IV TRAMADOL

PROTOCOL AVE-901-102

Tramadol Infusion - Postoperative Pain in Orthopedic Surgery

A PHASE 3, MULTICENTER, RANDOMIZED, DOUBLE BLIND, THREE-ARM STUDY TO EVALUATE THE EFFICACY AND SAFETY OF TRAMADOL INFUSION (AVE-901) VERSUS PLACEBO IN THE MANAGEMENT OF POSTOPERATIVE PAIN FOLLOWING BUNIONECTOMY

Sponsor:
Avenue Therapeutics, Inc.
New York, NY 10019

IND #: 108124

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APPROVAL SIGNATURES

Protocol Number:	AVE-901-102
Protocol Title:	A Phase 3, Multicenter, Randomized, Double Blind, Three-Arm Study to Evaluate the Efficacy and Safety of Tramadol Infusion (AVE-901) versus Placebo in the Management of Postoperative Pain Following Bunionectomy

_____ _____
Date

Lucy Lu, MD
CEO, Avenue Therapeutics

INVESTIGATOR'S AGREEMENT

I have received and read the Investigator's Brochure for Tramadol Infusion. I confirm that I have read this protocol. I understand it, and I will work according to the protocol and moral, ethical, and scientific principles governing clinical research as set out in the Declaration of Helsinki and the principles of ICH guidelines for GCP and according to applicable local regulatory requirements. I agree to maintain the confidentiality of all information received or developed in connection with this protocol.

_____

Printed Name of Investigator

_____

Signature of Investigator

_____

Date

EMERGENCY CONTACTS

Table 1. Emergency Contact Information (Study AVE-901-102)

Role in Study	Name	Contact Information
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1. SYNOPSIS

Name of Sponsor/Company: Avenue Therapeutics, Inc.	
Name of Investigational Product: AVE-901	
Name of Active Ingredient: Tramadol for intravenous infusion	
Title of Study: A Phase 3, Multicenter, Randomized, Double Blind, Three-Arm Study to Evaluate the Efficacy and Safety of Tramadol Infusion (AVE-901) versus Placebo in the Management of Postoperative Pain Following Bunionectomy	
Study center: Approximately 6 centers in the United States	
Studied period: Estimated date first patient enrolls: Q3 2017 Estimated date last patient enrolls: Q2 2018 Estimated date primary analysis: Q2 2018	**Phase of development:** 3

Objectives:

The primary objective of this study is to evaluate the analgesic efficacy of intravenous (IV) tramadol (AVE-901) compared to placebo in the management of postoperative pain following orthopedic (bunionectomy) surgery. The Sum of Pain Intensity Differences (SPID) through 48 hours post first dose (SPID48) measured at rest will be used as the primary measure of efficacy.

The secondary efficacy endpoints are:

- SPID through 24 hours post first dose (SPID24) measured at rest
- Total consumption of rescue (supplemental) analgesia. This is the total amount of rescue analgesia given to the patient after first dose of study medication through 48 hours post-first dose.
- Patient Global Assessment of efficacy at 24 and 48 hours post first dose using a 5-point scale. The question to be posed is "How would you rate the study medication in terms of its effectiveness in controlling your pain?" (0=poor; 1=fair; 2=good; 3=very good; 4=excellent).

Additional analgesia endpoints are:

- Time-specific pain intensity profile over time
- Time (in minutes) to first rescue analgesia from the time of first dose of study medication.
- Number (percent) of patients who require no rescue analgesia from T0-T48.
- Rate of consumption of rescue analgesia. The total amount of rescue analgesia will be expressed as a function of duration of treatment
- Time (in minutes) to meaningful pain relief after first dose.
- Time (in minutes) to perceptible pain relief after first dose.

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Safety endpoints will include:

- Adverse events (classified by the MedDRA System Organ Class (SOC) and preferred term), including assessment of AE's related to substance abuse.
- Clinical laboratory, vital sign, PE, and ECG changes
- Use of anti-emetics and other concomitant medications
- Local tolerability at the infusion site via pain, swelling, tenderness, and erythema.

Population pharmacokinetic outcomes will be assessed through collection of plasma samples for a subset of the randomized patients. Exposure-response relationships will be assessed for safety outcomes.

Study Design:

This study is a Phase 3, multicenter, double-blind, three-arm, randomized, placebo-controlled, multiple-dose, parallel-group trial to evaluate the safety, tolerability and the efficacy of IV Tramadol (AVE-901) versus placebo in the management of postoperative pain in consenting patients undergoing a unilateral primary first metatarsal bunionectomy surgery. The treatment groups will be:

- AVE-901 50 mg, given at Hours 0, 2, 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, and 44.
- AVE-901 25 mg, given at Hours 0, 2, 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, and 44.
- Placebo, given at Hours 0, 2, 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, and 44.

Approximately 405 patients will be randomized (135 per treatment group).

Each patient will undergo the Screening Visit (Day -28 to Day -1), the pre-operative assessment (within 24 hours prior to surgery start time), the Surgical/Treatment Visit, the first day of which is when the bunionectomy will be performed (Day 0), the primary treatment period through Hour 48 (the last on treatment assessment, and the time of End of Treatment visit assessments), and the Follow-up Visit (Day 14). Confinement is anticipated to be a 4-day 3 night stay from Day -1 to hour 48.

Screening will occur up to 28 days prior to surgery. Following the pre-operative assessments, after the patient has met eligibility criteria, patients will be randomized in a double-blinded fashion, stratified by study center, in a 1:1:1 ratio to the treatment groups.

Following surgery, patients that have met post-surgical dosing criteria will receive his or her assigned study medication infusion regimen over a period of 48 hours. Patients will be confined at the healthcare facility during study drug administration, and will be discharged only if clinically stable.

Efficacy will be assessed by patient reports of pain intensity (PI) performed at rest immediately prior to T0 (the first administration of study drug) and at 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, and 48 hours after first treatment (ie, post T0). PI is reported by the patient on a numeric rating scale for pain (NPRS Pain, See Appendix 1) anchored at 0 for no pain and 10 for the worst pain imaginable. In addition, efficacy and safety will be measured by other parameters.

Rescue medication (Ibuprofen 400 mg Q4 PRN) will be available any time after the initial dose of study medication to keep the patient comfortable. However, patients will be encouraged to wait at least 60 minutes after the initial dose of study medication before they receive rescue therapy. A pain

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intensity measure will be obtained immediately prior to each administration of rescue medication. Patient controlled analgesia (PCA) will not be allowed in this study.

Study Periods and Methodology

There are 5 periods in this study: Screening, Preoperative, Operative, Post-operative/Treatment Period, and Follow-up. Procedures for each study period are described below.

Screening:

Screening will occur from Day -28 through Day -1 and will be conducted as a clinic visit. Patients will have the purpose and procedures of the study explained to them and, those who elect to participate in the study, will provide informed consent and be screened for participation according to the eligibility criteria. Patients will undergo protocol specific education including but not limited to the various post-treatment efficacy measures. Screening will include eligibility assessment, medical history, physical examination, demographics, height and weight, BMI, vital signs (heart rate, systolic blood pressure, and diastolic blood pressure, respiratory rate, temperature, pulse oximetry), ASA Physical Status, 12-lead electrocardiogram (ECG), hematology panel, chemistry panel, coagulation tests, infectious disease testing, urinalysis, urine drug screen, Alcohol breath test, serum pregnancy test (in females of childbearing potential), and prior/current treatments. All screening laboratory evaluations must be within acceptable limits as determined by the investigator prior to randomization.

Preoperative:

Preoperative assessments to confirm the patient's eligibility will be performed within 24 hours of scheduled surgery start time. This visit will include reassessment of eligibility criteria, interim medical history, vital signs, brief physical exam, ASA Physical Status, chemistry panel, hematology panel, coagulation tests, urinalysis, urine drug screen, Alcohol breath test, urine pregnancy test (in females of childbearing potential), and concomitant treatments. These safety laboratory tests will not be used for determining eligibility. Patients with Baseline laboratory values that are subsequently found to meet any exclusion criteria may be discontinued from the trial at the discretion of the investigator, medical monitor and sponsor. A blood sample will be obtained during the preoperative period and stored for possible analysis of CYP2D6 metabolizer status, in case such information is deemed to be of interest during the analysis. If all preoperative assessments meet eligibility (with the exception of the laboratory values noted) the subject will be randomized prior to surgery.

Operative:

Operative procedures are described in the Surgical and Anesthetic Regimen.

Post-operative/Treatment Period:

In addition to meeting all eligibility criteria prior to surgery, patients must ALSO meet all postsurgical dosing criteria. Treatment will occur from Hour 0 through Hour 44 (T0-T44).

- Following surgery, patients will have their popliteal block withdrawn approximately between 4 and 5 AM. Post removal of the popliteal block, patients must be assessed for the dosing eligibility criteria prior to dosing. Patients must be awake and alert, and must have a pain intensity of 5 or greater on the NPRS and report a score of moderate or severe on a 4-point categorical rating scale (with categories of none, mild, moderate, or severe) just before the first dose of the study drug. Patients who do not report pain at this level within 8 hours of the removal of the block will be discontinued from the study.
- Antiemetic treatment with Reglan or Emend (not Zofran or other $5HT_3$ antagonists) is allowed.

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T0 will be time of start of infusion of first study drug administration. Pain intensity will be recorded using a NPRS from 0 to 10, where 0 is no pain and 10 is the worst pain imaginable (See Appendix 1). Pain intensity assessments will be recorded immediately prior to first dose (baseline) and at the times indicated in the Schedule of Assessments. The patient must rest for 15 minutes (+/- 5 minutes) prior to NPRS assessments. The Patient Global Assessment (PGA) of efficacy will be completed by the patient at 24 hours and 48 hours post T0. Ratings will be assessed as: 0=poor; 1=fair, 2=good, 3=very good or 4=excellent. Two stopwatches will be started at the start of infusion of first study drug dose. Patients will be instructed to stop the first stopwatch when pain relief is first perceptible and the second when pain relief is considered meaningful. Safety will be assessed by recording vital signs including: respiratory rate, pulse oximetry reading, temperature, heart rate and blood pressure throughout the study period. At the end of treatment (post T48), patients will undergo End of Treatment assessments, including a brief physical examination, vital signs measurements, electrocardiogram (ECG) NPRS, PGA, and clinical laboratory evaluations for safety. AEs and concomitant medication use will be recorded. If feasible, any patient who prematurely discontinues study medication or the study prior to the T48 will undergo the procedures required at the end of treatment. Post T48, pain management will be per investigator standard of care preference. **Follow up:** A final safety assessment via telephone call will be conducted on Day 14 (± 2 days); this will be a telephone contact. **Rescue medication:** Inadequate analgesia may be treated with 400 mg Q4 of ibuprofen PRN administered by a trained nurse or study clinician to the patients who request rescue medication any time after administration of the initial dose of study medication. However, patients will be encouraged to avoid rescue medications for at least 60 minutes after their first dose of study drug. A NPRS will be obtained immediately (approximately 5 minutes) prior to administering any rescue medication. Patients will continue to take study medication for the remainder of the 48-hour period even if they take rescue medication. Ibuprofen dosing is allowed: 400 mg PRN every 4 hours up to 2400 mg per day. The time of rescue medication must be recorded in the case report form. Investigator study team personnel will monitor the patient carefully for 48 hours following the surgery to assess the patient's condition and provide rescue medication whenever requested, within the above limits. This will minimize the patient's inconvenience caused due to pain. **Pharmacokinetics**: Approximately 33% of the total number of patients will be enrolled in the pharmacokinetic (PK) portion of this study at select sites. Blood will be collected for analysis of tramadol and O-desmethyltramadol (active metabolite) at Hour 0, Hour 0.25, Hour 0.5, Hour 2.25, Hour 2.5, Hour 4, Hour 4.25, Hour 4.5, Hour 8, Hour 8.25, Hour 24.5, Hour 44, Hour 44.25, Hour 44.5, and Hour 48.
Number of patients (planned): A total of approximately 405 patients in United States (US) undergoing bunionectomy who meet all of the inclusion and none of the exclusion criteria, randomized, have at least one pain intensity measurement and receive at least a partial dose of study drug will be included in the study population. Patients will be randomized to one of three treatment arms (AVE-901 50 mg, AVE-901 25 mg, or

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placebo) in a 1:1:1 ratio (approximately 135 patients per treatment group). Randomization will be stratified by study center.
Diagnosis and main criteria for inclusion: **Inclusion Criteria:** 1. The patient is male or female 18-75 years of age undergoing unilateral first metatarsal bunionectomy surgery 2. The patient or legal representative has voluntarily signed and dated an Institutional Review Board (IRB)/Independent Ethics Committee (IEC) approved written informed consent. 3. Female patients must be of non-childbearing potential (surgically sterile or post-menopausal for at least 1 year) or be practicing a highly effective contraception method from consent to at least 7 days after the last dose of study medication. Highly effective contraception methods include: vasectomized partner (at least 6 months prior to dosing); double barrier (diaphragm with spermicide; condoms with spermicide); intrauterine device; implanted or intrauterine hormonal contraceptives in use for at least 6 consecutive months prior to study dosing and throughout the study duration; oral, patch, or injected contraceptives in use for at least 3 consecutive months prior to study dosing. 4. Female patients of childbearing potential have a negative pregnancy test (serum human chorionic gonadotropin [HCG]) during screening and a negative pregnancy test (urine) ≤ 24 hours prior to surgery, and must not be lactating. 5. The patient must be willing to be housed in a healthcare facility and able to receive parenteral analgesia for at least 72 hours after surgery. 6. The patient meets definition of American Society of Anesthesiologists (ASA) Physical Class 1, or 2. 7. The patient is willing and able to understand the study procedures and the use of pain scales, to communicate meaningfully with the study personnel, and to comply with the study protocol. **Exclusion Criteria:** 1. Patient is not expected to receive a continuous infusion nerve block as described in the Post-Op anesthetic procedures protocol. 2. Patient is currently using, or is expected to receive gabapentin, pregabalin, ketamine or other peri- or postoperative analgesic adjuncts or any analgesics not allowed in the Surgical and Anesthesia protocol. 3. Patient is undergoing bilateral or revision bunionectomy surgery. 4. Patient has a history of primary or metastatic bone cancer or Paget's disease.

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5. The patient has current or historical evidence of any clinically significant disease or condition that might increase the risk of surgery or complicate the patient's postoperative course.
6. The patient has allergy or hypersensitivity (or is intolerant) to opioids or tramadol.
7. The patient has known physical dependence on opioids.
8. Patients who have taken any opioids, including tramadol for a total of 3 days, within 30 days prior to surgery.
9. The patient has a recent (within 5 years) and/or current history of chronic analgesic, opiate or tranquilizer abuse or dependence or is a user of illicit drugs, or has had a recent history (within 2 years) of drug or alcohol abuse.
10. The patient has another painful physical condition that, in the opinion of the investigator, may confound the assessments of postoperative pain.
11. The patient has taken other prior/concurrent chronic medications that have not been at a stable dose for at least 2 weeks prior to screening.
12. The patient is taking herbal or dietary supplements or medications that are moderate or strong inhibitors of CYP2D6 or CYP3A4 (e.g., fluoxetine, paroxetine, amitriptyline, quinidine, ketoconazole, erythromycin, grapefruit juice) or inducers of CYP3A4 (e.g., carbamazepine, rifampin, St. John's Wort) and cannot go through a minimum washout period of 7 days prior to surgery.
13. The patient has taken monoamine oxidase (MAO) inhibitors, trazodone, or cyclobenzaprine within 14 days prior to surgery.
14. The patient has a history of epilepsy, or is known to be susceptible to seizures.
15. The patient cannot be withdrawn from medications (at least 7 days prior to surgery) that may lower the seizure threshold (e.g. anti-psychotic agents, MAOI inhibitors) or which increase serotonergic tone (e.g. selective serotonin reuptake inhibitors (SSRIs), serotonin norepinephrine reuptake inhibitors (SNRIs), tricyclic antidepressants, triptans, amphetamines).
16. The patient has a history of Long QT Syndrome or a relative with this condition.
17. The patient has had a recent cardiovascular event or a clinically significant abnormal ECG at screening.
18. The patient has used (within 30 days of surgery), is currently using or is anticipated to use chronic corticosteroids.
19. The patient has expressed suicidal ideation or is considered to be at risk of suicide.
20. The patient is morbidly obese (body mass index [BMI] $\geq$ 40 kg/m2) or has documented sleep apnea requiring pharmacological or device intervention.

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21. The patient has a history of cardiopulmonary, neurological or psychiatric condition that may confound the assessments of efficacy or safety.
22. The patient has cirrhosis, moderate or severe hepatic impairment or an alanine aminotransferase (ALT) or aspartate aminotransferase (AST) value > 3X upper limit of normal (ULN) at Screening.
23. The patient has severe renal impairment or a serum creatinine value of > 2.0 mg/dL at Screening.
24. The patient has potassium, sodium, calcium or magnesium levels outside of the normal range, or any other clinically significant abnormalities in blood chemistry at Screening.
25. The patient has a hemoglobin level at screening which, in the judgment of the Investigator, is not suitable for participation in this study.
26. The patient used narcotics or alcohol within 24 hours prior to surgery.
27. The patient was administered an investigational product within 30 days prior to Screening.
28. The patient has previously participated in a clinical study with AVE-901.

POST SURGICAL DOSING CRITERIA

The patient is to be evaluated post-operatively prior to the initial treatment with the study drug and will be given the study drug only if all of the following criteria are met:

1. The patient reports a score of moderate or severe on a 4-point categorical rating scale (with categories of none, mild, moderate, or severe) and has a NPRS pain score of ≥5 (on a scale from 0 to 10) within 8 hours after removal of popliteal block.
2. The patient is alert and oriented. She/he is able to answer questions and follow commands and has appropriate cognitive function to properly interpret and answer protocol mandated assessments.
3. The patient has no evidence of respiratory insufficiency, such as a respiratory rate that is less than 8 breaths per minute or arterial oxygen saturation by pulse oximetry of less than 90% with supplemental oxygen.
4. The surgical procedure from incision to closure was not longer than 180 min.
5. There have been no significant deviations from the surgical or anesthetic protocol that would, in the opinion of the investigator, put the patient at risk of participation in the trial, confound the analgesic endpoints of the trial or cause concern regarding the patient's ability to participate in the trial.
6. There have been no use of gabapentanoids, or 5-HT_3 antagonists (e.g. ondansetron, granisetron, palonosetron) preoperatively, intraoperatively, or postoperatively.

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7. Subject has not experienced complications during the surgical procedure and in the opinion of the investigator is a candidate to continue in the study.

Investigational product, dosage and mode of administration:

AVE-901 50 mg, or 25 mg will be added to a 50 ml saline bag and administered intravenously approximately over 15 minutes +/- 2 minutes via infusion pump.

A trained health care professional will flush the line with normal saline at the end of each infusion.

Duration of treatment:

Study drug will be administered as 13 infusions, given at T0, T2, T4 and one infusion every 4 hours thereafter. T0 is defined as the start of the infusion of the first dose. The last dose will be administered at 44 hours following initiation of the first dose.

Total patient participation in this study, from initial screening through the Day 14 final assessment, is expected to be between 3 and 6 weeks.

Reference therapy, dosage and mode of administration:

Placebo (saline) (51 mL) will be administered in the same manner as the investigational product.

Criteria for evaluation:

Efficacy:

Primary Endpoint

- SPID48 for pain at rest

Key Secondary Endpoints

- SPID through 24 hours post first dose (SPID24) at rest
- Total consumption of rescue (supplemental) analgesia. This is the total amount of rescue analgesia given to the patient after first dose of study medication through 48 hours post-first dose.
- Patient Global Assessment of efficacy at 24 and 48 hours

Tertiary Endpoints

Additional analgesia endpoints are:

- Time-specific pain intensity profile over time
- Clock time (in minutes) to first use of rescue medication from the time of first dose of study medication.
- Number (percent) of patients who require no rescue analgesia from T_0-T_{48}
- The rate of consumption of rescue analgesia
- Time (in minutes) to meaningful pain relief after first dose
- Time (in minutes) to perceptible pain relief after first dose

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Safety: • AEs, including assessment of infusion site local reactions (skin and vein) • AEs related to substance abuse • Clinical laboratory tests (hematology panel, chemistry panel, coagulation and urinalysis) • Vital signs • Physical examination • 12-lead ECG. Of special note, an ECG will be performed between 20 and 30 minutes following the start of EACH dose during the first 24 hours of treatment. • Use of anti-emetics and other concomitant medications **PK:** • Plasma sampling in a subset (approximately 33%) of the population is planned at designated time points. Exposure-safety relationships will be assessed (including but not necessary limited to ECG, adverse event, and concomitant therapy/drug-drug interaction outcomes).

Statistical methods:

Sample Size Considerations:

A sample size of 135 patients per arm (405 patients in total) provides over 90% power to detect a SPID48 difference of 250 between treatment with placebo and treatment with AVE-901, assuming a common SPID48 standard deviation of 600 (and thus an approximate 40% effect size), an alpha of 0.05 using a two-sided test of mean differences.

General Statistical Methods

The baseline for all variables will be the last measurement obtained prior to the patient receiving the first dose of randomized study drug.

Patient disposition (including the number and percent of patients who are randomized, who receive randomized treatment, who prematurely discontinue and reasons for discontinuation, and who complete the study) will be tabulated by treatment group. The number (%) of patients by exposure will be tabulated.

Baseline comparability among the two treatment groups with respect to demographic and other baseline characteristics will be assessed for quantitative variables with a two-way analysis of variance (ANOVA) with treatment group and study center as main effects. For categorical variables, the Cochran-Mantel-Haenszel test will be used, stratified by center.

Study Populations

- The Full Analysis Set (FAS) population is defined as all randomized patients who receive study medication. Patients will be analyzed according to the treatment group they were randomized to.
- The Safety Population is defined as all patients who receive study medication. Patients will be analyzed according to the actual treatment they receive.

Alpha Levels

There are 2 pairwise comparisons of interest:

- High-dose tramadol (50 mg) vs placebo
- Low-dose tramadol (25 mg) vs placebo

All inferential assessments will be 2-sided tests performed at the 0.05 alpha level. A hierarchical alpha testing strategy will be utilized to control for the overall experiment-wise alpha. As there are multiple tests being performed (the single primary efficacy variable pairwise test and the 3 key secondary efficacy tests), the following strategy will be applied:

The high-dose tramadol arm vs placebo comparison will be assessed for the primary endpoint at the 0.05 alpha level. If and only if the p-value is ≤ 0.05 for this pairwise comparison will the lower dose tramadol arm vs placebo comparison be assessed. If the p-value for the high-dose arm is NOT significant, all testing will cease and it will be concluded that neither tramadol treatment arm provides better pain relief than placebo.

If the primary endpoint is significant for the AVE-901 vs. placebo comparison (in favor of the tramadol arm) for one or both pair-wise tests, then statistical testing will proceed to the secondary endpoints within each pair-wise grouping, to be tested in the following order:

- SPID24
- Total consumption of rescue analgesia

- Patient Global Assessment of efficacy at 24 and 48 hours (the two time points will be tested simultaneously)

If a statistical test within each pairwise comparison is significant at the nominal 0.05 level, two-sided (in favor of the tramadol arm), then testing will proceed to the next endpoint in the list. Once a non-significant test occurs, endpoints lower in the list are considered not statistically significant.

Note that while inferential comparison of the high dose to the low dose is not a key aspect of the statistical analysis, assessment of the dose-response will be performed across the 3 doses in order to allow visual comparison of the outcomes for the primary and key secondary endpoints.

Subgroups

Analysis of the primary and key secondary endpoints, as well as treatment-emergent AE and serious AE incidence, will be provided by the following subgroups:

- Gender
- Race
- Age (using the study median age)
- Investigational center

Efficacy

Primary Endpoint

$SPID_{48}$ (the sum of the time-weighted pain intensity differences for the time period 0-48 hours) is the primary efficacy endpoint.

Handling of Missing Data

For the primary causal estimate in this study (to reflect the true treatment effects of tramadol compared to placebo), missing values within a patient during the 48-hour treatment period will be handled in the following manner:

- When rescue medication is used, the last pain intensity measure prior to the use of rescue medication will be used (imputed) for the subsequent protocol-specified time points for measurement of pain intensity through 4-hours after the time of the rescue medication.

All other missing data will be imputed utilizing multiple imputation methods. Multiple imputation provides a useful strategy for analyzing data sets with missing values. Instead of filling in a single value for each missing value, Rubin's (1976) multiple imputation strategy replaces each missing value with a set of plausible values that represent the uncertainty about the correct value to impute.

For patients with arbitrary missing data patterns (missing at random, MAR), a pattern-mixture approach will be taken to the imputation. The first step will be to apply a Markov Chain Monte Carlo (MCMC) method (Schafer 1997) that assumes multivariate normality will be used to impute all missing values to make the imputed (resulting) data sets have strictly monotone missing patterns. The resulting monotone missing pattern will then, in a second imputation step, be used to impute the remaining missing values; specifically, a regression-based method for continuous variables will be applied.

For patients with missing data as a result of discontinuation due to adverse event (missing not at random, MNAR), the pattern-mixture approach will also be taken to the imputation (as for the

monotone MAR data), with the difference that while a similar regression-based method for continuous variables will be applied, a scaled (constant) adjustment to the imputed values will be made such that the outcomes that are imputed are distributionally 'worse' than those imputed for the monotone MAR data. This scaled adjustment will increase each imputed value (from the regression) for these MNAR data by a constant of '1', eg, if the regression provides an imputed value of 4.6 for a given missing value for a patient at a given timepoint, the MNAR imputed value will be set to 4.6 + 1 = 5.6 for that patient at that time point. This will, in effect, guarantee that the overall *distribution* of missing values for discontinuations due to adverse event is WORSE than that for other reasons.

The SAS software system will be used to perform this imputation. Because the imputation of missing data is a key aspect to the analysis of pain data, explicit details regarding this imputation are provided via sample SAS code that is intended to demonstrate the application of these strategies. Variable definitions are:

- TRT=treatment group (1 or 0)
- Pain_1 is the first time point for the pain score
- Pain_2 is the second time point for the pain score
- Pain_last is the last time point for the pain score. Additional pain scores (between Pain_1 and Pain_last) would be included in this model according to the time points for collection.

The first step will be to impute partially in order to obtain a monotone missing data pattern.

```
proc mi data=DATAIN out=DATAIN_MONO nimpute=100 seed=123;
    var TRT Pain_1 Pain_2 …. Pain_last;
    mcmc chain=multiple impute=monotone;
    run;
```

The second step will be to impute the remaining (monotone) missing data that is MAR for each of the 100 imputed datasets from the first step.

```
proc mi data=DATAIN_MONO out=DATAIN_REG seed=465 nimpute=1;
    by _Imputation_;
    var TRT Pain_1 Pain_2 …. Pain_last;
    class TRT;
    monotone regression;
    run;
```

In the case of missing data due to an adverse event, the following step will be used:

```
proc mi data=DATAIN_MONO out=DATAIN_REG seed=465 nimpute=1;
    by _Imputation_;
    var TRT Pain_1 Pain_2 …. Pain_last;
    class TRT;
    monotone regression (pain1 pain2 … Pain_last);
    mnar adjust (pain1 pain2 … Pain_last / shift=1);
    run;
```

Note that the regressions do not include the treatment variable, and thus the imputed data at each time point will be based on the distribution of all data rather than treatment-group specific distributions. A total of 100 imputed datasets will be created using these imputed data and analyzed according to the primary method of statistical analysis.

Primary Method of Statistical Analysis

Each of the 100 imputed datasets will be analyzed as follows:

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- An analysis of covariance (ANCOVA) model will be used to test the primary efficacy endpoint. The model will use treatment and study center as main effects, and baseline pain intensity (NPRS scale 0 to 10) as the covariate (See Appendix 1). Data from all three treatment groups will be included in the same ANCOVA model for purposes of the testing procedures.

The 100 resulting treatment effect parameters and standard errors from these ANCOVA will be combined to provide a distribution of parameters (and standard errors) upon which the primary hypothesis of treatment effect will be determined.

Key Secondary Endpoints

The key secondary endpoints will be assessed as follows:

- 24-hour pain intensity (SPID24) will be assessed in a similar manner as the primary endpoint.
- Total consumption of rescue analgesia will be analyzed nonparametrically using the Wilcoxon rank sum test.
- Patient Global Assessment at scheduled evaluations will be assessed for treatment group differences using an ANCOVA comparison with center and the baseline pain score as the stratification factor.

Tertiary endpoints

- Time-specific pain intensity profile over time will be assessed using a mixed models repeated measures (MMRM), with fixed effects for treatment, time, the treatment by time interaction, and random patient effect. Pairwise comparisons at each time point will be determined from the MMRM, and model-based means (LSMeans) and standard errors will be plotted for visual comparison of the treatment group outcomes.
- The time to pain relief (meaningful and perceptible), as well as time to first rescue medication, will be assessed using a log-rank statistic, stratified by center. A Kaplan-Meier plot will be provided for each of these outcomes. The time to event analyses will include the times to events of death or withdrawal from study as endpoints.

Name of Sponsor/Company: Avenue Therapeutics, Inc.

Name of Investigational Product: AVE-901

Name of Active Ingredient: Tramadol for intravenous infusion

Patients will be censored at 4 hours for time to pain relief, while time to first rescue medication will be censored at 48 hours.

- The number (%) of patients who require no rescue analgesia during the 48-hour period following T0 will be assessed using a CMH test stratified by center.
- The rate of consumption of rescue analgesia will be analyzed nonparametrically using the Wilcoxon rank sum test.

Pharmacokinetics

For the subset of patients undergoing PK blood sampling, descriptive statistics will be calculated for the plasma concentrations of tramadol and O-desmethyltramadol collected at the designated sample collection times. An assessment of the exposure-response profile for key safety outcomes (ECG changes, select adverse events, drug-drug interactions) will be performed for both parent and metabolite. Population pharmacokinetic methods (via exploration of potential intrinsic and extrinsic factors) may be applied if numerical assessments demonstrate potential associations between exposure and response.

Safety

All AEs will be classified with respect to the MedDRA System Organ Class (SOC) and preferred term. The number and percent of patients who report treatment-emergent adverse events (TEAEs) will be summarized for each treatment group. Additional summaries by severity, relationship, and subgroup will be presented. Serious AEs (SAEs) will be summarized similarly.

Exploratory analyses of use of anti-emetics may be performed. Other safety data presentations will be descriptive in nature and no formal statistical tests will be performed.

ECG results will be analyzed on an ongoing basis by a central ECG reader on an individual patient level and as well as by group analysis

Clinical laboratory and vital signs will be summarized descriptively by time point. Local tolerability at the infusion site will be assessed for pain, swelling, tenderness, and erythema. Adverse events suggestive of abuse liability will be summarized separately. These abuse-related AEs include euphoria-type adverse events, hallucinations and inappropriate affect, and withdrawal events after abrupt discontinuation of treatment.

2. TABLE OF CONTENTS, LIST OF TABLES, AND LIST OF FIGURES

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3. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

The following abbreviations and specialist terms are used in this study protocol.

Table 2 Abbreviations and Specialist Terms (Study AVE 901-102)

Abbreviation or special term	Explanation
°C	Degrees Celsius
°F	Degrees Fahrenheit
µM	Micromolar
5-HT_3	5-Hydroxytryptamine
AE	Adverse Event
AIDS	Acquired Immunodeficiency Syndrome
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
AUC	Area under the concentration-time curve
$AUC_{0\text{-tlast}}$	Area under the concentration-time curve from time 0 to the last measurable concentration; calculated using linear trapezoid rule
$AUC_{0\text{-inf}}$	Area under the concentration-time curve from time 0 to infinity
$AUC_{0\text{-}24}$	Area under the plasma concentration vs. time curve from time 0 to 24 hours
BLQ	Below the Lower Limit of Quantitation
BMI	Body Mass Index
BP	Blood Pressure
BUN	Blood Urea Nitrogen
C_{12}	Plasma concentration at 12 hours after oral drug administration
C_{24}	Plasma concentration at 24 hours after oral drug administration
C_{max}	Maximum plasma concentration
C_{min}	Minimum plasma concentration
CFR	Code of Federal Regulations
CHF	Congestive Heart Failure
CL/F	Oral clearance
CNS	Central nervous system
Conmed	Concomitant Medication
CRA	Clinical Research Associate
CRO	Clinical Research Organization
CRU	Clinical Research Unit
CV	Coefficient of variance
dL	Deciliter(s)
DMP	Data Management Plan
ECG	Electrocardiogram

Abbreviation or special term	Explanation
eCRF	Electronic Case Report Form
EDTA	Ethylenediaminetetraacetic Acid
FOB	Functional Observational Battery
FU	Follow-up
g	Gram
GCP	Good Clinical Practice
GGT	Gamma-glutamyl-transferase
HBsAg	Hepatitis B Surface Antigen
Hct	Hematocrit
HCV	Hepatitis C virus
HIV, HIV-1	Human Immunodeficiency Virus
Hgb	Hemoglobin
HPBL	Human Peripheral Blood Lymphocytes
hr(s)	Hour(s)
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IND	Investigational New Drug Application
IRB	Institutional Review Board
IUD	Intrauterine device
kg	Kilogram
L	Liter
LDL	Low Density Lipoprotein
MAOI	Monoamine Oxidase Inhibitors
mL	Milliliter
MedDRA	Medical Dictionary for Regulatory Activities
min(s)	Minute(s)
mg	Milligram
mL	Milliter
mm	Millimeter
msec	Millisecond
N/A	Not Applicable
ng	Nanogram
NOAEL	No Observed Adverse Effect Level
pH	Hydrogen Ion Concentration
PHI	Personal Health Information

Abbreviation or special term	Explanation
PI	Protease inhibitor
PIS	Patient Information Sheet(s)
PK	Pharmacokinetic
PR	Pulse Rate
QTc	The QTc interval is the corrected QT interval, adjusted for heart rate
RBC	Red Blood Cell
rpm	Revolutions Per Minute
RR	Respiratory Rate
RTV	Ritonavir
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SDV	Source Document Verification
SSRI	Selective serotonin reuptake inhibitor
$t_{1/2}$	Plasma elimination half-life
t_{lag}	Time to the first measurable plasma concentration
t_{max}	Time to reach peak plasma concentration
UDS	Urine Drug Screen
ULN	Upper limit of normal
V_{ss}	Apparent Volume of Distribution
WBC	White Blood Cell
WHO	World Health Organization

4. INTRODUCTION

Effective postoperative pain control is a critical need as most patients undergoing surgical procedures experience pain immediately following the procedure, and require treatment for several days. For instance, patients undergoing total knee arthroplasty (TKA) or bunionectomy typically demonstrate a need for short-term analgesia, which is critical for earlier mobilization and rehabilitation. In this setting, reducing pain intensity without providing extensive medical oversight required for some methods of treatment (such as neuraxial anesthesia) and prevention of effects such as opiate-induced respiratory depression and dependency would be highly beneficial (Sinatra et al., 2002).

Several options are available for postoperative pain management (Singelyn et al., 1998; Sinatra et al., 2002). Options include intermittent "on-demand" analgesia, including oral and via patient controlled analgesia (PCA) or by nurse-administered bolus injections of systemic opioids such as morphine. Second, continuous epidural analgesia with opioids and/or local anesthetics is effective, although this requires continued presence of the epidural catheter and oversight by an anesthesiologist. A third alternative is to provide a combination of nerve blocks with long-acting local anesthetics and/or opioids initiated intra-operatively and continued into the immediate postoperative period.

4.1. Tramadol Infusion

Tramadol is a centrally acting synthetic analgesic with a dual mechanism of action, comprised of μ-opioid activity and monoamine (serotonin and noradrenalin) reuptake inhibition. Tramadol is an analog of the phenanthrene group of opium alkaloids, which includes morphine and codeine, and is structurally related to these opioids (Grond and Sablotzki, 2004). Like codeine, there is a substitution of the methyl group on the phenol ring that imparts a relatively weak affinity for opioid receptors.

Tramadol was originally developed by the German pharmaceutical company Grünenthal GmbH in the late 1970s and is marketed globally under the trade names TRAMAL® and others outside of the United States (US). The approved doses of tramadol are 50 mg or 100 mg administered as a slow injection every 4-6 hours (Tramadol Core Product Label, 2008).

In the US, tramadol is approved by the Food and Drug Administration (FDA) and marketed as an oral tablet (the only available formulation) for moderate to moderately severe pain in adults. Tramadol was first approved in the US in April 1995 under the trade name ULTRAM® (Ortho-McNeil-Janssen Pharmaceuticals, Inc.). Tramadol is also an active agent in an extended release (ER) product, Ultram® ER, and as a combination product with acetaminophen, ULTRACET®. In the US, tramadol is only available as immediate release tablets or extended release tablets.

Tramadol injection (IV/IM/SC) is approved and used for the management of moderate to severe acute postoperative pain in several regions, including Europe, India and Australia/New Zealand; however, this dosage form is not available in the US. Tramadol ampoules or vials for parenteral (intravenous [IV], intramuscular [IM] and subcutaneous [SC]) administration and preservative-free solutions for injection by the various spinal routes (epidural, intrathecal, caudal, etc.) are available forms in these regions. Other tramadol formulations approved in several countries include tablets, capsules, effervescent powders, and suppositories (Grond and Sablotzki, 2004; Rosenberg, 2009).

There is extensive data demonstrating that tramadol use is not associated with the classic-opioid side effects seen with more potent opioids. There are numerous reports of the safety and efficacy of tramadol in this setting (Scott and Perry, 2000; Grond and Sablotzki, 2004).

4.2. Nonclinical Summary

Tramadol is a centrally-acting synthetic analgesic of the aminocyclohexanol group with opioid-like effects. Tramadol is extensively metabolized following administration, which results in a number of enantiomeric metabolites that display different opioid-receptor binding properties, and monoaminergic reuptake inhibition (Grond and Sablotzki, 2004).

Both enantiomers of tramadol and (+)-M1 are responsible for the analgesic effect. The primary metabolite [(+)-M1 or (+)-O-desmethyltramadol] of tramadol confers significant μ-opioid activity; (+)-tramadol confers weak μ-opioid activity and significant serotonin reuptake inhibition; and (−)-tramadol is responsible for the inhibition of noradrenaline re-uptake (Gillen et al., 2000; Raffa, 2008). Nonclinical studies have shown that antinociception induced by tramadol is only partially antagonized by the opiate antagonist, naloxone, indicating that non-opioid mechanisms are also involved in its pharmacodynamic action (Collart et al., 1992).

Consistent with the known clinical effects of opioids, non-clinical safety pharmacology studies have shown that tramadol at high doses affects the central nervous system (CNS), producing sedation, impaired mobility, vomiting (dogs), decreased activity, and convulsions (Matthiesen et al., 1998). Also consistent with clinical effects, changes in blood pressure have been observed in cardiovascular studies in rats at high doses (Raimundo et al., 2006).

The toxicity of tramadol has been summarized by Matthiesen et al. (1998). The single dose toxicity of tramadol was similar in all species tested, independent of the route of administration. Notable acute findings included restlessness, unsteady gait, reduced spontaneous activity, exophthalmus, mydriasis, salivation, vomiting (dog), tremor, convulsions, slight cyanosis and dyspnea. The principle findings in repeat-dose toxicity studies in rats and dogs were behavioral/clinical signs and convulsions at doses of ≥25 mg/kg/day. The kidney and liver were identified as potential target organs in rats, with mild effects (minimal tubular vacuolization and perivenular hydropic degeneration, respectively) following repeat intraperitoneal dosing at high doses of tramadol.

There was no evidence of genotoxic potential for tramadol in standard in vitro and in vivo studies (Matthiesen et al., 1998). Carcinogenicity bioassays in mice and rats showed no evidence of carcinogenic potential. An extensive reproductive and teratology program revealed no safety concerns with respect to fertility or teratogenic effects after oral administration (Matthiesen et al., 1998; Yamamoto et al., 1972). Toxicity to offspring only occurred at doses associated with maternal toxicity.

In conclusion, none of the results in non-clinical toxicity studies indicated a safety concern regarding administration of AVE-901 at the intended clinical dose.

4.3. Pharmacokinetic Profile

Following oral administration, tramadol is rapidly and almost completely absorbed. The pharmacokinetics (PK) of tramadol were evaluated in healthy male volunteers (n=10) in a crossover design using 100 mg (PO) or IV doses (Lintz et al., 1986). Peak serum concentrations

(Tmax) were reached approximately 2 hours after oral dosing and the peak serum concentration (Cmax) for PO tramadol was 280±49 ng/mL. The terminal half-life was 5.1 hours for PO and 5.2 hours for IV administration. The area under the serum tramadol concentration-time curve (AUC) was 2488±774 ng·h/mL for PO and 3709±977 ng·h/mL for IV administration. Total clearance was 467±124 mL/min for PO and 710±174 mL/min for IV administration. The absolute bioavailability of the oral dose was 68±13%, based on comparison of the AUC values, while the estimated absorption of the oral dose was 86-88%. The difference between absorption and bioavailability was attributed to first pass metabolism, which was estimated to be ~20%. However, the absolute bioavailability approaches 90-100% with continuous dosing, probably due to saturation of first pass metabolism (Liao et al., 1992). Other studies have corroborated these findings (Grond and Sablotzki, 2004).

Tramadol undergoes hepatic metabolism and both the parent drug and the active metabolite are excreted by the kidneys. The only known active metabolite, M1 (O-desmethyltramadol), is produced by the action of cytochrome P450 CYP2D6 isozyme of the cytochrome P450 enzyme system. It has a half-life of approximately 6.7 hours after oral administration (single dose of 100 mg), compared to a half-life of 5.6 hours for tramadol. Hepatic impairment results in decreased metabolism of both the parent compound and the active metabolite. Elimination half-life increases approximately 2-fold in patients with renal or hepatic impairment. Patients who metabolize drugs poorly via CYP2D6 (Caucasian population prevalence ~ 8%) may obtain reduced benefit from tramadol due to reduced formation of M1 (Ultram® Prescribing Information, Ortho McNeil-Janssen, 2009).

A Phase 1 study was performed to determine a treatment regimen of IV tramadol that would be comparable to the approved 100 mg dose of oral tramadol at steady state [Study title: "A Phase 1, Open-Label, Single Center, Three-Period, Multi-dose Crossover Study to Evaluate the Pharmacokinetics of Two Difference AVE-901 (Tramadol Infusion) Regimens versus Oral Tramadol Tablets" (Study AVE-901-101)].

The two different IV regimens were evaluated and compared to the oral regimen. A total of 18 patients, ages 24 to 55 years (inclusive) were enrolled (3 patients to each of the 6 sequences). The study included 11 males and 7 females. 17 patients completed all 3 treatment sequences.

The treatment regimens evaluated were:

1. 75 mg IV REGIMEN: IV tramadol 75 mg administered at Hour 0, followed by 75 mg at Hour 3 and Hour 6, and 75 mg every 6 hours thereafter through Hour 42
2. 50 mg IV REGIMEN: IV tramadol 50 mg administered at Hour 0, followed by 50 mg at Hour 2, 50 mg at Hour 4, and 50 mg every 4 hours thereafter through Hour 44
3. ORAL REGIMEN: Oral tramadol 100 mg (50 mg tablets x 2) at Hour 0, 6, and every 6 hours thereafter through Hour 42

Examination of the parent (tramadol) as well as the primary metabolite, M1 (O-desmethyltramadol), was performed over the 48-hour treatment period. A focus of the analysis was on assessment of C_{max} values (to ensure the C_{max} for the IV formulation was similar to that of the oral formulation) as well as on early concentrations during the first doses (to ensure adequate medication would be provided during the initial 6 to 12 hours of treatment as the drugs

reached steady-state concentrations). Overall exposure to tramadol was estimated primarily from average trough plasma concentrations.

Figure 1 provides the mean plasma tramadol time-concentration profiles for the 100 mg oral, 50 mg IV, and 75 mg IV regimens from Study AVE 901-101. Mean plasma tramadol concentrations were higher after the 75 mg IV regimen compared to the 50 mg IV regimen and 100 mg PO q6h. As evidenced from the trough/pre-dose samples between 24 and 42 h, the mean tramadol trough concentrations were very similar for the 50 mg IV regimen and 100 mg PO q6h but somewhat lower for the 75 mg IV regimen.

Tramadol peak and trough concentrations for the 50 mg IV and the 100 mg PO q6h regimens were very similar at the end of the pharmacokinetic sampling period, between approximately 44 and 48 h.

Figure 1: Mean Plasma Tramadol Time-Concentration Profiles for 100 mg oral, 50 mg IV, and 75 mg IV Regimens (Study AVE-901-101)

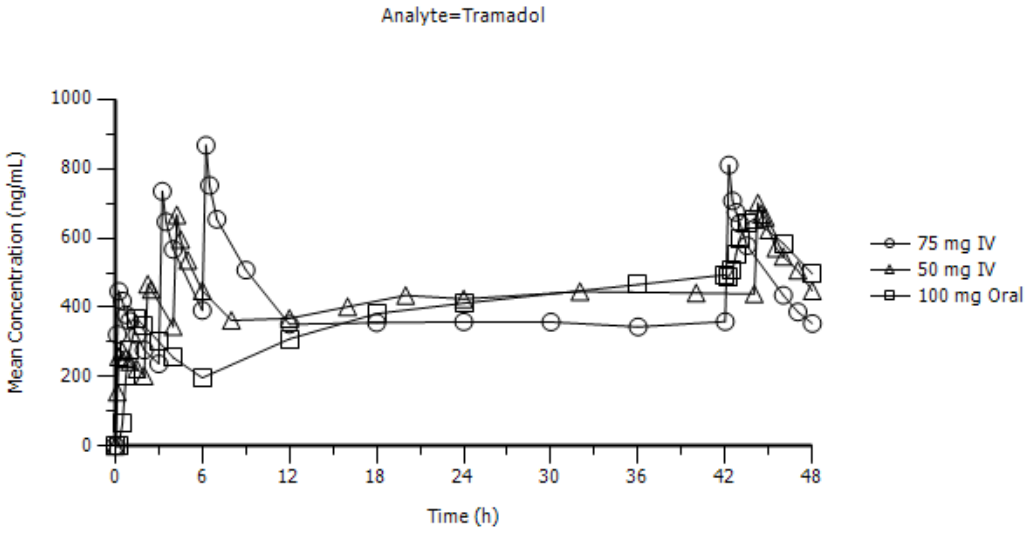
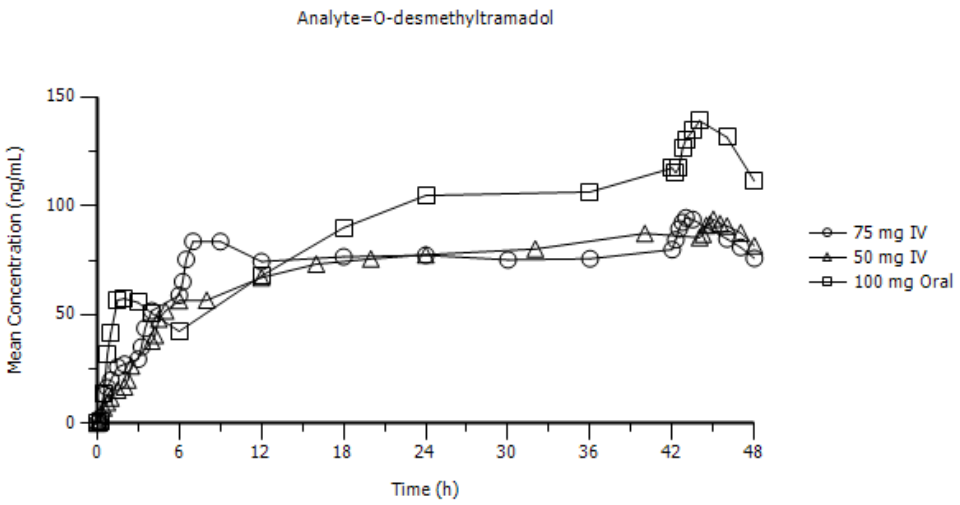


Figure 2 provides the mean plasma O-desmethyltramadol time-concentration profiles for the 100 mg oral, 50 mg IV, and 75 mg IV regimens from Study AVE 901-101. The mean plasma O-desmethyltramadol concentrations were higher for the 75 mg IV regimen following the 3rd dose at 6 h, but there was appreciable overlap of the trough concentrations for the 75 mg IV and 50 mg IV regimens between 24 and 42 h. The pre-dose concentrations as well as the concentrations after the last dose at 42 h were higher for 100 mg PO q6h compared to both IV arms, presumably due to first pass metabolism which results in a higher fraction of the active metabolite in systemic circulation after oral administration.

Figure 2: Mean Plasma O-desmethyltramadol Time-Concentration Profiles for 100 mg oral, 50 mg IV, and 75 mg IV Regimens (Study AVE-901-101)



Select pharmacokinetic parameters (overall C_{max}, C_{max} at steady state, trough at steady state, AUC over the last dosing interval for each regimen, i.e., $AUC_{tau\ n}$) for tramadol are summarized in Table 3.

Table 3: Additional Plasma Pharmacokinetic Parameters of Tramadol (Study AVE-901-101)

Parameter	75 mg IV				50 mg IV				100 mg Oral			
	n	Mean	SD	CV%	n	Mean	SD	CV%	n	Mean	SD	CV%
C_{max} (ng/mL)	14	932	199	21.30	14	736	152	20.60	17	701	178	25.44
$C_{max(42-48)}$ (ng/mL)	14	827	234	28.24	-	-	-	-	17	701	178	25.44
$C_{max(44-48)}$ (ng/mL)	-	-	-	-	14	711	152	21.40	-	-	-	-
T_{48} (ng/mL)	14	354	85.9	24.31	14	448	131	29.36	17	497	144	29.09
Css (ng/mL)	14	506	101	20.03	14	557	131	23.60	17	579	150	25.96

The 50 mg IV regimen provided favorable C_{max} and AUC values over the full 48 hour treatment period. Specifically:

- Overall C_{max} was comparable between the 50 mg IV and 100 mg PO regimens but was achieved earlier with the IV regimen
- Exposure to tramadol at steady state (or near steady state, in the case of the oral regimen), based on C_{max} and AUC, was also comparable between the 50 mg IV regimen and 100 mg PO q6h
- The 50 mg IV regimen, as compared to the 75 mg IV regimen, resulted in less peak to trough fluctuation with lower C_{max}

- Exposure to O-desmethyltramadol was higher after 100 mg PO q6h compared to either IV treatment, 50 mg IV or 75 mg IV regimens, based on AUC and C_{max} values

Based on these findings, the 50 mg IV regimen will be studied in this current protocol.

4.4. Rationale for this Study

This double-blind efficacy and safety study will be conducted in addition to one other efficacy Phase 3 study and one open-label safety study. This efficacy study is being performed in an orthopedic model (AVE-901-102), while the other Phase 3 efficacy study is being performed in a soft tissue model (AVE-901-103). The safety study (AVE-901-104) will be performed in a range of surgical types.

Data from this current study will be combined with data from the other studies into an overall safety and efficacy database for tramadol infusion.

5. TRIAL OBJECTIVES AND PURPOSE

The primary objective of this study is to evaluate the analgesic efficacy of intravenous (IV) tramadol (AVE-901) compared to placebo in the management of postoperative pain following orthopedic (bunionectomy) surgery.

The Sum of Pain Intensity Differences (SPID) through 48 hours post first dose (SPID48) measured at rest will be used as the primary measure of efficacy.

Secondary efficacy endpoints are:

- SPID through 24 hours post first dose (SPID24) measured at rest
- Total consumption of rescue (supplemental) analgesia. This is the total amount of rescue analgesia given to the patient after first dose of study medication through 48 hours post-first dose.
- Patient Global Assessment of efficacy at 24 and 48 hours post first dose using a 5-point scale. The question to be posed is "How would you rate the study medication in terms of its effectiveness in controlling your pain?" (0=poor; 1=fair; 2=good; 3=very good; 4=excellent).

Additional analgesia endpoints are:

- Time-specific pain intensity profile over time
- Time (in minutes) to first rescue analgesia from the time of first dose of study medication.
- Number (percent) of patients who require no rescue analgesia from T0-T48.
- Rate of consumption of rescue analgesia. The total amount of rescue analgesia will be expressed as a function of duration of treatment
- Time (in minutes) to meaningful pain relief after first dose.
- Time (in minutes) to perceptible pain relief after first dose.

Safety endpoints will include:

- Adverse events (classified by the MedDRA System Organ Class (SOC) and preferred term), including assessment of AE's related to substance abuse.
- Clinical laboratory, vital sign, PE and ECG changes
- Use of anti-emetics and other concomitant medicine
- Local tolerability at the infusion site via pain, swelling, tenderness, and erythema.

Population pharmacokinetic outcomes will be assessed through collection of plasma samples for a subset of the randomized patients. Exposure-response relationships will be assessed for safety outcomes.

6. INVESTIGATIONAL PLAN

6.1. Overall Study Design

This study is a Phase 3, multicenter, double-blind, three-arm, randomized, placebo-controlled, multiple-dose, parallel-group trial to evaluate the safety, tolerability and the efficacy of IV Tramadol (AVE-901) versus placebo in the management of postoperative pain in consenting patients undergoing a unilateral primary first metatarsal bunionectomy surgery. The treatment groups will be:

- AVE-901 50 mg, given at Hours 0, 2, 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, and 44.
- AVE-901 25 mg, given at Hours 0, 2, 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, and 44.
- Placebo, given at Hours 0, 2, 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, and 44.

Approximately 405 patients will be randomized (135 per treatment group).

Each patient will undergo the Screening Visit (Day -28 to Day -1), the pre-operative assessment (within 24 hours prior to surgery start time), the Surgical/Treatment Visit, the first day of which is when the bunionectomy will be performed (Day 0), the primary treatment period through Hour 48 (the last on treatment assessment, and the End of Treatment visit), and the Follow-up Visit (Day 14).

Screening will occur up to 28 days prior to surgery. Following the pre-operative assessments, and after the patient has met eligibility criteria, patients will be randomized in a double-blinded fashion, stratified by study center, in a 1:1:1 ratio to the treatment groups.

Following surgery, patients that have met the post-surgical dosing criteria will receive his or her assigned study medication infusion regimen over a period of 48 hours. Patients will be confined at the healthcare facility during study drug administration, and will be discharged only if clinically stable.

Efficacy will be assessed by patient reports of pain intensity (PI) performed immediately prior to T0 (the first administration of study drug) and at 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, and 48 hours after first treatment (ie, post T0). PI is reported by the patient on a numeric rating scale for pain (NPRS Pain) anchored at 0 for no pain and 10 for the worst pain imaginable (See Appendix 1). In addition, efficacy and safety will be measured by other parameters.

Rescue medication (Ibuprofen 400 mg Q4 PRN) will be available any time after the initial dose of study medication to keep the patient comfortable. However, patients will be encouraged to wait at least 60 minutes after the initial dose of study medication before they receive rescue therapy. An NPRS measure will be obtained immediately (approximately 5 minutes) prior to each administration of rescue medication. Patient controlled analgesia (PCA) will not be allowed in this study.

Approximately 33% of the total number of patients will be enrolled in the pharmacokinetic (PK) portion of this study at select sites. Blood will be collected for analysis of tramadol and O-desmethyltramadol (active metabolite) at Hour 0, Hour 0.25, Hour 0.5, Hour 2.25, Hour 2.5, Hour 4, Hour 4.25, Hour 4.5, Hour 8, Hour 8.25, Hour 24.5, Hour 44, Hour 44.25, Hour 44.5, and Hour 48.

6.2. Number of Patients

A total of approximately 405 patients in United States (US) undergoing elective orthopedic surgery who meet all of the inclusion and none of the exclusion criteria, randomized, have at least one pain intensity measurement and receive at least a partial dose of study drug will be included in the study population.

6.3. Treatment Assignment and Randomization

Patients will be randomized to one of three treatment arms (AVE-901 50 mg AVE-901 25 mg, or placebo) in a 1:1:1 ratio (approximately 135 patients per treatment group). Randomization will be stratified by study center.

6.4. Criteria for Study Termination

If the Investigator, Study Medical Monitor, or Avenue Therapeutics discovers conditions arising during the study, which indicate that the clinical investigation should be halted, the study must be terminated after appropriate consultation between Avenue Therapeutics, Study Medical Monitor, and the Investigators. Conditions that may warrant termination include, but are not limited to:

- The finding of an unacceptable risk to the patients enrolled in the study,
- Failure to enroll patients at an acceptable rate,
- Insufficient adherence to protocol requirements and good clinical practices, or;
- A decision on the part of the Avenue Therapeutics to suspend or discontinue development of the drug.

6.4.1. Individual Patient Stopping Rules

If a patient experiences a serious or severe adverse event assessed as possibly, probably or definitely related to study drug, the Medical Monitor and Investigator will review the patient's medical record and determine whether the patient should have study treatment either temporarily interrupted or permanently discontinued.

6.5. Schedule of Events

Table 4 provides the schedule of events. Note that patients discontinuing prematurely from the study will have all End of Treatment assessments performed.

Table 4. Schedule of Assessments for each Study Period (Study AVE 901-102)

Assessments	Screen Day -28 to -1	Pre-op Day -1/ 0	Surgery Day 0[1]	Treatment Hours Post Start of First Infusion Hour 0 to Hour 48 : Assessments must be done after 15 minutes of rest. The assessments must be performed +/- 10 minutes from the targeted time point.																													Phone Call Day 14 ± 2
				0	0.5	1	2	3	4	5	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40	42	44	46	48/ EOT	
Informed Consent	X																														
Medical History	X	X																													
Demographic Data	X																														
Height and Weight	X																														
Physical Examination	X	X																												X[8]	
ASA Physical Status	X	X																													
Vital Signs	X	X		X	X	X	X	X	X	X	X	X	X	X		X		X		X		X		X		X		X		X		X	
Pregnancy Test[2]	X	X																												X	
Lab blood draw[3]	X	X																												X	
Urinalysis	X	X																												X	
CYP2D6 blood sample		X																													
Urine Drug Screen[4]	X	X																													
Alcohol Breath Test	X	X																													
12-Lead ECG	X			X (Collect ECG 20 to 30 minutes after the start of each dose for the first 24 hours)																												X	
Inclusion/Exclusion Assessment	X	X																													
Patient Education Video	X	X																													
Patient Pain Assessment Training	X																														
Post-Op Dosing criteria assessment			X																												
Randomization[5]		X																													
Surgery			X																												
4-Point Pain Scale				X																											
Study Drug Admin				X[6]			X		X			X		X		X		X		X		X		X		X		X		X			
Pain Intensity NPRS[7]				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Initiate 2 stopwatches				X																											
Patient Global Assess																				X											X	
PopPK Sampling				Blood will be collected Hour 0, Hour 0.25, Hour 0.5, Hour 2.25, Hour 2.5, Hour 4, Hour 4.25, Hour 4.5, Hour 8, Hour 8.25, Hour 24.5 Hour 44, Hour 44.25, Hour 44.5, and Hour 48.																										
Record rescue med				Ongoing																											
Adverse Events				Ongoing																											

[1] Preoperative assessments to confirm the patient's eligibility will be performed within 24 hours of scheduled surgery start time.
[2] Pre-Op Pregnancy test (urine) performed on-site for female patients of childbearing potential. Serum pregnancy test is performed at Screening and End of Treatment
[3] Hem, Chem, Coag, Urinalysis Pre-op labs only needed for patients whose screening laboratory tests were performed >7 days prior to randomization
[4] Urine drug screen should be performed at screening and Day 0 (on-site)
[5] Randomization is to occur pre surgery after all eligibility criteria are met
[6] First dose of study drug at first report of moderate or severe pain from a 4 point scale and a score of ≥5 on NPRS pain intensity, See Appendix 1
[7] In addition Pain Intensity Assessment must be completed prior to each rescue medication use or dosing. Note there is no rest period required for NPRS prior to rescue drug administration
[8] Hour 48 PE can be done at any time prior to discharge (± 10 minute window not applicable)

Prior/Conmeds	X	X	Ongoing

6.6. Description of Visits

There are 5 distinct periods in this study:

- Screening (Days -28 to -1)
- Pre-operative (within 24 hours of scheduled surgery)
- Operative (Day 0)
- Post-operative/Treatment Period 48 hours
- Follow–up (approximately Day 14)

Procedures for each study period are described below.

6.6.1. Screening

Screening will occur from Day -28 through Day -1 and will be conducted as a clinic visit. Patients will have the purpose and procedures of the study explained to them and, those who elect to participate in the study, will provide written informed consent and be screened for participation according to the eligibility criteria. Patients will undergo protocol specific education including but not limited to the various post-treatment efficacy and safety measures. The following information and procedures will be performed and documented as part of the screening assessment:

- Demographics, including gender, race and ethnic origin, date of birth, height, weight and calculated BMI.
  - BMI Calculation: Sites should use the NIH website BMI calculator: https://www.nhlbi.nih.gov/health/educational/lose_wt/BMI/bmicalc.htm
- Inclusion/exclusion eligibility criteria
- Physical examination
- ASA Physical Status
- Medical history
- Prior and ongoing medications (taken in previous 30 days)
- Vital signs: blood pressure, heart rate, respiratory rate, and oral temperature (after seated or supine for 5 minutes) and SpO2
- 12-lead ECG (after supine for 5 minutes)
- Blood samples for hematology, clinical chemistry, coagulation, HIV-1/HIV-2 antibody, hepatitis B surface antigen, hepatitis C antibody, Pregnancy test for women of child-bearing potential (serum)
- Urine sample for urinalysis
- Urine drug screen for drugs of abuse (onsite)
- Alcohol breath Test

- Patient Education Video and Patient Pain Assessment Training

Compliance with inclusion criteria and exclusion criteria will be verified against information collected and documented in the source documents and the eCRF.

6.6.2. Preoperative

Preoperative assessments to confirm the patient's eligibility will be performed within 24 hours of scheduled surgery start time. This visit will include reassessment of:

- Inclusion/exclusion eligibility criteria
- Brief Physical examination (symptom driven)
- ASA Physical Status
- Medical history
- Prior and ongoing medications (taken in previous 30 days)
- Vital signs: blood pressure, heart rate, respiratory rate, and oral temperature (after seated or supine for 5 minutes) and SpO2
- Blood samples for hematology, clinical chemistry, coagulation (not required if pre-op visit is within 7 days of screening)
- A blood sample will be obtained and stored for possible analysis of CYP2D6 metabolizer status, in case such information is deemed to be of interest during the analysis. Collection and processing of these samples will be addressed in a lab manual.
- Urine Sample for urinalysis
- Urine Pregnancy test for women of child-bearing potential (onsite)
- Urine drug screen for drugs of abuse (onsite)
- Alcohol breath Test (onsite)
- Patient Education Video Training

The safety laboratory tests done centrally will not be used for determining eligibility; however, they may be used to exclude a patient from receiving further study medication at the discretion of the investigator, medical monitor and sponsor.

Randomization may occur at any time after confirmation that all inclusion and exclusion criteria have been met and before surgery. Dosing will occur once the subject has met all post-operative dosing criteria

6.6.3. Operative

Surgery will occur on Day 0. Operative procedures are described in the Surgical and Anesthetic Regimen.

6.6.4. Post-operative/Treatment Period

In addition to meeting all eligibility criteria prior to surgery, patients must ALSO meet all postsurgical dosing criteria. Treatment will occur from Hour 0 through Hour 44 (T0-T44).

- Following surgery, patients will have their popliteal block withdrawn at approximately between 4 AM and 5 AM. Post removal of the popliteal block, Patients must be assessed for the dosing eligibility criteria prior to dosing. Patients must be awake and alert, and must have a pain intensity of 5 or greater and report a score of moderate or severe on a 4-point categorical rating scale (with categories of none, mild, moderate, or severe) just before the first dose of the study drug. Patients who do not report pain at this level within 8 hours of the removal of the block will be discontinued (ie, not treated).
- Antiemetic treatment with Reglan or Emend (not Zofran or other 5HT3 antagonists) is allowed.

During the Treatment phase, patients will undergo:

- NPRS - T0 will be time of start of infusion of first study drug administration. Pain intensity will be recorded using a NPRS from 0 to 10, where 0 is no pain and 10 is the worst pain imaginable. Pain intensity assessments will be recorded immediately prior to first dose (baseline) and at the times indicated in the Schedule of Assessments. The patient must rest for 15 minutes (+/- 5 minutes) prior to assessments.
- The Patient Global Assessment (PGA) of efficacy will be completed by the patient at 24 hours and 48 hours post T0. Ratings will be assessed as: 0=poor; 1=fair, 2=good, 3=very good or 4=excellent.
- Two stopwatches will be started at the start of infusion of first study drug dose. Patients will be instructed to stop the first stopwatch when pain relief is first perceptible and the second when pain relief is considered meaningful.
- Vital signs: blood pressure, heart rate, respiratory rate, and oral temperature (after seated or supine for 5 minutes) and SpO2 as per the schedule of events
- Study Drug dosing at 0, 2, 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, and 44 hours
- 12 lead ECG (after supine for 5 minutes) at 20 to 30 minutes after the start of each dose for the first 24 hours
- AEs
- Concomitant medication use
- Rescue medication use
- For those patients enrolled in the PK portion of this study (at select sites), blood will be collected for analysis of tramadol and O-desmethyltramadol (active metabolite) at:

  Hour 0, Hour 0.25, Hour 0.5, Hour 2.25, Hour 2.5, Hour 4, Hour 4.25, Hour 4.5, Hour 8, Hour 8.25, Hour 24.5, Hour 44, Hour 44.25, Hour 44.5, and Hour 48.

At the End of Treatment or Early Termination, patients will undergo:

- A brief (symptom driven) physical examination (may be completed any time prior to discharge)
- Vital signs: blood pressure, heart rate, respiratory rate, and oral temperature (after seated or supine for 5 minutes) and SpO2
- 12-lead ECG (after supine for 5 minutes)
- Blood samples for hematology, clinical chemistry, urinalysis, and pregnancy test (serum)
- Pain Intensity NPRS and Patient Global Assessment
- AEs
- Concomitant medication use
- For those patients enrolled in the PK portion of this study (at select sites), blood will be collected for analysis of tramadol and O-desmethyltramadol (active metabolite)

At time points when multiple assessments are required, the order of assessments will be: Pain assessment, vital signs, blood draw, and then study drug administration

If feasible, any patient who prematurely discontinues study medication or the study prior to the T48 will undergo the procedures required at the end of treatment. Post T48, pain management will be per investigator standard of care preference.

6.6.4.1. Rescue medication during the Treatment Period

Inadequate analgesia may be treated with 400 mg of ibuprofen q 4 hours PRN administered by a trained nurse or study clinician to the patients who request rescue medication after administration of the initial dose of study medication. However, patients will be encouraged to avoid rescue medications for at least 60 minutes after their first dose of study drug. A NPRS measure will be obtained immediately (approximately 5 minutes) prior to administering any rescue medication. Patients will continue to take study medication for the remainder of the 48-hour period even if they take rescue medication. Ibuprofen dosing is allowed: 400 mg PRN every 4 hours up to 2400 mg per day. The time of rescue medication must be recorded in the case report form. Investigator study team personnel will monitor the patient carefully for 48 hours following the surgery to assess the patient's condition and provide rescue medication whenever requested, within the above limits. This will minimize the patient's inconvenience due to pain.

6.6.5. Follow up

A final safety assessment will be conducted on Day 14 (± 2 days) via a telephone call.

7. SELECTION AND WITHDRAWAL OF PATIENTS

7.1. Patient Inclusion Criteria

The following are patient inclusion criteria for this study; each patient must meet all inclusion criteria in order to be enrolled into this study.

1. The patient is male or female 18-75 years of age undergoing unilateral first metatarsal bunionectomy surgery
2. The patient or legal representative has voluntarily signed and dated an Institutional Review Board (IRB)/Independent Ethics Committee (IEC) approved written informed consent.
3. Female patients must be of non-childbearing potential (surgically sterile or post-menopausal for at least 1 year) or be practicing a highly effective contraception method from consent to at least 7 days after the last dose of study medication. Highly effective contraception methods include: vasectomized partner (at least 6 months prior to dosing); double barrier (diaphragm with spermicide; condoms with spermicide); intrauterine device; implanted or intrauterine hormonal contraceptives in use for at least 6 consecutive months prior to study dosing and throughout the study duration; oral, patch, or injected contraceptives in use for at least 3 consecutive months prior to study dosing.
4. Female patients of childbearing potential have a negative pregnancy test (serum human chorionic gonadotropin [HCG]) during screening and negative pregnancy test (urine) within 24 hours prior to surgery, and must not be lactating.
5. The patient must be willing to be housed in a healthcare facility and able to receive parenteral analgesia for at least 72 hours after surgery.
6. The patient meets definition of American Society of Anesthesiologists (ASA) Physical Class 1 or 2.
7. The patient is willing and able to understand the study procedures and the use of pain scales, to communicate meaningfully with the study personnel, and to comply with the study protocol.

7.2. Patient Exclusion Criteria

The following are patient exclusion criteria for this study; each patient must **not** meet any of these exclusion criteria in order to be enrolled into this study.

1. Patient is not expected to receive a continuous infusion nerve block as described in the Post-Op anesthetic procedure protocol.
2. Patient is currently using, or is expected to receive gabapentin, pregabalin, ketamine or other peri- or postoperative analgesic adjuncts or any analgesics not allowed in the Surgical and Anesthesia protocol.
3. Patient is undergoing bilateral or revision bunionectomy surgery.
4. Patient has a history of primary or metastatic bone cancer or Paget's disease.

5. The patient has current or historical evidence of any clinically significant disease or condition that might increase the risk of surgery or complicate the patient's postoperative course.
6. The patient has allergy or hypersensitivity (or is intolerant) to opioids or tramadol.
7. The patient has known physical dependence on opioids.
8. Patients who have taken any opioids, including tramadol for a total of 3 days, within 30 days prior to surgery.
9. The patient has a recent (within 5 years) and/or current history of chronic analgesic, opiate or tranquilizer abuse or dependence or is a user of illicit drugs, or has had a recent history (within 2 years) of drug or alcohol abuse.
10. The patient has a painful physical condition that, in the opinion of the investigator, may confound the assessments of postoperative pain.
11. The patient has taken other prior/concurrent chronic medications that have not been at a stable dose for at least 2 weeks prior to screening.
12. The patient is taking herbal or dietary supplements or medications that are moderate or strong inhibitors of CYP2D6 or CYP3A4 (e.g., fluoxetine, paroxetine, amitriptyline, quinidine, ketoconazole, erythromycin, grapefruit juice) or inducers of CYP3A4 (eg, carbamazepine, rifampin, St. John's Wort) and cannot go through a minimum washout period of 7 days prior to surgery.
13. The patient has taken monoamine oxidase (MAO) inhibitors, trazodone or cyclobenzaprine within 14 days prior to surgery.
14. The patient has a history of epilepsy, or is known to be susceptible to seizures.
15. The patient cannot be withdrawn from medications (at least 7 days prior to surgery) that may lower the seizure threshold (e.g. anti-psychotic agents, MAOI inhibitors) or which increase serotonergic tone (e.g. selective serotonin reuptake inhibitors (SSRIs), serotonin norepinephrine reuptake inhibitors (SNRIs), tricyclic antidepressants, triptans, amphetamines).
16. The patient has a history of Long QT Syndrome or a relative with this condition.
17. The patient has had a recent cardiovascular event or a clinically significant abnormal ECG at screening.
18. The patient has used (within 30 days of surgery), is currently using or is anticipated to use chronic corticosteroids .
19. The patient has expressed suicidal ideation or is considered to be at risk of suicide.
20. The patient is morbidly obese (body mass index [BMI] $\geq$ 40 kg/m2) or has documented sleep apnea requiring pharmacological or device intervention.
21. The patient has a history of cardiopulmonary, neurological or psychiatric condition that may confound the assessments of efficacy or safety.

22. The patient has cirrhosis, moderate or severe hepatic impairment or an alanine aminotransferase (ALT) or aspartate aminotransferase (AST) value > 3X upper limit of normal (ULN) at Screening.
23. The patient has severe renal impairment or a serum creatinine value of > 2.0 mg/dL at Screening.
24. The patient has potassium, sodium, calcium or magnesium levels outside of the normal range, or any other clinically significant abnormalities in blood chemistry at Screening.
25. The patient has a hemoglobin level at screening which, in the judgment of the Investigator, is not suitable for participation in this study.
26. The patient used narcotics or alcohol within 24 hours prior to surgery.
27. The patient was administered an investigational product within 30 days prior to Screening.
28. The patient has previously participated in a clinical study with AVE-901.

7.2.1. Post-Surgical Dosing Criteria

The patient is to be evaluated post-operatively prior to the initial treatment with the study drug and will be given the study drug only if all of the following criteria are met:

1. The patient reports a score of moderate or severe on a 4-point categorical rating scale (with categories of none, mild, moderate, or severe) and has a NPRS pain score of ≥5 (on a scale from 0 to 10) within 8 hours after removal of popliteal block.
2. The patient is alert and oriented. She/he is able to answer questions and follow commands and has appropriate cognitive function to properly interpret and answer protocol mandated assessments.
3. The patient has no evidence of respiratory insufficiency, such as a respiratory rate that is less than 8 breaths per minute or arterial oxygen saturation by pulse oximetry of less than 90% with supplemental oxygen.
4. The surgical procedure from incision to closure was not longer than 180 min.
5. There have been no significant deviations from the surgical or anesthetic protocol that would, in the opinion of the investigator, put the patient at risk of participation in the trial, confound the analgesic endpoints of the trial or cause concern regarding the patient's ability to participate in the trial.
6. There have been no use of gabapentanoids, or 5-HT_3 antagonists (e.g. ondansetron, granisetron, palonosetron) preoperatively, intraoperatively, or postoperatively.
7. Subject has not experienced complications during the surgical procedure and in the opinion of the investigator is a candidate to continue in the study.

7.3. Patient Withdrawal Criteria

If a patient is discontinued from the study prematurely, the Investigator must select the primary reason for discontinuation on the End of Study eCRF. In addition, every effort should be made to complete the assessments listed under the End of Treatment column on the Schedule of Assessments.

Patients withdrawn from the study will be considered evaluable for statistical assessment.

A patient may be removed from the study for the following medical or administrative reasons:

- Adverse Event: If a patient experiences an adverse event that the patient finds unacceptable or that, in the judgment of the Investigator or the Medical Monitor presents an unacceptable consequence or risk to the patient, the patient may be discontinued from further participation in the study.
- Administrative Discontinuation: After consultation with the Investigator or Medical Monitor, a patient may be discontinued from the study for failure to comply with protocol requirements. All instances of noncompliance must be documented in the eCRF.
- Refusal of Assessments: If for any reason, following dosing, the patient refuses further assessment during the study, the patient shall be discontinued from the study and the reasons for refusal documented. Reasonable efforts shall be made to monitor the patient for adverse events following such discontinuation. Such efforts shall be documented.

8. TREATMENT OF PATIENTS

8.1. Description of Study Drug

Investigational product will be packaged in identical ampoules containing 1ml of AVE-901. Each ampoule of AVE-901 contains 50 mg of tramadol hydrochloride and sodium acetate trihydrate as buffering agent diluted in water. Placebo ampules contain only the liquid vehicle.

Study drugs will be distributed to the clinic using a designated distribution center. The Sponsor will provide the investigator with adequate quantities of investigational product and supplies to conduct the study. Specific details regarding investigational product supplies, dose preparation, and accountability will be described in a pharmacy manual at the clinic.

Table 5 provides a summary description of the drug products, including the dosage form, the unit dose, and a physical description of the products.

Table 5: Investigational Product (Study AVE-901-102)

	Investigational Product		
Product Name:	Tramadol for infusion 50 mg	Tramadol for infusion 25 mg	Placebo
Dosage Form:	Liquid	Liquid	Liquid
Unit Dose:	50 mg/1mL ampoule	50 mg/1mL ampoule	1mL ampoule
Route of Administration:	Intravenous infusion	Intravenous infusion	Intravenous infusion
Physical Description:	Clear solution	Clear solution	Clear solution
Manufacturer:	Pharmaceutical Works PolPharma S. A.	Pharmaceutical Works PolPharma S. A.	Pharmaceutical Works PolPharma S.A.

8.2. Concomitant Medications

All concomitant medications, whether prescription, over-the-counter, herbal treatments or other therapy, taken or used by the patient within 30 days of screening through end of study assessments (f/u phone call Day 14) will be recorded in the patient's medical record and in the Concomitant Medications eCRF. Concomitant medications will be coded using the WHO Drug Dictionary.

Rescue medication (Ibuprofen 400 mg Q4 PRN) will be available q 4 hours after the initial dose of study medication to keep the patient comfortable. However, patients will be encouraged to wait at least 60 minutes after the initial dose of study medication before they receive rescue therapy. The rescue medication is the only analgesic allowed within the study. Other non-pain

relieving medications deemed necessary for the patient's welfare are permitted, other than medications specified in the inclusion/exclusion criteria. Postoperative adverse events (AEs) such as nausea, vomiting and pruritus should be managed using standard of care, excluding medications specifically noted in the study protocol.

If medication is needed to treat any adverse events during the study, patient study continuation will be evaluated.

8.3. Treatment Compliance

Treatment compliance with study medication during the treatment periods is expected to be high, as patients will be dosed directly in the clinic under well-controlled conditions. The date and start/stop time of study drug administration will be recorded on the eCRF.

8.4. Overdose

Tramadol products in excessive doses, either alone or in combination with other CNS depressants, including alcohol, are a cause of drug-related deaths. Acute over dosage with tramadol can be manifested by respiratory depression, somnolence progressing to stupor or coma, skeletal muscle flaccidity, cold and clammy skin, constricted pupils, seizures, bradycardia, hypotension, cardiac arrest, and death. Serious potential consequences of over dosage with tramadol are CNS depression, respiratory depression and death. Some deaths have occurred as a consequence of the accidental ingestion of excessive quantities of tramadol alone or in combination with other drugs, while others were associated with abuse of tramadol. Review of case reports has indicated that the risk of fatal overdose is further increased when tramadol is abused concurrently with alcohol or other CNS depressants, including other opioids (Ultram® Prescribing Information, 2009).

Of note, while naloxone will reverse some, but not all, symptoms caused by over dosage with tramadol, the risk of seizures is also increased with naloxone administration. In animals convulsions following the administration of toxic doses of tramadol could be suppressed with barbiturates or benzodiazepines but were increased with naloxone. Naloxone administration did not change the lethality of an overdose in mice. Hemodialysis is not expected to be helpful in an overdose because it removes less than 7% of the administered dose in a 4-hour dialysis period (Ultram® Prescribing Information, 2009).

Additional details may be found in the Investigator's Brochure.

8.5. Randomization, Blinding and Unblinding

Patients will be randomized to one of 3 treatment groups, as follows:

- AVE-901 50 mg, given at Hours 0, 2, 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, and 44.
- AVE-901 25 mg, given at Hours 0, 2, 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, and 44.
- Placebo, given at Hours 0, 2, 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, and 44.

Patients will be randomized to one of the three treatment arms (AVE-901 50 mg, AVE-901 25 mg, or placebo) in a 1:1:1 ratio (approximately 135 patients per treatment group). Randomization will be stratified by study center. The study will remain blinded until all patients have completed their treatment period and completed all study procedures and the clinical

database has been locked. Procedures for emergency unblinding will be described in the Study Blinding Plan.

9. STUDY DRUG MATERIALS AND MANAGEMENT

Study drug will be provided to the investigational centers.

9.1. Study Drug Packaging and Labeling

Single-use, glass ampoules with labels and an outer fiberboard carton with a single panel label will have the following information:

1. Protocol #: AVE-901-102
2. Each 1ml ampoule contains 50 mg of IV Tramadol (C-IV) or placebo
3. Intravenous injection administration only. Use as directed per protocol.
4. Store at 20°C to 25°C (68°F to 77°F). Store away from heat sources and direct sunlight.
5. Caution: New Drug – Limited by Federal (United States) Law to Investigational Use.
6. Sponsor: Avenue Therapeutics, New York, NY 10019
7. Manufactured by: Pharmaceutical Works PolPharma S. A.
8. Lot number: _____
9. Kit number:_____

The clinical trial supply labels will be in accordance with ICH GCP and local requirements for investigational product labelling.

9.2. Packaging, Labeling, and Storage of Clinical Supplies

Investigational products are for investigational use only and the study drug supplied for this study is intended for use only within the context of this study. The study drug supplied for this study should be stored in a secure place and maintained under adequate security until dispensed for patient use or returned to the Sponsor. Tramadol is classified as a Schedule IV controlled drug and security requirements as per 21CFR 1301.71-77 are to be followed at the clinic.

Investigational products should be stored at room temperature (20-25°C or 68-77°F). Investigational products should be stored away from heat sources and direct sunlight.

The Investigator, pharmacist, or their designee, will verify that study drug supplies are received intact and in the correct amounts by signing and dating the investigational product receipt log. The person receiving the supplies must verify that the shipment contains all the items noted in the shipment inventory. Any damaged or unusable drug in a given shipment will be documented in the study files. The Investigator must notify the Sponsor or designee of any damaged or unusable investigational product supplied to the Investigator's site.

The site will maintain a Drug Inventory Log (includes, but not limited to, the following: lot number, number of units received and number of ampoules/tablets dispensed. The site will also maintain patient-specific drug dispensing logs.

An overall accountability of investigational product will be performed and verified throughout the study and at the site closeout visit. Upon completion of the study, copies of the investigational product accountability records will be returned to the Sponsor. All used and unused study drug supplies will be inventoried, accounted, and returned to the Sponsor at the end of the study. By signing the Investigator Agreement page of this protocol, the investigator or

named sub-investigator agrees not to supply study drug to any person(s) not enrolled in the study.

9.3. Study Drug Preparation and Administration

Study drug will be dispensed by the qualified, licensed study personnel and administered to the patient by a licensed designated staff member. The designated personnel will prepare the treatments for administration (according to the randomization allocation) and maintain accountability records. Study treatment for each period will be prepared according to the treatment sequence assignments.

AVE-901 will be provided in ampoules containing 1 mL of IV Tramadol. Directions for preparation of study medication will be recorded in the pharmacy manual prior to initiation of the study. The pharmacy manual will provide procedures for preparation that correspond to the doses of study medication used in this study.

Unblinded personnel will be designated to prepare the treatment according to the randomization allocation. Study blinding and unblinding will be described in the Study Blinding Plan.

9.4. Study Drug Accountability

Patients will be treated at the investigational center and therefore the unblinded Pharmacist or other unblinded investigational staff via documentation of receipt of the study drug and dosing/treatment given will perform drug accountability.

9.5. Study Drug Handling and Disposal

Records of receipt, dispensing records and inventory forms, as applicable, will be examined and reconciled during and at the end of the study. Both the investigational drug that is used during the course of the study, as well as any remaining unused investigational drug, must be accounted for on a drug accountability record provided to the PI by the Sponsor or its designee. Drug destruction will be completed following the clinical sites SOP on destruction and a destruction certificate will be maintained at the clinic.

If directed, at the end of the study, all unused investigational drug, accompanied by a packing slip will be shipped to a designee provided by the sponsor.

In addition, a copy of all completed drug accountability records must be retained in the Investigators' Study Files, with a copy sent to the Sponsor or its designee.

The products are to be stored in a safe place (locked facility) at the appropriate temperature and without exposure to freezing.

10. PHARMACOKINETIC ASSESSMENTS

Approximately 33% of the total number of patients will be enrolled in the pharmacokinetic (PK) portion of this study at select sites. Blood will be collected for analysis of tramadol and O-desmethyltramadol (active metabolite) at Hour 0, Hour 0.25, Hour 0.5, Hour 2.25, Hour 2.5, Hour 4, Hour 4.25, Hour 4.5, Hour 8, Hour 8.25, Hour 24.5 Hour 44, Hour 44.25, Hour 44.5, and Hour 48. A separate population PK analysis plan will be written to describe the assessment of the PK data. PK sample processing will be addressed in the lab manual.

11. ASSESSMENT OF EFFICACY

The primary endpoint in this study is SPID48 for pain at rest. Pain intensity will be recorded using a NPRS from 0 to 10, where 0 is no pain and 10 is the worst pain imaginable (See Appendix 1).

Key secondary endpoints are:

- SPID through 24 hours post first dose (SPID24) at rest
- Total consumption of rescue (supplemental) analgesia. This is the total amount of rescue analgesia given to the patient after first dose of study medication through 48 hours post-first dose.
- Patient Global Assessment of efficacy at 24 and 48 hours. The Patient Global Assessment (PGA) of efficacy will be completed by the patient at 24 hours and 48 hours post T0. Ratings will be assessed as: 0=poor; 1=fair, 2=good, 3=very good or 4=excellent.

Tertiary endpoints (additional analgesia endpoints) are:

- Time-specific pain intensity profile over time
- Clock time (in minutes) to first use of rescue medication from the time of first dose of study medication.
- Number of patients who require no rescue analgesia from T_0-T_{48}
- The rate of consumption of rescue analgesia
- Time (in minutes) to meaningful pain relief after first dose. Two stopwatches will be started at the start of infusion of first study drug dose. Patients will be instructed to stop the first stopwatch when pain relief is first perceptible and the second when pain relief is considered meaningful.
- Time (in minutes) to perceptible pain relief after first dose

12. ASSESSMENT OF SAFETY

12.1. Safety Parameters

Safety assessments will include collection of adverse events. In addition, safety assessments include clinical laboratory tests, vital signs, physical examination, concomitant medications, and 12-lead ECGs.

12.1.1. Demographic/Medical History

Demographic information and medical history will be collected at Screening and pre-operatively for determination of eligibility.

12.1.2. Vital Signs

Vital sign assessments include blood pressure, pulse, temperature, respiratory rate, and SpO2, and are collected at varied times as per the Schedule of Events. Vitals will be performed after the patient has rested sitting or supine for approximately 5 minutes.

12.1.3. Weight and Height

Height and weight will be captured as per the Schedule of Events. BMI will be calculated using the NIH website BMI calculator http://www.nhlbi.nih.gov/health/educational/lose_wt/BMI/bmi-m.htm.

12.1.4. Electrocardiogram (ECG)

All scheduled ECGs will be performed after the patient has rested supine for approximately 5 minutes.

12.1.5. Physical Examination

A physical examination will be conducted and abnormalities will be described. After the initial physical exam, only symptom-driven examinations will be performed according to the treating physician.

12.1.6. Laboratory Assessments

All laboratory assessments will be collected as per the schedule of events.

12.1.6.1. Hematology and Blood Chemistry

Clinical Laboratories (Hematology and Serum Chemistry) will be performed as described below and as indicated in the schedule of events.

- CLINICAL CHEMISTRY - Total protein, albumin, serum creatinine, blood urea nitrogen (BUN), uric acid, bilirubin (total & direct), alkaline phosphatase, alanine aminotransferase (ALT, SGPT), aspartate aminotransferase (AST, SGOT), creatine phosphokinase (CPK), glucose, calcium, magnesium, phosphorus, sodium, potassium, chloride, bicarbonate

- HEMATOLOGY - White blood cell (WBC) count, differential white cell count (lymphocytes, monocytes, basophils, eosinophils, neutrophils), red blood cell (RBC) count, hematocrit, hemoglobin and platelet count.

12.1.6.2. Urinalysis

Urinalysis will be performed.

- URINALYSIS - Color, specific gravity, pH, glucose, ketones, blood, protein, nitrates, leukocyte esterase, appearance.

Instruct patient to obtain a "clean-catch" urine sample, collected in midstream.

12.1.6.3. Infectious Disease Testing

Infectious disease testing at screening will include: HIV-1/HIV-2 antibody, hepatitis B surface antigen, hepatitis C antibody.

12.1.6.4. Coagulation Factor Testing

Coagulation Factor Testing will be performed at screening and pre-op in accordance with the schedule of events.

12.1.6.5. Pregnancy Screen

Pregnancy screening will be performed for all females of childbearing potential in accordance with the schedule of events via central lab blood test at screening and EOT and via urine test onsite at pre-op.

12.1.6.6. Urine Drug Screen

Drug screening will minimally include: THC, Opiates, Amphetamines, Cocaine, Barbiturates and Benzodiazepines and will be performed onsite in accordance with the schedule of events.

12.1.6.7. Alcohol Breath Test

Alcohol Breath Test will be performed onsite in accordance with the schedule of events.

12.2. Adverse and Serious Adverse Events

12.2.1. Definition of Adverse Events

12.2.1.1. Adverse Event (AE)

Adverse event means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. Adverse events may include safety findings considered to be clinically significant by the Investigator. An adverse drug event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not it is related to the medicinal product. Reporting an adverse event does not necessarily reflect a conclusion by the Investigator that the event is causally related to the drug.

All adverse events should be captured and documented along with any supporting documentation. Adverse events should be spontaneously reported or elicited by non-suggestive probing. Signs or symptoms associated with a worsening in either severity or frequency as compared to a baseline condition should be evaluated by the Investigator for clinical significance and adverse event reporting.

Each adverse event in this study will be assessed for Grade, where Grade of an AE refers to the severity of the AE. Grade will be assessed according to CTCAE Version 4.03 or higher. The CTCAE displays Grades 1 through 5 with unique clinical descriptions of severity for each AE that are based on this general guideline. Table 6 provides the CTCAE grades and grade descriptions to be used in this study.

Table 6: CTCAE Grade (Study AVE 901-102)

CTCAE Grade	CTCAE Grade Description
Grade 1: Mild	Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
Grade 2: Moderate	Minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL[1].
Grade 3: Severe	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL[2].
Grade 4: Life-threatening	Life-threatening consequences; urgent intervention indicated.
Grade 5: Death	Death related to the AE.

Activities of Daily Living (ADL)
[1] Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.
[2] Self-care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.
National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE), Version 4.03 will be used for AE grading. A complete CTCAE list can be downloaded at http://evs.nci.nih.gov/ftp1/CTCAE/About.html.

12.2.1.2. Serious Adverse Event (SAE)

A serious adverse event is an AE occurring during the study that fulfills one or more of the following:

- Results in death
- It is immediately life-threatening
- It requires in-patient hospitalization or prolongation of existing hospitalization
- It results in persistent or significant disability or incapacity
- Results in a congenital abnormality or birth defect

- It is an important medical event that may jeopardize the patient or may require medical intervention to prevent one of the outcomes listed above

Reporting serious adverse events requires additional detailed reports and follow-up, depending upon the Investigator's estimate of a causal relationship between the test agent and the adverse event(s), and whether the adverse event(s) is identified in nature, severity, and frequency in the Investigator's Brochure or other risk information supplied to the Investigator.

All serious adverse events (SAEs) should be submitted promptly to the Institutional Review Board/Independent Ethics Committee (IRB/IEC). The investigator must make an effort to obtain all hospital medical records including discharge summary confirming final diagnosis. The death of a patient must be immediately (within 24 hours) reported to the IRB. All serious and non-serious adverse events should be thoroughly documented and followed out by the Investigator until the event resolves or until the termination visit. The event may be followed longer, if deemed necessary. For any death occurring during the trial, the medical condition that led to the death should also be noted. The "outcome" status should be noted as "death" in these cases of SAEs that resulted in death. In addition, all SAEs that occur within 1 week following early termination visit should be recorded and reported as noted previously.

12.3. Relationship to Study Drug

An Investigator who is qualified in medicine must make the determination of relationship to the investigational product for each AE. The Investigator should decide whether, in his or her medical judgment, there is a reasonable possibility that the event may have been caused by the investigational product. For purposes of the definitions below, "temporal sequence" is defined as an association between administration of a drug and the observed reaction or event such that the drug was present prior to the reaction or event.

DEFINITE - The adverse event:

- follows a reasonable temporal sequence from drug administration,
- abates upon discontinuation of the drug (dechallenge), AND
- is confirmed by reappearance of the reaction on repeat exposure (rechallenge).

PROBABLE - The adverse event:

- follows a reasonable temporal sequence from drug administration,
- abates upon discontinuation of the drug (dechallenge), and
- cannot be reasonably explained by the known characteristics of the patient's clinical state.

POSSIBLE - The adverse event:

- follows a reasonable temporal sequence from drug administration, and;
- could have been produced by the patient's clinical state or by other modes of therapy administered to the patient.

REMOTE

- The temporal sequence between the adverse event and the drug administration is such that the drug is not likely to have had any reasonable association with the observed event.

DEFINITELY NOT – The adverse event:

- is definitely produced by the patient's clinical state or by other modes of therapy administered to the patient.

12.4. Recording Adverse Events

Adverse events spontaneously reported by the patient/caregiver and/or in response to an open question from the study personnel or revealed by observation will be recorded during the study at the investigational site. The AE term should be reported in standard medical terminology when possible. For each AE, the Investigator will evaluate and report the onset (date and time), resolution (date and time), severity, causality, action taken, serious outcome (if applicable), and whether or not it caused the patient to discontinue the study.

It is important to distinguish between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined by the criteria under Section 12.2.1.2. An AE of severe intensity may not be considered serious.

Should a pregnancy occur, it must be reported and recorded on a pregnancy reporting form. Pregnancy in itself is not regarded as an AE unless there is a suspicion that an investigational product may have interfered with the effectiveness of a contraceptive medication.

The outcome of all pregnancies (spontaneous miscarriage, elective termination, normal birth or congenital abnormality) must be followed up and documented even if the patient was discontinued from the study.

All reports of congenital abnormalities/birth defects are SAEs. Spontaneous miscarriages should also be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs.

12.5. Reporting Adverse Events

Information about AEs and SAEs will be collected from treatment (i.e. from time of first dose) through the follow-up phone call. Events occurring prior to first dose of study treatment will be recorded as medical history. AEs or SAEs occurring after the first dose of study treatment will be treatment-emergent (S)AEs. Any SAEs considered at least possibly related to the investigational product and discovered by the Investigator at any time after the study should be reported. The Investigator must complete, sign and date the SAE pages, verify the accuracy of the information recorded on the SAE pages with the corresponding source documents.

Additional follow-up information, if required or available, should all be communicated within one business day of receipt and this should be completed on a follow-up SAE form and placed with the original SAE information and kept with the appropriate section of the eCRF and/or study file.

The Sponsor is responsible for notifying the relevant regulatory authorities of certain events. It is the Investigator's responsibility to notify the IRB of all SAEs that occur at his or her site.

Investigators will also be notified of all unexpected, serious, drug-related events (7/15 Day Safety Reports) that occur during the clinical trial. Each site is responsible for notifying its IRB or IEC of these additional SAEs.

13. STATISTICS

This is a double-blind, 3-arm, placebo-controlled treatment study to determine the efficacy and safety of tramadol infusion in patients undergoing bunionectomy. The statistical methods to be used in the analysis are consistent with both the study design and the study objectives.

13.1. Sample Size Considerations

A sample size of 135 patients per arm (405 patients in total) provides over 90% power to detect a SPID48 difference of 250 between treatment with placebo and treatment with AVE-901, assuming a common SPID48 standard deviation of 600 (and thus an approximate 40% effect size), an alpha of 0.05 using a two-sided test of mean differences.

13.2. General Statistical Methods

Data will be tabulated (by treatment group) using descriptive and inferential statistics, where specified. A comprehensive statistical analysis plan (SAP) will be written and approved prior to unblinding. This SAP will detail how missing values, windows for study visits, and other analysis considerations will be addressed.

13.3. Analysis Populations

Populations identified for purposes of the statistical analysis are listed below.

- The Full Analysis Set (FAS) population is defined as all randomized patients who receive study medication. Patients will be analyzed according to the treatment group they were randomized to.
- The Safety Population is defined as all patients who receive study medication. Patients will be analyzed according to the actual treatment they receive.
- The All Enrolled Population will include the FAS and subset of patients who are randomized but not treated due not meeting post-surgical dosing criteria. The handling of these patients will be described in the Statistical Analysis Plan.

13.4. Baseline Characteristics

Baseline characteristics will be tabulated descriptively (e.g., number and percent of patients for each category for categorical parameters, and the number, mean, standard deviation, and range for continuous parameters). Baseline comparability among the treatment groups with respect to demographic and other baseline characteristics will be assessed for quantitative variables with a two-way analysis of variance (ANOVA) with treatment group and study center as main effects. For categorical variables, the Cochran-Mantel-Haenszel test will be used, stratified by center.

13.5. Patient Disposition

Patient disposition (including the number and percent of patients who are randomized, who receive randomized treatment, who prematurely discontinue and reasons for discontinuation, and who complete the study) will be tabulated by treatment group. The number (%) of patients by exposure will be tabulated.

13.6. Efficacy Analyses

13.6.1. Primary Endpoint

SPID48 (the sum of the time-weighted pain intensity differences for the time period 0-48 hours) is the primary efficacy endpoint.

13.6.2. Handling of Missing Efficacy Data

For the primary causal estimate in this study (to reflect the true treatment effects of tramadol compared to placebo), missing values within a patient during the 48-hour treatment period will be handled in the following manner:

- When rescue medication is used, the last pain intensity measure prior to the use of rescue medication will be used (imputed) for the subsequent protocol-specified time points for measurement of pain intensity through 4-hours after the time of the rescue medication.

All other missing data will be imputed utilizing multiple imputation methods. Multiple imputation provides a useful strategy for analyzing data sets with missing values. Instead of filling in a single value for each missing value, Rubin's (1976,) multiple imputation strategy replaces each missing value with a set of plausible values that represent the uncertainty about the correct value to impute.

For patients with arbitrary missing data patterns (missing at random, MAR), a pattern-mixture approach will be taken to the imputation. The first step will be to apply a Markov chain Monte Carlo (MCMC) method (Schafer 1997) that assumes multivariate normality will be used to impute all missing values to make the imputed (resulting) data sets have strictly monotone missing patterns. The resulting monotone missing pattern will then, in a second imputation step, be used to impute the remaining missing values; specifically, a regression-based method for continuous variables will be applied.

For patients with missing data as a result of discontinuation due to adverse (missing not at random, MNAR), the pattern-mixture approach will also be taken to the imputation (as for the monotone MAR data),with the difference that while a similar regression-based method for continuous variables will be applied, a scaled (constant) adjustment to the imputed values will be made such that the outcomes that are imputed are distributionally 'worse' than those imputed for the monotone MAR data. This scaled adjustment will increase each imputed value (from the regression) for these MNAR data by a constant of '1', e.g., if the regression provides an imputed value of 4.6 for a given missing value for a patient at a given timepoint, the MNAR imputed value will be set to 4.6 + 1 = 5.6 for that patient at that time point. This will, in effect, guarantee that the overall distribution of missing values for discontinuations due to adverse event is WORSE than that for other reasons.

The SAS software system will be used to perform this imputation. Because the imputation of missing data is a key aspect to the analysis of pain data, explicit details regarding this imputation are provided via sample SAS code that is intended to demonstrate the application of these strategies. Variable definitions are:

- TRT=treatment group (1 or 0)

- Pain_1 is the first time point for the pain score
- Pain_2 is the second time point for the pain score
- Pain_last is the last time point for the pain score. Additional pain scores (between Pain_1 and Pain_last) would be included in this model according to the time points for collection.

The first step will be to impute partially in order to obtain a monotone missing data pattern.

```
proc mi data=DATAIN out=DATAIN_MONO nimpute=100 seed=123;
    var TRT Pain_1 Pain_2 …. Pain_last;
    mcmc chain=multiple impute=monotone;
    run;
```

The second step will be to impute the remaining (monotone) missing data that is MAR for each of the 100 imputed datasets from the first step.

```
proc mi data=DATAIN_MONO out=DATAIN_REG seed=465 nimpute=1;
    by _Imputation_;
    var TRT Pain_1 Pain_2 …. Pain_last;
    class TRT;
    monotone regression;
    run;
```

In the case of missing data due to an adverse event, the following step will be used:

```
proc mi data=DATAIN_MONO out=DATAIN_REG seed=465 nimpute=1;
    by _Imputation_;
    var TRT Pain_1 Pain_2 …. Pain_last;
    class TRT;
    monotone regression (pain1 pain2 … Pain_last);
    mnar adjust (pain1 pain2 … Pain_last / shift=1);
    run;
```

Note that the regressions do not include the treatment variable, and thus the imputed data at each time point will be based on the distribution of all data rather than treatment-group specific distributions. A total of 100 imputed datasets will be created using these imputed data and analyzed according to the primary method of statistical analysis.

13.6.3. Primary Method of Statistical Analysis

Each of the 100 imputed datasets will be analyzed as follows:

- An analysis of covariance (ANCOVA) model will be used to test the primary efficacy endpoint. The model will use treatment and study center as main effects, and baseline pain intensity (NPRS scale 0 to 10) as the covariate. Data from all three treatment groups will be included in the same ANCOVA model for purposes of the testing procedures.

The 100 resulting treatment effect parameters and standard errors from these ANCOVA will be combined to provide a distribution of parameters (and standard errors) upon which the primary hypothesis of treatment effect will be determined.

13.6.4. Key Secondary Endpoints

The key secondary endpoints will be assessed as follows:

- 24-hour pain intensity (SPID24) will be assessed in a similar manner as the primary endpoint.
- Total consumption of rescue analgesia will be analyzed nonparametrically using the Wilcoxon rank sum test.
- Patient Global Assessment at scheduled evaluations will be assessed for treatment group differences using an ANCOVA comparison with center and the baseline pain score as the stratification factor.

13.6.5. Tertiary endpoints

Tertiary endpoints will be assessed as follows:

- Time-specific pain intensity profile over time will be assessed using a mixed models repeated measures (MMRM), with fixed effects for treatment, time, the treatment by time interaction, and random patient effect. Pairwise comparisons at each time point will be determined from the MMRM, and model-based means (LSMeans) and standard errors will be plotted for visual comparison of the treatment group outcomes.
- The time to pain relief (meaningful and perceptible), as well as time to first rescue medication, will be assessed using a log-rank statistic, stratified by center. A Kaplan-Meier plot will be provided for each of these outcomes. The time to event analyses will include the times to events of death or withdrawal from study as endpoints. Patients will be censored at 4 hours for time to pain relief, while time to first rescue medication will be censored at 48 hours.
- The number (%) of patients who require no rescue analgesia during the 48-hour period following T0 will be assessed using a CMH test stratified by center.
- The rate of consumption of rescue analgesia will be analyzed nonparametrically using the Wilcoxon rank sum test.

13.7. Alpha Levels

There are 2 pairwise comparisons of interest:

- High-dose tramadol (50 mg) vs placebo
- Low-dose tramadol (25 mg) vs placebo

All inferential assessments will be 2-sided tests performed at the 0.05 alpha level. A hierarchical alpha testing strategy will be utilized to control for the overall experiment-wise alpha. As there are multiple tests being performed (the single primary efficacy variable pairwise test and the 2 key secondary efficacy tests), the following strategy will be applied:

The high-dose tramadol arm vs placebo comparison will be assessed for the primary endpoint at the 0.05 alpha level. If and only if the p-value is ≤ 0.05 for this pairwise comparison will the lower dose tramadol arm vs placebo comparison be assessed. If the p-value for the high-dose arm is NOT significant, all testing will cease and it will be concluded that neither tramadol treatment arm provides better pain relief than placebo.

If the primary endpoint is significant for the AVE-901 vs. placebo comparison (in favor of the tramadol arm) for one or both pair-wise tests, then statistical testing will proceed to the secondary endpoints within each pair-wise grouping, to be tested in the following order:

- SPID24
- Total consumption of rescue analgesia
- Patient Global Assessment of efficacy at 24 and 48 hours (the two time points will be tested simultaneously)

If a statistical test within each pairwise comparison is significant at the nominal 0.05 level, two-sided (in favor of the tramadol arm), then testing will proceed to the next endpoint in the list. Once a non-significant test occurs, endpoints lower in the list are considered not statistically significant.

Note that while inferential comparison of the high dose to the low dose is not a key aspect of the statistical analysis, assessment of the dose-response will be performed across the 3 doses in order to allow visual comparison of the outcomes for the primary and key secondary endpoints.

13.8. Subgroups

Analysis of the primary and key secondary endpoints, as well as treatment-emergent AE and serious AE incidence, will be provided by the following subgroups:

- Gender
- Race
- Age (using the study median age)
- Investigational center

13.9. Safety

The safety analysis will be descriptive in nature. All safety data will be listed, and data will be tabulated by treatment group where the data warrant. Safety data include:

- AEs, including assessment of infusion site local reactions (skin and vein)
- Clinical laboratory tests (hematology panel, chemistry panel and urinalysis) pre-treatment and discharge
- Vital signs including: respiratory rate, heart rate, and blood pressure, as per the schedule of events
- Physical examination pre-treatment and discharge
- 12-lead ECG at protocol specified timepoints
- Concomitant treatments

Exploratory analyses of use of anti-emetics may be performed. Exploratory analyses of use of anti-emetics may be performed. Other safety data presentations will be descriptive in nature and no formal statistical tests will be performed.

ECG results will be analyzed on an ongoing basis by a central ECG reader on an individual patient level and as well as by group analysis.

13.9.1. Adverse Events

Adverse events will be coded using the MedDRA coding dictionary; patient incidence of each system organ class and unique term will be tabulated. AE incidence will also be tabulated according to relationship to study medication and severity. Serious AEs and AEs resulting in premature discontinuation will be tabulated.

Adverse events starting before the first dose of treatment will be reported as Medical History Adverse events starting after the first dose of treatment will be considered treatment-emergent adverse events.

Local tolerability at the infusion site will be assessed for pain, swelling, tenderness, and erythema.

13.9.2. Prior and Concomitant Medications

Prior and concomitant medications will be reviewed and coded using the WHO Drug Dictionary, and tabulated by treatment.

13.9.3. Clinical Laboratories

Clinical laboratory observed values and changes from pre-treatment to on-treatment time points may be tabulated for continuous parameters, as warranted.

13.9.4. Vital Signs

Vital sign parameter outcomes (including SpO2) will be assessed for clinical significance; observed values and changes from pre-treatment to on-treatment time points may be tabulated for continuous parameters, as warranted.

13.9.5. Physical Examination

Physical examination outcomes will be listed in data listings.

14. STUDY MONITORING, AUDITS, IRB, AND QUALITY

14.1. Study Monitoring

Before an investigational site can enter a patient into the study, a Sponsor representative will visit the investigational study site to:

- Determine the adequacy of the facilities
- Discuss with the investigator(s) and other personnel their responsibilities with regard to protocol adherence, and the responsibilities of the Sponsor or its representatives. This will be documented in a Clinical Study Agreement between the Sponsor (or its delegate) and the investigator.

During the study, a monitor or Sponsor representative will have regular contacts with the investigational site, for the following:

- Provide information and support to the investigator(s)
- Confirm that facilities remain acceptable
- Confirm that the investigational team is adhering to the protocol, that data are being accurately recorded in the eCRF, and that investigational product accountability checks are being performed
- Perform source data verification. This includes a comparison of the data in the eCRF with the patient's medical records at the hospital or practice, and other records relevant to the study. This will require direct access to all original (or faxed/copied, if requested) records for each patient (e.g. clinic charts) which may include access to medical records for purposes of remote (i.e. not on-site at the Investigator's clinic) source data verification
- Record and report any protocol deviations not previously sent to the Sponsor (or its delegate)
- Confirm AEs and SAEs have been properly documented on eCRFs and confirm any SAEs have been forwarded to Sponsor (or its delegate) and those SAEs that met criteria for reporting have been forwarded to the IRB.

The monitor will be available between visits if the investigator(s) or other staff needs information or advice.

14.2. Audits and Inspections

Authorized representatives of the Sponsor, its delegate, a regulatory authority, an Independent Ethics Committee or an Institutional Review Board may visit the site to perform audits or inspections, including source data verification. The purpose of an audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, Good Clinical Practice guidelines of the International Conference on Harmonization, and any applicable regulatory requirements. The

investigator should contact the Sponsor or its delegate immediately if contacted by a regulatory agency about an inspection.

14.3. Institutional Review Board/ Independent Ethics Committee

The Investigator must obtain appropriate IRB approval prior to study initiation. A copy of the written approval from the IRB and a copy of the approved ICF should be sent to the Sponsor or its delegate. It is also necessary to submit a list of the IRB members (including their Institution affiliations, gender makeup, and occupations) or supply a statement from the IRB specifying that the membership comply with applicable regulations.

The study protocol, patient information and consent form, the Investigator Brochure, available safety information, patient recruitment procedures (e.g., advertisements), information about payments and compensation available to the patients and documentation evidencing the Investigator's qualifications should be submitted to the IRB/Ethics Committee for ethical review and approval according to local regulations, prior to the study start. The written approval should identify all documents reviewed by name and version.

14.4. Quality Control and Quality Assurance

The investigator is responsible for all quality control and quality assurance for the performance of the study.

15. ETHICS

15.1. Ethics Review

The final study protocol, including the final version of the Informed Consent Form, must be approved or given a favorable opinion in writing by an IRB or IEC as appropriate. The investigator must submit written approval to the Sponsor or its delegate before he or she can enroll any patient into the study.

The Principal Investigator is responsible for informing the IRB or IEC of any amendment to the protocol in accordance with local requirements. In addition, the IRB or IEC must approve all advertising used to recruit patients for the study. The protocol must be re-approved by the IRB or IEC upon receipt of amendments and annually, as local regulations require.

The Principal Investigator is also responsible for providing the IRB with reports of any reportable serious adverse drug reactions from any other study conducted with the investigational product. The Sponsor or its delegate will provide this information to the Principal Investigator.

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

15.2. Ethical Conduct of the Study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with ICH/GCP, applicable regulatory requirements and the Sponsor or its delegate's policy on Bioethics.

15.3. Written Informed Consent

The Investigator(s) will ensure that the patient is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study. Patients must also be notified that they are free to discontinue from the study at any time. The patient should be given the opportunity to ask questions and allowed time to consider the information provided.

The patient's signed and dated ICF and assent if applicable must be obtained before conducting any study procedures.

The Investigator(s) must maintain the original, signed ICF. A copy of the signed ICF must be given to the patient.

16. DATA HANDLING AND RECORDKEEPING

16.1. Inspection of Records

The Sponsor or its delegate will be allowed to conduct site visits to the investigation facilities for the purpose of monitoring any aspect of the study. The Investigator agrees to allow the monitor to inspect the drug storage area, study drug stocks, drug accountability records, patient charts and study source documents, and other records relative to study conduct.

16.2. Retention of Records

The Principal Investigator must maintain all documentation relating to the study for a period of 2 years after the last marketing application approval, or if not approved 2 years following the discontinuance of the test article for investigation. If it becomes necessary for the Sponsor, its delegate, or the Regulatory Authority to review any documentation relating to the study, the Investigator must permit access to such records.

16.3. Data Capture and Processing

Data will be captured on source documents and will be entered into an electronic data capture system via electronic case report forms (eCRFs) and will be processed according to a data management plan. The database will be cleaned and 'locked' according to that data management plan prior to the final statistical analysis being performed.

eCRFs will be completed for each study patient. It is the investigator's responsibility to ensure the accuracy, completeness, and timeliness of the data entered in each patient's eCRF. Source documentation supporting the eCRF data should indicate the patient's participation in the study and should document the dates and details of study procedures, adverse events, and patient status.

The investigator, or designated representative, should complete the eCRF as soon as possible after information is collected. Any outstanding entries must be entered immediately after the final examination. An explanation should be given for all missing data.

17. PUBLICATION POLICY

All information concerning the product, as well as any matter concerning the operation of the Sponsor or its delegate, such as clinical indications for the drug, its formula, methods of manufacture, and other scientific data relating to it, that have been provided by the Sponsor or its delegate and are unpublished, are confidential and must remain the sole property of the Sponsor or its delegate. The Investigator will agree to use the information only for the purposes of carrying out this study and for no other purpose unless prior written permission from the Sponsor or its delegate is obtained. The Sponsor has full ownership of the eCRFs completed as part of the study.

All publications and presentations of the results of the Study are governed by the applicable provisions of the Clinical Trial Agreement between the Sponsor (or its delegate) and the institution. By signing the study protocol, the investigator agrees that the results of the study may be used for the purposes of national and international registration, publication, and information for medical and pharmaceutical professionals by the Sponsor or its delegate. If necessary, the authorities will be notified of the Investigator's name, address, qualifications, and extent of involvement. The Investigator may not publish or present any information on this study without the express written approval of the Sponsor or its delegate. Additionally, the Sponsor or its delegate may, for any reason, withhold approval for publication or presentation. Such manuscript or materials should be provided for Sponsor/delegate review only after the final database, which has been approved by Quality Assurance, is available.

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APPENDIX 1

Numerical Pain Rating Scale (NPRS)

Pain Intensity - Numerical Pain Rating Scale (NPRS)
Instructions to Study Subject: Please respond to the question below. When completed, please initial at the bottom of the assessment.
On a scale of 0-10, please rate your pain by marking an 'X' in the appropriate box that best describes your pain NOW. ☐0 ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9 ☐10 *No Pain* *Worst pain imaginable* **Subject Initials:** _____