4) that is not older than six months prior to the date of screening".

*Patients will complete 7 daily pain score assessments up to and including the Baseline and Week 12 visits to allow calculation of WOMAC A weekly pain scores. The Screening and first Pre-Baseline daily pain score collection may occur on the same day, in office or at home, as appropriate.

**Urine will only be collected at Baseline if necessary to conduct a pregnancy test; collection to occur as applicable

***The WOMAC may occur in office or at home, and may serve as the 7th WOMAC Daily Pain collection

7 DISCONTINUATION CRITERIA

7.1 Early Discontinuation of the Study

It is agreed that for reasonable cause, either the investigator or the Sponsor may terminate this study, provided a written notice is submitted at a reasonable time in advance of intended termination; if by the investigator notice is to be submitted to Ampio Pharmaceuticals, Inc., and if by the Sponsor, notice will be provided to each investigator.

If a severe local reaction or drug-related SAE occurs at any time during the study, the Safety Monitoring Committee will review the case immediately.

The study will be immediately suspended and no additional Ampion treatments administered pending review and discussion of all appropriate study data by the SMC if one or more patients develop any of the following adverse events deemed to be possibly, probably, or definitely related to Ampion by the Investigator and/or Medical Monitor, based upon close temporal relationship or other factors:

- Death
- Anaphylaxis (angioedema, hypotension, shock, bronchospasm, hypoxia, or respiratory distress) Induction of autoimmune arthritis
- Hepatic failure
- Aplastic anemia

The study will not be restarted until all parties have agreed to the course of action to be taken and the IRB/EC has been notified.

7.2 Early Discontinuation of Individual Patients

Patients are to be withdrawn from the study for any of the following reasons:

- Withdrawal of informed consent
- Patient is lost to follow-up

Patients will also be withdrawn at any time if the investigator concludes that it would be in the patient's best interest for any reason. Protocol violations do not lead to patient withdrawal unless they constitute a significant risk to the patient's safety.

Patients can voluntarily withdraw from the trial for any reason at any time. They are to be considered withdrawn if they state an intention to withdraw, fail to return for visits, or become lost to follow up for any reason. Patients withdrawing from the study because of an AE should be followed for at least 30 days, resolution of the AE or until no further improvement is expected, whichever comes first.

8 TREATMENT

Eligible patients will receive a single IA injection into one knee of either Ampion or a saline injection, dependent on study arm.

Patients will be allocated to a treatment in accordance with the randomization schedule following confirmation of eligibility before treatment.

8.1 Dosing and administration of study medication

Appropriately trained site personnel should administer the study treatment.

If both knees are osteoarthritic, then the Investigator at Screening should select the knee that best satisfies the requirements for the study (see inclusion criteria) as the study knee. The study knee should be treated in accordance with the randomization schedule.

It is recommended that the study treatment be administered into the knee joint space under sterile prep conditions (i.e. the knee should be cleaned with an antiseptic). The treatment area may be anesthetized with local anesthesia including a topical anesthesia, injection of lidocaine, or nothing, as determined by the Principal Investigator. The recommended needle of choice for this procedure is a 25-gauge needle that is 1.5 inches long. The needle may be passed obliquely towards the trochlear groove. If an effusion is present, aspiration should not be performed, and the effusion should be noted in the study source notes/eCRF. Injection should proceed easily. Failure to easily inject should be documented. The same sized needle should be used for both Ampion and saline administration and recorded in the study source notes/eCRF. The date of administration should be recorded in the study source notes/eCRF.

8.2 Drug Storage and Accountability

Study drug should be stored at controlled room temperature (59º – 77ºF or 15º – 25ºC). Each site shipment of study drug will include temperature monitors and instructions to download the temperature data to Ampio Pharmaceuticals, Inc. If the study drug is stored at the clinical site, temperature should be monitored at least daily until use to ensure storage at the defined temperature.

The Investigator, the clinical site pharmacist, or other personnel authorized to store and dispense investigational product is responsible for ensuring that the investigational product used in the clinical study is securely maintained as specified by the Sponsor and in accordance with the applicable regulatory requirements.

All investigational product is to be dispensed in accordance with the Investigator's prescription and it is the Investigator's responsibility to ensure that an accurate record is maintained of investigational product issued and returned.

If any quality issue is noticed upon the receipt or use of an investigational product (i.e. deficiencies in condition, packaging, appearance, associated documentation, labeling, etc.), Ampio Pharmaceuticals, Inc. must be promptly notified.

Under no circumstances may the Investigator supply investigational product to a third party, allow the investigational product to be used other than as directed by this clinical study protocol, or dispose of investigational product in any other manner.

Ampio, or designated representative, will instruct sites on study kit returns and/or destruction of study drug. If returned, study kits must be returned to Ampio Pharmaceuticals, Inc., or designated representative after study completion.

8.3 Concomitant Treatments and Rescue Medication

The following medications / therapies are NOT allowed during this clinical study:

1. No IA injected pain medications in the study knee during the study.
2. No analgesics containing opioids.
3. No NSAID use during the 48 hours prior to efficacy evaluations.
4. No clinically significant increase in NSAID use above baseline/pre-study dosing levels to treat worsened symptoms in the treated knee.
    a. NSAID use during the study may be continued at baseline/pre-study dosing levels and may be increased or decreased if clinically indicated; acetaminophen is also available as a rescue medication during the study from the provided supply.
5. No non-pharmacological treatment targeting OA started or changed during the study.
6. No topical treatment on osteoarthritis index knee during the study.
7. No significant anticoagulant therapy (e.g. Heparin or Lovenox) during the study (treatment such as Aspirin or Plavix is allowed).
8. No systemic treatments that may interfere with safety or efficacy assessments during the study.
9. No immunosuppressants.
10. No use of corticosteroids > 10 mg prednisolone equivalent per day.
    a. If corticosteroid use is ≤ 10 mg prednisolone equivalent per day, and if clinically indicated, subjects should be allowed to decrease their corticosteroid use. Additionally, some subjects may need to increase their steroid dose to treat worsened symptoms in the treated knee, and subjects who increase their corticosteroid dose above their starting dose of corticosteroid during the study will be treated as “treatment failures” for efficacy analysis.
11. No human albumin treatment during the study.

Any medication used during the study should be recorded. All concomitant medication start and stop dates, total daily dose, route and indication should to be recorded.

The only allowed rescue medication is 500 mg of acetaminophen (paracetamol), one tablet every four hours as required by the patient.

8.4 Treatment Compliance

The injection of study drug will be performed by the unblinded injector(s). A 5 mL syringe and 25-gauge needle will be provided. Compliance with treatment is thus assured.

9 ADVERSE EVENTS

9.1 Definition of an adverse event

An adverse event (AE) is defined as any undesired medical occurrence in a patient or clinical investigation patient receiving a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable sign or unintended sign (including an abnormal laboratory finding), symptom, or disease temporarily associated with the use of a study drug, whether or not it is related to the study drug.

Assessment of severity of an AE will be rated according to categories listed in Table 9.1 .

Table 9.1 Definitions of AE Severity

Grade 1 (MILD): The symptom is barely noticeable to the study patient and does not influence performance or functioning. Concomitant medication is not ordinarily indicated for relief of mild AEs.
Grade 2 (MODERATE): The symptom is of sufficient severity to make the study patient uncomfortable and to influence performance of daily activities. Concomitant medication may be indicated for relief of moderate AEs.
Grade 3 (SEVERE): The symptom causes severe discomfort, sometimes of such severity that the study patient cannot continue in the study. Daily activities are significantly impaired or prevented by the symptom. Concomitant medication may be indicated for relief of severe AEs.

Determination of the relationship between the AE and the study drug will be made using the guidelines presented in Table 9.2.

Table 9.2 Guidelines for Determining the Relationship (if any) Between Adverse Event and the Study Drug

Unrelated	The adverse event is unlikely to have been caused by study drug.
Possibly related	It is unclear whether the adverse event may have been caused by study drug.
Related	The adverse event is likely to have been caused by study drug.

9.2 Definition of a Serious Adverse Event

A Serious Adverse Event (SAE) is any untoward medical occurrence that occurs at any dose that:

- Results in death
- Is life-threatening (patient is at immediate risk of death from the event as it occurred)
- Requires in-patient hospitalization (formal admission to a hospital for medical reasons) or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Results in a congenital anomaly/birth defect

Important medical events that may not result in death, are not life-threatening, or do not require hospitalization may be considered SAEs when, based on appropriate medical judgment, they

may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in in-patient hospitalization, or the development of drug dependency or drug abuse.

Hospitalizations for elective surgery or other medical procedures that are not related to a treatment-emergent AE are not considered SAEs.

9.3 Recording of adverse events and serious adverse events

Recording and reporting of adverse events should be in accordance with the FDA final "Guidance for Industry and Investigators Safety Reporting Requirements for INDs and BA/BE Studies" of December 2012.

Any AE is to be recorded in the eCRF. In order to avoid vague, ambiguous, or colloquial expressions, the AE should be recorded in standard medical terminology rather than the patient's own words. Whenever possible, the investigator should combine signs and symptoms that constitute a single diagnosis.

The existence of an AE may be concluded from a spontaneous report of the patient, from the physical examination, or from special tests (e.g. laboratory assessments, where applicable, or other study-specified tests [source of AE]).

The reporting period begins from the time that the patient receives IA injection at the Baseline visit through including patient's Final Visit at 24 weeks. Any events continuing at study exit will be followed for 30 days or to resolution, or until no improvement is expected, whichever comes first. Any SAE occurring after the reporting period must be promptly reported if a causal relationship to the investigational drug is suspected. If the patient begins a new therapy, the safety reporting period ends at the time the new treatment is started, however, death must always be reported when it occurs during the 24-week study period irrespective of intervening treatment.

Each AE is to be evaluated for duration, severity, seriousness, and causal relationship to the investigational drug. Any action(s) taken, and the outcome must also be recorded.

9.3.1 AE Follow-up

All AEs occurring during the study are to be followed up in accordance with good medical practice until they are resolved, stabilized or judged no longer clinically significant or, if a chronic condition, until fully characterized. Any AEs that are considered drug-related (possibly related, definitely related) must be followed for 30 days, or to resolution, or until no improvement is expected, whichever occurs first.

9.3.2 Overdose

No information on treatment of overdose of Ampion is currently available. In the case of overdose, the patient should be followed as an AE and appropriate supportive medical treatment instigated.

9.4 Serious Adverse Event Reporting

9.4.1 Reporting Requirements

Unexpected serious suspected adverse reactions are subject to expedited reporting to FDA. All SAEs must be entered into the eCRF within 24 hours of first knowledge of the event by study personnel. It is important that the investigator provide his/her assessment of relationship to study drug at the time of the initial report. Entry of an SAE into the eCRF triggers an automatic alert to the CRO safety team. The following information must be reported:

- Protocol number
- Site and/or Investigator number
- Patient number
- Demographic data
- Brief description of the event
- Onset date and time
- Resolution date and time, if the event resolved
- Current status, if event not yet resolved
- Any concomitant treatment and medication
- Investigator's assessment of whether the SAE was related to Investigative product or not

The CRO Safety Associate will contact the site for clarification of data entered onto the eCRF, or to obtain missing information. In the event of questions regarding SAE reporting, the site may contact the appropriate individual as in Section 9.4.2.

9.4.2 SAE Contact Information

Ampio Main Line: 1 (720) 437-6500

Ampio Pharmaceuticals, Inc., or their designee CRO, is responsible for submitting reports of AEs associated with the use of the drug that are both serious and unexpected to FDA according to 21 CFR 312.32 and the final guidance (2012). All investigators participating in ongoing clinical studies with the study medication will receive copies of these reports for prompt submission to their Institutional Review Board (IRB) or Ethics committee (EC).

10 STATISTICAL METHODS

10.1 General Considerations

This section describes the rules and conventions to be used in the presentation and analysis of the data. A comprehensive presentation of the data management and statistical analysis plan will be approved by Ampio Pharmaceuticals, Inc. prior to unblinding of study data.

10.2 Study Objectives

The co-primary trial objectives are to evaluate the greater efficacy for pain improvement and function improvement of 4 mL Ampion versus 4 mL saline IA injection when applied to patients suffering from severe OAK. Efficacy will primarily be assessed with WOMAC A pain and WOMAC C function scores.

The secondary trial objectives include the following evaluations of an intra-articular injection of Ampion, as compared to saline, when administered to patients suffering from severe OAK: improvement in the 100 mm VAS Pain scale; assessment of safety; improvement in the Patient Global Assessment; the amount of rescue analgesia.

Exploratory objectives are to analyze the effect, if any, of the efficacy of Ampion, as compared to saline, on stiffness; OMERACT-OARSI responder status; proportion of treatment failures; change in pain and function will be examined as a CDF plot for Ampion and saline treated patients; and a Bayesian meta-analysis for LK WOMAC A pain sub score including KL 4 patients from studies AP-003-A, AP-003-B, AP-003-C, AP-004, and AP-013. Analysis with biomarkers, such as those for analgesic use and chemokines and cytokines associated with inflammation and cartilage regeneration, may be analyzed to compare differences between treatment groups using urine samples collected at Week 12.

10.3 Analysis Populations

10.3.1 Safety Analysis Population:

The safety analysis population is defined as all patients who are randomized and receive study medication (Ampion or saline). Patients will be analyzed as treated.

10.3.2 Intent-to-treat Population:

The intent-to-treat (ITT) analysis population is defined as all patients who are randomized. All efficacy analyses will be performed in the ITT population. Patients will be analyzed as randomized.

10.3.3 Per Protocol Population:

The per protocol analysis population is defined as all patients included in the ITT analysis who met all entry criteria and had no major protocol violations. All efficacy analyses will be repeated

in the per-protocol population. These analyses will be supportive of the ITT analysis. Patients will be analyzed as treated.

10.3.4 Statistical Analysis of Co-Primary Effectiveness Endpoints

A mixed-effects model with repeated measures (MMRM) will be used to test the main effect of change in WOMAC A weekly pain score (determined as the average of Daily Pain measurements taken at Baseline and Week 12) from Baseline of Ampion vs. saline at 12 weeks. The model will include change from Baseline measures at 2, 4, 6, 8, 10, and 12 weeks. Treatment assignment, week, and Baseline WOMAC A measure will be included as fixed effects. Patient ID will be included to capture repeated measures using a compound symmetric covariance matrix for residuals. The primary hypothesis test for pain is the change in pain at 12-weeks at the one-sided $\alpha = 0.025$ level.

The same model will be used to test the effect of WOMAC C function change from Baseline of Ampion vs. saline at 12 weeks. WOMAC C will be tested at one sided $\alpha = 0.025$. Because both endpoints have to achieve statistical significance, this pairing ensures the one-sided Type I error control at the 0.025 level.

10.3.5 Primary Hypotheses

The trial has co-primary hypotheses – requiring success on both for a successful trial. First, pain reduction must be demonstrated for Ampion vs. saline control. Next a function improvement must be shown on Ampion vs. saline control.

Reduction in Pain, as measured by a change in the WOMAC A pain sub score as measured by the 5-point Likert scale between Baseline and Week 12, will be greater in patients treated with Ampion than with saline:

- Null Hypothesis: $\mu A = \mu S$
- Alternate Hypothesis: $\mu A < \mu S$

Where μA = mean pain reduction in Ampion arm; μS = mean pain reduction in saline arm. Lower mean change scores indicate greater pain reduction.

Improvement in function, as measured by a reduction in the WOMAC C function sub score as measured by the 5-point Likert scale between Baseline and Week 12, will be greater in patients treated with Ampion than with saline:

- Null Hypothesis: $\mu A = \mu S$
- Alternate Hypothesis: $\mu A < \mu S$

Where μA = mean function reduction in Ampion arm; μS = mean function reduction in saline arm.

10.3.6 Definition of Study Visits

This clinical trial has a total of 11 study visits, including telephone contacts, during the 24-week study (see Table 6.1). The time-on-study for each patient observation will be defined relative to Day 0, the day of the initial dose. For analysis, the Baseline measure is the latest measure prior to initiation of treatment. For analysis of Baseline WOMAC A pain, the Baseline pain score is the average of all scores measured during days -6 to 0, prior to injection of treatment. For analysis of Week 12 WOMAC A pain, the Week 12 pain score is the average of all scores measured during the seven days prior to and including the Week 12 office visit.

10.3.7 Number of patients to receive study drug

This study is designed to enroll 1,034 patients, 517 per study arm, randomized 1:1 for Ampion and saline. There will be an interim analysis at 724 patients, 362 per study arm, randomized 1:1, Ampion and saline.

Following the interim analysis, the trial will either a) continue up to a total of 1,034 patients, 517 patients per study arm, randomized 1:1 for Ampion and saline IA injection; b) allow for sample size re-estimation up to 1.5 times the original trial size for a total of 1,552 patients, 776 per study arm, randomized 1:1 for Ampion and saline IA injection.

10.3.8 Disposition of patients

Disposition of patients, including study completion status and response to therapy as measured by WOMAC pain sub scores, will be summarized by treatment group (Ampion and saline), age group, race and gender for each of the analysis populations.

10.3.9 Interim analysis

There will be one interim analysis once 724 subjects have reached the primary endpoint assessment at Week 12.

At the interim analysis, the conditional probability based upon the observed treatment WOMAC C function score will be calculated. The minimal conditional probability that allows a sample size increase without inflating Type I error under the promising zone for a sample size increase up to 150% is 0.391. Any conditional power < 0.391 results in going to 1,034 patients, randomized 1:1 for Ampion and saline IA injection. Sample size re-estimation according to the promising zone is done for conditional powers between 0.391 and 0.90 with a sample size increase capped to a maximum of 1,552 total patients, randomized 1:1 for Ampion and saline IA injection. A conditional power > 90% results in enrolling to 1,034 patients.

The final analysis, defined by a sample size established using the promising zone, will use the critical value with one-sided $\alpha = 0.025$ for WOMAC A (Ampion vs. saline control) and WOMAC C (Ampion vs. saline control).

10.3.10 Blinding and randomization

Patients will be assigned to treatment by a randomization schedule generated by an independent statistician. Ampion and saline will be provided in study vials labeled with the appropriate information and packed into patient kits.

Where required, safety personnel may be unblinded to a particular patient's treatment assignment to meet reporting requirements to Regulators. Unblinding may occur by contacting Ampio Pharmaceuticals, Inc.

A final Statistical Analysis Plan will be issued prior to unblinding at the interim analysis of data to adjust for any changes to the protocol or unexpected issues in study conduct and data that affect the planned analyses.

10.3.11 Data presentation

10.3.11.1 Demographic and Baseline Characteristics

Demographic (e.g., age, sex, race, ethnicity) and Baseline characteristics (e.g., weight, height) summarized using descriptive statistics, overall and by treatment group for the ITT analysis population.

10.3.11.2 Medical History and Physical Examination

The number and percent of patients with past and current medical disorders at the time of randomization will be presented overall and by treatment group for the ITT analysis population. Results of any abnormalities documented from the abbreviated physical examination at Baseline and Week 12 and Week 24, will be summarized overall and by treatment group for the safety and ITT analysis populations.

10.3.11.3 Concomitant Medications or Treatments

The number and percent of patients receiving concomitant medications or treatments prior to, during the study and at the final visit will be tabulated and presented overall and by treatment group for the ITT analysis population. Concomitant medications/treatments will be summarized using descriptive statistics and will be presented by type of drug (WHO drug classification) overall and by treatment group for the safety and ITT analysis populations.

Treatment failures are defined as patients who receive additional medications or surgical procedures in order to treat worsened symptoms in the treated knee during the study; specifically, treatment failures are any patients who:

- Increase their corticosteroid dose of ≤ 10 mg above their starting dose of corticosteroid during the study to treat worsened symptoms in the treated knee;
- Increase use of NSAID pain medications by a clinically significant amount during the study to treat worsened symptoms in the treated knee;
- Begin opioid treatment during the study to treat worsened symptoms in the treated knee;
- Receive intra-articular injection of pain medicine in the treated knee during the study to treat worsened symptoms in the treated knee;

- Undergo total knee replacement or other surgical procedure in the treated knee prior to assessment of the primary efficacy endpoint.

10.3.11.4 Safety data

Safety data will be evaluated by changes in vital sign measurements and the frequency and severity of AEs. Concomitant medication will be recorded for safety.

Adverse events:

The Investigator is responsible for monitoring the safety of patients who have enrolled in the study. All AEs considered to be possibly related to Ampion will be followed until the event resolves or stabilized without further change. Patients will be followed for the occurrence of AEs until 12 weeks after the first dose of study medication.

Investigators are required to document all AEs occurring during the clinical trial, commencing with the first day of treatment and including the protocol-defined post-treatment follow-up period on the appropriate CRF pages.

The severity of AEs (mild, moderate, severe), relatedness (related, possibly related, unrelated) along with the duration, action taken, and outcome (e.g., study withdrawal) will also be recorded. In addition, events meeting the criteria of a SAE) must be reported to the Sponsor within 24 hours on the SAE reporting forms.

10.3.11.5 Efficacy data:

All efficacy variables will be assessed at Baseline (Day 0), Week 2 (Day 14 ± 7), Week 4 (Day 30 ± 7), Week 6 (Day 42 ± 7), Week 8 (Day 56 ± 7), Week 10 (Day 70 ± 7) and Week 12 (Day 84 ± 7). For analysis of baseline WOMAC A pain, the baseline pain score is the average of all scores measured during days -7 to 0, prior to injection of treatment. For analysis of week 12 WOMAC A pain, the week 12 pain score is the average of all scores measured during the seven days prior to and including the Week 12 office visit.

All statistical tests will be two-sided, except the co-primary endpoints which require superiority in pain vs. saline control in pain and superiority to baseline in function.

Unless otherwise specified, continuous variables will be summarized with the number of non-missing observations, mean, standard deviation, median, minimum, and maximum displayed. Categorical data will be summarized as counts and percentages. All efficacy assessments will be summarized as the measured value and as the change from Baseline by treatment at each timepoint. Summary statistics will include number of observations, mean, standard deviation, median, minimum and maximum. Change from Baseline will also include a 95% confidence interval.

Except where otherwise specified, missing data will not be estimated or carried forward in any of the descriptive analyses. No multiple comparison adjustment will be made for the secondary

efficacy analyses. Data transformation or use of rank-based tests may be used if endpoints depart substantially from a normal distribution.

Baseline is defined as the last pre-treatment assessment.

10.4 STUDY ENDPOINTS

10.4.1 Co-Primary Endpoints

1) Change in the WOMAC Osteoarthritis Index 3.1 pain sub score as measured by the 5-point Likert scale between Baseline and Week 12.

The primary variable is the average score of the five WOMAC A (pain) subscale questions:

In the last 12 hours, how much pain have you had in the study knee:

1. When walking on a flat surface?
2. When going up or down stairs?
3. At night while in bed? (that is - pain that disturbs your sleep)
4. While sitting or lying down?
5. While standing?

The primary analysis will include the repeated measures of WOMAC A pain sub score from Baseline to Weeks 2, 4, 6, 8, 10, and 12 and will use a mixed-effects model with repeated measures MMRM model, with Treatment assignment, Week, and Baseline WOMAC A pain sub score as fixed effects, and including patient ID as a repeated measure with a compound symmetric covariance matrix for residuals.

2) Change in the WOMAC Osteoarthritis Index 3.1 function sub score as measured by the 5-point Likert scale between Baseline and Week 12.

The primary variable is the average score of the seventeen WOMAC C (function) subscale questions.

The primary analysis will include the repeated measures of WOMAC C function sub score from Baseline to Weeks 2, 4, 6, 8, 10, and 12 and will use a mixed-effects model with repeated measures, with Week as a fixed effect, and including patient ID as a repeated measure with a compound symmetric covariance matrix for residuals.

10.4.2 Secondary Endpoints

- Change in PGA between Baseline and Week 12
- Change in the 100 mm VAS Pain Scale between Baseline and Week 12
- Use of rescue analgesia (amount of acetaminophen used between Baseline and Week 12)
- Incidence and severity of treatment-emergent adverse events (TEAEs)

A serial gatekeeping approach will be used to test the secondary endpoints in a sequential manner (i.e. PGA, 100 mm VAS, rescue analgesia).

The difference between treatment groups in the mean change in PGA from Baseline to Week 12 will be analyzed using a similar MMRM model, with Treatment assignment, Week, and Baseline value as fixed effects and including patient as a repeated measure with a compound symmetric covariance matrix for residuals.

The difference between treatment groups in the mean change in 100 mm VAS from Baseline to Week 12 will be analyzed using a similar MMRM model, with Treatment assignment, and Baseline value as fixed effects and including patient as a repeated measure with a compound symmetric covariance matrix for residuals.

The difference between treatment groups in the amount of acetaminophen used between Baseline and Week 12 will be analyzed with a Wilcoxon rank-sum test.

All secondary efficacy analyses will be reported with one sided p-values and successively compared to a critical level of 0.025 if each preceding test was significant.

10.4.3 Exploratory Endpoints

- Change in WOMAC B stiffness sub score between Baseline and Weeks 2, 4, 6, 8, 10, 12 and 24
- Response status based on the OMERACT-OARSI criteria at Weeks 2, 4, 6, 8, 10, 12 and 24
- Proportion of treatment failures at Week 12
- Change in pain and function CDF plot for Ampion and saline patients
- A Bayesian meta-analysis for LK WOMAC A pain sub score including KL 4 patients from studies AP-003-A, AP-003-B, AP-003-C, AP-004, and AP-013
- Change in WOMAC A pain sub score between Baseline and Weeks 2, 4, 6, 8, 10, and 24
- Change in PGA between Baseline and Weeks 2, 4, 6, 8, 10, and 24
- Change in WOMAC C physical function sub score between Baseline and Weeks 2, 4, 6, 8, 10, and 24
- If collected, difference in urine analysis between treatment groups at Week 12

The Bayesian meta-analysis methodology is described in the Statistical Analysis Plan.

Percent responders using the OMERACT-OARSI criteria will be analyzed using separate logistic regression models for each visit.

Proportion of treatment failures will be analyzed using Fisher's exact tests.

Analysis with exploratory biomarkers, such as those for analgesic use and chemokines and cytokines associated with inflammation and cartilage regeneration may be analyzed to compare differences between treatment groups using urine samples collected at Week 12.

All other exploratory endpoints will be analyzed as the difference between treatment groups at each time point extracted from the MMRM model, adjusted for Baseline value.

10.4.4 Subset Analyses

The two co-primary endpoints and three efficacy secondary endpoints will be re-analyzed separately for (a) ages <60, [60, 70), and ≥70, (b) men and women, (c) white and other, (d) Non-Hispanic and Hispanic.

10.4.5 Sensitivity Analysis

The two co-primary endpoints and three efficacy secondary endpoints will be re-analyzed separately in the per-protocol population to exclude treatment failures.

10.5 Missing and Spurious Data

All data collected under this study protocol will be included in the assessment of patient safety. Missing or incomplete AE data will assume greatest relationship to study drug and/or severity.

For the co-primary effectiveness analysis of WOMAC pain change and WOMAC C function change, missing change scores will be imputed when the ITT analysis population is used. Missing data will be imputed by the fully conditional specification predictive mean matching multiple imputation approach of Berglund and Heeringa (2014). A sensitivity analysis will be conducted in which the missing 12-week endpoint is replaced by the Baseline WOMAC pain score.

Per the WOMAC User Guide, the patient's response will be regarded as invalid, and the subscale(s) deficient, in the event that two pain, both stiffness, or more than four physical function items are omitted. Further, if one pain, one stiffness, or 1-3 physical function units are missing, the average value for the subscale will be substituted in lieu of the missing item value(s).

11 REGULATORY, ETHICAL AND LEGAL OBLIGATIONS

11.1 Declaration of Helsinki

The Principal Investigator will ensure that this Study is conducted in accordance with the most recent revision of the Declaration of Helsinki.

11.2 Good Clinical Practice

The Study will be conducted according to the study protocol and to Standard Operating Procedures (SOPs) that meet the guidelines provided by the International Conference on Harmonisation (ICH) for Good Clinical Practice in clinical studies.

11.3 Institutional Review Boards/Ethics Committees

Before implementing this study, the protocol, the proposed patient informed consent forms and other information for the patients, must be reviewed by a properly constituted committee or committees responsible for approving clinical studies. The IRB/IEC written, signed approval letter/form must contain approval of the designated investigator, the protocol (identifying protocol title, date and version number), and of the patient informed consent form (date, version).

Any change or addition to the protocol can only be made in a written protocol amendment that must be approved by the Sponsor, the IRB/IEC and the Health Authorities.

11.4 Regulatory Authority Approval

Before this study is implemented, the protocol must be approved by the relevant regulatory authority.

11.5 Informed Consent

The investigator must fully inform the patient of all pertinent aspects of the trial including the written information approved/favorably assessed by the IRB/IEC.

Prior to the start of the pre-study examination, the written informed consent form must be signed and personally dated by the patient and by the physician who conducted the informed consent discussion. One copy of the written information and signed consent form must be given to each patient and one copy must be retained in the investigator's study records.

11.6 Patient Confidentiality and Disclosure

Data on patients collected on eCRFs during the trial will be documented in an anonymous fashion and the patient will only be identified by the patient number, and by his/her initials. As an exception, if it is necessary for safety or regulatory reasons to identify the patient, all parties are bound to keep this information confidential.

The investigator will guarantee that all persons involved will respect the confidentiality of any information concerning the trial patients. All parties involved in the study will maintain strict confidentiality to assure that neither the person nor the family privacy of a patient participating in the trial is violated. Likewise, the appropriate measures shall be taken to prevent access of non-authorized persons to the trial data.

11.7 Collection, Monitoring and Auditing Study Documentation, and Data Storage

11.7.1 Collection of Data and Monitoring Procedures

This study will use a 21 CFR Part 11 compliant electronic data capture system (eDC). An electronic case report form (eCRF) is used for data recording. All data requested on the eCRF must be entered and all missing data must be accounted for.

The data will be checked for completeness and correctness as it is entered by the real-time online checks applied by the eDC system. Off-line checks will also be run to perform any additional data review required. Discrepancy reports will be generated accordingly and transferred to the study center for resolution by the investigator or his/her designee.

Accurate and reliable data collection will be assured by verification and cross–check of the eCRF against the investigator's records by the study monitor (source document verification), and the maintenance of a study drug–dispensing log by the investigator.

Before study initiation, at a site initiation visit or at an investigator's meeting, a Sponsor representative will review the protocol and case report forms with the investigators and their staff. During the study a monitor will visit the site regularly to check the completeness of patient records, the accuracy of entries on the case report forms, the adherence to the protocol and to Good Clinical Practice, the progress of enrollment. The monitor will ensure during on-site visits that study medication is being stored, dispensed and accounted for according to specifications. Key trial personnel must be available to assist the monitors during these visits.

The investigator must give the monitor access to relevant hospital or clinical records, to confirm their consistency with the case report form entries. No information in these records about the identity of the patients will leave the study center. Monitoring standards require full verification for the presence of informed consent, adherence to the inclusion/exclusion criteria, documentation of SAEs and the recording of primary efficacy and safety variables. Additional checks of the consistency of the source data with the eCRFs are performed according to the study-specific monitoring plan.

11.7.2 Auditing Procedure

In addition to the routine monitoring procedures the Sponsor or the regulatory authority can conduct an audit or an inspection (during the study or after its completion) to evaluate compliance with the protocol and the principles of Good Clinical Practice.

The investigator agrees that representatives of the Sponsor and Regulatory Authorities will have direct access, both during and after the course of this study, to audit and review all study-relevant medical records.

11.7.3 Retention of Documents

The investigator must maintain source documents for each patient in the study, consisting of all demographic and medical information, including laboratory data, x-ray, etc., and keep a copy of the signed informed consent form. All information on case report forms must be traceable to these source documents in the patient's file. Data without a written or electronic record will be defined before trial start and will be recorded directly on the case report forms, which will be documented as being the source data.

11.8 Disclosure of Information

All information provided to the investigator by Ampio Pharmaceuticals, Inc. or their designee, will be kept strictly confidential. No disclosure shall be made except in accordance with a right of publication granted to the investigator.

No information about this study or its progress will be provided to anyone not involved in the study other than to Ampio Pharmaceuticals, Inc., or its authorized representatives, or in confidence to the IRB, or similar committee, except if required by law.

11.9 Discontinuation of the Study

It is agreed that, for reasonable cause, either the investigator or Ampio Pharmaceuticals, Inc., may terminate the investigator’s participation in this study after submission of a written notice. Ampio Pharmaceuticals, Inc., may terminate the study at any time upon immediate notice for any reason, including the Sponsor’s belief that discontinuation of the study is necessary for the safety of patients.

11.10 Study Report, Publication Policy and Archiving of Study Documentation

11.10.1 Study Report and Publication Policy

An ICH-compliant integrated clinical and statistical report will be prepared upon completion of the study and data analysis. The results of the study will be published in a relevant peer-reviewed journal, with authorship status and ranking designated according to the acknowledged contributions of participating investigators, institutions and the Sponsor.

11.10.2 Study Documents

The investigator must maintain source documents for each patient in the study, consisting of all demographic and medical information, questionnaires, including laboratory data, MRI (as applicable), etc., and keep a copy of the signed informed consent form. All information on the e-case report forms must be traceable to these source documents in the patient's file. Data without a written or electronic record will be defined before trial start and will be recorded directly on the e-case report forms, which will be documented as being the source data.

11.10.3 Archiving of Documents

Essential documents, as listed below, must be retained by the investigator for as long as needed to comply with national and international regulations. The Sponsor will notify the investigator(s)/institution(s) when the study-related records are no longer required. The investigator agrees to adhere to the document retention procedures by signing the protocol. Essential documents include:

- IRB/IEC/REB approvals for the study protocol and all amendments
- All source documents and laboratory records
- CRF copies (electronic copies on a CD-ROM)
- Patients' informed consent forms (with study number and title of trial)
- FDA form 1572
- Any other pertinent study document

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