Official Title: A Phase 2B, Randomized, Double Blind, Active Comparator, Multicenter, Safety, and Efficacy Trial of ATX-101 in Subjects Undergoing Total Knee Arthroplasty (SPARK)
NCT Number: NCT05260008
Document Date: 02 June 2023

Protocol No.	ATX-101-TKA-003
Title:	A Phase 2B, Randomized, Double Blind, Active Comparator, Multicenter, Safety, and Efficacy Trial of ATX-101 in Subjects Undergoing Total Knee Arthroplasty (SPARK)
Short Title/Acronym	Study Assessing Pain Relief after Replacement of the Knee / SPARK
Protocol Version Number:	Amendment 03
Issue Date:	02 June 2023
Investigational Product:	ATX-101
Drug Development Phase:	2B
Sponsor:	Allay Therapeutics, Inc. 2720 Zanker Road San Jose, CA 95134
Local Sponsor in Australia:	Foundry Therapeutics 1 Pty. Ltd a wholly owned subsidiary of Allay Therapeutics, Inc. Level 19 HWT Tower 40 City Road Southbank, VIC 3006
Medical Monitor:	[redacted]

PROTOCOL CONTACTS

Key Roles	Name/Address	Telephone
Sponsor	Allay Therapeutics, Inc. 2720 Zanker Road, San Jose, CA 95134	[redacted]
Medical Monitor/Safety Monitor [redacted]	[redacted]	[redacted]
Bioanalytical Laboratory	[redacted]	[redacted]
Central Laboratory	[redacted]	[redacted]
	[redacted]	[redacted]
	[redacted]	[redacted]

PROTOCOL SIGNATURE PAGE

A Phase 2B, Randomized, Double Blind, Active Comparator, Multicenter, Safety, and Efficacy Trial of ATX 101 in Subjects Undergoing Total Knee Arthroplasty

ATX-101-TKA-003

Version: Amendment 03

Issue Date: 02 June 2023

Sponsor Statement

This protocol was subject to critical review and has been approved by the following persons:

[redacted] June 2, 2023
Date

[redacted] June 2, 2023.
Date

INVESTIGATOR SIGNATURE PAGE

A Phase 2B, Randomized, Double Blind, Active Comparator, Multicenter, Safety, and Efficacy Trial of ATX 101 in Subjects Undergoing Total Knee Arthroplasty

ATX-101-TKA-003

Version: Amendment 03

Issue Date: 02 June 2023

Investigator Agreement

I have read the above-mentioned protocol and am aware of my responsibilities as Investigator for this trial. As such, I agree to:

- Personally supervise the conduct of this trial.
- Conduct the trial in accordance with International Conference on Harmonization E6 Good Clinical Practice (ICH GCP) Consolidated Guidance, applicable regulatory requirements, and the protocol.
- Comply with the procedures for data recording and reporting as required by the regulatory authorities and the Sponsor.
- Permit monitoring, auditing, and inspection of trial records as required by ICH GCP.
- Retain the essential clinical trial documents as required by ICH GCP and the Sponsor.

The information contained in this protocol is proprietary and provided to me in confidence, and may not be disclosed to any other party, in any form, without prior authorization from Allay Therapeutics, except to the extent necessary for conduct of the trial.

_____ _____

Investigator Signature Date

_____

Investigator Name (Print)

HISTORY OF PROTOCOL CHANGES

Version	History of Changes	Date
Original	Not Applicable	21 December 2021
Amendment 01	Updated Sponsor address, emergency contact, additional contact information, and administrative changes. Removed tramadol testing from the drug screen. Modification of medication names for multiple regions (epinephrine is now epinephrine/adrenaline, acetaminophen is now acetaminophen/paracetamol). Clarification was provided on the frequency of rescue opioid medication and the dosage of aspirin being based upon regionally approved doses of 75-100 mg. The exclusion criteria has been updated to allow previous surgery on the contralateral knee and to exclude further contraindications to celecoxib, as well as clarify CYP3A inhibitors and inducers in Appendix M.	13 May 2022
Amendment 02	Added short title and acronym. Changed the saline placebo arm in Part A to a bupivacaine HCl active comparator arm and removed the saline placebo arm in Part B. Updated applicable sections (ie objectives, methodology, reference product, and statistics) for the change in comparator arms. Increased the number of subjects per treatment group due to the change in study design. Updated exclusionary laboratory values for renal function at screening. Added ECG pre-surgery in Part B. Clarified rescue opioid medication information. Administrative changes to update study contacts with reformatted table.	6 December 2022
Amendment 03	Updated Protocol Contact information. Added information to allow for an interim analysis for Part A in the applicable sections (ie synopsis and section 10 Statistics). Correction of typographical error on Exclusion Criteria 12. Added clarification of acetaminophen/ paracetamol dose to include the option of either as well as the 500 mg every 4 hours or 1000 mg every 6 to 8 hours, for the maximum dose not to exceed 3000 mg per any 24-hours in appropriate section. Updated exclusion 11: Body Mass Index (BMI) $\geq$40 kg/m^2 to a BMI $\geq$45 kg/m^2. Minor editorial changes.	02 June 2023

1.0 TRIAL SYNOPSIS

NAME OF SPONSOR

Allay Therapeutics, Inc.

NAME OF INVESTIGATIONAL DRUG PRODUCT

ATX-101

NAME OF ACTIVE INGREDIENT

Bupivacaine

PROTOCOL/TRIAL TITLE

A Phase 2B, Randomized, Double Blind, Active Comparator, Multicenter, Safety, and Efficacy Trial of ATX-101 in Subjects Undergoing Total Knee Arthroplasty

SHORT TITLE AND ACRONYM

Study Assessing Pain Relief after Replacement of the Knee (SPARK**)**

PROTOCOL NUMBER

ATX-101-TKA-003

INVESTIGATORS & TRIAL CENTERS

The trial is planned to be conducted at approximately 20 multi-national clinical centers

CLINICAL PHASE

2B

OBJECTIVES

- To compare the efficacy of ATX-101 (1,000 mg or 1,500 mg) with that of bupivacaine hydrochloride (HCl) in subjects undergoing primary unilateral total knee arthroplasty (TKA)
- To evaluate the safety and tolerability of ATX-101
- To characterize the pharmacokinetic (PK) profile following administration of ATX-101 (Part A only)
- To compare opioid consumption of subjects administered ATX-101 with that of bupivacaine HCl (Part B only)
- To estimate the sample size, determine dose, and primary endpoint duration from Part A needed for Part B
- To compare the efficacy of ATX-101 with that of bupivacaine HCl

METHODOLOGY

This is a two-part (Part A and Part B) Phase 2B, randomized, double blind, active comparator (bupivacaine HCL) (Part B only) multicenter trial in subjects undergoing primary unilateral TKA. Part A will be used to determine the sample size, dose, and primary endpoint duration for Part B of the trial.

Subjects will participate in the trial for up to approximately 12 weeks, including a 30-day screening period. All subjects will be screened within 30 days prior to surgery. Subjects who meet eligibility criteria will be randomized up to 1 business day prior to the day of surgery. On the day of surgery (Day 1), subjects who continue to meet the eligibility criteria will undergo primary unilateral TKA under bupivacaine spinal anesthesia without adjunct medications used in the spinal anesthesia. The surgical operating team, including the surgeon, will be unblinded to the assigned treatment. All other research staff (unless expressly specified in the site's blinding plan that is approved by Allay), and the subject will remain blinded until the end of the trial. The Sponsor will remain blinded during the treatment phase of Part A and Part B.

Total enrollment will be split into Part A and Part B, where Part A is expected to randomize approximately 165 subjects and Part B up to 140 subjects. In Part A (ATX-101, or active comparator and Part B (ATX-101 or active comparator) investigational product will be administered to each subject intraoperatively. Part A will include a

one-time dose of ATX-101 (either 1,000 mg or 1,500 mg), whereas Part B will include a one-time dose of ATX-101 at a dose level determined from data analysis of Part A.

Part A

Approximately 165 subjects will be randomized to one (1) of the following three (3) treatment groups in a 1:1:1 ratio with approximately 55 subjects per group:

- ATX-101, 1,000 mg dose (two ATX-101 implants)
- ATX-101, 1,500 mg dose (three ATX-101 implants)
- Bupivacaine HCl 0.25% without epinephrine/adrenaline, maximum dose of 2 mg/kg, via local periarticular infiltration or adductor canal block, or in combination

Part B

Up to 140 subjects (total number will be determined from Part A) will be randomized to one (1) of the following two (2) treatment groups in a 1:1 ratio with up to 70 subjects per group:

- ATX-101 dose to be determined from Part A
- Bupivacaine HCl 0.25% without epinephrine/adrenaline, maximum dose of 2 mg/kg, via local periarticular infiltration or adductor canal block, or in combination

Before surgery, all subjects will be administered acetaminophen/paracetamol 1,000 mg and celecoxib 200 mg [1]. Spinal anesthesia with bupivacaine (with or without dextrose) will be used in all subjects; no other adjunct medication may be used in the spinal anesthesia. General anesthesia is optional, but if used must be in conjunction with spinal anesthesia. Local nerve blocks (eg adductor canal blocks) are prohibited in the ATX-101 treatment group (but permissible in the bupivacaine HCl group), however topical anesthetics such as lidocaine can be used to aid in intravenous (IV) access and subcutaneously for spinal needle placement. At the time of induction, the use of fentanyl (no more than 300 µg) for airway management is permitted when general anesthesia is used. Other than fentanyl for airway management, no other opioids may be administered during surgery.

Surgical drains are not permitted. Two doses of IV tranexamic acid (1 g each) will be administered to the subject. One dose of tranexamic acid will be administered prior to investigational product administration and the second dose after investigational product administration, with the timing at the discretion of the treating Investigator. A one-time dose of IV dexamethasone (4 mg) will be administered during the surgical procedure, with the timing at the discretion of the treating Investigator. Intravenous or oral antibiotic regimen may also be given per the center's standard of care. One dose of prophylactic ondansetron will be given before or during surgery, with the timing at the discretion of the treating investigator. All other doses of antiemetics will be given only after an adverse event has been observed or reported.

Subjects should only receive rescue opioid medication upon request for pain control, as needed, after surgery. Rescue opioid medication may be given/taken for pain treatment, but not for pain prophylaxis. Prior to the administration of any rescue opioid medication, from surgery through the Day 30 Visit, a Numeric Rating Score at Rest (NRS-R) assessing the subject's pain intensity must be obtained.

If required to treat pain, the following standardized rescue opioid medication protocol will be used. Preferred rescue opioid medication will consist of oral immediate-release oxycodone (5 mg or 10 mg). If a subject cannot tolerate oral medication, morphine may be administered IV (including via patient-controlled analgesia [PCA] pump) or IM as needed (morphine is preferred, however hydromorphone can also be used with the dose determined by the treating physician). If a subject cannot tolerate the medication or the breakthrough pain is not responsive to oral oxycodone, then oral hydromorphone (2 mg or 4 mg) may be given for rescue opioid medication. Should the subject have an adverse event (AE) and is unable to tolerate the rescue opioid medications of oral oxycodone (5 mg or 10 mg), IV morphine, oral hydromorphone (2 mg or 4 mg), and IV hydromorphone, then hospital standard of care may be utilized. No long-lasting opioids should be administered. (See Section 7.6.3.1 for further information on rescue opioids.)

The subjects will be administered celecoxib (200 mg) twice a day for 30 days, unless an AE occurs that requires discontinuation. Acetaminophen/paracetamol 500 mg should be taken every 4 hours or 1,000 mg should be taken every 6 to 8 hours not to exceed 3,000 mg per any 24-hour period for a minimum of 21 days. Deep vein thrombosis (DVT) prophylaxis is required, and 75-100 mg aspirin (based upon regional approved dose) is recommended twice a day, unless otherwise contraindicated for the subject, for 30 days. Celecoxib, acetaminophen/paracetamol, and aspirin may be provided by the Sponsor.

Following surgery, subjects will be transferred to the post-anesthesia care unit (PACU). Subjects in Part A of the trial will remain under in-patient observation until Hour 96. While under observation subjects will have assessments of vital signs, neurological assessments, pain scores, ECGs, PK sampling, and AE/concomitant medication review as per the study schedule. Subjects in Part B of the trial will remain under observation until Hour 24 and then may be discharged per standard of care.

Regardless of in-patient observation/hospitalization or discharge status, all procedures in the Schedule of Events will be followed. Home visits as detailed in Appendix A and Appendix B may be completed at the subject's home, a physical therapy facility, in the clinic/hospital/facility, or any other place agreed upon by the trial staff and the subject. In clinic visits may also be completed at a physical therapy facility or in the clinic/hospital/facility as long as all procedures can be conducted.

During trial participation subjects will utilize an electronic diary (e-diary) to capture pain intensity using the NRS-R and Numeric Rating Scale with Activity (NRS-A) and location of pain. The e-diary will also be utilized to collect medication and trial questionnaires/surveys.

The Day 6 and Day 8 Visit may occur in the hospital/clinic/facility, at the subject's home, at a physical therapy facility, or any other place agreed upon by the subject and site staff. Subjects will return to the clinic at Day 15, Day 22, Day 30, and Day 56 and at any visit where the subject is not able to be seen at their home or alternate location by a home health care nurse/the trial staff.

Pharmacokinetic Sampling
For Part A, venous blood samples for plasma PK analysis are drawn before surgery (before bupivacaine spinal), during surgery prior to administration of ATX-101 or the bupivacaine HCl active comparator, then approximately 6 hours after surgical closure 12 hours after surgical closure, and on Days 2 (Hour 24), 3 (Hour 48), 4 (Hour 72), 5 (Hour 96), 6, 8, 15, 22, and 30.

For Part A and Part B, PK sampling will be done for bupivacaine monitoring anytime there is a Serious Adverse Event (SAE) or Adverse Events of Special Interest (AESI).

SAFETY REVIEW & STOPPING CRITERIA

Safety Monitoring Committee

A Safety Monitoring Committee (SMC) will review blinded summary safety data in Part A after 25%, 50%, and 100% enrollment of the study and in Part B after 25%, 50%, and 75% enrollment of the study. The SMC will be composed of independent members and Sponsor representatives. Representatives from the Sponsor will include clinical, regulatory and the study medical monitor. The biostatistician, anesthesiologist, cardiologist and orthopedic surgeon will be independent members. The SMC will operate under a written, detailed SMC plan.

The SMC will evaluate study data including adverse events, pharmacokinetic data, cardiac information, and safety laboratory results from subjects. The SMC will investigate all reports of PK greater than or equal to 1,000 ng/mL of plasma bupivacaine. Should data arise where further information is needed to address a safety concern, the SMC will escalate the data review to an unblinded, independent statistician and physician.

Stopping Rules:

The SMC may recommend that the trial be stopped and evaluated at any time. The trial will also be stopped should there be:

- One death where a clear alternative cause (other than ATX-101) is not identified or
- Two non-fatal SAE's where a clear alternate cause (other than ATX-101) is not identified or
- Five moderate cardiac or central nervous system AESI's with a PK concentration of more than 1,000 ng/mL and a clear alternate cause (other than ATX-101) is not identified or
- Three severe cardiac or central nervous system AESI's with a PK concentration of more than 1,000 ng/mL and a clear alternate cause (other than ATX-101) is not identified.

In these instances, the study blind may be broken for the SMC for the identified subjects.

NUMBER OF SUBJECTS

This trial will enroll approximately 165 subjects in Part A and up to 140 subjects in Part B.

DIAGNOSIS & MAIN CRITERIA FOR INCLUSION

This trial will evaluate the treatment of postsurgical pain in TKA subjects.

Inclusion Criteria

1. Primary indication of TKA is knee pain due to osteoarthritis or post-traumatic arthritis.
2. Scheduled to undergo primary unilateral TKA with a cemented prosthesis, without use of a surgical drain, and under bupivacaine spinal anesthesia (dextrose is permitted).
3. American Society of Anesthesiology (ASA) Physical Classification System of class 1, 2 or 3.
4. Male or female, ≥18 and ≤80 years of age at the Screening Visit.
5. Female subjects only: Postmenopausal, surgically sterile, or willing to use acceptable means of contraception from the Screening Visit through the Day 56 Visit.
    a. Medically acceptable methods of contraception that may be used by the subject include birth control pills, diaphragm with spermicide, intrauterine device (IUD), condom with spermicide, vaginal spermicidal suppository, or progestin implant or injection (used consistently for ≥3 months at the time of screening).

   b. Female subjects who are not of childbearing potential must have a medical history recorded of surgical sterilization (≥6 months post-surgery at the time of screening) or post-menopausal (not experienced a menstrual period ≥2 years at the time of screening).
6. Capable, able, and willing to comply with all trial visits and procedures. Subject must also be able to use trial required electronic applications (eg electronic diary) for patient reported outcome measures.
7. English speaking, willing, and capable of providing written informed consent.

Exclusion Criteria:

1. Has a planned concurrent surgical procedure (eg bilateral TKA) at the time of surgery or a planned surgical procedure any time before the Day 56 Visit.
2. Has had any previous arthroplasty, unicompartmental knee arthroplasty or TKA in the study knee or previous arthroplasty, unicompartmental knee arthroplasty, or TKA in the contralateral knee within 6 months prior to screening.
3. Has been administered any type of intra-articular injection within 3 months of surgery in the trial knee.
4. Has a pre-existing concurrent, acute, or chronic, painful/restrictive physical condition for which they routinely take opioid analgesics and are expected to require opioid analgesics in the postsurgical period that is not strictly related to the trial knee osteoarthritis/post-traumatic arthritis and/or trial knee surgery.
5. Unable to abstain from opioid use for knee pain (including codeine) within 2 weeks (14 days) of surgery.
6. Has been administered systemic steroids within 14 days prior to surgery. (Note: Steroids provided on the day of surgery as part of a standard surgical medication regimen are permitted. Local steroids such as inhalers or ophthalmologic drops are permitted).
7. Is unwilling or unable to discontinue use of medications or products that can impact pain control from the Screening Visit until the Day 56 Visit (eg cannabidiol (CBD) oil, Kratom, herbal supplements). Non-Steroidal Anti-Inflammatory Drugs (NSAIDS) and acetaminophen/paracetamol are permitted prior to surgery at the treating Investigator's discretion. Subjects prescribed gabapentin and/or pregabalin will require medical monitor approval.
8. Has been administered any anesthetic including but not limited to bupivacaine, ropivacaine, or lidocaine within 5 days prior to the scheduled surgery. See protocol Section 7.6.1 for uses and permitted anesthetics on the day of surgery.
9. Requires medication that will impact the metabolism, absorption, or excretion of bupivacaine. Epinephrine/adrenaline should be used with caution. Strong inhibitors and inducers of CYP3A4 should be avoided (See Appendix M). Coadministration of ATX-101or bupivacaine HCl with local anesthetics is prohibited.
10. Has a contraindication or a known or suspected history of allergy, hypersensitivity, or idiosyncratic reaction to trial medications including but not limited to those used during surgery or post-surgery (eg anesthesia, IV dexamethasone, IV tranexamic acid, antibiotics, ondansetron, and opioids such as morphine, oxycodone, and hydromorphone), also to medications taken prior to surgery and/or dispensed for home use (ie acetaminophen/paracetamol and celecoxib), or to the investigational product or its components (bupivacaine, poly[DL-lactide-co-glycolide] (PLGA), and/or polysorbate).
11. Has a Body Mass Index (BMI) ≥45 kg/m^2.
12. Has laboratory values at screening that will exclude enrollment including:
    a. Bilirubin >2 times the upper normal limit unless due to Gilberts syndrome
    b. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), or alkaline phosphatase (ALP) >1.5 times the upper normal limit
    c. Creatinine > 2 times the upper normal limit
    d. Estimated serum creatinine clearance <30 mL/min
    e. Hemoglobin below the lower normal limit

13. Screening electrocardiograms (ECG) with significant abnormalities such as left bundle-branch blocks, bifascicular blocks, second- or third-degree AV block, possible current ischemia, or other findings associated with significant heart disease. The average QTc of the screening triplicate ECGs must not be >450 ms in males or >470 ms in females. Significant findings are to be clearly documented.
14. Has symptomatic central nervous system (CNS) injury or disorder including epilepsy.
15. Has any chronic condition or disease that would compromise neurological or vascular assessments.
16. Immunocompromised or has a known history of Hepatitis B, human immunodeficiency virus (HIV), or Hepatitis C.
17. Has a medical condition or receiving medication such that, in the opinion of the Investigator, participating in the trial would pose a health risk to the subject or confound the postsurgical assessments or might confound or interfere with the outcome of the trial. Examples include but are not limited to inability to ambulate, diabetic neuropathy, complex regional pain syndrome, prior infection in operative joint, known or suspected coagulopathy, uncontrolled anticoagulation, long COVID diagnosis, history of thromboembolic events, active gastrointestinal ulcers/bleeding, or cerebrovascular bleeding.
18. Has uncontrolled depression, anxiety, psychiatric, or neurological disorder that might interfere with trial assessments or might confound or interfere with the outcome of the trial.
    a. Subjects currently taking anti-psychotic medication will be excluded.
    b. Anti-depressant medication (selective serotonin reuptake inhibitors only) is allowed if the subject has been on a stable dose for at least 30 days prior to screening and no dose changes are planned during the conduct of the trial.
19. At any time prior to randomization, subjects with moderately severe or severe depression, as defined by
    a. Patient Health Questionnaire (PHQ-9) total score of ≥10; or
    b. Suicidal ideation, as defined by a PHQ-9 score of ≥1 in response to question 9, "thoughts that you would be better off dead, or hurting yourself."
20. Has a known or suspected history of drug or alcohol abuse. A subject with a history of alcohol use disorder that has ≥10 years sobriety will be permitted.
21. Has a positive drug screen at the Screening Visit or on the day of surgery including positive results from prescribed medications. The exception being a positive drug screen resulting from infrequent use of short acting benzodiazepines that can be washed out 7 days prior to surgery and not utilized during the trial duration.
22. Has received/used an investigational drug, product, or device for a clinical trial within 30 days of screening. COVID-19 vaccines (approved or under emergency use authorization locally) are permitted if the subject is not in a clinical trial for the vaccine.
23. Pregnant, breastfeeding, or planning to become pregnant during the trial or before the Day 56 Visit.

In addition, the subject will not have met eligibility if the following occurs during surgery.

24. Any clinically significant (CS) event or condition uncovered during surgery (eg excessive bleeding, femur fracture), which occurs before investigational product administration, that might render the subject medically unstable or complicate the subject's postsurgical course.
25. [redacted]

TEST PRODUCT, DOSE AND MODE OF ADMINISTRATION

ATX-101 (bupivacaine) implant, 500 mg, implant.

Each bioabsorbable implant contains 500 mg bupivacaine free base (equal to 563 mg bupivacaine HCl or 594 mg bupivacaine HCl monohydrate and hereafter referred to as bupivacaine).

In Part A of this trial, subjects will receive a single-dose administration of 1,000 mg of ATX-101 (2 implants each containing 500 mg bupivacaine), or 1,500 mg of ATX-101 (3 implants each containing 500 mg bupivacaine).
In Part B of this trial, subjects will receive a single dose level of ATX-101 determined from the analysis of Part A.

A one-time administration of a single dose level of ATX-101 will be placed in the subject's knee capsule following fixation of the knee prosthesis when tissue will not be disrupted any further by surgery, after any betadine or saline rinse, and after suction has occurred, but prior to initiation of surgical closure. ATX-101 will be placed in the suprapatellar pouch, medial gutter, and/or lateral gutter according to the Dosage and Administration Instructions.

REFERENCE PRODUCT, DOSE AND MODE OF ADMINISTRATION

Bupivacaine HCl 0.25% without epinephrine/adrenaline, maximum dose of 2 mg/kg, via local periarticular infiltration or adductor canal block, or in combination
Bupivacaine HCl may be provided by the Sponsor for trial use.

TRIAL ENDPOINTS AND CRITERIA FOR EVALUATION

Primary Efficacy Endpoint (evaluated in Part B): Area under the curve (AUC) for the NRS-R of pain intensity from 30 Minutes through a time point to be determined from Part A.

The primary efficacy endpoint for Part A is AUC for the NRS-R of pain intensity from 30 Minutes through Hour 168 (Day 8 of the trial).

Key Secondary Endpoints (evaluated in Part B):

- Area under the curve for the NRS-R of pain intensity from 30 Minutes through Hour 168 (Day 8 of the trial), Hour 240 (Day 11 of the trial), and Hour 336 (Day 15 of the trial).
- Percentage of subjects who remain opioid free from Hour 72 through Day 30.
- Total post-surgical consumption of opioid medications from surgical closure through Day 30.

Secondary Endpoints:

- Area under the curve for the NRS-R of pain intensity for each 24-hour period through Hour 336 (Day 15 of the trial)
- Percentage of subjects who remain opioid free
- Total post-surgical consumption of rescue opioid medications
- Time to first postsurgical use of rescue opioid medication

Safety Endpoints:

- Incidence of AEs, AESIs, and SAEs
- Wound healing assessment using the Southampton Wound Scoring System
- Characterization of the bupivacaine PK from ATX-101

Exploratory Endpoints:

- Percentage of subjects reporting being pain free on the NRS-R of pain intensity
- Time to various degrees of flexion and extension
- Reduction/incidence of opioid-related AEs
- Evaluation of Quality of Recovery 15 Scale (QOR-15)
- Evaluation of Knee Injury and Osteoarthritis Outcome Score for Joint Replacement (KOOS, JR)
- Difference in pain intensity scores (NRS-R and NRS-A) between treatment groups
- If a statistical difference in AUC of pain intensity NRS-R at 336 Hours (Day 15 of the trial) is observed,

the AUC for the NRS-R of pain intensity will be further evaluated between Days 16 and 30
- Evaluation of the Knee Society Score

For consideration of efficacy, safety, and PK assessments the time of surgical closure (skin closure) will be considered Time 0 for all assessments.

STATISTICAL METHODS

Sample Size Calculation:

Part A

Approximately 165 subjects will be randomized to one (1) of the following three (3) treatment groups in a 1:1:1 ratio with approximately 55 subjects per group:

- ATX-101, 1,000 mg dose (two ATX-101 implants)
- ATX-101, 1,500 mg dose (three ATX-101 implants)
- Bupivacaine HCl 0.25% without epinephrine/adrenaline, maximum dose of 2 mg/kg, via local periarticular infiltration or adductor canal block, or in combination

Based on the literature review of other products used in TKA and discussion with experts in post-operative pain, it is believed that 55 subjects per group would provide sufficient information to inform dose response and dose selection for Part B.

Part B

Up to 140 subjects will be randomized to one (1) of the following two (2) treatment groups in a 1:1 ratio with up to 70 subjects per group:

- ATX-101, dose to be determined from Part A
- Bupivacaine HCl 0.25% without epinephrine/adrenaline, maximum dose of 2 mg/kg, via local periarticular infiltration or adductor canal block, or in combination

The current planned sample size for Part B is up to 140 subjects. The actual number of subjects to be randomized will be determined based on data from Part A.

Analysis Populations:

- Full Analysis Set (FAS): all subjects who are randomized and administered ATX-101or bupivacaine HCl. The FAS will be used for the efficacy analyses and subject demographic and baseline characteristics.
- Safety Analysis Set: all subjects who are randomized and administered ATX-101 or bupivacaine HCl. The Safety Analysis Set will be used for all safety analyses.
- PK Analysis Set: all subjects who provide enough plasma samples for PK assessment of bupivacaine that allows for generation of at least one PK parameter of T_{max}, C_{max}, or AUC.

Efficacy Evaluations:

The primary efficacy endpoint (evaluated in Part B) is the AUC for the NRS-R of pain intensity from 30 Minutes through a time point to be determined from Part A. The time-weighted AUC will be calculated using the trapezoidal rule based on the available NRS pain scores. Missing values will not be imputed with the exception of the final time point. Prior to administration of rescue opioid medications, NRS-R scores will be recorded. NRS-R values will be censored for the duration of the effectiveness of rescue opioid medications before the trapezoidal rule is applied.

The primary efficacy endpoint will be analyzed using an Analysis of Variance (ANOVA) model with a main effect of the treatment group. Summary statistics will be reported as well as the least square (LS) means, difference in the LS means, 95% confidence intervals (CI) and p-value for the contrast comparing the LS means.

For Part B, the primary comparison will be ATX-101 versus bupivacaine HCl. A two-sided alpha 0.05 will be spent on the primary comparison.

To control the type I error for the analyses of key secondary endpoints, a stepdown procedure will be used. If the primary comparison for the primary endpoint is statistically significant, comparisons between ATX-101 and bupivacaine HCl for the key secondary endpoints will be carried out in the order as shown below. Comparisons will be stopped once any proceeding comparison is not statistically significant.

- Area Under the Curve for NRS-R of pain intensity from 30 Minutes through Hour 168 (Day 8 of the trial)
- Area Under the Curve for NRS-R of pain intensity from 30 Minutes through Hour 240 (Day 11 of the trial)
- Area Under the Curve for NRS-R of pain intensity from 30 Minutes through Hour 336 (Day 15 of the trial)
- Percentage of subjects who remain opioid free from Hour 72 through Day 30
- Total post-surgical consumption of opioid medications from surgical closure through Day 30

For Part A, each single dose level of ATX-101 will be compared to the bupivacaine HCl active comparator group. In addition, the LS mean of each dose level group will be plotted with 95% CI to evaluate dose response.

For Part A, an interim analysis may be performed when approximately 50% of the initially planned enrollment has completed Day 30. Enrollment will continue during the interim analysis. This interim analysis will be performed by the unblinded statistician separate from the team responsible for the conduct and analysis of the study. A Review Committee of at least two unblinded statisticians and a designated sponsor representative will review the data and recommend one of the following:

- Keep the current sample size and continue Part A as planned
- Stop Part A for efficacy (no further enrollment)
- Increase the sample size in Part A

The basis of this decision will be whether sufficient data have been collected to plan Part B, taking into account both safety and efficacy (including outcomes other than the primary). All part A data will be evaluated. In particular, data pertaining to selection of Part B primary endpoint, eg treatment effect and primary endpoint variability, will be evaluated for the planning of Part B.

The Sponsor may make adjustments other than those detailed above; however, the blinded Sponsor team will receive no information other than the recommendations listed. If the interim analysis demonstrates sufficient data have been collected to plan Part B, then Part A may be stopped, unblinded and the final analysis will be completed on the population enrolled to date.

Full details of the interim analysis will be described in a separate document including data available to the Review Committee, timing, and communication procedures. Details for an interim analysis are provided in Protocol Section 10.8.7.

For the analysis of other efficacy endpoints, refer to Protocol Sections 10.4 and 10.5 for details.

Safety Evaluations: All subjects who have received ATX-101 or bupivacaine HCl (regardless of whether or not they completed the trial) will be included in the safety evaluation.

Adverse events will be collected from the time of administration of ATX-101 or active comparator through the Day 56 Visit and will be summarized by System Organ Class (SOC) using the Medical Dictionary for Regulatory Activities (MedDRA). Central Nervous System and Cardiovascular AESIs will also be summarized by SOC using MedDRA. Adverse Events of Special Interest include **CNS Symptoms/Events:** tongue and perioral numbness, tinnitus, muscle fasciculations, tremors, seizures, global CNS depression, apnea (not sleep apnea); as well as the following **Cardiovascular Symptoms/Events:** sinus bradycardia, atrioventricular blocks, conduction defects (prolonged PR or prolonged QRS), ventricular dysrhythmias, cardiac arrest, or asystole. Additional AESIs regarding **Wound Healing Symptoms/Events** include wound dehiscence, wound pus/purulent discharge, deep wound infection, or cellulitis near the incision site.

Prior and concomitant medication usage is to be recorded from 30 days before the Screening Visit through the Day 56 Visit. Safety evaluations, including vital signs, physical examinations, neurological assessments, ECGs, and laboratory tests are performed as indicated in the Schedule of Events.

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3.0 LIST OF ABBREVIATIONS

Abbreviation	Definition
AE	Adverse Event
AESI	Adverse Event of Special Interest
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
ANOVA	Analysis of Variance
ASA	American Society of Anesthesiology
AST	Aspartate Aminotransferase
ATX-101	Allay Therapeutics Investigational Product
AUC	Area Under the Curve
$AUC_{0-\infty}$	Area Under the Concentration-Time Curve Through Infinity
AUC_{last}	Area Under the Concentration-Time Curve up to the Time of Last Quantifiable Concentration
BMI	Body Mass Index
BUN	Blood Urea Nitrogen
CBD	Cannabidiol
CDC	Center of Disease Control
CI	Confidence Intervals
C_{max}	Maximum Observed Plasma Concentration
CNS	Central Nervous System
COVID-19	Coronavirus disease of 2019
CRF	Case Report Form
CS	Clinically Significant
CYP3A4	Cytochrome P450 3A4
dL	Deciliter
DSMB	Data Safety Monitoring Board
DVT	Deep Vein Thrombosis
EC	Ethics Committee
ECG	Electrocardiogram
EDC	Electronic Data Capture
e-diary	Electronic Diary
FAS	Full Set Analysis
FDA	Food and Drug Administration

Abbreviation	Definition
G	Gram
GCP	Good Clinical Practice
GGT	Gamma-glutamyl Transferase
HCl	Hydrochloride
HIV	Human Immunodeficiency Virus
IB	Investigator's Brochure
ICH	International Conference on Harmonization
IRB	Investigational Review Board
IUD	Intrauterine Device
IV	Intravenous
KOOS, JR	Knee Injury and Osteoarthritis Outcome Score for Joint Replacement
LAST	Local Anesthetic Systemic Toxicity
LS	Least Square
MDMA	3,4-Methylenedioxymethamphetamine or Ecstasy
MedDRA	Medical Dictionary for Regulatory Activities
mg	Milligram
mL	Milliliter
mm	Millimeter
MME	Milligram-Morphine Equivalents
ms	Millisecond
ng	Nanogram
NRS	Numeric Rating Scale
NRS-A	Numeric Rating Scale with Activity
NRS-R	Numeric Rating Scale at Rest
NSAID	Non-steroidal Anti-inflammatory Drug
OA	Osteoarthritis
PACU	Post-Anesthesia Care Unit
PHQ-9	Patient Health Questionnaire 9
PK	Pharmacokinetic(s)
PLGA	Poly(DL-lactide-co-glycolide)
PS20	Polysorbate 20
QOR-15	Quality of Recovery 15 Scale
RA	Rheumatoid Arthritis

Abbreviation	Definition
ROM	Range of Motion
SAE	Serious Adverse Event
SD	Standard Deviation
SMC	Safety Monitoring Committee
SOC	System Organ Class
SUSAR	Suspected Unexpected Serious Adverse Reaction
TEAE	Treatment Emergent Adverse Events
TKA	Total Knee Arthroplasty
$T_{1/2}$	Terminal Elimination Half-Life
T_{max}	Observed Time to Reach Maximum Concentration
µg	Microgram

4.0 BACKGROUND

Allay Therapeutics, Inc. (Allay) is developing an extended-release bupivacaine bioabsorbable implant designed to provide postsurgical analgesia following total knee arthroplasty (TKA). Primary TKA is most commonly performed for knee joint failure caused by osteoarthritis (OA). Other indications for TKA include rheumatoid arthritis (RA), osteonecrosis, and other types of inflammatory arthritis [2]. Total knee arthroplasty is performed after other non-operative treatment options (eg weight loss, aerobic exercise, physical therapy, pain relievers, nonsteroidal anti-inflammatory drugs, and corticosteroid injections) [3] have failed. The goals of TKA include pain relief and improvement in function and mobility. While the goal of a TKA is pain relief, following surgery the patient experience is often moderate to severe post-operative pain that can last for days or weeks and mild to moderate pain lasting for weeks to months following surgery. The current post-operative pain management solutions described below are not adequate to address longer duration of pain during recovery.

Potential complications following TKA include infection, deep vein thrombosis (DVT), joint stiffness, and early failure. To reduce the risk of DVT and to facilitate rehabilitation and recovery, early mobilization, including full compliance with the prescribed regimen of physical therapy exercises, is encouraged [4]. However, significant immediate post-operative pain and pain during recovery associated with TKA can compromise the rehabilitation process and adversely affect outcomes and extends recovery times [5]. Pain following TKA is currently addressed by a multi-modal strategy comprising at least one of several short-term local therapies followed by the use of oral pain management products. Pre-operatively, several options are available, including epidural and spinal anesthetics with adjuncts such as long-acting morphine and/or peripheral nerve blocks. Intra-operative injections of bupivacaine (eg, MARCAINE) or other local anesthetics may be administered at the surgical site. Post-operatively, peripheral nerve blocks, and multi-modal pain control which combines two or more analgesics that act by different mechanisms may be used.

The 2 to 3 days of postoperative analgesia typically achieved by the aforementioned approaches are inadequate for the weeks to months of pain following a TKA. Within hours of the TKA surgery patients are expected to be ambulatory and articulating the prosthetic joint. Walk distance and range of motion (ROM) tests, a variety of therapeutic exercises and a rigorous physical therapy regimen are quite painful, but necessary to the successful rehabilitation and recovery process. Since poor compliance may result in loss of joint function, due to development of scar tissue, requiring additional procedures, surgeons will typically prescribe several weeks of oral opioids with instructions to use prior to the onset of any breakthrough pain.

Although prescription opioids have made pain treatable, medical use of opioids can lead to addiction and dependence. Namba et al. indicated that 41.5% (9,914 of 24,105 patients studied) continued using opioids after the initial 90-day post-operative period following TKA for OA [6]. Prevention of opioid addiction has become an urgent public health emergency [7]. Of several highly invasive surgeries studied, it was determined that TKA puts patients at the highest risk of chronic opioid use [8]. Moreover, generous prescriptions written following TKA put a

tremendous amount of opioids into circulation. More effective local pain management after TKA will preclude the need for opioids.

ATX-101 is intended to provide sustained post-surgical analgesia for a targeted several week duration with bupivacaine as the active pharmaceutical ingredient. Bupivacaine, a sodium ion channel blocker, is the active, non-opioid, pharmaceutical ingredient in US-approved MARCAINE (NDA 016964), which has been in use as an infiltrative and intravenous (IV) local anesthetic or analgesia since 1972. Marcaine has a half-life of 2.7 hours and a maximum recommended daily dose of 400 milligrams (mg). Bupivacaine is widely used in surgical procedures. Additional dosage forms of bupivacaine approved for postsurgical local anesthesia include: Exparel® (US 2011, EU 2020) a slower-releasing bupivacaine liposome suspension approved for doses up to 266 mg; Xaracoll (US 2020) a bupivacaine implant approved for doses up to 300 mg; most recently Posimir® (US 2021), a bupivacaine solution for infiltration for doses up to 660 mg and Zynrelef® (EU 2020, US 2021) an extended-release solution of bupivacaine and meloxicam with bupivacaine doses up to 400 mg for TKA. The safety and efficacy of bupivacaine for postsurgical analgesia, therefore, has been well characterized in humans and various animal models.

ATX-101 is intended to be placed at the conclusion of the TKA procedure utilizing a straightforward technique, which differs from the injection, infiltration, and instillation techniques of the current local bupivacaine treatments that can typically yield significant, technique-dependent variability in outcomes. ATX-101 will be placed by the orthopedic surgeon in predetermined locations under direct visualization. It is anticipated that sustained delivery of bupivacaine from ATX-101 will achieve a sustained, local analgesic effect that reduces pain, minimizes opioid use, decreases the length of hospitalization, facilitates rehabilitation and recovery, and avoids concerns over systemic toxicity.

4.1 BUPIVACAINE PHARMACOLOGY

The pharmacology and pharmacokinetics (PK) of bupivacaine have been evaluated in guinea pigs and rabbits (MARCAINE NDA 016964) and other animals in a large, publicly available body of research from multiple labs. Following absorption, bupivacaine is rapidly and widely systemically distributed with higher concentrations detected in highly perfused organs such as the brain, lungs, heart, liver, and kidney. After direct IV injection, bupivacaine kinetics fit a three-compartment model, with the first compartment characterized by intravascular distribution, the second compartment resulting from distribution and equilibration to highly perfused organs, and the third compartment due to distribution and equilibration in poorly perfused tissues [9]. Bupivacaine is known to be highly protein bound (95%), and binding is associated with the α1-acid glycoprotein and albumin fractions in human plasma [10,11,12]. In humans, the half-life is 2.7 hours after IV administration.

Bupivacaine is extensively metabolized, primarily by Cytochrome P450 3A4 (CYP3A4) in the liver with only approximately 6% of unchanged bupivacaine excreted in urine by humans [9].

The major metabolite in humans is desbutylbupivacaine (pipecoloxylidide), while the major route of excretion in humans is in urine.

4.2 BUPIVACAINE EXPOSURE AND CLINICAL SAFETY

Most of the available literature data on the safety and toxicity of bupivacaine are based on information available from use of bupivacaine through needle infiltration and instillation. Cases of systemic toxicity often result from accidental injection of bupivacaine into or around a blood vessel or acute injection of an excessive dose, the majority of which absorbs into the blood stream. In contrast, ATX-101 is surgically placed and cannot be injected like other formulations of bupivacaine. Moreover, the release of bupivacaine from ATX-101 is gradual and sustained over time with minimal systemic concentrations throughout the duration of the drug release.

Bupivacaine is a nonselective voltage-gated sodium ion channel blocker. Blockade of voltage-gated sodium ion channels inhibits depolarization and thus conduction of pain signaling. Uncontrolled high plasma levels of bupivacaine can lead to cardiotoxicity and neurotoxicity. These adverse drug reactions typically occur due to accelerated absorption from the injection site or unintentional intravascular injection. Injectable bupivacaine products have a maximum recommended human dose of 400 mg/day [9]. However, multiple studies, with a few summarized herein, have demonstrated that doses higher than 400 mg from extended-release products are safe due to their slower release of bupivacaine over an extended period of time. Moreover, the knee capsule provides a built-in compartment to isolate the bupivacaine delivered or released within the joint capsule from the blood stream [13]. Goyal et al provided 75 TKA patients with a continuous intra-articular infusion of bupivacaine solution at a rate of 600 mg/day (5 mL/hour of 0.5% bupivacaine solution) via an elastomeric pain pump for two consecutive days with no adverse events (AEs) related to systemic toxicity [14].

The maximum tolerated plasma concentration with respect to central nervous system (CNS) effects in healthy volunteers administered bupivacaine in solution has been reported to be 2,100 ng/mL (2.1 µg/mL, range of 0.8-4.5 µg/mL) [15] and 2,250 ng/mL (2.25 µg/mL) [16]. Onset of CNS toxicity has been reported in the range of 2,000-4,000 ng/ml (2-4 µg/mL) [17] with serious risk of toxicity possibly at 2,300-5,000 ng/mL (2.3-5 µg/mL) [18].

4.3 OVERALL DOSE SAFETY SUMMARY AND ATX-101 DOSE JUSTIFICATION

Though CNS associated AEs can occur as low as 2,000 ng/mL, safe levels of plasma bupivacaine concentrations have been reported up to 9,270 ng/mL (9.27 µg/mL) in humans and more frequently at approximately 4,500 ng/mL (4.5 µg/mL) [19]. While extended bupivacaine delivery from ATX-101 is designed to deliver lower systemic exposure over a greater duration as compared with infusion and infiltration approaches, safety thresholds for systemic exposure may be adequately derived from the human infusion and infiltration experience. Human clinical data from bupivacaine delivered via infusion or infiltration are summarized in the Investigator's Brochure (IB). The first in human trial FT1-TKA-001 administered 252 mg, 756 mg, and 1,512 mg of bupivacaine free base to 4, 3, and 15 subjects respectively and it appeared to be well

tolerated. Based upon tolerability in FT1-TKA-001, this clinical trial will plan to evaluate ATX-101 at 1,000 mg and at 1,500 mg.

4.4 ATX-101 CLINICAL EXPERIENCE

Trial FT1-TKA-001 was a Phase 1/2A safety trial in humans evaluating ATX-101. The trial was an open-label ascending dose PK, safety and tolerability clinical trial that was conducted in Australia. The primary objective of the trial was to characterize the PK concentration profile associated with bupivacaine via ATX-101 (formerly referred to as the TKAine System). The secondary objectives were to evaluate the safety and tolerability as well as the dose-related response of ATX-101.

Three dose groups were evaluated in the trial. Before escalating to the next dose, a Data Safety Monitoring Board (DSMB) reviewed PK concentrations, cardiac data, and the AE profile. Depending upon the Cohort, a single dose level of ATX-101 was implanted in the knee capsule following the TKA procedure when tissue was not disrupted any further by surgery but prior to surgical closure. ATX-101 was placed in the suprapatellar pouch, medial gutter alongside capsular tissue, and/or lateral gutter alongside capsular tissue under direct visualization (Table 1).

Table 1. FT1-TKA-001 Dosage, Number of Subjects, and Placement

Cohort	Dose (bupivacaine free base)	Dose (bupivacaine HCl monohydrate)	Number of Subjects	Location of Implants
Cohort 1	252 mg	300 mg	4	Medial Gutter
Cohort 2	756 mg	900 mg	3	Medial Gutter and Suprapatellar Pouch
Cohort 3	1512 mg	1800 mg	15	Medial Gutter, Suprapatellar Pouch, and Lateral Gutter

HCl = Hydrochloride

The Australian patient population enrolled in the FT1-TKA-001 clinical trial had similar surgical techniques, standard perioperative care, and demographics to US population receiving TKAs (refer to the Investigator's Brochure for additional information). In trial FT1-TKA-001 there were no deaths. There were no withdrawals in the trial for any reason. There were two (2) Serious Adverse Events (SAEs) and three (3) Adverse Events of Special Interest (AESIs). One subject experienced an SAE of drug (acetaminophen/paracetamol) induced liver injury that started about one month after the TKA surgery. The SAE was determined by the Investigator as unlikely related to the trial drug and not related to the trial procedure.

One subject experienced an SAE of acute spontaneous urticaria with angioedema (preferred term urticaria) that started about 11 days after the TKA surgery. The SAE was determined by the Investigator as not related to the trial drug and not related to the trial procedure. The event was

likely triggered from aspirin, non-steroidal anti-inflammatory drugs (NSAIDS), or opioids per the medical records.

Three AESIs were identified. Two events were asymptomatic sinus bradycardia both indicated as unlikely related to the trial drug and unlikely related to the trial procedure. One event was symptomatic sinus bradycardia and was indicated as not related to the trial drug and not related to the trial procedure.

The most common AEs in the trial were nausea (54.5%), constipation (27.3%), haematoma (27.3%), dizziness (22.7%), diarrhoea (18.2%), vomiting (18.2%), and peripheral oedema (18.2%).

Adverse events were classified by the Investigator as Not Related, Unlikely Related, Possibly Related, Probably Related or Definitely Related to the study drug (bupivacaine) or to the TKA surgical procedure.

The most common AE indicated as related (Possibly, Probably, or Definitely Related) to the study drug, ATX-101 was paraesthesia (9.1%).

The most common AEs indicated as related (Possibly, Probably, or Definitely Related) to the TKA surgical procedure include haematoma (22.7%) nausea, (13.6%), peripheral oedema (13.6%), hypotension (13.6%), and erythema (9.1%).

The majority of AEs were mild or moderate in severity. There were 3 severe AEs (syncope, urticaria, and drug [acetaminophen/paracetamol] induced liver injury).

5.0 TRIAL OBJECTIVES AND ENDPOINTS

5.1 OBJECTIVES

The objectives of this Phase 2B trial are:

- To compare the efficacy of ATX-101 (1,000 mg or 1,500 mg) with that of bupivacaine hydrochloride (HCl) in subjects undergoing primary unilateral TKA
- To evaluate the safety and tolerability of ATX-101
- To characterize the PK profile following administration of ATX-101 (Part A only)
- To compare opioid consumption of subjects administered ATX-101 with that of bupivacaine HCl (Part B only)
- To estimate the sample size, determine dose, and primary endpoint duration from Part A needed for Part B
- To compare the efficacy of ATX-101 with that of bupivacaine HCl in this trial population

5.2 ENDPOINTS

5.2.1 Primary Endpoints

The primary efficacy endpoint (evaluated in Part B) is to determine the area under the curve (AUC) for the Numeric Rating Scale at Rest (NRS-R) of pain intensity from 30 Minutes through a time point to be determined from Part A.

The primary efficacy endpoint for Part A is AUC for the NRS-R of pain intensity from 30 Minutes through Hour 168 (Day 8 of the trial).

5.2.2 Key Secondary Endpoints (Evaluated in Part B)

Key secondary endpoints are to evaluate the:

- Area under the curve for the NRS-R of pain intensity from 30 Minutes through Hour 168 (Day 8 of the trial), Hour 240 (Day 11 of the trial), and Hour 336 (Day 15 of the trial).
- Percentage of subjects who remain opioid free from Hour 72 through Day 30.
- Total post-surgical consumption of opioid medications from surgical closure through Day 30.

5.2.3 Secondary Endpoints

Secondary endpoints for both Part A and Part B are to evaluate the:

- Area under the curve for the NRS-R of pain intensity for each 24-hour period through Hour 336 (Day 15 of the trial)
- Percentage of subjects who remain opioid free
- Total post-surgical consumption of rescue opioid medications
- Time to first postsurgical use of rescue opioid medication

5.2.4 Safety Endpoints

Safety endpoints for both Part A and Part B are to evaluate the:

- Incidence of AEs, AESIs, and SAEs
- Wound healing assessment using the Southampton Wound Scoring System
- Characterization of the bupivacaine PK from ATX-101

5.2.5 Exploratory Endpoints

Exploratory endpoints for both Part A and Part B will evaluate the:

- Percentage of subjects reporting being pain free on the NRS-R of pain intensity
- Time to various degrees of flexion and extension
- Reduction/incidence of opioid-related AEs

- Evaluation of Quality of Recovery 15 Scale (QOR-15)
- Evaluation of Knee Injury and Osteoarthritis Outcome Score for Joint Replacement (KOOS, JR)
- Difference in pain intensity scores (NRS-R and Numeric Rating Scale with Activity [NRS-A]) between treatment groups
- If a statistical difference in AUC of pain intensity NRS-R at Hour 336 (Day 15 of the trial) is observed, the AUC for the NRS-R of pain intensity will be further evaluated between Days 16 and 30
- Evaluation of the Knee Society Score

6.0 INVESTIGATIONAL PLAN

6.1 OVERALL TRIAL DESIGN

This is a two-part (Part A and Part B) Phase 2B, randomized, double-blind, active comparator multicenter trial in subjects undergoing primary unilateral TKA. Part A will be used to determine the sample size, dose, and primary endpoint duration for Part B of the trial.

In Part A of the trial, approximately 165 subjects will be randomized to one (1) of the following three (3) treatment groups in a 1:1:1 ratio with approximately 55 subjects per group:

- ATX-101, 1,000 mg dose (two ATX-101 implants)
- ATX-101, 1,500 mg dose, (three ATX-101 implants)
- Bupivacaine HCl 0.25% without epinephrine/adrenaline, maximum dose of 2 mg/kg, via local periarticular infiltration or adductor canal block, or in combination

In Part B of the trial, up to 140 subjects (total number will be determined from Part A) will be randomized to one (1) of the following two (2) treatment groups in a 1:1 ratio with up to 70 subjects per group:

- ATX-101 dose to be determined from Part A
- Bupivacaine HCl 0.25% without epinephrine/adrenaline, maximum dose of 2 mg/kg, via local periarticular infiltration or adductor canal block, or in combination

6.1.1 Trial Procedures

Subjects will participate in the trial for up to approximately 12 weeks, including a 30-day screening period. All subjects will be screened within 30 days prior to surgery. Subjects who meet eligibility criteria will be randomized up to 1 business day prior to the day of surgery.

Unless otherwise specified, all procedures are applicable for both Part A and Part B of the trial.

In addition to the below trial procedures, all subject visits to the emergency department, urgent care and/or clinic will be documented and indicated if the visit was related to pain. Throughout

the trial all phone calls or digital interactions (eg email) with the subject will be documented in the source documentation.

6.1.1.1 Screening Visit

Subjects will attend the hospital/clinic/facility up to 30 days prior to the scheduled surgical procedure. After obtaining informed consent from the subject, as documented by the signed informed consent form, the following assessments will be conducted:

- Demographic data collection
- Review of medical history
- Review of trial entry criteria
- Vital signs
- Physical examination including an orthopedic evaluation of Kellgen Lawrence Classification, varus, valgus, and an evaluation of ipsilateral hip osteoarthritis.
- Height and weight
- 12-lead electrocardiogram (ECG)
- Laboratory testing (Chemistry, Hematology, Drug Screen, and Pregnancy Test for females of childbearing potential)
- ROM assessment
- Patient Health Questionnaire 9 (PHQ-9)
- Neurological assessments
- Electronic (e-diary) training
- Numeric Rating Scale (NRS) pain score training for pain intensity
- Prior and concomitant medication review
- Knee Society Score
- Subject self-administered questionnaires/survey completion (NRS-R and location of pain; QOR-15; KOOS, JR; Pain Manageability; and NRS-A and location of pain)

Subjects will complete the self-administered questionnaires/surveys after being trained on the e-diary and NRS pain score training for pain intensity. The subject may utilize an e-diary maintained by the site for completion of the questionnaires/survey. For further information please see Section 8.9 and 8.10.

When possible, subjects should stop taking gabapentin and/or pregabalin before the TKA surgery. If a subject is otherwise a good candidate for the trial and unable or unwilling to stop taking gabapentin and/or pregabalin as of the Screening Visit, then medical monitor approval will be required prior to permitting enrollment into the trial.

Subjects should be encouraged to stop taking opioids from the Screening Visit and are not considered eligible for the trial if unable to abstain from taking opioids at least 14 days prior to surgery.

Confirmation of eligibility based upon cardiac and laboratory data must be confirmed prior to surgery. Availability of receipt of this data may vary based upon the site location to the cardiac laboratory and central labs and should be planned for accordingly.

6.1.1.2 Day of Surgery (Day 1)

6.1.1.2.1 Before Surgery

On the day of surgery, before surgery and before bupivacaine spinal is administered, the following assessments will be conducted:

- Medical history will be confirmed and updated as appropriate
- Review of trial entry criteria
- Vital signs
- 12-lead ECG
- Laboratory testing (Drug Screen only for opioids and Pregnancy Test for females of childbearing potential)
- Neurological assessments
- e-diary training
- e-diary dispensation if the subject is not using their own device
- NRS pain score training for pain intensity
- Prior and concomitant medication review
- Randomization and investigational product allocation
- Pharmacokinetic sampling before bupivacaine spinal (Part A only)

Randomization and investigational product allocation may occur up to one business day before surgery. Subjects who fail to meet eligibility criteria during screening (any time before randomization) will be considered screen failures. Subjects who fail to meet eligibility from the time of randomization through surgery will be considered randomization failures. In Part A subjects will be randomized to one of three treatment groups: 1,000 mg ATX-101, 1,500 mg ATX-101, or bupivacaine HCl.

In Part B subjects will be randomized to one of two treatment groups: either ATX-101 (dose determined after Part A) or bupivacaine HCl. All needed investigational product will be brought to the operating room. It is recommended that spare investigational products be brought to the operating room in the event the originally assigned product is broken, damaged, dropped outside of the sterile field, or the product becomes unusable. All reasons for utilizing the spare product

will be documented on unblinded records and only viewable to unblinded staff and unblinded monitors. Section 7.8 contains further information regarding blinding.

After checking in for surgery on Day 1, the subject will receive acetaminophen/paracetamol 1,000 mg and celecoxib 200 mg before surgery.

In addition, the subject will complete the self-administered e-diary questionnaire/survey which will include:

- NRS-R and location of pain

6.1.1.2.2 During Surgery

Subjects will undergo primary unilateral TKA surgical procedure completed under spinal anesthesia. Spinal anesthesia with bupivacaine (with or without dextrose) will be used in all subjects; no other adjunct medications such as morphine or ketamine may be used in the spinal anesthesia. General anesthesia is optional, but if used must be in conjunction with spinal anesthesia. Local nerve blocks (eg adductor canal blocks) are prohibited in the ATX-101 treatment group (but permissible in the bupivacaine HCl group), however topical anesthetics such as lidocaine can be used to aid in IV access and subcutaneously for spinal needle placement.

At the time of induction, the use of fentanyl (no more than 300 µg) for airway management is permitted when general anesthesia is used. Other than fentanyl for airway management, no other opioids may be administered during surgery. If not administered before surgery, one dose of prophylactic ondansetron will be given. All other doses of antiemetics will be given only after an adverse event has been observed or reported.

[redacted]

Surgical drains are not permitted. Two doses of IV tranexamic acid (1 g each) will be administered to the subject. One dose of tranexamic acid will be administered prior to investigational product administration and the second dose after investigational product administration, with the timing at the discretion of the treating Investigator. A one-time dose of IV dexamethasone (4 mg) will be administered during the surgical procedure, with the timing at the discretion of the treating Investigator. Intravenous or oral antibiotic regimen may also be given per the center's standard of care. Antibiotic powders or beads in the knee joint are prohibited.

Subjects will not be administered the investigational product if any clinically significant (CS) event or condition is uncovered during surgery (eg excessive bleeding, femur fracture), that occurs before investigational product administration and might render the subject medically unstable or complicate the subject's postsurgical course.

If randomized to ATX-101, a one-time dose of a single dose level of ATX-101 will be placed in the subject's knee capsule under direct visualization following fixation of the knee implant prosthesis when tissue will not be disrupted any further by surgery, after any betadine or saline

rinse, and after any suction has occurred, but prior to initiation of surgical closure. ATX-101 will be placed in the suprapatellar pouch, medial gutter, and/or lateral gutter by a trained surgeon according to the Dosage and Administration Instructions.

If randomized to active comparator, bupivacaine HCl 0.25% without epinephrine/adrenaline maximum dose of 2 mg/kg via local periarticular infiltration or an adductor canal block, or in combination will be administered during surgery. Bupivacaine HCl alone will be used for infiltration or adductor canal block, additional anesthetics or medications are not allowed.

Subjects who fail to meet the entry criteria at the time of the surgical procedure will not be enrolled in the trial. Subjects who are randomized but do not receive investigational product (eg did not meet exclusion criterion #24 or #25), will be considered randomization failures.

The surgical staff will not be blinded to the trial treatment, all other research staff (unless expressly specified in the site's blinding plan that is approved by Allay) and the subject will remain blinded throughout the trial. Section 7.8 contains further information regarding blinding.

The following activities/assessments will be conducted during the surgery:

- Review of entry criteria and confirmation of eligibility prior to investigational product administration
- In Part A, a PK blood sample will be taken during surgery before investigational product administration (within -10 minutes before ATX-101 administration or first administration of bupivacaine HCl)
- 12-lead ECG (Part A only)
- Investigational product administration of ATX-101, or bupivacaine HCl
- Concomitant medication collection
- Recording of AEs after the investigational product administration

The time of surgical closure (skin closure/final stitch placed) will be documented and considered Time 0 for all assessments conducted.

6.1.1.2.3 After Surgery

Following surgery, subjects will be transferred to the post-anesthesia care unit (PACU). The time the subject arrives and departs PACU will be recorded. The time the subject first ambulates will also be recorded.

While in the hospital/facility subjects will have regular assessments of vital signs, neurological assessments, pain scores, AEs, and concomitant medication review.

In Part A only, the subject must remain under observation until Hour 96. Subjects may remain in the hospital/facility for 96 hours. Alternatively, subjects may be discharged from the

hospital/facility per standard of care and moved to another unit until the conclusion of the 96-hour in-patient monitoring period. In Part B, subjects will remain under observation until Hour 24 and then may be discharged per standard of care.

Subjects should only receive rescue opioid medication upon request for pain control, as needed, after surgery. Rescue opioid medication may be given/taken for pain treatment, but not for pain prophylaxis. Prior to the administration of any rescue opioid medication from surgery through the Day 30 Visit, a NRS-R assessing the subject's pain intensity and location of pain must be obtained.

If required to treat pain, the following standardized rescue opioid medication protocol will be used. Preferred rescue opioid medication will consist of oral immediate-release oxycodone (5 mg or 10 mg). If a subject cannot tolerate oral medication, IV (including patient-controlled analgesia pump) or IM medication may be administered as needed (morphine is preferred, however hydromorphone can also be used with the dose determined by the treating physician). If a subject cannot tolerate the medication or the breakthrough pain is not responsive to oral oxycodone (5 mg or 10 mg), then oral hydromorphone (2 mg or 4 mg) may be given for rescue opioid medication. Should the subject have an AE and is unable to tolerate the rescue opioid medications of oral oxycodone (5 mg or 10 mg), IV morphine, oral hydromorphone (2 mg or 4 mg), and IV hydromorphone, then hospital standard of care may be utilized, however, local anesthetic nerve blocks may not be administered after surgery. (See Section 7.6.3.1 for further information on rescue opioids.)

The subjects will be administered celecoxib (200 mg) twice a day for 30 days, unless an AE occurs that requires discontinuation. Acetaminophen/paracetamol 500 mg should be taken every 4 hours or 1,000 mg every 6 to 8 hours not to exceed 3,000 mg per any 24-hour period for a minimum of 21 days. Deep vein thrombosis (DVT) prophylaxis is required, and 75-100 mg aspirin (based upon regional approved dose) is recommended twice a day for 30 days. Aspirin is recommended in trial subjects unless there are additional risks of thromboembolic events identified requiring a different DVT prophylaxis. Celecoxib, acetaminophen/paracetamol, and aspirin may be provided by the Sponsor.

Should the subject experience an SAE or AESI, refer to Section 6.1.1.12 for required assessments.

The following activities/assessments will be conducted on Day 1 after surgery:

- Vital signs
- 12-lead ECG (Part A only)
- Neurological assessments
- Concomitant medication review
- PK sampling (Part A only)

- Recording of AEs

See Appendix A (Table: Part A Schedule of Events for Day of Surgery through Hour 96 (Day 5)) and Appendix B (Table: Part B Schedule of Events for Day of Surgery through Hour 24 (Day 2)) for specific timing of assessments. After surgical closure the subject will be completing self-administered e-diary questionnaires/surveys which will include:

- NRS-R and location of pain, multiple times a day as specified in Appendix C
- Rescue opioid medication questions, any time before an opioid is consumed and assessment of medication compliance
- Physical therapy survey

The subject will continue to complete self-administered e-diary questionnaires/surveys per Appendix C on the trial visit day and in-between visits.

6.1.1.3 Part A: Days 2, 3, 4, & 5 During 96 Hour Observation

Subjects will remain under observation for 96 hours following surgery. Subjects may remain in the hospital/facility for 96 hours. Alternatively, subjects may be discharged from the hospital/facility per standard of care and moved to another unit until the conclusion of the 96-hour monitoring period. All procedures in the Schedule of Events (Appendix A) will be followed. Once the subject is ready to leave the facility, the date and time of discharge will be recorded. Upon discharge, the location/facility where subjects are being discharged will be recorded (eg home, rehabilitation facility, facility for 96-hour in-patient monitoring). Subjects may have more than one discharge date and time if they were transferred to a different location for the in-patient monitoring period.

Site staff should confirm the subject has been regularly completing the self-administered questionnaires and surveys. As needed, the site staff should conduct any training to ensure compliance with medication consumption, rescue opioid medication, e-diary completion/use, and/or prescribed physical therapy regimens.

The following assessments will be conducted on Days 2, 3, 4, & 5:

- Vital signs
- 12-lead ECG
- ROM assessment
- Neurological assessments
- Subject NRS pain score training for pain intensity (Days 2 and 3 only)
- Concomitant medication review
- PK sampling
- Recording of AEs

See Appendix A (Table: Part A Schedule of Events for Day of Surgery through Hour 96 (Day 5)) for specific timing of assessments. In addition, the subject will continue to complete self-administered e-diary questionnaires/surveys which will include:

- NRS-R and location of pain, multiple times a day as specified in Appendix C
- QOR-15
- NRS-A and location of pain
- Rescue opioid medication questions, any time before an opioid is consumed and assessment of medication compliance
- Physical therapy survey

6.1.1.4 Part A, Day 6

The Day 6 Visit may occur in the hospital/clinic/facility, at the subject's home, at a physical therapy facility, or any other place agreed upon by the subject and site staff.

Before or during the visit, site staff should confirm the subject has been regularly completing the self-administered questionnaires and surveys. As needed, the site staff should conduct any training to ensure compliance with medication consumption, rescue opioid medication, e-diary completion/use, and/or prescribed physical therapy regimens.

The following assessments will be conducted on Day 6:

- Vital signs
- 12-lead ECG
- ROM assessment
- Neurological assessments
- Concomitant medication review
- PK sampling
- Recording of AEs

In addition, the subject will continue to complete self-administered e-diary questionnaires/surveys which will include:

- NRS-R and location of pain, multiple times a day as specified in Appendix C
- QOR-15
- NRS-A and location of pain
- Rescue opioid medication questions, any time before an opioid is consumed and assessment of medication compliance
- Physical therapy survey

The subject will continue to complete self-administered e-diary questionnaires/surveys per Appendix C on the trial visit day and in-between visits.

6.1.1.5 Part B: Day 2

All procedures in the Schedule of Events (Appendix B) will be followed. For Part B, subjects will remain under observation for a minimum of one overnight (approximately 24 hours) and then may be discharged per standard of care after the Day 2 procedures have been conducted. The date and time of discharge will be recorded. Upon discharge, the location/facility where subjects are being discharged to will be recorded (eg home, rehabilitation facility).

Site staff should confirm the subject has been regularly completing the self-administered questionnaires and surveys. As needed, the site staff should conduct any training to ensure compliance with medication consumption, rescue opioid medication, e-diary completion/use, and/or prescribed physical therapy regimens.

The following assessments will be conducted on Day 2:

- Vital signs
- ROM assessment
- Neurological assessments
- Subject NRS pain score training for pain intensity
- Concomitant medication review
- Recording of AEs

See Appendix B (Table: Part B Schedule of Events for Day of Surgery through Hour 24 (Day 2)) for specific timing of assessments.

In addition, the subject will continue to complete self-administered e-diary questionnaires/surveys which will include:

- NRS-R and location of pain, multiple times a day as specified in Appendix C
- QOR-15
- NRS-A and location of pain
- Rescue opioid medication questions, any time before an opioid is consumed and assessment of medication compliance
- Physical therapy survey

The subject will continue to complete self-administered e-diary questionnaires/surveys per Appendix C on the trial visit day and in-between visits.

6.1.1.6 Part B: Days 3, 4, 5, & 6

For Part B, following the Day After Discharge Visit the scheduled visits on Days 3, 4, 5, & 6 will be conducted. All procedures in the Schedule of Events (Appendix B) will be followed.

Trial visits on Days 3, 4, 5, & 6 may occur in the hospital/clinic/facility, at the subject's home, at a physical therapy facility, or any other place agreed upon by the subject and site staff.

Before or during the visit, site staff should confirm the subject has been regularly completing the self-administered questionnaires and surveys. As needed, the site staff should conduct any training to ensure compliance with medication consumption, rescue opioid medication, e-diary completion/use, and/or prescribed physical therapy regimens.

The following assessments will be conducted on Days 3, 4, 5, & 6:

- Vital signs
- ROM assessment
- Neurological assessments
- Subject NRS pain score training for pain intensity (Day 3 only)
- Concomitant medication review
- Recording of AEs

In addition, the subject will continue to complete self-administered e-diary questionnaires/surveys which will include:

- NRS-R and location of pain, multiple times a day as specified in Appendix C
- QOR-15
- NRS-A and location of pain
- Rescue opioid medication questions, any time before an opioid is consumed and assessment of medication compliance
- Physical therapy survey

The subject will continue to complete self-administered e-diary questionnaires/surveys per Appendix C on the trial visit day and in-between visits.

6.1.1.7 Day 8

The Day 8 Visit (±1 day) may occur in the hospital/clinic/facility, at the subject's home, at a physical therapy facility, or any other place agreed upon by the subject and site staff.

Before or during the visit, site staff should confirm the subject has been regularly completing the self-administered questionnaires and surveys. As needed, the site staff should conduct any training to ensure compliance with medication consumption, rescue opioid medication, e-diary completion/use, and/or prescribed physical therapy regimens.

The following assessments will be conducted on Day 8:

- Vital signs
- 12-lead ECG (Part A only)
- ROM assessment
- Neurological assessments
- Concomitant medication review
- PK Sampling (Part A only)
- Recording of AEs

In addition, the subject will continue to complete self-administered e-diary questionnaires/surveys which will include:

- NRS-R and location of pain, multiple times a day as specified in Appendix C
- QOR-15
- KOOS, JR
- Pain manageability survey
- NRS-A and location of pain
- Rescue opioid medication questions, any time before an opioid is consumed and assessment of medication compliance
- Physical therapy survey

The subject will continue to complete self-administered e-diary questionnaires/surveys per Appendix C on the trial visit day and in-between visits.

6.1.1.8 Day 15 and Day 22 Visits

The Day 15 Visit (±3 days) and Day 22 Visit (±3 days) may occur in the hospital/clinic or at a physical therapy facility.

Before or during the visit, site staff should confirm the subject has been regularly completing the self-administered questionnaires and surveys. As needed, the site staff should conduct any training to ensure compliance with medication consumption, rescue opioid medication, e-diary completion/use, and/or prescribed physical therapy regimens.

The following assessments will be conducted on Day 15 and Day 22:

- Vital signs
- 12-lead ECG (Part A only)
- Laboratory testing (drug screen) (Day 15 only)
- ROM assessment
- Neurological assessments
- Subject NRS pain score training for pain intensity (Day 15 only)
- Concomitant medication review. Opioid accountability at Day 15 only
- PK sampling (Part A only)
- Wound healing assessment
- Knee Society Score (Day 15 only)
- Recording of AEs

In addition, the subject will continue to complete self-administered e-diary questionnaires/surveys which will include:

- NRS-R and location of pain, multiple times a day as specified in Appendix C
- QOR-15
- KOOS, JR
- Pain manageability survey
- NRS-A and location of pain
- Rescue opioid medication questions, any time before an opioid is consumed and assessment of medication compliance
- Physical therapy survey

The subject will continue to complete self-administered e-diary questionnaires/surveys per Appendix C on the trial visit day and in-between visits.

6.1.1.9 Day 30 Visit

The Day 30 Visit (±3 days) may occur in the hospital/clinic or at a physical therapy facility.

Before or during the visit, site staff should confirm the subject has been regularly completing the self-administered questionnaires and surveys. As needed the site staff should conduct any training to ensure compliance with medication consumption, rescue opioid medication, e-diary completion/use, and/or prescribed physical therapy regimens.

The following assessments will be conducted on Day 30:

- Vital signs
- 12-lead ECG (Part A only)
- ROM assessment
- Neurological assessments
- e-diary return if the subject used a provisioned device
- Concomitant medication review including opioid accountability
- PK sampling (Part A only)
- Wound healing assessment
- Knee Society Score
- Recording of AEs

If not completed in advance of the Day 30 Visit, then prior to the conclusion of the Day 30 Visit, the subject should complete all Day 30 e-diary questionnaires/surveys and return the e-diary to the site staff (if using a provisioned device). The self-administered e-diary questionnaires/surveys will include:

- NRS-R and location of pain
- QOR-15
- KOOS, JR
- Pain manageability survey
- NRS-A and location of pain
- Rescue opioid medication questions, any time before an opioid is consumed and assessment of medication compliance
- Physical therapy survey

If the subject did not complete the Day 30 self-administered questionnaires/surveys before the visit and did not return the provisioned e-diary during the Day 30 Visit, then they may utilize an e-diary maintained by the site for completion of the self-administered questionnaires/surveys. The subject will be instructed to return the e-diary to the site before or during the Day 56 Visit.

6.1.1.10 Day 56 Visit

The Day 56 Visit (±7 days) may occur in the hospital/clinic or at a physical therapy facility.

The following assessments will be conducted on Day 56:

- Vital signs
- Physical examination

- Weight
- 12-Lead ECG
- Laboratory testing (Chemistry, Hematology, and Pregnancy Test for females of childbearing potential)
- ROM assessment
- Neurological assessments
- Concomitant medication review
- Wound healing assessment
- Knee Society Score
- Recording of AEs

The subject may utilize an e-diary maintained by the site for completion of the final self-administered questionnaires/surveys. The questionnaires/surveys will include:

- NRS-R and location of pain
- QOR-15
- KOOS, JR
- Pain manageability survey
- NRS-A and location of pain

If the subject did not return the provisioned e-diary at the Day 30 Visit, it should be returned to the site staff during this visit.

6.1.1.11 Early Termination Visit

The Early Termination Visit may occur in the hospital/clinic or at a physical therapy facility.

If a subject early terminates, when possible and with subject consent, all end of trial (Day 56) procedures should be completed. In addition to the Day 56 assessments, if the subject early terminates on or before Day 30, a PK sample should be obtained (Part A only) and opioid accountability should occur.

If used, the subject should return the provisioned e-diary during this visit if not previously returned.

The subject may utilize an e-diary maintained by the site or their assigned e-diary for completion of the final self-administered questionnaires/surveys. The questionnaires/surveys will include:

- NRS-R and location of pain
- QOR-15

- KOOS, JR
- Pain manageability survey
- NRS-A and location of pain
- Rescue opioid medication questions, any time before an opioid is consumed and assessment of medication compliance (if early terminated on or before the Day 30 Visit)
- Physical therapy survey (if early terminated on or before the Day 30 Visit)

6.1.1.12 Serious Adverse Event or Adverse Event of Special Interest

In the event of a SAE or AESI, the subject will be asked to come to the hospital/clinic or at a physical therapy facility and the following assessments will be conducted:

- Vital signs
- 12-Lead ECG
- Laboratory testing (Blood Chemistry and Hematology)
- Neurological assessments
- Concomitant medication review
- PK sampling
- Recording of AEs

For further information regarding treatment of SAEs and AESIs please see Section 9.5.1 and 9.5.2.

6.2 NUMBER OF SUBJECTS

This trial will enroll approximately 165 subjects in Part A and up to 140 subjects in Part B at up to 20 multinational clinical centers.

6.3 TRIAL POPULATION

6.3.1 Subject Inclusion Criteria

To be eligible for trial participation subjects must meet the following inclusion criteria:

1. Primary indication of TKA is knee pain due to osteoarthritis or post-traumatic arthritis.
2. Scheduled to undergo primary unilateral TKA with a cemented prosthesis, without use of a surgical drain, and under bupivacaine spinal anesthesia (dextrose is permitted).
3. American Society of Anesthesiology (ASA) Physical Classification System of class 1, 2, or 3.
4. Male or female, ≥18 and ≤80 years of age at the Screening Visit.

5. Female subjects only: Postmenopausal, surgically sterile, or willing to use acceptable means of contraception from the Screening Visit through the Day 56 Visit.
    a. Medically acceptable methods of contraception that may be used by the subject include birth control pills, diaphragm with spermicide, intrauterine device (IUD), condom with spermicide, vaginal spermicidal suppository, or progestin implant or injection (used consistently for ≥3 months at the time of screening).
    b. Female subjects who are not of childbearing potential must have a medical history recorded of surgical sterilization (≥6 months post-surgery at the time of screening) or post-menopausal (not experienced a menstrual period ≥2 years at the time of screening).
6. Capable, able, and willing to comply with all trial visits and procedures. Subject must also be able to use trial required electronic applications (eg electronic diary) for patient reported outcome measures.
7. English speaking, willing, and capable of providing written informed consent.

It is recommended that participating subjects are vaccinated for COVID-19 per local requirements.

6.3.2 Subject Exclusion Criteria

Exclude subjects from this trial if they do not fulfill the inclusion criteria, or if any of the following conditions are observed:

1. Has a planned concurrent surgical procedure (eg bilateral TKA) at the time of surgery or a planned surgical procedure before the Day 56 Visit.
2. Has had any previous arthroplasty, unicompartmental knee arthroplasty or TKA in the study knee or previous arthroplasty, unicompartmental knee arthroplasty, or TKA in the contralateral knee within 6 months prior to screening.
3. Has been administered any type of intra-articular injection within 3 months of surgery in the trial knee.
4. Has a pre-existing concurrent, acute, or chronic, painful/restrictive physical condition for which they routinely take opioid analgesics and are expected to require opioid analgesics in the postsurgical period that is not strictly related to the trial knee osteoarthritis/post-traumatic arthritis and/or trial knee surgery.
5. Unable to abstain from opioid use for knee pain (including codeine) within 2 weeks (14 days) of surgery.
6. Has been administered systemic steroids within 14 days prior to surgery. (Note: Steroids provided on the day of surgery as part of a standard surgical medication regime are permitted. Local steroids such as inhalers or ophthalmologic drops are permitted).
7. Is unwilling or unable to discontinue use of medications or products that can impact pain control from the Screening Visit until the Day 56 Visit (eg cannabidiol (CBD) oil,

Kratom, herbal supplements). Non-Steroidal Anti-Inflammatory Drugs (NSAIDS) and acetaminophen/paracetamol are permitted prior to surgery at the treating Investigators discretion. Subjects prescribed gabapentin and/or pregabalin will require medical monitor approval.

8. Has been administered any anesthetic including but not limited to bupivacaine, ropivacaine, or lidocaine within 5 days prior to the scheduled surgery. See protocol Section 7.6.1 for uses and permitted anesthetics on the day of surgery.
9. Requires medication that will impact the metabolism, absorption, or excretion of bupivacaine. Epinephrine/adrenaline should be used with caution. Strong inhibitors and inducers of CYP3A4 should be avoided (See Appendix M). Coadministration of ATX-101, or bupivacaine HCl with local anesthetics is prohibited.
10. Has a contraindication or a known or suspected history of allergy, hypersensitivity, or idiosyncratic reaction to trial medications including but not limited to those used during surgery or post-surgery (eg anesthesia, IV dexamethasone, IV tranexamic acid, antibiotics, ondansetron, and opioids such as morphine, oxycodone, and hydromorphone), also to medications taken prior to surgery and/or dispensed for home use (ie acetaminophen/paracetamol and celecoxib), or to the investigational product or its components (bupivacaine, poly[DL-lactide-co-glycolide] (PLGA), and/or polysorbate).
11. Has a Body Mass Index (BMI) ≥45 kg/m^2.
12. Has laboratory values at screening that will exclude enrollment including:
    a. Bilirubin >2 times the upper normal limit unless due to Gilberts syndrome
    b. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), or alkaline phosphatase (ALP) >1.5 times the upper normal limit
    c. Creatinine > 2 times the upper normal limit
    d. Estimated serum creatinine clearance <30 mL/min
    e. Hemoglobin below the lower normal limit
13. Screening ECGs with significant abnormalities such as left bundle-branch blocks, bifascicular blocks, second- or third-degree AV block, possible current ischemia, or other findings associated with significant heart disease. The average QTc of the screening triplicate ECGs must not be >450 ms in males or >470 ms in females. Significant findings are to be clearly documented.
14. Has symptomatic central nervous system (CNS) injury or disorder including epilepsy.
15. Has any chronic condition or disease that would compromise neurological or vascular assessments.
16. Immunocompromised or has a known history of Hepatitis B, human immunodeficiency virus (HIV), or Hepatitis C.
17. Has a medical condition or receiving medication such that, in the opinion of the Investigator, participating in the trial would pose a health risk to the subject or confound the postsurgical assessments or might confound or interfere with the outcome of the trial. Examples include but are not limited to inability to ambulate, diabetic neuropathy,

complex regional pain syndrome, prior infection in operative joint, known or suspected coagulopathy, uncontrolled anticoagulation, long COVID diagnosis, history of thromboembolic events, active gastrointestinal ulcers/bleeding, or cerebrovascular bleeding.

18. Has uncontrolled depression, anxiety, psychiatric, or neurological disorder that might interfere with trial assessments or might confound or interfere with the outcome of the trial.
    a. Subjects currently taking anti-psychotic medication will be excluded.
    b. Anti-depressant medication (selective serotonin reuptake inhibitors only) is allowed if the subject has been on a stable dose for at least 30 days prior to screening and no dose changes are planned during the conduct of the trial.
19. At any time prior to randomization, subjects with moderately severe or severe depression, as defined by
    a. Patient Health Questionnaire (PHQ-9) total score of ≥10; or
    b. Suicidal ideation, as defined by a PHQ-9 score of ≥1 in response to question 9, "thoughts that you would be better off dead, or hurting yourself."
20. Has a known or suspected history of drug or alcohol abuse. A subject with a history of alcohol use disorder that has ≥10 years sobriety will be permitted.
21. Has a positive drug screen at the Screening Visit or on the day of surgery including positive results from prescribed medications. The exception being a positive drug screen resulting from infrequent use of short acting benzodiazepines that can be washed out 7 days prior to surgery and not utilized during the trial duration.
22. Has received/used an investigational drug, product, or device for a clinical trial within 30 days of screening. COVID-19 vaccines (approved or under emergency use authorization locally) are permitted if the subject is not in a clinical trial for the vaccine.
23. Pregnant, breastfeeding, or planning to become pregnant during the trial or before the Day 56 Visit.

In addition, the subject will not have met eligibility if the following occurs during surgery.

24. Any clinically significant (CS) event or condition uncovered during surgery (eg excessive bleeding, femur fracture), which occurs before investigational product administration, that might render the subject medically unstable or complicate the subject's postsurgical course.
25. [redacted]

6.3.3 Subject Withdrawal Criteria

Subjects must be withdrawn from the clinical trial if they wish to do so or if they are unwilling or unable to comply with the procedures of the clinical trial. The reasons for the subject's withdrawal from the clinical trial must be recorded in the subject's records, which should be

completed as soon as possible after the subject's withdrawal is determined. Any subject who withdraws from the clinical trial must complete all protocol Early Termination Visit assessments whenever possible.

When an AE or SAE is the cause of withdrawal, the Investigator should record this in the case report form (CRF). The subject should be followed and treated by the Investigator until the AE has resolved or until the Day 56 Visit whichever occurs first.

Subjects are free to withdraw from the trial at any time and for any reason and will document their understanding by signing the informed consent form.

6.4 SAFETY MONITORING COMMITTEE

A Safety Monitoring Committee (SMC) will review blinded safety data in Part A after 25%, 50%, and 100% enrollment of the study and in Part B after 25%, 50%, and 75% of the study. The SMC will be composed of independent members and Sponsor representatives. Representatives from the Sponsor will include clinical, regulatory and the study medical monitor. The biostatistician, anesthesiologist, cardiologist and orthopedic surgeon will be independent members. The SMC will operate under a written, detailed SMC plan.

The SMC will evaluate study data including adverse events, pharmacokinetic data, cardiac information, and safety laboratory results from subjects. The SMC will investigate all reports of PK greater than or equal to 1,000 ng/mL of plasma bupivacaine. Should data arise where further information is needed to address a safety concern, the SMC will escalate the data review to an unblinded, independent statistician and physician.

6.5 STOPPING RULES

The SMC may recommend that the trial be stopped based upon identification of any relevant PK and/or safety data at any time. The trial will also be stopped should there be:

- One death where a clear alternative cause (other than ATX-101) is not identified or
- Two non-fatal SAEs where a clear alternate cause (other than ATX-101) is not identified or
- Five moderate cardiac or central nervous system AESIs with a PK concentration of more than 1,000 ng/mL and a clear alternate cause (other than ATX-101) is not identified or
- Three severe cardiac or central nervous system AESI's with a PK concentration of more than 1,000 ng/mL and a clear alternate cause (other than ATX-101) is not identified.

In these instances, the study blind may be broken for the SMC for the identified subjects.

6.6 CRITERIA FOR TRIAL TERMINATION

In the event that the Investigator is unable to continue the trial at the site, another suitable person will be designated as Investigator, and documentation testifying to this will be submitted to Allay within 10 days. The new Investigator must be approved by Allay and the Investigational Review Board (IRB)/Ethics Committee (EC) before the trial can be continued.

If Allay and/or the Investigator should discover conditions arising during the trial that indicate that the trial should be terminated at their site, an appropriate schedule for termination will be instituted. If the Investigator terminates the trial at their site, an explanatory letter will be provided to Allay.

Allay also reserves the right to discontinue this trial for administrative reasons at any time.

7.0 TRIAL TREATMENTS

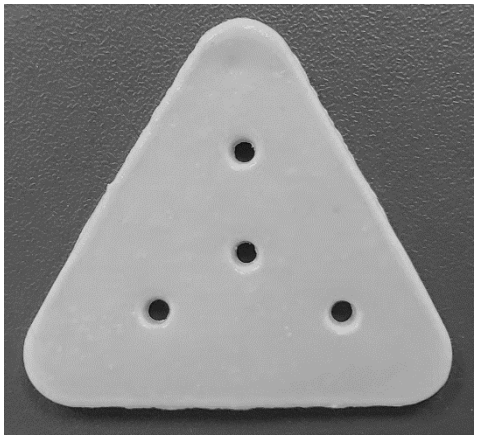
7.1 DESCRIPTION OF INVESTIGATIONAL PRODUCT (ATX-101)

ATX-101 is a bioabsorbable implant. Each implant contains 500 mg bupivacaine free base (equal to 563 mg bupivacaine HCl or 594 mg bupivacaine HCl monohydrate).

In this trial, subjects will be randomized to receive a single-dose administration of 1,000 mg or 1,500 mg of ATX-101, corresponding to 2 or 3 implants, respectively. ATX-101 will be placed under direct visualization in the knee capsule, per the Dosage and Administration Instructions.

Each ATX-101 contains the drug bupivacaine, a poly (DL-lactide-co-glycolide) polymer (PLGA), and polysorbate 20 (PS20). The dosage form for ATX-101 is a triangular implant, nominally 28 millimeters (mm) along each side and 2 mm thick with four suture holes in the approximate center and near each tip of the triangle and is white to light yellow or light tan in color (Figure 1).

Figure 1: Example of the ATX-101



7.1.1 ATX-101 Packaging, Labeling & Storage

Each ATX-101 implant will be provided [redacted] The foil pouch will be provided in an outer box. ATX-101 must be stored in an ambient (15-25°C/59-77°F), secure location with access limited to the pharmacist and/or authorized staff.

At a minimum, the ATX-101 packaging will include the following information:

- Sponsor Name
- Protocol Number
- Product Name/Drug Code
- Dose/Product Strength
- Route of Administration
- Lot Number
- Storage Temperature/Conditions.

Any additional information and specific cautionary statements will be included according to local law.

7.2 DESCRIPTION OF ACTIVE COMPARATOR (BUPIVACAINE HCL)

Bupivacaine HCl 0.25% without epinephrine/adrenaline maximum dose of 2 mg/kg will be used via local periarticular infiltration or adductor canal block or in combination per the administration instructions in section 8.14.2. Do not exceed a total daily dosage of 400 mg [9].

7.3 DOSE ADJUSTMENT CRITERIA

The trial includes a one-time dose of ATX-101 or bupivacaine HCl administered during surgery. Doses of investigational product will not be able to be adjusted after administration.

7.4 INVESTIGATIONAL PRODUCT HANDLING AND DISPOSAL

7.4.1 Safety and Handling

Although exposure of healthcare workers to bupivacaine from ATX-101 should be minimal, care should be taking to avoid unnecessary contact with ATX-101. Proper surgical and sterile techniques should be used when handling ATX-101.

Standard of care procedures should be employed when handling the 0.25% bupivacaine HCl. Proper surgical and sterile technique should be used when handling 0.25% bupivacaine HCl.

7.4.2 Disposal

Allay will provide instructions regarding the destruction and/or return of all investigational product.

If ATX-101 has not been used but is not able to be placed in a subject (eg dropped on the floor), disposal should be handled per hospital procedures through the pharmacy and/or biohazard disposal for bupivacaine. Disposal should be done in such a way as to not unblind members of the site staff that are to remain blinded.

7.5 INVESTIGATIONAL PRODUCT ACCOUNTABILITY

Complete receipt, inventory, accountability, reconciliation, and return records will be maintained for all used and unused investigational products and trial provided medications. Documentation will include lot numbers, dates and quantity of shipment, dates and subject identification of dispensation, reconciliation, returns, and destruction.

The packaging lot numbers will be recorded on the unblinded investigational product accountability records and on each subject's unblinded drug administration source documentation and unblinded electronic form. Unblinded documentation will only be available to the specific individuals that are unblinded in the trial. Section 7.8 contains further information on blinding.

7.6 CONCOMITANT MEDICATIONS

Prior and concomitant medication usage will be recorded from 30 days before the Screening Visit through the Day 56 Visit.

Prohibited medications are listed in Appendix M.

7.6.1 Pre-Surgical Medications

Before surgery, subjects will be administered acetaminophen/paracetamol 1,000 mg and celecoxib 200 mg [1]. Spinal anesthesia with bupivacaine (with or without dextrose) will be used in all subjects; no other adjunct medication such as morphine or ketamine may be used in the spinal anesthesia. General anesthesia is optional, but if used must be in conjunction with spinal anesthesia. Local nerve blocks (eg adductor canal blocks) are prohibited in the ATX-101 treatment group, however topical anesthetics such as lidocaine can be used to aid in IV access and subcutaneously for spinal needle placement.

7.6.2 Surgical Concomitant Medications

At the time of induction, the use of fentanyl (no more than 300 µg) for airway management is permitted when general anesthesia is used. Other than fentanyl for airway management, no other opioids may be administered during surgery.

Two doses of IV tranexamic acid (1 g each) will be administered to the subject. One dose of tranexamic acid will be administered prior to investigational product administration and the second dose after investigational product administration, with the timing at the discretion of the treating Investigator. Intravenous or oral antibiotic regimen may also be given per the center's standard of care. Antibiotic powders or beads in the knee joint are prohibited. A one-time dose of IV dexamethasone (4 mg) will be administered during the surgical procedure, with the timing at the discretion of the treating Investigator. One dose of prophylactic ondansetron will be given before or during surgery, with the timing at the discretion of the treating investigator. All other doses of antiemetics will be given only after an adverse event.

7.6.3 Post-Surgical Concomitant Medications

All trial subjects will receive a standardized approach for managing postsurgical pain that includes a scheduled multimodal regimen of celecoxib (200 mg) twice a day for 30 days, unless an AE occurs that requires discontinuation. Acetaminophen/paracetamol 500 mg should be taken every 4 hours or 1,000 mg every 6 to 8 hours not to exceed 3,000 mg per any 24-hour period for a minimum of 21 days.

Deep vein thrombosis prophylaxis is required, and 75-100 mg aspirin (based upon regional approved dose) is recommended two times a day for 30 days. Aspirin is recommended in trial subjects unless there are additional risks of thromboembolic events identified requiring a different DVT prophylaxis. Celecoxib, acetaminophen/paracetamol, and aspirin may be provided by the Sponsor.

7.6.3.1 Rescue Opioid Medications

Subjects should only receive rescue opioid medication upon request for pain control, as needed, after surgery. Rescue opioid medication may be given/taken for pain treatment, but not for pain prophylaxis. If required to treat pain, the following standardized postsurgical rescue opioid medication protocol will be used.

From Day 1 after Surgery through Day 30 every time a rescue opioid medication is required, prior to opioid administration, the subject must complete the NRS-R scale for pain intensity and record information about the opioid consumed.

7.6.3.1.1 In Hospital Rescue Opioid Medications

Preferred rescue opioid medication will consist of oral immediate-release oxycodone (5 mg or 10 mg). The prescription should indicate the patient take 1 to 2 pills (5 mg to 10 mg) every 4-6 hours as needed. The prescription must indicate that substitutions with any other opioid-containing product are not permitted, including combination opioid/non-opioid products. If a subject cannot tolerate oral medication, morphine may be administered IV (including via patient-controlled analgesia [PCA] pump) or IM as needed (morphine is preferred, however hydromorphone can also be used with the dose determined by the treating physician). If a subject cannot tolerate the medication or the breakthrough pain is not responsive to oral oxycodone (5

mg or 10 mg), then oral hydromorphone (2 mg or 4 mg) may be given for rescue opioid medication. The prescription should indicate the patient take 1 to 2 pills (2 mg to 4 mg) every 4-6 hours as needed. The prescription must indicate that substitutions with any other opioid-containing product are not permitted, including combination opioid/non-opioid products. Should the subject have an AE and is unable to tolerate requested rescue opioid medications of oral oxycodone (5 mg or 10 mg), IV morphine, oral hydromorphone (2 mg or 4 mg), and IV hydromorphone, then hospital standard of care may be utilized however, local anesthetic nerve blocks may not be administered after surgery. No long-lasting opioids should be administered.

7.6.3.1.2 Post-Discharge Rescue Opioid Medications

Preferred rescue opioid medication will consist of oral immediate-release oxycodone (5 mg or 10 mg). If a subject cannot tolerate the oral oxycodone or the breakthrough pain is not responsive to oral oxycodone (5 mg or 10 mg), then oral hydromorphone (2 mg or 4 mg) may be given for rescue opioid medication. Should the subject have an AE and is unable to tolerate requested rescue opioid medications of oral oxycodone (5 mg or 10 mg), or oral hydromorphone (2 mg or 4 mg), then hospital standard of care may be utilized, however, local anesthetic nerve blocks may not be administered after surgery

No more than thirty (30) 5 mg immediate-release oxycodone tablets (or as limited by local law) will be provided as part of the first prescription. The prescription should indicate the patient take 1 to 2 pills (5 mg to 10 mg) every 4-6 hours as needed. The prescription must indicate that substitutions with any other opioid-containing product are not permitted, including combination opioid/non-opioid products. Additional prescriptions can be provided at Investigator discretion, with instructions the same as the first prescription.

If the subject has been prescribed hydromorphone, no more than thirty (30) 2 mg immediate-release hydromorphone tablets (or as limited by local law) will be provided as part of the first prescription. The prescription should indicate the patient take 1 to 2 pills (2 mg to 4 mg) every 4-6 hours as needed. The prescription must indicate that substitutions with any other opioid-containing product are not permitted, including combination opioid/non-opioid products. Additional prescriptions can be provided at Investigator discretion, with instructions the same as the first prescription.

If a subject is prescribed both 5 mg immediate release oxycodone and 2 mg immediate release hydromorphone, no more than 30 total tablets combined may be prescribed.

All unused, prescribed opioids should be returned to the clinical site for accountability during the Day 15 and Day 30 in-clinic visits. If the subject has a need to continue to take opioids after the accountability visit, the opioids may be returned to the subject.

Total number of opioid prescriptions during the trial will be documented.

7.7 RANDOMIZATION

A randomization schedule will be computer generated.

Subjects will be randomized in a 1:1:1 ratio in Part A to a one-time administration of 1,000 mg ATX-101, 1,500 mg of ATX-101, or bupivacaine HCl.

Subjects will be randomized in a 1:1 ratio in Part B to a one-time administration of ATX-101 (1,000 mg or 1,500 mg determined from Part A) or bupivacaine HCl.

7.8 BLINDING

In the event of a medical emergency when knowledge of the actual treatment becomes medically necessary to affect treatment options, prior to the end of trial unblinding, the Investigator will be able to obtain details of the treatment assigned to a subject. The Medical Monitor is to be informed immediately that the blind has been broken.

The method for unblinding through the electronic data capture (EDC) system includes:

- The Investigator will consult with the Medical Monitor if possible.
- The Investigator/Medical Monitor accesses the Code Break Module in the EDC.
- The Investigator/Medical Monitor retrieves the treatment assignment from the EDC Code Break Module
- An unblinded email notification will be sent to the Investigator or Medical Monitor who broke the code.

The surgical staff will not be blinded to the trial treatment, all other research staff (unless expressly specified in the site's blinding plan that is approved by Allay) and the subject will remain blinded throughout the trial. The trial Sponsor will remain blinded during the treatment phase of Part A and Part B unless individual subjects need to be unblinded due to safety after a SMC meeting. Trained and pre-identified monitors will be unblinded throughout the trial to ensure compliance with trial protocols and investigational product accountability. Further information regarding accountability can be found in Section 7.5. Blinded monitors will be assessing safety and efficacy data at the site.

8.0 TRIAL PROCEDURES

A signed and dated informed consent will be obtained from trial subjects using the consent form approved by the IRB/EC, prior to any trial procedures being performed. A copy will be provided to the subject.

For each subject, AEs will be collected from the time of investigational product administration through the Day 56 Visit. Prior and concomitant medication usage will be collected throughout the trial from 30 days prior to the Screening Visit until end of trial participation or at the Day 56 Visit.

Refer to the Schedule of Events (Appendix A for Part A and Appendix B for Part B) for complete details regarding the timing of trial procedures. Subject self-administered procedures are detailed in Appendix C.

8.1 DEMOGRAPHIC/MEDICAL HISTORY

The subject's demographic information will be collected at the Screening Visit. The subject's medical history will be collected at the Screening Visit and confirmed/updated prior to surgery on Day 1 before surgery. Any medical events that occur prior to investigational product administration will be considered medical history.

8.2 VITAL SIGNS

Vital signs will be measured at the Screening Visit, on the day of surgery before surgery and after surgery (Assessment timing will be based on surgery closure which will be time 0) at Hours 3 and 6 (±30 minutes); Hours 9 and 12 (±1 hour). Starting at Hour 18 vital signs will be required every 6 hours (±2 hours) unless the subject is sleeping. In Part A vital signs will be taken through Hour 96 (Day 5). In Part B after Hour 18, vital signs will be taken every 6 hours (±2 hours) unless the subject is sleeping until Hour 24 or discharge, whichever is later. In a 24-hour period, vital signs can only be missed one time for the subject sleeping.

Upon discharge (Part A only), vital signs will be measured on trial visit Days 6, 8, 15, 22, 30, and 56.

Upon discharge (Part B only), vital signs will be measured Days 3, 4, 5, 6, 8, 15, 22, 30 and 56.

Vital Signs are required anytime a subject has an SAE or AESI.

The following vital signs will be measured after the subject has been supine for 5 minutes: pulse, temperature, respiratory rate, systolic/diastolic blood pressure, and oxygen saturation.

8.3 PHYSICAL EXAMINATION AND HEIGHT/WEIGHT

A physical exam will be performed at the Screening Visit and at the Day 56 Visit. At the screening visit, the physical exam must include a Kellgren Lawrence Classification, (varus evaluation, valgus evaluation, and evaluation of ipsilateral hip osteoarthritis). The subject's weight will be measured during the physical exam at the Screening Visit and at the Day 56 Visit. The subject's height will be measured only at the Screening Visit.

8.3.1 Kellgren Lawrence Classification

Kellgren Lawrence Classification is used to classify the severity of knee osteoarthritis [20]. The Kellgren Lawrence Classification will occur on the subject's proposed trial knee and one of five grades (Grade 0 through Grade 4). Grades include:

- **Grade 0 (none)**: definite absence of x-ray changes of osteoarthritis

- **Grade 1 (doubtful)**: doubtful joint space narrowing and possible osteophytic lipping
- **Grade 2 (minimal)**: definite osteophytes and possible joint space narrowing
- **Grade 3 (moderate)**: moderate multiple osteophytes, definite narrowing of joint space and some sclerosis and possible deformity of bone ends
- **Grade 4 (severe)**: large osteophytes, marked narrowing of joint space, severe sclerosis and definite deformity of bone ends

8.4 ELECTROCARDIOGRAM

For Part A, triplicate 12-lead ECGs will be performed at the Screening Visit, on Day 1 prior to receiving the bupivacaine spinal, during surgery before ATX-101 or bupivacaine HCl administration, Hour 6 and Hour 12 after the surgical procedure, Days 2 (Hour 24), 3 (Hour 48), 4 (Hour 72), 5 (Hour 96), 6, 8, 15, 22, 30, and 56.

For Part B, triplicate 12-lead ECGs will be performed at the Screening Visit, on Day 1 prior to receiving the bupivacaine spinal, and Day 56.

Triplicate 12-lead ECGs are required any time a subject has an SAE or AESI.

Triplicate ECGs will be taken 2 minutes apart (±1 minute) for each nominal timepoint. Subjects will be at rest in a supine or semi-recumbent position for 10 minutes before the ECGs are obtained. No TV, video games, etc. will be allowed during the rest or ECG period. The ECGs will be taken BEFORE PK sampling.

ECGs will be analyzed by a central cardiac core lab and reports will be provided from the cardiac core lab to the clinical site for medical review. The Investigator or delegated medically qualified site staff will immediately review the ECGs for safety concerns, as well as later review the ECG reports and determine if any abnormal findings are considered clinically significant in their medical judgement and therefore required to be entered into the subject's medical history (from the Screening Visit or the Day 1 before bupivacaine spinal) or recorded as adverse events.

8.5 LABORATORY ASSESSMENTS

Laboratory Assessments will be performed at the Screening Visit, before surgery on Day 1, on Day 15, and at the Day 56 Visit. Specific laboratory tests are detailed below. Laboratory assessments are required any time a subject has an SAE or AESI.

Screening laboratory values that are out of range will be identified and may be repeated at the Investigator's discretion with Allay's approval. The Investigator will determine if any out-of-range laboratory values that emerge after treatment are clinically significant and record these as an AE. All clinically significant out-of-range laboratory values that emerge after treatment will be followed until resolution or the Day 56 Visit, whichever occurs first. See Appendix K for further information.

8.5.1 Hematology

The following hematologic assessments will be performed: hemoglobin, hematocrit, white blood cell count (total and differential), red blood cell count and platelet count.

Hematology assessments will occur at the Screening Visit and at the Day 56 Visit. A hematology assessment is required anytime a subject has an SAE or AESI.

8.5.2 Chemistry

The following chemistry assessments will be performed: sodium, potassium, creatinine, creatinine clearance, albumin, ALP, total and direct bilirubin, AST, ALT, blood urea nitrogen (BUN), creatinine kinase, gamma-glutamyl transferase (GGT), and phosphate.

Blood chemistry assessments will occur at the Screening Visit and at the Day 56 Visit. A chemistry assessment is required anytime a subject has an SAE or AESI.

8.5.3 Drug Screen

At the Screening Visit a urine drug screen will be performed for opiates (including oxycodone), amphetamines, methadone, barbiturates, benzodiazepines, cocaine, phencyclidine, methamphetamine, ecstasy (3,4-Methylenedioxymethamphetamine or MDMA), tricyclic antidepressant, and cannabinoids. If a subject has a positive urine drug screen at the Screening Visit, a screen may be repeated one time at the Sponsor's discretion to confirm non-chronic use. The subject may be considered eligible for the trial if a repeat urine drug screen is negative.

On Day 1 before surgery a urine drug screen for opioids will be performed. If the subject tests positive for opioids and the treating Investigator feels this is a false positive result, the medical monitor will be contacted to determine if the subject may be enrolled in the trial. The subject may only be enrolled if approved by the medical monitor. If enrollment is permitted, a confirmatory blood or urine sample (as applicable) from the subject on Day 1 before surgery will be sent to the blood and urine central lab.

At the Day 15 Visit a urine drug screen will be performed for opiates (including oxycodone), amphetamines, methadone, barbiturates, benzodiazepines, cocaine, phencyclidine, methamphetamine, ecstasy (3,4-Methylenedioxymethamphetamine or MDMA), tricyclic antidepressant, and cannabinoids. It is expected that subjects taking rescue opioid medications will test positive for opioids at this visit.

8.5.4 Pregnancy Test

A pregnancy test will occur at the Screening Visit, before surgery on Day 1, and at the Day 56 Visit for females of childbearing potential. Pregnant females identified at the Screening Visit or on the day of surgery will not be eligible for the trial.

A urine pregnancy test from the Screening Visit and Day 56 Visit will be analyzed. A urine or serum pregnancy test will occur on the day of surgery (Day 1), before surgery, per standard of care at the clinical site.

Female subjects who are not of childbearing potential must have a medical history recorded of surgical sterilization (≥6 months post-surgery at the time of screening) or post-menopausal (not experienced a menstrual period ≥2 years at the time of screening).

8.6 RANGE OF MOTION ASSESSMENT

The ROM assessment will occur at the Screening Visit, on Days 2 (Hour 24), 3 (Hour 48), 4 (Hour 72), 5 (Hour 96), 6, 8, 15, 22, 30, and 56.

Range of motion will be conducted using a goniometer to measure flexion and extension for the subject per Sponsor provided ROM training.

8.7 PATIENT HEALTH QUESTIONNAIRE 9

The PHQ-9 (Appendix G) will be conducted at the Screening Visit. The PHQ-9 questionnaire assesses for depression.

If the subject exhibits signs of moderately severe or severe depression, as defined by a PHQ-9 total score of ≥10; or suicidal ideation, as defined by a PHQ-9 score of ≥1 in response to question 9, then the subject will not be eligible for the trial.

If the subject exhibits signs of moderately severe or severe depression, they should be informed of their result. The hospital/clinic staff should recommend the best course of action for the subject (eg counseling, referral to a specialist, hospitalization etc.) and document the outcome and advice in the subject records.

8.8 NEUROLOGICAL ASSESSMENTS

The neurological assessments will be conducted during the Screening Visit, on Day 1 before surgery, and after surgery (assessment timing will be based on surgery closure which will be time 0) at Hours 3 and 6 (±30 minutes); Hours 9 and 12 (±1 hour); Hours 18 and 24 (±2 hours) unless the subject is sleeping. In the 24-Hour period, the neurological assessment can only be missed one time for the subject sleeping.

The neurological assessments will be conducted on Days 3 (Hour 48), 4 (Hour 72), 5 (Hour 96), 6, 8, 15, 22, 30, and 56.

Neurological assessments are required any time a subject has an SAE or AESI.

A gross motor and sensory exam will be conducted with a focus on the lower extremities. Motor examinations will focus on distal lower extremities dorsi flexion and plantar flexion of each foot

(ankles and toes) against resistance. The gross sensory examination should focus on intact sensory examination to touch in the dorsal and plantar side of each foot.

The subject will be queried on abnormal senses (ears/hearing, sight, touch, smell, and taste).

Any abnormal, clinically significant findings from the neurological assessments will be recorded as an AE.

8.9 E-DIARY TRAINING

The subject will be trained on the use of the e-diary at the Screening Visit and on Day 1 before surgery. Throughout the trial e-diary questions and concerns from the subject will be addressed and re-training will occur if needed at any time during the trial.

If subject is temporarily unable to complete the e-diary questionnaires, a paper-based questionnaire may be utilized. Paper questionnaires will also be used if an Early Termination Visit is conducted.

8.10 E-DIARY DISPENSATION/RETURN

The provisioned e-diary will be dispensed to the subject on Day 1 before surgery and will be returned at the Day 30 Visit. If the subject's own device is used, the site staff will work with the subject to have the e-diary set-up before the subject has surgery.

Questionnaires and surveys completed at the Screening Visit and the Day 56 Visit may utilize e-diaries maintained by the clinical site. If the subject does not return the provisioned e-diary at the Day 30 Visit or an Early Termination Visit before Day 30, then a site-maintained e-diary may be used to complete the self-administered questionnaires and surveys.

8.11 SUBJECT PAIN SCORE TRAINING

The subject will be trained on the completion of the NRS pain intensity scales at the Screening Visit, on Day 1 before surgery, Day 2, Day 3, and after Day 15 (on Day 16). Standardized pain score training will be conducted by the site staff or through training videos (Appendix H).

8.12 RANDOMIZATION AND INVESTIGATIONAL PRODUCT ALLOCATION

Randomization for allocation of investigational product may occur up to one business day before surgery. Randomization will occur through a web-based tool. In Part A, subjects who are deemed randomization failures (subjects who are randomized but do not receive investigational product), will be replaced. In Part B subjects will not be replaced.

8.13 BUPIVACAINE PHARMACOKINETIC ASSESSMENT

Pharmacokinetic samples are centrifuged within 2 hours of collection, and the supernatant aspirated and stored at -20˚C or -80˚C until shipped for testing at an external bioanalytical laboratory. Specific processing details will be provided in a PK/laboratory manual.

8.13.1 Blood Sample Collection for Pharmacokinetic Analysis

For Part A, venous blood samples (4 mL) for plasma PK analysis are drawn on Day 1 before surgery (before bupivacaine spinal), during surgery prior to administration of ATX-101 or bupivacaine then 6 hours ±30 minutes and 12 hours ± 1 hour after surgical closure. PK samples will also be collected at Day 2 (Hour 24 ±2 hours), Day 3 (Hour 48 ±2 hours), Day 4 (Hour 72 ±2 hours), Day 5 (Hour 96 ±2 hours), on Days 6, 8, 15, 22, and 30. It is requested, if possible, that the PK samples from Days 6 to 30 are collected at approximately the time of surgical closure on Day 1.

In Part A and Part B, a PK sample is required for bupivacaine monitoring anytime a subject has an SAE or AESI. A PK sample is also required if a subject terminates the study early on or before Day 30 (Part A only).

The ECGs will be taken BEFORE PK sampling.

8.13.2 Bupivacaine Concentration Analysis

Plasma bioanalysis of bupivacaine from PK samples collected in the trial will be performed by [redacted]

8.14 INVESTIGATIONAL PRODUCT ADMINISTRATION

8.14.1 ATX-101

A one-time administration of a single dose level of ATX-101 will be placed under direct visualization in the subject's knee capsule following fixation of the knee implant prosthesis when tissue will not be disrupted any further by surgery, after any betadine or saline rinse, and after suction has occurred, but prior to initiation of surgical closure. ATX-101 will be placed in the suprapatellar pouch, medial gutter, and/or lateral gutter according to the Dosage and Administration Instructions.

8.14.2 Bupivacaine Hydrochloride-Active Comparator

Active comparator bupivacaine HCl 0.25% without epinephrine/adrenaline, maximum dose of 2 mg/kg, will be administered via local periarticular infiltration or adductor canal block (Ultrasound guided saphenous nerve block), or in combination during surgery.

The bupivacaine HCl may be provided by the Sponsor for use in the trial.

8.15 WOUND HEALING ASSESSMENT

The wound healing assessment will occur on Days 15, 22, 30, and 56 using the Southampton Wound Scoring System (Appendix I).

Any score of Grade 2 or greater from the Southampton Wound Scoring System will be recorded as an AE and may be documented with photographic evidence. So as to be able to distinguish TKA incision/wound events from other events, the Investigator must report these events as "surgical wound" preceded by the description (eg surgical wound purulent discharge).

8.16 KNEE SOCIETY SCORE

The Knee Society Score (Appendix J) will occur at the Screening Visit, on Days 15, 30, and 56.

The Knee Society Score assesses knee joint and knee function.

8.17 SUBJECT SELF-ADMINISTERED E-DIARY ASSESSMENTS

Subjects will use their own device or be provided with an e-diary as specified in Protocol Section 8.10 to complete self-administered questionnaires and surveys as well as track opioid consumption.

8.17.1 Numeric Rating Scale at Rest for Pain Intensity & Location of Pain

The NRS-R for pain intensity (Appendix H) is an 11-item scale from 0-10 where subjects rank the level of pain intensity. The NRS scale goes from No Pain (0) to Worst Imaginable Pain (10). The NRS-R will be collected during the Screening Visit, on Day 1 before surgery, approximately 6 times on Day 1 after surgery, and approximately 3 times a day from Day 2 through Day 15. After the Day 15 Visit the NRS-R will be collected one time daily through Day 30 and again Day 56.

The NRS-R will also be collected post-surgically through Day 30 every time the subject requires an opioid medication administration.

Prior to obtaining the NRS-R it is recommended subjects be in a comfortable resting position (either seated or lying down) for at least 5 minutes prior to obtaining the pain intensity score.

If the subject records a pain intensity score of 1 or greater, they will be asked to indicate the single area of their knee with the most intense pain (See Appendix L).

8.17.2 Numeric Rating Scale with Activity for Pain Intensity & Location of Pain

The NRS with activity (NRS-A) for pain intensity (Appendix H) is an 11-item scale from 0-10 where subjects rank the level of pain intensity after an activity. The NRS scale goes from No Pain (0) to Worst Imaginable Pain (10). The NRS-A will be conducted at the Screening Visit, once a day from Day 2 until the Day 30 Visit, and at Day 56.

Prior to the NRS-A collection the subject should complete one set of 8 to 10 repetitions of knee flexion/extension by sitting on the edge of a chair/bed and extending the leg straight.

If the subject records a pain intensity score of 1 or greater, they will be asked to indicate the single area of their knee with the most intense pain (See Appendix L).

8.17.3 Quality of Recovery 15 Scale

The QOR-15 (Appendix D) will be collected during the Screening Visit, one time daily from Day 2 through Day 30, and at the Day 56 Visit.

The QOR-15 is a 15-item questionnaire designed to evaluate the early post-operative health status of subjects following surgery and anesthesia.

8.17.4 Knee Injury and Osteoarthritis Outcome Scale for Joint Replacement

The KOOS, JR will be conducted at the Screening Visit, on Day 8, 15, 22, 30, and 56.

The KOOS, JR questionnaire (Appendix E) is used to assess the subject's opinion about their knee and associated problems. The assessments are taken directly from the parent KOOS questionnaire and focus on the three categories: joint pain, stiffness, and function in daily living.

8.17.5 Pain Manageability Assessment

The pain manageability assessment (Appendix F) will be conducted at the Screening Visit, on Day 8, 15, 22, 30, and 56.

8.17.6 Recording Rescue Opioid Medication and Medication Compliance

Each time a subject consumes an opioid from Day 1 after surgery until Day 30, a NRS-R for pain intensity must be completed along with opioid type, dose, and time.

From Day 1 after surgery until Day 30 subjects will be asked daily about compliance with study provided medications (celecoxib and acetaminophen/paracetamol).

8.17.7 Physical Therapy Survey

Each day, at the end of the day, starting on Day 1 through Day 30 a physical therapy survey will be completed including the type (eg in person, home exercises) of physical therapy completed.

Subjects from some sites may be eligible for physical therapy through a digital platform. Allay may collect physical therapy information on these subjects.

9.0 REPORTING SAFETY INFORMATION

9.1 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

9.1.1 Definition of Adverse Event

An AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship to this treatment. An AE can, therefore, be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational product, whether or not related to the investigational product.

For each subject, AEs will be collected from the time of the investigational product administration through the Day 56 Visit. Adverse events will be followed until resolution of AE(s) or the Day 56 Visit, whichever occurs first. All AEs must be recorded regardless of the severity or relationship to investigational product. It is important that Investigators also report all AEs whether serious or non-serious. Events occurring prior to administration of the investigational product should be recorded as medical history.

An AE does include a/an:

- Exacerbation of a pre-existing illness
- Increase in frequency or intensity of a pre-existing episodic event or condition
- Continuous persistent disease or symptoms present at baseline which worsens following the start of the trial
- New post-surgical findings such as erythema and swelling of the surgical site

An AE does not include:

- Planned medical or surgical procedures (eg, surgery, endoscopy, tooth extraction, transfusion); the condition that leads to the procedure is an AE. Planned surgical measures permitted by the clinical trial protocol and the condition(s) leading to these measures are not AEs.
- Pre-existing diseases or conditions present or detected prior to the administration of the investigational product that do not worsen.
- Situations where an untoward medical occurrence has not occurred (eg, social and/or convenience admissions).
- The disease or disorder (post-TKA trial knee pain) being studied unless it represents an excessive exacerbation of the condition.

Definitions for considering sinus bradycardia and respiratory depression AEs are in Appendix K.

9.1.2 Procedures for Eliciting Adverse Events

The Investigator shall report all directly observed AEs, all spontaneously reported AEs, and AEs discovered during the physical exam, laboratory testing, and/or other means. At each visit the Investigator or properly documented delegate will ask the subject a non-specific question (eg, "Have you noticed anything different since your last visit?") to assess whether any AEs have been experienced since the last report or visit.

9.1.3 Reporting a Diagnosis, Not Signs or Symptoms

In general, the use of a unifying diagnosis is preferred to listing individual symptoms. Grouping symptoms into a diagnosis should be done if each sign and/or symptom is a medically confirmed component of a diagnosis. If any aspect of a sign or symptom does not fit into the diagnosis, report the individual symptom as a separate AE. Two examples follow:

- Fever, cough, rhinitis, and headache are diagnosed to be symptoms of an upper respiratory tract infection
- Urinary frequency, flank pain, and fever are diagnosed to be symptoms of a mild renal infection.

9.1.4 Definition of Serious Adverse Event

An SAE is any untoward medical occurrence that at any dose results in death, is life-threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity or is a congenital anomaly/birth defect. An AE occurring at any dose or time during the trial should be classified as SERIOUS if the event results in any of the following:

- Fatality: The AE resulted in death. All deaths are reported whether or not suspected of being related to trial treatment. Death is an outcome of an event. The event that resulted in the death should be recorded and reported. However, if the event resulting in death is not initially known, please report the death immediately and continue to seek additional information, including the cause of death.
- Life-threatening condition: The AE was immediately life-threatening (ie, the AE placed the subject at immediate risk of death; it does not apply to an AE that hypothetically might have caused death if it were more severe).
- Inpatient hospitalization (initial or prolonged): "Inpatient hospitalization" does not imply that the subject must have had an overnight stay in the hospital. If the subject was admitted to the hospital for less than a day for the purpose of treatment or observation, the definition of "Inpatient hospitalization" is met. Brief treatment in an outpatient clinic or Emergency department does not constitute "inpatient hospitalization." For an event to meet the criteria for prolonged hospitalization, the subject must be in the hospital prior to the onset of the AE and that hospitalization was lengthened due to the AE. The following circumstances are NOT considered SAEs by the criterion described above: hospitalizations for diagnostic or elective

medical/surgical procedures (ie, scheduled prior to trial enrollment or during the course of the trial) scheduled treatments and routine check-ups.

- Disability: The AE was disabling (ie, the AE resulted in a significant, persistent, or permanent change, impairment, damage, or disruption in the subject's body function/structure, physical activities, or quality of life).
- Congenital anomaly: The AE was a congenital anomaly/birth defect (ie, an adverse outcome in a child or fetus of a subject exposed to trial treatment prior to conception or during pregnancy).
- Other important medical condition: An AE that may not result in death, be life-threatening, or require hospitalization, but based upon appropriate medical judgement may jeopardize the subject or subject may require medical or surgical intervention to prevent one of the outcomes defined above. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse. A new diagnosis of cancer should be considered serious and should be reported on the SAE form.

9.1.5 Medical Emergency

In a medical emergency requiring immediate attention, trial site staff will apply appropriate medical intervention, according to current standards of care, and contact Allay medical monitor as soon as possible to alert them of the situation.

Should severe cardiotoxic or neurotoxic events of local anesthetic systemic toxicity (LAST) occur and standard methods of treatment (eg oxygen, medication) fail, a lipid emulsion should be available in the event the hospital/clinic/facility staff assess it is in the best interest of the subject. Should an AESI present after the subject has been discharged from the hospital/clinic/facility the subject should return to the hospital/clinic/facility for further evaluation.

9.2 RELATIONSHIP TO INVESTIGATIONAL PRODUCT

The Investigator will assess the causality of the investigational product to the AE using the following definitions:

"Definitely related", "Probably related" and "Possibly related" will be considered as "related" to the investigational product.

Relationship	Assessment Criteria	Description
Definitely related:	• Event or laboratory test abnormality, with plausible time relationship to investigational product intake and • Cannot be explained by disease or other drugs.	• A clinical event, including laboratory test abnormality, occurring in a plausible time relationship to investigational product administration, and which cannot be explained by concurrent disease or other drugs or chemicals.
Probably related:	• Event or laboratory test abnormality, with reasonable time relationship to investigational product intake and • Unlikely to be attributed to disease or other drugs.	• A clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the investigational product, unlikely to be attributed to concurrent disease or other drugs or chemicals.
Possibly related:	• Event or laboratory test abnormality, with reasonable time relationship to investigational product intake but • Could also be explained by disease or other drugs.	• A clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the investigational product, but which could also be explained by concurrent disease or other drugs or chemicals.

"Unlikely related" and "Not related" will be considered as "not related" to the investigational product.

Relationship	Assessment Criteria	Description
Unlikely related:	• Event or laboratory test abnormality, with a time to investigational product intake that makes a relationship improbable (but not impossible) and • Disease or other drugs provide plausible explanations	• A clinical event, including laboratory test abnormality, with a temporal relationship to investigational product administration which makes a causal relationship improbable, and in which other drugs, chemicals or underlying disease provide plausible explanations.

Relationship	Assessment Criteria	Description
Not related:	• The AE is clearly not related to the investigational agent/procedure	• Another cause of the event is most plausible; and/or a clinically plausible temporal sequence is inconsistent with the onset of the event and the trial intervention and/or a causal relationship is considered biologically implausible.

Assessment of the relationship of the AE to investigational product will not be conducted by the treating surgeon since they are unblinded to trial treatment.

9.3 RELATIONSHIP TO THE TKA SURGERY

The Investigator will assess the causality of the TKA surgery to the AE using the following definitions:

"Definitely related" and "Probably related" and "Possibly related" will be considered as "related" to the TKA surgery.

Relationship	Assessment Criteria	Description
Definitely related:	• Event or laboratory test abnormality, with plausible time relationship to TKA surgery and • Cannot be explained by disease or other drugs.	• A clinical event, including laboratory test abnormality, occurring in a plausible time relationship to TKA surgery, and which cannot be explained by concurrent disease or other drugs or chemicals.
Probably related:	• Event or laboratory test abnormality, with reasonable time relationship to TKA surgery and • Unlikely to be attributed to disease or other drugs.	• A clinical event, including laboratory test abnormality, with a reasonable time sequence to TKA surgery, unlikely to be attributed to concurrent disease or other drugs or chemicals.
Possibly related:	• Event or laboratory test abnormality, with reasonable time relationship to TKA surgery intake but • Could also be explained by disease or other drugs.	• A clinical event, including laboratory test abnormality, with a reasonable time sequence to TKA surgery, but which could also be explained by concurrent disease or other drugs or chemicals.

"Unlikely related" and "Not related" will be considered as "not related" to the TKA surgery.

Relationship	Assessment Criteria	Description
Unlikely related:	• Event or laboratory test abnormality, with a time to TKA surgery that makes a relationship improbable (but not impossible) and • Disease or other drugs provide plausible explanations	• A clinical event, including laboratory test abnormality, with a temporal relationship to TKA surgery which makes a causal relationship improbable, and in which other drugs, chemicals or underlying disease provide plausible explanations.
Not related:	• The AE is clearly not related to the TKA surgery	• Another cause of the event is most plausible; and/or a clinically plausible temporal sequence is inconsistent with the onset of the event and the TKA surgery and/or a causal relationship is considered biologically implausible.

Assessment of the relationship of the AE to TKA surgery will not be conducted by the treating surgeon since they are unblinded to trial treatment.

Direct relationship to the TKA surgery is required. As an example, if a subject is taking opioids due to the TKA surgical procedure and the subject has constipation as a result of the opioids, the constipation is not related to the TKA surgical procedure, but instead related to the opioid consumption.

9.4 RECORDING ADVERSE EVENTS

Any AE occurrence during the trial must be recorded on source documentation at the site and on the AE CRF. Adverse events will be collected from the time of investigational product administration and through the Day 56 Visit.

The trial site should observe the following guidelines when recording AEs:

- Whenever possible, use recognized medical terms when recording events. Do not use colloquialisms and/or abbreviations.
- The Investigator will evaluate the severity of each AE using the following definitions:
  - Mild: The event may be noticeable to the subject; does not influence daily activities; usually does not require intervention.
  - Moderate: The event may be of sufficient severity to make the subject uncomfortable, performance of daily activities may be influenced; intervention may be needed.

- Severe: The event may cause severe discomfort; usually interferes with daily activities: subject may not be able to continue in the trial; treatment or other intervention usually needed.

- Medication administered to relieve symptoms of the AE will be recorded and the outcome of the AE will be recorded.

For information on AEs from neurological assessments, laboratory findings, and wound healing refer to Sections 8.8, 8.5, and 8.15 respectively.

9.5 REPORTING ADVERSE EVENTS

9.5.1 Serious Adverse Event Reporting

The Sponsor is under obligation to report certain SAEs to regulatory authorities related to investigational products in clinical trials. The Sponsor representative (medical monitor) must be notified within 24 hours when the Investigator determines that an AE meets the protocol definition (Section 9.1.4) of a SAE.

The procedure for reporting SAEs is as follows:

1) For fatal or life-threatening events, contact the Sponsor representative immediately by telephone:

[redacted]

2) Within 24 hours of the Investigator's knowledge of the event, [redacted] must be informed about all SAEs. A completed SAE report must be emailed to [redacted] within this 24-hour period.

After receiving the SAE form, the Sponsor or [redacted] may request additional information from the Investigator to ensure the timely completion of accurate safety reports.

9.5.2 Adverse Events of Special Interest

Adverse Events of Special Interest (AESI) associated with LAST will also be collected in this trial and include the following:

Central Nervous System Symptoms/Events:

- Tongue and perioral numbness
- Tinnitus
- Muscle fasciculations
- Tremors
- Seizures
- Global CNS depression
- Apnea (not sleep apnea)

Cardiovascular Symptoms/Events:

- Sinus bradycardia
- Atrioventricular blocks
- Conduction defects (prolonged PR or prolonged QRS)
- Ventricular dysrhythmias
- Cardiac arrest
- Asystole

Wound Healing Symptoms/Events

- Wound dehiscence
- Wound pus/purulent discharge (Grade 4 from Southampton Wound Scoring System)
- Deep wound infection (Grade 5 from Southampton Wound Scoring System)
- Cellulitis near the incision site (Grade 5 from Southampton Wound Scoring System)

Should any AESI occur, the time of the event should be recorded, and a PK, Blood Chemistry and Hematology sample should be obtained as well as vital signs, neurological assessments, and a triplicate 12 lead ECG. Additionally, a consult with a doctor should occur.

Should cardiotoxic events of LAST occur, a lipid emulsion should be available in the event the hospital/clinic/facility staff assess it is in the best interest of the subject. Should an AESI present after the subject has been discharged from the hospital/clinic the subject should return to the hospital/clinic/facility for further evaluation.

9.5.3 Suspected Unexpected Serious Adverse Reactions

The Sponsor will determine if an SAE is a suspected, unexpected and serious adverse reaction (SUSAR). A SUSAR is a report of a SAE assessed to be possibly related to investigational medicinal product by either the Investigator or the clinical trial Sponsor and assessed to be unexpected (ie the nature or severity is not expected from the information provided) by the Sponsor.

The Sponsor is required to submit a report about a SUSAR to regulatory/competent authorities and to Investigators participating in clinical trials where subjects are exposed to the investigational medicinal product. Reporting will be done in accordance with the applicable regulatory requirements.

9.5.4 Reporting of Adverse Events and Serious Adverse Events to the Investigational Review Board/Ethics Committee

The Investigator must also comply with applicable requirements concerning reporting of SAEs to the IRB/EC that are new and have an impact on the continued ethical acceptability of the trial, or that may indicate the need for amendments to the trial protocol, including altered monitoring of safety.

9.6 PREGNANCY REPORTS

An Investigator should immediately notify [redacted] at [redacted] about pregnancy in a female clinical trial subject.

Pregnancy will be captured on a pregnancy-specific form.

The pregnancy must be followed up through the delivery or other fetal outcome. The Investigator shall promptly report to [redacted] any abnormal fetal outcome, including congenital anomaly or birth defect, spontaneous or therapeutic abortion, still birth, premature birth, or other outcome other than live normal birth on an SAE form.

9.7 POSSIBLE ADVERSE EVENTS

9.7.1 Due to Bupivacaine

Possible AEs: these events have been reported as side effects from bupivacaine use:

- Allergic reaction (rare and may occur as a result of sensitivity to the local anesthetic)
- Anaphylactic symptoms, severe asthmatic episodes
- Angioneurotic edema (including laryngeal edema)
- Anxiety
- Atrioventricular block
- Blurred vision
- Bradycardia
- Cardiac arrest
- Cardiovascular collapse
- Cardiovascular stimulations or cardiovascular depression
- Changes in cardiac conduction
- Chills
- Confusion
- Constrictions of the pupils
- Convulsions
- Decreased cardiac output
- Depression
- Depression of the myocardium
- Dizziness
- Drowsiness
- Epinephrine/adrenaline response (increase in heart rate, and/or systolic blood pressure, circumoral pallor, palpitations. nervousness)
- Erythema
- Excitability
- Fatality
- Headache
- Heart block
- Hypotension
- Hypertension
- Impaired renal function
- Nausea
- Paresthesia
- Peripheral vascular resistance
- Persistent anesthesia
- Prolonged severe hypertension
- Pruritus
- Refractoriness
- Respiratory arrest
- Respiratory depression
- Respiratory paralysis
- Restlessness
- Severe persistent hypertension
- Shivering
- Sneezing
- Syncope
- Tachycardia
- Tinnitus
- Tremors
- Unconsciousness
- Under-ventilation or apnea
- Urticaria
- Ventricular arrhythmias
- Ventricular fibrillation
- Ventricular tachycardia
- Vomiting
- Weakness

These events have been reported as side effects from bupivacaine use and are likely a result of the way bupivacaine was administered (eg injection) or due to the location of injection (eg head/neck area), they are unlikely to be associated with the use in this protocol; however, they have been listed below:

- Arachnoiditis
- Backache
- Cranial nerve palsies
- Fecal and urinary incontinence
- Loss of cerebrospinal fluid
- Loss of perineal sensation and sexual function
- Loss of sphincter control
- Meningism/meningismus
- Paralysis
- Paralysis of the lower extremities
- Septic meningitis
- Urinary retention

Additional possible AEs related to anesthetic use include those in Section 9.5.2.

9.7.2 Due to General Surgery, TKA and Blood Collections

Adverse Events related to general surgery, TKA, and blood collections are listed below:

- Adhesions
- Allergic reaction to suture or dressing
- Altered leg length
- Artificial joint failure or wear out
- Bursitis
- Bleeding/hemorrhage
- Blood clots
- Blood vessel (vascular) damage or injury
- Broken bones (Femur, Tibia, Patella)
- Bruising
- Cellulitis
- Complications for surgical anesthesia
- Damage to neurovascular structures
- Death
- Delayed wound healing
- Deep vein thrombosis (DVT)
- Early failure
- Erythema
- Faintness
- Hematoma
- Incision site or knee infection
- Infection
- Infection at the blood draw site
- Inflammation of the vein
- Inflammation of the surgical site
- Injury to muscle or soft tissue of the knee
- Joint stiffness
- Keloid like scar formation
- Knee joint instability
- Lung collapse
- Myocardial infarction
- Numbness
- Nerve damage or injury
- Pain
- Pain or bruising at the site where blood is drawn
- Prosthesis dislocation
- Prosthesis failure
- Pulmonary embolism
- Thrombosis
- Reaction to a blood transfusion
- Re-operation and/or manipulation on the knee joint
- Stroke
- Stitch abscess (pus formation)
- Swelling
- Synovitis
- Wound infection
- Wound swelling, draining, or delayed healing

10.0 STATISTICS

A comprehensive Statistical Analysis Plan will be developed for this trial. Demographic and baseline characteristics will be summarized descriptively by treatment group. Analysis methods presented in this statistics section applies to both Part A and Part B. Data from Part A and Part B will be analyzed separately.

10.1 SAMPLE SIZE CALCULATION

Part A

Approximately 165 subjects will be randomized with one (1) of the following three (3) treatment groups in a 1:1:1 ratio with approximately 55 subjects per group:

- ATX-101, 1,000 mg dose (two ATX-101 implants)
- ATX-101, 1,500 mg dose (three ATX-101 implants)
- Bupivacaine HCl 0.25% without epinephrine/adrenaline, maximum dose of 2 mg/kg, via local periarticular infiltration or adductor canal block, or in combination

Based on the literature review and discussion with experts in post-operative pain, it is believed that 55 subjects per group would provide sufficient information to inform dose response and dose selection for Part B.

Part B

Up to 140 subjects will be randomized to one (1) of the following two (2) treatment groups in a 1:1 ratio with up to 70 subjects per group:

- ATX-101 dose to be determined from Part A
- Bupivacaine HCl 0.25% without epinephrine/adrenaline, maximum dose of 2 mg/kg, via local periarticular infiltration or adductor canal block, or in combination

The current planned sample size for Part B is up to 140 subjects. The actual number of subjects to be randomized will be determined based on data from Part A.

10.2 ANALYSIS POPULATIONS

The Full Analysis Set (FAS) includes all subjects who are randomized and administered ATX-101 or bupivacaine HCl. Randomization failures (subjects who are randomized but did not receive investigational product) will be excluded from the FAS. The FAS will be used for the efficacy analyses and subject demographic and baseline characteristics.

The Safety Analysis Set includes all subjects who are randomized and administered ATX-101 or bupivacaine HCl. Randomization failures (subjects who are randomized but did not receive investigational product) will be excluded from the Safety Analysis Set.

The Safety Analysis Set will be used for all safety analyses.

The PK Analysis Set includes all subjects who provide enough plasma samples for PK assessment of bupivacaine that allows for generation of at least one PK parameter of T_{max}, C_{max}, or AUC.

10.3 PRIMARY ENDPOINT ANALYSIS

The primary efficacy endpoint will occur in Part B of the study and is the AUC for the NRS-R of pain intensity from 30 Minutes through a time point to be determined from Part A. The time-weighted AUC will be calculated using the trapezoidal rule based on the available NRS pain scores. Missing values will not be imputed with the exception of the final time point. The trapezoidal rule performs a linear interpolation between the 2 observed values on either side of a missing value. Actual times of each NRS-R score will be used for calculating the AUC.

Prior to administration of rescue opioid medications, NRS-R scores will be recorded. The primary endpoint of AUC will be calculated with censoring pain scores after opioid use. NRS-R values will be censored for the duration of the effectiveness of rescue opioid medications. Actual durations of censoring will be dependent on each type of rescue opioid medication used. Detailed censoring durations for each type of rescue opioid medication will be documented in the Statistical Analysis Plan.

The primary efficacy endpoint will be analyzed using an Analysis of Variance (ANOVA) model with a main effect of the treatment group. Summary statistics will be reported as well as the least square (LS) means, difference in the LS means, 95% confidence intervals (CI) and p-value for the contrast comparing the LS means.

For Part B, the primary comparison will be between ATX-101 and bupivacaine HCl. A two-sided alpha 0.05 will be spent on the primary comparison.

For Part A, each dose of ATX-101 will be compared to the bupivacaine HCl group. In addition, the LS mean of each dose level group will be plotted with 95% CI to evaluate dose response. The AUC for the NRS-R of pain intensity from 30 Minutes through Hour 168 (Day 8 of the trial) will be evaluated.

10.4 KEY SECONDARY ENDPOINT ANALYSIS FOR PART B

10.4.1 Type I Error Control Procedure

A stepdown procedure will be used to control the type I error. If the primary comparison for the primary endpoint is statistically significant, comparisons between ATX-101 and bupivacaine HCl for the key secondary endpoints will be carried out in the order shown below. Comparisons will be stopped once any proceeding comparison is not statistically significant.

1. Area under the curve for NRS-R of pain intensity from 30 Minutes through Hour 168 (Day 8 of the trial)

2. Area under the curve for NRS-R of pain intensity from 30 Minutes through Hour 240 (Day 11 of the trial)

3. Area under the curve for NRS-R of pain intensity from 30 Minutes through Hour 336 (Day 15 of the trial)

4. Percentage of subjects who remain opioid free from Hour 72 through Day 30

5. Total post-surgical consumption of opioid medications from surgical closure through Day 30

10.4.2 Area Under the Curve for Numeric Rating Scale at Rest of Pain Intensity from 30 Minutes through Hours 168, 240, and 336

The AUC of the NRS-R will be calculated from 30 Minutes through Hour 168 (Day 8 of the trial), Hour 240 (Day 11 of the trial), and Hour 336 (Day 15 of the trial) using the trapezoidal method as described in the primary endpoint section. Data will be analyzed using the same ANOVA model as in the analysis of the primary endpoint. Comparisons between ATX-101 and bupivacaine HCl will be carried out in the following order: Hour 168, Hour 240, and Hour 336.

10.4.3 Percentage of Subjects Who Remain Opioid Free from Hour 72 Through Day 30

The percentage of subjects opioid free from Hour 72 through Day 30 will be analyzed using Fisher's exact test. The number of subjects, percentage, and percentage difference between treatment groups will be summarized and their associated 95% CI will also be provided.

10.4.4 Total Post-Surgical Consumption of Opioid Medications from Surgical Closure Through Day 30

For total post-surgical consumption of opioid medication, all opioids will be converted to an equianalgesic parenteral morphine amount (milligram morphine equivalent or MME) using standard conversion factors as laid out by the Center of Disease Control (CDC) [21]. Total post-surgical consumption of rescue opioid medications from surgical closure through Day 30 will be analyzed using the Wilcoxon rank-sum test.

10.5 SECONDARY ENDPOINT ANALYSIS

Secondary endpoints will be analyzed in Part A and Part B of the trial.

10.5.1 Area Under the Curve for Numeric Rating Scale at Rest for Each 24-Hour Period Through Hour 336

The AUC of the NRS-R for pain intensity will be calculated for each day and cumulatively through the end of each day using the trapezoidal method as described in the primary endpoint section. Data will be analyzed using the same ANOVA model as in the analysis of the primary endpoint.

10.5.2 Percentage of Subjects Opioid Free

The percentage of subjects who remain opioid free from surgical closure to Day 30, from Hour 72 (Day 4) to Day 30, Hour 96 (Day 5) to Day 30, Hour 168 (Day 8) to Day 30, Hour 336 (Day 15) to Day 30 and Hour 504 (Day 22) to Day 30 will be evaluated. Opioid free data will be analyzed using Fisher's exact test. The number of subjects, percentage, and percentage difference between treatment groups will be summarized by treatment group and associated 95% CI will be provided.

10.5.3 Total Post-Surgical Consumption of Opioids

Opioid consumption will be summarized descriptively every 24-hour period through Day 30 and total opioids consumed over 30 days. In addition, opioid consumption will also be analyzed from the time of surgical closure to Hour 48 (Day 3), Hour 72 (Day 4), Hour 96 (Day 5), Hour 168 (Day 8), Hour 336 (Day 15), and Day 30 using the Wilcoxon rank-sum test.

10.5.4 Time to First Rescue Opioid Medication

The time to the first post-surgical rescue opioid medication will be calculated. Data will be summarized using the Kaplan Meier method and displayed graphically where appropriate. Confidence intervals for the 25th, 50th, and 75th percentiles will be reported. The Cox proportional hazards model will be fitted to compute the treatment hazard ratios and the corresponding 95% CI.

10.6 SAFETY ENDPOINTS

Safety endpoints will be analyzed in Part A and Part B of the trial.

10.6.1 Adverse Events

AEs verbatim descriptions will be coded using a current version of the Medical Dictionary for Regulatory Activities (MedDRA). A treatment emergent adverse event (TEAE) is defined as an AE that is new or worsening after the subject has received the investigational product.

Summaries of incidence rates (frequencies and percentages) of TEAEs by MedDRA system organ class (SOC) and preferred term, by maximum severity, and by strongest relationship to investigational product and TKA surgical procedure will be provided. SAEs by MedDRA SOC and preferred term will also be provided. A subject will be counted only once by the highest severity grade within a SOC and preferred term, even if the subject experienced more than 1 TEAE within a specific SOC and preferred term.

Summaries of incidence rates (frequencies and percentages) of individual AESIs by MedDRA SOC and preferred term will be provided.

All AEs, SAEs, and AESIs will be listed by subject.

10.6.2 Wound Healing Assessment

The Southampton Wound Scoring System will be used to evaluate wound healing. Wound healing grades on Day 15, 22, 30, and 56 will be summarized descriptively.

10.6.3 Pharmacokinetic Analysis

Individual concentrations of bupivacaine will be listed, graphed, and summarized by scheduled sampling time. Bupivacaine concentration will be summarized at each sampling timepoint using descriptive statistics.

The PK parameters will be estimated using non-compartmental PK analysis for each subject with sufficient data to characterize the administered dose. The overall PK parameters for plasma bupivacaine will be presented (AUC_{last}, $AUC_{0-\infty}$, C_{max}, T_{max}, $T_{1/2}$) as well as dose specific PK parameters (C_{max}, AUC) for each ATX-101 dose. Additional parameters will be detailed in the PK analysis plan. Actual sampling times will be used to calculate plasma-derived PK parameters. These parameters will be summarized by dose for comparison and a mean concentration figure with plots for each level of the ATX-101 doses and will be produced both with a linear y-axis and with a log y-axis. A PK analysis plan will detail the specific PK analyses.

The PK analyses and PK report will be presented in a separate analytical report (Bioanalytical Report) and PK report.

10.7 EXPLORATORY ENDPOINTS

Exploratory endpoints will be analyzed in Part A and Part B of the trial. Exploratory endpoints are defined below. Continuous endpoints will be summarized using descriptive statistics (number of participants, mean, standard deviation [SD], median, and maximum values) for by treatment group and trial visit, as appropriate. Categorical endpoints will be summarized as the number and percentage of participants per category by treatment group and trial visit, as appropriate. Time-to-event endpoints will be summarized using the Kaplan Meier method by treatment group.

- Percentage of subjects reporting being pain free on the NRS-R of pain intensity
- Time to various degrees of flexion and extension
- Reduction/incidence of opioid-related AEs
- Evaluation of Quality of Recovery (QOR-15)
- Evaluation of Knee Injury and Osteoarthritis Outcome Score for Joint Replacement (KOOS, JR)
- Difference in pain intensity scores (NRS-R and NRS-A) between treatment groups
- If a statistical difference in AUC of pain intensity NRS-R at 15 days is observed, the AUC for the NRS-R of pain intensity will be further evaluated between Days 16 and 30
- Evaluation of the Knee Society Score

10.8 OTHER SAFETY ANALYSIS

10.8.1 Clinical Laboratory Tests

Clinical laboratory (e.g., hematology and clinical chemistry) values will be evaluated for each laboratory parameter as appropriate. Abnormal laboratory values will be flagged and identified as outside (above or below) the normal range. Reference (normal) ranges for laboratory parameters will be provided.

Descriptive summary statistics (eg, n, mean, SD, median, minimum, and maximum for continuous variables; n [%] for categorical variables) for laboratory parameters and their changes from baseline will be calculated. Laboratory values will be summarized by visit.

10.8.2 Electrocardiograms

ECG data (12-lead) will be summarized and will be listed by subject and visit.

10.8.3 Vital Signs

Descriptive summary statistics for vital sign parameters (systolic and diastolic blood pressure, pulse rate, body temperature, and oxygen saturation) and changes from baseline will be presented by visit.

Vital signs data will be listed by subject and visit.

10.8.4 Physical Examination and Neurological Assessment Findings

Physical examination and neurological assessment findings will be displayed in listings.

10.8.5 Patient Health Questionnaire 9

Data from the screening PHQ-9 questionnaire will be presented in a subject listing.

10.8.6 Treatment Exposure and Disposition

Exposure to treatment will be summarized descriptively. Summary tables for subject disposition and population assignment will be provided.

10.8.7 Interim Analysis

For Part A, an interim analysis may be performed when approximately 50% of the initially planned enrollment has completed Day 30. Enrollment will continue during the interim analysis. This interim analysis will be performed by the unblinded statistician separate from the team responsible for the conduct and analysis of the study. A Review Committee of at least two unblinded statisticians and a designated sponsor representative will review the data and recommend one of the following:

- Keep the current sample size and continue Part A as planned
- Stop Part A for efficacy (no further enrollment)
- Increase the sample size in Part A

The basis of this decision will be whether sufficient data have been collected to plan Part B, taking into account both safety and efficacy (including outcomes other than the primary). All part A data will be evaluated. In particular, data pertaining to selection of Part B primary endpoint, eg treatment effect and primary endpoint variability, will be evaluated for the planning of Part B.

The Sponsor may make adjustments other than those detailed above; however, the blinded Sponsor team will receive no information other than the recommendations listed. If the interim analysis demonstrates sufficient data have been collected to plan Part B, then Part A may be stopped, unblinded and the final analysis will be completed on the population enrolled to date.

Full details of the interim analysis will be described in a separate document including data available to the Review Committee, timing, and communication procedures. The designated sponsor representative will not participate in ongoing study conduct once unblinded to study results and will not have access to individual patient treatment assignments. Should Part A continue to full enrollment they likewise will be blinded to assignments and results until the final unblinding and analysis.

11.0 ETHICS

This trial will be conducted in the hospital/facility, clinic, in the subject's home, physical therapy facility, or other similar location, as agreed to by the subject and the trial staff under the supervision of medical personnel experienced in conducting clinical studies. The trial will be conducted in accordance with the International Conference on Harmonization (ICH) Guideline E6: Good Clinical Practice (GCP).

11.1 ETHICAL CONDUCT OF THE TRIAL

The trial will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki (2013 revision) and are consistent with ICH Guideline E6: GCP and applicable regulatory requirements.

11.2 INVESTIGATIONAL REVIEW BOARD/ ETHICS COMMITTEEREVIEW

The Investigator shall assure that an IRB/EC, constituted in accordance with ICH Guideline E6: GCP, or other federal or local guidelines, will provide initial and continuing review of the trial. The final trial protocol, including the final version of the informed consent, must be approved or given a favorable opinion in writing by an IRB/EC as appropriate. Prior to shipment of the investigational product and enrollment of trial subjects, documented IRB/EC approval of the

protocol, informed consent, subject questionnaires/surveys, subject training, and any advertisement for subject recruitment (if used) must be obtained and provided to Allay or its designee(s). The written consent form which describes the trial with sufficient information for the subject to make an informed decision about his/her participation will be obtained before that subject begins any trial specific procedure. It must be signed by the subject and the Investigator-designated healthcare professional obtaining the consent, and a copy of the signed document provided to the subject.

The Investigator is responsible for informing the IRB/EC in accordance with local requirements of any amendment to the protocol or any changes in research activity (ie, completion, termination, or discontinuation of a trial) prior to implementation. In addition, the IRB/EC must approve all advertising used to recruit subjects for the trial. The protocol must be re-approved by the IRB/EC upon receipt of amendments and annually, as local regulations require.

The Investigator is also responsible for providing the IRB/EC with reports of any reportable serious adverse reactions from any other trial conducted with the investigational product. Allay will provide this information to the Investigator.

Progress reports and notifications of serious adverse drug reactions will be provided to the IRB/EC according to local regulations and guidelines.

12.0 ADMINISTRATIVE CONSIDERATIONS

12.1 RECORDING DATA

The Investigator is required to initiate and maintain an adequate and accurate case history for each subject, capturing all clinical trial observations and other data. Data must be recorded in the designated CRFs approved by Allay. The Investigator or his/her qualified designee will enter all applicable data from the source documents into the appropriate portions of the CRFs. All information recorded on the CRFs for this clinical trial must be consistent with the subject's source documentation.

An electronic data capture (EDC) system will be used for entry of the data into CRFs. Entry and changes to the data will be made only by users authorized by Allay, and data entries and changes will be captured in an electronic audit trail. An explanation of any data change should be recorded in the CRF.

The clinical trial data will be entered into a secure, validated data processing system, and a backup will be maintained. Any changes to the clinical trial data will be documented.

12.2 DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

12.2.1 Trial Monitoring

Allay or its designee will assign a Monitor to maintain contact with the Investigator and will visit the trial site for the purpose of discussing and/or reviewing data.

A site initiation visit will be conducted by Allay or designee and, if available, the Monitor to discuss the protocol and the obligations of both the Sponsor and the Investigator. The Investigator must allow the Monitor to perform periodic, interim monitoring visits. The purposes of these visits are to:

- Verify that signed and dated informed consent was obtained prior to each subject's participation in the trial
- Assess the progress of the trial
- Review compliance with the trial protocol
- Determine whether all AEs were appropriately reported
- Determine whether the Investigator is maintaining the essential documents
- Discuss any emergent problem
- Check the CRF for accuracy and completeness
- Validate the contents of the CRF against source documents
- Perform drug accountability and assess the status of investigational product and storage, dispensing and destruction
- Ensure the blind has been maintained

All data required by the protocol must be reported accurately on the CRF and must be consistent with the source documents. Source documents are original documents, data and records (eg, hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, X-rays, ECGs or other diagnostic images, subject files, pharmacy records, and laboratory records). The Investigator will make available the source documentation for inspection. The information will be considered confidential and treated as such.

The Monitor will perform a close-out visit at the conclusion of the Investigator's involvement in the trial.

The Clinical Monitoring Plan contains more complete details with regard to monitoring of this trial and specific duties of the blinded and unblinded monitors.

12.2.2 Audits and Inspection

The trial may be evaluated by representatives of the US Food and Drug Administration (FDA), other government agencies, or national health agencies (where applicable), the IRB/EC, other national health authorities or the Sponsor who will be allowed access to trial documents.

All authorized personnel, including health authority inspector(s), Sponsor and designees, monitor(s), and auditor(s) will be given direct access to source data and documentation (eg, medical records, laboratory results, etc.) for source data verification, provided that subject confidentiality is maintained.

The Investigator should promptly notify Allay of any audits scheduled or results of any unscheduled investigations performed by regulatory authorities.

12.3 QUALITY CONTROL AND QUALITY ASSURANCE

The protocol shall be conducted as described unless there is a change that is intended to eliminate an apparent immediate hazard to the subject. Any such change must be reported immediately to Allay and the IRB/EC in accordance with IRB/EC requirements.

Allay may conduct a quality assurance audit at its discretion.

12.3.1 General Responsibilities

The trial will be conducted in full compliance with the Declaration of Helsinki (2013 revision), ICH Guideline E6: GCP, and any applicable national and local laws and regulations.

The Investigator is responsible for distributing trial information and documentation to all appropriate staff members prior to and during the course of the trial as updated information becomes available.

The Investigator is responsible for ensuring the privacy, health, and welfare of the subjects during and after the clinical trial and must ensure that fully functional resuscitation equipment and personnel trained in its use are immediately available in the event of an emergency.

The Investigator must be familiar with the background and requirements of the trial and with the properties of the investigational product as described in the current version of the IB and prescribing information for the active comparator.

The Investigator has the overall responsibility for the conduct and administration of the trial at the clinical site and for contacts with the Sponsor, IRB/EC, and local authorities.

The Investigator is responsible for performing the trial in accordance with the protocol and the above guidelines and regulations, and for collecting, documenting, and reporting the data accurately.

Data relating to the trial will be recorded in CRFs prepared by Allay or its designee. Data must be entered into the CRFs in English. The CRFs are to be completed at the time of the subject's visit, with the exception of results of tests performed outside of the Investigator's office, so that they always reflect the latest observations on the subjects participating in the trial. All CRFs should be entered in a timely fashion after each assessment.

The Investigators must verify that all data entries in the CRF are accurate and correct. All CRF entries, corrections and alterations must be made by the Investigator or other, authorized, trial-site personnel and only by individuals who have been trained on the CRF completion requirements for this trial, and who are using their own password/passcode for the electronic CRF system.

The completed CRFs will be reviewed against source documents by the monitor at each monitoring visit. If any data, signatures, or form are missing or incorrect, the Investigator will be informed and corrections will be made.

12.3.2 Retention of Records

The Investigator shall retain all trial related documentation, including the following:

- Source data, including tracings, computer discs, or tapes
- Investigator's Brochure
- Source Documents
- Completed SAE forms
- Protocol and amendment(s)
- Drug accountability records
- Regulatory documents and correspondence
- Signed informed consents
- Subject identification lists
- Correspondence

The Investigator will retain the trial documents for at least 2 years after the approval date for marketing the product in an ICH region and until there are no pending or contemplating marketing applications in any region or until 2 years after the investigational use of the investigational product is discontinued or 25 years, whichever is longer. If the Investigator leaves the institution, the records shall be transferred to an appropriate designee who accepts the responsibility for records retention. Written notice of transfer of documentation shall be provided to Allay. The Investigator must obtain written permission from Allay before destroying any trial documents.

All changes to trial records should be documented and traceable. The media used to store trial documents should be in a form that keeps the documents complete and legible through the required retention period. All trial documents should be readily available, upon request, to Allay or designee and regulatory authorities.

12.3.3 Protocol Compliance

This trial will be conducted in compliance with the protocol approved by the IRB/EC and according to current ICH Guideline E6: GCP standards. No deviation from the protocol will be implemented without the prior review and approval of the Sponsor except where it may be necessary to eliminate an immediate hazard to a research subject. In such a case, the deviation will be reported to Allay and the IRB/EC as soon as possible.

Protocol amendments will be prepared and approved by the Sponsor or designee. All protocol amendments will be signed by the Investigator and submitted to the IRB/EC for review and approval prior to implementation. Documentation of IRB/EC approval must be forwarded to the Sponsor or designee.

If an amendment significantly alters the trial design, increased potential risk to the subject, or otherwise affects statements in the informed consent, it must be revised accordingly and submitted to the IRB/EC for review and approval. The approved consent form must be signed by all new subjects and ongoing subjects if they are affected by the amendment or if the amendment contains new safety information or as required by the IRB/EC.

12.3.4 Subject Informed Consent

The consent form must be approved by the IRB/EC and must contain all elements required by federal, state, local, and institutional regulations or requirements.

The trial, including its objective, methods, and potential risks of subjects, will be completely explained to each prospective trial subject. It will also be explained to each subject that s/he is free to refuse entry or to withdraw from the trial at any time without prejudice to future treatment. Voluntary informed consent must be obtained from each eligible subject prior to screening for the trial (ie, before any protocol-defined procedures are performed).

The subject's willingness to participate will be documented in writing on the IRB/EC approved informed consent form, which will be signed and dated by the subject and the trial Investigator or designee. The Investigator will keep the original signed informed consent form, and a copy will be given to the subject.

12.4 LABORATORY ACCREDITATION

A central laboratory facility will be used for analysis of clinical laboratory samples obtained under this protocol. The laboratory will demonstrate adequate licensure and accreditation. Reference ranges for all parameters will be available.

12.5 CONFIDENTIALITY REGARDING TRIAL SUBJECTS

The Investigator must assure that the privacy of the subjects, including their identity and all personal medical information, will be maintained at all times. In the CRFs and other documents

(laboratory reports, etc) submitted to the Sponsor, subjects will not be identified by name but by an identification code (eg, initials and subject identification numbers).

Personal medical information will always be treated as confidential.

12.6 PUBLICATION POLICY

By conducting this trial, the Investigator affirms to Allay that s/he will maintain, in strict confidence, information furnished by Allay or any representatives, including data generated from this trial, except as exempted for regulatory purposes.

All data generated during the conduct of this trial is owned by Allay and may not be used by the Investigator or affiliates without the express written consent of Allay.

Allay intends to have all trial results published in due course after completion of the trial and regulatory submission. All manuscripts, abstracts, or other presentation materials will be reviewed, and approved by Allay, prior to submission. Allay will have 90 calendar days to review and approve (in writing) the release of manuscripts, abstracts, and other presentation materials. This will allow Allay to protect proprietary information and allow both parties to provide comments based upon information that may not yet be available to the other.

13.0 APPENDICES

APPENDIX A. SCHEDULE OF EVENTS FOR PART A

Part A Schedule of Events for Screening, Day 6 Through Day 56										
Procedure	**Screening ≤ 30 Days Before Surgery**	**Day 1 to Day 5 See Below Table for Schedule of Events**	**Day 6**	**Day 8 (±1 Day)**	**Day 15 (±3 Days)**	**Day 22 (±3 Days)**	**Day 30 (±3 Days)**	**Day 56 (±7 Days)**	**Early Termination**	**In Event of an SAE/ AESI**
In Clinic Visit Required[a]	X				X	X	X	X	X	X
96 Hour In-Patient Period		X								
In Clinic or Home Visit[b]			X	X						
Informed Consent	X									
Demographics/Medical History	X									
Update Medical History										
Review Entry Criteria	X									
Vital Signs[c]	X		X	X	X	X	X	X	X	X
Physical Examination[d]	X							X	X	
Height	X									
Weight	X							X	X	
12 Lead ECG[e]	X		X	X	X	X	X	X	X	X
Chemistry/Hematology Safety Labs[f]	X							X	X	X
Drug Screen[g]	X				X					
Serum or Urine Pregnancy Test[h]	X							X	X	
Range of Motion Assessment[i]	X		X	X	X	X	X	X	X	
Patient Health Questionnaire-9	X									
Neurological Assessments[j]	X		X	X	X	X	X	X	X	X
e-diary Training[k]	X									
e-diary Dispensation/Return[l]							X		X	
Subject NRS Pain Score Training for Pain Intensity[m]	X				X					
Concomitant Medications and Opioid Accountability[n]	X		X	X	X	X	X	X	X	X
Randomization and Investigational Product Allocation[o]										
Pharmacokinetic Sampling[p]			X	X	X	X	X		X	X
Wound Healing Assessments[q]					X	X	X	X	X	
Knee Society Score[r]	X				X		X	X	X	
Adverse Events[s]			X	X	X	X	X	X	X	X
Subject Self-Administered Questionnaires/Surveys[t]	Refer to Appendix C for frequency of self-administered e-diary questionnaires/surveys									

a. In clinic visits may also be completed at a physical therapy facility or in the clinic/hospital/facility as long as all procedures can be conducted.
b. Home visits may be completed at the subject's home, a physical therapy facility, in the clinic/hospital/facility, or any other place agreed upon by the trial staff and the subject.
c. Vitals signs include pulse, temperature, respiratory rate, systolic/diastolic blood pressure and oxygen saturation. Vital signs will be collected after the subject has been supine for 5 minutes.
d. Physical examination is conducted at the Screening Visit and Day 56 Visit. The Screening Visit will record Kellgren Lawrence Classification (to classify severity of knee osteoarthritis), varus, valgus, and evaluation of ipsilateral hip osteoarthritis.
e. ECGs will be taken in triplicate (2 minutes apart [±1 minute]) after the subject has been at rest in a supine position for 10 minutes without any other assessments being conducted. ECGs will be taken BEFORE PK sampling.
f. Chemistry/Hematology are non-fasting and will be analyzed at a central lab. Chemistry tests include sodium, potassium, creatinine, creatinine clearance, albumin, ALP, total and direct bilirubin, AST, ALT, BUN, creatinine kinase, GGT, and phosphate. Hematology tests include hemoglobin, hematocrit, white blood cell count (total and differential), red blood cell count and platelet count.
g. Urine Drug Screen is performed to include but not limited to opiates (including oxycodone), amphetamines, methadone, barbiturates, benzodiazepines, cocaine, phencyclidine, methamphetamine, ecstasy/MDMA, tricyclic antidepressant, and cannabinoids at the Screening Visit and at the Day 15 Visit. On Day 1, the subject will be tested for opiates only by urine dipstick (Section 8.5.3).
h. The pregnancy test is only for females of child-bearing potential. The Screening Visit and Day 56 pregnancy test will be via urine.
i. Range of motion assessment will be conducted using a goniometer and will evaluate flexion and extension of the knee.
j. Neurological Assessments will determine if the patient has any symptoms of LAST by asking if they have had any changes to their senses (ears/hearing, sight, touch, smell, or taste). The neurological assessments will have a gross motor and sensory exam conducted with a focus on the lower extremities. Motor examinations will focus on distal lower extremities dorsi flexion and plantar flexion of the foot (ankles and toes) against resistance. The gross sensory examination should focus on intact sensory examination to touch in the dorsal and plantar side of the foot.
k. Formal e-diary training must occur prior to surgery. Through the duration of the trial e-diary questions and concerns from the subject will be addressed and re-training will occur if needed at any time during the trial.
l. If a provisioned e-diary is used by the subject, it will be dispensed on Day 1 before surgery and returned on the Day 30 Visit. The assessments conducted on the e-diary at the Screening Visit and Day 56 Visit may occur using an e-diary maintained by the site. If the subject early terminates before the Day 30 Visit, the e-diary will be returned at the Early Termination Visit. If the subject's own device is used, the site staff will work with the subject to have the e-diary set-up before the subject has surgery.
m. Subject pain score training will occur at the Screening Visit, on Day 1 before surgery, on Day 2, Day 3, and after Day 15. If needed, pain score training can occur anytime during the trial that the subject has questions or as determined by the site staff. Standardized pain score training will be conducted by the site staff or through training videos.
n. Prior and concomitant medication usage is recorded from 30 days before the Screening Visit through Day 56 or Early Termination. At the Day 15 Visit, Day 30 Visit, and Early Termination Visit (if on or before Day 30) accountability of prescribed opioids will occur.
o. Randomization for allocation of investigational product may occur up to one business day before surgery.

p. Pharmacokinetic sampling will occur on Day 1 before surgery (before bupivacaine spinal), during surgery prior to administration of ATX-101 or the bupivacaine HCl (-10 minutes), then after surgical closure at Hour 6 (± 30 minutes), Hour 12 (± 1 hour), and on Days 2 (24 Hour), 3 (48 Hour), 4 (72 Hour), 5 (96 Hour), 6, 8, 15, 22, and 30. PK sampling will also occur at the Early Termination Visit if on or before the Day 30, as well as anytime the subject has an SAE or AESI**.** It is requested, if possible, that the PK samples from Days 6 to 15 are collected at approximately the time of surgical closure on Day 1.

q. The Southampton Wound Scoring System (Appendix I) will be used to evaluate the TKA surgical wound for each subject.

r. The Knee Society Score (Appendix J) will be used to evaluate the subject's knee joint and function.

s. Adverse Events are collected from the time of investigational product administration and through Day 56 or Early Termination. If an AESI or SAE occurs, then neurological assessment, vital signs, 12-lead ECG, Chemistry, Hematology, and PK sampling must occur.

t. Subject self-administered e-diary questionnaires/surveys will be completed. NRS pain intensity scales include both at rest (NRS-R) and activity (NRS-A). Each time rescue opioid medications are taken, the NRS-R scale for pain intensity must be completed along with opioid type, dose, and time. Refer to Appendix C for further details on frequency of collection.

Part A Schedule of Events for Day of Surgery Through Hour 96 (Day 5)															
Procedure		**Day of Surgery (Day 1)**							**Day 2**		**Day 3**		**Day 4**		**Day 5**
	Before Surgery	**During Surgery**	**30 Min & 1 Hour**	**3 Hour**	**6 Hour**	**9 Hour**	**12 Hour**	**18 Hour**	**24 Hour**	**30, 36 & 42 Hour**	**48 Hour**	**54, 60, 66 Hour**	**72 Hour**	**78, 84, 90 Hour**	**96 Hour**
Window	Before Spinal	-10 min	±10 min	±30 min	±30 min	±1 hour	±1 hour	±2 hour	±2 hour	±2 hour	±2 hour	±2 hour	±2 hour	±2 hour	±2 hour
96 Hour In-Patient Period[a]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Update Medical History	X														
Review Entry Criteria	X	X													
Vital Signs[b]	X			X	X	X	X	X	X	X	X	X	X	X	X
12 Lead ECG[c]	X	X			X		X		X		X		X		X
Drug Screen[d]	X														
Serum or Urine Pregnancy Test[e]	X														
Range of Motion Assessment[f]									X		X		X		X
Neurological Assessments[g]	X			X	X	X	X	X	X		X		X		X
e-diary Training[h]	X														
e-diary Dispensation/Return[i]	X														
Subject NRS Pain Score Training for Pain Intensity[j]	X								X		X				
Concomitant Medications and Opioid Accountability[k]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Randomization and Investigational Product Allocation[l]	X														
Pharmacokinetic Sampling[m]	X	X			X		X		X		X		X		X
Investigational Product Administration[n]		X													
Adverse Events[o]		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Subject Self-Administered Questionnaires/Surveys[p]	X		X	X	X	X	X	X	X	Refer to Appendix C for frequency of self-administered e-diary questionnaires/surveys					

a. Subjects will remain under observation until Hour 96. Subjects may remain in the hospital/facility for 96 hours or may be discharged from the hospital/facility per standard of care and moved to another unit until the conclusion of the 96-hour in-patient monitoring period.
b. Vitals Signs include pulse, temperature, respiratory rate, systolic/diastolic blood pressure and oxygen saturation. In a 24-hour period, vital signs can only be missed one time for the subject sleeping. Vital signs will be collected after the subject has been supine for 5 minutes.
c. ECGs will be taken in triplicate (2 minutes apart [±1 minute]) after the subject has been at rest in a supine position for 10 minutes without any other assessments being conducted. ECGs will be taken BEFORE PK sampling.
d. Urine Drug Screen is performed on Day 1 before surgery for opiates only by urine dipstick (Section 8.5.3).
e. The pregnancy test is only for females of child-bearing potential. The Day 1 pregnancy test may be done via serum or urine per hospital standard of care. If females of child-bearing potential would not normally be tested on the day of surgery, then a urine pregnancy test will be conducted.
f. Range of motion assessment will be conducted using a goniometer and will evaluate flexion and extension of the knee.
g. Neurological Assessments will determine if the patient has any symptoms of LAST by asking if they have had any changes to their senses (ears/hearing, sight, touch, smell, or taste). The neurological assessments will have a gross motor and sensory exam conducted with a focus on the lower extremities. Motor examinations will focus on distal lower extremities dorsi flexion and plantar flexion of the foot (ankles and toes) against resistance. The gross sensory examination should focus on intact sensory examination to touch in the dorsal and plantar side of the foot. In the first 24-hour period, the neurological assessments can only be missed one time for the subject sleeping.
h. Formal e-diary training must occur prior to surgery. Through the duration of the trial e-diary questions and concerns from the subject will be addressed and re-training will occur if needed at any time during the trial.
i. If a provisioned e-diary is used by the subject, it will be dispensed on Day 1 before surgery and returned on the Day 30 Visit. If the subject early terminates before the Day 30 Visit, the e-diary will be returned at the Early Termination Visit. If the subject's own device is used, the site staff will work with the subject to have the e-diary set-up before the subject has surgery.
j. Subject pain score training will occur at the Screening Visit, on Day 1 before surgery, on Day 2, Day 3, and after the Day 15. If needed, pain score training can occur anytime during the trial that the subject has questions or as determined by the site staff. Standardized pain score training will be conducted by the site staff or through training videos.
k. Prior and concomitant medication usage is recorded from 30 days before the Screening Visit through Day 56 or Early Termination.
l. Randomization for allocation of investigational product may occur up to one business day before surgery.
m. Pharmacokinetic sampling will occur on Day 1 before surgery (before bupivicaine spinal), during surgery prior to administration of ATX-101 or the bupivacaine HCl (-10 minutes), then after surgical closure at Hour 6 (±30 minutes), Hour 12 (±1 hour), and on Days 2 (Hour 24), 3 (Hour 48), 4 (Hour 72), 5 (Hour 96), 6, 8, 15, 22, and 30. PK sampling will also occur at the Early Termination Visit if on or before the Day 30, as well as anytime the subject has an SAE or AESI**.** It is requested, if possible, that the PK samples from Days 6 to 15 are collected at approximately the time of surgical closure on Day 1.
n. ATX-101 or bupivacaine HCl administration will only occur during the surgical procedure and prior to initiating capsule closure. ATX-101 will be placed in the knee capsule following fixation of the knee implant prosthesis when tissue will not be disrupted any further by surgery, after any betadine or saline rinse and after suction has occurred.
o. Adverse Events are collected from the time of investigational product administration and through Day 56 or Early Termination.

p. Subject self-administered e-diary questionnaires/surveys will be completed. NRS pain intensity scales include both at rest (NRS-R) and activity (NRS-A). Each time rescue opioid medications are taken, the NRS-R scale for pain intensity must be completed along with opioid type, dose, and time. Refer to Appendix C for further details on frequency of collection.

APPENDIX B. SCHEDULE OF EVENTS FOR PART B

Part B Schedule of Events for Screening, Day 6 Through Day 56										
Procedure	**Screening ≤ 30 Days Before Surgery**	**Day 1 to Day 2 (Hour 24) See Below Table for Schedule of Events**	**Days 3, 4, 5, & 6**	**Day 8 (±1 Day)**	**Day 15 (±3 Days)**	**Day 22 (±3 Days)**	**Day 30 (±3 Days)**	**Day 56 (±7 Days)**	**Early Termination**	**In Event of an SAE/ AESI**
In Clinic Visit Required[a]	X				X	X	X	X	X	X
24 Hour In-Patient Period		X								
In Clinic or Home Visit[b]			X	X						
Informed Consent	X									
Demographics/Medical History	X									
Update Medical History										
Review Entry Criteria	X									
Vital Signs[c]	X		X	X	X	X	X	X	X	X
Physical Examination[d]	X							X	X	
Height	X									
Weight	X							X	X	
12 Lead ECG[e]	X							X	X	X
Chemistry/Hematology Safety Labs [f]	X							X	X	X
Drug Screen[g]	X				X					
Serum or Urine Pregnancy Test[h]	X							X	X	
Range of Motion Assessment[i]	X		X	X	X	X	X	X	X	
Patient Health Questionnaire-9	X									
Neurological Assessments[j]	X		X	X	X	X	X	X	X	X
e-diary Training[k]	X									
e-diary Dispensation/Return[l]							X		X	
Subject NRS Pain Score Training for Pain Intensity[m]	X		X[m]		X					
Concomitant Medications and Opioid Accountability[n]	X		X	X	X	X	X	X	X	X
Wound Healing Assessment[o]					X	X	X	X	X	
Knee Society Score[p]	X				X		X	X	X	

Part B Schedule of Events for Screening, Day 6 Through Day 56										
Procedure	**Screening ≤ 30 Days Before Surgery**	**Day 1 to Day 2 (Hour 24) See Below Table for Schedule of Events**	**Days 3, 4, 5, & 6**	**Day 8 (±1 Day)**	**Day 15 (±3 Days)**	**Day 22 (±3 Days)**	**Day 30 (±3 Days)**	**Day 56 (±7 Days)**	**Early Termination**	**In Event of an SAE/ AESI**
Adverse Events[q]			X	X	X	X	X	X	X	X
Pharmacokinetic Sampling[r]										X
Subject Self-Administered Questionnaires/Surveys[s]	Refer to Appendix C for frequency of self-administered e-diary questionnaires/surveys									

a. In clinic visits may also be completed at a physical therapy facility or in the clinic/hospital/facility as long as all procedures can be conducted.
b. Home visits may be completed at the subject's home, physical therapy facility, in the clinic/hospital/facility, or any other place agreed upon by the trial staff and the subject.
c. Vitals Signs include pulse, temperature, respiratory rate, systolic/diastolic blood pressure and oxygen saturation. Vital signs will be collected after the subject has been supine for 5 minutes.
d. Physical examination is conducted at the Screening Visit and Day 56 Visit. The Screening Visit will record Kellgren Lawrence Classification (to classify severity of knee osteoarthritis), varus, valgus, and evaluation of ipsilateral hip osteoarthritis.
e. ECGs will be taken in triplicate (2 minutes apart [±1 minute]) after the subject has been at rest in a supine position for 10 minutes without any other assessments being conducted. ECGs will be taken BEFORE PK sampling.
f. Chemistry/Hematology are non-fasting and will be analyzed at a central lab. Chemistry tests include sodium, potassium, creatinine, creatinine clearance albumin, ALP, total and direct bilirubin, AST, ALT, BUN, creatinine kinase, GGT, and phosphate. Hematology tests include hemoglobin, hematocrit, white blood cell count (total and differential), red blood cell count and platelet count.
g. Urine Drug Screen is performed to include but not limited to opiates (including oxycodone), amphetamines, methadone, barbiturates, benzodiazepines, cocaine, phencyclidine, methamphetamine, ecstasy/MDMA, tricyclic antidepressant, and cannabinoids at the Screening Visit and Day 15 Visit. On Day 1, the subject will be tested for opiates only by urine dipstick (Section 8.5.3).
h. The pregnancy test is only for females of child-bearing potential. The Screening Visit and Day 56 pregnancy test will be via urine.
i. Range of motion assessment will be conducted using a goniometer and will evaluate flexion and extension of the knee.
j. Neurological assessments will determine if the patient has any symptoms of LAST by asking if they have had any changes to their senses (ears/hearing, sight, touch, smell, or taste). The neurological assessments will have a gross motor and sensory exam conducted with a focus on the lower extremities. Motor examinations will focus on distal lower extremities dorsi flexion and plantar flexion of the foot (ankles and toes) against resistance. The gross sensory examination should focus on intact sensory examination to touch in the dorsal and plantar side of the foot.
k. Formal e-diary training must occur prior to surgery. Through the duration of the trial e-diary questions and concerns from the subject will be addressed and re-training will occur if needed at any time during the trial.

l. If a provisioned e-diary is used by the subject it will be dispensed on Day 1 before surgery and returned on the Day 30 Visit. The assessments conducted on the e-diary at the Screening Visit and Day 56 Visit may occur using an e-diary maintained by the site. If the subject early terminates before the Day 30 Visit the e-diary will be returned at the Early Termination Visit. If the subject's own device is used the site staff will work with the subject to have the e-diary set-up before the subject has surgery.

m. Subject pain score training will occur at the Screening Visit, on Day 1 before surgery, on Day 2, Day 3, and after the Day 15. If needed, pain score training can occur anytime during the trial that the subject has questions or as determined by the site staff. Standardized pain score training will be conducted by the site staff or through training videos.

n. Prior and concomitant medication usage is recorded from 30 days before the Screening Visit through Day 56 or Early Termination. At the Day 15 Visit, Day 30 Visit, and Early Termination Visit (if on or before the Day 30) accountability of prescribed opioids will occur.

o. The Southampton Wound Scoring System (Appendix I) will be used to evaluate the TKA surgical wound for each subject.

p. The Knee Society Score (Appendix J) will be used to evaluate the subject's knee joint and function.

q. Adverse Events are collected from the time of first investigational product administration and through the Day 56 or Early Termination Visit.

r. PK sampling is required any time a subject has an SAE or AESI. ECGs will be taken BEFORE PK sampling.

s. Subject self-administered e-diary questionnaires/surveys will be completed. NRS pain intensity scales include both at rest (NRS-R) and activity (NRS-A). Each time rescue opioid medications are taken, the NRS-R scale for pain intensity must be completed along with opioid type, dose, and time. Refer to Appendix C for further details on frequency of collection.

Part B Schedule of Events for Day of Surgery Through Hour 24 (Day 2)							
Procedure	**Day of Surgery (Day 1)**						**Day 2**
	Before Surgery	**During Surgery**	**30 Min & 1 Hour**	**3 & 6 Hour**	**9 & 12 Hour**	**18 Hour**	**24 Hour**
Window	Before Spinal	-10 min	±10 min	±30 min	±1 hour	±2 hour	±2 hour
24 Hour In-Patient Period[a]	X	X	X	X	X	X	X
Update Medical History	X						
Review Entry Criteria	X	X					
Vital Signs[b]	X			X	X	X	X
12-Lead ECG	X						
Drug Screen[c]	X						
Serum or Urine Pregnancy Test[d]	X						
Range of Motion Assessment[e]							X
Neurological Assessments[f]	X			X	X	X	X
e-diary Training[g]	X						
e-diary Dispensation/Return[h]	X						
Subject NRS Pain Score Training for Pain Intensity[i]	X						X
Concomitant Medications and Opioid Accountability[j]	X	X	X	X	X	X	X
Randomization and Investigational Product Allocation[k]	X						
Investigational Product Administration[l]		X					
Adverse Events[m]		X	X	X	X	X	X
Subject Self-Administered Questionnaires/Surveys[n]	X		X	X	X	X	X

a. Subjects will remain under observation until Hour 24.

b. Vitals Signs include pulse, temperature, respiratory rate, systolic/diastolic blood pressure and oxygen saturation. In the 24-hour period, vital signs can only be missed one time for the subject sleeping. Vital signs will be collected after the subject has been supine for 5 minutes.

c. Urine Drug Screen is performed on Day 1 before surgery for opiates only by urine dipstick (Section 8.5.3).

d. The pregnancy test is only for females of child-bearing potential. The Day 1 pregnancy test may be done via serum or urine per hospital standard of care. If females of child-bearing potential would not normally be tested on the day of surgery, then a urine pregnancy test will be conducted.

e. Range of motion assessment will be conducted using a goniometer and will evaluate flexion and extension of the knee.

f. Neurological Assessments will determine if the patient has any symptoms of LAST by asking if they have had any changes to their senses (ears/hearing, sight, touch, smell, or taste). The neurological assessments will have a gross motor and sensory exam conducted with a focus on the lower extremities. Motor examinations will focus on distal lower extremities dorsi flexion and plantar flexion of the foot (ankles and toes) against resistance. The gross sensory examination should focus on intact sensory examination to touch in the dorsal and plantar side of the foot. In the first 24-hour period, the neurological assessments can only be missed one time for the subject sleeping.

g. Formal e-diary training must occur prior to surgery. Through the duration of the trial e-diary questions and concerns from the subject will be addressed and re-training will occur if needed at any time during the trial.

h. If a provisioned e-diary is used by the subject, it will be dispensed on Day 1 before surgery and returned on the Day 30 Visit. If the subject early terminates before the Day 30 Visit, the e-diary will be returned at the Early Termination Visit. If the subject's own device is used, the site staff will work with the subject to have the e-diary set-up before the subject has surgery.

i. Subject pain score training will occur at the Screening Visit, on Day 1 before surgery, on Day 2, Day 3, and after the Day 15. If needed, pain score training can occur anytime during the trial that the subject has questions or as determined by the site staff. Standardized pain score training will be conducted by the site staff or through training videos.

j. Prior and concomitant medication usage is recorded from 30 days before the Screening Visit through Day 56 or Early Termination.

k. Randomization for allocation of investigational product may occur up to one business day before surgery.

l. ATX-101 or bupivacaine HCl administration will only occur during the surgical procedure and prior to initiating capsule closure. ATX-101 will be placed in the knee capsule following fixation of the knee implant prosthesis when tissue will not be disrupted any further by surgery, after any betadine or saline rinse and after suction has occurred.

m. Adverse Events are collected from the time of investigational product administration and through Day 56 or Early Termination.

n. Subject self-administered e-diary questionnaires/surveys will be completed. NRS pain intensity scales include both at rest (NRS-R) and activity (NRS-A). Each time rescue opioid medications are taken, the NRS-R scale for pain intensity must be completed along with opioid type, dose, and time. Refer to Appendix C for further details on frequency of collection.

APPENDIX C. SUBJECT SELF-ADMINISTERED E-DIARY PROCEDURES

Visit Day	Pain Intensity NRS-Resting (Frequency)[a] & Location of Pain	Quality of Recovery 15	KOOS, JR & Pain Manageability	Pain Intensity NRS-Activity (Frequency) & Location of Pain	Recording Rescue Opioid Medication Use & Assessment of Medication Compliance[b]	Physical Therapy Survey[c]
Screening[c]	X	X	X	X		
Day 1 Before Surgery	X					
Day 1 After Surgery	30 minutes, 1, 3, 6, 9, 12, & 18 hours after Time 0				X	
Day 2	Hour 24 and/or mid-day, & night[d]	X		X	X	X
Day 3	Three times (morning, mid-day, & night)	X		X	X	X
Day 4	Three times (morning, mid-day, & night)	X		X	X	X
Day 5	Three times (morning, mid-day, & night)	X		X	X	X
Day 6	Three times (morning, mid-day, & night)	X		X	X	X
Day 7	Three times (morning, mid-day, & night)	X		X	X	X
Day 8	Three times (morning, mid-day, & night)	X	X	X	X	X
Day 9	Three times (morning, mid-day, & night)	X		X	X	X
Day 10	Three times (morning, mid-day, & night)	X		X	X	X
Day 11	Three times (morning, mid-day, & night)	X		X	X	X
Day 12	Three times (morning, mid-day, & night)	X		X	X	X
Day 13	Three times (morning, mid-day, & night)	X		X	X	X
Day 14	Three times (morning, mid-day, & night)	X		X	X	X
Day 15	Three times (morning, mid-day, & night)	X	X	X	X	X
Day 16	X	X		X	X	X
Day 17	X	X		X	X	X
Day 18	X	X		X	X	X
Day 19	X	X		X	X	X
Day 20	X	X		X	X	X
Day 21	X	X		X	X	X
Day 22	X	X	X	X	X	X
Day 23	X	X		X	X	X
Day 24	X	X		X	X	X
Day 25	X	X		X	X	X
Day 26	X	X		X	X	X

Visit Day	Pain Intensity NRS-Resting (Frequency)[a] & Location of Pain	Quality of Recovery 15	KOOS, JR & Pain Manageability	Pain Intensity NRS-Activity (Frequency) & Location of Pain	Recording Rescue Opioid Medication Use & Assessment of Medication Compliance[b]	Physical Therapy Survey[c]
Day 27	X	X		X	X	X
Day 28	X	X		X	X	X
Day 29	X	X		X	X	X
Day 30[e]	X	X	X	X	X	X
Day 56[e,f]	X	X	X	X		

X=One time daily.
[a]For the first 24 hours after surgery, pain assessments will be conducted at 30 minutes and 1 hour (±10 min); 3 and 6 hours (±30 minutes); 9 and 12 hours (±1 hour); 18 and 24 hours (±2 hours). After the first 24 hours from Day 2 to 15 subjects will move to a morning, mid-day, and evening assessment period where the morning assessment will be conducted from 3 am until 10:59 am, the mid-day assessment will occur from 11:00 am until 4:59 pm and the night assessment from 5:00 pm until 2:59 am.
[b]Prior to consuming an opioid after surgery through the Day 30 Visit, subjects will be required to complete an NRS-R for pain intensity and indicate the opioid, dose, time, and type of opioid consumed. Each day subjects will be asked about consumption of other study medications.
[c]At the end of the day, starting on Day 1 through Day 30 a physical therapy survey will be completed including the type (eg in person, home exercises) of physical therapy completed.
[d]All subjects will skip the morning report on Day 2 as the assessments will be completed as part of the 24 Hour Visit. Depending upon the time of surgical closure on (Day 1) some subjects may not complete all assessments on Day 2 (eg the afternoon report).
[e]If a subject early terminates on or before Day 30, all Day 30 self-administered questionnaires should be completed. If a subject early terminates after Day 30 all Day 56 self-administered questionnaires should be completed.
[f]Questionnaires and surveys completed at the Screening Visit and the Day 56 Visit may utilize e-diaries maintained by the clinical site.

APPENDIX D. QUALITY OF RECOVERY-15 PATIENT SURVEY

PART A

How have you been feeling in the last 24 hours?

(0 to 10, where: 0 = none of the time [poor] and 10 = all of the time [excellent])

1.	Able to breathe easily	None of the time	0	1	2	3	4	5	6	7	8	9	10	All of the time
2.	Been able to enjoy food	None of the time	0	1	2	3	4	5	6	7	8	9	10	All of the time
3.	Feeling rested	None of the time	0	1	2	3	4	5	6	7	8	9	10	All of the time
4.	Have had a good sleep	None of the time	0	1	2	3	4	5	6	7	8	9	10	All of the time
5.	Able to look after personal toilet and hygiene unaided	None of the time	0	1	2	3	4	5	6	7	8	9	10	All of the time
6.	Able to communicate with family or friends	None of the time	0	1	2	3	4	5	6	7	8	9	10	All of the time
7.	Getting support from hospital doctors and nurses	None of the time	0	1	2	3	4	5	6	7	8	9	10	All of the time
8.	Able to return to work or usual home activities	None of the time	0	1	2	3	4	5	6	7	8	9	10	All of the time
9.	Feeling comfortable and in control	None of the time	0	1	2	3	4	5	6	7	8	9	10	All of the time
10.	Having a feeling of general well-being	None of the time	0	1	2	3	4	5	6	7	8	9	10	All of the time

PART B

Have you had any of the following in the last 24 hours?

(10 to 0, where: 10 = none of the time [excellent] and 0 = all of the time [poor])

11.	Moderate pain	None of the time	10	9	8	7	6	5	4	3	2	1	0	All of the time
12.	Severe pain	None of the time	10	9	8	7	6	5	4	3	2	1	0	All of the time
13.	Nausea or vomiting	None of the time	10	9	8	7	6	5	4	3	2	1	0	All of the time
14.	Feeling worried or anxious	None of the time	10	9	8	7	6	5	4	3	2	1	0	All of the time
15.	Feeling sad or depressed	None of the time	10	9	8	7	6	5	4	3	2	1	0	All of the time

Reference: Stark PA, Myles PS, Burke JA. Development and Psychometric Evaluation of a Postoperative Quality of Recovery Score. Anesthesiology 2013; 118(6):1332-1340 [22].

APPENDIX E. KNEE INJURY AND OSTEOARTHRITIS OUTCOME SCORE FOR JOINT REPLACEMENT

HSS

Knee injury and Osteoarthritis Outcome Score for Joint Replacement (KOOS, JR.)

English version 2.0

Instructions

This survey asks for your view about your knee. This information will help us keep track of how you feel about your knee and how well you are able to do your usual activities. Answer every question by ticking the appropriate box, only one box for each question. If you are unsure about how to answer a question, please give the best answer you can.

Stiffness

1. How severe is your knee stiffness after first wakening in the morning?

   None ☐ Mild ☐ Moderate ☐ Severe ☐ Extreme ☐

Pain

What amount of knee pain have you experienced in the **last week** during the following activities?

2. Twisting/pivoting on your knee

   None ☐ Mild ☐ Moderate ☐ Severe ☐ Extreme ☐

3. Straightening knee fully

   None ☐ Mild ☐ Moderate ☐ Severe ☐ Extreme ☐

4. Going up or down stairs

   None ☐ Mild ☐ Moderate ☐ Severe ☐ Extreme ☐

5. Standing upright

   None ☐ Mild ☐ Moderate ☐ Severe ☐ Extreme ☐

(Continue on next page for *Function, daily living*)

Function, daily living

The following questions concern your physical function. By this we mean your ability to move arounc and to look after yourself. For each of the following activities please indicate the degree of difficulty you have experienced in the last week due to your knee.

6. Rising from sitting

   None ☐ Mild ☐ Moderate ☐ Severe ☐ Extreme ☐

7. Bending to floor/pick up an object

   None ☐ Mild ☐ Moderate ☐ Severe ☐ Extreme ☐

APPENDIX F. ABILITY TO MANAGE PAIN

APPENDIX G. PATIENT HEALTH QUESTIONNAIRE 9

PATIENT HEALTH QUESTIONNAIRE-9 (PHQ-9)

Over the last 2 weeks, how often have you been bothered by any of the following problems? *(Use "✔" to indicate your answer)*	Not at all	Several days	More than half the days	Nearly every day
1. Little interest or pleasure in doing things	0	1	2	3
2. Feeling down, depressed, or hopeless	0	1	2	3
3. Trouble falling or staying asleep, or sleeping too much	0	1	2	3
4. Feeling tired or having little energy	0	1	2	3
5. Poor appetite or overeating	0	1	2	3
6. Feeling bad about yourself — or that you are a failure or have let yourself or your family down	0	1	2	3
7. Trouble concentrating on things, such as reading the newspaper or watching television	0	1	2	3
8. Moving or speaking so slowly that other people could have noticed? Or the opposite — being so fidgety or restless that you have been moving around a lot more than usual	0	1	2	3
9. Thoughts that you would be better off dead or of hurting yourself in some way	0	1	2	3

FOR OFFICE CODING 0 + _____ + _____ + _____

=Total Score: _____

If you checked off any problems, how difficult have these problems made it for you to do your work, take care of things at home, or get along with other people?

Not difficult at all	Somewhat difficult	Very difficult	Extremely difficult
☐	☐	☐	☐

Developed by Drs. Robert L. Spitzer, Janet B.W. Williams, Kurt Kroenke and colleagues, with an educational grant from Pfizer Inc. No permission required to reproduce, translate, display or distribute.

APPENDIX H. PAIN INTENSITY ASSESSMENTS USING THE NUMERIC RATING SCALE

The NRS will be used for pain intensity at both Rest (NRS-R) and with Activity (NRS-A).

0 1 2 3 4 5 6 7 8 9 10

No Pain Worst Imaginable Pain

The subject should be asked about their pain scores on a scale from 0-10. Subjects will be asked at the Screening Visit and multiple times a day from Day 1 until the Day 15 Visit for NRS-R and for all NRS-A assessments "on a scale of 0 to 10, with 0 being no pain at all and 10 being the worst pain imaginable, how would you rate your pain RIGHT NOW?".

After the Day 15 Visit subjects will be asked to indicate for NRS-R assessments "on a scale of 0 to 10, with 0 being no pain at all and 10 being the worst pain imaginable, how would you rate your USUAL (AVERAGE) pain in the last 24 hours?".

Subjects will be trained on how to record their pain scores using the information below. Standardized pain score training will be conducted by the site staff or through training videos.

Scale		**Description**
0		No Pain
Mild	1	Pain is uncomfortable, nagging and annoying
	2	
	3	
Moderate	4	Pain is bothering, troubling, interrupts concentration and you are considering or requesting medication
	5	
	6	
Severe	7	Pain is preoccupying thoughts and is distressing; you may be unable to perform simple tasks or can barely speak you may have low groaning or wincing.
	8	
	9	
	10	Pain is immobilizing; retching/crying

The NRS-R for pain intensity will also be obtained just prior to consumption of every rescue opioid medication until the Day 30 Visit. When an opioid is consumed the subject will also indicate the type, dose, and time of consumption.

Screening Visit to Day 15 Guidance Subject Pain Intensity Score Training

Subjects will be trained on trial days as specified in Appendix A and Appendix B.

Subjects will be told: In this clinical trial, you will be asked to rate your pain at different times using a number. When you rate your pain, we would like you to answer according to the pain you are experiencing in your **surgical knee** right now, not pain you might have experienced earlier in the day or at another point in time, not pain you are experiencing in another part of your body, and not pain from your surgical incision. You will score your pain from **0-10,** with 0 being no pain at all and 10 being the worst pain imaginable.

You will be asked "on a scale of 0 to 10, with 0 being no pain at all and 10 being the worst pain imaginable, how would you rate your pain RIGHT NOW?".

"0" is no pain. "1, 2, or 3" is mild pain. This means pain that is uncomfortable, nagging, and annoying. "4, 5, or 6" is moderate pain. This means pain that is bothering, troubling, interrupts your concentration, and you are considering or requesting medication. "7, 8, or 9" is severe pain. This means pain that is preoccupying your thoughts and is distressing; you may be unable to perform simple tasks or can barely speak. You may have low groaning or wincing. "10" is also severe pain. This means pain that is immobilizing, and you are retching and crying.

You will be asked to rate your pain at different times, after resting and being active.

When you are asked to rate your pain after resting, you will be asked to be in a comfortable position, either seated or lying down for at least 5 minutes before scoring your pain. You will be asked to rate your pain before surgery and multiple times a day for the first two weeks after your surgery. You will also be asked to rate your pain after resting each time you take an opioid medication. If you take an opioid you will also record the type, dose, and time that you took the opioid.

When you are asked to rate your pain after activity, you will be asked to complete a set of 8 to 10 repetitions of a knee exercise where you will sit on the edge of a chair or bed and extend your leg straight off the floor and then back down to the floor. You will be asked to rate your pain with activity before surgery and once a day for the first two weeks after your surgery.

Please ask the study staff if you have any questions regarding pain score completion.

Day 16 to Day 56 Guidance for Subject Pain Intensity Score Training

Subjects will be trained on trial days as specified in Appendix A and Appendix B.

Subjects will be told: In this clinical trial, you will be asked to rate your pain at different times using a number. When you rate your pain, we would like you to answer according to the usual or average pain you have experienced in the last 24 hours in your surgical knee. You should not consider pain you have experienced or are experiencing in another part of your body and not pain from your surgical incision. You will score your pain from 0-10, with 0 being no pain at all and 10 being the worst pain imaginable.

You will be asked "on a scale of 0 to 10, with 0 being no pain at all and 10 being the worst pain imaginable, how would you rate your USUAL (AVERAGE) pain in the last 24 hours?".

"0" is no pain. "1, 2, or 3" is mild pain. This means pain that is uncomfortable, nagging, and annoying. "4, 5, or 6" is moderate pain. This means pain that is bothering, troubling, interrupts concentration, and you are considering or requesting medication. "7, 8, or 9" is severe pain. This means pain that is preoccupying your thoughts and is distressing; you may be unable to perform simple tasks or can barely speak. You may have low groaning or wincing. "10" is also severe pain. This means pain that is immobilizing, and you are retching and crying.

You will be asked to rate your pain at different times, after resting and being active.

When you are asked to rate your pain after resting, you will be asked to be in a comfortable position, either seated or lying down for at least 5 minutes before scoring your pain. You will be asked to rate your pain one time a day for one month after your surgery. You will also be asked to rate your pain after resting each time you take an opioid medication. If you take an opioid, you will also record the type, dose, and time that you took the opioid.

When you are asked to rate your pain after activity, you will be asked to complete a set of 8 to 10 repetitions of a knee exercise where you will sit on the edge of a chair or bed and extend your leg straight off the floor and then back down to the floor. You will be asked to rate your pain with activity one time a day for one month after your surgery.

Please ask the study staff if you have any questions regarding pain score completion.

APPENDIX I. WOUND HEALING ASSESSMENT - SOUTHAMPTON WOUND SCORING SYSTEM

WOUND HEALING GRADE and APPEARANCE

Column A		Column B	
Grade the wound healing by reviewing the definitions and ticking one (1) box below. Then choose one (1) additional and most severe description experienced from column B for that Grade.		Tick the box that further describes the chosen Grade assessment from Column A (Grade 1- 4 only). If Grade 0 or 5 were chosen, then leave column B blank.	
☐	**Grade 0** = Normal Healing		
☐	**Grade 1** = Normal Healing with Mild Bruising or Erythema	☐ ☐ ☐	Some bruising Considerable bruising Mild erythema
☐	**Grade 2** = Erythema Plus Other Signs of Inflammation	☐ ☐ ☐ ☐	At one point Around sutures Along wound Around wound
☐	**Grade 3** = Clear or Haemoserous Discharge	☐ ☐ ☐ ☐	At one point only (< 2 cm) Along wound (>2 cm) Large volume Prolonged > 3 days
☐	**Grade 4** = Pus/Purulent Discharge	☐ ☐	At one point only (< 2 cm) Along wound (> 2 cm)
☐	**Grade 5** = Deep or Severe Wound Infection, With or Without Tissue Breakdown		

References:
Alam SI, Khan MY, Gul A, Jan QA. Surgical site infection. *Professional Med J*. 2014;21(2):377-38 [23].
Bailey I. Community surveillance of complications after hernia surgery. *BMJ*. 1992;304:469-471 [24].

APPENDIX J. KNEE SOCIETY SCORE

The Knee Society Score's shown below are representative samples, the official questionnaire may have modifications. The pre-operative and post-operative questionnaires are shown. Demographics information found on page 1 of the questionnaire will not be completed by the subject and surgeon assessment will not be completed. Only the questions being completed are shown below.

8099569400

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SYMPTOMS (To be completed by patient)

1- Pain with level walking (10 - Score)

0	1	2	3	4	5	6	7	8	9	10
none										severe

2- Pain with stairs or inclines (10 - Score)

0	1	2	3	4	5	6	7	8	9	10
none										severe

3- Does this knee feel "normal" to you? (5 points)

○ Always (5 pts) ○ Sometimes (3 pts) ○ Never (0 pts)

Maximum total points (25 points)

PATIENT SATISFACTION

1- Currently, how satisfied are you with the pain level of your knee while sitting? (8 points)

○ Very Satisfied (8 pts) ○ Satisfied (6 pts) ○ Neutral (4 pts) ○ Dissatisfied (2 pts) ○ Very Dissatisfied (0 pts)

2- Currently, how satisfied are you with the pain level of your knee while lying in bed? (8 points)

○ Very Satisfied (8 pts) ○ Satisfied (6 pts) ○ Neutral (4 pts) ○ Dissatisfied (2 pts) ○ Very Dissatisfied (0 pts)

3- Currently, how satisfied are you with your knee function while getting out of bed? (8 points)

○ Very Satisfied (8 pts) ○ Satisfied (6 pts) ○ Neutral (4 pts) ○ Dissatisfied (2 pts) ○ Very Dissatisfied (0 pts)

4- Currently, how satisfied are you with your knee function while performing light household duties? (8 points)

○ Very Satisfied (8 pts) ○ Satisfied (6 pts) ○ Neutral (4 pts) ○ Dissatisfied (2 pts) ○ Very Dissatisfied (0 pts)

5- Currently, how satisfied are you with your knee function while performing leisure recreational activities? (8 points)

○ Very Satisfied (8 pts) ○ Satisfied (6 pts) ○ Neutral (4 pts) ○ Dissatisfied (2 pts) ○ Very Dissatisfied (0 pts)

Maximum total points (40 points)

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PATIENT EXPECTATIONS (To be completed by patient)

What do you expect to accomplish with your knee replacement:

1- Do you expect your knee joint replacement surgery will relieve your knee pain? **(5 points)**

- O no, not at all (1 pt)
- O yes, a little bit (2 pts)
- O yes, somewhat (3 pts)
- O yes, a moderate amount (4 pts)
- O yes, a lot (5 pts)

2- Do you expect your surgery will help you carry out your normal activities of daily living? **(5 points)**

- O no, not at all (1 pt)
- O yes, a little bit (2 pts)
- O yes, somewhat (3 pts)
- O yes, a moderate amount (4 pts)
- O yes, a lot (5 pts)

3- Do you expect you surgery will help you perform leisure, recreational or sports activities? **(5 points)**

- O no, not at all (1 pt)
- O yes, a little bit (2 pts)
- O yes, somewhat (3 pts)
- O yes, a moderate amount (4 pts)
- O yes, a lot (5 pts)

Maximum total points (15 points) ☐

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FUNCTIONAL ACTIVITIES (To be completed by patient)

WALKING AND STANDING (30 points)

1 - Can you walk without any aids (such as a cane, crutches or wheelchair)? **(0 points)**

O Yes O No

2 - If no, which of the following aid(s) do you use? **(-10 points)**

O wheelchair (-10 pts) O walker (-8 pts) O crutches (-8 pts) O two canes (-6 pts)

O one crutch (-4 pts) O one cane (-4 pts) O knee sleeve / brace (-2 pts)

O other

3 - Do you use these aid(s) because of your knees? **(0 points)**

O Yes O No

4 - For how long can you stand (with or without aid) before sitting due to knee discomfort? **(15 points)**

O cannot stand (0 pts) O 0-5 minutes (3 pts) O 6-15 minutes (6 pts)

O 16-30 minutes (9 pts) O 31-60 minutes (12 pts) O more than an hour (15 pts)

5 - For how long can you walk (with or without aid) before stopping due to knee discomfort? **(15 points)**

O cannot walk (0 pts) O 0-5 minutes (3 pts) O 6-15 minutes (6 pts)

O 16-30 minutes (9 pts) O 31-60 minutes (12 pts) O more than an hour (15 pts)

Maximum points (30 points)

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STANDARD ACTIVITIES (30 points)

How much does your knee bother you during each of the following activities?	no bother 5	slight 4	moderate 3	severe 2	very severe 1	cannot do (because of knee) 0	I never do this	
1 - Walking on an uneven surface	○	○	○	○	○	○	○	
2 - Turning or pivoting on your leg	○	○	○	○	○	○	○	
3 - Climbing up or down a flight of stairs	○	○	○	○	○	○	○	
4 - Getting up from a low couch or a chair without arms	○	○	○	○	○	○	○	
5 - Getting into or out of a car	○	○	○	○	○	○	○	
6 - Moving laterally (stepping to the side)	○	○	○	○	○	○	○	

Maximum points (30 points) ☐

ADVANCED ACTIVITIES (25 points)

1 - Climbing a ladder or step stool	○	○	○	○	○	○	○	☐
2 - Carrying a shopping bag for a block	○	○	○	○	○	○	○	☐
3 - Squatting	○	○	○	○	○	○	○	☐
4 - Kneeling	○	○	○	○	○	○	○	☐
5 - Running	○	○	○	○	○	○	○	☐

Maximum points (25 points) ☐

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DISCRETIONARY KNEE ACTIVITIES (15 points)

Please check 3 of the activities below that you consider *most important* to you.

(Please do not write in additional activities)

Recreational Activities	Workout and Gym Activities
☐ Swimming	☐ Weight-lifting
☐ Golfing (18 holes)	☐ Leg Extensions
☐ Road Cycling (>30mins)	☐ Stair-Climber
☐ Gardening	☐ Stationary Biking / Spinning
☐ Bowling	☐ Leg Press
☐ Racquet Sports (Tennis, Racquetball, etc.)	☐ Jogging
☐ Distance Walking	☐ Elliptical Trainer
☐ Dancing / Ballet	☐ Aerobic Exercises
☐ Stretching Exercises (stretching out your muscles)	

Please copy all 3 checked activities into the empty boxes below.

How much does your knee bother you during each of these activities?

Activity (Please write the 3 activites from list above)	no bother 5	slight 4	moderate 3	severe 2	very severe 1	cannot do (because of knee) 0	
1.	○	○	○	○	○	○	
2.	○	○	○	○	○	○	
3.	○	○	○	○	○	○	
						Maximum points (15 points)	

Maximum total points (100 points)

9572547313

SYMPTOMS (To be completed by patient)

1- Pain with level walking (10 - Score)

0	1	2	3	4	5	6	7	8	9	10

none severe

2- Pain with stairs or inclines (10 - Score)

0	1	2	3	4	5	6	7	8	9	10

none severe

3- Does this knee feel "normal" to you? (5 points)

O Always (5 pts) O Sometimes (3 pts) O Never (0 pts)

Maximum total points (25 points)

PATIENT SATISFACTION

1- Currently, how satisfied are you with the pain level of your knee while sitting? (8 points)

O Very Satisfied (8 pts) O Satisfied (6 pts) O Neutral (4 pts) O Dissatisfied (2 pts) O Very Dissatisfied (0 pts)

2- Currently, how satisfied are you with the pain level of your knee while lying in bed? (8 points)

O Very Satisfied (8 pts) O Satisfied (6 pts) O Neutral (4 pts) O Dissatisfied (2 pts) O Very Dissatisfied (0 pts)

3- Currently, how satisfied are you with your knee function while getting out of bed? (8 points)

O Very Satisfied (8 pts) O Satisfied (6 pts) O Neutral (4 pts) O Dissatisfied (2 pts) O Very Dissatisfied (0 pts)

4- Currently, how satisfied are you with your knee function while performing light household duties? (8 points)

O Very Satisfied (8 pts) O Satisfied (6 pts) O Neutral (4 pts) O Dissatisfied (2 pts) O Very Dissatisfied (0 pts)

5- Currently, how satisfied are you with your knee function while performing leisure recreational activities? (8 points)

O Very Satisfied (8 pts) O Satisfied (6 pts) O Neutral (4 pts) O Dissatisfied (2 pts) O Very Dissatisfied (0 pts)

Maximum total points (40 points)

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PATIENT EXPECTATION (To be completed by patient)

Compared to what you expected before your knee replacement:

1- My expectations for pain relief were... **(5 points)**

- O Too High- "I'm a lot worse than I thought" (1 pt)
- O Too High- "I'm somewhat worse than I thought" (2 pts)
- O Just Right- "My expectations were met" (3 pts)
- O Too Low- "I'm somewhat better than I thought" (4 pts)
- O Too Low- "I'm a lot better than I thought" (5 pts)

2- My expectations for being able to do my normal activities of daily living were... **(5 points)**

- O Too High- "I'm a lot worse than I thought" (1 pt)
- O Too High- "I'm somewhat worse than I thought" (2 pts)
- O Just Right- "My expectations were met" (3 pts)
- O Too Low- "I'm somewhat better than I thought" (4 pts)
- O Too Low- "I'm a lot better than I thought" (5 pts)

3- My expectations for being able to do my leisure, recreational or sports activities were... **(5 points)**

- O Too High- "I'm a lot worse than I thought" (1 pt)
- O Too High- "I'm somewhat worse than I thought" (2 pts)
- O Just Right- "My expectations were met" (3 pts)
- O Too Low- "I'm somewhat better than I thought" (4 pts)
- O Too Low- "I'm a lot better than I thought" (5 pts)

Maximum total points (15 points) ☐

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FUNCTIONAL ACTIVITIES (To be completed by patient)

WALKING AND STANDING (30 points)

1 - Can you walk without any aids (such as a cane, crutches or wheelchair)? **(0 points)**

O Yes O No

2 - If no, which of the following aid(s) do you use? **(-10 points)**

O wheelchair (-10 pts) O walker (-8 pts) O crutches (-8 pts) O two canes (-6 pts)

O one crutch (-4 pts) O one cane (-4 pts) O knee sleeve / brace (-2 pts)

O other

3 - Do you use these aid(s) because of your knees? **(0 points)**

O Yes O No

4 - For how long can you stand (with or without aid) before sitting due to knee discomfort? **(15 points)**

O cannot stand (0 pts) O 0-5 minutes (3 pts) O 6-15 minutes (6 pts)

O 16-30 minutes (9 pts) O 31-60 minutes (12 pts) O more than an hour (15 pts)

5 - For how long can you walk (with or without aid) before stopping due to knee discomfort? **(15 points)**

O cannot walk (0 pts) O 0-5 minutes (3 pts) O 6-15 minutes (6 pts)

O 16-30 minutes (9 pts) O 31-60 minutes (12 pts) O more than an hour (15 pts)

Maximum points (30 points)

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STANDARD ACTIVITIES (30 points)

How much does your knee bother you during each of the following activities?	no bother 5	slight 4	moderate 3	severe 2	very severe 1	cannot do (because of knee) 0	I never do this	
1 - Walking on an uneven surface	○	○	○	○	○	○	○	
2 - Turning or pivoting on your leg	○	○	○	○	○	○	○	
3 - Climbing up or down a flight of stairs	○	○	○	○	○	○	○	
4 - Getting up from a low couch or a chair without arms	○	○	○	○	○	○	○	
5 - Getting into or out of a car	○	○	○	○	○	○	○	
6 - Moving laterally (stepping to the side)	○	○	○	○	○	○	○	

Maximum points (30 points) ☐

ADVANCED ACTIVITIES (25 points)

1 - Climbing a ladder or step stool	○	○	○	○	○	○	○	
2 - Carrying a shopping bag for a block	○	○	○	○	○	○	○	
3 - Squatting	○	○	○	○	○	○	○	
4 - Kneeling	○	○	○	○	○	○	○	
5 - Running	○	○	○	○	○	○	○	

Maximum points (25 points) ☐

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DISCRETIONARY KNEE ACTIVITIES (15 points)

Please check 3 of the activities below that you consider *most important* to you.

(Please do not write in additional activities)

Recreational Activities

- ☐ Swimming
- ☐ Golfing (18 holes)
- ☐ Road Cycling (>30mins)
- ☐ Gardening
- ☐ Bowling
- ☐ Racquet Sports (Tennis, Racquetball, etc.)
- ☐ Distance Walking
- ☐ Dancing / Ballet
- ☐ Stretching Exercises (stretching out your muscles)

Workout and Gym Activities

- ☐ Weight-lifting
- ☐ Leg Extensions
- ☐ Stair-Climber
- ☐ Stationary Biking / Spinning
- ☐ Leg Press
- ☐ Jogging
- ☐ Elliptical Trainer
- ☐ Aerobic Exercises

Please copy all 3 checked activities into the empty boxes below.

How much does your knee bother you during each of these activities?

Activity (Please write the 3 activites from list above)	no bother 5	slight 4	moderate 3	severe 2	very severe 1	cannot do (because of knee) 0	
1.	○	○	○	○	○	○	
2.	○	○	○	○	○	○	
3.	○	○	○	○	○	○	
					Maximum points (15 points)		

Maximum total points (100 points)

APPENDIX K. ADVERSE EVENT TOXICITY GRADING SCALE

Vital Signs	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Fever[a] (°C) (°F)	38.0 – 38.4 100.4 – 101.1	38.5 – 38.9 101.2 – 102.0	>39.0 >102.1
Tachycardia - beats per minute	101– 115	116 – 130	>130
Bradycardia - beats per minute (bpm)[b]	50 – 54	45 – 49	<45
Sinus Bradycardia	<50 bpm with patient aware, but no adverse symptoms	< 50 bpm with symptoms (eg lightheaded) and medication required	<50 bpm with syncope or symptomatic hypertension
Hypertension (systolic) - mm Hg[c, d]	141 – 150	151 – 155	>155
Hypertension (diastolic) - mm Hg[3c, d]	91 – 95	96 – 100	>100
Hypotension (systolic) – mm Hg	85 – 89	80 – 84	<80
Respiratory Depression	Drop in oxygen less than 90% and requiring intermittent supplemental oxygen or requiring an intervention (eg jaw lift)	Drop in oxygen less than 90% and requiring continuous (>24 hours) supplemental oxygen	Drop in oxygen less than 90% and requiring an advanced intervention (eg intubation)

[a] Temperature here is based upon oral. Non-oral measurements should be based upon clinical judgement when characterizing.

[b] When resting heart rate is not between 60-100 beats per minute, use clinical judgement

[c] When a patient has a history of elevated blood pressure or hypertension, clinical judgement should be used when characterizing a worsening of elevated blood pressure or hypertension.

[d] For individual readings of >140 or >90 clinical judgement will be used to determine if there is an adverse event.

The laboratory values provided in the tables serve as guidelines and are dependent upon central laboratory normal parameters. Central laboratory normal reference ranges will be provided.

Chemistry[a]	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Sodium – Hyponatremia mmol/L	132 – 134	130 – 131	<130
Sodium – Hypernatremia mmol/L[b]	144 – 145	146 – 147	>147
Potassium – Hyperkalemia mmol/L[b]	5.1 – 5.2	5.3 – 5.4	>5.4
Potassium – Hypokalemia mmol/L[b]	3.5 – 3.6	3.3 – 3.4	<3.3
Blood Urea Nitrogen BUN mg/dL[b]	23 – 26	27 – 31	>31
Creatinine – mg/dL[b]	1.5 - 1.7	1.8 – 2.0	>2.0
Phosphorous (Phosphate)– hypophosphatemia mg/dL	2.3 - 2.5	2.0-2.2	<2.0
Albumin – Hypoalbuminemia g/dL	2.8 – 3.1	2.5 – 2.7	<2.5
Alkaline phosphate U/L – increase by factor[b]	1.1 – 2.0 x ULN	2.1 – 3.0 x ULN	>3.0 x ULN
Aspartate Aminotransferase (ALT) U/L – increase by factor[b]	1.1 – 2.5 x ULN	2.6 – 5.0 x ULN	>5.0 x ULN

Chemistry[a]	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Alanine Aminotransferase (AST) U/L – increase by factor[b]	1.1 – 2.5 x ULN	2.6 – 5.0 x ULN	>5.0 x ULN
Total Bilirubin – when accompanied by any increase in Liver Function Test increase by factor	1.1 – 1.25 x ULN	1.26 – 1.5 x ULN	>1.5 x ULN
Total Bilirubin – when Liver Function Test is normal; increase by factor	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	>2.0 x ULN

[a] Out of range screening values should be recorded as medical history. Clinical judgement should be utilized in determining post-enrollment laboratory adverse events.

[b] See central laboratory normal reference ranges which may differ for values and gender.

Hematology[a]	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Hemoglobin - gm/dL (Female)[b]	11.0 – 12. 0	9.5 – 10.9	<9.5
Hemoglobin - gm/dL (Male)	12.5 – 13.5	10.5 – 12.4	<10.5
WBC Increase - 10^3/uL[b]	10.8 – 15.0	15.1-20.0	>20.0
WBC Decrease - 10^3/uL	2.5 – 3.5	1.5 – 2.4	<1.5
Lymphocytes Decrease - 10^3/uL[b]	7.5 – 10.0	5.0 – 7.4	<5.0
Neutrophils Decrease - 10^3/uL[b]	1.5 – 2.0	1.0 – 1.4	<1.0
Eosinophils - 10^3/uL	0.65 – 1.5	1.6 – 5.0	>5.0
Platelets Decreased - 10^3/uL[b]	125 – 140	100 – 124	<100

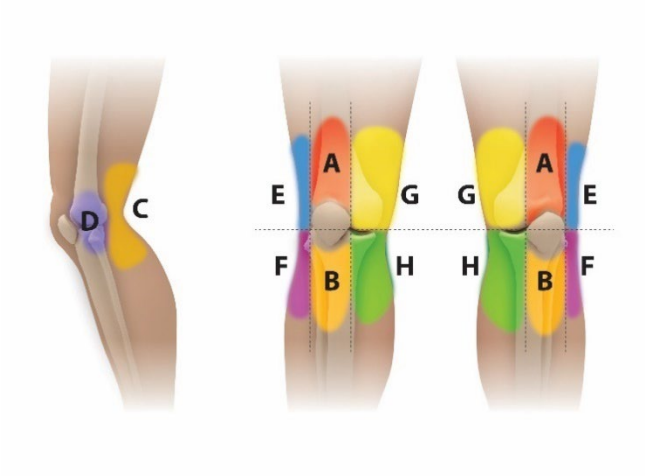
[a] Out of range screening values should be recorded as medical history. Clinical judgement should be utilized in determining post-enrollment laboratory adverse events.

[b] See central laboratory normal reference ranges which may differ for values and gender.

APPENDIX L. LOCATION OF PAIN

After subjects complete the NRS-R or NRS-A if they indicated a pain intensity score of 1 or greater, they will be asked to indicate a single location of the most intense pain. Subjects will be shown the below diagram of the knee and indicate if the pain is:

- Above my kneecap
- Below my kneecap
- Behind my knee on the back of my leg
- Deep inside of my knee (under my kneecap)
- The side of my knee (away from my body) and above the joint line
- The side of my knee (away from my body) and below the joint line
- The side of my knee (toward my other knee) and above the joint line
- The side of my knee (toward my other knee) and below the joint line



APPENDIX M. CYP3A INHIBITORS AND INDUCERS

A global list has been provided. The following medications should be avoided during the study.

Potent CYP3A Inhibitors:

- Amiodarone
- Aprepitant
- Chloramphenicol
- Cimetidine
- Ciprofloxacin
- Clarithromycin
- Cobicistat
- Cyclosporin
- Diltiazem
- Dronedarone
- Erythromycin
- Fluconazole
- Fluvoxamine
- Imatinib
- Itraconazole
- Ketoconazole
- Miconazole,
- Nefazodone
- Posaconazole
- Protease inhibitors
- Telithromycin
- Valerian
- Verapamil
- Voriconazole

Potent CYP3A Inducers:

- Anticonvulsants
- Apalutamide
- Barbiturates
- Capsaicin
- Efavirenz
- Enzalutamide
- Glucocorticoids
- Modafinil
- Nevirapine
- Phenytoin
- Pioglitazone
- Quercetin
- Rifabutin
- Rifampicin
- St. John's Wort

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