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STATISTICAL ANALYSIS PLAN

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

Protocol Number: ATX-101-TKA-003

Sponsor: Allay Therapeutics, Inc.
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Approval	Date
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Revision History

Version and Date	Reason of Revision	Comments
Version 1.0, 15Aug2023	Initial document	
Amendment 1, 20Feb2024	Addition to Study Design for placebo group prior to Protocol Amendment 03 Updated KOOS Jr analysis. Updated the sensitivity analyses. Updated opioid related AEs	Clarification Corrected per the protocol. Added analyses. Clarification.

ABBREVIATIONS

Abbreviation	Definition
ADAM	Analysis Data Model
AE	Adverse Event
AESI	Adverse Event of Special Interest
ATC	Anatomical Therapeutic Chemical
AUC	Area Under the Curve
BMI	Body Mass Index
CI	Confidence Interval
CDISC	Clinical Data Interchange Standards Consortium
cm	centimeters
CRA	Clinical Research Associate
ECG	Electrocardiogram
FAS	Full Analysis Set
HCl	Hydrogen Chloride
IV	Intravenous
kg	kilograms
KOOS JR	Knee Injury and Osteoarthritis Outcome Score
LOCF	Last Observation Carried Forward
m^2	Meters squared
MedDRA	Medical Dictionary for Regulatory Activities
mg	Milligram

MME	Morphine Milligram Equivalent
n	Number
NRS	Numeric Rating Scale
NRS-A	Numeric Rating Scale with Activity
NRS-R	Numeric Rating Scale at Rest
PACU	Post-Anesthesia Care Unit
PCA	Patient Controlled Anesthesia
PHQ-9	Patient Health Questionnaire 9
PI	Pain Intensity
PK	Pharmacokinetic
QoR-15	Quality of Recovery 15 Scale
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SDTM	Study Data Tabulation Model
SOC	System Organ Class
SPI	Time Weighted Sum of Pain Intensity
SPI-R	Time Weighted Sum of Pain Intensity Area Under the Curve
TFLs	Tables, Figures, and Listings
TKA	Total Knee Arthroplasty
WHO-DD	World Health Organization Drug Dictionary
WOCF	Worst Observation Carried Forward

wWOCF	Windowed Worst Observation Carried Forward

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1.0 INTRODUCTION TO THE STATISTICIAL ANALYSIS PLAN

The statistical analysis plan (SAP) is based on protocol ATX-101-TKA-003: A Phase 2B, Randomized, Double Blind, Active Comparator, Multicenter, Safety, and Efficacy Trial of ATX-101 in Subjects Undergoing Total Knee Arthroplasty (SPARK), Amendment 03 dated 02 June 2023. This SAP details the methodology to be used in analyzing the data and outlines the specifications in the Tables, Figures and Listings (TFLs) for data to be included for executing the final statistical analyses for this study.

Pharmacokinetic (PK) and triplicate ECG data analyses are not included in the scope of this plan.

The analyses specified in this document supersede any high-level analysis plan described in the protocol.

The study data collected in the Electronic Data Capture system will be mapped to Clinical Data Interchange Standards Consortium (CDISC) Study Data Tabulation Model (SDTM) standard and analysis data will be derived following CDISC Analysis Data Model (ADaM) data standard.

2.0 INTRODUCTION TO THE STUDY

2.1 Study Objectives

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

2.2 Study Endpoints

2.2.1 Primary Endpoint and Primary Estimand

Primary Endpoint

- The primary efficacy endpoint for Part A is AUC for the NRS-R of pain intensity from 30 Minutes through Hour 168 (Day 8 of the trial).
- The primary efficacy endpoint (evaluated in Part B) is to determine the area under the curve (AUC) for the Numeric Rating Scale at Rest (NRS-R) of pain intensity from 30 Minutes through a time point to be determined from Part A.

Primary Estimand (evaluated in Part B)

Population

- The analysis population will be Part B subjects meeting the protocol inclusion/exclusion criteria that were randomized and administered IP.

Variable

- The primary endpoint will be the AUC of the NRS-R from 30 Minutes through a time point to be determined from Part A.

Intercurrent Events

- A hypothetical strategy will be used in handling of intercurrent events.
- The intercurrent event of use of opioid rescue medication will be handled by obtaining a NRS-R score prior to rescue medication administration and censoring subsequent scheduled NRS-R values that fall within the duration of efficacy of the rescue medication. The exception to this will be instances where the scheduled NRS values

recorded within the censoring period are higher than the pre-rescue value; these will not be censored and will be included in calculation of the AUC. Censoring periods for each opioid rescue will be detailed in the SAP.

- For the intercurrent event of sporadic missing values, no special handling or imputation will be performed; the trapezoidal rule is equivalent to performing a linear interpolation between the 2 observed values on either side of a missing value.
- For the intercurrent event of withdrawal, values will be imputed based on the reason for withdrawal. Only the last time point will be imputed for calculating the AUC; in this instance, the trapezoidal rule will calculate area by connecting the last observed value to the imputed last time point for the chosen AUC. For subjects withdrawing for adverse events (other than COVID), the value imputed will be the worst value observed prior to withdrawal. All other subjects (including those that withdraw **solely** for COVID) will have their last observation carried forward.

Population-Level Summary

- The population-level summary will be the difference in least-square means between treatment arms (analyzed as randomized) for the AUC chosen from Part A. This analysis will use an Analysis of Variance (ANOVA) model with a main effect of the treatment group.
- The primary analysis will be followed by several sensitivity analyses. Further details on sensitivity analyses can be found in Section 8.1.2.

2.2.2 Key Secondary Endpoints (Evaluated in Part B)

Key secondary endpoints are to evaluate the:

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

2.2.3 Secondary Endpoints

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

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

2.2.4 Safety Endpoints

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

Incidence of AEs, AESIs, and SAEs

- Wound healing assessment using the Southampton Wound Scoring System
- Characterization of the bupivacaine PK from ATX-101.

2.2.5 Exploratory Endpoints

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

- Percentage of subjects reporting being pain free on the NRS-R of pain intensity
- Time to various degrees of flexion and extension
- Reduction/incidence of opioid-related AEs
- Evaluation of Quality of Recovery 15 Scale (QOR-15)
- Evaluation of Knee Injury and Osteoarthritis Outcome Score for Joint Replacement (KOOS, JR)
- Difference in pain intensity scores (NRS-R and Numeric Rating Scale with Activity [NRS-A]) between treatment groups
- If a statistical difference in AUC of pain intensity NRS-R at Hour 336 (Day 15 of the trial) is observed, the AUC for the NRS-R of pain intensity will be further evaluated between Days 16 and 30
- Evaluation of the Knee Society Score.

2.3 Study Design

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

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

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

The trial was initiated under Protocol Version 00, and subjects were enrolled under Version 00 and Protocol Amendment 01, which included a Saline Placebo control which was changed to a

Bupivacaine HCl active comparator in Protocol Amendment 03. The Placebo group is included in the Part A study analyses.

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

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

Refer to Appendix A and Appendix B and the study protocol for further details of the schedule of events and procedures.

3.0 RANDOMIZATION AND BLINDING

A randomization schedule will be computer generated.

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

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

In the event of a medical emergency when knowledge of the actual treatment becomes medically necessary to affect treatment options, prior to the end of the data base lock (Part A and Part B), the Investigator will be able to break the blind and obtain details of the treatment assigned to a subject. The Medical Monitor is to be informed immediately that the blind has been broken.

The surgical staff will not be blinded to the trial treatment, all other research staff (unless expressly specified in the site-specific Blinding Plan and the subject will remain blinded throughout the trial. Allay will remain blinded during the treatment phase of both Part A and Part B of the study until database lock occurs for Part A and Part B.

There will be unblinded clinical research associates (CRAs)/monitors who will be unblinded throughout the trial to ensure compliance with the trial protocol and investigational product accountability. Further information regarding investigational drug accountability can be found in Section 7.6. Blinded monitors will be monitoring safety and efficacy data at the sites.

4.0 SAMPLE SIZE CONSIDERATIONS

Part A

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

- ATX-101, 1,000 mg dose (two ATX-101 implants)
- ATX-101, 1,500 mg dose (three ATX-101 implants)
- 0.25% bupivacaine HCl 125 mg

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

Part B

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

- ATX-101 dose to be determined from Part A
- 0.25% bupivacaine HCl 125 mg

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

5.0 ANALYSIS POPULATIONS

5.1 Full Analysis Set (FAS)

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

5.2 Safety Analysis Set

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

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

5.3 PK Analysis Set

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

6.0 GENERAL STATISTICAL CONSIDERATIONS AND DEFINITONS

6.1 Baseline and Visit Windows

Unless otherwise specified in this section, baseline, which is defined as the last non missing assessment prior to receiving the study treatment, will be used in baseline and change from baseline analyses.

The summary tables by visit will use the values from the scheduled timepoints/visits.

6.2 Study Day

For the purpose of analysis, Day 1 is defined as the surgery day.

6.3 Summary Statistics

Analysis will be summarized by Part A and Part B and overall, unless specified otherwise.

Descriptive statistics (the number of subjects [n], mean, standard deviation [SD], median, minimum, and maximum) will be used to summarize continuous variables. Means and medians will be presented to one more decimal place than the recorded data. Standard deviations will be presented to two more decimal places than the recorded data. Minimum and maximum values will be presented using the same number of decimal places as the recorded data. Percentages will be presented to one decimal place.

Frequency distributions (number [n] and percentage of subjects [%]) will be used to summarize categorical or qualitative variables. In general, the denominator for the percentage calculation will be based upon the total number of subjects in the study population for the treatment groups, unless otherwise specified.

All statistical tests will be performed against a two-sided alternative hypothesis with a significance level of 5% ($\alpha = 0.05$), and all confidence intervals (CIs) calculated will be two-sided 95% CIs. All tests will be declared to be statistically significant if the calculated p-value is ≤ 0.05 unless specified otherwise. Tests for binomial proportions will be conducted.

(1) using a normal approximation whenever each classification cell for each group to be compared contains expected cell counts of 5 or more subjects, or (2) using an exact method whenever any one classification cell for any one group to be compared contains expected cell counts of fewer than 5 subjects.

All analyses will be performed using SAS® software Version 9.4 or later.

For the listings, subjects will be listed under their randomized treatment, regardless of actual treatment received. Subjects who are randomized but do not receive study drug will be

included in the listings with the treatment group identified as "ATX-01 Not Treated" or 'Bupivacaine, Not Treated' depending on their randomized treatment. Subject listings of data will be presented for all enrolled subjects unless specified otherwise.

6.4 Calculation of AUC or NRS

Area under the curve (AUC) will be calculated using the trapezoidal method with the actual NRS pain intensity scores reported by the subject as follows.

$$SPI - R = \frac{(NRS - R_1 + NRS - R_2)(t_2 - t_1)}{2}$$

The trapezoidal rule performs a linear interpolation between the 2 observed values on either side of a missing value. Actual times of each NRS-R score will be used for calculating the AUC.

Missing values prior to early termination (ET) will be imputed according to the trapezoidal rule, and missing values after ET will be imputed.

- using the last observation carried forward if the primary reason is not AE
- using the worst observation carried forward if the primary reason is AE

Details are described in Section 6.7. Actual times not scheduled times, when available will be used in the calculations. Linear interpolation will be used to calculate the pain intensity score at the end of an interval.

6.5 Calculation Of Post-Surgical Opioid Consumption Compliance

The total number of opioid pills dispensed will be compared to those that are returned and consumed (eDiary data). Opioid accountability will be done for Day 15, Day 30 and ET (if before Day 30). The percentage will be calculated using the following:

(Opioid Accountability / Opioid Pills Dispensed – Opioid Pills Consumed) * 100

6.6 Data Handling Rules

Opioid consumption will be converted to morphine equivalence in milligrams (MME) according to the conversion factor in Appendix C. Numeric Rating Scale (NRS) pain scores taken after an opioid as rescue medication will be censored for the amount of time according to the NRS Censoring Time (Hours) in Table 1:

TABLE 1: NRS Censoring Rules

Opioid Type*	NRS Censoring Time (Hours)
Endone/Oxycodone (oral)	4
Hydromorphone (oral)	4
Hydromorphone (IM)	3
Hydromorphone (IV)	3
Fentanyl (IV)	0.5
Morphine (oral)	3
Morphine (IM)	2
Morphine (via PCA pump)	3
Palexia/Tapentadol (oral)	4
Panadeine Forte/Codeine (oral)	4
Targin/Oxycodone & Naloxone (oral)	12
Tramadol (oral)	4

*Extended release opioid not allowed per protocol.

6.7 Imputation of NRS Pain Intensity Scores

For calculation of AUC of NRS pain intensity scores, data will be handles as follows:

- Trapezoidal Rule:

  For the intercurrent event of sporadic missing values, no special handling or imputation will be performed; the trapezoidal rule is equivalent to performing a linear interpolation between the 2 observed values on either side of a missing value.

- Windowed Worst Observation Carried Forward (wWOCF) with Rescue Medication

The intercurrent event of use of opioid rescue medication will be handled by obtaining a NRS-R score prior to rescue medication administration and censoring subsequent scheduled NRS-R values that fall within the duration of efficacy of the rescue medication as described in Table 1. The exception to this will be instances where the scheduled NRS values recorded within the censoring period are higher than the pre-rescue value; these will not be censored and will be included in calculation of the AUC. This method of imputation is referred to as wWOCF. If no NRS score is available prior to the first rescue the worst observation from all available NRS scores from the subject will be used instead.

- Last Observation Carried Forward (LOCF)

For the intercurrent event of withdrawal, values will be imputed based on the reason for withdrawal. Only the last time point will be imputed for calculating the AUC; in this instance, the trapezoidal rule will calculate area by connecting the last observed value to the imputed last time point for the chosen AUC.

For subjects withdrawing for adverse events (other than COVID), the value imputed will be the worst value observed prior to withdrawal. All other subjects (including those that withdraw **solely** for COVID) will have their last observation carried forward.

6.8 Interim Analysis

For Part A, an interim analysis may be performed when approximately 50% of the initially planned enrollment has completed Day 30. Enrollment will continue during the interim analysis.

This interim analysis will be performed by the unblinded statistician separate from the team responsible for the conduct and analysis of the study. A Review Committee of at least two unblinded statisticians and a designated sponsor representative will review the data and recommend one of the following:

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

Analyses to be included in the interim analysis will be the following at a minimum:

- Subject disposition
- Demographic and baseline characteristics
- Primary analysis of the AUC of pain intensity at rest from 30 minutes post-surgery.
- AUC of pain intensity at rest for Each 24-Hour Period Through Hour 336 AUC of active pain intensity at by assessed time points

- Summary of Post-Surgical Consumption of Opioid in Morphine Equivalent Milligram Dose
- Safety analysis including lab results, vital signs, TEAE, SAE and AESI

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

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

The designated sponsor representative will not participate in ongoing study conduct once unblinded to study results and will not have access to individual patient treatment assignments. Should Part A continue to full enrollment they likewise will be blinded to assignments and results until the final unblinding and analysis.

The same IA will be repeated when 100% of subjects complete D30 visit.

6.9 Reporting Plan

There will be two reporting events for this study, in addition to the interim analysis. One will be at the end of Part A and another one will be at the end of the study for Part B reporting.

The end of Part A analysis of data will be for all subjects who have completed Day 56 visit. Data that will be reviewed will be cleaned data according to edit checks in the Data Validation Specification and Data Review Plan and the Part A database locked. The purpose of the Part A analysis is to inform the development plan and Part B study design.

End of Part B analysis of data will include all data in Part B portion of study.

7.0 STATISTICAL ANALYSES

7.1 Subject Disposition and Withdrawals

The number and percentages of subjects screened, enrolled, received treatment (FAS), safety analysis set, PK analysis set, completed the study and discontinued from the study will be reported, along with the primary reason for discontinuation.

Percentages will be out of the safety population. Only counts will be reported for enrolled subjects.

7.2 Demographic and Baseline Characteristics

Demographic/baseline characteristics collected at the Screening Visit and during the Pre-Surgical Baseline Period will be summarized and presented. Descriptive statistics will be presented based on FAS population.

Variables to be summarized include:

- Age (years)
- Sex (male, female)
- Race
- Height (cm)
- Weight (kg)
- BMI (kg/m^2)

All demographics and baseline characteristics will be provided in a subject listing.

7.3 Medical History

Medical history will be coded using Medical Dictionary for Regulatory Activities (MedDRA). Conditions will be summarized by system organ class (SOC) and preferred term. In addition, conditions will be listed by the verbatim investigator description of the relevant medical condition, the coded terms (SOC, preferred term), start date, end date, and whether the condition is resolved or ongoing.

7.4 Prior and Concomitant Medications

Prior and concomitant medications (including intra-surgical and post-surgical concomitant medications) will be coded with the World Health Organization Drug Dictionary (WHO-DD).

Prior and concomitant medication will be summarized using the FAS Population. Medications will be reported by drug class (Anatomical Therapeutic Chemical [ATC] Level 3) and preferred term; a subject will be counted only once for each medication.

Prior and concomitant medications will be listed.

7.5 Protocol Deviations

All protocol deviations will be presented in a listing. All major deviations will be provided in a separate listing

7.6 Study Drug Administration

For Part A, the total number of ATX-101 implants, total ATX-101 dose administered, and the total dose for active comparator will be summarized.

For Part B, the total number of ATX-101 implants, total dose administered for active comparator will be summarized.

8.0 EFFICACY ENDPOINT EVALUATION

8.1 Primary Endpoint – Time weighted AUC (Part B)

The primary efficacy endpoint will occur in Part B of the study and is the AUC for the NRS-R of pain intensity from 30 Minutes through a time point x (hour x or day x) which is to be determined from Part A.

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

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

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

Assuming that a subject has a 24-hour nominal value, SPI-R will be normalized to exactly 24hrs. Should the subject be missing the nominal 24-hour value, but have a subsequent NRS-R recorded, linear interpolation will be performed to impute the 24-hour value by connecting the prior and subsequent values and calculating where the 24-hour value would fall on that line.

AUC will be calculated according to the handlings specified in Section 6.7.

Censored NRS-R values will be retained in the database but will not be utilized for the SPI-R calculations.

8.1.1 Subgroup Analysis

The primary endpoint will be analyzed using the same ANOVA model by subgroups of subjects who previously received TKA and those who did not.

8.1.2 Sensitivity Analysis

In Part A,

- Additional analysis for the primary endpoint and other efficacy measures as well as opioid consumption will be conducted comparing each dose of ATX-101 to combined group of bupivacaine HCl and Placebo using FAS.
- The primary analysis will be repeated for sites who have enrolled >=10 subjects.

8.2 Key Secondary Endpoint Analysis for Part B

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

1. Area under the curve for NRS-R of pain intensity from 30 Minutes through Hour 168 (Day8 of the trial)
2. Area under the curve for NRS-R of pain intensity from 30 Minutes through Hour 240 (Day 11 of the trial)
3. Area under the curve for NRS-R of pain intensity from 30 Minutes through Hour 336 (Day 15 of the trial)
4. Percentage of subjects who remain opioid free from Hour 72 through Day 30
5. Total post-surgical consumption of opioid medications from surgical closure through Day 30.

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

For total post-surgical consumption of opioid medication, all opioids will be converted to an equianalgesic parenteral morphine amount (milligram morphine equivalent or MME) using standard conversion factors as laid out in Appendix 11.3. Subject's total post-surgical consumption of rescue opioid medications from surgical closure through Day 30 will be analyzed using the Wilcoxon rank-sum test.

8.3 Secondary Endpoint Analysis

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

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

8.3.2 Percentage of Subjects Opioid Free

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

8.3.3 Total Post-Surgical Consumption of Opioids

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

8.3.4 Time to First Rescue Opioid Medication

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

8.4 Exploratory Endpoints

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

- Pain Free on the NRS-R , which is defined as NRS-R=0, will be summarized.
- Time to pain free, which is defined as NRS-R=0, from surgery closure to Day 30 will be analyzed using KM method.
- Time to the first rescue of opioid medication will be analyzed using KM method.
- Time to Opioid free will be analyzed using Kaplan Meier method.
- Number of subjects who are opioid free at 1 week, 2 weeks, 3 weeks and 4 weeks will be summarized.
- NRS-R and NRS-A as collected (without imputation) will be summarized by assessment timepoints. In addition, the group mean scores will be graphed by assessment timepoints in categories of
    - No Pain – 0
    - Mild Pain – 1-3
    - Moderate Pain – 4-6

- Severe Pain – 7-10

- NRS-R and NRS-A as collected (without imputation) will be summarized by location of pain by assessment timepoints.
- Other endpoints such as time to various degrees of flexion and extension and reduction/incidence of opioid-related AEs may be explored.

8.4.1 Evaluation of Quality of Recovery (QOR-15)

The Quality of Recovery 15 Scale (QoR-15) outcome will be summarized descriptively as a continuous variable for each scale, by each assessment visit. Change from baseline values will be calculated, listed, and summarized similarly. The total score of the 15 questions will be calculated and included in the analysis.

8.4.2 Evaluation of Knee Injury and Osteoarthritis Outcome Score for Joint Replacement (KOOS, JR)

KOOS, JR contains the subscales of Stiffness (Question 1), Pain (Question 2-5) and Function, daily living (Question 6-7).

The responses will first be scored to 0-4 for 0 being None and 4 being Extreme.

KOOS, JR is scored by summing the raw response (range 0-28) and then converting it to an interval score using the table provided below. The interval score ranges from 0 to 100 where 0 represents total knee disability and 100 represents perfect knee health.

Table 2: Converting Koos Score to Interval Score

Raw summed score (0-28)	Interval score (0 to 100 scale)
0	100.000
1	91.975
2	84.600
3	79.914
4	76.332
5	73.342
6	70.704
7	68.284
8	65.994
9	63.776
10	61.583
11	59.381
12	57.140
13	54.840
14	52.465

15	50.012
16	47.487
17	44.905
18	42.281
19	39.625
20	36.931
21	34.174
22	31.307
23	28.251
24	24.875
25	20.941
26	15.939
27	8.291
28	0.000

KOOS JR total scores will be summarized descriptively by subscales and assessment time point and change from baseline values will be calculated. In addition, the interval scores and change from baseline interval scores will be summarized descriptively by assessment time. A separate listing will be presented for the KOOS JR raw scores, total score, and interval scores.

8.4.3 Physical Therapy Survey

The scores collected from the ability to manage pain will be listed.

8.4.4 Range of Motion (ROM)

Range of motion is defined as the degree of flexion minus the degree of extension.

ROM data will be summarized by the extension and flexion limb by assessment time point.

In addition, the calculated ROM, as defined above, will be summarized descriptively by assessment time point.

Number of subjects that achieve 90 degrees of flexion will be summarized similarly by assessment time point.

Time to reach 90 degrees of flexion will be explored using KM method.

8.4.5 PHQ-9

PHQ-9 data for Q1-Q9 will be summarized by each question and the total score. All data collected will be listed.

8.4.6 Evaluation of the Knee Society Score

Maximum total points collected from the Knee Society score will be summarized by categories evaluated. All data collected on the questionnaire will be listed.

9.0 SAFETY ENDPOINT EVALUATION

All summaries of the safety data will use the safety analysis set.

9.1 Adverse Events

All AEs will be coded and summarized by system organ class (SOC) and preferred terms based on the Medical Dictionary for Regulatory Activities (MedDRA) coding dictionary.

All AEs will be evaluated for those subjects who were administered any opioid and Opioid-related AEs will be determined prior to the database lock (DBL).

Incidence (frequencies and percentages) of TEAEs by SOC and preferred terms will be summarized for the following:

- TEAEs
- TEAEs by maximum severity
- TEAEs leading to study discontinuation TEAEs by the strongest relationship to:
    - Investigational product
    - TKA surgical procedure
- Serious AEs (SAEs)
- AESI
- Opioid-related AE.

Each subject will be counted only once within each summation level (SOC; preferred term). If a subject experiences more than one AE within a given summation level, the AE with the strongest relationship or the maximum severity, as appropriate, will be included in the summaries of relationship and severity.

Additionally, an overall summary table of TEAEs that include the total number of events as well of incidences of TEAE, TEAE by severity, AE by relationship, AEs leading to study discontinuation and SAEs will be provided.

In the AE data listings, all AEs will be displayed and AESI flagged.

9.2 Wound Healing Assessment

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

9.3 Clinical Laboratory Results

Descriptive summaries for chemistry and hematology laboratory tests of observed (absolute) values and changes from baseline values will be presented for clinical laboratory values at each time point.

Laboratory values will be listed with the reference range. Any values outside of reference range will be flagged.

Urine pregnancy tests, and urine drug screen data will be presented in a listing

9.4 12-Lead ECG

12-Lead ECG results will be presented in a subject listing. As it is sometimes not possible to collect 3 ECGs (triplicate) at a specified timepoint, the values at that timepoint will include the average of the two ECGs, or the values of the single ECG that is collected at that timepoint. If one variable is not available, it will not preclude the inclusion of other variables in the analysis.

The number and percentage of subjects with normal, abnormal but not clinically significant, or abnormal and clinically significant will be summarized by assessment timepoints.

All other parameters of HR, QT, QTcF etc. will be summarized descriptively by assessment timepoints.

9.5 Vital Signs

Vital signs measurements (systolic and diastolic blood pressure, pulse rate, body temperature, and oxygen saturation) will be summarized and listed by assessment visit. Additionally, the changes from baseline will be calculated and presented descriptively.

Vital signs data will be listed by subject and visit.

9.6 Physical Examination and Neurological Assessment Findings

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

10.0 DEVIATIONS FROM STATISTICAL PLAN AND OTHER ISSUES

During the analysis and reporting process, any deviations from the statistical plan designed for this protocol will be described and justified in the clinical study report.

11.0 APPENDICES

11.1 Appendix A: SCHEDULE OF EVENTS FOR PART A

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

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

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

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

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

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

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

Part A Schedule of Events for Day of Surgery Through Hour 96 (Day 5)

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

a. Subjects will remain under observation until Hour 96. Subjects may remain in the hospital/facility for 96 hours or may be discharged from the hospital/facility per standard of care and moved to another unit until the conclusion of the 96-hour in-patient monitoring period.

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

c. ECGs will be taken in triplicate (2 minutes apart [±1 minute]) after the subject has been at rest in a supine position for 10 minutes without any other assessments being conducted. ECGs will be taken BEFORE PK sampling.

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

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

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

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

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

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

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

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

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

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

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

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

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

11.2 Appendix B: SCHEDULE OF EVENTS FOR PART B

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

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

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

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

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

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

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

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

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

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

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

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

a. Subjects will remain under observation until Hour 24.

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

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

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

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

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

11.3 Appendix C: Opioid Oral Morphine Milligram Equivalent (MME) Conversion Factors

Opioid Oral Morphine Milligram Equivalent (MME) Conversion Factors[i,ii]

Type of Opioid (strength units)	MME Conversion Factor
Buprenorphine film/tablet[iii] (mg)	
Buprenorphine patch[iii] (mcg/hr)	
Buprenorphine film [iii] (mcg)	
Butorphanol (mg)	7
Codeine (mg)	0.15
Dihydrocodeine (mg)	0.25
Fentanyl buccal or SL tablets, or lozenge/troche[iv] (mcg)	0.13
Fentanyl film or oral spray[v] (mcg)	0.18
Fentanyl nasal spray[vi] (mcg)	0.16
Fentanyl patch[vii] (mcg)	7.2
Hydrocodone (mg)	1
Hydromorphone (mg)	4
Levorphanol tartrate (mg)	11
Meperidine hydrochloride (mg)	0.1
Methadone[viii] (mg)	3
>0, <= 20	4
>20, <=40	8
>40, <=60	10
>60	12
Morphine (mg)	1
Opium (mg)	1
Oxycodone (mg)	1.5
Oxymorphone (mg)	3
Pentazocine (mg)	0.37
Tapentadol[ix] (mg)	0.4
Tramadol (mg)	0.1

[i] The MME conversion factor is intended only for analytic purposes where prescription data are used to calculate daily MME. Use the formula: Strength per Unit X (Number of Units/ Days Supply) X MME conversion factor = MME/Day. This value does not constitute clinical guidance or recommendations for converting patients from one form of opioid analgesic to another. Please consult the manufacturer's full prescribing information for such guidance. Use of this file for the purposes of any clinical decision-making warrants caution. This is particularly true with regard to methadone (see viii below).

[ii] National Center for Injury Prevention and Control. CDC compilation of benzodiazepines, muscle relaxants, stimulants, zolpidem, and opioid analgesics with oral morphine milligram equivalent conversion factors, 2017 version. Atlanta, GA: Centers for Disease Control and Prevention; Available at https://www.cdc.gov/drugoverdose/resources/data.html . For more information, send an email to Mbohm@cdc.gov.

[iii] Buprenorphine products are listed but do not have an associated MME conversion factor. These buprenorphine products, as partial opioid agonists, are not expected to be associated with overdose risk in the same dose-dependent manner as doses for

full agonist opioids. The conversion factors for drugs prescribed or provided as part of medication-assisted treatment for opioid use disorder should not be used to benchmark against dosage thresholds meant for opioids prescribed for pain.

[iv] The MME conversion factor for fentanyl buccal tablets, sublingual tablets, and lozenges/troche is 0.13. This conversion factor should be multiplied by the number of micrograms in a given tablet or lozenge/troche.

[v] The MME conversion factor for fentanyl film and oral spray is 0.18. This reflects a 40% greater bioavailability for films compared to lozenges/tablets and 38% greater bioavailability for oral sprays compared to lozenges/tablets.

[vi] The MME conversion factor for fentanyl nasal spray is 0.16, which reflects a 20% greater bioavailability for sprays compared to lozenges/tablets.

[vii] The MME conversion factor for fentanyl patches is based on the assumption that one milligram of parenteral fentanyl is equivalent to 100 milligrams of oral morphine and that one patch delivers the dispensed micrograms per hour over a 24 hour day. Example: 25 ug/hr fentanyl patch X 24 hrs = 600 ug/day fentanyl = 60 mg/day oral morphine milligram equivalent.
In other words, the conversion factor not accounting for days of use would be 60/25 or 2.4.
However, since the fentanyl patch remains in place for 3 days, we have multiplied the conversion factor by 3 (2.4 X 3 = 7.2). In this example, MME/day for ten 25 µg/hr fentanyl patches dispensed for use over 30 days would work out as follows:
Example: 25 ug/hr fentanyl patch X (10 patches/30 days) X 7.2 = 60 MME/day. Please note that because this allowance has been made based on the typical dosage of one fentanyl patch per 3 days, you should first change all Days Supply in your prescription data to follow this standard, i.e., Days Supply for fentanyl patches= # of patches X 3.

[viii] The CDC MME conversion factor to calculate morphine milligram equivalents of methadone is 3. Calculating MME for methadone in clinical practice often involves a sliding-scale approach whereby the conversion factor increases with increasing dose since the conversion factor of 3 for methadone could underestimate MME for a given patient. CMS uses this conversion factor when analyzing Medicare population opioid use. CMS uses the graduated methadone MME conversion factors to calculate MME within the Overutilization Monitoring System (OMS) for identifying and reporting potential opioid overutilizers. https://www.cdc.gov/drugoverdose/pdf/calculating_total_daily_dose-a.pdf.

[ix] Tapentadol is a mu receptor agonist and norepinephrine reuptake inhibitor. Oral MMEs are based on degree of mu receptor agonist activity, but it is unknown if this drug is associated with overdose in the same dose-dependent manner as observed with medications that are solely mu receptor agonists.

12.0 REFERENCES

1. Center for Disease Control and Prevention (CDC) clinical guidance on dosage of opioids for treatment of chronic pain factsheet: *Calculating Total Daily Dose of Opioids for Safer Dosage*.

2. Centers for Medicare & Medicaid Services (CMS) Opioid Products Indicated for Pain Management. KMAP General Bulletin 18101, April 2018

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