Clinvest Research, LLC.
Statistical Analysis Plan

Study Title:	**A Multicenter, Randomized, Double-Blind, Placebo-Controlled Study Evaluating the Efficacy and Safety of ISIS 546254 for Preventive Treatment of Chronic Migraine**
Protocol Number:	**15-001IS**
Sponsor:	Timothy R. Smith MD, RPh, FACP 4350 S. National Ave. Suite C100 Springfield, MO 65810 Phone: (417) 883-7889 Email: timothy.smith.md@gmail.com
Medical Monitor:	Timothy R. Smith MD, RPh, FACP 4350 S. National Ave. Suite C100 Springfield, MO 65810 Phone: (417) 883-7889 Email: timothy.smith.md@gmail.com
Trial Biostatistician	Jim Sly, MS 4350 S. National Ave. Suite C100 Springfield, MO 65810 Phone: (417) 841-3673 Email: jsly@clinvest.com
SAP Author	Jim Sly, MS
Date of Original SAP (Version No. 1.0):	17-Jan-2018

Approved by:	**Date:**	**Signature:**
Jim Sly, MS	_____	_____

Confidential Information

The information contained in this document is the property or under the control of Clinvest Research, LLC., and is provided to you in confidence as an Investigator, potential Investigator, or consultant for review by you, your staff and applicable Institutional Review Board. You shall not disclose this information to others without prior written authorization from Clinvest Research, LLC., except to the extent necessary to obtain informed consent from those persons to whom the drug may be administered, and except as may be required by federal, state, or local laws or regulations.

Table of Contents

1 Abbreviations and Definitions

AE	Adverse Event
ANOVA	Analysis of Variance
BOCF	Baseline Observation Carried Forward
C-SSRS	Columbia-Suicide Severity Rating Scale
ECG	Electrocardiogram
EDC	Electronic Data Capture
HIV	Human immunodeficiency virus (HIV)
ICHD	International Classification of Headache Disorders
IRB	Institutional Review Board
IP	Investigational Product
LOCF	Last Observation Carried Forward
MedDRA	Medical Dictionary of Regulatory Affairs
mITT	Modified Intent-to-Treat
MO	Medication Overuse
MSQ	Migraine Specific Quality of Life Questionnaire
NSAE	Non-Serious Adverse Event
PK	Pharmacokinetics
PGIC	Physician Global Impression of Change
SAP	Statistical Analysis Plan
SAE	Serious Adverse Event
SC	Subcutaneous
SGIC	Subject Global Impression of Change

2 Introduction

2.1 Study Purpose

To evaluate the safety, tolerability, and changes in the number of migraine and headache days with repeated subcutaneous administration of ISIS 546254 or placebo in subjects with chronic migraine.

2.2 Summary of Study Design

This is a double-blind, placebo-controlled, randomized, multi-center study in subjects with chronic migraine. The study will consist of 7 office visits, 6 sample collection visits and 3 phone call assessments. Subjects agreeing to participate in the study and meeting the entry criteria assessed at the screening visit, will begin a 28-day baseline period to confirm their diagnosis, and establish a baseline frequency of migraine and headache days. During the baseline period, subjects will continue treating their migraines in their usual manner. They will monitor headache activity, migraine related symptoms, and medication usage with an electronic daily headache diary.

Subjects who, after completing the baseline, continue to meet entrance criteria will be eligible to enter into the 4-month treatment phase. They will be randomized according to the Clinvest generated randomization schedule. A total of 30 randomized subjects will enter the treatment phase receiving ISIS 546254 (SC) or placebo in a 1:1 design. Study drug or placebo will be administered weekly for 16 weeks. A short phone call to assess any treatment related adverse events will take place 1 and 2 days after randomization. Daily electronic diary assessments will collect headache frequency and severity, associated migraine symptoms, acute medication usage, and the emergence of unusual symptoms and adverse events. Subjects will return to the site at weeks 4, 8, and 12 for investigational product (IP) accountability/dispensing, medication and medication updates, biomarker/lab sample collection, and assessment of adverse events. An end of treatment visit will take place 16 weeks after randomization. Subjects will have a follow-up safety visit one month after their last dosage of IP) for assessment of any adverse events (AE) and satisfaction and a final safety phone call 2 months following their last office visit (3 months after last dose of IP) for assessment of any adverse events (AE). Subjects will continue to complete headache diaries through Visit 7. Subjects will also have hematology samples collected every other week starting after Visit 2 through Day 154.

The study consists of 3 phases (Table 1):

Baseline Phase:	Visit 1 (Screening/Baseline Period – Day -28)
Treatment Phase:	Visit 2 (Randomization/Treatment Month 1/Day 0)
	Phone Call Visit 1 (Randomization/Treatment Month 1/Day 1)
	Phone Call Visit 2 (Randomization/Treatment Month 1/Day 2)
	Sample Collection – Day 14
	Visit 3 (Treatment Period Month 2/Day 28)
	Sample Collection – Day 42
	Visit 4 (Treatment Period Month 3/Day 56)
	Sample Collection – Day 70
	Visit 5 (Treatment Period Month 4/Day 84)

	Sample Collection – Day 98
Follow-up Phase:	Visit 6 (End of Treatment/ET/Day 112)
	Sample Collection – Day 126
	Visit 7 (Follow up one month after last IP dosage/Day 140)
	Sample Collection – Day 154
	Phone Call Visit (3 months after last IP dosage/Day 196)

Safety and tolerability will be monitored by the Investigators. Patients who discontinue study treatment prematurely should complete any follow-up visits associated with the most recent dose and should move into and complete the Follow-up Phase.

Subjects will undergo sampling for (pharmacokinetics) PK, coagulation, chemistry, hematology, and optional future biomedical research, as specified in the schedule of procedures.

2.3 Power Analysis

A power analysis, using GPower Version 3.1.9.3, indicated a total sample size of 30 subjects would be needed to detect a medium effect (partial eta squared = .07) with 80% power using a 2 X 2 mixed factorial analysis of variance (ANOVA) test to include within-between interactions with alpha being set at .05.

2.4 Inclusion-Exclusion Criteria

Subjects who meet all of the following inclusion criteria and none of the exclusion criteria will be enrolled.

2.4.1 Inclusion Criteria

Potential subjects must meet the following criteria at the screening visit to enter this study:

1. male or female, in otherwise good health, 18 to 65 years of age.
2. history of chronic migraine meeting the diagnostic criteria listed in the International Classification of Headache Disorders (ICHD-III beta version, 2013; see Appendix A), as follows:
    a. History of frequent headaches suggestive of chronic migraine (15 or greater days of qualifying headaches per month) for at least three months prior to screening
    b. Verification of headache frequency through prospectively collected baseline information during the 28-day run-in phase demonstrating headaches on at least 15 days, with at least 8 days per month fulfilling any ONE of the following;
        i. Qualify as being a migraine attack
        ii. Relieved by migraine specific acute medications
3. onset of migraine before age 50.
4. stable pattern of migraine pattern for at least 3 months prior to screening.

5. not currently taking a migraine preventive **OR** has been taking a stable dose of a preventive for at least 30 days prior to screening and agrees to not start, stop, or change medication and/or dosage during the study period.
    i. Subjects on migraine preventative should have stable headache pattern
    ii. Injections of onabotulinumtoxinA are allowable if subject has completed at least 2 injection cycles and agrees to maintain a regular injection cycle for the duration of the study
6. females must be either surgically sterile (e.g., tubal occlusion such as bilateral tubal ligation, hysterectomy, bilateral salpingectomy, bilateral oophorectomy) **OR** post-menopausal (>12 months since last period) **OR** if of child-bearing potential, using an acceptable contraceptive method during, and for 97 days (approximately 5 half-lives of ISIS 546254) after the last dose of study drug.
7. males must be surgically sterile, abstinent, **OR** if engaged in sexual relations of child-bearing potential, the subject must be using an acceptable contraceptive method as defined in Section 14 during and for at least 97 days after the last dose of study drug.
8. completion of online diary must be ≥ 80 % compliance, unless otherwise approved by Clinvest.
9. must have given written, informed consent and obtain any authorizations required by local law.
10. be able and willing to comply with all study requirements.

2.4.2 Exclusion Criteria

Potential subjects meeting any of the following criteria will be excluded from entering this study:

1. unable to understand the study requirements, the informed consent, or complete headache records as required per protocol.
2. pregnant, actively trying to become pregnant, or breast-feeding.
3. history of medication overuse (MO) of opioids, or butalbital, as defined by ICHD-3 beta criteria and/or MO during baseline period (Appendix C).
4. history of substance abuse and/or dependence, in the opinion of the Investigator.
5. unstable neurological condition or a significantly abnormal neurological examination with focal signs or signs of increased intracranial pressure.
6. suffers from a serious illness, or an unstable medical condition, one that could require hospitalization, or could increase the risk of adverse events.
7. any psychiatric condition with psychotic features, and/or any other psychiatric disorder not stable or well controlled, that would interfere in the ability to complete study activities.
8. history of thrombocytopenia.
9. history of bleeding, diathesis or coagulopathy

10. use of any anticoagulant
11. received any investigational agents within 30 days prior to Visit 1, or 5 half-lives of study drug, whichever is longer.
12. has significant risk of suicide, defined as a "yes" answer to any of the following questions on the Columbia-Suicide Severity Rating Scale (C-SSRS), either at the screening visit (when assessing the prior 12 months) or at visit 2 (when assessing time since the screening visit):
    a. Questions 4 or 5 on the suicidal ideation section
    b. Any question on any item in the suicidal behavior section
13. plans to participate in another clinical study at any time during this study.
14. malignancy within 5 years, except for basal or squamous cell carcinoma of the skin or carcinoma in situ of the cervix that has been successfully treated.
15. treatment with any non-Ionis oligonucleotide (including siRNA) at any time or prior treatment with an Ionis oligonucleotide within 6 months of screening. Subjects that have previously received only a single dose of an ISIS oligonucleotide as part of a clinical study may be included as long as a duration ≥4 months elapsed since dosing.
16. unwillingness or inability to comply with study procedures, including follow-up, as specified by this protocol.
17. electrocardiogram (ECG) showing a clinically significant abnormality at screening.
18. screening laboratory results that would render a subject unsuitable for study participation including, but not limited to:
    a. ALT/AST > 1.5 x ULN
    b. Bilirubin ≥ 1.2 x ULN
        i. Study Medical Monitor may allow subjects with bilirubin ≥ 1.2 x ULN on study **if all of the following are satisfied**:
            1. indirect bilirubin only is elevated
            2. ALT/AST is ≤ ULN
            3. genetic testing confirms Gilbert's disease
    c. Platelets < 150 x 109/L
    d. Alkaline phosphatase > 2.0 x ULN
    e. Cockcroft-Gault calculated GFR < 60 mL/min
    f. INR > 1.2
    g. aPTT > ULN
    h. PT > ULN
    i. Serum creatinine > 1.1 x ULN
    j. Urine protein/creatinine (P/C) ratio ≥ 0.2 mg/mg.
        i. In the event of P/C ratio above this threshold eligibility may be confirmed by a quantitative total urine protein measurement of < 200 mg/24hr with prior Sponsor approval
    k. Urine blood ≥ trace by dipstick
        i. Subjects with positive dipsticks are eligible if urine microscopy shows ≤ 5 red blood cells per high power field.

19. have any other conditions, which, in the opinion of the Investigator would make the subject unsuitable for inclusion, or could interfere with the subject participating in or completing the study.
20. active infection requiring systemic antiviral or antimicrobial therapy that will not be completed prior to Study Day 1
21. positive urine drug screen for substances not otherwise prescribed.

suffers from human compromised immune system including immunodeficiency virus (HIV), Hepatitis B or C

2.5 Randomization and Blinding

Eligible subjects will be randomized 1:1 to receive ISIS 546254, 200 mg/mL, 1 mL or placebo at Visit 2 by unblinded staff. If at any time during the study the subject withdrawals from the study, they will be instructed to return all used packaging and unused medication to unblinded staff. All doses (200mg ISIS546254 or placebo will be given by subcutaneous (SC) injection at a standardized volume of 1.00 mL ISIS 546254 or placebo weekly through week 16. All dosing after the first injection at the site may be self-administered per dosing schedule if desired by the subject. All subjects will have the option to return to the site for injections administered by the unblinded staff if they desire. Subjects will receive training on proper administration of IP, storage requirements, and will be asked to return all used/partially used/unused medication containers at the next office visit to unblinded staff. Any subject self-administering IP will call site to confirm most recent lab report values are within allowable rages dosing.

Adjustments of dose and/or treatment schedule should occur only on rare occasions. Adjustments in the dose and/or treatment schedule may be allowed only with consent of the study medical monitor following discussion with the investigator for subjects that are unable to tolerate the once weekly dose.

At randomization (Visit 2), neither the subject nor the investigator will be aware to which treatment group the subject has been assigned. Investigational product will be drawn and administered by an unblinded member of the staff. If needed, for safety and proper treatment of the subject, the investigator can unblind the subject's treatment assignment to determine which treatment has been assigned and institute appropriate follow-up care.

2.6 Study Procedures

	Screening	Randomization	Treatment							
	Visit 1 Day -28	Visit 2 Day 0 (+3)	Phone Call 1&2 Day 1 & 2	Sample Collection Day 14 (+/- 3)	Visit 3 Day 28 (+/- 3)	Sample Collection Day 42 (+/- 3)	Visit 4 Day 56 (+/- 3)	Sample Collection Day 70 (+/-3)	Visit 5 Day 84 (+/-3)	Sample Collection Day 98 (+/-3)
Informed Consent	X									
Physical/Neurological Exam	X									
Vital Signs	X	X			X		X		X	
Verify Inclusion/Exclusion	X	X								
Subject Randomization		X								
Medical History	X									
Migraine History	X									
Medication History	X									
Update Concomitant Medications		X	X		X		X		X	
Urine Drug Screen	X	X								
Clinical Labs*	X	X		X†	X	X†	X	X†	X	X†
Plasma Collection for ADA Screening		X			X				X	
ECG	X	X			X		X		X	
Serology (HIV/ Hep B and C)	X									
Pregnancy Test	X	X			X		X		X	
SGIC										
PGIC										
Dispense Study Medication		X			X		X		X	
Drug Accountability					X		X		X	
Headache Diary	X	X			X		X		X	
Review Diary		X			X		X		X	
Collect Adverse Events		X	X		X		X		X	
Administer MSQ		X								
Administer C-SSRS	X	X			X		X		X	
Follow-Up Phone Call			X							

	EOT/ET	Follow-Up			
	Visit 6 Day 112 (+/- 3)	Sample Collection Day 126 (+/-3)	Visit 7 Day 140 (+/- 3)	Sample Collection Day 154 (+/-3)	Phone Call 3 Day 196 (+/- 3)
Informed Consent					
Physical/Neurological Exam	X				
Vital Signs	X		X		
Verify Inclusion/Exclusion					
Subject Randomization					
Medical History					
Migraine History					
Medication History					
Update Concomitant Medications	X		X		X
Urine Drug Screen					
Clinical Labs*	X	X†	X	X†	
Plasma Collection for ADA Screening	X				
ECG	X		X		
Serology (HIV/ Heb B and C)					
Pregnancy Test	X		X		
SGIC	X				
PGIC	X				
Dispense Study Medication					
Drug Accountability	X				
Headache Diary					
Review Diary	X				
Collect Adverse Events	X		X		X
Administer MSQ	X		X		X
Administer C-SSRS	X		X		
Follow-Up Phone Call					X

***Clinical chemistry, hematology, inflammation panel, PK and PD biomarkers (see table 2). Any missing or unreportable value should be repeated as soon as possible (within 1 week) using a clinic local to the patient if necessary.

†Hematology panel only. Any missing or unreportable value should be repeated as soon as possible (within 1 week) using a clinic local to the patient if necessary

3 Analysis Populations

3.1 Full Analysis Population

The full analysis population will include all subjects who were enrolled. The full analysis population will be used for demographic reporting.

3.2 Intent-to-Treat Population (ITT) Population

The intent-to-treat (ITT) population will include all randomized subjects who received at least one dose of study drug. The ITT population will be used for both primary endpoint analyses (Safety: adverse event and laboratory abnormality endpoints analyses, and Efficacy: change in the monthly migraine days.

3.3 Modified Intent-to-Treat Population (mITT)

The modified intent-to-treat (mITT) population will include all randomized subjects who received at least one dose of study drug and obtained at least one full endpoint measurement (e.g., completion of one month treatment eDiary). The mITT population will be used for all primary and secondary efficacy endpoint analyses.

4 Endpoints

4.1 Primary Endpoints

1. Compare the number of adverse events and laboratory abnormalities through the study for subjects treated with ISIS 546254 vs. placebo.
2. Compare the efficacy of ISIS 546254 in the preventive treatment of chronic migraine, measured by mean change in the monthly migraine days comparing baseline to the final month of the 4-month treatment period for subjects treated with ISIS 546254 vs. placebo.

4.2 Secondary Endpoints

1. Evaluate the efficacy of ISIS 546254 in the preventive treatment of chronic migraine, measured by mean change in the monthly headache severity comparing baseline to the final month of the 4-month treatment period for subjects treated with ISIS 546254 vs. placebo.
2. Compare the efficacy of ISIS 546254 in the preventive treatment of chronic migraine, measured by mean change in the monthly headache days comparing baseline to the final month of the 4-month treatment period for subjects treated with ISIS 546254 vs. placebo.
3. Compare the proportion of patients meeting 50% response criteria, response defined as a $\geq$ 50% reduction in the number of headaches from baseline to the final month of the 4-month treatment period for subjects treated with ISIS 546254 vs. placebo.
4. Compare the change in frequency in the number of headache days requiring use of medication for the treatment of migraine or headache pain (i.e., acute and rescue or

breakthrough medication use) from baseline to the final month of the 4-month treatment period for subjects treated with ISIS 546254 vs. placebo.

5. Compare change from baseline to the final month of the 4-month treatment period in the Migraine Specific Quality of Life (MSQ) Questionnaire for subjects treated with ISIS 546254 vs. placebo.
6. Compare the end of treatment month 4 in the physician global impress of change (PGIC) for subjects treated with ISIS 546254 vs. placebo.
7. Compare the end of treatment month 4 in the subjects' global impression of change (SGIC) for subjects treated with ISIS 546254 vs. placebo.

5 Collection and Derivation of Endpoint Variables

Data required for the evaluation of endpoints will be recorded for the duration of the study using electronic data capture (EDC) and include subject reported outcomes. Subjects will record data into the web-based EDC using a daily eDiary. Subjects will be provided instruction and training regarding daily eDiary entry and compliance requirements. On each day, the subject will be asked to record their diary data for the previous 24-hour period. The headache variables will be derived from variables collected daily using an electronic headache diary.

Clinic visits will be scheduled to occur every 28 days throughout the study; however, in practice, there may or may not be an exact 28-day duration between 2 consecutive visits. For this reason, subjects will be given access to 31 days of eDiary to complete between clinic visits. For the collection and derivation of endpoint variables and analyses, subject's eDiary information will be used as follows:

- eDiary information entered during the first 28 continuous days of the run-in period will serve as the "baseline" for calculating change from baseline for 28-day periods subsequent to each office visit.

- If a subject enters more than 28 days of eDiary information between clinic visits during the treatment phase of the study, the last (most recent) 28 eDiary entries will be used for analyses. For example, if a subject has a duration of 31 days between Visit 2 and Visit 3, and the subject entered eDiary information for all 31 days, the last 28 days of eDiary information will be used to calculate and derive endpoint variables, with the first three eDiary entries being discarded from the analysis datasets.

- Each subject-entered eDiary record will be used and analyzed as a separate study day, regardless of the subject's entered diary date (value entered from the subject as the date of diary data), actual date of eDiary entry (EDC timestamp), or perceived duplicate entry.

5.1 Primary Efficacy Variable

The variable used to measure the primary efficacy endpoint of change in frequency of migraine headache days will be derived from subjects self-reported data entered into the eDiary. The time points for this variable are baseline month and treatment month 4. This variable is derived from the eDiary and is based on the count of days with migraine headaches for both 28-day time points (baseline and treatment month 4). Migraine headaches are defined in this study as an attack within a calendar day (00:00 to 23:59) that meet at least one of the following two criteria:

1. A headache attack that includes all of the following criteria:
    A. Headache attacks lasting 4-72 hours (untreated or unsuccessfully treated)

    B. Headache has at least two of the following four characteristics:

        1. unilateral location

        2. pulsating quality

        3. moderate or severe pain intensity

        4. aggravation by or causing avoidance of routine physical activity (e.g. walking or climbing stairs)

    C. During headache at least one of the following:

        1. nausea and/or vomiting

        2. photophobia and phonophobia

2. A headache attack of any duration with the use of migraine-specific acute medication(s).

    Migraine-specific acute medication(s) are defined as any of the following pre-populated options available on the eDiary dropdown question "*What was the name of the medication taken?*":

    - Amerge (Naratriptan)
    - Axert (Almotriptan)
    - Butabitol
    - Cafergot (Ergotamine Tartrate/Caffeine)
    - Cambia (Diclofenac Potassium)
    - DHE 45 (Dihydroergotamine)
    - Excedrin Migraine
    - Fiorinal
    - Frova (Frovatriptan)
    - Imitrex (Sumatriptan)
    - Indocin (Indomethacin)

- Maxalt (Rizatriptan)
- Midrin (Isometheptene Mucate, Dichloralphenazone and Acetaminophen)
- Migerot (Ergotamine Tartrate/Caffeine Suppository)
- Migranol
- Migril (Ergotamine, Cylizine, and Caffeine)
- Onzetra (Sumatriptan Nasal Powder)
- Relpax (Electriptan)
- Sumavel (Sumatriptan Needleless Injection)
- Tivorbex (Indomethacin)
- Treximet (Sumatriptan/Naproxen)
- Zecuity (Sumatriptan Dermal Patch)
- Zembrace (Sumatriptan injection)
- Zomig (Zolmitriptan)
- Zorvolex (Diclofenac)

5.2 Secondary Efficacy Variables

1. The variable used to measure the secondary efficacy endpoint of mean change in headache severity will be derived from subjects self-reported data entered into the eDiary. Headache severity will be determined from the eDiary question "*What was the greatest severity of head pain that you experienced yesterday?*" and is coded as: 0 = *No Pain*, 1 = *Mild Pain*, 2 = *Moderate Pain*, 3 = *Severe Pain*. The time points for this variable are baseline month and treatment month 4. Average headache severity will be calculated via the arithmetic mean for both 28-day time points (baseline and treatment month 4).
2. The variable used to measure the secondary efficacy endpoint of change in frequency of headache days will be derived from subjects self-reported data entered into the eDiary. The time points for this variable are baseline month and treatment month 4. This variable is derived from the eDiary and is based on the count of days with headaches for both 28-day time points (baseline and treatment month 4). Headache days are defined as any subject self-reported answer other than *No Pain* (0) to the eDiary question "*What was the greatest severity of head pain that you experienced yesterday?*" Answer options for this question are coded as: 0 = *No Pain*, 1 = *Mild Pain*, 2 = *Moderate Pain*, 3 = *Severe Pain*.
3. The variable used to measure the secondary efficacy endpoint of proportion of subjects meeting 50% response criteria (responders) will be derived from subjects self-reported data entered into the eDiary. The time points for this variable are baseline month and treatment month 4. This variable is derived from the eDiary and is based on the count of days with headaches for both 28-day time points (baseline and treatment month 4). Headache days are defined as any subject self-reported answer other than *No Pain* (0) to the eDiary question "*What was the greatest severity of head pain that you experienced yesterday?*" Answer options for this question are coded as: 0 = *No Pain*, 1 = *Mild Pain*, 2 = *Moderate Pain*, 3 = *Severe Pain*. Responders are defined as any subject with ≥ 50%

reduction in the number of headaches from baseline to the final month of the 4-month treatment period.

4. The variable used to measure the secondary efficacy endpoint of change in frequency of headache days requiring medication usage will be derived from subjects self-reported data entered into the eDiary. The time points for this variable are baseline month and treatment month 4. This variable is derived from the eDiary and is based on the total number of days that any acute, rescue, or breakthrough medication use is recorded in the eDiary. Acute, rescue, or breakthrough medication use is defined as any entry in the eDiary dropdown question "*What was the name of the medication taken?*".
5. The variable used to measure the secondary efficacy endpoint of change in Migraine Specific Quality of Life (MSQ) score will be derived from subjects self-reported data entered into the Migraine Specific Quality of Life (MSQ) questionnaires administered at Visit 2 (Randomization) and Visit 6 (End of Treatment). The MSQ total score is derived from the summation of all fourteen questions on each MSQ. Answer options for the MSQ are coded as: 1 = *None of the time*, 2 = *A little bit of the time*, 3 = *Some of the time*, 4 = *A good bit of the time*, 5 = *Most of the time*, 6 = *All of the time*. The range of total MSQ score for each time point is 14 to 84 (with higher scores indicating more effects of migraine on the subject's daily activities).
6. The variables used to measure the secondary efficacy endpoint of comparison in Physician's Global Impression of Change (PGIC) scores by treatment arm will be derived from the physician's self-reported data entered into the PGIC at Visit 6 (End of Treatment). The PGIC is a one item questionnaire asking, "*Since the beginning of treatment in this study, how would you describe this patient's change (if any) in their overall status?".* The answer option for the PGIC is coded as: 3 = *Very Much Improved*, 2 = *Much Improved*, 1 = *Minimally Improved*, 0 = *No Change*, -1 = *Minimally Worse*, -2 = *Much Worse*, -3 = *Very Much Worse*.
7. The variables used to measure the secondary efficacy endpoint of comparison in Subject's Global Impression of Change (SGIC) scores by treatment arm will be derived from the subjects self-reported data entered into the SGIC at Visit 6 (End of Treatment). The SGIC is a one item questionnaire asking, "*Since the beginning of treatment in this study, how would you describe the change (if any) in your overall status?".* The answer option for the SGIC is coded as: 3 = *Very Much Improved*, 2 = *Much Improved*, 1 = *Minimally Improved*, 0 = *No Change*, -1 = *Minimally Worse*, -2 = *Much Worse*, -3 = *Very Much Worse*.

5.3 Safety Variables

Non-Serious adverse events (NSAEs) and Serious adverse events (SAEs) will be recorded in the EDC system by study staff and/or physicians and be coded to Medical Dictionary of Regulatory Affairs (MedDRA) preferred terms by the Clinvest Research Data Management team in consultation with the Medical Monitor.

6 Missing Data

A baseline observation carried forward (BOCF) method will be utilized to impute missing values for all ITT population subjects that discontinue the study prior to obtaining at least one full primary endpoint measurement (e.g., completion of one month treatment eDiary). For example, if a subject has a missing *frequency of migraine headache days* value (primary endpoint) at Treatment Month 1 due to discontinuation, the baseline value will be utilized for all further migraine headache days values through the end of study, and the subject will be considered a non-responder.

A last observation carried forward (LOCF) method will be utilized to impute missing values for all mITT population subjects with missing values for a specific efficacy analysis. This type of imputation replaces the missing value with the last observation value obtained for the subject.

Missing values on any item where there is only one measurement (e.g., PGIC and SGIC) will be imputed with the mean value of all eligible subjects for that particular analysis. If an eDiary day is not recorded by an active subject (i.e., a subject that has not early terminated the study), the day will be considered a non-headache day.

7 Timing of Final Analyses

The final analyses will be performed after:

- the finalization and approval of this statistical analysis plan (SAP) document
- all randomized subjects have exited the study by completing Follow-Up Phone Call 3 or early terminated prior to completion
- the EDC database has been locked

8 Efficacy Analyses

All statistical tests will be two-tailed, and an alpha of .05 will be used for statistical significance. All analyses of variance (ANOVAs) will be followed by univariate post-hoc tests as appropriate. Multiple comparison adjustments will be made if needed.

8.1 Primary Efficacy Analysis

Data for the primary endpoint will be statistically analyzed via a 2 X 2 mixed factorial ANOVA. The analysis will compare the mean change in the frequency of migraine headache days from baseline month to treatment month 4 between subjects treated with ISIS 546254 versus placebo.

8.2 Secondary Efficacy Analyses

8.2.1 Headache severity

Data for this secondary endpoint will be statistically analyzed via a 2 X 2 mixed factorial ANOVA. The analysis will compare the mean change in headache severity from baseline month to treatment month 4 between subjects treated with ISIS 546254 versus placebo.

8.2.2 Frequency of headache days

Data for this secondary endpoint will be statistically analyzed via a 2 X 2 mixed factorial ANOVA. The analysis will compare the mean change in the frequency of headache days from baseline month to treatment month 4 between subjects treated with ISIS 546254 versus placebo.

8.2.3 50% Responders - Headaches

Data for this secondary endpoint will be statistically analyzed via a Chi-squared analysis. The analysis will compare the proportion of Responders (defined above) from baseline month to treatment month 4 between subjects treated with ISIS 546254 versus placebo.

8.2.4 Headache days requiring medication usage

Data for this secondary endpoint will be statistically analyzed via a 2 X 2 mixed factorial ANOVA. The analysis will compare the mean change in the frequency of headache days requiring use of medication for the treatment of migraine or headache pain (i.e., acute and rescue or breakthrough medication use) from baseline month to treatment month 4 between subjects treated with ISIS 546254 versus placebo.

8.2.5 Migraine Specific Quality of Life (MSQ) scores at Visits 2 and 6

Data for this secondary endpoint will be statistically analyzed via a 2 X 2 mixed factorial ANOVA. The analysis will compare the mean change in total MSQ scores at Visit 2 (Randomization) and Visit 6 (End of Treatment) between subjects treated with ISIS 546254 versus placebo.

8.2.6 Physician Global Impression of Change (PGIC) at Visit 6

Data for this secondary endpoint will be statistically analyzed via an independent samples *t*-test. The analysis will compare the mean difference of PGIC scores between subjects treated with ISIS 546254 versus placebo.

8.2.7 Subject Global Impression of Change (SGIC) at Visit 6

Data for this secondary endpoint will be statistically analyzed via an independent samples *t*-test. The analysis will compare the mean difference of SGIC scores between subjects treated with ISIS 546254 versus placebo.

9 Safety Analysis

9.1 Adverse Events

Data for this safety endpoint will be statistically analyzed via a Chi-squared analysis. The analysis will compare the proportion of subjects experiencing adverse events throughout the study, between treatment arms (ISIS 546254 and placebo).

9.2 Laboratory Abnormalities

Data for this safety endpoint will be statistically analyzed via a Chi-squared analysis. The analysis will compare the proportion of subjects with laboratory abnormalities throughout the study, between treatment arms (ISIS 546254 and placebo).

10 APPENDIX A

Proposed Revised International Headache Society Criteria

for Migraine Without and With Aura

Headache Classification Committee: J Olesen, et al. The International Classification of Headache Disorders, 3rd edition (beta version). *Cephalalgia*. 2013;33:629-808.

1.1 Migraine without aura

Description:

Recurrent headache disorder manifesting in attacks lasting 4-72 hours.

Diagnostic criteria:

A. At least five attacks1 fulfilling criteria B–D

B. Headache attacks lasting 4-72 hours (untreated or unsuccessfully treated)

C. Headache has at least two of the following four characteristics:

1. unilateral location
2. pulsating quality
3. moderate or severe pain intensity
4. aggravation by or causing avoidance of routine physical activity (e.g. walking or climbing stairs)

D. During headache at least one of the following:

1. nausea and/or vomiting
2. photophobia and phonophobia

E. Not better accounted for by another ICHD-3 diagnosis.

1.2 Migraine with aura

Description:

Recurrent attacks, lasting minutes, of unilateral fully reversible visual, sensory or other central nervous system symptoms that usually develop gradually.

Diagnostic criteria:

A. At least two attacks fulfilling criteria B and C

B. One or more of the following fully reversible aura symptoms:

1. visual
2. sensory
3. speech and/or language
4. motor
5. brainstem
6. retinal

C. At least two of the following four characteristics:

1. at least one aura symptom spreads gradually over ≥5 minutes, and/or two or more symptoms occur in succession
2. each individual aura symptom lasts 5-60 minutes
3. at least one aura symptom is unilateral
4. the aura is accompanied, or followed within 60 minutes, by headache

D. Not better accounted for by another ICHD-3 diagnosis, and transient ischemic attack has been excluded.