

Official Title: A Phase 2B, Randomized, Double Blind, Placebo and Active Comparator Controlled, Multicenter, Safety, and Efficacy Trial of ATX101 in Adults Undergoing Total Knee Arthroplasty (SPARK 2)

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CLINICAL PROTOCOL

Protocol No.	ATX-101-TKA-004
Title:	A Phase 2B, Randomized, Double Blind, Placebo and Active Comparator Controlled, Multicenter, Safety, and Efficacy Trial of ATX101 in Adults Undergoing Total Knee Arthroplasty
Short Title/Acronym:	Study Assessing Pain Relief After Replacement of the Knee/ SPARK 2
Version/Date:	Amendment 5 / 11 July 2025
Regulatory Agency Identifier Number(s):	IND 139725
Investigational Product:	ATX101; ATX-101 (bupivacaine) Implant
Drug Development Phase:	2B
Sponsor:	Allay Therapeutics, Inc. 2720 Zanker Road, San Jose, CA 95134
Medical Monitor:	

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PROTOCOL SIGNATURE PAGE

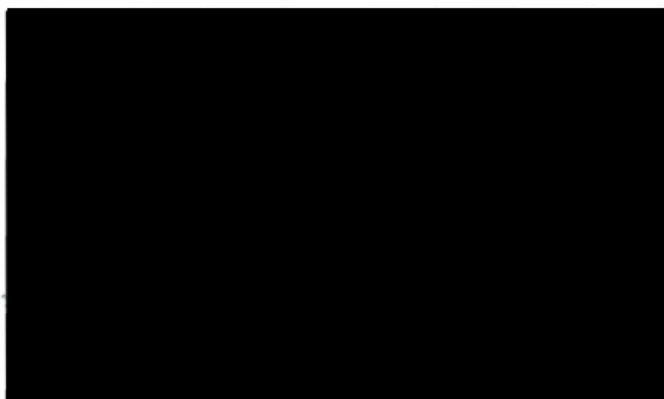
**A Phase 2B, Randomized, Double Blind, Placebo and Active Comparator Controlled,
Multicenter, Safety, and Efficacy Trial of ATX101 in Adults Undergoing Total Knee
Arthroplasty**

Protocol: ATX-101-TKA-004

Version: Amendment 5

Date: 11 July 2025

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



July 11, 2025
Date

July 11, 2025
Date

INVESTIGATOR AGREEMENT PAGE

A Phase 2B, Randomized, Double Blind, Placebo and Active Comparator Controlled, Multicenter, Safety, and Efficacy Trial of ATX101 in Adults Undergoing Total Knee Arthroplasty

Protocol: ATX-101-TKA-004

Version: Amendment 5

Date: 11 July 2025

I will provide copies of the protocol, any subsequent protocol amendments and access to all information provided by Allay to the trial personnel under my supervision. I will discuss this material with them to ensure that they are fully informed about the investigational drug and the trial protocol.

I agree to conduct this clinical trial according to the attached protocol, except when mutually agreed to in writing. I also agree to conduct this trial in compliance with all federal, state, and local regulations, as well as with the requirements of the appropriate Institutional Review Board and any other institutional requirements.

Investigator Signature

Date

Investigator Name (Print)

SUMMARY OF CHANGES

Protocol: ATX-101-TKA-004

Protocol Version: Amendment 5

Issue Date: 11 July 2025

The following table summarizes the changes made in this amendment:

Section	Change Made
1.7 Trial Population 3.0 Trial Design 4.0 Selection and Withdrawal of Participants 7.2 Participant Population(s) for Analysis 7.4 Interim Analysis	The overall sample size for the study will be increased from 200 to 240 participants in order to account for a higher rate of missing data observed in the earlier post-operative timepoints than what was expected during the planning of the study. Edits were made throughout the protocol to 240 participants will be randomized and dosed to one of the three treatment groups in a 2:2:1 ratio with approximately 96 participants each in the ATX101 1,500 mg and saline groups and 48 participants in the bupivacaine active comparator group
4.2 Exclusion	Reverted to previous exclusion parameter of Gamma-glutamyl transferase (GGT) >1.5 times the upper normal limit based on FDA's recommendation and Protocol Administrative Change Letter dated 17 Apr 2025
7.2 Participant Population(s) for Analysis	Fixed typographical error to clarify the Safety Analysis Set includes all participants who are randomized and administered ATX101 1,500 mg, saline placebo, or bupivacaine active comparator
Throughout the Protocol	Administrative edits throughout the protocol to keep consistency

TABLE OF CONTENTS

PROTOCOL SYNOPSIS	8
LIST OF ABBREVIATIONS	18
1.0 BACKGROUND	21
1.1 Investigational Agent	22
1.2 Preclinical Data	22
1.3 Clinical Data	23
1.4 Risk/Benefits	24
1.5 Dose Rationale	25
1.6 Trial Conduct	25
1.7 Trial Population	25
2.0 TRIAL OBJECTIVES.....	26
3.0 TRIAL DESIGN.....	26
3.1 Trial Endpoints.....	26
3.1.1 Primary Endpoint	26
3.1.2 Key Secondary Endpoints	26
3.1.3 Secondary Endpoints.....	26
3.1.4 Safety Endpoints	28
3.1.5 Exploratory Endpoints	28
3.2 Trial Design	28
3.2.1 Visit Procedures	28
3.2.2 Description of Procedures	38
3.3 Randomization	44
3.4 Unblinding	44
3.5 Trial Treatment	44
3.5.1 Investigational Product (ATX101).....	45
3.5.2 Placebo (Normal Saline)	46
3.5.3 Active Comparator (bupivacaine hydrochloride 0.25%)	47
3.6 Duration	47
3.7 Discontinuation	47
3.8 Stopping Rules	47
3.9 Product Accountability	48
3.10 Data Identification	48
4.0 SELECTION AND WITHDRAWAL OF PARTICIPANTS	48
4.1 Inclusion Criteria	48

4.2	Exclusion Criteria	49
4.3	Participant Withdrawal	52
5.0	TREATMENT OF PARTICIPANT	53
5.1	Concomitant Medications	53
5.1.1	Pre-Surgical Medications	53
5.1.2	Surgical Medications.....	53
5.1.3	Post-Surgical Medications	53
5.2	Prohibited Medications and Procedures.....	54
6.0	SAFETY MONITORING	55
6.1	Safety Monitoring Committee	55
6.2	Method and Timing.....	55
6.3	Definitions of Adverse Event.....	55
6.3.1	Adverse Event (AE)	55
6.3.2	Serious Adverse Event (SAE)	56
6.3.3	Suspected Unexpected Serious Adverse Reactions (SUSAR).....	57
6.3.4	Adverse Events of Special Interest (AESI)	57
6.3.5	Opioid Related Adverse Events	59
6.3.6	Relationship to Investigational Product	60
6.3.7	Relationship to the TKA Surgery	61
6.4	Adverse Event Reporting.....	62
6.5	Adverse Event Follow-up	64
6.6	Pregnancy and Postpartum Reporting.....	64
7.0	STATISTICAL PLAN.....	64
7.1	Statistical Methods.....	64
7.2	Participant Population(s) for Analysis	64
7.2.1	Primary and Key Secondary Endpoint Analysis.....	65
7.2.2	Subgroup Analysis	70
7.2.3	Sensitivity Analysis.....	70
7.2.4	Secondary Endpoints.....	71
7.2.5	Safety Endpoints	73
7.2.6	Other Safety Analysis	73
7.2.7	Exploratory Endpoints	74
7.3	Significance.....	74
7.4	Interim Analysis.....	74
7.5	Termination Criteria.....	76
7.6	Deviation Reporting.....	76

8.0	DIRECT ACCESS TO SOURCE DATA/DOCUMENTATION	76
9.0	QUALITY CONTROL AND QUALITY ASSURANCE	77
10.0	ETHICAL CONSIDERATIONS.....	78
11.0	DATA HANDLING AND RECORD KEEPING.....	79
12.0	PUBLICATION PLAN	80
13.0	APPENDICES	80
13.1	Appendix A: Schedule of Events.....	81
13.2	Appendix B: Patient Health Questionnaire 9.....	84
13.3	Appendix C: Knee Society Score	85
13.4	Appendix D: Wound Healing Assessment - Southampton Wound Scoring System.....	96
13.5	Appendix E: Pain Intensity Assessments Using the Numeric Rating Scale.....	97
13.6	Appendix F: Location of Pain.....	98
13.7	Appendix G: Sleep Quality Scale	99
13.8	Appendix H: Knee Injury and Osteoarthritis Outcome Score for Joint Replacement.....	100
13.9	Appendix I: Physical Therapy Questionnaire	101
13.10	Appendix J: Adverse Event Toxicity Grading Scale	102
13.11	Appendix K: Moderate and Strong CYP3A4 Inhibitors.....	104
14.0	REFERENCES.....	105
15.0	HISTORY OF PROTOCOL CHANGES.....	107

PROTOCOL SYNOPSIS

Protocol Title:	ATX-101-TKA-004: A Phase 2B, Randomized, Double Blind, Placebo and Active Comparator Controlled, Multicenter, Safety, and Efficacy Trial of ATX101 in Adults Undergoing Total Knee Arthroplasty
Version/Date:	Amendment 5 / 11 July 2025
Sponsor:	Allay Therapeutics, Inc
Phase of Development:	2B
Trial Treatment:	1,500 mg ATX101 (3 implants 500 mg each), saline placebo (multiple periarticular injections), and bupivacaine active comparator
Trial Objectives:	The objectives of the trial are: • To compare the efficacy of ATX101 1,500 mg with that of saline placebo and bupivacaine active comparator in participants undergoing primary unilateral total knee arthroplasty • To compare opioid consumption of participants administered ATX101 with that of participants administered saline placebo and bupivacaine active comparator • To evaluate the safety and tolerability of ATX101
Trial Endpoints:	<u>Primary Endpoint:</u> The primary efficacy endpoint is area under the curve (AUC) for the Numerical Rating Scale at Rest (NRS-R) of pain intensity from 30 minutes through 168 hours (Day 8) compared to placebo. <u>Key Secondary Endpoints:</u> • AUC for the NRS-R of pain intensity from 30 minutes through 336 hours (Day 15) compared to placebo • AUC for the NRS-R of pain intensity from 30 minutes through 504 hours (Day 22) compared to placebo • Total post-surgical consumption of opioid medications from 30 minutes through 336 hours (Day 15) compared to placebo • AUC for the NRS-R of pain intensity from 30 minutes through 336 hours (Day 15) compared to active comparator • Difference in NRS-A pain intensity scores during the Timed Up and Go (TUG) Test at Day 15 compared to placebo <u>Secondary Endpoints:</u>

	• Difference in NRS-A pain intensity scores during the TUG Test at Day 8 compared to placebo and active comparator • Difference in NRS-A pain intensity scores during the TUG Test at Day 15 compared to active comparator • Difference in NRS-A pain intensity scores during the TUG Test at Day 22 compared to placebo and active comparator • AUC for the NRS-R of pain intensity from 30 minutes through 168 hours (Day 8) compared to active comparator • AUC for the NRS-R of pain intensity from 30 minutes through 504 hours (Day 22) compared to active comparator • Total post-surgical consumption of opioid medications from 30 minutes through 168 hours (Day 8) compared to placebo and active comparator • Total post-surgical consumption of opioid medications from 30 minutes through 504 hours (Day 22) compared to placebo and active comparator • Total post-surgical consumption of opioid in morphine equivalent milligram dose from 30 minutes to Day 8, Day 9 to Day 15, Day 16 to Day 22, and Day 23 to Day 30 compared to placebo • Time to discontinuation of opioid analgesia compared to placebo and active comparator • Proportion of participants who are opioid free starting at Day 8, Day 15, Day 22, and Day 30 compared to placebo and active comparator • Proportion of participants who remain opioid free from 30 minutes through Day 8, Day 15, Day 22, and Day 30 compared to placebo and active comparator • AUC for the NRS-R of pain intensity from 168 hours (Day 8) through 336 hours (Day 15) compared to placebo and active comparator • AUC for the NRS-R of pain intensity from 336 hours (Day 15) through 504 hours (Day 22) compared to placebo and active comparator • AUC for the NRS-R of pain intensity from 30 minutes to 696 hours (Day 30) compared to placebo and active comparator
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	• Time-weighted sum of pain intensity from 30 minutes to Day 8 (time interval in the primary endpoint), Day 9 to Day 15, Day 16 to Day 22, and Day 23 to Day 30 compared to placebo • Total post-surgical consumption of ibuprofen from 30 minutes through Day 30 compared to placebo and active comparator • AUC for the NRS of Worst Pain in the last 24 hours compared to placebo and active comparator from 30 minutes to Day 8, Day 15, Day 22, and Day 30 • AUC of Sleep Quality Scale compared to placebo and active comparator • Difference in Knee Injury and Osteoarthritis Outcome Score for Joint Replacement (KOOS, JR) compared to placebo and active comparator • Difference in the Knee Society Score (KSS) compared to placebo and active comparator • Difference in percentage of participants reporting being pain free on the NRS-R of pain intensity compared to placebo and active comparator • Difference in NRS-A pain intensity scores during the TUG Test by visit compared to placebo and active comparator. • Time to 90 Degrees of Flexion compared to placebo and active comparator • Time to -5 Degrees of Extension compared to placebo and active comparator <u>Safety Endpoints:</u> • Incidence of AEs, AESIs, and SAEs compared to placebo and active comparator • Reduction in the incidence of opioid-related AEs compared to placebo and active comparator • Wound healing assessment using the Southampton Wound Scoring System compared to placebo and active comparator <u>Exploratory Endpoint:</u> • Difference in the duration of TUG Test compared to placebo and active comparator
Trial Population and Design:	This trial will enroll male and female participants aged 18 through 80, inclusive, undergoing primary unilateral TKA due to knee pain caused by osteoarthritis or post-traumatic arthritis. Approximately

	240 participants will be randomized and dosed to one of the three treatment groups in a 2:2:1 ratio with approximately 96 participants each in the ATX101 1,500 mg and saline groups and 48 participants in the bupivacaine active comparator group. The duration of the clinical trial for participants is up to approximately 15 weeks, including a 60-day screening. All participants will be screened within 60 days prior to surgery. Those who meet eligibility criteria will be randomized up to one business day prior to the day of TKA.
Safety Evaluation:	The Safety Analysis Set includes all participants who are randomized and administered investigational product. Randomization failures (participants 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. <u>Safety Monitoring Committee:</u> A Safety Monitoring Committee (SMC) will review blinded safety data after approximately 25%, 50%, and 75% of the planned enrollment has completed Day 30. The SMC will hold an immediate ad hoc meeting in the event that a trial stopping criterion is met. The SMC will be composed of 3 independent voting members with pertinent clinical expertise including an anesthesiologist, cardiologist, and orthopedic surgeon. The SMC will operate under a written, detailed SMC plan. The SMC will evaluate trial data including adverse events, cardiac information, and safety laboratory results from participants. Should data arise where further information is needed to address a safety concern, the SMC will escalate the data review to an unblinded biostatistician and an unblinded physician. <u>Stopping Rules:</u> 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 ATX101) is not identified or • Two non-fatal SAEs where a clear alternate cause (other than ATX101) 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 ATX101) 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 ATX101) is not identified or • Five wound healing AEsIs for which a clear alternate cause (other than ATX101) is not identified. In these instances, the trial blind may be broken for the SMC for the identified participants.
Efficacy Evaluation:	Based on the first Phase 2B trial (ATX-101-TKA-003 Part A) results, an effect size in the range of 0.50 to 0.55 was deemed to be plausible with 0.52 chosen for an even number of 96 participants per group. This sample size yields greater than 90% power, for the primary comparison of ATX101 vs. placebo, with and two-sided alpha of 5%. After the first 50% of participants have been treated and have either reached the Day 30 timepoint or have exited the trial, an optional interim analysis may be performed to re-estimate the sample size. The Full Analysis Set (FAS) includes all participants who are randomized and administered ATX101 1,500 mg, saline placebo, or bupivacaine active comparator. Randomization failures (participants 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 participant demographic and baseline characteristics.
Inclusion and Exclusion criteria:	<u>Inclusion Criteria:</u> To be eligible for trial participation, participants 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 participants only: Postmenopausal, surgically sterile, or willing to use acceptable means of

	contraception from the Screening Visit through the last trial visit. a. Medically acceptable methods of contraception that may be used by the participant 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 participants 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. Participant must also be able to use trial required e-diary and demonstrate completion compliance ($\geq 80\%$) during the screening period. 7. English or Spanish speaking, willing, and capable of providing written informed consent. It is recommended that participants are vaccinated for COVID-19 per local requirements. <u>Exclusion Criteria:</u> A participant is excluded 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 (e.g. bilateral TKA) at the time of surgery or a planned surgical procedure before the last trial visit. 2. Has had any previous open surgery, (e.g., ORIF for fracture, osteotomy, arthroplasty, unicompartamental knee arthroplasty, or TKA), in the trial knee at any time in the past or arthroscopic surgery within 12 months. Has had any previous surgery in the contralateral knee within 6 months prior to screening.
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	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 last trial visit (e.g. cannabidiol (CBD) oil, Kratom). Non-Steroidal Anti-Inflammatory Drugs (NSAIDS) and acetaminophen/paracetamol are permitted prior to surgery. 8. Has been administered any anesthetic including but not limited to bupivacaine, ropivacaine, or lidocaine within 5 days prior to the scheduled surgery. 9. Requires medication that will impact the metabolism, absorption, or excretion of bupivacaine. Concurrent use of moderate or strong inhibitors of CYP3A4 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 (e.g. anesthesia, IV tranexamic acid, antibiotics, and opioids such as morphine, oxycodone, and hydromorphone), also to medications taken prior to surgery and/or dispensed for home use (i.e. ibuprofen, oxycodone, acetaminophen/paracetamol and celecoxib), or to the
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	investigational product or its components (bupivacaine, poly[DL lactide-co-glycolide], and/or polysorbate). 11. Has a Body Mass Index (BMI) ≥ 45 kg/m². 12. Has laboratory values at screening that will exclude enrollment including: a. Total 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 f. Gamma-glutamyl transferase (GGT) >1.5 times the upper normal limit These labs may be repeated once. 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. The Fridericia Method will be used to calculate QTc (refer to Section 3.2.2.2.5). 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
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	has not been definitively treated for Hepatitis C (i.e., cured). 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 participant 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 or other neuropathies, 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, cerebrovascular bleeding, or hypertension (defined by triplicate average systolic >165 mmHg or diastolic >100 mmHg blood pressure measurement at Screening). 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. Medication to treat depression is allowed if the participant 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. Participants currently taking anti-psychotic medication for indications other than depression will be excluded. 19. Participant with moderately severe or severe depression, as defined by - Patient Health Questionnaire (PHQ-9) total score of ≥ 10; or - 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. In the opinion of the investigator, has a history of illicit drug use or prescription medicine or alcohol abuse (regularly drinks >4 units of alcohol per day, where 1 unit = 8 ounces beer, 3 ounces of wine, or 1 ounce of
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	spirits) within the past year (12 months) or a history of cannabis use within the last 3 months. 21. Has a positive drug screen at the Screening Visit or on the day of surgery. If drug screen is positive for opioids, cannabinoids, or benzodiazepines, participant may repeat the drug screen after appropriate washout period and again 72 hours before planned surgery. If both repeat drug screens are negative, participant would be eligible. 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 participant is not in a clinical trial for the vaccine. 23. Has participated in an ATX101 clinical trial. 24. Pregnant, breastfeeding, or planning to become pregnant during the trial or before the last trial visit. 25. Has received gabapentin or pregabalin within 2 weeks of planned surgery date. In addition, the participant will not have met eligibility if the following occurs during surgery. 26. Any event or condition uncovered during surgery (e.g. excessive bleeding, femur fracture), which occurs before investigational product administration, that might render the participant medically unstable or complicate the participant's postsurgical course. 27. [REDACTED]
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LIST OF ABBREVIATIONS

Abbreviation or Term	Definition/Explanation
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
ATX101	Allay Therapeutics Investigational Product
AUC	Area Under the Curve
AUC _{0-∞}	Area Under the Concentration-Time Curve Through Infinity
BMI	Body Mass Index
BUN	Blood Urea Nitrogen
C	Celsius
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
CRA	Clinical Research Associate
CRF	Case Report Form
CS	Clinically Significant
CYP3A4	Cytochrome P450 3A4
dL	Deciliter
DVT	Deep Vein Thrombosis
EC	Ethics Committee
ECG	Electrocardiogram
EDC	Electronic Data Capture
e-diary	Electronic Diary
F	Fahrenheit
FAS	Full Analysis Set

Abbreviation or Term	Definition/Explanation
FDA	Food and Drug Administration
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
ICF	Informed Consent Form
IRB	Institutional Review Board
IRT	Interactive Response Technology
IUD	Intrauterine Device
IV	Intravenous
KOOS, JR	Knee Injury and Osteoarthritis Outcome Score for Joint Replacement
kg	Kilogram
L	Liter
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
PHQ-9	Patient Health Questionnaire 9

Abbreviation or Term	Definition/Explanation
PK	Pharmacokinetic(s)
PS20	Polysorbate 20
QOR-15	Quality of Recovery 15 Scale
RA	Rheumatoid Arthritis
ROM	Range of Motion
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SC	Subcutaneous
SD	Standard Deviation
SMC	Safety Monitoring Committee
SOC	System Organ Class
SUSAR	Suspected Unexpected Serious Adverse Reaction
t _{1/2}	Terminal elimination phase half-life
TEAE	Treatment Emergent Adverse Events
TKA	Total Knee Arthroplasty
TUG	Timed Up and Go Test
µg	Microgram

1.0 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 nonoperative treatment options (e.g. weight loss, aerobic exercise, physical therapy, pain relievers, nonsteroidal anti-inflammatory drugs, and corticosteroid injections) have failed [3]. 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 postoperative pain that can last for days or weeks and mild to moderate pain lasting for weeks to months following surgery [3, 4]. The current postoperative 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 [5]. However, significant immediate postoperative pain and pain during recovery associated with TKA can compromise the rehabilitation process and adversely affect outcomes and extends recovery times [6]. 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. Preoperatively, several options are available, including epidural and spinal anesthetics with adjuncts such as long-acting morphine and/or peripheral nerve blocks. Intraoperative injections of bupivacaine (e.g., MARCAINE) or other local anesthetics may be administered at the surgical site. Postoperatively, peripheral nerve blocks, and multimodal 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 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 (e.g., manipulation under anesthesia), surgeons will typically prescribe several weeks of oral opioids with instructions to use prior to the onset of any breakthrough or worsening of pain.

Although prescription opioids have made pain treatable, medical use of opioids can lead to significant side effects including 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 postoperative period following TKA for OA [7]. Prevention of opioid addiction has become an urgent public health emergency [8]. Of several highly invasive surgeries studied, it was determined that TKA

patients are at the highest risk of chronic opioid use [9]. Moreover, generous prescriptions written following TKA put significant quantities of opioids into circulation. More effective local pain management after TKA will decrease or eliminate the need for opioids.

1.1 Investigational Agent

ATX101 is an extended-release bupivacaine bioabsorbable implant intended to provide sustained postsurgical analgesia for several weeks 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 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.

ATX101 contains the drug bupivacaine, a poly(DL-lactide-co-glycolide) polymer, and polysorbate 20. The dose for each implant is 500 milligrams (mg) bupivacaine (equivalent to 594 mg bupivacaine hydrochloride monohydrate or 563 mg bupivacaine hydrochloride). Multiple ATX101 implants will be utilized to obtain the one-time total dose to be used in this clinical trial. ATX101 is 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. ATX101 will be placed by the orthopedic surgeon in predetermined locations under direct visualization. It is anticipated that sustained delivery of bupivacaine from ATX101 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 local anesthetic systemic toxicity.

1.2 Preclinical Data

The pharmacology, pharmacokinetics, and toxicology of bupivacaine has been extensively characterized by intravenous administration to support approval of MARCAINE. 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. In humans, the half-life is 2.7 hours after IV administration. Bupivacaine is extensively metabolized, primarily by CYP3A4 in the liver. The major metabolite in humans is desbutylbupivacaine (pipercoloxylidide), while the major route of excretion in humans is in urine.

Allay has conducted two pivotal GLP single dose toxicity studies in rabbits and sheep and supportive non-GLP implantation studies in minipigs and sheep to determine the pharmacokinetics of ATX101. The PK/TK results for these studies demonstrated safe systemic absorption and sustained delivery of bupivacaine for several weeks from ATX101 for different routes of administration at doses up to 7,140 mg bupivacaine HCl monohydrate (equivalent to 6,000 mg bupivacaine free base). In a GLP 16-week single dose toxicity study in rats, sustained bupivacaine delivery of high doses of

bioabsorbable implant was generally well tolerated and demonstrated the systemic safety of bupivacaine exposure for extended durations.

For additional information on the nonclinical studies of ATX101, refer to the Investigator's Brochure. For information on the active pharmaceutical ingredient bupivacaine also refer to the local product labels.

1.3 Clinical Data

Trial FT1-TKA-001. ATX101 has been evaluated in a completed, Phase 1/2A open-label ascending dose, pharmacokinetic (PK), safety, and tolerability clinical trial, Protocol FT1-TKA-001, conducted in Australia. Total doses of 252 mg, 756 mg, and 1,512 mg of bupivacaine were administered in a dose ascending manner to 4, 3, and 15 participants, respectively. Participants in the FT1-TKA-001 clinical trial were monitored for safety and tolerability through 60 days after the TKA. Doses of ATX101 up to 1,512 mg were well-tolerated with no attributable SAEs, no attributable signs of local anesthetic systemic toxicity (LAST), and all systemic PK bupivacaine concentrations were within an acceptable range. There was a dose response across the three doses of ATX101 with respect to the use of opioid analgesics and pharmacokinetics concentration. The most common AEs in the trial were nausea 12 (54.5%), constipation 6 (27.3%), haematoma 6 (27.3%), dizziness 5 (22.7%), diarrhoea 4 (18.2%), vomiting 4 (18.2%), and peripheral oedema 4 (18.2%). The majority of AEs were mild or moderate in severity. There were 3 severe AEs (syncope, urticaria, and drug [acetaminophen/paracetamol] induced liver injury. The FT1-TKA-001 trial results and the nonclinical GLP toxicity studies support the safety of the investigational ATX101 doses of 1,000 and 1,500 mg.

Trial ATX-101-TKA-003, was a randomized, double blind, 2-part, placebo and active comparator, multicenter trial to evaluate efficacy and safety of ATX101 implants placed in the knee capsule following a TKA. Part A was an exploratory trial to inform the trial design for the Part B adequate and well controlled trial. This ATX-101-TKA-004 Phase 2B protocol replaces Part B of the previous Protocol ATX-101-TKA-003 and is intended to be an adequate and well controlled trial to support future marketing application for ATX101.

Part A of the Phase 2B trial was an exploratory trial of 1,000 mg and 1,500 mg intended to provide data to select the dose of ATX101 to be used in Part B; the timing of the primary endpoint for Part B; and the population size needed to ensure that Part B would be an adequate and well controlled clinical trial. In addition, the trial was intended to provide further information on safety and tolerability and further characterize the PK of ATX101. Part A was conducted in Australia, Canada, and the United Kingdom. Based on this interim analysis, Part A was completed with 112 participants (37 in ATX101 1,000 mg group, 37 in ATX101 1,500 mg group, 34 in 0.25% bupivacaine group, and 4 in saline placebo group). One participant in the ATX101 1,500 mg group discontinued early per physician's decision at Day 30 due to patient burden associated with participation in the trial. In trial ATX-101-TKA-003 there were no deaths.

Part A demographics were similar across treatment groups. The trial enrolled more females (56.3%, 63/112) than males (43.8%, 49/112). The participants that were enrolled had an average age (mean, SD) of 67.8 (7.69) BMI of 31.42 (4.93), and 91.1% were white/Caucasian (102/112), 0.9% American Indian or Alaskan Native, (1/112), 0% Native Hawaiian or other Pacific Islander (0/112), 1.8% Black or African American (2/112) 3.6% Asian (4/112) and 2.7% other 3/112).

There was a total of 87 (77.7%) AEs with fewer occurring in the 1,500 mg dose 25 (67.6%) versus the bupivacaine 28 (82.4%), ATX101 1,000 mg 30 (81.1%) or placebo groups 4 (100%). The most common AEs (>5 in any treatment group) were nausea, constipation, procedural pain, contusion, dizziness, joint swelling, arthralgia, vomiting, diarrhea, implant site swelling, pain in extremity, fatigue, muscle spasm, and peripheral swelling. There was 1 (0.9%) participant with an AESI in the ATX101 group with tinnitus which was considered mild in severity and unlikely related to IP or TKA.

Of the 112 participants 9 (8.0%) participants experienced an SAE. This includes 3 participants in the 1,000 mg, 4 participants in 1,500 mg, 2 participants in bupivacaine group, and 0 participants in the placebo group. SAEs included 2 incidences of pulmonary embolism, and 1 incident each duodenal ulcer, atrial fibrillation, incision site discharge/worsening of leaky wound, hepatic enzyme increase, liver injury, diarrhea, hemarthrosis, and delirium.

There have been no suspected unexpected serious adverse reactions (SUSARs) reported for ATX101 in the above clinical trials. Refer to the Investigator's Brochure for additional information.

1.4 Risk/Benefits

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, ATX101 is surgically placed and cannot be injected like other formulations of bupivacaine. Moreover, the release of bupivacaine from ATX101 is gradual and sustained over time with minimal systemic concentrations throughout the duration of the drug release.

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 [10] Local anesthetic systemic toxicity symptoms that should be carefully monitored for include: tongue and perioral numbness, tinnitus, muscle fasciculations, tremors, seizures, apnea (not sleep apnea), sinus bradycardia, atrioventricular blocks, conduction defects (prolonged PR or prolonged QRS), ventricular dysrhythmias, cardiac arrest, and asystole. Refer to the Investigator's Brochure and local bupivacaine product label for additional information.

Other potential risks from administration of ATX101 may include wound healing and infection. Use of ATX101 is contraindicated in participants with hypersensitivity to bupivacaine or participants that required medication that will impact the metabolism, absorption, or excretion of bupivacaine. Concurrent use of moderate and strong inhibitors of CYP3A4 is also contraindicated.

This trial is designed to evaluate the safety and potential benefits such as postoperative pain analgesia for up to 30 days after surgery, improved recovery, and function.

1.5 Dose Rationale

The Phase 2B trial ATX-101-TKA-003 Part A administered ATX101 at 1,000 mg and at 1,500 mg in 37 participants per dose group, 74 participants administered ATX101 in total. Based on the efficacy, safety, and PK results of Part A, the 1,500 mg dose was selected for the current protocol. The pain intensity over time curve showed that the 1,500 mg dose group separates from ATX101 1,000 mg, bupivacaine, and placebo dose groups in the setting of a small sample size and multimodal therapy. Based on the NRS-R AUC the 1,500 mg dose demonstrated similar efficacy to bupivacaine over the first 24 hours and trend toward superiority over control groups from 24 hours to Day 8 ($p=0.1225$), Day 15 ($p=0.0645$), Day 22 ($p=0.0825$), and Day 30 ($p=0.0889$). The 1,500 mg dose also showed a trend for using less opioid versus the control group for opioid consumption at 15 days with $p = 0.0788$. The PK profile is also supportive with an exposure over 2 weeks greater than that of the 1,000 mg dose. Importantly, the 1,500 mg C_{max} is below levels of serum bupivacaine associated with the development of symptoms of local anesthetic systemic toxicity (LAST) and the adverse effect profile supports the overall safety and tolerability of the 1,500 mg dose. Based upon safety and tolerability in ATX-101-TKA-003 Part A trial, this clinical trial will evaluate ATX101 at 1,500 mg dose.

1.6 Trial Conduct

This trial will be conducted in compliance with the protocol approved by the applicable Institutional Review Boards (IRB)/Ethics Committees (EC), and according to the principles of International Conference of Harmonisation (ICH), Good Clinical Practice (GCP), Declaration of Helsinki, and all applicable national and local regulations governing clinical studies.

1.7 Trial Population

This trial will enroll male and female participants aged 18 through 80, inclusive, undergoing primary unilateral TKA due to knee pain caused by osteoarthritis or post-traumatic arthritis. Approximately 240 participants will be randomized and dosed to one of the three treatment groups. Participants will be randomized in a 2:2:1 ratio to either ATX101 1,500 mg (three ATX101 500 mg implants, 96 participants), placebo (normal saline periarticular infiltration, 96 participants) or active comparator (bupivacaine hydrochloride 0.25% periarticular infiltration, 48 participants).

2.0 TRIAL OBJECTIVES

The objectives of the trial are:

- To compare the efficacy of ATX101 1,500 mg with that of saline placebo and bupivacaine active comparator in participants undergoing primary unilateral total knee arthroplasty
- To compare opioid consumption of participants administered ATX101 with that of participants administered saline placebo and bupivacaine active comparator.
- To evaluate the safety and tolerability of ATX101

3.0 TRIAL DESIGN

This is a Phase 2B, randomized, double-blind, placebo and active comparator controlled, multicenter trial in participants undergoing primary unilateral TKA.

3.1 Trial Endpoints

3.1.1 Primary Endpoint

The primary efficacy endpoint is area under the curve (AUC) for the Numerical Rating Scale at Rest (NRS-R) of pain intensity from 30 minutes through 168 hours (Day 8) compared to placebo.

3.1.2 Key Secondary Endpoints

The key secondary efficacy endpoints include the following:

- AUC for the NRS-R of pain intensity from 30 minutes through 336 hours (Day 15) compared to placebo
- AUC for the NRS-R of pain intensity from 30 minutes through 504 hours (Day 22) compared to placebo
- Total post-surgical consumption of opioid medications from 30 minutes through 336 hours (Day 15) compared to placebo
- AUC for the NRS-R of pain intensity from 30 minutes through 336 hours (Day 15) compared to active comparator
- Difference in NRS-A pain intensity scores during the Timed Up and Go (TUG) Test at Day 15 compared to placebo

3.1.3 Secondary Endpoints

The secondary efficacy endpoints include the following:

- Difference in NRS-A pain intensity scores during the TUG Test at Day 8 compared to placebo and active comparator
- Difference in NRS-A pain intensity scores during the TUG Test at Day 15 compared to active comparator

- Difference in NRS-A pain intensity scores during the TUG Test at Day 22 compared to placebo and active comparator
- AUC for the NRS-R of pain intensity from 30 minutes through 168 hours (Day 8) compared to active comparator
- AUC for the NRS-R of pain intensity from 30 minutes through 504 hours (Day 22) compared to active comparator
- Total post-surgical consumption of opioid medications from 30 minutes through 168 hours (Day 8) compared to placebo and active comparator
- Total post-surgical consumption of opioid medications from 30 minutes through 504 hours (Day 22) compared to placebo and active comparator
- Total post-surgical consumption of opioid in morphine equivalent milligram dose from 30 minutes to Day 8, Day 9 to Day 15, Day 16 to Day 22, and Day 23 to Day 30 compared to placebo
- Time to discontinuation of opioid analgesia compared to placebo and active comparator
- Proportion of participants who are opioid free starting at Day 8, Day 15, Day 22, and Day 30 compared to placebo and active comparator
- Proportion of participants who remain opioid free from 30 minutes through Day 8, Day 15, Day 22, and Day 30 compared to placebo and active comparator
- AUC for the NRS-R of pain intensity from 168 hours (Day 8) through 336 hours (Day 15) compared to placebo and active comparator
- AUC for the NRS-R of pain intensity from 336 hours (Day 15) through 504 hours (Day 22) compared to placebo and active comparator
- AUC for the NRS-R of pain intensity from 30 minutes to 696 hours (Day 30) compared to placebo and active comparator
- Time-weighted sum of pain intensity from 30 minutes to Day 8 (time interval in the primary endpoint), Day 9 to Day 15, Day 16 to Day 22, and Day 23 to Day 30 compared to placebo
- Total post-surgical consumption of ibuprofen from 30 minutes through Day 30 compared to placebo and active comparator
- AUC for the NRS of Worst Pain in the last 24 hours compared to placebo and active comparator from 30 minutes to Day 8, Day 15, Day 22, and Day 30. AUC of Sleep Quality Scale compared to placebo and active comparator
- Difference in Knee Injury and Osteoarthritis Outcome Score for Joint Replacement (KOOS, JR) compared to placebo and active comparator
- Difference in the Knee Society Score (KSS) compared to placebo and active comparator

- Difference in percentage of participants reporting being pain free on the NRS-R of pain intensity compared to placebo and active comparator
- Difference in NRS-A pain intensity scores during the TUG Test by visit compared to placebo and active comparator compared to placebo and active comparator
- Time to 90 Degrees of Flexion compared to placebo and active comparator
- Time to -5 Degrees of Extension compared to placebo and active comparator

3.1.4 Safety Endpoints

The safety endpoints include the following:

- Incidence of AEs, AESIs, and SAEs compared to placebo and active comparator
- Reduction in the incidence of opioid-related AEs compared to placebo and active comparator
- Wound healing assessment using the Southampton Wound Scoring System compared to placebo and active comparator

3.1.5 Exploratory Endpoints

The exploratory efficacy endpoint is the difference in the duration of TUG test compared to placebo and active comparator

3.2 Trial Design

The duration of the clinical trial for participants is up to approximately 15 weeks, including a 60-day screening period. All participants will be screened within 60 days prior to surgery. Those who meet eligibility criteria will be randomized up to one business day prior to the day of TKA.

3.2.1 Visit Procedures

Before trial entry and throughout the trial, various clinical and diagnostic laboratory evaluations are performed. The purpose of obtaining these detailed measurements is to ensure adequate assessment of safety and tolerability. Clinical evaluations and laboratory assessments may be repeated more frequently if clinically indicated. The Schedule of Events are provided in [Appendix A](#).

3.2.1.1 Screening Visit ($\leq$ 60 Days Before Surgery)

Participants will visit the hospital/clinic up to 60 days prior to the scheduled TKA procedure. After the informed consent form (ICF) has been reviewed and signed by the participant, the following assessments will be conducted:

- Demographic data collection
- Medical history review

- Prior (medications 30 days before the screening) and concomitant medication review
- Entry criteria review. If the participant has not been previously examined by the trial surgeon, an examination will be performed by the trial surgeon to confirm eligibility for TKA procedure. Diagnostic X-rays may be performed if unable to obtain recent historical X-rays.
- Physical examination
- Vital signs
- Height and weight
- Triplicate 12-lead electrocardiogram (ECG)
- Laboratory testing (Chemistry, Hematology, Coagulation profile, Drug Screen, and Pregnancy Test for females of childbearing potential)
- Range of Motion (ROM) assessment
- Neurological assessments
- Electronic diary (e-diary) training
- E-diary device dispensed if the participant is not using their own device
- Numeric Rating Scale (NRS) pain score training
- TUG test with duration and Pain NRS-A with location of pain

During the screening visit, the site staff will train the participant on how to use the e-diary to complete the self-administered questionnaires/surveys. Participants will complete at least 5 days of assessments using the e-diary during the screening period for training and compliance purposes.

Participants self-administered questionnaires to complete during clinic visit:

- Patient Health Questionnaire 9 (PHQ-9)
- Knee Society Score
- KOOS, JR

Participants self-administered questionnaires to complete at home:

- NRS with location of pain (three times a day)
- Worst Pain Over Last 24 Hours
- Sleep Quality Scale

Confirmation of eligibility based upon e-diary completion and laboratory data must be confirmed prior to surgery. Participants must have an $\geq 80\%$ compliance rate with e-diary completion during

the 5 days of entries during the Screening Period to remain eligible. Compliance <80% will result in a screen failure.

Participants who fail to meet eligibility criteria during screening (any time before randomization) will be considered screen failures. Participants who fail to meet eligibility from the time of randomization through surgery will be considered randomization failures.

3.2.1.2 Day 1 (Day of Surgery)

All procedures in the Schedule of Events ([Appendix A](#)) will be followed.

Before Surgery:

On the day of surgery, before surgery and before bupivacaine spinal is administered, the site staff will conduct the following assessments:

- Medical history review
- Prior and concomitant medication review
- Entry criteria review
- Vital signs
- Directed physical exam
- Triplicate 12-lead electrocardiogram (ECG)
- Laboratory testing (Chemistry, Hematology, Drug Screen and Pregnancy Test for females of childbearing potential)
- Neurological assessments
- E-diary training
- NRS pain score training for pain intensity
- TUG test with duration and Pain NRS-A with location of pain
- Participant completes the e-diary: NRS-R with location of pain
- Randomization and investigational product allocation

Randomization and investigational product allocation may occur up to one business day before surgery. Randomization will be computer generated using an electronic IRT system. Participants will be randomized to either ATX101 1,500 mg, saline placebo, or bupivacaine active comparator.

All needed investigational product will be brought to the operating room. It is recommended that spare investigational products (up to 3 additional implants) 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 Clinical Research Associate (CRA).

Pre-Surgical medications:

After checking in for surgery on Day 1, the participant will receive acetaminophen/paracetamol 1,000 mg and celecoxib 200 mg before surgery. Spinal anesthesia will be limited up to bupivacaine 15 mg intrathecal (with or without dextrose); no other adjunct medication may be used in the spinal anesthesia. See [Section 5.1.1](#) Pre-Surgical Medications for medications allowed.

During Surgery:

Participants will undergo primary unilateral TKA surgical procedure completed under spinal anesthesia.

The surgical staff will not be blinded to the trial treatment, all other site staff (unless expressly specified in the Allay pre-approved site blinding plan), and the participant will remain blinded throughout the trial.

Required: Cemented prostheses are required for all participants. [REDACTED]. See [Section 5.1.2](#) Surgical Concomitant Medications for medications allowed.

Not Permitted: Surgical drains are not permitted. Analgesic mixtures/cocktails are not allowed. See [Section 5.2](#) for Prohibited Medications and Procedures.

Participants will not be administered the investigational product if any event or condition is uncovered during surgery (e.g. excessive bleeding, femur fracture), that might render the participant medically unstable or complicate the participant's postsurgical course.

If randomized to ATX101, a one-time dose of ATX101 1,500 mg, corresponding to 3 implants (500 mg each) will be placed in the participant'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. ATX101 will be placed in the suprapatellar pouch, medial gutter, and lateral gutter (1,500 mg dose) by a trained unblinded surgeon according to the Pharmacy Manual.

If randomized to placebo, normal saline will be administered by a trained unblinded surgeon during surgery by infiltration; additional anesthetics or medications (e.g. cocktail) are not allowed. Instructions on the administration of saline are detailed in [Section 3.5.2](#) and the Pharmacy Manual.

If randomized to active comparator, bupivacaine hydrochloride 0.25% (125 mg in 50 mL) will be administered by a trained unblinded surgeon during surgery by infiltration; additional

anesthetics or medications (e.g. cocktail) are not allowed. Instructions on the administration of bupivacaine hydrochloride 0.25% are detailed in [Section 3.5.3](#) and the Pharmacy Manual.

The site staff will conduct the following assessments during surgery:

- Concomitant medication review
- Recording of AEs

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

After Surgery:

Following surgery, participants will be transferred to the post anesthesia care unit. The site staff will conduct the following assessments after surgery:

- Vital signs (three times per day during inpatient period)
- Neurological assessments
- Concomitant medication review
- Recording of AEs

After surgical closure, the participant will complete the self-administered e-diary questionnaires/surveys during the inpatient period in the hospital/clinic which will include:

- NRS-R with location of pain:
For the first 24 hours after surgery/Time 0, pain assessments will be completed at 30 minutes and 1 hour (+10 min); 3 and 6 hours (+30 minutes); 9, 12, 18, and 24 hours (+1 hour)
- Rescue pain/opioid medication questions (any time before ibuprofen or opioid is consumed)
- Worst pain of last 24 hours
- Sleep Quality Scale
- Physical therapy survey
- LAST-related Symptoms Survey

Participants should only receive rescue pain medication (ibuprofen or opioid medication) upon request for pain control, as needed, after surgery (as defined in [Section 5.1.3.1](#) Rescue Pain Medications).

Rescue pain medication may be given/taken for pain treatment, but not for pain prophylaxis. Prior to the administration of any rescue pain medication from surgery through the Day 30 Visit, participants will complete an NRS-R in the e-diary assessing the participant's pain intensity and location of pain.

Participants will be prescribed Deep vein thrombosis (DVT) prophylaxis as per standard of care.

Participants will remain under observation for a minimum of 72 hours and then may be discharged per standard of care. Should the participant experience an SAE or AESI, refer to [Section 6.3](#) for required assessments.

3.2.1.3 Day 2, Day 3, Day 4 (Inpatient Period)

All procedures in the Schedule of Events ([Appendix A](#)) will be followed. Participants will remain under observation for a minimum of 72 hours after surgery and then may be discharged per standard of care. Trial procedures and assessments during the inpatient period that will be performed are listed below.

Site staff should review and confirm the participant has been regularly completing the self-administered e-diary questionnaires and surveys. The site staff should conduct re-training to ensure compliance with medication consumption, rescue pain/opioid medication, and e-diary completion.

The site staff will conduct the following assessments on Day 2 through Day 4:

- Vital signs (three times per day during inpatient period around mealtimes)
- Laboratory testing (Chemistry and Hematology)
- Neurological assessments (approximately the same time every day)
- E-diary review and training
- NRS pain score training for pain intensity
- TUG test with duration and NRS-A with location of pain
- Range of Motion assessment (Day 4 only)
- Wound healing assessment (Day 4 only)
- Concomitant medication review
- Recording of AEs
- Trial required co-medication (rescue pain medication) needed for home use post-surgery will be prescribed.
- Physical therapy with therapist (Day 2 and Day 3)
- Prior to discharge an at home physical therapy regimen will be prescribed to the participant per standard of care.

- Prior to discharge, participants will be provided with a wallet card stating that, “This subject is participating in a clinical research trial and may have been given an extended-release anesthetic (bupivacaine) for post-TKA that is delivered over multiple weeks from the date of TKA surgery. If this subject is experiencing symptoms of LAST (Local anesthetic system toxicity) treat per standard of care and contact the trial doctor listed on the reverse side of the card.” Information is also provided on the card with the investigator contact information (business hours and after hours).

The participant will continue to complete the self-administered e-diary questionnaires/surveys during the inpatient period in the hospital/clinic and at home after discharge in-between visits:

- NRS-R with location of pain:
After 24 hours, pain assessment will be completed every 6 hours through 72 hours (± 2 hours). At home after discharge, pain assessment will be completed three times a day.
- Rescue pain/opioid medication questions (any time before ibuprofen or opioid is consumed)
- Worst pain of last 24 hours
- Sleep Quality Scale
- Physical therapy survey
- LAST-related Symptoms Survey

3.2.1.4 Day 8 Visit

All procedures in the Schedule of Events ([Appendix A](#)) will be followed. Before and during the visit, site staff should confirm the participant has been regularly completing the e-diary. The site staff should conduct re-training to ensure compliance with medication consumption, rescue pain/opioid medication, and e-diary completion and prescribed physical therapy regimens as needed.

The following assessments will be conducted on Day 8 (± 1 day):

- Vital signs
- Laboratory testing (Chemistry and Hematology)
- Neurological assessments
- E-diary review and training
- NRS pain score training for pain intensity
- TUG test with duration and NRS-A with location of pain
- ROM assessment
- Wound healing assessment
- Concomitant medication review

- Rescue medications review
- Recording of AEs
- Trial required co-medication (rescue pain medication) needed for home use post-surgery will be prescribed.
- Physical Therapy with therapist conducted once between Day 4 and Day 8 inclusive

The participant will continue to complete the self-administered questionnaires/surveys on the trial visit day and at home in-between visits:

- Knee Society Score (complete during hospital/clinic visit)
- KOOS, JR (complete during hospital/clinic visit)
- NRS-R with location of pain (three times a day)
- Rescue pain/opioid medication questions (any time before ibuprofen or opioid is consumed)
- Worst pain of last 24 hours
- Sleep Quality Scale
- Physical therapy survey
- LAST-related Symptoms Survey

3.2.1.5 Day 15, Day 22 and Day 30 Visit

All procedures in the Schedule of Events ([Appendix A](#)) will be followed. Before and during the visit, site staff should confirm the participant has been regularly completing the e-diary. The site staff should conduct re-training to ensure compliance with medication consumption, rescue pain/opioid medication, and e-diary completion and prescribed physical therapy regimens as needed.

The following assessments will be conducted on Day 15, Day 22 and Day 30 (± 3 days):

- Vital signs
- Laboratory testing (Chemistry and Hematology)
- Neurological assessments
- E-diary review and training
- NRS pain score training for pain intensity
- TUG test with duration and Pain NRS-A with location of pain
- ROM assessment
- Wound healing assessment
- Concomitant medication review

- Rescue medications review
- Recording of AEs
- Trial required co-medication (rescue pain medication) needed for home use post-surgery will be prescribed.
- Physical therapy with therapist conducted twice per week until the end of trial/Day 45 Visit.

The participant will continue to complete the self-administered e-diary questionnaires/surveys on the trial visit day and at home in-between visits:

- Knee Society Score (complete during hospital/clinic visit)
- KOOS, JR (complete during hospital/clinic visit)
- NRS-R with location of pain (three times a day)
- Rescue pain/opioid medication questions (any time before ibuprofen or opioid is consumed)
- Worst pain of last 24 hours
- Sleep Quality Scale
- Physical therapy survey
- LAST-related Symptoms Survey

Participants will stop completing the daily e-diary after Day 30 and return to clinic for the last visit on Day 45. If a participant is using a provisioned e-diary device, the site staff will instruct the participant to return the e-diary to the site at the Day 45 Visit.

3.2.1.6 Day 45 Visit (End of Trial)

All procedures in the Schedule of Events ([Appendix A](#)) will be followed. The following assessments will be conducted on Day 45 (± 7 days):

- Vital signs
- Laboratory testing (Chemistry and Hematology)
- Weight
- Physical examination
- Neurological assessments
- TUG test with duration and NRS-A with location of pain
- ROM assessment
- Wound healing assessment
- Concomitant medication review
- Rescue medications review

- Recording of AEs

The participants will complete the self-administered questionnaires/surveys during the visit:

- Knee Society Score
- KOOS, JR

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

3.2.1.7 Early Termination Visit

Participants who withdraw from the trial prior to the Day 45 visit will be asked to complete all end of trial procedures (See [Appendix A](#)). If the participant withdraws on or before Day 30 Visit, rescue medications review should occur.

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

3.2.1.8 Unscheduled Visit

In the event of an SAE or AESI after the participant has been discharged (after 72 hours), the participant will be asked to return to the hospital/clinic for an Unscheduled Visit for further evaluation. For further information regarding treatment of SAEs and AESIs see [Section 6.3](#). If an Unscheduled Visit is needed at a minimum the following procedures should be performed:

- Vital signs
- Laboratory testing (Chemistry and Hematology)
- Triplicate ECGs
- Neurological assessments
- Concomitant medication review
- Recording of AEs
- Any other procedure listed at the Screening or End of Trial Visit as necessary to support reason for Unscheduled Visit.
- PK sampling is required for all Unscheduled Visits that are due to an AESI or LAST-related symptom. PK sampling may be obtained at the Investigator's discretion at Unscheduled Visits occurring for other reasons.

3.2.2 Description of Procedures

3.2.2.1 Screening Procedures

3.2.2.1.1 Informed Consent

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

AEs collection will start from the time of signed informed consent until end of trial participation or at the last trial visit.

3.2.2.1.2 Demographic/Medical History/Prior Medications

The participant's demographic information will be collected at the Screening Visit. The participant's medical history will be collected at the Screening Visit and confirmed/updated prior to surgery on Day 1. Prior and concomitant medications will be collected throughout the trial from 30 days prior to the Screening Visit until end of trial participation or at the last trial visit.

3.2.2.1.3 Patient Health Questionnaire 9

The PHQ-9 will be conducted at the Screening Visit. The PHQ-9 questionnaire assesses depression.

If the participant 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 participant will not be eligible for the trial.

If the participant exhibits signs of moderately severe or severe depression, they should be informed of their result. The site staff should recommend the best course of action for the participant (e.g. counseling, referral to a specialist, hospitalization etc.) and document the outcome and advice in the participant records. See [Appendix B](#).

3.2.2.2 Safety Procedures

3.2.2.2.1 Laboratory Assessments

All laboratory samples are collected non-fasting. The Investigator will determine if any out-of-range laboratory values are clinically significant and record the diagnosis as an AE. All clinically significant out-of-range laboratory values that emerge after treatment will be followed until resolution. See [Appendix J](#) for more information.

3.2.2.2.1.1 Hematology

At all scheduled visits and each day while an inpatient, the following hematologic assessments will be performed: hemoglobin, hematocrit, white blood cell count (total and differential), red blood cell count and platelet count. Safety lab testing is also performed at all Unscheduled Visits

that are due to an AESI or LAST-related symptom and for any Unscheduled Visit for which the Investigator deems lab testing needed.

3.2.2.2.1.2 Chemistry

At all scheduled visits and each day while an inpatient, 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. Safety lab testing is also performed at all Unscheduled Visits that are due to an AESI or LAST-related symptom and for any Unscheduled Visit for which the Investigator deems lab testing needed.

Ibuprofen will be discontinued if an elevation of LFTs is greater than 2.5 times the upper limit of normal or at the discretion of the Investigator.

3.2.2.2.1.3 Pharmacokinetic Sampling

A PK sample is required for bupivacaine monitoring for an AESI or LAST-related Symptom.

If LAST-related Symptom is reported before discharge from inpatient setting, PK sample will be collected every day until discharge and at all regularly scheduled visits.

If LAST-related Symptom is reported after discharge, PK sample will be promptly collected (Unscheduled or scheduled visit) and additional PK samples collected at all regularly scheduled visits following the event.

Collection and handling of PK samples is described in the Laboratory Manual.

3.2.2.2.1.4 Drug Screen

At the Screening Visit a drug screen will be performed for opioids (including oxycodone), amphetamines, methadone, barbiturates, benzodiazepines, cocaine, phencyclidine, methamphetamine, ecstasy (3,4-Methylenedioxymethamphetamine or MDMA), and cannabinoids. If a participant has a positive drug screen for opioids, cannabinoids, or benzodiazepines, the participant may repeat the drug screen after appropriate washout period and again 72 hours before planned surgery. If both repeat drug screens are negative, the participant would be eligible. A drug screen will also be performed on Day 1 before surgery.

3.2.2.2.1.5 Pregnancy Test

A pregnancy test will occur at the Screening Visit for females of childbearing potential. A urine or serum pregnancy test will occur on Day 1 before surgery per standard of care at the clinical site. Pregnant females identified at the Screening Visit or on the day of surgery will not be eligible for the trial.

Female participants 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).

3.2.2.2.1.6 Coagulation Profile Test

At the Screening Visit coagulation profile testing will be performed for prothrombin time (PT), international normalized ratio (INR), and partial thromboplastin time (PTT).

3.2.2.2.2 Vital Signs

The following vital signs will be measured after the participant has been supine for 5 minutes: pulse, temperature, respiratory rate, systolic/diastolic blood pressure, and oxygen saturation. Vital signs are done three times daily (morning, mid-day, and night around mealtimes) during the hospitalization/inpatient period from Day 1 through Day 4. On Day 4, vital signs may not include all three timepoints depending on the time of discharge.

3.2.2.2.3 Physical Examination and Height/Weight

A complete physical exam will be performed at the Screening Visit. An abbreviated/directed physical exam will be performed to include an assessment of participant's symptoms as well as auscultation of heart and lungs on Day 1 and at the last trial visit on Day 45 or Early Termination. The participant's weight will be measured during the physical exam at the Screening Visit and at the Day 45/ET visit. The participant's height will be measured only at the Screening Visit.

3.2.2.2.4 Neurological Assessments

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. Neurological assessments are once daily (approximately the same time every day) during the hospitalization/inpatient period from Day 1 through Day 4. On Day 4, neurological assessments should be conducted prior to discharge.

The participant 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.

3.2.2.2.5 Electrocardiogram (ECG)

Triplicate ECGs will be taken 2 minutes apart (± 1 minute) for each nominal timepoint. Participants will be at rest in a supine position for 5 minutes before the ECGs are obtained.

The corrected QT interval (QTc) will be calculated using the Fridericia Method to determine trial eligibility:

$$QTc = QT / RR^{1/3}$$

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

3.2.2.2.6 Wound Healing Assessment

The wound healing assessment will be the Southampton Wound Scoring System (See [Appendix D](#)). Any score of Grade 2 or greater from the Southampton Wound Scoring System will be recorded as an AE. The Investigator must report these events as “surgical wound” preceded by the description (e.g. surgical wound purulent discharge).

3.2.2.3 Efficacy Procedures

3.2.2.3.1 Range of Motion (ROM) Assessment

Range of motion will be conducted using a goniometer to measure flexion and extension for the participant per Allay’s provided ROM training.

3.2.2.3.2 Timed up and go (TUG) Test and NRS-A with Location of Pain

For the Timed Up and Go Test participants will be timed (stopwatch) on getting up from a chair, walking 10 feet, turn around, walk back to their chair, and sit down. The NRS-A with location will be completed immediately after the TUG Test in which they will then be asked about the amount of pain they had with this activity and the location of pain.

The NRS with activity (NRS-A) for pain intensity is an 11-item scale from 0 to 10 where participants rank the level of pain intensity after an activity. The NRS scale goes from No Pain (0) to Worst Imaginable Pain (10). See [Appendix E](#) and [Appendix F](#).

3.2.2.3.3 E-diary Training/Dispensation/Return

A provisioned e-diary device may be dispensed to the participant during the Screening Visit and will be returned at the Day 45 Visit or Early Termination Visit, whichever is first. The participant will have an option to use his/her own device if desired. If the participant’s own device is used, the site staff will work with the participant to have the e-diary set-up at the Screening Visit.

The participant will be trained on the use of the e-diary at the Screening Visit and on Day 1 before surgery. Participants will complete at least 5 days of assessments starting the day of Screening Visit for training and compliance purposes. If a participant is less than 80% compliant, then the participant will be a screen failure.

Throughout the trial, e-diary questions and concerns from the participants will be addressed. Retraining will occur at every visit and if needed at any time during the trial. The site staff should contact the participant if he/she has not been completing the e-diary at home.

3.2.2.3.4 Participant Pain Score Training

Standardized NRS-R with location pain score training will be conducted by the site staff or through training videos.

3.2.2.3.5 Participant Self-Administered Questionnaires/Surveys

Participants will complete 5 days of assessments during the Screening period for training and compliance purposes. Participants will complete the e-diary before the spinal and then post-surgical and at multiple timepoints on Day 1 through Day 30 and in the clinic at Day 45 Visit. A participant may choose to use a provisioned device or their own device to complete the e-diary questionnaires/survey.

3.2.2.3.5.1 Knee Society Score

The Knee Society Score assesses knee joint and knee function. See [Appendix C](#).

3.2.2.3.5.2 Numeric Rating Scale at Rest (NRS-R) for Pain Intensity with Location of Pain

The NRS-R for pain intensity is an 11-item scale from 0 to 10 where participants rank the level of pain intensity. The NRS scale goes from No Pain (0) to Worst Imaginable Pain (10). If the participant 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 E](#) and [Appendix F](#).

Participants should wait one hour from completion of ice/cold therapy to record their daily pain intensity scores. Prior to obtaining the NRS-R it is recommended participants be in a comfortable resting position (either seated or lying down) for at least 5 minutes prior to obtaining the pain intensity score.

Participants will complete the NRS-R with location of pain questionnaire in the e-diary three times daily for 5 days during the Screening period for training and compliance purposes.

For the first 24 hours after surgery/Time 0, pain assessments will be completed at 30 minutes and 1 hour (+10 min); 3 and 6 hours (+30 minutes); 9, 12, 18, 24 hours (+1 hour). After 24 hours, pain assessment will be completed every 6 hours through 72 hours (± 2 hours). On Day 4 through Day 30, participants will move to a morning, mid-day, and evening and as needed prior to taking any rescue medications (ibuprofen or opioid) for pain.

3.2.2.3.5.3 Recording Rescue Pain Medication Consumption and Compliance

Participants will complete self-administered questionnaires/surveys in the e-diary to track ibuprofen and opioid consumptions and compliance. Prior to the administration of any rescue pain medication from surgery through the Day 30 Visit, participants will complete an NRS-R in the e-diary assessing the participant's pain intensity and location of pain. Participants should complete the NRS-R with location of pain prior to taking any rescue medications at any time.

3.2.2.3.5.4 Numeric Rating Scale for Worst Pain over Last 24 hours with Location of Pain

The NRS for Worst Pain in over the last 24 hours is an 11-item scale from 0 to 10 where participants rank their most severe/worst level of pain intensity over the last 24 hours. The NRS scale goes from No Pain (0) to Worst Imaginable Pain (10). If the participant 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 E](#) and [Appendix F](#).

3.2.2.3.5.5 Physical Therapy

Physical therapy with a therapist will be conducted on Day 2 and Day 3 during the inpatient period. Thereafter, physical therapy with a therapist will be conducted twice per week until the end of trial/Day 45 Visit. Physical therapy with a therapist may be conducted more frequently at the discretion of the investigator per standard of care but is not a requirement of this protocol. Prior to discharge an at home physical therapy regimen will be prescribed to the participant per standard of care. Participants should be instructed to follow a PT regimen that is tailored to prevent injury in the postoperative period. Caution should be exercised so that participants do not push themselves too hard during the recovery period. Participants will complete questions related to physical therapy after TKA in the e-diary. Participants should be trained to record any type of exercise on their own or with a physical therapist as a form of physical therapy. See [Appendix I](#).

3.2.2.3.5.6 LAST-related Symptoms Survey

Starting on Day 1 after surgery through Day 30, participants will complete a daily questionnaire asking if they have experienced any LAST-related symptoms. Prior to discharge, participants must be provided with detailed education regarding symptoms concerning LAST, and instructions to immediately contact the site staff should they develop any potential LAST-related symptoms. To ensure adequate monitoring for LAST, should any potential LAST-related symptom occur when a participant is completing the daily e-diary, the e-diary will alert the participant to call the site staff. If a participant does not complete the daily e-diary questionnaire for two consecutive days, the site staff will be alerted to call the participant.

Refer to [Section 6.3.4.1](#) for details on LAST and LAST-related symptom management.

3.2.2.3.5.7 Knee Injury and Osteoarthritis Outcome Scale for Joint Replacement

The KOOS, JR questionnaire is used to assess the participant'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. The questionnaire will be completed in the e-diary during the clinic visits. See [Appendix H](#).

3.2.2.3.5.8 Sleep Quality

Participants will be asked in the morning upon awakening to rate their quality of sleep using the Sleep Quality Scale in the e-diary. See [Appendix G](#) for the Sleep Quality Scale.

3.3 Randomization

A randomization schedule will be computer generated using an IRT web-based system. Participants will be randomized in a 2:2:1 ratio to a one-time dose of ATX101 1,500 mg, saline placebo, or bupivacaine active comparator, administered during surgery. Participants who are deemed randomization failures (participants who are randomized but do not receive investigational product), will not be replaced.

3.4 Unblinding

The Investigator should consult with the Medical Monitor if possible, prior to any unblinding. In the event of a medical emergency when knowledge of the actual treatment becomes medically necessary to affect treatment options, the Investigator will be able to obtain details of the treatment assigned to a participant using the Code Break Module in the IRT system. The Medical Monitor is to be informed immediately that the blind has been broken. Instructions for the unblinding process are detailed in the IRT's Site Manual.

The surgical staff and pharmacist dispensing assigned treatment 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 participant will remain blinded throughout the trial. Allay will remain blinded during the treatment phase of trial unless individual participants need to be unblinded due to safety concerns. Trained and pre-identified monitors will be unblinded throughout the trial to ensure compliance with trial protocols and investigational product accountability. Blinded monitors will be monitoring all other blinded data at the site.

Participants and blinded staff will need to refrain from reviewing any post-surgical X-ray or X-ray report to prevent unblinding.

3.5 Trial Treatment

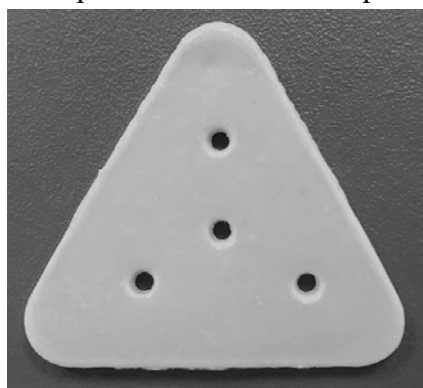
The trial includes a one-time dose of ATX101 1,500 mg, saline placebo, or bupivacaine active comparator, administered during surgery.

3.5.1 Investigational Product (ATX101)

Participants that are randomized to ATX101 group will receive a single-dose administration of 1,500 mg of ATX101, corresponding to 3 implants (500 mg each). ATX101 will be placed under direct visualization 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, but prior to initiation of surgical closure. ATX101 will be placed in the suprapatellar pouch, medial gutter, and lateral gutter according to the Pharmacy Manual.

ATX101 is a bioabsorbable implant, containing a poly (DL-lactide-co-glycolide) polymer, and polysorbate 20 (PS20). The dosage form for ATX101 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. Each ATX101 implant contains 500 mg bupivacaine (free base equivalent; equal to 563 mg bupivacaine HCl or 594 mg bupivacaine HCl monohydrate).

Example of the ATX101 Implant:



3.5.1.1 ATX101 Packaging, Labeling, and Storage

Each ATX101 implant will be provided [REDACTED]

[REDACTED] The foil pouch will be provided in an outer box. ATX101 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 ATX101 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.

3.5.1.2 ATX101 Safety Handling and Disposal

Although exposure of healthcare workers to bupivacaine from ATX101 should be minimal, care should be taken to avoid unnecessary contact with ATX101. Proper surgical and sterile techniques should be used when handling ATX101.

Allay will provide instructions regarding the destruction or return of all investigational product in the Pharmacy Manual.

If ATX101 has not been used but is not able to be placed in a participant (e.g. 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.

3.5.2 Placebo (Normal Saline)

Participants that are randomized to placebo group will be administered normal saline (0.9%) sodium chloride via local infiltration using a moving-needle technique during surgery. The saline placebo will be administered at a total volume of up to 17 mL via multiple periarticular injections. Injection of normal saline should follow the surgeon's usual technique for injection of local anesthetic with intent to equally distribute placebo into the various regions. The following guide for injection can be utilized.

Before placement of the prosthesis:

- Posterior capsule: One half of the volume (8 mL) with approximately 4-6 injection points.

After the placement of the prosthesis:

- Capsular regions and periosteum of the femur and tibia both medially and laterally: 4 mL injected 2 mL per side.
- Peripatellar area including all anterior joint capsular tissues: 2 mL
- Patellofemoral capsule: 2 mL
- Deep tissues on either side of the skin incision prior to surgical wound closure: 0.8 mL Avoid administration into the shallow subdermal areas.

3.5.3 Active Comparator (bupivacaine hydrochloride 0.25%)

Participants that are randomized to the active control group will be administered bupivacaine hydrochloride 0.25% without epinephrine/adrenaline at a dose of 125 mg in 50 mL via local periarticular infiltration. Surgeons will allocate the 50 mL of bupivacaine to the following regions per their standard practice.

The following guide can be used for infiltration of bupivacaine.

Location before placement of the prosthesis:

- Posterior capsule

Locations after the placement of the prosthesis:

- Capsular regions and periosteum of the femur and tibia both medially and laterally
- Peripatellar area including all anterior joint capsular tissues
- Patellofemoral capsule
- Deep tissues on either side of the skin incision prior to surgical wound closure:
- Avoid administration into the shallow subdermal areas.

3.6 Duration

Participants will participate in the trial for approximately 15 weeks, including a 60-day screening period. All participants will be screened within 60 days prior to surgery. Participants who meet eligibility criteria will be randomized up to one business day prior to the day of TKA.

3.7 Discontinuation

Allay reserves the right to terminate the trial at any time. 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. Allay and the Investigator will assure that adequate consideration is given to the protection of the participants' interests.

3.8 Stopping Rules

The Safety Monitoring Committee (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 ATX101) is not identified or
- Two non-fatal SAEs where a clear alternate cause (other than ATX101) 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 ATX101) 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 ATX101) is not identified or
- Five wound healing AESIs for which a clear alternate cause (other than ATX101) is not identified.

In these instances, the trial blind may be broken for the SMC for the identified participants.

3.9 Product Accountability

Complete receipt, inventory, accountability, reconciliation, and return records will be maintained for all used and unused investigational products. Documentation will include lot numbers, dates and quantity of shipment, dates and participant identification of dispensation, reconciliation, returns, and destruction. Further information on IP accountability is detailed in the Pharmacy Manual.

3.10 Data Identification

The Investigator is required to initiate and maintain an adequate and accurate case history for each participant, capturing all clinical trial observations and other data. The Investigator or his/her qualified designee will enter all applicable data from the source documents into the appropriate portions of the Case Report Form (CRF). All information recorded on the CRFs for this clinical trial must be consistent with the participant's source documentation.

An electronic data capture (EDC) system will be used for entry of the clinical trial data into the CRFs. 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. Entry and changes to the data will be made only by authorized users, and data entries and changes will be captured in an electronic audit trail.

4.0 SELECTION AND WITHDRAWAL OF PARTICIPANTS

Approximately 240 participants will be randomized and dosed to one of the three treatment groups in a 2:2:1 ratio with approximately 96 participants each in the ATX101 1,500 mg and saline placebo groups and 48 participants in the bupivacaine active comparator group. Eligible participants will be randomized prior to TKA.

4.1 Inclusion Criteria

To be eligible for trial participation, participants 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 participants only: Postmenopausal, surgically sterile, or willing to use acceptable means of contraception from the Screening Visit through the last trial visit.
 - a. Medically acceptable methods of contraception that may be used by the participant 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 participants 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. Participant must also be able to use trial required e-diary and demonstrate completion compliance ($\geq 80\%$) during the screening period.
7. English or Spanish speaking, willing, and capable of providing written informed consent.

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

4.2 Exclusion Criteria

A participant is excluded 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 (e.g. bilateral TKA) at the time of surgery or a planned surgical procedure before the last trial visit.
2. Has had any previous open surgery (e.g., ORIF for fracture, osteotomy, arthroplasty, unicompartmental knee arthroplasty, or TKA), in the trial knee at any time in the past or arthroscopic surgery within 12 months. Has had any previous surgery 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 last trial visit (e.g. cannabidiol (CBD) oil, Kratom). Non-Steroidal Anti-Inflammatory Drugs (NSAIDS) and acetaminophen/paracetamol are permitted prior to surgery.
8. Has been administered any anesthetic including but not limited to bupivacaine, ropivacaine, or lidocaine within 5 days prior to the scheduled surgery.
9. Requires medication that will impact the metabolism, absorption, or excretion of bupivacaine. Concurrent use of moderate or strong inhibitors of CYP3A4 is prohibited (See [Appendix K](#)).
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 (e.g. anesthesia, IV tranexamic acid, antibiotics, and opioids such as morphine, oxycodone, and hydromorphone), also to medications taken prior to surgery and/or dispensed for home use (i.e. ibuprofen, oxycodone, acetaminophen/paracetamol and celecoxib), or to the investigational product or its components (bupivacaine, poly[DL lactide-co-glycolide], and/or polysorbate).
11. Has a Body Mass Index (BMI) ≥ 45 kg/m².
12. Has laboratory values at screening that will exclude enrollment including:
 - a. Total 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
- f. Gamma-glutamyl transferase (GGT) >1.5 times the upper normal limit

These labs may be repeated once.

- 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. The Fridericia Method will be used to calculate QTc (refer to [Section 3.2.2.2.5](#)).
- 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 has not been definitively treated for Hepatitis C (i.e., cured).
- 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 participant 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 or other neuropathies, 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, cerebrovascular bleeding, or hypertension (defined by triplicate average systolic >165 mmHg or diastolic >100 mmHg blood pressure measurement at Screening).
- 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. Medication to treat depression is allowed if the participant 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. Participants currently taking anti-psychotic medication for indications other than depression will be excluded.
- 19. Participant 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. In the opinion of the investigator, has a history of illicit drug use or prescription medicine or alcohol abuse (regularly drinks >4 units of alcohol per day, where 1 unit = 8 ounces beer, 3 ounces of wine, or 1 ounce of spirits) within the past year (12 months) or a history of cannabis use within the last 3 months.
21. Has a positive drug screen at the Screening Visit or on the day of surgery. If drug screen is positive for opioids, cannabinoids, or benzodiazepines, participant may repeat the drug screen after appropriate washout period and again 72 hours before planned surgery. If both repeat drug screens are negative, participant would be eligible.
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 participant is not in a clinical trial for the vaccine.
23. Has participated in an ATX101 clinical trial.
24. Pregnant, breastfeeding, or planning to become pregnant during the trial or before the last trial visit.
25. Has received gabapentin or pregabalin within 2 weeks of planned surgery date.

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

26. Any event or condition uncovered during surgery (e.g. excessive bleeding, femur fracture), which occurs before investigational product administration, that might render the participant medically unstable or complicate the participant’s postsurgical course.
27. [REDACTED]

4.3 Participant Withdrawal

Participants 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 participant’s withdrawal from the clinical trial must be recorded in the participant’s records, which should be completed as soon as possible after the participant’s withdrawal is determined. Any participant 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). Refer to [Section 6.4](#) and [Section 6.5](#) for AE/SAE reporting and follow up guidance.

Participants are free to withdraw from the trial at any time and for any reason and will document their understanding by signing the ICF.

5.0 TREATMENT OF PARTICIPANT

Prior and concomitant medication usage will be recorded from 30 days before the Screening Visit through the last trial visit. Prohibited medications and procedures are listed in [Section 5.2](#).

5.1 Concomitant Medications

5.1.1 Pre-Surgical Medications

Before surgery, all participants will be administered acetaminophen/paracetamol 1,000 mg and celecoxib 200 mg [1]. Spinal anesthesia will be limited up to bupivacaine 15 mg intrathecal (with or without dextrose); no other adjunct medication may be used in the spinal anesthesia. General anesthesia is optional. If spinal anesthesia fails or cannot be performed, participant may receive general anesthesia only. Topical anesthetics such as lidocaine can be used to aid in intravenous (IV) access and subcutaneously for spinal needle placement. Antiemetics can be used at the discretion of the investigator.

5.1.2 Surgical Medications

The use of fentanyl (no more than 300 µg) for airway management is permitted when general anesthesia is used. Other than fentanyl, no other opioids may be administered during surgery. Two doses of IV tranexamic acid (1 g each) will be administered to the participant. Tranexamic acid will be administered in accordance with the standard of care at the site. Intravenous or oral antibiotic regimen may also be given per the center's standard of care.

5.1.3 Post-Surgical Medications

5.1.3.1 Rescue Pain Medications

Rescue pain medication may be given for pain treatment, but not for pain prophylaxis.

Rescue pain medication after surgery during inpatient stay will include ibuprofen (600 mg) as needed not more than every 6 hours and a short acting opioid analgesic. The preferred opioid analgesic is oral oxycodone.

If pain relief is insufficient or is not well tolerated, a subsequent medication may be provided if in the physician's judgement it is safe to do so. Elapsed time should be considered.

The following stepwise approach is suggested but investigator has the discretion to start with IV rescue initially and then move to oral therapy prior to discharge.

1. The following short acting opioid analgesic will be allowed in this trial: oral immediate-release oxycodone (5 mg or 10 mg) as needed not more than every 4 hours.
2. If a participant cannot tolerate oral oxycodone or pain is not adequately managed, then oral hydromorphone (2 mg-4 mg) will be used not more often than every 4 hours.
3. If oral opioid analgesia is not tolerated or pain is not adequately managed, then IV or IM/SC opioid as needed may be used. Morphine IV is preferred; however, hydromorphone IV or fentanyl IV can also be used with the dose determined by the treating physician. The use of IV PCA pumps is discouraged.
4. If a participant cannot tolerate either IV, IM/SC, or oral medication or the participant continues to have uncontrolled pain, then the hospital standard of care may be utilized. The pain management decision in this case should be discussed with the Medical Monitor within 24 hours.

Rescue medication following discharge will include only two rescue pain medications: ibuprofen and a short acting opioid medication. Participants will be instructed to take the rescue medication as needed for the acute treatment of pain and not for the prevention (prophylaxis) of pain. Participants will be instructed to take ibuprofen (600 mg) as needed but not more than every 6 hours. They will also be able to take immediate-release oxycodone (5 mg or 10 mg) as needed not more than every 4 hours. If during hospitalization the participant cannot tolerate oral oxycodone, they will be discharged on oral hydromorphone (2 mg-4 mg) not more often than every 4 hours.

If a participant cannot tolerate ibuprofen or oxycodone or hydromorphone or continues to have uncontrolled pain, the participant will contact the clinical site for further evaluation by the investigator and consideration will be given to an alternative analgesic regimen. The pain management decision in this case should be discussed with the Medical Monitor within 24 hours.

5.1.3.2 Antiemetics

The use of antiemetics as needed is per standard of care.

5.1.3.3 DVT Prophylaxis

Deep vein thrombosis prophylaxis is required per standard of care.

5.2 Prohibited Medications and Procedures

The following are medications and procedures are prohibited during the trial:

- Surgical drains during surgery
- Local nerve blocks (e.g. adductor canal blocks)

- Antibiotic powders or beads in the knee joint
- Analgesic mixtures (cocktails)
- Long-lasting opioids
- Combination opioids analgesics with acetaminophen or NSAID
- Moderate and strong CYP3A4 inhibitors (See [Appendix K](#))

6.0 SAFETY MONITORING

6.1 Safety Monitoring Committee

A Safety Monitoring Committee (SMC) will review blinded safety data after approximately 25%, 50%, and 75% of the planned enrollment has completed Day 30. The SMC will hold an immediate ad hoc meeting in the event that a trial stopping criterion is met. The SMC will be composed of 3 independent voting members with pertinent clinical expertise including, an anesthesiologist, cardiologist, and orthopedic surgeon. The SMC will operate under a written, detailed SMC plan.

The SMC will evaluate trial data including adverse events, cardiac information, and safety laboratory results from participants. Should data arise where further information is needed to address a safety concern, the SMC will escalate the data review to an unblinded biostatistician and an unblinded physician.

6.2 Method and Timing

Timely, accurate, and complete reporting and analysis of safety information from clinical studies are crucial for the protection of participants, investigators, and the sponsor, and are mandated by regulatory agencies. Allay has established processes in conformity with regulatory requirements to ensure appropriate reporting of safety information; all clinical trials conducted by Allay or its affiliates will be conducted in accordance with those procedures. The investigator is responsible for ensuring that all AEs and SAEs that are observed during the trial are reported and recorded according to the sections below.

6.3 Definitions of Adverse Event

6.3.1 Adverse Event (AE)

An AE is any untoward medical occurrence in a patient or clinical investigation participant administered to 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.

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 (e.g. 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 (e.g. 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 J](#).

6.3.2 Serious Adverse Event (SAE)

A serious adverse event (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 (i.e., the AE placed the participant 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 participant must have had an overnight stay in the hospital. If the participant 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 participant 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 (i.e., scheduled prior to trial enrollment or during the course of the trial) scheduled treatments and routine check-ups.

- Disability: The AE was disabling (i.e., the AE resulted in a significant, persistent, or permanent change, impairment, damage, or disruption in the participant's body function/structure, physical activities, or quality of life).
- Congenital anomaly: The AE was a congenital anomaly/birth defect (i.e., an adverse outcome in a child or fetus of a participant 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 participant or participant 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.

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

6.3.3 Suspected Unexpected Serious Adverse Reactions (SUSAR)

Allay 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 (i.e. the nature or severity is not expected from the information provided) by Allay.

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

6.3.4 Adverse Events of Special Interest (AESI)

Adverse Events of Special Interest (AESI) including LAST-related symptoms will also be collected in this trial and include the following:

Central Nervous System Symptoms/Events

- Tongue and perioral numbness
- Metallic taste

- Dysarthria
- Tinnitus
- Muscle fasciculations
- Tremors
- Seizures
- Global CNS depression
- Apnea (not sleep apnea)
- Severe dizziness
- Severe drowsiness
- Severe change in mental status

Cardiovascular Symptoms/Events

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

Investigations

- Elevated liver enzymes (Grade 3 per [Appendix J](#))

Renal and Urinary Disorders

- Moderate chronic urinary retention

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 an AESI be present after the participant has been discharged from the hospital/clinic, the participant should call the site staff.

Should severe cardiotoxic or neurotoxic events of LAST occur and standard methods of treatment (e.g. oxygen, medication) fail, a lipid emulsion should be available in the event the hospital/clinic staff assess it is in the best interest of the participant.

6.3.4.1 Daily Collection of LAST-related Symptoms in E-diary and Management of LAST-related Symptoms

Participants will be instructed to go to the Emergency Room or a medical facility or alternatively to promptly come to the investigational site for an Unscheduled Visit for evaluation of potential LAST-related symptoms for immediate work-up, treatment, and PK sample.

Participants will complete a daily questionnaire asking about the following LAST-related symptoms in the e-diary.

- Severe dizziness
- Severe drowsiness
- Tongue and perioral numbness
- Metallic taste
- Tinnitus
- Dysarthria
- Severe change in mental status
- Any significant Cardiovascular symptoms/events

To ensure adequate monitoring for LAST, should any potential LAST-related symptom occur when a participant is completing the daily e-diary, the e-diary will alert the participant to call the site staff and it will also alert the site staff to follow up with the participant. If a participant does not complete the daily e-diary questionnaire for two consecutive days, the site staff will be alerted to call the participant.

6.3.5 Opioid Related Adverse Events

- Constipation
- Nausea
- Vomiting
- Urinary retention
- Pruritis

- Delirium
- Hallucinations
- Sedation
- Respiratory depression
- Paralytic Ileus

6.3.6 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.
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.

6.3.7 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.

Relationship	Assessment Criteria	Description
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 participant is taking opioids due to the TKA surgical procedure and the participant 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.

6.4 Adverse Event Reporting

The Investigator must comply with applicable requirements concerning reporting of AEs/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.

The Investigator shall report all directly observed adverse events (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 participant a non-specific question (e.g., “Have you noticed anything different since your last visit?”) to assess whether any AEs have been experienced since the last report or visit.

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.

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 signed informed consent until the end of trial participation or at the last trial 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 participant; does not influence daily activities; usually does not require intervention.
- Moderate: The event may be of sufficient severity to make the participant uncomfortable, performance of daily activities may be influenced; intervention may be needed.
- Severe: The event may cause severe discomfort; usually interferes with daily activities; participant 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.

Allay is under obligation to report certain serious adverse events (SAEs) to regulatory authorities related to investigational products in clinical trials. Allay's representative must be notified when the Investigator determines that an adverse event meets the definition of an SAE. The SAE must be reported within 24 hours of first learning of the event to [REDACTED]

Once an SAE form has been submitted, the site staff will receive a confirmation sent via email by [REDACTED]. The confirmation email should be filed in the trial binder with the Pregnancy form. If the email was not successfully transmitted, and a second attempt is unsuccessful, contact the CRA.

After receiving the SAE report, Allay's representative may request additional information from the Investigator to ensure the timely completion of accurate safety reports.

6.5 Adverse Event Follow-up

For each participant, AEs will be collected from the time of the investigational product administration through the last trial visit. Adverse events will be followed until resolution of AE(s) or the last trial visit, whichever occurs first. All AEs must be recorded regardless of the severity or relationship to investigational product. All SAEs will be followed until resolution, stable, or lost to follow-up. 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.

6.6 Pregnancy and Postpartum Reporting

All initial reports of pregnancy must be reported within 24 hours of first learning of the event using the Pregnancy form. Once the applicable sections on the Pregnancy form have been completed, submit the form to [REDACTED]

Once a Pregnancy form has been submitted, the site staff will receive a confirmation sent via email by [REDACTED]. The confirmation email should be filed in the trial binder with the Pregnancy form. If the email was not successfully transmitted, and a second attempt is unsuccessful, contact the CRA.

A pregnancy must be followed up through the delivery or other fetal outcome. The Investigator shall promptly report 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.

7.0 STATISTICAL PLAN

7.1 Statistical Methods

A comprehensive Statistical Analysis Plan (SAP) will be developed for this trial. Demographic and baseline characteristics will be summarized descriptively by treatment group.

7.2 Participant Population(s) for Analysis

Approximately 240 participants will be randomized and dosed to one of the three treatment groups in a 2:2:1 ratio to either ATX101 1,500 mg (three ATX101 500 mg implants; 96

participants), placebo (normal saline periarticular infiltration; 96 participants), or active comparator (bupivacaine hydrochloride 0.25% periarticular infiltration; 48 participants)

Based on the Part A results, an effect size in the range of 0.50 to 0.55 was deemed to be plausible with 0.52 chosen for an even number of 96 participants per group. This sample size yields greater than 90% power, for the primary comparison of ATX101 vs. placebo, with two-sided alpha of 5%. After the first 50% of participants have been treated and have either reached the Day 30 timepoint or have exited the trial, an optional interim analysis may be performed to re-estimate the sample size.

The Full Analysis Set (FAS) includes all participants who are randomized and administered ATX101 1,500 mg, saline placebo, or bupivacaine active comparator. Randomization failures (participants 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 participant demographic and baseline characteristics.

The Safety Analysis Set includes all participants who are randomized and administered ATX101 1,500 mg, saline placebo, or bupivacaine active comparator. Randomization failures (participants 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.

7.2.1 Primary and Key Secondary Endpoint Analysis

The primary and key secondary efficacy endpoint of the trial is the Area under the curve (AUC) for the NRS-R of pain intensity. A fixed sequence hierarchical testing procedure will be used to control the type I error. If the primary endpoint (1. below) is statistically significant, comparisons for the key secondary endpoints will be carried out in the order shown below. Nominal p-values will be reported for all endpoints but will be declared statistically non-significant once any proceeding comparison is not statistically significant.

1. AUC for the NRS-R of pain intensity from 30 minutes through Hour 168 (Day 8) compared to placebo (primary endpoint)
2. AUC for the NRS-R of pain intensity from 30 minutes through 336 hours (Day 15) compared to placebo
3. AUC for the NRS-R of pain intensity from 30 minutes through 504 hours (Day 22) compared to placebo
4. Total post-surgical consumption of opioid medications from 30 minutes through 336 hours (Day 15) compared to placebo
5. AUC for the NRS-R of pain intensity from 30 minutes through 336 hours (Day 15) compared to active comparator
6. Difference in NRS-A pain intensity scores during the TUG Test at Day 15 compared to placebo

7.2.1.1 Primary Estimand

Population

Patients requiring analgesia following primary unilateral TKA due to knee pain caused by osteoarthritis or post-traumatic arthritis.

Variable

The primary endpoint will be the Pain NRS area under the curve from 30 minutes through 168 hours ($AUC_{30m-168h}$), calculated using the trapezoidal rule as described below.

Intercurrent Events

A hypothetical strategy will be employed to handle intercurrent events.

- For participants who receive rescue medication, an NRS score for the current pain will be recorded just prior to the administration of rescue medication; scheduled assessments collected while the rescue medication would be expected to influence the participant's NRS score will be censored and replaced with pre-rescue values. See below for full details of the rescue medication handling.
- For the intercurrent event of intermittent missing data, these values will not be imputed; under the trapezoidal rule, this is equivalent to linear interpolation and assumes that pain scores track linearly from one observed score to the next.
- For intercurrent events of Treatment Related Discontinuation, treatment discontinuation due to AE or lack of efficacy (LOE), missing data will be imputed via multiple imputation (MI), under the assumption that participants are missing not at random (MNAR) and will have poor outcomes for the unobserved values; these values are assumed to follow the distribution of the worst values through the time point being imputed. For Unrelated Study Discontinuation, missing data following trial withdrawal due to reasons other than AE or LOE are assumed to be missing at random (MAR) and will be imputed using the distribution of the pain scores within treatment group for the time point being imputed. Participant narratives will be produced for each participant that withdraws from the study and reviewed for possible indications that the withdrawal was related to adverse events or lack of efficacy; if these cannot be ruled out, the participant's imputation will be handled as described above for AEs and LOE (MNAR). All determinations of MNAR and MAR will be made prior to unblinding and narratives will note the determination.
- If the initial pain score at 30 minutes post-surgical closure is missing, then the missing data are assumed to be missing at random (MAR) and are imputed using the distribution of NRS pain scores within the treatment group for the 30-minute timepoint.

Population-Level Summary

The population-level summary will be the difference in least-square (LS) means between treatment arms (analyzed as randomized) and placebo from the primary analysis of variance (ANOVA) model.

7.2.1.2 Secondary Estimands

For the key secondary endpoints 2 and 3 as defined above, the estimand elements will be identical to those of the primary estimand, with the substitution of the corresponding AUC duration for the variable.

Likewise for the key secondary endpoint 4, the estimand elements will be identical to those of the primary estimand, with the substitution of the active comparator for placebo as the contrast of interest.

For the key secondary endpoint 5, the variable will be the total post-surgical consumption of opioid medications measured in morphine milligram equivalents (MME), from 30 minutes through 336 hours (Day 15). The intercurrent event of withdrawal from the trial will be handled with a hypothetical strategy. Participants will be assumed to consume opioid medications at the same level as participants in their randomized treatment arm on corresponding days and values will be imputed via MI. A sensitivity analysis will be conducted using MI for Treatment Related Discontinuation: treatment discontinuation due to AE or LOE as if they consume opioid medications at the same level as placebo recipients on corresponding days. The population and population-level summary will be identical to the primary estimand.

For the key secondary endpoint 6, the variable will be the NRS-A pain intensity score during the TUG Test at Day 15. The intercurrent event of rescue medication will be handled with a treatment policy strategy, however, participants should not take rescue medications prior to the administration of the test. Other intercurrent event handling, the population, and population-level summary will be identical to the primary estimand.

Full details will be provided in the SAP.

7.2.1.3 Calculation and Analysis of the Primary Endpoint

The time-weighted AUC will be calculated using the trapezoidal rule (formula below) based on the available NRS pain scores. Intermittent missing values will not be imputed however, the trapezoidal rule implicitly 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 medications (ibuprofen and opioids), NRS-R scores will be recorded. Participants will be allowed to use either ibuprofen or an opioid as rescue medication. The primary endpoint of AUC will be calculated by censoring scheduled pain scores after the use of rescue medications and substituting them with the pre-rescue value. NRS-R values will be censored/substituted for the duration of the effectiveness of the rescue medications. The

censoring for ibuprofen will be 6 hours. For opioids, the actual duration of censoring will be dependent on each type of rescue opioid medication used.

In cases where multiple rescues result in overlapping censoring periods, (e.g., in the immediate postoperative setting when multiple opioids might be used), then the pain intensity value prior to the last rescue medication will be used in place of the censored value. The censoring period will continue until the end of the duration effect of all overlapping analgesics, such that:

- If ibuprofen is followed by a short-acting opioid, and the opioid's censoring period expires while the ibuprofen is still active, the pre-rescue NRS-R score for the ibuprofen will be used until the start of the opioid's censoring period, upon which the pre-rescue NRS-R score for the opioid will be used until the end of the censoring period for all overlapping analgesics.
- If the censoring periods for ibuprofen and a short-acting opioid expire at the same time, the pre-rescue NRS-R score for the last administered rescue medication will be used. This ensures that the most recent pain experience prior to the last rescue medication is accurately reflected.
- If the ibuprofen censoring period expires while a short-acting opioid is still active, the pre-rescue NRS-R score for the opioid will be used until the end of the opioid's censoring period.

To ensure accurate reflection of the patient's pain experience and avoid bias, the pain score prior to the administration of any rescue medication will be recorded and used for censoring. This approach will prevent the false decrease in pain experience in the comparator groups and maintain the assay sensitivity of the primary endpoint. Detailed censoring durations for each type of rescue opioid medication will be documented in the Statistical Analysis Plan.

Monotonic missing values will be imputed with multiple imputation (MI) based on the reason for missingness. Missing values due to participant dropout for lack of efficacy or adverse events will be imputed from the distribution of worst values observed through each timepoint, conditioned on the non-missing values. All other missing values will be imputed within treatment group assuming that they are missing at random. At least 20 repeats of the MI will be generated; this may be increased to ensure stable results if a large number of participants have missing values imputed with MI.

When performing the multiple imputation, only scheduled timepoints will be used, but for the purposes of the imputation algorithm, the values censored for rescue use will be substituted with the pre-rescue NRS prior to performing the multiple imputation process; the monotonic missing values will be imputed as described and used to augment the original dataset and the AUCs calculated within each MI repeat.

Once the NRS values have been established (taking into account the rescue medication algorithm and MI) the AUC will be calculated for each participant and each set of MI values. The following formula will be used for AUC:

$$\sum_{i=1}^{i=n-1} \frac{(NRS_{i+1} + NRS_i)}{2} * (time_{i+1} - time_i)$$

Where NRS_i values 1 to n are those that fall within the period of the AUC being calculated and an additional final calculated value placed exactly at the end of the nominal AUC period (see below); Time values are the actual times of NRS collection and calculations will be done at the precision that the time values are collected, then converted to hours. . For the first 24 hours after surgery/Time 0, pain assessments will be completed at 30 minutes and 1 hour (+10 min); 3 and 6 hours (+30 minutes); 9, 12, 18, 24 hours (+1 hour). After 24 hours, pain assessment will be completed every 6 hours through 72 hours (±2 hours). On Day 4 through Day 30, participants will move to reporting morning, mid-day, and evening (with actual times recorded). Because there is some protocol-allowed variability in the actual times of collection resulting in different possible total areas for the same nominal AUC, a linear interpolation approach will be used to achieve a uniform amount of time. A value will be calculated at exactly the end of the AUC timeframe by connecting a line between the NRS values on either side of the target time and finding the NRS value that falls on that line where it crosses the target time. The NRS values used will be those that have been determined after taking into account rescue and imputation. Thus, the calculated AUC_{30m-168h} will span exactly 167.5 hours of area and the range will be 0 to 1675 (167.5h*10 maximum NRS).

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. In the case that multiple imputation is utilized, this model will be run within each MI repeat and combined using Rubin's method [18].

The primary comparison will be between ATX101 1,500 mg and saline placebo. A two-sided alpha 0.05 will be spent on the primary comparison.

In the case of a sample size increase, the interim and post-interim cohorts will be combined using the methodology of Cui, Hung, and Wang [11]:

$$\begin{aligned} Z_1 &= \Phi^{-1}(1 - p_1) \\ \text{and } Z_2 &= w_1 Z_1 + w_2 \Phi^{-1}(1 - p_2) \end{aligned} \quad (1)$$

Where:

Z_1 = the Z statistic for the first stage

Z_2 = the combination test statistic at the end of the second stage

$w_1, w_2 = \sqrt{0.5}$

p_1 = the first stage one-sided p-value

p_2 = the second stage one-sided p-value based on second stage participants

$\Phi^{-1}()$ = the inverse normal function

The weights are defined prospectively according to the square root of the planned proportion of participants in the two stages, relative to the preplanned total enrollment of 160 participants for ATX101 and placebo groups, as $w_i = \sqrt{0.5}$. To control the type-I error, adaptive changes of the stagewise sample sizes will not lead to changes of the weights [12].

For each difference of interest, the combined point estimate of the difference and adjusted 95% CI will be provided [13]. The formula for the inferential statistics are given below.

The combined point estimate of the treatment difference is provided in formula (2) and its adjusted confidence interval is provided in formula (3).

$$\hat{\delta} = \frac{r_1 \hat{\delta}^{(1)} + \sqrt{1-r_1} \sqrt{N^*-r_1} \hat{\delta}^{(2)}}{r_1 + \sqrt{1-r_1} \sqrt{N^*-r_1}} \quad (2)$$

$$\hat{\delta} \pm \left(\left| \frac{\hat{\delta}}{Z_2} \right| 1.96 \right) \quad (3)$$

Where:

$\hat{\delta}^{(1)}$: LS Means difference from stage 1 analysis (interim cohort analysis)

$\hat{\delta}^{(2)}$: LS Means difference from stage 2 analysis (post-interim cohort analysis)

r_1 : total number of participants planned to be included at the interim analysis, divided by the total number of participants originally planned (planned sample size) (i.e., 0.5)

N^* : final total number of participants divided by the total number of participants originally planned

Z_2 : combination test statistic at the end of the second stage obtained in equation (1)

The above adjustments for the interim will apply to both the primary and key secondary endpoints listed in [Section 7.2.1](#).

7.2.2 Subgroup Analysis

There is no subgroup analysis planned for this trial.

7.2.3 Sensitivity Analysis

Two sensitivity analyses will be performed under alternative hypothetical estimands. The first will impute all missing values due to dropout, regardless of reason for trial withdrawal, from the distribution of worst values observed through each timepoint, conditioned on the non-missing values under the assumption participants that discontinue receive no further benefit from their trial arm and receive no further rescue medication; the distribution of worst values are representative of what they would experience. The second sensitivity analysis will impute all missing values from the placebo distribution conditioned on the non-missing values under the assumption that participants that dropout will continue to have access to PRN medication similar to the rescue available to the placebo group and these values are representative of what they would experience. All other estimand elements will be identical to the primary estimand.

7.2.4 Secondary Endpoints

- Difference in NRS-A pain intensity scores during the TUG Test at Day 8 compared to placebo and active comparator
- Difference in NRS-A pain intensity scores during the TUG at Day 15 compared to active comparator
- Difference in NRS-A pain intensity scores during the TUG Test at Day 22 compared to placebo and active comparator
- AUC for the NRS-R of pain intensity from 30 minutes through 168 hours (Day 8) compared to active comparator
- AUC for the NRS-R of pain intensity from 30 minutes through 504 hours (Day 22) compared to active comparator
- Total post-surgical consumption of opioid medications from 30 minutes through 168 hours (Day 8) compared to placebo and active comparator
- Total post-surgical consumption of opioid medications from 30 minutes through 504 hours (Day 22) compared to placebo and active comparator
- Total post-surgical consumption of opioid in morphine equivalent milligram dose from 30 minutes to Day 8, Day 9 to Day 15, Day 16 to Day 22, and Day 23 to Day 30 compared to placebo
- Time to discontinuation of opioid analgesia compared to placebo and active comparator:

The time to the discontinuation of post-surgical rescue opioid medication will be calculated as their last reported use of opioid medications. Participants that discontinue prior to Day 30 will be considered to have halted opioids at the last reported time of use as long as they did not report any opioid use within 24h of discontinuation. In the case that they did use opioids in that 24h window, they will be censored at Day 30, as well as those participants that report any opioid use within 24h hours of their Day 30 assessments. 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.
- Proportion of participants who are opioid free starting at Day 8, Day 15, Day 22, and Day 30 compared to placebo and active comparator:

The percentage of participants who remain opioid free from surgical closure to Day 30, from 72 hours (Day 4) to Day 30 from 168 hours (Day 8) to Day 30, 336 hours (Day 15) to Day 30 and 504 hours (Day 22) to Day 30 will be evaluated. Opioid free data will be analyzed using Fisher's exact test. The number of participants, percentage, and percentage difference between treatment groups will be summarized by treatment group and associated 95% CI will be provided.
- Proportion of participants who remain opioid free from 30 minutes through Day 8, Day 15, Day 22, and Day 30 compared to placebo and active comparator:

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

- AUC for the NRS-R of pain intensity from 168 hours (Day 8) through 336 hours (Day 15) compared to placebo and active comparator
- AUC for the NRS-R of pain intensity from 336 hours (Day 15) through 504 hours (Day 22) compared to placebo and active comparator
- AUC for the NRS-R of pain intensity from 30 minutes to 696 hours (Day 30) compared to placebo and active comparator
- Time-weighted sum of pain intensity from 30 minutes to Day 8 (time interval in the primary endpoint), Day 9 to Day 15, Day 16 to Day 22, and Day 23 to Day 30 compared to placebo
- Total post-surgical consumption of ibuprofen from 30 minutes through Day 30 compared to placebo and active comparator:
- For total post-surgical consumption of ibuprofen, all doses during the period will be summed. The total mg from surgical closure through Day 8, Day 15, Day 22, and Day 30 will be reported with descriptive statistics and compared between groups using the Wilcoxon rank-sum test.
- AUC for the NRS of Worst Pain in the last 24 hours compared to placebo and active comparator from 30 minutes to Day 8, Day 15, and Day 30:

Participants will be asked to rate their pain on the NRS pain scale regarding the worst pain they have had in the previous 24 hours. Data will be analyzed using the same ANOVA model as in the analysis of the primary endpoint.

- AUC of Sleep Quality Scale compared to placebo and active comparator:

Participants will be asked to rate the quality of their sleep upon awakening in the morning. Data will be analyzed using the same ANOVA model as in the analysis of the primary endpoint.

- Difference in Knee Injury and Osteoarthritis Outcome Score for Joint Replacement (KOOS, JR) compared to placebo and active comparator
- Difference in the Knee Society Score (KSS) compared to placebo and active comparator
- Difference in percentage of participants reporting being pain free on the NRS-R of pain intensity compared to placebo and active comparator
- Difference in NRS-A pain intensity during the TUG Test by visit compared to placebo and active comparator
- Time to 90 Degrees of Flexion compared to placebo and active comparator
- Time to -5 Degrees of Extension compared to placebo and active comparator

7.2.5 Safety Endpoints

- Incidence of AEs, AESIs, and SAEs compared to placebo and active comparator:

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 participant 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 participant will be counted only once by the highest severity grade within a SOC and preferred term, even if the participant 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. Summaries of incidence rates of opioid related AEs will be provided.

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

- Reduction in the incidence of opioid-related AEs compared to placebo and active comparator:

Opioid related AEs (refer to [Section 6.3.5](#))

- Wound Healing Assessment compared to placebo and active comparator:

The Southampton Wound Scoring System will be used to evaluate wound healing. Wound healing grades will be summarized descriptively.

7.2.6 Other Safety Analysis

7.2.6.1 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.

7.2.6.2 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 participant and visit.

7.2.6.3 Physical Examination and Neurological Assessment Findings

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

7.2.6.4 Treatment Exposure and Disposition

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

7.2.7 Exploratory Endpoints

Exploratory endpoints will be analyzed in the trial. Comparisons among the treatment groups not explicitly noted above in the primary, key secondary, and secondary endpoints will be considered exploratory. Exploratory endpoints are defined below. Continuous endpoints will be summarized using descriptive statistics (number of participants, mean, standard deviation [SD], median, and maximum values) 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.

- Difference in the duration of TUG test

7.3 Significance

All tests will be performed at two-sided alpha of 0.05. Due to the nature of some of the statistical functions required in the calculations cited, one-sided p-values may be required where noted, but all conversions and formulas will be applied in a manner to maintain the two-sided alpha of 0.05.

7.4 Interim Analysis

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 an unblinded Statistical Review Committee (SRC) separate from the team responsible for the conduct and analysis of the trial. The SRC will review the conditional power and recommend one of the following:

- Keep the current sample size and continue as planned, or
- Increase the sample size in by up to 50% (100 participants)

The conditional power will be calculated based on the first 50% of participants, using the following formula [14]

$$CP(Z_1, \tilde{n}_2) = 1 - \Phi\left(\frac{1.96\sqrt{\tilde{n}_2} - Z_1\sqrt{n_1}}{\sqrt{\tilde{n}_2}} - \frac{Z_1\sqrt{\tilde{n}_2}}{\sqrt{n_1}}\right)$$

Where:

Z_1 is the value of the Z score for the primary estimand at the interim; this will be calculated by taking the inverse normal of the one-sided p-value of the comparison, such that a large,

positive Z corresponds to a finding in favor of active treatment.

n_1 is the actual sample size at the interim

n_2 is the total planned sample size

$\tilde{n}_2$ is the total planned sample size minus the actual sample size at the interim

$\Phi()$ is the probability from a normal distribution

The above conditional power will be calculated based on the primary and the key secondary endpoints. These will be examined sequentially to determine the sample size recommendation.

Endpoint	Conditional Power (CP)	Recommendation
AUC _{30M-D8}	$\geq 90\%$	Check AUC _{30M-D15} CP
onAUC _{30M-D8}	$< 90\%$ and $\geq 40\%$	Increase sample size in blocks of 10 participant by the smallest amount that raises the CP of AUC _{30M-D8} over 90%, capped at 100 participants total increase
AUC _{30M-D8}	$< 40\%$	No change to sample size
AUC _{30M-D15}	$\geq 90\%$	Check AUC _{30M-D22} CP
AUC _{30M-D15}	$< 90\%$ and $\geq 40\%$	Increase sample size in blocks of 10 participant by the smallest amount that raises the CP of AUC _{30M-D15} over 90%, capped at 100 participants total increase
AUC _{30M-D15}	$< 40\%$	No change to sample size
AUC _{30M-D22}	$\geq 90\%$	No change to sample size
AUC _{30M-D22}	$< 90\%$ and $\geq 40\%$	Increase sample size in blocks of 10 participant by the smallest amount that raises the CP of AUC _{30M-D22} over 90%, capped at 100 participants total increase
AUC _{30M-D22}	$< 40\%$	No change to sample size

AUC_{30M-D15} conditional power will only be considered in the case that the AUC_{30M-D8} has conditional power $\geq 90\%$; likewise, AUC_{30M-D22} conditional power will only be considered in the case that the AUC_{30M-D8} and AUC_{30M-D15} have conditional power $\geq 90\%$. The sequence will be stopped with the first endpoint that falls under 90% conditional power at the planned sample size and then either the sample size will be kept the same (conditional power for that endpoint $< 40\%$) or increased (conditional power $< 90\%$ and $\geq 40\%$).

The trial team will only be informed of the non-binding recommendation to maintain the original sample size or to increase the sample size (by a fixed, pre-specified amount), as well as the endpoint that was the basis of the recommendation. All of the above calculations will be performed on the Full Analysis Set, and relative to the Full Analysis Set's planned sample size of 240 participants.

7.5 Termination Criteria

If Allay and/or the Investigator or regulatory agencies should discover conditions arising during the trial that indicate that the trial should be terminated or that a site should be closed, 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.

Reasons for terminating the trial early include, but are not limited to, the following

- If after suspension of the trial enrollment, further investigation shows that any death or met stopping criteria (refer to section 3.8 Stopping Rules) are determined by the Sponsor to be related to trial drug and pose an unacceptable risk to the trial participants, the trial will be terminated.
- Sponsor decision. In addition, if any non-anticipated safety issues arise the trial may be terminated.

If the trial is terminated early by the Sponsor, written notification documenting the reason for trial termination will be provided to the Investigator and regulatory authorities. The Investigator will promptly inform the EC and provide the reason(s) for trial termination. The Sponsor will provide written notification of early termination documenting the reason to the Investigator and regulatory authorities. The Investigator will promptly inform the IRB/EC of early termination for the trial or the site with the reason for termination.

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

7.6 Deviation Reporting

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 Allay except where it may be necessary to eliminate an immediate hazard to a research participant. In such a case, the deviation will be reported to Allay and the IRB/EC as soon as possible.

8.0 DIRECT ACCESS TO SOURCE DATA/DOCUMENTATION

Allay or its designee will assign a CRA 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 CRA to discuss the protocol and the obligations of both Allay and the Investigator. The Investigator must allow the CRA 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 participant'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 (e.g., hospital records, clinical and office charts, laboratory notes, memoranda, participants' e-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, participant 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.

9.0 QUALITY CONTROL AND QUALITY ASSURANCE

Allay shall implement and maintain quality control and quality assurance procedures to ensure that the trial is conducted, and data are generated, documented, and reported in compliance with the protocol, GCP, and applicable regulatory requirements. This trial will be conducted in accordance with US and international standards of Good Clinical Practice (FDA regulations 21 CFR 312 for IND studies and FDA guidance E6).

10.0 ETHICAL CONSIDERATIONS

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 participants, documented IRB/EC approval of the protocol, informed consent, participant questionnaires/surveys, participant training, and any advertisement for participant 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 participant to make an informed decision about his/her participation will be obtained before that participant begins any trial specific procedure. It must be signed by the participant and the Investigator-designated healthcare professional obtaining the consent, and a copy of the signed document provided to the participant.

Protocol amendments will be prepared and approved by Allay 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 Allay or designee.

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

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 (i.e., completion, termination, or discontinuation of a trial) prior to implementation. In addition, the IRB/EC must approve all advertising used to recruit participants 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.

All participants for this trial will be provided a consent form describing this trial and providing sufficient information for participants to make an informed decision about their participation in this trial. This consent form will be submitted with the protocol for review and approval by the IRB. The formal consent of a participant, using the IRB-approved consent form, will be obtained before that participant is submitted to any trial procedure. This consent form must be signed by

the participant or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

11.0 DATA HANDLING AND RECORD KEEPING

The Investigator is required to initiate and maintain an adequate and accurate case history for each participant, capturing all clinical trial observations and 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 participant'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.

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.
- Participant 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.

12.0 PUBLICATION PLAN

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

13.1 Appendix A: Schedule of Events

Schedule of Events													
Procedure	Screening	Day 1			Day 2,3	Day 4 Before Discharge ⁱ	Day 8	Day 15	Day 22	Day 30	Day 45	Early Termination	Unscheduled Visit ^l
Window	≤60 Days Before Surgery	Before Spinal	During Surgery ^j	After Surgery	Inpatient Period		±1 Days	±3 Days	±3 Days	±3 Days	±7 Days		
Informed Consent	X												
Demographics/Medical History/Prior Medication Review	X	X											
Concomitant Medication Review	X	X	X	X	X	X	X	X	X	X	X	X	X
Entry Criteria Review	X	X											
Physical Examination ^k	X	X									X	X	
Vital Signs	X	X		X ^a	X ^a	X ^a	X	X	X	X	X	X	X
Weight (Height at Screening Only)	X										X	X	
12 Lead ECG (triplicate)	X	X											X
Coagulation Profile	X												
Safety Labs (Chemistry and Hematology)	X	X			X	X	X	X	X	X	X	X	X
PK Samples ^l		For any AESI or LAST-related Symptom identified during a routine or unscheduled visit, and at all following visits											
Drug Screen	X ^b	X											
Pregnancy Test ^c	X	X											
Neurological Assessments	X	X		X ^d	X ^d	X ^d	X	X	X	X	X	X	X
E-diary Review/Training	X ^e	X		X	X	X	X	X	X	X		X	
E-diary Dispensation/Return	X										X	X	
NRS Pain Score Training	X	X			X	X	X	X	X	X			
TUG Test with duration and NRS-A with location of pain	X	X			X	X	X	X	X	X	X	X	
Range of Motion Assessment	X					X	X	X	X	X	X	X	
Wound Healing Assessment						X	X	X	X	X	X	X	
Physical Therapy					X	Physical therapy with a physical therapist twice a week							
Rescue Medications Prescribed (as needed)					X	X	X	X	X	X			

	Schedule of Events												
Procedure	Screening	Day 1			Day 2,3	Day 4 Before Discharge ⁱ	Day 8	Day 15	Day 22	Day 30	Day 45	Early Termination	Unscheduled Visit ^l
Window	≤60 Days Before Surgery	Before Spinal	During Surgery ^j	After Surgery	Inpatient Period		±1 Days	±3 Days	±3 Days	±3 Days	±7 Days		
Rescue Medications Review				X	X	X	X	X	X	X	X	X	X
Randomization		X											
Investigational Product Administration			X										
Adverse Events	X	X	X	X	X	X	X	X	X	X	X	X	X
Participant Self-Administered Questionnaires: During Inpatient Period and At Home													
NRS-R with location of pain	X	X		Daily completion ^{f,g,h}									
Rescue Pain Medication Usage				As needed after NRS-R ^h									
Worst Pain Over Last 24 Hours	X			Daily completion									
Physical Therapy questions				Daily completion									
LAST-related Symptoms Survey				Daily completion									
Sleep Quality Scale	X			Daily completion									
Participant Self-Administered Questionnaires: In Clinic													
Patient Health Questionnaire-9	X												
Knee Society Score	X						X	X	X	X	X	X	
KOOS, JR	X						X	X	X	X	X	X	

- ^a Vital signs are done three times daily (morning, mid-day, and night around mealtimes) during the hospitalization/inpatient period from Day 1 through Day 4. On Day 4, vital signs may not include all three timepoints depending on the time of discharge.
- ^b If a participant has a positive drug screen for opioids, cannabinoids, or benzodiazepines, the participant may repeat the drug screen after appropriate washout period and again 72 hours before planned surgery. If both repeat drug screens are negative, participant would be eligible.
- ^c The pregnancy test is only for females of child-bearing potential.
- ^d Neurological assessments are done once daily (approximately the same time every day) during the hospitalization/inpatient period from Day 1 through Day 4. On Day 4, neurological assessments will be prior to time of discharge which may be before the evening mealtime.
- ^e Participants will complete at least 5 days of assessments starting the day of Screening Visit for training and compliance purposes. If a participant is less than 80% compliant, then the participant will be a screen failure.

- ^f Participants should wait one hour from completion of ice/cold therapy to record their daily pain intensity scores. Prior to obtaining the NRS-R it is recommended participants be in a comfortable resting position (either seated or lying down) for at least 5 minutes prior to obtaining the pain intensity score.
- ^g For the first 24 hours after surgery/Time 0, pain assessments will be completed at 30 minutes and 1 hour (+10 min); 3 and 6 hours (+30 minutes); 9, 12, 18, 24 hours (+1 hour). After 24 hours, pain assessment will be completed every 6 hours through 72 hours (± 2 hours). On Day 4 through Day 30, participants will move to a morning, mid-day, and evening and as needed prior to taking any rescue medications (ibuprofen or opioid) for pain.
- ^h Prior to the administration of any rescue pain medication from surgery through the Day 30 Visit, participants will complete an NRS-R in the e-diary assessing the participant's pain intensity and location of pain. Participants should complete the NRS-R with location of pain prior to taking any rescue medications at any time.
- ⁱ Prior to discharge, participants must be provided with detailed education regarding LAST-related symptoms, and instructions to immediately contact the site staff should they develop any potential LAST-related symptoms. To ensure adequate monitoring for LAST, should any potential LAST-related symptom occur when a participant is completing the daily e-diary, the e-diary will immediately alert the participant to call the site staff and it will also alert the site staff to follow up with the participant. If a participant does not complete the daily e-diary questionnaire for two consecutive days, the site staff will be alerted to call the participant.
- ^j The time of surgical closure (skin closure/final stitch placed) will be documented and considered Time 0 for all assessments conducted.
- ^k A complete physical exam will be performed at the Screening Visit. An abbreviated/directed physical exam will be performed to include an assessment of participant's symptoms as well as auscultation of heart and lungs on Day 1 and at the last trial visit on Day 45 or Early Termination.
- ^l At any Unscheduled Visit due to AESI or LAST-related Symptom or a routine visit when an AESI or LAST-related symptom has been identified, in addition to the assessment defined in the Schedule of Events, safety labs and PK sampling will be obtained.

13.2 Appendix B: Patient Health Questionnaire 9

PATIENT HEALTH QUESTIONNAIRE-9 (PHQ-9)				
Over the <u>last 2 weeks</u> , 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.

13.3 Appendix C: 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 and Objective Knee Indicators questions on page 2 of the questionnaires will not be completed. Only the questions being completed are shown below.

Pre-operative

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Page 3/7

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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Page 4/7

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)

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

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

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

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

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

Maximum total points (15 points)

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Page 5/7

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)? ☐ Yes ☐ No	(0 points)
2 - If no, which of the following aid(s) do you use? ☐ wheelchair (-10 pts) ☐ walker (-8 pts) ☐ crutches (-8 pts) ☐ two canes (-6 pts) ☐ one crutch (-4 pts) ☐ one cane (-4 pts) ☐ knee sleeve / brace (-2 pts) ☐ other	(-10 points)
3 - Do you use these aid(s) because of your knees? ☐ Yes ☐ No	(0 points)
4 - For how long can you stand (with or without aid) before sitting due to knee discomfort? ☐ cannot stand (0 pts) ☐ 0-5 minutes (3 pts) ☐ 6-15 minutes (6 pts) ☐ 16-30 minutes (9 pts) ☐ 31-60 minutes (12 pts) ☐ more than an hour (15 pts)	(15 points)
5 - For how long can you walk (with or without aid) before stopping due to knee discomfort? ☐ cannot walk (0 pts) ☐ 0-5 minutes (3 pts) ☐ 6-15 minutes (6 pts) ☐ 16-30 minutes (9 pts) ☐ 31-60 minutes (12 pts) ☐ more than an hour (15 pts)	(15 points)
Maximum points (30 points)	

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Page 6/7

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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Page 7/7

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 activities from list above)	no bother	slight	moderate	severe	very severe	cannot do (because of knee)	
	5	4	3	2	1	0	
1.	☐	☐	☐	☐	☐	☐	
2.	☐	☐	☐	☐	☐	☐	
3.	☐	☐	☐	☐	☐	☐	
Maximum points (15 points)							

Maximum total points (100 points)

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Post-operative

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Page 3/7

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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Page 4/7

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)

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

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

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

Maximum total points (15 points)

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Page 5/7

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)? ☐ Yes ☐ No	(0 points)																				
2 - If no, which of the following aid(s) do you use? ☐ wheelchair (-10 pts) ☐ walker (-8 pts) ☐ crutches (-8 pts) ☐ two canes (-6 pts) ☐ one crutch (-4 pts) ☐ one cane (-4 pts) ☐ knee sleeve / brace (-2 pts) ☐ other <table border="1" style="display: inline-table; border-collapse: collapse; text-align: center;"> <tr> <td style="width: 20px; height: 20px;"></td> <td style="width: 20px; height: 20px;"></td> <td style="width: 20px; height: 20px;"></td> <td style="width: 20px; height: 20px;"></td> <td style="width: 20px; height: 20px;"></td> <td style="width: 20px; height: 20px;"></td> <td style="width: 20px; height: 20px;"></td> <td style="width: 20px; height: 20px;"></td> <td style="width: 20px; height: 20px;"></td> <td style="width: 20px; height: 20px;"></td> <td style="width: 20px; height: 20px;"></td> <td style="width: 20px; height: 20px;"></td> <td style="width: 20px; height: 20px;"></td> <td style="width: 20px; height: 20px;"></td> <td style="width: 20px; height: 20px;"></td> <td style="width: 20px; height: 20px;"></td> <td style="width: 20px; height: 20px;"></td> <td style="width: 20px; height: 20px;"></td> <td style="width: 20px; height: 20px;"></td> <td style="width: 20px; height: 20px;"></td> </tr> </table>																					(-10 points)
3 - Do you use these aid(s) because of your knees? ☐ Yes ☐ No	(0 points)																				
4 - For how long can you stand (with or without aid) before sitting due to knee discomfort? ☐ cannot stand (0 pts) ☐ 0-5 minutes (3 pts) ☐ 6-15 minutes (6 pts) ☐ 16-30 minutes (9 pts) ☐ 31-60 minutes (12 pts) ☐ more than an hour (15 pts)	(15 points)																				
5 - For how long can you walk (with or without aid) before stopping due to knee discomfort? ☐ cannot walk (0 pts) ☐ 0-5 minutes (3 pts) ☐ 6-15 minutes (6 pts) ☐ 16-30 minutes (9 pts) ☐ 31-60 minutes (12 pts) ☐ more than an hour (15 pts)	(15 points)																				
Maximum points (30 points)																					

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Page 6/7

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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Page 7/7

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 activities 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)

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13.4 Appendix D: 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 [16].
Bailey I. Community surveillance of complications after hernia surgery. *BMJ*. 1992;304:469-471 [17].

13.5 Appendix E: Pain Intensity Assessments Using the Numeric Rating Scale

The NRS will be used for pain intensity.

0	1	2	3	4	5	6	7	8	9	10
No Pain					Worst Imaginable Pain					

Participants will be asked about their pain scores on a scale from 0-10.

Participants will be trained on how to record their pain scores. Standardized pain score training will be conducted by the site staff and through an online training system.

NRS-Rest (NRS-R)

Participants will be asked at the Screening Visit and multiple times a day from Day 1 until the Day 30 Visit to indicate NRS-R “on a scale of 0 to 10, with 0 being no pain and 10 being the worst pain imaginable, how would you rate your pain RIGHT NOW?”.

NRS for Worst Pain

The participants will be asked once a day to indicate on the NRS scale their worst pain over the last 24 hours “on a scale of 0 to 10, with 0 being no pain and 10 being the worst pain imaginable, how would you rate your WORST pain in the last 24 hours?”.

NRS-Activity (NRS-A)

Participants will be asked to rate their pain with activity following the TUG test on days “on a scale of 0 to 10, with 0 being no pain and 10 being the worst pain imaginable, how would you rate your pain while doing the TUG test?”.

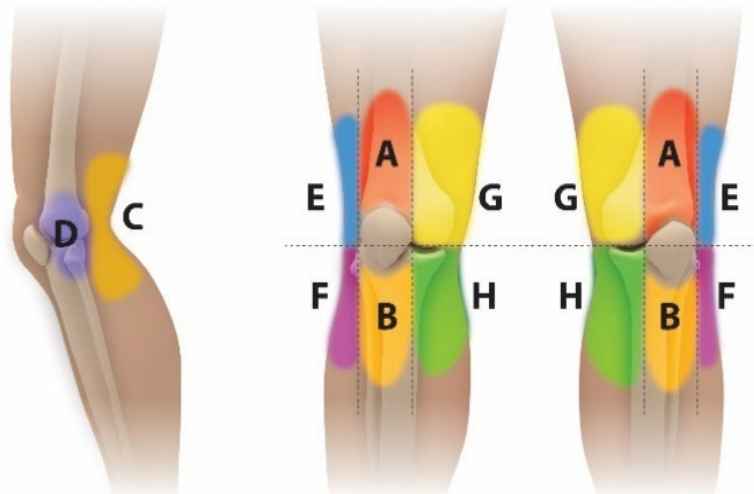
NRS during physical therapy

Participants will be asked to rate their pain with physical therapy on a scale of 0 to 10, with 0 being no pain and 10 being the worst pain imaginable, how would you rate your pain while doing the physical therapy regimen?”.

13.6 Appendix F: Location of Pain

After participants complete the NRS-R, NRS-A, or Worst Pain in the Last 24 hours, 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. Participants will be shown the below diagram of the knee and indicate if the pain is:

- A- Above my kneecap
- B- Below my kneecap
- C- Behind my knee on the back of my leg
- D- Deep inside of my knee (under my kneecap)
- E- The side of my knee (away from my body) and above the joint line
- F- The side of my knee (away from my body) and below the joint line
- G- The side of my knee (toward my other knee) and above the joint line
- H- The side of my knee (toward my other knee) and below the joint line



13.7 Appendix G: Sleep Quality Scale

Participants will be asked in the morning upon awakening to rate on a scale of 0-10 about their quality of sleep. Participants will be asked to report the quality of their sleep on a numeric scale from 0 ("best possible sleep") to 10 ("worst possible sleep") during the Screening period and from Day 1 through Day 30 after TKA.

0	1	2	3	4	5	6	7	8	9	10
Best Possible Sleep						Worst Possible Sleep				

Reference:

Cappelleri, J.C., Bushmakina, A.G., McDermott, A.M. et al. Psychometric properties of a single-item scale to assess sleep quality among individuals with fibromyalgia. *Health Qual Life Outcomes* 7, 54 (2009). <https://doi.org/10.1186/1477-7525-7-54> [19]

13.8 Appendix H: Knee Injury and Osteoarthritis Outcome Score for Joint Replacement



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*)

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Function, daily living

The following questions concern your physical function. By this we mean your ability to move around 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 ☐

13.9 Appendix I: Physical Therapy Questionnaire

Participants will complete questions related to physical therapy after TKA in the e-diary. Participants will be trained to record any type of exercise on their own or with a physical therapist as a form of physical therapy. The following questions focus on the pain the knee the participant had surgery on.

Did you participate in physical therapy today? ____ Yes/No

Did pain limit your ability to participate in physical therapy? ____ Yes/No

Were you not able to participate in physical therapy today because of pain? ____ Yes/No

How many total minutes did you participate in physical therapy today? ____ (minutes)

What was your worst pain during physical therapy?

0	1	2	3	4	5	6	7	8	9	10
No pain										Worst imaginable pain

13.10 Appendix J: 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
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

Chemistry^a	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
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
Gamma-glutamyl transferase (GGT) increased	>ULN - 2.5 x ULN if baseline was normal; 2.0 - 2.5 x baseline if baseline was abnormal	>2.5 - 5.0 x ULN if baseline was abnormal	>5.0 - 20.0 x ULN if baseline was normal; >5.0 - 20.0 x baseline if baseline was abnormal
^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.			

13.11 Appendix K: Moderate and Strong CYP3A4 Inhibitors

The following is a list of moderate and strong CYP3A4 inhibitors that are prohibited during the trial. This list is not an inclusive list but provides commonly used inhibitors.

Moderate CYP3A4 Inhibitors:	Strong CYP3A4 Inhibitors:
• Amiodarone • Aprepitant • Berostralstat • Cimetidine • Conivaptan • Crizotinib • Cyclosporine • Diltiazem • Duvelisib • Dronedarone • Erythromycin • Fedratinib • Fluconazole • Fosamprenavir • Fosaprepitant • Fosnetupitant-palonosetron • Grapefruit juice • Imatinib • Isavuconazole (isavuconazonium sulfate) • Lefamulin • Letermovir • Netupitant • Nilotinib • Nirogecestat • Ribociclib • Schisandra • Verapamil	• Adagrasib • Atazanavir • Ceritinib • Clarithromycin • Cobicistat and cobicistat-containing coformulations • Darunavir • Idelalisib • Indinavir • Itraconazole • Ketoconazole • Levoketoconazole • Lonafarnib • Lopinavir • Mifepristone • Nefazodone • Nelfinavir • Nirmatrelvir-ritonavir • Ombitasvir-paritaprevir-ritonavir • Ombitasvir-paritaprevir-ritonavir plus dasabuvir • Posaconazole • Ritonavir and ritonavir-containing coformulations • Saquinavir • Tucatinib • Voriconazole

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15.0 HISTORY OF PROTOCOL CHANGES

Version	History of Changes	Date
Original	Not Applicable	17 April 2024
Amendment 1	Revised protocol title “and active comparator” to reflect the addition of an active comparator treatment group. Updated trial ATX-101-TKA-003 information with final data from the Preliminary Draft Clinical Study Report, April 22, 2024. Updated 160 to 200 participants for the additional active comparator group of 40 participants and randomization 1:1 ratio to 2:2:1 ratio to reflect the addition of the bupivacaine treatment group. Updated trial endpoints to reflect addition of active comparator and clarification of endpoints to placebo or comparator groups. Added Wound healing assessment as a Safety Endpoint and as an assessment on Day 4 and Day 8. Updated Statistical Plan and added sections on subgroup, sensitivity and other analysis. Added additional chemistry and hematology to laboratory testing to every study visits. Removed ketamine as an allowed pre-surgical medication. Added wording on in-clinic and at-home physical therapy per standard of care. Added verbiage regarding a wallet card to be provided to participant prior to discharge. Added clarification regarding PK sampling to be collected for and AESI or LAST-related symptom. Clarified safety lab testing for all Unscheduled Visits that are due to an AESI or LAST-related symptoms and for any Unscheduled Visit for which the Investigator deems lab testing needed. Added instruction on Ibuprofen management to discontinue if an elevation of LFTs is greater than 2.5 times the upper limit of normal or at the discretion of the Investigator. Added stopping rule for wound healing. Clarification on exclusion criteria 2, 13, and 18. Added gamma-glutamyl transferase (GGT) >1.5 times the upper normal limit to exclusion criteria 1. Allowed cannabinoids to be retested after appropriate washout to exclusion criteria 21.	10 June 2024

	Removed exclusion criteria 28. Removed ketamine as an allowed pre-surgical medication. Removed tramadol and tapentadol from prohibited medications list. Added that the SMC will hold an immediate ad hoc meeting in the event that a trial stopping criterion is met. Clarified SMC membership. Updated AESI's for safety monitoring. Made updates to section to be consistent with other sections. Updated the Schedule of Events throughout the table to match the changes to the protocol. Administrative edits throughout the protocol to keep consistency.	
Amendment 2	Updated trial ATX-101-TKA-003 information with final data from the Preliminary Draft Clinical Study Report, April 22, 2024. Updated Trial Endpoints and Statistical Analysis Plan. Added coagulation profile to laboratory testing at Screening and removed Brief Pain Inventory questionnaire. Clarified bupivacaine hydrochloride dose. Removed ondansetron from exclusion criteria 10 and corrected typographical error on exclusion criteria 12 to total bilirubin. Clarified SMC membership. Clarified adverse events will be collected from the time of signed informed consent. Administrative edits throughout the protocol to keep consistency.	31 July 2024
Amendment 3	Updated Key Secondary and Secondary Endpoints. Clarified that Drug Screen will be done on Day 1 before Surgery. Clarified that physical therapy with a therapist may be conducted more frequently at the discretion of the investigator per standard of care. Revised exclusion criterion 2 and 10. Revised celecoxib dose to 200 mg and removed IV dexamethasone 8 mg to be administered during surgery. Administrative edits throughout the protocol to keep consistency.	11 December 2024
Amendment 4	Clarified Secondary Endpoints. Changed screening window from 30 days to 60 days and duration of the clinical trial for participants to 15 weeks. Removed Day 2 through Day 4 assessment window. Clarified neurological assessments to be conducted	04 March 2025

	approximately the same time every day. Changed participants will be at rest in a supine position from 10 minutes to 5 minutes before the ECGs are obtained. Revised exclusion criterion 17 to include hypertension defined by triplicate average systolic >165 mmHg or diastolic >100 mmHg blood pressure measurement at Screening. Clarified spinal anesthesia will be limited up to bupivacaine 15 mg. Clarified if spinal anesthesia fails or cannot be performed, participant may receive general anesthesia only. Removed “at the time of induction” for the use of fentanyl for airway management. Clarified that investigator has the discretion to start with IV rescue initially and then move to oral therapy prior to discharge. Administrative edits of LAST-related symptom monitoring language to keep consistent throughout the protocol. Clarification for Full Analysis Population (FAS) to include bupivacaine active comparator. Updated language around censoring of pre-rescue NRS-R values. Added language around censoring of missing NRS-R pain score at 30-minute post-surgical closure time point	
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