Clinvest Research, LLC.
Clinical Study Protocol

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**
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Date of Original Protocol (Version No. 1.0):	13-Dec-2016
Date of Amendment 1 (Version No. 1.1):	12-Jun-2017

Confidential Information

INVESTIGATOR SIGNATURE PAGE

Title: A Multicenter, Randomized, Double-Blind, Placebo-Controlled Study Evaluating the Efficacy and Safety of ISIS 546254 for Preventive Treatment of Chronic Migraine

I agree to:

- Implement and conduct this study diligently and in strict compliance with the protocol, good clinical practices and all applicable laws and regulations.
- Maintain all information supplied by Clinvest Research, LLC. in confidence and, when this information is submitted to an Institutional Review Board (IRB), it will be submitted with a designation that the materials are confidential.

I have read and agree to follow this protocol.

_____	_____	_____
Investigator Printed Name	Signature	Date

Sponsor Protocol Approval

_____ **Date:** _____

Timothy R. Smith, MD, RPh, FACP

Medical Director, Clinvest Research, LLC.

SUMMARY OF CHANGES FOR 15-001IS PROTOCOL

Amendment 1 (Version No. 1.1, 12-Jun-2017)

Section	Pg	Change From	Change To	Justification
Header	All	12/13/2016	**Protocol No.**: 15-001IS **Title**: A Multicenter, Randomized, Double-Blind, Placebo-Controlled Study Evaluating the Efficacy and Safety of ISIS 546254 for Preventive Treatment of Chronic Migraine **Date**: 12-Jun-2017	Clarification of study information across all pages.
Footer	All	[Current page number]	**Version No.**: 1.1 **CONFIDENTIAL** [Current page number] of 54	Clarification of study information across all pages, as well as total page count.
Cover page	1			Revised Protocol Template Cover page to better detail Sponsor/Medical Monitor contact information, and protocol version history.
Investigator signature page	2	Separate Investigator and Sponsor signature pages (pp. 2-3)	Combined Investigator and Sponsor signature pages (p. 2)	Consolidate all required signatures on one page.
Summary of changes for 15-001IS Protocol	3-8			Addition of changes to protocol by revision number.
6. Study Drug	14	Subjects will receive Investigational Drug Product ISIS 546254, 200 mg/mL, 2 mL (to be provided by Ionis Pharmaceuticals, Inc.) or matching placebo, which will be provided by the sponsor. All doses (200mg) will be given by subcutaneous (SC) injection at a volume of 1.00 mL (200mg) ISIS 546254 or placebo and will be administered at 200mg weekly thereafter through week 16. Those randomized to ISIS 546254 will receive subcutaneous injections	Subjects will receive study drug (ISIS 546254, 200 mg/mL, 1 mL or placebo) which will be provided by Ionis Pharmaceuticals, Inc. All doses will be given by subcutaneous (SC) injection at a volume of 1.00 mL and will be administered at the same volume weekly thereafter through week 16. Those randomized to ISIS 546254 will receive subcutaneous injections containing 1.00 mL (200mg) weekly for weeks 1-16 of ISIS 546254.	Clarification of study drug units, and procedures.

		containing 1.00 mL (200mg) weekly for weeks 1-16 of ISIS 546254. Those randomized to placebo will receive 1.00 mL during weeks 1-16 subcutaneous injections of 0.9% sterile saline. Unblinded personal will label and package all study kits provided to subejcts following standard operating proceudes. The investigational product (IP) and matching placebo will be drawn by an unblinded staff member. The first dose of investigational product (IP) will be administered under supervision of the unblinded staff at the study site. Thereafter the IP 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 by the unblinded staff member if they prefer.	Those randomized to placebo will receive 1.00 mL during weeks 1-16 Unblinded personnel will label and package all study kits provided to subjects following standard operating procedures. The study drug will be drawn by an unblinded staff member. The first dose of study drug will be administered under supervision of the unblinded staff at the study site. Thereafter the study drug 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 by the unblinded staff member if they prefer	
7. Background & Study Rationale	17	CGRP	calcitonin gene-related peptide (CGRP)	Full name at first instance of abbreviation.
12.2 Visit 2 - Randomization (Day 0)	23	8. Clinical laboratory sample collection for testing hematology, chemistry, PK, biomarkers and urinalysis .	8. Clinical laboratory sample collection for testing hematology, chemistry, PK, biomarkers, plasma for anti-drug antibody screening, and urinalysis.	Addition of plasma for anti-drug antibody screening at Visit 2.
12.2 Visit 2 - Randomization (Day 0)	24	13. Dispense and instruct subjects on how to inject study drug. Subjects will also be educated on prohibited medications, dosage	13. Dispense and instruct subjects on how to inject study drug. Subjects will also be educated on prohibited medications, dosage	Addition of “unblinded staff.”

		limitations of study drug, and storage requirements. Subjects will be instructed to return all used/partially used/unused study drug at next office visit and drug reconciliation will be performed.	limitations of study drug, and storage requirements. Subjects will be instructed to return all used/partially used/unused study drug at next office visit to the unblinded staff and drug reconciliation will be performed.	
12.6 Visit 3 - End of Treatment Month 1 (Day 28 +/- 3 Days)	24	6. Clinical laboratory sample collection for testing hematology, chemistry, PK, biomarkers and urinalysis .	6. Clinical laboratory sample collection for testing hematology, chemistry, PK, biomarkers, plasma for anti-drug antibody screening, and urinalysis.	Addition of plasma for anti-drug antibody screening at Visit 3.
12.6 Visit 3 - End of Treatment Month 1 (Day 28 +/- 3 Days)	24	8. Perform drug accountability.	8. Unblinded staff to perform drug accountability.	Addition of "unblinded staff."
12.6 Visit 3 - End of Treatment Month 1 (Day 28 +/- 3 Days)	24	10. Dispense and review how to inject study drug, prohibited medications, dosage limitations of study drug, and storage requirements. Subjects will be instructed to return all used/partially used/unused study drug at next office visit.	10. Dispense and review how to inject study drug, prohibited medications, dosage limitations of study drug, and storage requirements. Subjects will be instructed to return all used/partially used/unused study drug at next office visit to unblinded staff .	Addition of "to unblinded staff."
12.8 Visit 4 - End of Treatment Month 2 (Day 56 +/- 3 Days)	25	• Collect unused study drug and used packaging.	7. Unblinded staff to collect unused study drug and used packaging.	Addition of "Unblinded staff to."
12.8 Visit 4 - End of Treatment Month 2 (Day 56 +/- 3 Days)	25	• Perform drug accountability.	8. Unblinded staff to perform drug accountability.	Addition of "Unblinded staff to."
12.8 Visit 4 - End of Treatment Month 2 (Day 56 +/- 3 Days)	25	• Dispense and review how to inject study drug, prohibited medications, dosage limitations of study drug, and storage requirements. Subjects will be instructed to return all used/partially used/unused study drug at next office visit.	10. Dispense and review how to inject study drug, prohibited medications, dosage limitations of study drug, and storage requirements. Subjects will be instructed to return all used/partially used/unused study drug at next office visit to unblinded staff.	Addition of "to unblinded staff."
12.10 Visit 5 – End Of Treatment Month 3 (Day 84 +/- 3 Days)	25	6. Clinical laboratory sample collection for testing hematology,	6. Clinical laboratory sample collection for testing hematology,	Addition of plasma for anti-drug antibody screening at Visit 5.

		chemistry, PK, biomarkers and urinalysis .	chemistry, PK, biomarkers, plasma for anti-drug antibody screening, and urinalysis.	
12.10 Visit 5 – End Of Treatment Month 3 (Day 84 +/- 3 Days)	25	7. Collect unused study drug and used packaging.	7. Unblinded staff to collect unused study drug and used packaging.	Addition of "Unblinded staff to."
12.10 Visit 5 – End Of Treatment Month 3 (Day 84 +/- 3 Days)	25	8. Perform drug accountability.	8. Unblinded staff to perform drug accountability.	Addition of "Unblinded staff to."
12.10 Visit 5 – End Of Treatment Month 3 (Day 84 +/- 3 Days)	25	10. Dispense and review how to inject study drug, prohibited medications, dosage limitations of study drug, and storage requirements. Subjects will be instructed to return all used/partially used/unused study drug at next office visit.	10. Dispense and review how to inject study drug, prohibited medications, dosage limitations of study drug, and storage requirements. Subjects will be instructed to return all used/partially used/unused study drug at next office visit to unblinded staff.	Addition of "to unblinded staff."
12.12 Visit 6 – End Of Treatment/Et (Day 112 +/- 3 Days)	26	7. Clinical laboratory sample collection for testing hematology, chemistry, PK, biomarkers and urinalysis .	7. Clinical laboratory sample collection for testing hematology, chemistry, PK, biomarkers, plasma for anti-drug antibody screening, and urinalysis.	Addition of plasma for anti-drug antibody screening at Visit 5.
12.12 Visit 6 – End Of Treatment/Et (Day 112 +/- 3 Days)	26	8. Collect unused study drug and used packaging.	8. Unblinded staff to collect unused study drug and used packaging.	Addition of "Unblinded staff to."
12.12 Visit 6 – End Of Treatment/Et (Day 112 +/- 3 Days)	26	10. Perform drug accountability.	10. Unblinded staff to perform drug accountability.	Addition of "Unblinded staff to."
12.14 Visit 7 – Follow Up Visit (Day 140 +/- 3 Days)	26	4. Administer Columbia-Suicide Severity Rating Scale (C-SSRS).	4. Administer Columbia-Suicide Severity Rating Scale (C-SSRS), and MSQ.	Addition of MSQ to Visit 7.
12.16 Phone Call 3 – Safety Follow Up (Day 196 +/- 3 Days)	26	1. Record any AEs.	1. Record any AEs. 2. Administer MSQ.	Addition of MSQ to Phone Call 3
Schedule of Events Table	27-30			Addition of Plasma Collection for ADA Screening to Visit 2, 3, 5, and 6
Schedule of Events Table	27-30			Addition of MSQ to Visit 7 and Phone Call 3

15 Study Drug Randomization and Training	31-32	Eligible subjects will be randomized 1:1 to receive ISIS 546254, 200 mg/mL, 2 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. 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. 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	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	Addition of "to unblinded staff."

		consent of the study medical monitor following discussion with the investigator for subjects that are unable to tolerate the once weekly dose.	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.	
28. Storage Requirements	39-40	Study drug will be inventoried and accounted for throughout the study. Drug Accountability will be maintained for subjects randomized in the study. Study staff will record the amount of study drug dispensed and amount returned at each appropriate office visit. Any missing devices or discrepancies will be addressed and reconciled as needed.	Study drug will be inventoried and accounted for throughout the study. Drug Accountability will be maintained for subjects randomized in the study. Unblinded study staff will record the amount of study drug dispensed and amount returned at each appropriate office visit. Any missing devices or discrepancies will be addressed and reconciled as needed.	Addition of "Unblinded study."
36.6 List of Laboratory Analytes	45	Hematology, clinical chemistry, urinalysis laboratory, urine drug screen, HIV, HbsAg, and HCV serology will be performed at sites local laboratory. Reference ranges will be supplied by local labratory and used by the Investigator to assess the laboratory data for clinical significance and pathological changes.	Hematology, clinical chemistry, urinalysis laboratory, urine drug screen, HIV, HbsAg, and HCV serology will be performed at sites local laboratory. Plasma samples for anti-drug-antibody screening will also be collected and stored for potential future analysis. Reference ranges will be supplied by local laboratory and used by the Investigator to assess the laboratory data for clinical significance and pathological changes.	Addition of ". Plasma samples for anti-drug-antibody screening will also be collected and stored for potential future analysis."

Table of Contents

1 ABBREVIATIONS

Abbreviation or Term	Definition/Explanation
ADD	Attention-Deficit Disorder
ADHD	Attention-Deficit/Hyperactivity Disorder
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
BK	Bradykinin
CV	Curriculum Vitae
DSM-IV-TR	The Diagnostic and Statistical Manual of Mental Disorders Version 4 Text Revision
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
ET	Early Termination
FDA	Food And Drug Administration
g/kg	Gram Per Kilogram
GCP	Good Clinical Practices
HbsAg	Hepatitis B Surface Antigen
HCV	Hepatitis C Virus
HIPPA	Health Insurance Portability and Accountability Act of 1996
HIV	Human Immunodeficiency Virus
HMWK	High Molecular Weight Kininogen
ICF	Informed Consent Form
ICH	International Conference on Harmonization
ICHD	Classification Of Headache Disorders
IP	Investigational Product
IRB	Institutional Review Board
LOCF	Last Observation Carried Forward
MAO-A	Monoamine Oxidase A
MOH	Medication Overuse Headache
mg	Milligram
mg/dL	Milligrams Per Deciliter
mITT	Modified Intent To Treat
mmHg	Millimeter of Mercury
NSAE	Non-Serious Adverse Event
QTcF	Fridericia's Corrected QT Interval
SAE	Serious Adverse Event
SC	Subcutaneous
SOP	Standard Operating Procedure

2 INDICATION

The prophylaxis of chronic migraine in adults ages 18 - 65

3 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.

4 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.

5 POPULATION SAMPLE

Subjects included in the study are those:

- who have at least a 3 month history of chronic migraine meeting the diagnostic criteria listed in the International Classification of Headache Disorders (ICHD)-3 beta (Appendix B)
- who migraine started prior to the age of 50.
- not currently taking a migraine preventive or has been taking preventive for at least 30 days prior to screening and agrees to not start, stop, or change any medications and/or dosage during the study period.

6 STUDY DRUG

Subjects will receive study drug (ISIS 546254, 200 mg/mL, 1 mL or placebo) which will be provided by Ionis Pharmaceuticals, Inc. All doses will be given by subcutaneous (SC) injection at a volume of 1.00 mL and will be administered at the same volume weekly thereafter through week 16.

Those randomized to ISIS 546254 will receive subcutaneous injections containing 1.00 mL (200mg) weekly for weeks 1-16 of ISIS 546254.

Those randomized to placebo will receive 1.00 mL during weeks 1-16

Unblinded personnel will label and package all study kits provided to subjects following standard operating procedures. The study drug will be drawn by an unblinded staff member. The first dose of study drug will be administered under supervision of the unblinded staff at the study site. Thereafter the study drug 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 by the unblinded staff member if they prefer.

7 BACKGROUND & STUDY RATIONALE

Migraine is a highly prevalent neurovascular disorder that affects a significant proportion of the adult population worldwide: up to 6% of males and 18% of females, with the highest prevalence occurring between 25 and 55 years of age. Migraine represents an enormous socio-economic burden to the individual as well as to the society and affects the quality of life. A migraine attack is characterized by recurrent, severe, debilitating headache associated with nausea, vomiting, photophobia, phonophobia, and/or osmophobia, and fatigue, along with other disturbances in autonomic, mental, sensory and motor functions. Migraine attacks typically last 4 to 72 hours and may be precipitated by endogenous factors (i.e., hormonal changes, psychosocial stress, sleep deficit or surplus, hunger), or by exogenous factors (i.e., certain kinds of food; stimulation of different sensory modalities). Migraine attacks can be preceded by abnormal visual, sensory, motor and/or speech functions (migraine with aura) or start with no warning signs (migraine without aura).

Chronic migraine (CM), defined as migraine occurring on ≥ 15 days per month, is a disabling neurological condition with an estimated global prevalence of up to 4 - 5%, and represents approximately half of all cases of chronic primary headache (Stovner et al. 2006; Stovner et al. 2007; Diener et al. 2012; Paemeleire et al. 2014). Episodic migraine (EM) has a complex relationship with CM as approximately 2.5–3 % of EM patients per year evolve into a CM state (‘‘progression’’) and CM can remit back to EM, with a 2-year remission rate of around 25%(Bigal 2008; Manack 2011). CM patients are most commonly females in their 40s and have longer attacks, experience greater pain severity, are more disabled, and more likely to have a lower quality of life than patients with EM (Paemeleire et al. 2014). Mood and anxiety disorders, respiratory disorders and cardiac risk factors, including hypertension, diabetes mellitus and high cholesterol, were also reported significantly more by CM patients (Buse et al. 2010).

Treatment of Chronic Migraine

The goal of CM treatment is to reduce the frequency and severity of attacks and return the patient to an episodic pattern and includes counseling on risk factors such as caffeine and analgesic use and stress management. Drug therapy for prevention of migraine revolves around a variety of nonspecific drugs that primarily reduce neuronal hyperexcitability, the putative pathophysiological hallmark for migraine.

Various pharmacologic classes of medication are used for migraine prophylaxis. Four of the more effective classes include effective classes include beta blockers, anticonvulsants, calcium antagonists, tricyclic antidepressants. In the US, topiramate and valproate sodium are approved for migraine prophylaxis, and botulinum toxin type A (BoNT-A) is approved for prophylaxis of CM. Regardless of class, effect sizes are modest and data from randomized, placebo-controlled trials (RCT) is scant. Topiramate, one of the best-studied medications, established efficacy at a daily dose of 100 mg during 16 weeks via two multicenter, parallel-group RCT in Europe (59 patients) and the USA (328 patients) (Diener et al. 2007; Silberstein et al. 2007). The topiramate studies demonstrated a mean reduction in monthly migraine days of 3.5 (versus a reduction of 0.2 for placebo) and a number needed-to-treat of 12.5. There is no standard definition for drug refractory CM, but generally at least three or four drugs should have been adequately tested

before a migraineur is declared refractory. Other therapies for CM include peripheral nerve stimulation (both invasive and non-invasive), central neurostimulation, transcranial magnetic stimulation, cranial osteopathic manipulative treatment, and cognitive-behavioral therapy.

The various effective preventive agents used in migraine prophylaxis, such as topiramate, valproate, b-blockers, and tricyclic antidepressants, appear to have in common the effect of suppressing cortical excitability.

Migraine Pathophysiology

CM is a chronic pain disorder where migraine attacks coexist with almost daily head pain: its pathophysiology is complex, with features of migraine attacks overlapping with those of acute head pain and chronic pain disorders. Structural, physiologic, and biochemical alterations in the brains of patients with CM have been documented. Migraine has a genetic basis in that common genetic variants set a ‘‘migraine threshold’’ that is modulated by endogenous and exogenous factors and characterized by an abnormal pattern of sensory processing. Neurophysiological studies have shown that for a number of different sensory modalities the migrainous brain is characterized by a lack of habituation to evoked responses (Coppola et al. 2007). Whether this is due to increased cortical hyperexcitability, possibly due to decreased inhibition, or to an abnormal responsivity of the cortex due a decreased preactivation level remains disputed. Sensitization of central trigeminovascular neurons in nucleus caudalis mediates cutaneous allodynia (Burnstein and Jakubowski 2005).

Kallikrein-Kinin System

Plasma prekallikrein (PKK) is a glycoprotein that is predominantly synthesized in the liver. Low levels of PKK mRNA are also found in the epithelial cells of the kidney, adrenal gland and placenta. PKK is secreted into the circulation as a single chain zymogen that has an apparent molecular weight of approximately 85-88 kDa. The major physiological activator of PKK, activated factor XII (FXIIa), cleaves a single peptide bond to generate the active form of plasma kallikrein. Kallikrein is anchored to cell surfaces by binding to its substrate HMWK (Björkqvist et al. 2013).

PKK is a key component of the kallikrein-kinin system (KKS) signaling cascade. Kallikrein cleaves HMWK to release the potent inflammatory vasodilator peptide hormone bradykinin (BK). BK acts through stimulation of G-protein coupled B1 receptors (B1R) or B2 receptors (B2R). B2R are constitutively expressed in multiple tissues, whereas B1R are typically expressed in very low numbers but rapidly increase in response to tissue injury, inflammation, or other pathophysiological events. B2R mediate bronchoconstriction, local blood flow regulation, hypotension, acute inflammatory reactions, pain, and hyperalgesia. B1R mediate short-lived arachidonic acid release and prostaglandin (PG) synthesis and B1R activation leads to elevation of intracellular free calcium activity by increasing calcium entry into the cell (Sharma et al. 2012). B1R affect natriuresis and glomerular filtration, contribute in the pathogenesis of diabetes, support leucocyte recruitment and the initiation of inflammatory responses and pain, and are mitogenic in fibrotic tissue (Sharma et al. 2012).

Bradykinin and Migraine

Increased production of BK is the main characteristic of the well-known genetic deficiency of C1-INH in HAE patients. BK might also influence migraine. As an inflammatory agent, it directly activates the trigeminal system when applied to the meninges. Furthermore, BK stimulates the increase of intracellular Ca2+. Ca2+ has a key function in hemiplegic migraines and in cortical spreading depression. Via calcium, the signaling cascade leads to increased nitric oxide (NO) production and calcitonin gene-related peptide (CGRP) release. Both NO and CGRP are intensely discussed elements of migraines. BK stimulates endothelial cell prostacyclin synthesis leading to inhibition of platelet function and the formation of nitric oxide (Palmer et al. 1987)

BK is involved in numerous signaling pathways, including involvement with g-coupled receptors that modulate pain and inflammation. Stimulation of the BK receptor leads to activation of a phosphoinositide signaling pathway, the release of intracellular calcium, and the activation of a kinase system. Various signal transduction mechanisms have been described for kinins, e.g. the activation of phospholipases A2, C, and D with the subsequent release of prostaglandins, NO, inositol phosphates and diacylglycerol leading to the mobilization of intracellular calcium and activation of protein kinase C (Engeli 2004). This leads to the opening of the nociceptors TRPV1 channels in the sensory nerve cell membrane, enhancing nociception. Generated during inflammation and tissue injury, BK is a prominent algogenic mediator and regulator of the noxious heat sensitivity of nociceptors. Two (2) receptor types have been described: B1 and B2. B2 is constitutive in numerous tissues, including neurons, whereas B1 is typically inducible and its activation can generate both pain and inflammation. BKs numerous physiological functions might also be implicated in migraine headache (Engeli 2004).

The activation of B2 receptors, constitutively expressed in primary sensory neurons, promotes polymodal nociceptor activation and hyperalgesia through the production of diacylglycerol and activation of protein kinase C (Engeli 2004). BK can sensitize nociceptors following the release of inflammatory mediators such as prostaglandins, cytokines and NO either from sensory neurons or endothelial and immune cells. BK is also known to facilitate the release of substance P and CGRP from rat sensory neurons in culture (Engeli 2004). However, the type of BK receptor responsible for pain and inflammation in humans has not been adequately defined.

Studies have substantiated the binding of BK to cortical astrocytes and indicated that stimulation with BK causes calcium turnover, suggesting a role for BK in central nervous transmission (Stephens et al. 1993). BK increases Ca2+-levels in sensory neurons (Linhart et al. 2003). BK activation simulates the release of Ca2+ from intracellular stores and Ca2+ supply through Ca2+ entry pathways in the plasma membrane. BK regulates systemic and local hemodynamics by turning on NO production (Engeli 2004).

HAE and Migraine

Chung and Kim reported on an HAE patient manifesting migraine-like episodes of headache, effectively prophylaxed with Danazol. The patient, a 44-yr-old Korean man, suffered from frequent attacks of migraine-like headache (3-7 per month), pulsating in nature, associated with nausea, and aggravated by activity. His headache had shown recent progression with abdominal pain. No remarkable findings were observed on radiologic examination, including brain MRI

and intracranial and extracranial MRA. Danazol 200 mg every other day prevented the migraine-like episodes for about 2 years. A single moderate migraine-like headache was resolved with administration of naratriptan 2.5 mg (Chung and Kim 2012). It suggests that pathogenic mechanism of headache in hereditary angioedema may be mediated by the neurogenic inflammatory-like physiology of migraine. Hoxha et al. reported on 38-old female HAE patient presenting facial paralysis and hemiparesis, persistent headache, nausea, and vomiting who also responded to Danazol with resolution of her symptoms (Hoxha et al. 2013).

Current clinical studies are underway investigating inhibitors of calcitonin gene-related peptide (CGRP) as prophylactic agents for chronic migraine. Due to the role that BK plays in the stimulating the release of CGRP via its increasing prostaglandin production through binding to BK BR2, it is thought that the systemic reduction of circulating PKK, and therefore the reduction of plasma's potential to produce BK, could greatly reduce the contribution of BK and its associated downstream effects in chronic migraine (Ceruti et al. 2011; Supowit et al. 2011; Petho and Reeh 2012; Gangadha et al. 2014). This, combined with the known long tissue half-life of ASOs, supports ISIS 546254 as a potentially attractive once-weekly, self-administered, subcutaneous treatment for the prevention, or amelioration, of symptoms associated with chronic migraine.

ISIS 546254

Antisense oligonucleotides are short synthetic strings of nucleotides that selectively bind to mRNA and prevent the expression of a targeted protein encoded by that specific segment of mRNA. These compounds bind to RNA with high affinity and selectivity. Antisense technologies offer a number of potential advantages over more traditional approaches for drug discovery and development including: (1) greatly improved specificity allowing discrimination between closely related gene products; (2) predictability due to some similarities in pharmacokinetics, toxicology and manufacturing; and (3) versatility allowing inhibition of "non-druggable" targets, such as transcription factors, adapter proteins and other proteins with no intrinsic enzymatic activity or proteins with unknown function. Antisense oligonucleotides have been tested clinically for the treatment of a wide variety of diseases including viral disease, cancer, cardiovascular, metabolic and inflammatory diseases.

The investigational product, ISIS 546254, is a chimeric 2′-*O*-(2-methoxyethyl) (2′-MOE) antisense oligonucleotide (ASO) targeted to human prekallikrein (PKK) mRNA. As such, it is a highly specific inhibitor of PKK synthesis which should result in reduced BK generation. The antisense concept is based upon Watson-Crick hybridization. Antisense oligonucleotides are designed to inhibit the translation of mRNA by binding with high affinity and selectivity to their RNA targets. Antisense oligonucleotides exert their inhibitory effects on mRNA translation by a number of mechanisms, the most studied of which includes breakdown of steady-state mRNA levels through the utilization of endogenous RNase H1 enzyme.(Monia et al. 1993) ISIS 546254 was designed to function through an RNase H1-dependent terminating mechanism. (Baker and Monia 1999)RNase H1 efficiently induces antisense-mediated cleavage of mRNA and is present in most mammalian cells. Antisense-mediated reduction of target mRNA levels is typically 85-90% of control levels.(Crooke and Bennett 1996) Furthermore, reduction in target mRNA levels

using this approach correlates directly with a subsequent reduction in target protein levels. ISIS 546254 is a very potent antisense inhibitor of human PKK expression and has demonstrated an ED_{50} of 2.5-5 mg/kg/week in transgenic animal models.

After systemic administration, the primary mechanism of ISIS 546254 is to reduce circulating prekallikrein (PKK) levels, therefore reducing the plasma's ability to produce kallikrein which in turn will reduce bradykinin formation and vascular permeability. Preclinical data indicate that reduction of PKK by administering a specific antisense oligonucleotide (ASO) targeting PKK mRNA (ISIS 546254) leads to reduced ability of treated plasma to produce bradykinin and reduced vascular permeability in animal models. (Revenkop et al. 2011)

This, combined with the known long tissue half-life of ASOs, supports ISIS 546254 as a potentially attractive self-administered, SC treatment for the prophylaxis of chronic migraine.

8 PRIMARY OBJECTIVES

1. To evaluate the safety and tolerability of ISIS 546254 in the preventive treatment of chronic migraine
2. To evaluate the efficacy of ISIS 546254 in the preventive treatment of chronic migraine, measured by change in migraine days

9 SECONDARY OBJECTIVES

1. To evaluate the efficacy of ISIS 546254 in the preventive treatment of chronic migraine, measured by change in average headache severity from baseline
2. To evaluate the efficacy of ISIS 546254 in the preventive treatment of chronic migraine, measured by change in headache days from baseline
3. To evaluate the proportion of patients reporting a $\geq$ 50% reduction in the number of migraine headaches from baseline
4. To evaluate the frequency of migraine headache days requiring use of acute and rescue migraine medication as compared to baseline
5. To evaluate changes in the Migraine Specific Quality of Life (MSQ) Questionnaire from baseline
6. To evaluate changes in physician global impress of change (PGIC) from baseline
7. To evaluate changes in subject global impress of change (SGIC) from baseline

10 SUBJECT SELECTION

The study population will be composed of approximately 30 randomized female and male patients, aged 18 to 65 years, with a history of migraine for at least 1 year and chronic migraine (CM) (as defined by International Classification of Headache Disorders, 3rd revision [ICHD-3] criteria [IHS 2013]) for at least 3 months prior to screening. The diagnosis will be prospectively confirmed via a review of headache data recorded in an electronic daily headache diary during a 28-day run-in period. Subjects on migraine preventative medications will be required to be on a

stable regimen for 30 days prior to and throughout the study period and to be experiencing ≥15 headaches per month despite prophylaxis.

11 INCLUSION/EXCLUSION CRITERIA

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

11.1 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), 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.

11.2 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.
22. suffers from human compromised immune system including immunodeficiency virus (HIV), Hepatitis B or C.

12 STUDY DESIGN

This is a double-blind, placebo-controlled, randomized, multi-center study in subjects with chronic migraine. Approximately 30 randomized female and male patients, aged 18 to 65 years, inclusive, with a history of migraine for at least 1 year and chronic migraine (CM) (as defined by International Classification of Headache Disorders, 3rd revision [ICHD-3] criteria [IHS 2013]) for at least 3 months prior to screening. Subjects will complete a 1-month baseline prior to randomization to confirm their diagnosis, as well as establish baseline migraine characteristics. Eligible subjects will enter a four month treatment phase receiving ISIS 546254 (200mg SC) or placebo in a 1:1 design. Following the treatment phase subjects will return to the site one month

after their last dosage of IP and receive a phone call at 3 months after last dose to complete for safety assessments. During the treatment and follow-up phases of the study, all patients will have samples taken every two weeks for hematology testing to monitor platelet levels.

12.1 VISIT 1 – SCREENING (Day -28)

The following will be completed at Visit 1:

1. Obtain written Informed Consent. The informed consent will be obtained in accordance with Good Clinical Practices (GCP) and all applicable regulatory requirements from each subject prior to participation in the study.
2. Verify Inclusion/Exclusion Criteria. Subjects will meet all the inclusion and none of the exclusion criteria.
3. Obtain demographics.
4. Obtain medical, medication, and headache history. Data collected will include medical history and diagnoses, age at onset of migraine and other pertinent migraine/headache history, history of acute and prophylactic headache medications within the past 30 days, and history of other recent/concomitant medications.
5. Perform urine pregnancy test, if appropriate. Results of the pregnancy test must be negative to continue in study.
6. Perform physical and neurological examinations.
7. Measure vital signs.
8. Perform electrocardiogram (ECG).
9. Perform urine drug screen.
10. Administer Columbia-Suicide Severity Rating Scale (C-SSRS).
11. Clinical laboratory sample collection for testing hematology, chemistry, PK, biomarkers and urinalysis.
12. Serology for human immunodeficiency virus (HIV), Hepatitis B and C.
13. Instruct subject on diary completion.

12.2 VISIT 2 – RANDOMIZATION (Day 0)

1. Verify Inclusion/Exclusion Criteria. Subjects must continue to meet all inclusion and none of the exclusion criteria.
2. Perform urine pregnancy test, if appropriate. Results of the pregnancy test must be negative to continue in study.
3. Perform brief physical and collect vital signs.
4. Record any changes to concomitant medications.
5. Record any Adverse Events (AE) since signing the Informed Consent.
6. Review Baseline Headache Diary for completeness and continuing eligibility.
7. Perform urine drug screen.
8. Clinical laboratory sample collection for testing hematology, chemistry, PK, biomarkers, plasma for anti-drug antibody screening, and urinalysis.
9. Randomize subject.
10. Review Diary instruction (same instructions as Baseline Headache Diary).
11. Administer Columbia-Suicide Severity Rating Scale (C-SSRS).
12. Administer first dose of study drug (1.00 mL).

13. Dispense and instruct subjects on how to inject study drug. Subjects will also be educated on prohibited medications, dosage limitations of study drug, and storage requirements. Subjects will be instructed to return all used/partially used/unused study drug at next office visit to the unblinded staff and drug reconciliation will be performed.
14. Administer MSQ.
15. Schedule Visit 3.

12.3 PHONE CALL 1 – SAFETY FOLLOW UP (DAY 1)

1. Record any changes to concomitant medications.
2. Record any Adverse Events.
3. Answer any subject questions on IP administration.

12.4 PHONE CALL 2 – SAFETY FOLLOW UP (DAY 2)

1. Record any changes to concomitant medications.
2. Record any AEs.
3. Answer any subject questions on IP administration.

12.5 HEMATOLOGY SAMPLE COLLECTION (DAY 14 +/- 3 DAYS)

1. Patient to submit sample through contract lab

12.6 VISIT 3 – END OF TREATMENT MONTH 1 (DAY 28 +/- 3 DAYS)

1. Perform urine pregnancy test, if appropriate. Results of the pregnancy test must be negative to continue in study.
2. Perform brief physical and collect vital signs.
3. Record any changes to concomitant medications.
4. Record any AEs.
5. Review Diary for completeness maintaining an 80% compliance and continuing eligibility.
6. Clinical laboratory sample collection for testing hematology, chemistry, PK, biomarkers, plasma for anti-drug antibody screening, and urinalysis.
7. Collect unused study drug and used packaging.
8. Unblinded staff to perform drug accountability.
9. Administer Columbia-Suicide Severity Rating Scale (C-SSRS).
10. Dispense and review how to inject study drug, prohibited medications, dosage limitations of study drug, and storage requirements. Subjects will be instructed to return all used/partially used/unused study drug at next office visit to unblinded staff .
11. Schedule Visit 4.

12.7 HEMATOLOGY SAMPLE COLLECTION (DAY 42 +/- 3 DAYS)

1. Patient to submit sample through contract lab

12.8 VISIT 4 – END OF TREATMENT MONTH 2 (DAY 56 +/- 3 DAYS)

1. Perform urine pregnancy test, if appropriate. Results of the pregnancy test must be negative to continue in study.
2. Perform brief physical and collect vital signs.

3. Record any changes to concomitant medications.
4. Record any AEs.
5. Review Diary for completeness maintaining an 80% compliance and continuing eligibility.
6. Clinical laboratory sample collection for testing hematology, chemistry, PK, biomarkers and urinalysis .
7. Unblinded staff to collect unused study drug and used packaging.
8. Unblinded staff to perform drug accountability.
9. Administer Columbia-Suicide Severity Rating Scale (C-SSRS).
10. Dispense and review how to inject study drug, prohibited medications, dosage limitations of study drug, and storage requirements. Subjects will be instructed to return all used/partially used/unused study drug at next office visit to unblinded staff.
11. Schedule visit 5.

12.9 HEMATOLOGY SAMPLE COLLECTION (DAY 70 +/- 3 DAYS)

1. Patient to submit sample through contract lab.

12.10 VISIT 5 – END OF TREATMENT MONTH 3 (DAY 84 +/- 3 DAYS)

1. Perform urine pregnancy test, if appropriate. Results of the pregnancy test must be negative to continue in study.
2. Perform brief physical and collect vital signs.
3. Record any changes to concomitant medications.
4. Record any AEs.
5. Review Diary for completeness maintaining an 80% compliance and continuing eligibility.
6. Clinical laboratory sample collection for testing hematology, chemistry, PK, biomarkers, plasma for anti-drug antibody screening, and urinalysis.
7. Unblinded staff to collect unused study drug and used packaging.
8. Unblinded staff to perform drug accountability.
9. Administer Columbia-Suicide Severity Rating Scale (C-SSRS).
10. Dispense and review how to inject study drug, prohibited medications, dosage limitations of study drug, and storage requirements. Subjects will be instructed to return all used/partially used/unused study drug at next office visit to unblinded staff.
11. Schedule visit 6

12.11 HEMATOLOGY SAMPLE COLLECTION (DAY 98 +/- 3 DAYS)

1. Patient to submit sample through contract lab

12.12 VISIT 6 – END OF TREATMENT/ET (DAY 112 +/- 3 DAYS)

1. Perform urine pregnancy test, if appropriate. Results of the pregnancy test must be negative to continue in study.
2. Perform physical and neurological examinations.
3. Measure vital signs.
4. Perform electrocardiogram (ECG).
5. Record any changes to concomitant medications.

6. Record any AEs.
7. Clinical laboratory sample collection for testing hematology, chemistry, PK, biomarkers, plasma for anti-drug antibody screening, and urinalysis.
8. Unblinded staff to collect unused study drug and used packaging.
9. Administer Columbia-Suicide Severity Rating Scale (C-SSRS).
10. Unblinded staff to perform drug accountability.
11. Administer MSQ, PGIC, and SGIC.
12. Schedule Visit 7

12.13 HEMATOLOGY SAMPLE COLLECTION (DAY 126 +/- 3 DAYS)

1. Patient to submit sample through contract lab

12.14 VISIT 7 – FOLLOW UP VISIT (DAY 140 +/- 3 DAYS)

1. Perform urine pregnancy test, if appropriate. Results of the pregnancy test must be negative to continue in study.
2. Collect vital signs.
3. Record any changes to concomitant medications.
4. Administer Columbia-Suicide Severity Rating Scale (C-SSRS), and MSQ.
5. Record any AEs.
6. Clinical laboratory sample collection for testing hematology, chemistry, PK, biomarkers and urinalysis..

12.15 HEMATOLOGY SAMPLE COLLECTION (DAY 154 +/- 3 DAYS)

1. Patient to submit sample through contract lab

12.16 PHONE CALL 3 – SAFETY FOLLOW UP (DAY 196 +/- 3 DAYS)

1. Record any AEs.
2. Administer MSQ.

	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/ Heb 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							

*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

	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

13 CONTRACEPTION REQUIREMENTS

Male subjects must refrain from sperm donation and either be abstinent† or, if engaged in sexual relations with a female of child-bearing potential, they must use effective barrier contraception with their partner from the time of signing the informed consent form until at least *13* weeks after their last dose of Study Drug. Effective contraception includes a vasectomy with negative semen analysis at follow-up, or the use of condoms together with spermicidal foam/gel/film/cream/suppository. Male subjects engaged in sexual relations with a female of child-bearing potential must also encourage their female partner to use effective contraception from the time of signing the informed consent until *96 days* after the subject's last dose of study treatment.

Effective contraception for the female partner includes: surgical sterilization (e.g., bilateral tubal ligation), hormonal contraception, intrauterine contraception/device, or barrier methods (female condom*, diaphragm, sponge, cervical cap) together with spermicidal foam/gel/film/cream/suppository. Male subjects with partners that are pregnant must use condoms to ensure that the fetus is not exposed to the Study Drug.

†**Note:** Abstinence is only acceptable as true abstinence, i.e., when this is in line with the preferred and usual lifestyle of the patient. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods), declaration of abstinence for the duration of a trial and withdrawal are not acceptable methods of contraception.

***Note:** **A female condom and a male condom should not be used together as friction between the two can result in either or both products failing.**

14 INFORMED CONSENT

The investigator must obtain documented consent from each potential subject, or legally authorized representative, prior to any study related procedures being performed. Consent must be documented by the subject's dated signature on an Informed Consent Form (ICF) along with the dated signature of the persons conducting the consenting process. A copy of the signed and dated consent form should be given to the subject before participating in the study.

15 STUDY DRUG RANDOMIZATION AND TRAINING

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.

16 DOSING ERRORS

Study drug errors should be documented as protocol deviations. A brief description should be provided in the deviation, including whether the subject was symptomatic (list symptoms) or asymptomatic, and the event accidental or intentional.

17 RESCUE MEDICATION

Subjects will be instructed they may take their investigator approved rescue medications for headaches, if needed. Rescue medication usage and dosage will be recorded on the headache diary.

18 CONCOMITANT MEDICATIONS

Therapy considered necessary for the subject's welfare may be given at the discretion of the Investigator. Concomitant medication usage and dosage will be recorded on the concomitant medication form.

Routine medications, including migraine preventative medications, should be maintained on a stable dose and regimen for the duration of the study period. Additionally, any concomitant chronic therapies should be maintained at a stable dose and dose regimen during the study.

18.1 Allowed Medications

1. All acute migraine medications currently taken by subject at Visit 1 will be allowed during the study as a rescue medication or if a subject does not treat with study drug for that specific headache.
2. All migraine preventative medications, currently taken by subject at Visit 1, will be allowed if on a stable dose for at least 30 days prior to Visit 1 and subject agrees to continue at that dose throughout the study.

18.2 Prohibited Medications

1. Any anticoagulant during the study is prohibited

19 HEADACHE DIARY

The primary and much of the secondary endpoints will be derived from the electronic daily headache diary. Site personnel will be responsible for instructing subjects on the requirement for timely and daily completion of the electronic diary. Each day, the subject will be asked to record diary data for the previous day (24 hour period). If a subject does not experience a headache in the previous 24 hour period, the diary must still be completed and recorded as no headache. Subject's diaries may vary day to day based on their responses. Subjects will be asked to document all headaches experienced regardless of severity for the duration of the study. All subjects should have at least 80% compliance with diary completion throughout the length of the study. Subjects will record headache severity, symptoms, use of acute medications, as well as additional questions as required. Headache severity will be subjectively rated by the subject as

follows: no pain, mild pain, moderate pain, or severe pain. These ratings will be collected at pre-defined time points.

20 UNSCHEDULED VISITS

If a subject has an unscheduled visit, the unscheduled visit form must be completed. If the visit occurs for safety reasons, all relevant safety data should be captured and reported on the appropriate forms. If the unscheduled visit results in an early termination, all applicable final study visit procedures will be performed and the early termination form will need to be completed as well.

21 BLINDING/UNBLINDING

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. When possible, Sponsor and Clinvest should be notified prior to unblinding study drug.

Individual unblinding envelopes are shipped with study drug. Each study drug kit has a corresponding unblinding envelope. Unblinding will include matching the kit and unblinding envelope with the subject drug number.

To unblind a subject without breaking the blind for remaining subjects' treatment the following instructions described below will be followed:

- Obtain the security envelope containing the unblinding envelopes.
- Remove the unblinding envelope with the study drug number corresponding with the study drug kit dispensed to the subject.
- Break the seal of the unblinding envelope and remove the subjects' treatment label.
- Return the subjects' treatment label to the unblinding envelope.
- Return the unblinding envelope to the security envelope.
- Document on the unblinding form located in the eAdmin Binder.
- Email copy of unblinding form to study@clinvest.com within 24 hours of unblinding.
- Complete and email or fax the Sterling IRB, unanticipated problem report form, to Sterling IRB within 10 business days of unblinding.

22 DISCONTINUATION/WITHDRAWAL FROM STUDY

All subjects who withdraw from the study, for any reason, must return all study drug and supplies to the investigator or his/her delegate at the first available opportunity. Subjects may withdraw at any time or be dropped from the study at the discretion of the investigator or Clinvest if he/she violates the study plan or for administrative and/or safety reasons. When a subject discontinues/withdraws prior to study completion, all applicable activities scheduled for the final study visit should be performed at the time of discontinuation, as well as the completion of the early termination form and unscheduled visit form (if appropriate). Any adverse experiences which are present at the time of discontinuation/withdrawal should be followed in

accordance with the safety requirements outlined in the adverse event reporting section of this protocol.

23 ADVERSE EVENTS

The investigator will be responsible for the detection, collection, and evaluation of all events meeting the definition of an adverse event (AE). An adverse event is defined as any untoward medical occurrence in a subject or clinical investigation subject administered a medicinal product and which does not necessarily have a causal relationship with the study product. An adverse event can therefore be any unfavorable and unintended sign (including abnormal laboratory finding), symptom, or disease temporally associated with the use of the study product, whether or not related to the study product.

An adverse event observed after the initial dose of the study product will be considered a "treatment-emergent adverse event". Treatment-emergent adverse events will be analyzed and discussed in the clinical study report for this study. Adverse event terms should include a diagnosis, as available, in preference to the listing of individual signs and symptoms. If a diagnosis is not possible, each sign and symptom should be recorded as an individual adverse event.

All adverse events, whether or not related to the study drug, must be completely documented on the appropriate adverse event eCRF (electronic case report form) page. If a subject is withdrawn from the study due to an adverse event, this must also be recorded on the appropriate eCRF pages.

The site staff must record all directly observed AEs and all spontaneously reported AEs. At each visit, the site staff will ask the subject a non-specific question (e.g., "Have you noticed any change in your health since your last visit?") to assess AE occurrence since the last report or visit.

23.1 Non-Serious Adverse Event (NSAE)

Non-serious adverse events (NSAE) will be collected beginning after the first dose of study medication of treatment and will include any change from the subject's condition at Visit 2 though the last follow up phone call 3 months post treatment. These include physical findings, clinical signs and symptoms, or sequelae. Any worsening (i.e. any clinically significant adverse change in the frequency and/or intensity) of a preexisting condition which is temporally associated with the use of the provided product, is also considered to be an adverse event. Events such as elective medical/surgical procedures or anticipated day-to-day fluctuations of pre-existing conditions present at screening that do not worsen are not considered NSAE's.

All NSAE's noted will be captured on the non-serious adverse events eCRF. Information captured will include start date/time, end date/time, severity, relationship of causality to study drug, course of action taken, and outcome.

23.2 Serious Adverse Event (SAE)

An serious adverse event (SAE) is defined as any untoward medical occurring after signing of the informed consent and until cessation of the study which:

1. results in death.
2. is life threatening (Note: The term "life-threatening" in the definition of "serious" refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.).
3. requires subject hospitalization or prolongation of existing hospitalization.
4. results in persistent or significant disability/incapacity; or a congenital anomaly/birth defect.
5. Important medical events that may not result in death, are not life-threatening, or do not require hospitalization may also be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse

Medical or scientific judgment should be exercised in deciding whether reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the definition above. These should also be considered serious.

SAE's will be reported in compliance with all applicable safety reporting requirements as set forth in the Code of Federal Regulations. SAE's assessed as life threatening or death "possibly related to the study medication" will be reported to Clinvest, Sponsor, and Sterling IRB within 24 hours of knowledge of the event by study staff. All other SAE's (such as hospitalization, disability, congenital anomaly, and an important medical event) will be reported to Clinvest, Sponsor, and Sterling IRB within 48 hours of knowledge of the event by study staff. A MedWatch Form FDA 3500A will also be completed and forwarded to Sponsor and Clinvest.

Included in the SAE Report Form will be an assessment of the causal relationship between the Study IP and the SAE. SAE's will be followed by study staff until the event(s) have returned to normal, stabilized, or have been otherwise explained, for at least 2 weeks following the last dose of study drug.

If the investigator learns of any SAEs after a subject has been discharged from the study and he/she considers the event reasonably related to the investigational product, the investigator will notify Clinvest.

24 SAFETY MONITORING RULES

In addition to the standard monitoring of clinical safety parameters, the following guidelines are provided for the monitoring of selected parameters chosen based on preclinical and clinical observations.

Confirmation Guidance: At any time during the Study (Treatment or Post-Treatment Periods), the initial clinical laboratory results meeting the safety monitoring criteria presented below **must be confirmed** by performing measurements (ideally in the same laboratory that performed the initial measurement) on new specimens. All new specimen collections should take place as soon as possible (ideally within 3 days of the initial collection). For stopping rules, if the initial laboratory result is observed during the Treatment Period, the results from the retest **must be available** prior to administering the next dose of Study Drug (ISIS 546254 or placebo).

Re-dosing Guidance: Subjects with initial laboratory test values that reach a stopping rule must not be re-dosed until the re-test results are available. In general, subjects who do not meet the stopping rules based upon retest may continue dosing. However, the Investigator and study drug manufacturer (Ionis Pharmaceuticals Inc.) should confer as to whether additional close monitoring of the subject is appropriate. If any of the stopping criteria described below (Section 25) are met, the subject will be permanently discontinued from further treatment with Study Drug, evaluated fully as outlined below and in consultation Ionis Pharmaceuticals or appropriately qualified designee, and will be followed up in accordance with Section 23 of the protocol.

24.1 Safety Monitoring Rules for Liver Chemistry Tests

The following rules are adapted from the draft guidance for industry, "Drug-Induced Liver Injury: Premarketing Clinical Evaluation," issued by the U.S. Department of Health and Human Services, Food and Drug Administration, July 2009. For a definition of Baseline, please refer to guidance in Section 8.5 above.

In the event of an ALT or AST measurement that is > 3 x ULN (or the greater of 2 x baseline value or 3 x ULN if the baseline value was > ULN) at any time during the Study (Treatment or Post-Treatment Period), the initial measurement(s) should be confirmed as described above. Additional, confirmatory measurements should also be performed if ALT or AST levels increase to 5 x ULN.

Frequency of Repeat Measurements: Subjects with confirmed ALT or AST levels > 3 x ULN (or the greater of 2 x baseline value or 3 x ULN if the baseline value was > ULN) should have their liver chemistry tests (ALT, AST, ALP, INR and total bilirubin) retested at least once-weekly until ALT and AST levels become ≤ 1.2 x ULN *(* or 1.2 x baseline value if the baseline value was > ULN).

Further Investigation into Liver Chemistry Elevations: For subjects with confirmed ALT or AST levels > 3 x ULN (or the greater of 2 x baseline value or 3 x ULN if the baseline value was > ULN), the following evaluations should be performed:

1. Obtain a more detailed history of symptoms and prior and concurrent diseases
2. Obtain further history for concomitant drug use (including nonprescription medications, herbal and dietary supplement preparations), alcohol use, recreational drug use, and special diets
3. Obtain a history for exposure to environmental chemical agents and travel
4. Serology for viral hepatitis (HAV IgM, HBsAg, HCV antibody, CMV IgM, and EBV antibody panel)
5. Serology for autoimmune hepatitis (e.g., antinuclear antibody (ANA))

Additional liver evaluations, including gastroenterology/hepatology consultations, hepatic CT or MRI scans, may be performed at the discretion of the Investigator, in consultation with Ionis Pharmaceuticals. Repetition of the above evaluations should be considered if a subject's ALT and/or AST levels reach 5 x ULN.

24.2 Safety Monitoring Rules for Platelet Count Results

All patients will have platelet measures taken every two weeks for the duration of the study.

24.3 Safety Monitoring for Bleeding Events

Minor bleeding events are those that do not fulfill the criteria for major bleeding or clinically relevant, non-major bleeding events (which are defined below), for example excess bruising, petechiae, gingival bleeding on brushing teeth. If a minor bleeding event occurs, the Investigator should notify Ionis Pharmaceuticles and additional testing of coagulation parameters (aPTT, PT, INR) and platelet count should be performed.

24.3.1 Definition of Major Bleeding Events

1. Fatal bleeding, and/or
2. Symptomatic bleeding in a critical area or organ, such as intracranial, intraspinal, intraocular, retroperitoneal, intra-articular or pericardial, or intramuscular with compartment syndrome, and/or
3. Bleeding causing a fall in hemoglobin level of 2.0 mg/dL (1.24 mmol/L) or more within 24 hours, or leading to transfusion of two or more units of whole or red cells

24.3.2 Definition of Clinically Relevant, Non-Major Bleeding Events

1. Multiple-source bleeding
2. Spontaneous hematoma > 25 cm^2
3. Excessive wound hematoma (not injection site related)

4. Macroscopic hematuria (spontaneous or lasting > 24 hours if associated with an intervention)
5. Spontaneous rectal bleeding; epitastaxis, gingival bleeding, hemoptysis, hematemesis
6. Bleeding after venipuncture for > 5 minutes

25 STOPPING RULES

25.1 Liver Chemistry Elevations

In the event of laboratory results meeting the following criteria, **and the event is without an alternative explanation,** dosing of a subject with Study Drug will be stopped permanently; values that are not confirmed due failure to retest or missing lab values will be presumed confirmed:

1. ALT or AST > 8 x ULN, which is confirmed
2. ALT or AST > 5 x ULN, which is confirmed and persists for ≥ 2 weeks
3. ALT or AST > 3 x ULN (or the greater of 2 x baseline value or 3 x ULN if the baseline value was > ULN), which is confirmed **and** total bilirubin > 2 x ULN or INR > 1.5
4. ALT or AST > 3 x ULN (or the greater of 2 x baseline value or 3 x ULN if the baseline value was > ULN), which is confirmed, and the new appearance (i.e. onset coincides with the changes in hepatic enzymes) of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or concomitant eosinophilia (> ULN)

25.2 Renal Function Test Results

In the event of laboratory results for either of the following criteria, dosing of a subject with Study Drug (ISIS 546254 or placebo) will be stopped permanently:

1. Confirmed serum creatinine increase that is both ≥ 0.3 mg/dL (26.5 μmol/L) and ≥ 40% above Baseline creatinine values
2. Proteinuria, dipstick 2 + (confirmed by dipstick retest and then further confirmed by a quantitative total urine protein measurement of > 1.0 g/24 hour)

The follow-up schedule for any events meeting either of these stopping criteria will be determined by the Investigator **in consultation with** Ionis Pharmaceuticals or designee.

25.3 Platelet Count Results

In the event of a confirmed platelet count less than 100,000/mm^3, dosing of a subject with Study Drug (ISIS 546254 or placebo) will be stopped permanently. The follow-up schedule for any

events meeting this stopping criterion will be determined by the Investigator in consultation with Ionis Pharmaceuticles or designee.

26 PREGNANCY

All pregnancies of women participating in the study that occur during the study, are to be reported immediately, and the site must complete the pregnancy form.

Any female patient becoming pregnant during the study will immediately discontinue the study drug. All
patients who become pregnant will be monitored for the outcome of the pregnancy (including spontaneous or voluntary termination). If the pregnancy continues to term, the outcome (health of the infant up to 8 weeks of age), including details of birth and presence or absence of any birth defect, congenital abnormalities, or maternal and newborn complications, will be reported to the sponsor. Any complication of pregnancy during the study and any complication of pregnancy that the investigator becomes aware of after termination from the study will be reported as an adverse event or serious adverse event, as appropriate.
If the pregnancy does not continue to term, 1 of the following actions will be taken:

1. For a spontaneous abortion, report as a serious adverse event.
2. For an elective abortion due to developmental anomalies, report as a serious adverse event.
3. For an elective abortion not due to developmental anomalies, report on the pregnancy form; do not report as an adverse event.

For pregnancies of partners of men participating in the study, Clinvest will determine the procedure to appropriately follow up after notification as described above. All partners who become pregnant and provide appropriate consent will be monitored until the completion or termination of the pregnancy.

27 CLINICAL SUPPLIES

Clinical supplies will be packaged for subjects in accordance to an allocation schedule generated by Clinvest.

28 STORAGE REQUIREMENTS

The study drug and clinical supplies must be received by a designated person at the study site, handled and stored safely and properly, and kept in a secured location to which only the designated staff have access. Clinical supplies and medications are to be dispensed only as defined in the protocol. It is the Investigator's responsibility to keep accurate records of the supplies received, the amount dispensed to and returned by subjects, and the remaining amount at the end of the study. Study staff should not open individual study drug containers prior to dispensing to the subject.

Study drug will be inventoried and accounted for throughout the study. Drug Accountability will be maintained for subjects randomized in the study. Unblinded study staff will record the

amount of study drug dispensed and amount returned at each appropriate office visit. Any missing devices or discrepancies will be addressed and reconciled as needed.

Study drug should be kept in a secure location and stored according to the package insert.

The study drug storage area at the site must be monitored by the site staff for temperature consistency with the acceptable storage temperature range specified on study drug temperature log. Documentation of temperature monitoring should be maintained.

29 CONFIDENTIALITY

By signing this protocol, the investigator affirms to Clinvest information furnished to the Investigator by Clinvest will be maintained in confidence. Likewise, data generated by this study will be considered confidential by the Investigator, with the exception of information included in a publication or information shared with Ionis Pharmaceuticals.

The Investigator also agrees that Clinvest, Institutional Review Board (IRB), or Regulatory Agency representatives may consult and/or copy study documents in order to verify data. By signing the consent form, the subject agrees to this process. Signing of this protocol also means the Investigator agrees to treat all subject data used and disclosed in connection with this study in accordance will all applicable privacy laws, rules and regulations, including applicable provisions of Health Insurance Portability and Accountability Act (HIPPA).

30 COMPLIANCE WITH LAW, AUDIT, AND DEBARMENT

By signing the protocol, the Investigator agrees to conduct the study in a diligent manner and in conformance with the protocol, standards of the Declaration of Helsinki under its most recent amendment and including Good Clinical Practice (GCP) according to the International Conference on Harmonisation (ICH) guidelines, and all applicable federal, state, and local laws, rules and regulations relating to the conduct of the clinical study.

Prior to trial initiation, the Investigator at each site will provide Clinvest a fully executed and signed Food and Drug Administration (FDA) Form 1572 and curriculum vitae (CV).

The Investigator also agrees to allow monitoring, audits, IRB review, and regulatory agency inspection of trial-related documents and procedures. Centralized monitoring will be performed to verify accuracy of data entered into the electronic data capture system (EDC).

Additionally, the Investigator agrees not to seek reimbursement from subjects, their insurance providers, or from government programs for procedures included as part of the study that are reimbursed by Clinvest.

Persons debarred from conducting or working on clinical studies by any court or regulatory agency will not be allowed to conduct or work on Clinvest studies. The Investigator will immediately disclose in writing to Clinvest if any person who is involved in study conduct is debarred, or if any proceeding for debarment is pending or threatened.

31 QUALITY CONTROL/ASSURANCE

It is Clinvest's responsibility for implementing and maintaining quality control and assurance with written standard operating procedures (SOPs) to ensure the trial is conducted in compliance with GCP standards and all applicable federal, state, and local laws, rules, and regulations. Appropriate tools will be provided to the site to facilitate quality management of the subject and continuity through the trial.

Quality control should be applied by all parties and to each stage of subject management, investigational product management and data handling to ensure all data are reliable and have been processed correctly. All clinical data are to be generated and processed by personnel with relevant clinical and GCP knowledge. Subject generated data should be preceded with appropriate subject training and technical support for questions that arise during the trial.

Agreements, made by the sponsor, or their designee, with the investigator, institution and/or with any other parties involved with clinical trial should be in writing in a separate agreement.

The investigator agrees to be responsible for the integrity of all study conduct at their site.

During the trial Clinvest will conduct remote monitoring and safety oversight periodically. The clinical monitors may review eCRFs, etc. at intervals throughout the study to verify appropriate inclusion of subjects, adherence to the protocol, and completeness, correctness, and accuracy of eCRF entries. Any source upload should be reviewed for privacy elements and removed. Source uploads should only include the subject enrollment number as identifying information.

The study will be registered on www.clinicaltrials.gov by Clinvest.

32 STUDY DOCUMENTATION

Data for this trial will be primarily collected in a web-based electronic data capture (EDC) REDCap platform. The EDC access will be supplied by Clinvest with relevant training support to sites. Sites will be responsible for training study subjects in the EDC system. All data specified should be captured by the site personnel or subjects in the EDC system. All eCRFs are to be completely filled out by personnel administering the study procedures at the time of the visit. The eCRF will be considered the source document for all data collected other than laboratory and procedure findings and the ICF, which will be uploaded into the eCRF as source. All data must be reviewed and signed by the Investigator or Sub-Investigator at the conclusion of the study for each subject. The eCRFs should not be made available in any form to third parties, without written permission from the sponsor.

It is the Investigator's responsibility for the collection and reporting of all clinical, safety, and laboratory data entered on the eCRF and confirmation the data is accurate, authentic, attributable, complete, and consistent. The Investigator or Sub-Investigator must sign the eCRFs within the EDC system to attest the information contained with the eCRF is true and causality of any safety information has been assessed.

33 RECORD RETENTION

Study documentation includes all workbooks, worksheets, forms, lab reports, logs, signature pages, appointment schedules, investigator correspondence, electronic data (i.e. data stored on cds, flash drives, etc.), and regulatory documents. The original recording of an observation should be retained as the source document.

1. Investigator will maintain essential documents for the conduct of a clinical study and any other documentation as specified by applicable regulatory requirements.
2. Investigator will maintain a binder containing written informed consent records.
3. Conduct of study visits will be maintained on appropriate Source Documentation/Case Report Forms. Patient anonymity will be maintained on these forms by identification codes (i.e., subject initials and number). The Investigator's electronic signature will verify that all data entries in the CRF's (CRF) are complete and accurate.
4. Investigator will maintain a Subject Identification Log. Information such as full name of subject, address, contact information, and additional subject identifiers will be recorded. This log will be kept confidential and not copied.
5. Investigator will maintain a Subject Screening/Enrollment Record. This record will record chronologically subjects who were seen for Visit 1, randomized at Visit 2, additional visits conducted during study, and completion/discontinuation information.
6. Clinvest will maintain a Drug Assignment Log. This log will record chronologically study drug received, as well as drug returned or destroyed at the completion of the study.
7. Investigator will maintain a Site Drug Accountability Record. This record will record chronologically study drug received from the Sponsor, as well as drug returned or destroyed at the completion of the study.

Government agency regulation and directives require all study documentation pertaining to the conduct of a clinical trial must be retained by the investigator for at least 2 years after the last approval of a marketing application in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. The investigator will maintain all study documentation on file in a secure and safe location. Clinvest will notify the investigator in writing when retention is no longer necessary. No study records will be destroyed without prior agreement between Ionis Pharmaceuticals and Clinvest.

34 INSTITUTIONAL REVIEW BOARD

Clinvest is responsible for obtaining IRB approval of the protocol, informed consent document, written information provided to the subject, recruiting material, and all other appropriate documents. The trial will not be initiated until IRB approval of all trial documents. The investigator will provide Ionis Pharmaceuticals with documentation that the IRB has approved (such as the protocol, informed consent, and any study-related materials to be provided to the subject) before the study drug is shipped to the sites and study begins. In the event an amendment is needed to any document, Clinvest will also be responsible for the approval of all subsequent major changes. The investigator is responsible for obtaining initial and continuing

review (annually if necessary) of the study by an IRB. Written approval must be forwarded to Clinvest before clinical supplies will be shipped. For continuing studies, written approval from the IRB must be sent to Clinvest at intervals not to exceed 1 year. All other appropriate reports on the progress of the study will be made to the IRB and the Sponsor by Clinvest in accordance with applicable governmental regulations and in agreement with the policy established by the Sponsor and the IRB.

35 TRAINING

Each approved study site will participate in a protocol training program prior to enrollment of any subjects in the study. The program must be attended by the principal investigator at the site, and at least one research coordinator, including the coordinator who will have the lead responsibility for coordination of the study at the site.

Comprehensive training will be provided by Clinvest to site personnel. Training topics will include protocol, study design, study documents, electronic data capture system, reporting of AEs and pregnancy, and any other study related tasks.

36 SAFETY MEASURES

36.1 Adverse Events

Adverse events (AEs) will be monitored throughout the study. All reported AEs will be documented on the appropriate eCRF.

36.2 Physical Examination

At screening and study exit, the investigator will examine the patient for any detectable abnormalities of the following body systems: general appearance; neck (including thyroid); head, eyes, ears, nose, and throat; lungs; heart/cardiovascular; abdomen; neurologic; extremities; back; musculoskeletal; lymphatic; skin; and other. The neurologic examination should be conducted to detect the presence of any significant sensory/motor abnormalities. A digital rectal examination is not required. Gynecological examinations are not required.

Weight should be measured at screening, day 1, and end of study visit using the same scale for all patients at a given investigator site when possible.

Pregnancy tests for all females of childbearing potential. Urine pregnancy testing will be conducted at screening, on day 1, and at the end of study visit to confirm continued non-pregnant status prior to study drug administration or study completion.

36.3 Vital Signs

Vital signs will be measured at all study visits. Systolic and diastolic blood pressure and pulse rate over 30 seconds should be taken after patients have been at rest (seated) for at least 2 minutes. Blood pressure should be recorded in mmHg. Pulse rate should be measured in beats per minutes.

36.4 ECG

Twelve-lead ECG will be performed at the study site, per institutional guidelines, at all study visits. At each of these visits, 1 ECG reading will be collected and will be reviewed by the investigator. The overall interpretation and determination of the clinical relevance of ECG findings will be the responsibility of the investigator and will be recorded in the patient's eCRF.

36.5 Laboratory Assessments

Laboratory assessment samples are to be obtained at designated visits as detailed in the Schedule of Assessments.

36.6 List of Laboratory Analytes

Based on emerging data from this or future studies, additional tests not listed below may be performed on stored samples to better characterize the profile of ISIS 546254 or other similar oligonucleotides

Table 2. Laboratory assessments

Clinical Chemistry Panel	**Screening Tests**	**Hematology**	**Inflammatory**

• Sodium
• Potassium
• Chloride
• Bicarbonate
• Total protein
• Albumin
• Calcium
• Magnesium
• Phosphorus
• Glucose
• BUN
• Creatinine
• Cholesterol
• Uric Acid
• Total bilirubin
• Direct bilirubin (conjugated)
• Indirect bilirubin (unconjugated)
• ALT
• AST
	• Hepatitis B surface antigen
	• Hepatitis C antibody
	• HIV antibody
	• Drug/EtOH
	• C1 Esterase Inhibitor Functional Activity
	• Thyroid Panel
	
	Females only
	• FSH
	• Serum βhCG
	• Urine Pregnancy
	
	Coagulation
	• aPTT (sec)
	• PT (sec)
	• INR
		• Red blood cells
		• Hemoglobin
		• Hematocrit
		• MCV, MCH, MCHC
		• Platelets
		• White blood cells
		• WBC Differential (% and absolute)
		  • Neutrophils
		  • Eosinophils
		  • Basophils
		  • Lymphocytes
		  • Monocytes
		
		Pharmacokinetics
		1. ISIS 546254 levels in plasma
			1.Hs-CRP
			
			Urinalysis
			• Color
			• Appearance
			• Specific gravity
			• pH
			• P/C Ratio
			• Protein
			• Blood
			• Ketones
			• Urobilinogen
			• Glucose
			• Bilirubin
			• Leukocyte esterase
			• Nitrate
			• Microscopic examination[1]

- Alkaline phosphatase
- Creatinine kinase
- GGT

[1] Will be performed on abnormal findings unless otherwise specified

Hematology, clinical chemistry, urinalysis laboratory, urine drug screen, HIV, HbsAg, and HCV serology will be performed at sites local laboratory. Plasma samples for anti-drug-antibody screening will also be collected and stored for potential future analysis. Reference ranges will be supplied by local laboratory and used by the Investigator to assess the laboratory data for clinical significance and pathological changes.

37 FUTURE BIOMEDICAL RESEARCH

Blood samples will be collected from all patients who consent to participate in the optional Future Biomedical Research substudy. If possible, the samples to be used in the substudy will be obtained at the same time that the patient is scheduled to have blood drawn for clinical laboratory assessments as required at the Screening visit for the main study. All samples collected for the substudy will be sent to the designated central laboratory and later shipped to a biorepository for storage. Samples collected for the substudy may be stored at the biorepository for potential analysis under separate protocols for up to 15 years. Substudy samples may be stored for a longer time if a regulatory or governmental authority has active questions that are being answered. In this special circumstance, such substudy samples will be stored until these questions have been adequately addressed.
All patients enrolled in the clinical trial will be considered for enrollment in the Future Biomedical Research substudy; however, participation is optional and will require a separate informed consent form. A patient who initially consents can withdraw that consent at any time and have his or her substudy sample destroyed, including any by-products of the sample.

38 SUMMARY OF METHODS OF DATA COLLECTION

Data required for the evaluation of the primary and some secondary measures will be recorded by subjects using a daily e-diary. The following data will be collected daily with the e-diary:

- Onset and duration of headache
- Severity of headache (mild, moderate, or severe)
- Acute headache medication(s) usage
- Study drug usage
- Associated symptoms
- Unusual symptoms

Questionnaire data required to be directly collected from subject will be obtained at each appropriate visit using an electronic device as defined in the protocol. Questionnaires are to be completed by the subject and the answers to the questions on the questionnaires should come directly form the subject directly, not from family, friend or the study personnel.

Questionnaire data required to be collected directly from clinicians will be obtained at the appropriate visit and recorded in the eCRF per protocol.

39 STATISTICAL ANALYSIS

Subjects will be randomized 1:1 into the treatment groups. Randomization will not include any type of stratification.

An alpha of .05 will be used for statistical significance. All statistical tests will be two-tailed. Descriptive statistics will establish baseline characteristics and adverse event frequency. Data for the safety primary endpoint will be statistically analyzed via Chi-squared analyses and a 2-tailed Repeated Measures ANOVA for the efficacy primary endpoint. Data for each of the secondary outcome measures will be statistically analyzed via a 2-tailed Repeated Measures ANOVA, chi-squared, and/or independent or dependent t-tests as appropriate. All ANOVAs will be followed by univariate post-hoc tests as appropriate. Multiple comparison adjustments will be made if needed.

39.1 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 primary endpoint measurement after treating. The mITT population will be used for efficacy analyses.

39.2 Safety Population

The safety population will include all randomized subjects who received at least one dose of study drug.

A last observation carried forward (LOCF) method will be utilized to impute missing values within a single headache diary. This type of imputation replaces the missing value with the last observation value obtained. If an e-diary day is not recorded, the day will be considered a non-headache day.

40 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.

41 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 ≥ 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.

42 COLLECTION AND DERIVATION OF ENDPOINTS

Data required for the evaluation of endpoints will be recorded for the duration of the study using electronic data capture and include subject reported outcomes. The headache variables will be derived from variables collected daily using an electronic headache diary. On each day, the subject will be asked to record their diary data for the previous 24-hour period.

42.1 Primary Efficacy Variable

1. The variable used to measure mean change in migraine days will be derived from subjects self-reported data entered into the e-diary. It will be based on the number of days with migraine headaches, fulfilling ICHD-3 beta criteria, and/or headaches of any duration with the use of migraine-specific acute headache medication(s).

42.2 Secondary Efficacy Variables

1. The variable used to measure mean change in headache severity will be derived from subjects self-reported data entered into the e-diary. It will be based on the severity recorded for each headache.
2. The variable used to measure mean change in headache days will be derived from subjects self-reported data entered into the e-diary. It will be based on the number of days recorded as headache.
3. The variable used to measure the proportion of subjects experiencing a 50% reduction in the number of headaches will be derived from subjects self-reported data entered into the e-diary. It will be based on the number of days recorded as headache.

4. The variable used to measure change in frequency of headache days requiring medication usage will be derived from subjects self-reported data entered into the e-diary. It will be based on the total number of days that any acute, rescue, or breakthrough medication use is recorded in the e-diary.
5. The variable used to measure change in the MSQ score will be derived from the total score on the MSQ subject questionnaire.
6. The variables used to measure PGIC and SGIC scores will be derived from the total score on the PGIC and SGIC subject questionnaires.

43 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.

44 APPENDIX B

Proposed Revised International Headache Society Criteria for Chronic Migraine

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

1.3 Chronic migraine

Description:

Headache occurring on 15 or more days per month for more than 3 months, which has the features of migraine headache on at least 8 days per month.

Diagnostic criteria:

A. Headache (tension-type-like and/or migraine-like) on ≥15 days per month for >3 months and fulfilling criteria B and C
B. Occurring in a patient who has had at least five attacks fulfilling criteria B-D for 1.1 Migraine without aura and/or criteria B and C for 1.2 Migraine with aura
C. On ≥8 days per month for >3 months, fulfilling any of the following:
    1. criteria C and D for 1.1 Migraine without aura
    2. criteria B and C for 1.2 Migraine with aura
    3. believed by the patient to be migraine at onset and relieved by a triptan or ergot derivative
D. Not better accounted for by another ICHD-3 diagnosis.

45 APPENDIX C

Proposed Revised International Headache Society Criteria for Medication-Overuse Headache

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

8.2 Medication-overuse headache (MOH)

Description:

Headache occurring on 15 or more days per month developing as a consequence of regular overuse of acute or symptomatic headache medication (on 10 or more, or 15 or more days per month, depending on the medication) for more than 3 months. It usually, but not invariably, resolves after the overuse is stopped.

General comment:

In the criteria set out below for the various subtypes, the specified numbers of days of medication use considered to constitute overuse are based on expert opinion rather than on formal evidence.

Diagnostic criteria:

A. Headache occurring on >15 days per month in a patient with a pre-existing headache disorder
B. Regular overuse for >3 months of one or more drugs that can be taken for acute and/or symptomatic treatment of headache
C. Not better accounted for by another ICHD-3 diagnosis.

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