AOBIOME

CLINICAL TRIAL PROTOCOL
Phase II

COMPOUND:
B244

A Prospective, Randomized, Vehicle-Controlled, Double-Blind, Phase 2 Study to Assess the Safety, Tolerability, and Efficacy of B244 Delivered as an Intranasal Spray for Preventive Treatment in Subjects with Episodic Migraine

STUDY NUMBER: MGB244-001

VERSION DATE: June 22, 2018

Sponsor:
AOBiome LLC

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Revision Chronology

Original	January 19, 2018
Amendment 1	March 26, 2018
Amendment 2	June 22, 2018

SPONSOR APPROVAL

A Prospective, Randomized, Vehicle-Controlled, Double-Blind, Phase 2 Study to Assess the Safety, Tolerability, and Efficacy of B244 Delivered as an Intranasal Spray for Preventive Treatment in Subjects with Episodic Migraine

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INVESTIGATOR AGREEMENT

I have read this protocol and agree:

- To conduct the study as outlined herein, in accordance with Good Clinical Practices (GCPs), the Declaration of Helsinki and complying with the obligations and requirements of Clinical Investigators and all other requirements listed in 21 CFR part 312 and according to the study procedures provided by AOBiome LLC and local regulations.
- Not to implement any changes to the protocol without prior agreement from the Sponsor and prior review and written approval from the IRB or IEC, except as would be necessary to eliminate an immediate hazard to study participant (s), or for administrative aspects of the study.
- To ensure that all persons assisting me with the study are adequately informed about the investigational product(s) and of their study-related duties as described in the protocol.
- To completely inform all participants in this study concerning the pertinent details and purpose of the study prior to their agreement to participate in the study in accordance with GCP and regulatory authority requirements.
- To be responsible for maintaining each participant's consent form in a secure study file and providing each participant with a signed copy of the consent form.
- That I am thoroughly familiar with the appropriate use of the investigational product(s), as described in the protocol, and any additional information provided to me by, or on behalf of AOBiome LLC.

Investigator Printed Name: David Lindrow, MD

Signature: _____ **Date:** 6/29/18

Statement of Compliance

The study will be carried out in accordance with Good Clinical Practice (GCP) as required by the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46; 21 CFR Part 50, 21 CFR Part 56, and 21 CFR Part 312)
- ICH E6; 62 Federal Register 25691 (1997)

All key personnel (all individuals responsible for the design and conduct of this study) have completed Good Clinical Practice Training.

CLINICAL TRIAL SUMMARY

COMPOUND: B244 STUDY NUMBER: **MGB244-001**

TITLE:	A Prospective, Randomized, Vehicle-Controlled, Double-Blind, Phase 2 Study to Assess the Safety, Tolerability, and Efficacy of B244 Delivered as an Intranasal Spray for Preventive Treatment in Subjects with Episodic Migraine
INVESTIGATIONAL PRODUCT	B244
STUDY PHASE	2
STUDY ARMS	B244 (140ul per nostril; OD 0.5; $1x10^9$ cells/ml); B244 (140ul per nostril; OD 2.0; $4x10^9$ cells/ml); Vehicle (140ul per nostril);
PURPOSE:	To assess the safety, tolerability and efficacy of B244 relative to vehicle in subjects with episodic migraine.
OBJECTIVES:	**Primary Objective** • To assess the safety and tolerability of B244 relative to vehicle for the preventive treatment of episodic migraine. Safety and tolerability will be assessed by reporting of AEs, physical examination, neurological examination, and vital signs (blood pressure, heart rate, respiratory rate). **Secondary Objective(s)**

	• To evaluate the efficacy of B244 relative to vehicle for migraine prevention in subjects with episodic migraine. • To continue to assess safety during the 28 day follow-up period. **Exploratory Objective** • To evaluate the effect of B244 on changes in cytokine concentration in nasal fluid and blood.
ENDPOINTS:	**Primary** • Safety as assessed by incidence and severity of AEs, physical examination, neurological examination, and vital signs (blood pressure, heart rate, respiratory rate). **Secondary** • Mean change in monthly migraine days from baseline to 12 weeks of treatment. • Proportion of subjects experiencing a ≥50%, ≥75%, and 100% reduction in monthly migraine days from baseline to 12 weeks of treatment. • Mean change in monthly migraine attacks from baseline to 12 weeks of treatment. • Mean change in monthly migraine days from baseline to the 4-week period after first dose of study drug. • Mean change in monthly acute migraine specific medication days from baseline to 12 weeks of treatment.

	• Mean change in monthly moderate and severe headache days (migraine pain-intensity score) from baseline to 12 weeks of treatment. • Mean change in monthly headache days from baseline to 12 weeks of treatment. • Mean change in monthly headache hours from baseline to 12 weeks of treatment. • Mean change from baseline to 12 weeks of treatment in disability, as measured by the Migraine Disability Assessment (MIDAS) questionnaire. • Mean change from baseline to 12 weeks of treatment in monthly Headache Impact Test-6 (HIT-6) questionnaire. • Mean change from baseline to 12 weeks of treatment in monthly Migraine Specific Quality of Life questionnaire (MSQL) questionnaire. • Safety assessments after 28 day follow-up period. • Mean change in baseline Clinical Global Impression (CGI) score to end of treatment period. **Exploratory** • Changes in cytokine concentration in nasal fluid and blood from baseline to 12 weeks of treatment. • Mean change in migraine days from end of treatment (last month of 12 weeks of treatment) to follow-up.

	• Mean change in migraine associated symptoms: nausea/vomiting, photophobia and sonophobia from baseline to 12 weeks of treatment.
STUDY DESIGN:	This is a prospective, randomized, vehicle-controlled, double-blind, 3-arm parallel assignment study assessing the safety, tolerability, and efficacy of B244 delivered as an intranasal spray for preventive treatment in subjects with episodic migraine. This study will enroll 303 subjects. Enrolled subjects will undergo a 28 day baseline period to establish baseline headache frequency and characteristics. Subjects will be randomized at the end of the baseline period. Randomization will be 1:1:1 so that equal number of subjects will be treated in each Arm of the study. Subjects will be trained for in-home treatment/assessment on the use of the nasal spray, and apply their first dose of treatment under the supervision of study personnel on site. Subjects will remain under observation at the study site for 1 hour post dosing. Subjects will then be dispensed study medication for 12 weeks of at home dosing (either $1x10^9$ cell/ml; $4x10^9$ cells/ml; or vehicle). While at home, subjects will dose twice daily (am and pm) for 12 weeks during the prevention treatment phase. Subjects will be asked to visit the site every 4 weeks during the 12 week treatment period and 4 week follow-up. Daily assessments will be made during the 12 week double-blind treatment period for

	safety, tolerability and efficacy. Additional safety assessments will be made during the follow-up period 28 days after last treatment. Subjects will record their dosing, migraine incidence/severity/duration, and rescue (acute migraine specific) medications using a study diary.
NUMBER OF PATIENTS PLANNED FOR RANDOMIZATION:	Approximately 303 subjects will be enrolled in order to achieve a target sample size of 264 (i.e., 88 subjects per arm), allowing for a 10-15% drop-out rate.
STUDY POPULATION:	Male and female subjects, 18 to 65 years of age, with a history of episodic migraine are eligible for enrollment. Subjects must be willing to refrain from using any treatments that are or might be considered useful for migraine prevention including recognized pharmaceutical products, investigational products, herbal medicines or supplements while participating in this study. Use of acute treatment is allowed throughout the study with the study subjects' usual acute medication of choice (usually a triptan, possibly NSAID or analgesic) but not intranasal formulations. Subjects should not initiate a preventive medication during study.
MAIN INCLUSION/EXCLUSION CRITERIA:	**Inclusion Criteria** 1. Males and Females, 18 to 65 years of age. 2. In good general health as determined by a thorough medical history and physical examination, and vital signs. 3. At least a 1-year history of migraine with or without aura that

began before the age of 50 years old and consistent with a diagnosis of migraine with or without aura according to the International Classification of Headache Disorders, 3rd edition, beta version.

4. Experiences 4-14 migraine days per month and 3-14 migraine attacks per month and no more than 14 headache days per month (including migraine and non-migraine headache days) in the 3 months prior to screening.
5. Experiences 4-14 migraine headache days per month during the baseline period.
6. Ability and willingness to abstain from taking medications not allowed by the protocol or administering any foreign substance intranasally.
7. Ability and willingness to complete a migraine-history diary from screening to treatment with study drug and a migraine-treatment diary from prevention treatment through the remainder of the follow-up period.

Exclusion Criteria

Subjects who meet any of these criteria are not eligible for enrollment as study participants:

1. Headache on greater than 14 days/month in any of the three months (90 days) preceding entry into the study.
2. Use of acute migraine-specific medications (e.g., ergotamine, triptan) on more than 10 days per

	month in the previous 3 months and during study. 3. Use of intranasal migraine medications during study. 4. Use of any intranasally administered over-the-counter product or nasal irrigation (e.g., neti pot) during study. 5. Opioids/barbiturates used on more than 4 days per month in the previous 3 months and throughout the duration of the study. 6. Use of analgesics (including acetaminophen, nonsteroidal anti-inflammatory drugs [NSAIDs], acetylsalicylic acid, or combination analgesics) for migraine and non-migraine headaches on more than 14 days per month in the previous 3 months and during study. 7. Use of migraine prevention medication within two months prior to study and throughout the duration of the study. 8. Botulinum toxin injection within 3 months prior to screening or during study. 9. Anti-CGRP monoclonal antibody (e.g., erenumab, fremanezumab, galcanezumab, and eptinezumab) injection or infusion within 4 months prior to screening or during study. 10. Small molecule anti-CGRP medications in the 30 days prior to the screening visit. 11. Use of systemic antibiotics during study. 12. Pregnancy or breast-feeding. 13. Female of childbearing potential not using adequate contraceptive measures.

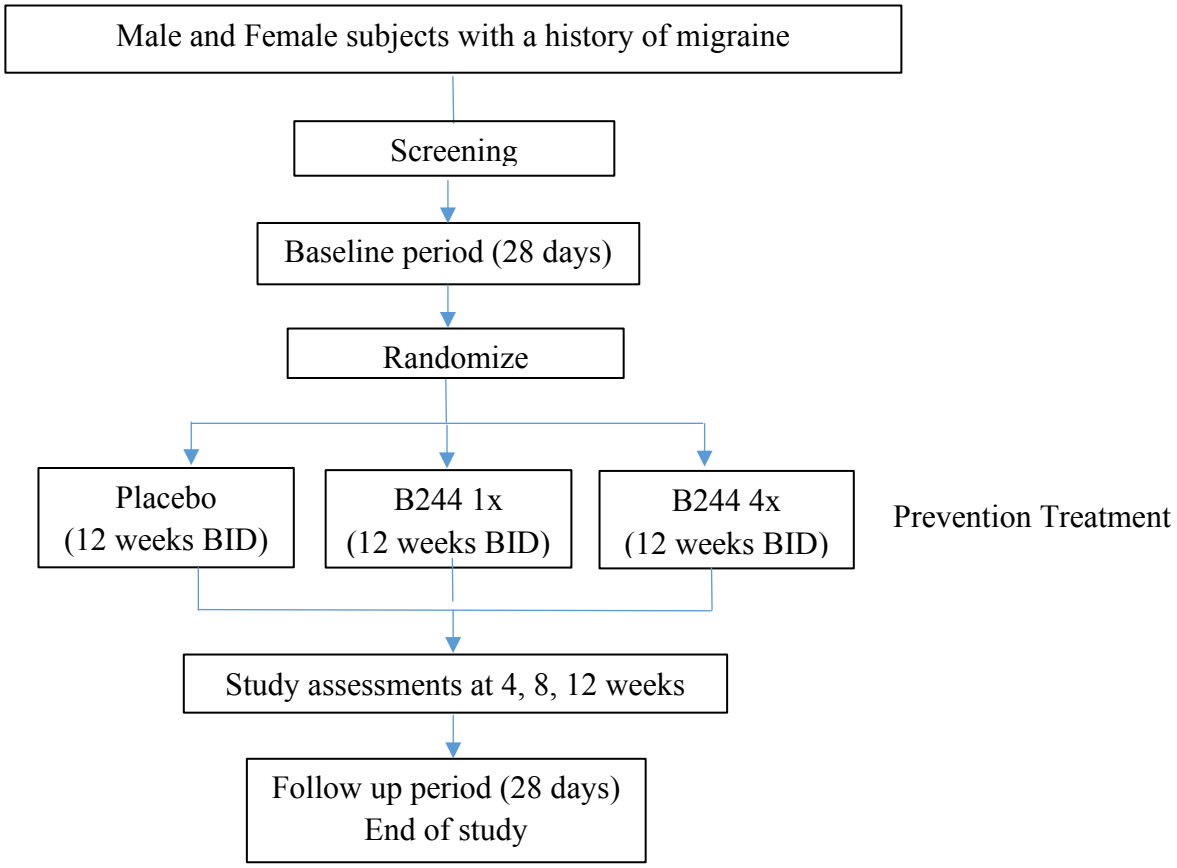
	14. Inability to give informed consent. 15. History of neurological, psychiatric, or any other medical condition that in the opinion of the investigator may compromise the subject's ability to safely participate in the study. 16. Subjects with any significant clinical abnormalities which may interfere with study participation. 17. Prior use of AO+ Mist. 18. Subjects with immunodeficiencies, nasal lesions, nasal polyps, or sinus infections. 19. Subjects who have participated in an investigational drug trial in the 30 days prior to the screening visit. 20. Inability to maintain at least 80% diary compliance during the study from baseline to follow-up.
DOSE REGIMEN:	After screening and recruitment, participants will be randomized to one of the following arms of the study: B244 (140ul per nostril; OD 0.5; $1x10^9$ cells/ml) twice-a-day for 12 weeks; B244 (140ul per nostril; OD 2.0; $4x10^9$ cells/ml) twice-a-day for 12 weeks; Vehicle (140ul per nostril) twice-a-day for 12 weeks;
ASSESSMENT SCHEDULE:	All subjects will attend a screening visit (V1) not more than 14 days prior to V2 (Day -28). Subjects with a history of migraine as defined in the inclusion/exclusion criteria who pass screening will return to clinic on Day -28 (V2) for baseline assessments and sent home to record their migraine events and AEs over the 28 day baseline period using a diary. Subjects will return to clinic on Day 0 (V3) to determine continued study

	eligibility. Subjects who continue to meet all inclusion and exclusion criteria will be randomized to receive study treatment. Subjects will then be trained for in-home treatment/assessment on the use of the nasal spray, and apply their first dose of treatment under the supervision of study personnel while on site (V3). Subjects will remain under observation at the study site for 1 hour post dosing. Subjects will then be dispensed study medication for 12 weeks of at home dosing (either $1x10^9$ cell/ml; $4x10^9$ cells/ml; or vehicle). While at home, subjects will dose twice daily (am and pm) for 12 weeks during the prevention treatment phase, starting with the second dose of the first day of treatment. Subjects will record their dosing, migraine events, and rescue medications daily and return to clinic every 4 weeks during the 12 week treatment period at Day 28 (V4), Day 56 (V5), and Day 84 (V6) for safety and additional prevention treatment assessments. Additional safety assessments will be made during the 28 day follow-up period and end of study visit at Day 112 (V7).
STATISTICAL CONSIDERATIONS:	Sample Size: Group sample sizes of 88 per arm achieve 80% power to reject the null hypothesis of equal means when the population mean difference $\mu_1 - \mu_2 = -3.8 - -2.3 = -1.5$, where -3.8 is the mean reduction in migraine headache days for an active treatment and -2.3 is the mean reduction in migraine headache days for placebo. The sample size calculation assumes a standard deviation for both groups of 4.0 and a Bonferroni adjusted significance level

	(alpha) of 0.05 using a one-sided two-sample equal-variance z-test for the comparison of each active treatment arm versus vehicle. Safety: The analysis of safety will be based on the safety population, which is defined as all subjects receiving at least 1 dose of B244 or Vehicle. Adverse events (AEs) will be coded using the Medical Dictionary for Regulatory Activities (MedDRA®) for purposes of summarization. All AEs occurring during the study will be included in by-subject data listings and tabulated. Events leading to death, serious AEs, and events resulting in study discontinuation will be summarized using standard descriptive statistics, and presented by treatment arm. Exposure to study drug and reasons for discontinuation of study treatment will be tabulated. Shift tables relative to the normal range will be provided for laboratory parameters. Additional analyses will be performed, if warranted, upon review of the data. Efficacy: Efficacy analyses will be performed on the intent-to-treat (ITT) population, which consists of all randomized subjects who receive at least 1 dose of study medication, and on the per protocol (PP) population, which consists of subjects who complete their Week 12 visit and have percent compliance (based on the weight of IP used). For the continuous endpoints of the change in migraine days, the change in migraine attacks, the change in days using rescue medication, the change in headache days, the change in headache hours, and the change in MIDAS, HIT-6, MSQL, and CGI scores, a one-sided two-sample

	equal-variance z-test will be used to compare treatment group means. Fisher's Exact test will be used to make treatment group comparisons on efficacy outcomes including the following binary outcomes: • Proportion of subjects experiencing a ≥50%, ≥75%, and 100% reduction in monthly migraine days from baseline to 12 weeks of treatment. Exploratory: Exploratory endpoints will be analyzed using the same method as for continuous efficacy endpoints. The analyses will be performed on the ITT and PP population.
INVESTIGATIONAL DRUG AND PLACEBO:	Investigational drug refers to B244. Investigational Product/treatment refers to either B244 or Vehicle.
PLANNED DURATION PER SUBJECT:	Up to 22 weeks
DURATION OF STUDY:	Estimated study duration (First Subject First Visit to Top Line Report) is 9.5 months.

1 STUDY SCHEMA



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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

AE	Adverse Event
AMO	Ammonia Monooxygenase
AOB	Ammonia Oxidizing Bacteria
BID	Twice-Daily
CGI	Clinical Global Impression
CRF	Case Report Form
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
ECG	Electrocardiogram
EOS	End of Study
E/T	Early Termination
FDA	Food and Drug Administration
HAO	NH_2OH oxidoreductase
HIT-6	Headache Impact Test-6
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IP	Investigational Product
IRB	Institutional Review Board
IWRS	Interactive Web Response System
MedDRA	Medical Dictionary for Regulatory Activities
MIDAS	Migraine Disability Assessment
MSQL	Migraine Specific Quality of Life
NH_2OH	Hydroxylamine
NH_3	Ammonia
NO	Nitric Oxide
NO_2-	Nitrite
PT	Preferred Term
SAE	Serious Adverse Event
SPM	Study Procedures Manual
SOC	System Organ Class
TEAE	Treatment Emergent Adverse Event
WOCBP	Women of Child Bearing Potential

2 INTRODUCTION

2.1 Background

Migraine is a chronic, recurrent disorder affecting an estimated 37 million Americans. Migraine is marked by recurring moderate to severe headache with throbbing pain that usually lasts from 4-72 hours, typically unilateral in location, is often accompanied by nausea, vomiting, and sensitivity to light or sound, and is sometimes preceded by an aura and is often followed by fatigue. Around 12% of adults have episodic migraine (headaches on less than 15 days per month) [3], while around 1% of the adult population suffers from migraine and headaches on more days than not [4], a condition termed chronic migraine. In addition, during a migraine attack, individuals experience impaired health-related quality of life and considerable disability [5, 6].

The treatment of migraine consists of acute/abortive therapies and preventive therapies. The acute treatment of migraine includes most commonly, migraine-specific medications such as triptans and ergotamines which, while effective in a majority of cases may be limited by side effects, the development of medication overuse/rebound and some contraindications including cardiovascular, cerebrovascular and peripheral vascular disease and hypertension. Preventive treatments include four medications approved by the FDA for migraine prevention: anti-epilepsy medications topiramate and divalproex sodium; and beta-adrenergic blockers propranolol and timolol. These drugs are not disease-specific and are plagued by a plethora of tolerance issues including cognitive side effects, weight loss or weight gain, depression, hypotension, etc., and therefore adherence to therapy is typically low. Botulinum Toxin A is approved for the narrow indication of chronic migraine prevention but is expensive and requires multiple injections in the face, scalp and neck. In addition, nearly half of individuals with episodic or chronic migraine who are in need of preventive therapies do not receive them [7].

Current models for the etiology of migraine headaches support the role of cortical spreading depression (CSD) and activation of the trigeminovascular system and its constituent neuropeptides, as well as about the importance of neuronal and glial ion channels and transporters that contribute to the putative cortical excitatory/inhibitory imbalance that renders migraineurs susceptible to an attack [8]. It is our hypothesis that under conditions of stress, cortical and trigeminovascular cells have reduced cellular ATP levels as a result of ischemic preconditioning. Intranasal administration of ammonia oxidizing bacteria (AOB) delivers physiologic levels of nitric oxide (NO) to these tissues through the oxidation of mucosal ammonia. It has been demonstrated [9] that increases in NO result in corresponding increases in cellular ATP levels though a soluble Guanylyl Cyclase (sGC) intermediate. Increased cellular ATP availability supports Na+/K+ ATPase function necessary to maintain membrane electrical stability, reducing the risk of excitatory/inhibitory imbalance and subsequent cortical spreading depression [10].

It is believed that intranasal AOB will restore physiologic levels of NO which reduce or reset the effects of stress induced ischemic preconditioning and subsequent cortical spreading depression associated with migraines. NO is also believed to modulate pain involving nociceptor inhibition and central sensitization. Migraine is an inflammatory state as observed by activation of macrophages and dendritic cells to an inflammatory state, degranulation of mast cells, and

upregulation of pro-inflammatory genes with the initiation of CSD. To this effect, AOB's anti-inflammatory and pain modulatory effects may further improve acute migraine symptoms and prevent recurrence of additional migraines.

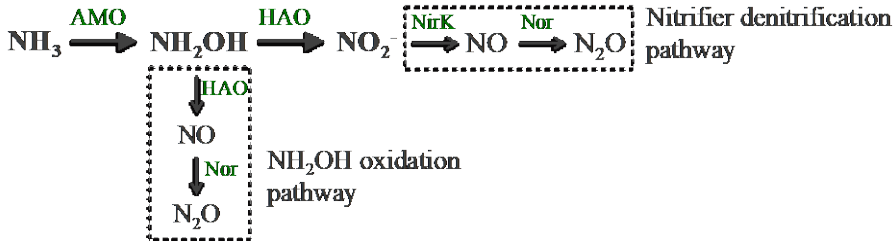

Figure 1 Nitrifier Denitrification Pathway

AOBs are essential for the initial step in environmental nitrification processes, specifically the oxidation of ammonia (NH_3) to nitrite (NO_2-). *Nitrosomonas* are Gram-negative chemolithoautotrophic betaproteobacteria that obtain energy solely from NH_3 oxidation, while fixing CO_2 for their carbon needs [11]. Oxidation of NH_3 proceeds in two steps (Figure 1) leading to sequential generation of hydroxylamine (NH_2OH) and NO_2- that require two enzyme complexes: the membrane-bound ammonia monooxygenase (AMO) comprised of subunits AmoA, AmoB and AmoC; and the periplasmic NH_2OH oxidoreductase (HAO). In addition to high NO_2- levels, NH_3 oxidation leads to nitric oxide (NO) and N_2O production through two independent pathways downstream of NH_2OH production: nitrifier denitrification and NH_2OH oxidation [12].

B244 is a purified a strain of *Nitrosomonas eutropha*, designated D23, originally isolated from soil samples. Sequencing of the B244 genome revealed a distinct genetic profile from that of other published *Nitrosomonas* strains and AOB genomes. Based on *in vitro* co-culture studies, B244 was able to reduce survival of pathogenic bacteria. Nitrite generation from ammonia concurrently with medium acidification by B244 led to strong antibacterial effects and a marked reduction (~100-fold) in viable counts of methicillin-resistant *Staphylcoccus aureus* and *Pseudomonas aeruginosa*, two pathogens frequently isolated from infected skin and wound sites. In contrast, control cultures with B244 in the absence of ammonium or with heat-killed B244 supplemented with ammonium, had no antibacterial effects. The unique metabolic and antimicrobial activity of *Nitrosomonas*, in combination with their lack of virulence render these bacteria as attractive candidates for topical delivery of nitrite and nitric oxide on human skin with potential to improve health in both normal and abnormal skin conditions or wound sites. NO-releasing drugs or NO donors have also shown activity against *Propionibacterium acnes* and other pathogenic bacteria, anti-inflammatory activity, and inhibition of lipogenesis by insulin-stimulated immortal sebocytes [13-15].

To date, there have been no reported infections or health risks associated with topical application or ingestion of *Nitrosomonas* species. The absence of any illnesses attributed to these bacteria despite our widespread exposure indicates that they pose a minimal health risk, if any at all. Infection or tissue damage by *Nitrosomonas* is unlikely, because the sequenced genomes of several *Nitrosomonas* and other AOB lack genes encoding cytotoxins, or other known bacterial virulence factors. Further, AOB are slow growing, as compared to most heterotrophic bacteria,

with optimum doubling times of 8 hours or higher. In particular, *Nitrosomonas* growth is rate limited by the availability of ammonia requiring the oxidation of 27 moles NH_3/mole CO_2 fixed. Due to their dependence on ammonia for their growth, the numbers of *Nitrosomonas* on the skin will be necessarily limited and naturally regulated by the amount of ammonia produced in sweat. This would ensure that the amount of nitrite and NO generated would be relatively low, without any adverse effects.

Currently, *Nitrosomonas eutropha* D23 in buffer at a concentration of $1x10^9$ cells/mL is available as a cosmetic product for topical application of a natural source of AOB and NO/NO_2 to improve the appearance of human skin. AOBiome plans to study *Nitrosomonas eutropha* D23 diluted in storage solution (50 mM Na_2HPO_4 and 2 mM $MgCl_2$, pH 7.6) at defined doses to be provided as an intranasal spray (B244) for therapeutic applications.

B244 is being developed under IND #16487 as a 'live topical' to provide a natural source of AOB and NO/NO_2 to the human skin. Under IND #16487, a phase 1b/2a clinical trial was completed in 2016 where 36 participants with clinical diagnosis of facial acne vulgaris were randomized to receive ascending doses of investigational product (IP) over 14 days. Safety analyses have been completed and there have been no attributable drug related SAEs reported. In addition, a Phase 2b/3 clinical trial in 372 patients with clinical diagnosis of facial acne has been completed with a positive safety and efficacy readout with no attributable drug related SAEs reported in this trial.

Additionally, other topical development programs with B244 include hypertension (IND #17086) and atopic dermatitis (IND #17485). Under IND #17086, a study titled, "A Prospective, Controlled, Double Blinded, Multicenter, Randomized, Vehicle controlled, Phase II Study of B244 delivered as a topical spray to Determine Safety and Efficacy in Subjects with elevated blood pressure" has been completed. Under IND #17485, a study titled, "A Prospective, Vehicle Controlled, Double Blind, Multicenter, Randomized, Phase II Study of B244 Delivered as a Topical Spray to Determine Safety and Efficacy in Subjects with Mild to Moderate Atopic Dermatitis" is planned.

Furthermore, B244 is being developed under IND #17085 as a nasal spray for intranasal delivery of B244. A rat toxicology study performed by intranasal administration of B244 twice daily for 28 days found that B244 was safe and well tolerated in rats at levels of up to the maximum dose $8x10^9$ cell/mL. Under IND #17085, a study titled, "A Prospective, Controlled, Double Blinded, Single Center, Randomized, 3 arm, Phase 1b/2a Study to Assess the Safety, Tolerability, and Preliminary Efficacy of B244 Delivered as an Intranasal Spray in Healthy Volunteers and Subjects with Seasonal Allergic Rhinitis" has been initiated.

The proposed clinical study is designed to evaluate the safety, tolerability, and efficacy of intranasally delivered B244 for preventive treatment of episodic migraine.

3 STUDY OBJECTIVES

3.1 Primary Objectives

- To assess the safety and tolerability of B244 relative to vehicle for the preventive treatment of episodic migraine. Safety and tolerability will be assessed by reporting of AEs, physical examination, neurological examination, and vital signs (blood pressure, heart rate, respiratory rate).

3.2 Secondary Objectives

- To evaluate the efficacy of B244 relative to vehicle for migraine prevention in subjects with episodic migraine
- To continue to assess safety during the 28 day follow-up period.

3.3 Exploratory Objectives

- To evaluate the effect of B244 on changes in cytokine concentration in nasal fluid and blood.

4 ENDPOINTS

4.1 Primary

- Safety as assessed by incidence and severity of AEs, physical examination, neurological examination, and vital signs (blood pressure, heart rate, respiratory rate).

4.2 Secondary

- Mean change in monthly migraine days from baseline to 12 weeks of treatment.
- Proportion of subjects experiencing a ≥50%, ≥75%, and 100% reduction in monthly migraine days from baseline to 12 weeks of treatment.
- Mean change in monthly migraine attacks from baseline to 12 weeks of treatment.
- Mean change in monthly migraine days from baseline to the 4-week period after first dose of study drug.
- Mean change in monthly acute migraine specific medication days from baseline to 12 weeks of treatment.
- Mean change in monthly moderate and severe headache days (migraine pain-intensity score) from baseline to 12 weeks of treatment.
- Mean change in monthly headache days from baseline to 12 weeks of treatment.
- Mean change in monthly headache hours from baseline to 12 weeks of treatment.
- Mean change from baseline to 12 weeks of treatment in disability, as measured by the Migraine Disability Assessment (MIDAS) questionnaire.
- Mean change from baseline to 12 weeks of treatment in monthly Headache Impact Test-6 (HIT-6) questionnaire.

- Mean change from baseline to 12 weeks of treatment in monthly Migraine Specific Quality of Life questionnaire (MSQL) questionnaire.
- Safety assessments after 28 day follow-up period.
- Mean change in baseline Clinical Global Impression (CGI) score to end of treatment period.

4.3 Exploratory

- Changes in cytokine concentration in nasal fluid and blood from baseline to 12 weeks of treatment.
- Mean change in migraine days from end of treatment (last month of 12 weeks of treatment) to follow-up.
- Mean change in migraine associated symptoms: nausea/vomiting, photophobia and sonophobia from baseline to 12 weeks of treatment.

5 STUDY DESIGN

- This is a prospective, randomized, vehicle-controlled, double-blind, 3-arm parallel assignment study assessing the safety, tolerability, and efficacy of B244 delivered as an intranasal spray for preventive treatment in subjects with episodic migraine.
- This study will enroll 303 subjects.
- Enrolled subjects will undergo a 28 day baseline period to establish baseline headache frequency and characteristics.
- Subjects will be randomized at the end of the baseline period. Randomization will be 1:1:1 so that equal number of subjects will be treated in each Arm of the study.
- Subjects will be trained for in-home treatment/assessment on the use of the nasal spray, and apply their first dose of treatment under the supervision of study personnel on site. Subjects will remain under observation at the study site for 1 hour post dosing. Subjects will then be dispensed study medication for 12 weeks of at home dosing (either $1x10^9$ cell/ml; $4x10^9$ cells/ml; or vehicle). While at home, subjects will dose twice daily (am and pm) for 12 weeks during the prevention treatment phase. Subjects will be asked to visit the site every 4 weeks during the 12 week treatment period and 4 week follow-up.
- Daily assessments will be made during the 12 week double-blind treatment period for safety, tolerability and efficacy. Additional safety assessments will be made during the follow-up period 28 days after last treatment. Subjects will record their dosing, migraine incidence/severity/duration, and rescue (acute migraine specific) medications using a study diary.

6 SELECTION OF STUDY PARTICIPANTS

6.1 Number of Participants Planned

Approximately 379 subjects will be consented and screened, allowing for a 20% screen fail rate. Approximately 303 subjects will be enrolled in order to achieve a target sample size of 264 (i.e., 88 subjects per arm), allowing for a 10-15% drop-out rate.

6.2 Study Population

Male and female subjects, 18 to 65 years of age, with a history of episodic migraine are eligible for enrollment. Subjects must be willing to refrain from using any treatments that are or might be considered useful for migraine prevention including recognized pharmaceutical products, investigational products, herbal medicines or supplements while participating in this study. Use of acute treatment is allowed throughout the study with the study subjects' usual acute medication of choice (usually a triptan, possibly NSAID or analgesic), but not intranasal formulations. Subjects should not initiate a preventive medication during study.

6.3 Inclusion Criteria

Deviations from inclusion criteria are not allowed because they can potentially jeopardize the scientific integrity of the study, regulatory acceptability or participant safety. Therefore, adherence to the criteria as specified in the protocol is essential.

Participants eligible for enrollment in the study must meet all the following criteria:

1. Males and Females, 18 to 65 years of age.
2. In good general health as determined by a thorough medical history and physical examination, and vital signs.
3. At least a 1-year history of migraine with or without aura that began before the age of 50 years old and consistent with a diagnosis of migraine with or without aura according to the International Classification of Headache Disorders, 3rd edition, beta version.
4. Experiences 4-14 migraine days per month and 3-14 migraine attacks per month and no more than 14 headache days per month (including migraine and non-migraine headache days) in the 3 months prior to screening.
5. Experiences 4-14 migraine headache days per month during the baseline period.
6. Ability and willingness to abstain from taking medications not allowed by the protocol or administering any foreign substance intranasally.
7. Ability and willingness to complete a migraine-history diary from screening to treatment with study drug and a migraine-treatment diary from prevention treatment through the remainder of the follow-up period.

6.4 Exclusion Criteria

Deviations from exclusion criteria are not allowed because they can potentially jeopardize the scientific integrity of the study, regulatory acceptability or participant safety. Therefore, adherence to the criteria as specified in the protocol is essential.

Participants will be excluded from the study if any of the following criteria are met:

1. Headache on greater than 14 days/month in any of the three months (90 days) preceding entry into the study.
2. Use of acute migraine-specific medications (e.g., ergotamine, triptan) on more than 10 days per month in the previous 3 months and during study.
3. Use of intranasal migraine medications during study.
4. Use of any intranasally administered over-the-counter product or nasal irrigation (e.g., neti pot) during study.
5. Opioids/barbiturates used on more than 4 days per month in the previous 3 months and throughout the duration of the study.
6. Use of analgesics (including acetaminophen, nonsteroidal anti-inflammatory drugs [NSAIDs], acetylsalicylic acid, or combination analgesics) for migraine or non-migraine headaches on more than 14 days per month in the previous 3 months and during study.
7. Use of migraine prevention medication within two months prior to study and throughout the duration of the study.
8. Botulinum toxin injection within 3 months prior to screening or during study.
9. Anti-CGRP monoclonal antibody (e.g., erenumab, fremanezumab, galcanezumab, and eptinezumab) injection or infusion within 4 months prior to screening or during study.
10. Small molecule anti-CGRP medications in the 30 days prior to the screening visit.
11. Use of systemic antibiotics during study.
12. Pregnancy or breast-feeding.
13. Female of childbearing potential not using adequate contraceptive measures.
14. Inability to give informed consent.
15. History of neurological, psychiatric, or any other medical condition that in the opinion of the investigator may compromise the subject's ability to safely participate in the study.
16. Subjects with any significant clinical abnormalities which may interfere with study participation.
17. Prior use of AO+ Mist
18. Subjects with immunodeficiencies, nasal lesions, nasal polyps, or sinus infections.
19. Subjects who have participated in an investigational drug trial in the 30 days prior to the screening visit.
20. Inability to maintain at least 80% diary compliance during the study from baseline to follow-up.

7 PARTICIPANT ENROLLMENT

7.1 Consenting Participants

Informed consent for participation in the study must be obtained before performing any study-specific procedures.

Informed Consent Forms for enrolled participants and for participants who are not subsequently enrolled will be maintained at the study site in secure study files. Consent will be obtained by trained research study staff trained in taking informed consent. The study will be explained with the opportunity for the participant to ask questions. If a participant wishes to enter the study, a consent form will be completed and signed.

7.2 Screening for Eligibility

Informed consent will be obtained prior to any screening assessments. Screening assessments (Visit 1; V1) will be performed within 2 weeks (-14 days) prior to the Baseline visit (V2) and will include: medical history and medication use, complete physical examination including height, weight, vital signs, and 12-lead ECG, a neurological examination, nasal inspection, laboratory testing, including blood sample for a comprehensive metabolic panel, complete blood count, chemistry, lipid panel, and liver function test, as well as urinalysis. Urine pregnancy test will be performed on WOCBP. All screening evaluations must be completed and reviewed to confirm that participants meet all eligibility criteria before entering baseline period on Day -28. Inclusion and exclusion criteria will be reviewed at Screening (V1), Baseline visit (V2), and Randomization visit (V3) to make sure nothing changed. Enrolled subjects will be asked to undergo a 4 week (Day -28 to 0) baseline period.

Eligible subjects will return to clinic on Day -28 (V2) for baseline assessments and will be sent home to record their migraine events and acute migraine medication use over the 28 day baseline period using a diary. Subjects will return to clinic on Day 0 (V3) for randomization upon confirming continued eligibility. Subjects are randomized at V3 upon confirming the inclusion/exclusion criteria and the entry criteria of 4-14 migraine headache days and 80% diary compliance during the baseline period.

For subjects who do not meet the 80% daily diary compliance but otherwise meet the 4-14 migraine days requirement during the baseline period, they can be provided additional opportunities to meet the 80% diary compliance requirement to enable continued eligibility for randomization. The following allowances can be made per investigator judgement on a case by case basis:

- If the anticipated diary compliance is slightly less than 80% or short by 4 daily diary entries by the day before the randomization visit, subjects can enter up to 4 additional days of diary entries (over the ±3 day visit window specified in the schedule of events in the protocol) as part of the baseline period and reschedule the randomization visit immediately after this period. This provides up to 7 additional days of diary entries to fulfill compliance. Twenty-eight days prior to the new randomization visit day can be re-evaluated for the baseline period.
- If the anticipated diary compliance is short by more than 4 daily diary entries by the day before the randomization visit, the investigator has the option to shift the baseline period (i.e., reset) to a randomization visit date that allows subjects to record the additional days of diary entries that fulfills 80% compliance and resets the baseline period 4 weeks back from the new randomization visit day.

- The investigator has the option to request subjects to repeat the baseline period (i.e., rebaseline) by entering 28 additional days of diary entries as part of a new baseline period and re-evaluate the baseline period 4 weeks back from the new randomization visit day.

All screening assessments are listed in the Schedule of Events Table (Appendix A). A participant must meet all inclusion criteria, and none of the exclusion criteria, to be enrolled and randomized in this study. The Investigator and team will maintain a screening log to record details of all persons screened and to confirm eligibility or record reasons for screening failure, as applicable.

7.3 Study Withdrawal and Withdrawal From Investigational Product and Stopping Criteria

A participant may withdraw from the study at any time at his/her own request, or may be withdrawn at any time at the discretion of the Investigator.

Reasons for withdrawal (participants who refuse to complete any remaining study visits) or discontinuation (participants who prematurely stop the application) at any time during the study may include, but are not limited, to the following:

- For safety reasons, either at the discretion of the Investigator or at the participant's request
- For protocol violations at the discretion of AOBiome
- Due to concomitant therapy that could interfere with the results of the study (the Investigator will report all such information on the CRFs and decide, in accordance with AOBiome, whether the participant is to be withdrawn).

The reason for participant study withdrawal will be recorded in the electronic Case Report Form (eCRF). Data from participants withdrawing from the study will be considered evaluable up to the point at which they are withdrawn using the same criteria for evaluability as for participants who complete the study.

7.4 Screen Failures

Data for screen and baseline failures will be collected in source documentation at the site but will not be transmitted to AOBiome.

7.5 Early Termination

Participants who have discontinued the study early will be evaluated by the Investigator at the Early Termination Visit. See the list of assessments to be performed at the Early Termination Visit in the Schedule of Events Table (Appendix A). Participants with ongoing AEs or SAEs believed to be possibly related to investigational product (IP) will continue to be followed until resolution or for 30 days as warranted by the nature of the AE.

8 STUDY TREATMENT

8.1 Investigational Product

Under normal conditions of handling and administration, IP is not expected to pose significant safety risks to site staff. A Material Safety Data Sheet describing the occupational hazards and recommended handling precautions will be provided to site staff if required by local laws or will otherwise be available from the Sponsor upon request.

Investigational product must be stored in a secure area under the appropriate physical conditions for the product. Access to and administration of the IP will be limited to the Investigator and authorized site staff. Investigational product must be dispensed or administered only to participants enrolled in the study and in accordance with the protocol.

The Investigator is responsible for study treatment accountability, reconciliation, and record maintenance. The Investigator or designated site personnel must maintain study treatment accountability records throughout the course of the study. The responsible person(s) will document the amount of study treatment received from and returned to the sponsor and the amount administered to participants. The required accountability unit for this study will be the bottle. Discrepancies are to be reconciled or resolved.

Product name:	B244, 30ml/bottle	B244, 30ml/bottle	Vehicle, 30ml/bottle
Dosage form:	B244 suspension	B244 suspension	Vehicle solution
Unit dose strength:	OD 0.5; $1x10^9$ cells/ml	OD 2.0; $4x10^9$ cells/ml	50nM Na_2HPO_4-2mM $MgCl_2$ (pH 7.6)
Route/administration/duration:	Intranasal application: 1 pump per nostril BID for 12 weeks	Intranasal application: 1 pump per nostril BID for 12 weeks	Intranasal application: 1 pump per nostril BID for 12 weeks
Dosing instruction:	Prime the spray with 5 actuations (pumps) into air prior to first use only. Apply 1 pump per nostril (2 pumps total) in the morning and repeat (2 additional pumps) at night for 12 weeks of treatment. Use 1 bottle per 4 weeks; 3 bottles per kit included.	Prime the spray with 5 actuations (pumps) into air prior to first use only. Apply 1 pump per nostril (2 pumps total) in the morning and repeat (2 additional pumps) at night for 12 weeks of treatment. Use 1 bottle per 4 weeks; 3 bottles per kit included.	Prime the spray with 5 actuations (pumps) into air prior to first use only. Apply 1 pump per nostril (2 pumps total) in the morning and repeat (2 additional pumps) at night for 12 weeks of treatment. Use 1 bottle per 4 weeks; 3 bottles per kit included.
Physical description:	Odorless, cloudy, light pink suspension	Odorless, cloudy, light pink suspension	Odorless, clear, and colorless suspension
Manufacturer/source of procurement:	AOBiome, LLC	AOBiome, LLC	AOBiome, LLC

The contents of the label will be in accordance with all applicable regulatory requirements. B244 and matching vehicle will be packaged in identical 30 ml white bottles.

8.2 Dose Changes

No dose changes are anticipated.

8.3 Storage Conditions

All investigational drug supplies in the study will be stored in a secure, refrigerated (2-8ºC) safe place, under the responsibility of the Investigator or other authorized individual prior to randomization and distribution to participants. All 3 bottles in each kit will be weighed prior to distribution to participants and first dosing. Subjects will be trained for in-home treatment/assessment on the use of the nasal spray, have their first dose of treatment on site (V3) with the first bottle, and have the bottle reweighed. Once subjects are sent home with investigational product at visit 3 (V3) for preventive treatment, subjects will take out the first bottle from the kit and store the investigational product (IP) on the counter (i.e., in room temperature 20-25ºC) during use at home or remotely for the first 4 weeks of preventive treatment, being mindful to avoid extreme temperatures and areas of excessive heat (e.g., inside the car or trunk during heat) or cold (i.e., do not freeze). The remaining 2 unused bottles in the kit will be stored refrigerated (2-8ºC) until use. A second bottle will be taken out of the refrigerator for the second 4 weeks of treatment, and a third bottle will be taken out of the refrigerator for the last 4 weeks of treatment. The new bottles can be used immediately after taking them out of the refrigerator for the initial dose or equilibrated in room temperature for 5-10 minutes prior to use. Subsequent doses will be administered at room temperature as the IP will be left on the counter during the 4 week treatment period. The used bottles from the previous 4 weeks of treatment will be returned at each interim visit (V4, V5, or V6) for IP compliance (weight measurements).

8.4 Description of Blinding Method

This study will be double-blinded: neither Investigator(s), nor study participants, nor those involved in the conduct of the trial (including sponsor staff) will be aware of the treatment the participants are receiving.

8.5 Treatment Assignments:

This is a double-blind study. Participants will be randomly assigned to one of three study treatment groups in a 1:1:1 ratio in accordance with the randomization schedule generated for the allocation of vehicle or the 2 dose levels of B244 prior to the initiation of the trial. Randomization will be centrally-based and performed using an appropriate randomization system.

Each participant scheduled to receive investigational product (IP) will receive a randomization number at the time of randomization. The randomization number will be used to identify the study medication kit assigned to the participant and indicate the treatment to be administered to that participant.

8.6 Treatment Compliance

Study treatment must be dispensed or administered according to procedures described herein. Only participants enrolled in the study may receive study treatment. Only authorized site personnel may supply study treatment. Participants will record use of the study medication utilizing the study diary at the time of use each day. Participants will review study medication compliance with the Investigator or designee. Any missed doses, timing, and reason for missed dose will be recorded in the eCRF. There should be no doubling of doses to make up for missed doses. If a dose is missed, the next dose of study medication should be taken as scheduled.

Each site participating in the trial will be instructed to assess subject's compliance by weighing the investigational product and obtaining the weight of the bottle in grams pre and post application during preventive treatment (all 3 unused bottles at visit V3 before the on-site first dose, after priming of first bottle at V3, after 4 weeks dosing of first bottle at V4, after 4 weeks dosing of second bottle at V5, and after 4 weeks dosing of third bottle at V6). Weight measurements may be performed with the protection cap and safety clip from each bottle removed. Sites will be provided scales, which will be calibrated prior to each use. Study personnel will be instructed to record measurements into the eCRF.

8.7 Treatment Application

Subjects will receive one kit containing three 30 ml bottles at the Randomization visit (Visit 3) for the 12 week application period, with each bottle assigned for 4 weeks of application. Subjects will be instructed in the use of the spray bottle and asked to self-administer the Investigational Product as follows:

- Spray 1 pump of medication into each nostril twice a day for 12 weeks

Instructions for Nasal Spray Administration:

- Shake bottle gently
- Prime nozzle if unused; Pump until mist appears (5 times)
- Place nozzle straight up one nostril and pump once
- Sniff gently after spraying
- Repeat other nostril
- Wipe spray nozzle with tissue when done
- Do not wash/rinse the application site or blow nose for 30 min after applying the mist

Preventive Treatment:

Subjects will be trained for in-home treatment/assessment on the use of the nasal spray at the end of the 4 week baseline period (at visit V3), and apply their first dose of treatment under the supervision of study personal while on site. Subjects will remain under observation at the study site for 1 hour post dosing. Subjects will then be dispensed study medication for 12 weeks of at home dosing (kit containing three 30 mL bottles of either dose of $1x10^9$ cells/ml; $4x10^9$ cells/ml or vehicle). While at home, subjects will dose twice daily (am and pm; morning and night) for 12 weeks during the prevention treatment phase, starting with the second dose of the first day of treatment.

- Treatment application: 1 pump per nostril (2 pumps) BID for 12 weeks; 2 pumps in the morning and 2 pumps at night; 4 pumps total per day; 336 total pumps during 12 weeks
- Subjects may not wash/rinse the application site or blow their nose after applying the IP for 30 minutes
- The spray bottle can be stored at room temperature during use for each 4 week interval. A new spray bottle will be taken out of the refrigerator for use during the next 4 weeks. After each 4 week use, the used spray bottle will be returned to the site for compliance measurement.

8.8 Treatment of Investigational Product Overdose

The sponsor does not recommend specific treatment for an overdose.

8.9 Product Accountability

In accordance with federal and local regulatory requirements, the Investigator and designated site personnel must document the amount of investigational product dispensed to study participants, the amount returned by study participants, and amount received and returned to the sponsor, when applicable. Product accountability records must be maintained throughout the course of the trial. Any quality issue noticed with the receipt or use of an IP (deficient IP in condition, appearance, pertaining documentation, labeling, expiry date, etc.) should be promptly reported to the Sponsor, who will initiate a complaint procedure.

All investigational product must be stored in a secure locked room with access limited to the Investigator and designated site personnel. Study product is to be stored in a refrigerator between 2-8 degrees C. Maintenance of a temperature log is required.

Under no circumstances will the Investigator allow IP to be used other than as directed by this Clinical Trial Protocol, or dispose of IP in any other manner.

8.10 Unblinding Procedures

The Investigator may unblind a participant's treatment assignment only in the case of emergency or in the event of a serious medical condition when knowledge of the study treatment is essential for the appropriate clinical management or welfare of the participant, as determined by the Investigator. It is preferred (but not required) that the Investigator first contacts the medical monitor to discuss options before unblinding the participant's treatment assignment. The Investigator must notify the Sponsor as soon as possible when a participant's treatment assignment is unblinded without revealing the treatment assignment of the unblinded participant unless that information is deemed important for the safety of participants currently in the study. The date and reason for the unblinding must be documented in the participant's study record.

The Medical Monitor may unblind the treatment assignment for any participant with an SAE. If the SAE requires that an expedited regulatory report be sent to one or more regulatory agencies, a copy of the report, identifying the participant's treatment assignment, may be sent to clinical Investigators in accordance with local regulations and/or sponsor policy.

8.11 Retrieval and Destruction of Investigational Product

All partially used or unused treatments will be returned to the site as brought by study participants. A detailed treatment log of the returned IP shall be established.

The site will not destroy unused IP unless the Sponsor provides written authorization to the contrary. All used and unused bottles will be shipped to the authorized drug depot at the end of the study.

8.12 Permitted Medications

All participants will be screened for concomitant medications prior to inclusion into the study. Any concomitant medication to treat adverse events will be recorded in the Concomitant Medication section of the eCRF.

8.13 Prohibited Medications

Subjects must be willing to refrain from using any treatments that are or might be considered useful for migraine prevention including recognized pharmaceutical products, investigational products, herbal medicines or supplements while participating in this study. A list of prohibited medications includes but is not limited to:

- Beta blockers: for example, propranolol, metoprolol, atenolol, bisoprolol, timolol, nadolol (Corgard)
- Calcium channel blockers or antiserotonergic agents: for example, verapamil, flunarizine, pizotifen
- Antidepressants (TCAs, SNRIs): for example, amitriptyline, nortriptyline, venlafaxine, duloxetine
- Angiotensin receptor blockers (ARBs) and angiotensin converting enzyme blockers (ACEs): for example, candesartan, lisinopril
- Onabotulinumtoxin A (Botox)
- Anticonvulsants: topiramate (Topamax), valproic acid (Depakote), divalproex sodium (Depakote), and gabapentin (These are the only ones not permitted; others are allowed if required for treatment of a seizure disorder. If a subject must use any of these prohibited drugs, the subject will not be eligible for the study.)
- Triptans and ergots if used as preventive/pre-emptive medications (These are allowed for acute treatment of migraine, as described and limited below in section 8.14.)
- NSAIDs used as preventive medications (daily basis for migraine or other indications) (These are allowed for acute treatment of migraine, as described and limited below in section 8.14.)
- Devices for migraine prevention
- Nerve blocks in the head and neck
- Anti-CGRP monoclonal antibody (for example, erenumab, fremanezumab, galcanezumab, and eptinezumab) injection or infusion
- Small molecule anti-CGRP medications

- Systemic antibiotics (oral or IV only)
- Intranasal formulations of acute/abortive rescue medications such as sumatriptan (Imitrex, Onzetra) and zolmitriptan (Zomig). All triptans are allowable for acute treatment of migraine as described and limited to below including Relpax (eletriptan), Maxalt (rizatriptan), Amerge (naratriptan), Imitrex (sumatriptan), Axert (almotriptan), Frova (frovatriptan) and Zomig (zolmitriptan)
- Any herbal medicines or supplements related to migraine prevention such as vitamin B2 (riboflavin), butterbur, feverfew, high dose magnesium, etc.

If needed, subjects are allowed to use their usual migraine specific medication of their choice to relieve their symptoms of an acute attack of migraine, although they must avoid medications that are sprayed into their nose. Subjects are prohibited from using any migraine-specific medications as preventive treatment between acute episodes. A list of medications that are allowed includes:

- All acute/abortive migraine medications (via mouth or injection only) such as triptans and ergot containing preparations (ergotamine tartrate, DHE 45) up to 10 days per month
- NSAIDs and analgesics (for example, acetaminophen, acetylsalicylic acid, combination analgesics) for migraine and non-migraine headaches up to 14 days per month
- Opioid/barbiturate containing medications of up to 4 days per month

8.14 Rescue Medications

Use of rescue medication is allowed throughout the study with subjects' usual acute migraine specific medication of choice to relieve their symptoms of an acute attack of migraine (usually a triptan, possibly NSAID or analgesic) and recorded in the eCRF. Subjects should avoid intranasal formulations of rescue medications. Subjects should not initiate a preventive medication during study and are prohibited from using any migraine-specific medications as preventive treatment between acute episodes. Subject may not start additional non-randomized medication and should immediately alert the study site PI. Subjects are encouraged not to start any new acute migraine/headache treatments during the course of the study and may be withdrawn from the study pending discussion with the PI and sponsor medical monitor in such cases. If discontinuation is necessary, this would be considered an early termination. A list of allowed rescue medications includes:

- All acute/abortive medications (excluding intranasal formulations) such as triptans (oral or injection route only) and ergot containing preparations (ergotamine tartrate, DHE 45 by IM, SQ, IV) up to 10 days per month
- NSAIDs and analgesics (e.g., acetaminophen, acetylsalicylic acid, combination analgesics) for migraine and non-migraine headaches up to 14 days per month
- Opioid/barbiturate containing medications of up to 4 days per month

8.15 Handling of Investigational Product

Subjects will receive a kit containing three 30 ml white bottles for the duration of the 12 weeks of preventive treatment. Each bottle will be used for 4 weeks for the preventive treatment phase,

and brought to all study appointments (V4, V5, V6, Unanticipated/Early Termination visit). Subjects will be asked to refrigerate bottles that are not in active use. The bottle which is being used for preventive treatment may be placed on the counter at room temperature to be used during its 4 week preventive treatment period. The remaining 2 unused bottles in the kit will be stored refrigerated (2-8ºC) until use. A second bottle will be taken out of the refrigerator for the second 4 weeks of treatment, and a third bottle will be taken out of the refrigerator for the last 4 weeks of treatment. The used bottles from the previous 4 weeks of treatment will be returned at each interim visit (V4, V5, or V6) for IP compliance (weight measurements).

Subjects will be asked not to expose the treatment kit to conditions which are unnatural or harmful to the product, such as excessive heat (temperatures over 77°F (25°C) and freezing temperatures (at 0ºC)). Subjects may travel with their study medication but should not leave it in the hot car, outside in the cold temperatures etc. Preferably, subjects will be asked to place the IP on the counter at room temperature at home for morning and nighttime dosing. Subjects will also be asked not to tamper or cause damage to IP.

9 CONTRACEPTION REQUIREMENTS

Effective contraception is required for all women physiologically capable of becoming pregnant during study participation. Women of child-bearing potential must agree to use an acceptable form of contraception for up to 2 weeks after the study completion. Women of childbearing potential are defined as any female who has experienced menarche and who is NOT permanently sterile or postmenopausal. Postmenopausal is defined as 12 consecutive months with no menses without an alternative medical cause.

Effective contraception methods include:

- Total abstinence (when this is in line with the preferred and usual lifestyle of the study participant). Periodic abstinence (i.e., calendar, ovulation, symptothermal, postovulation methods) and withdrawal are not acceptable methods of contraception.
- Use of oral, injected or implanted hormonal methods of contraception or placement of an IUD or IUS or other forms of hormonal contraception that have comparable efficacy, for example hormone vaginal ring or transdermal hormone contraception.
- Use of barrier methods (i.e., condom, diaphragm) used with a spermicide (i.e., foam, cream, or gel that kills sperm)

In case of use of oral contraception women should have been stable on the same pill for a minimum of 90 days before the baseline visit.

Male participants of the study who are having sexual intercourse with a woman who can become pregnant must use an acceptable form of birth control if not permanently sterile (e.g., from vasectomy) while participating in the study. Additionally, male participants are expected to let their female partners know of their participation in a research study of a drug, and that the effects of the drug on an unborn baby and on a pregnant woman are unknown. Male participants will also be expected to provide their female partners with the contraception requirements information previously described and the study doctor’s contact information for questions.

Payment for all aspects of obstetrical care, child-or related care will be the study participant's responsibility.

In case of pregnancy, Investigational Product should be discontinued and the Sponsor should be informed immediately. Follow-up of the pregnancy will be mandatory until the outcome is available.

10 STUDY PROCEDURES

10.1 Pre-screening Procedures

Study subjects will be recruited from among participating hospitals, clinics, and diagnostic centers, under the responsibility of a participating Investigator. Prior to initiation of the recruitment phase, participating Investigators will identify a pool of potential study subjects. Each of these centers will identify potentially eligible patients in advance, by either reviewing past medical records and diagnoses, screening in clinics, referral from other physicians, or other sources of recruitment, including online advertising and patient databases, to identify those aged 18 to 65 with clinical diagnosis of migraine meeting the inclusion criteria.

10.2 Informed Consent Procedures

Eligible participants may only be included in the study after providing a consent using the IRB-approved informed consent. Written informed consent must be obtained before conducting any study-specific procedures (i.e. all of the procedures described in the protocol). The process of obtaining informed consent should be documented in the participant's source documents. The date of signing of informed consent (and withdrawal, if later withdrawn) should be documented in the eCRF.

10.3 Study Assessments

Study activities will take place according to the Schedule of Events table (Appendix A).

All subjects will attend a screening visit (V1) not more than 14 days prior to V2 (Day -28). Subjects with a history of migraine as defined in the inclusion/exclusion criteria who pass screening will return to clinic on Day -28 (V2) for baseline assessments and sent home to record their migraine events and rescue (acute migraine treatment) medications over the 28 day baseline period using a diary. Subjects will return to clinic on Day 0 (V3) to determine continued study eligibility. Subjects who continue to meet all inclusion and exclusion criteria will be randomized to receive study treatment.

Subjects will then be trained for in-home treatment/assessment on the use of the nasal spray, and apply their first dose of treatment under the supervision of study personnel while on site (V3). Subjects will remain onsite for 1 hour post dosing for observation. Subjects will then be dispensed study medication for 12 weeks of at home dosing (either $1x10^9$ cell/ml; $4x10^9$ cells/ml; or vehicle). While at home, subjects will dose twice daily (am and pm) for 12 weeks during the prevention treatment phase, starting with the second dose of the first day of treatment.

Subjects will record their dosing, migraine events, and rescue medications daily and return to clinic every 4 weeks during the 12 week treatment period at Day 28 (V4), Day 56 (V5), and Day 84 (V6) for safety and additional prevention treatment assessments. Additional safety assessments will be made during the 28 day follow-up period and end of study visit at Day 112 (V7).

At the final visit (V7) or at the Early Termination visit due to study discontinuation or withdrawal, subjects may be requested to complete an optional, brief subject satisfaction survey.

10.4 Inclusion Procedures

Once all inclusion/exclusion criteria are fulfilled after the 28 day baseline period, the patient becomes eligible for randomization and inclusion into the treatment period. Treatment allocation will be performed as stated above in Section 8.5. Study medication will be delivered as stated in Section 8.7. Patients will be counseled on product application and diary completion.

10.5 Timing of Patient's Visits to the Clinic

Patients will be asked to report to the clinic for their scheduled appointments. If a subject is unable to schedule an appointment within the required time frame, study staff will be asked to reschedule the patient to a day when they are able to come in within a predetermined time frame.

10.6 Description by Type of Visit

10.6.1 Screening Visit (V1; Study Day -29) (-42 to -28 days visit window)

- informed consent completed and signed
- inclusion and exclusion criteria
- demographic data
- medical/surgical history
- smoking status
- record concomitant medications
- obtain in clinic blood pressure, heart rate, respiratory rate
- brief physical exam
- neurological exam
- body weight
- height measurement
- 12-lead ECG
- nasal inspection
- laboratory testing (blood for metabolic panel, hematology, clinical chemistry, lipid panel, liver function test, urinalysis)
- urine pregnancy test (for women of childbearing potential)
- study AEs
- study diary
- migraine pain intensity score

10.6.2 Baseline (Beginning of 4 Week Baseline Period) (V2; Study Day -28) (-31 to -27 Days Visit Window)

- inclusion and exclusion criteria
- record concomitant medications
- obtain in clinic blood pressure, heart rate, respiratory rate
- brief physical exam
- neurological exam
- body weight
- nasal inspection
- study AEs
- study diary
- migraine pain intensity score
- MIDAS
- HIT-6
- MSQL
- CGI

10.6.3 Randomization (End of 4 Week Baseline Period) (V3; Study Day 0) (±3 Days Visit Window)

- inclusion and exclusion criteria, including entry criteria of 4-14 migraine headache days during the baseline period
- record concomitant medications
- obtain in clinic blood pressure, heart rate, respiratory rate
- brief physical exam
- neurological exam
- body weight
- blood collection for biomarkers
- 12-lead ECG
- allocation of a randomized treatment kit number via randomization system
- nasal inspection
- delivery of the corresponding pack of Investigational Product
- Investigational Product application
- obtain study medication weight for Investigational Product compliance
- study counseling
- laboratory testing (blood for metabolic panel, hematology, clinical chemistry, lipid panel, liver function test, urinalysis)
- nasal fluid collection for biomarkers
- study AEs
- study diary
- migraine pain intensity score
- MIDAS

- HIT-6
- MSQL
- CGI

10.6.4 Prevention Treatment Week 4 (V4; Study Day 28) (±3 Days Visit Window)

- record concomitant medications
- obtain in clinic blood pressure, heart rate, respiratory rate
- brief physical exam
- neurological exam
- body weight
- blood collection for biomarkers
- nasal inspection
- Investigational Product application
- obtain study medication weight for Investigational Product compliance
- study counseling
- nasal fluid collection for biomarkers
- study AEs
- study diary
- migraine pain intensity score
- HIT-6
- MSQL
- CGI

10.6.5 Prevention Treatment Week 8 (V5; Study Day 56) (±3 Days Visit Window)

- record concomitant medications
- obtain in clinic blood pressure, heart rate, respiratory rate
- brief physical exam
- neurological exam
- body weight
- blood collection for biomarkers
- nasal inspection
- Investigational Product application
- obtain study medication weight for Investigational Product compliance
- study counseling
- nasal fluid collection for biomarkers
- study AEs
- study diary
- migraine pain intensity score
- HIT-6
- MSQL
- CGI

10.6.6 Prevention Treatment Week 12 (V6; Study Day 84) (±3 Days Visit Window)

- record concomitant medications
- obtain in clinic blood pressure, heart rate, respiratory rate
- brief physical exam
- neurological exam
- body weight
- blood collection for biomarkers
- 12-lead ECG
- nasal inspection
- Investigational Product application
- obtain study medication weight for Investigational Product compliance
- laboratory testing (blood for metabolic panel, hematology, clinical chemistry, lipid panel, liver function test, urinalysis)
- nasal fluid collection for biomarkers
- study AEs
- study diary
- migraine pain intensity score
- MIDAS
- HIT-6
- MSQL
- CGI

10.6.7 Follow-Up Week 16 (End of Study) (V7; Study Day 112) (±3 Days Visit Window)

Every attempt should be made to complete all visits during the defined window periods. Subjects who do not complete all required study visits and withdraw from the study will be asked to complete an End of Study Evaluation.

- record concomitant medications
- obtain in clinic blood pressure, heart rate, respiratory rate
- brief physical exam
- neurological exam
- body weight
- blood collection for biomarkers
- nasal inspection
- nasal fluid collection for biomarkers
- study AEs
- study diary
- migraine pain intensity score
- HIT-6
- MSQL
- CGI
- subject satisfaction survey

10.6.8 Unscheduled/Unanticipated or Early Termination Study Visit

If an event arises that requires patient to come in to the research center, subjects should be scheduled for the Unscheduled visit.

During the visit, the following will be obtained:

- record concomitant medications
- obtain in clinic blood pressure, heart rate, respiratory rate
- brief physical exam
- neurological exam
- body weight
- blood collection for biomarkers
- nasal inspection
- obtain study medication weight for Investigational Product compliance
- study counseling
- laboratory testing (blood for metabolic panel, hematology, clinical chemistry, lipid panel, liver function test, urinalysis)
- nasal fluid collection for biomarkers
- study AEs
- study diary
- migraine pain intensity score
- MIDAS
- HIT-6
- MSQL
- CGI

11 METHODS OF ASSESSMENTS

11.1 Blood Pressure Measurement

Blood pressure readings will be obtained at every visit as described in Appendix A (schedule of events) preferably during standardized times across sites (e.g., mornings). Subject should be asked to remove all clothing that covers the location of cuff placement. The individual should be comfortably seated in the chair, with the legs uncrossed, and the back and arm supported, such that the middle of the cuff on the upper arm is at the level of the right atrium (the mid-point of the sternum). Neither the patient nor the observer should talk during the measurement. After 5 minutes sitting, serial clinic BP measurements and heart (x3) rate will be performed, using the non-dominant arm with a calibrated electronic sphygmomanometer. The average of the second and third readings will be documented. Respiratory rate (number of breaths per minute) will also be recorded.

11.2 Brief Physical Examinations

Brief physical examination will be performed at Screening (V1), Baseline (beginning of 4 week baseline period; V2), Randomization (end of 4 week baseline period; V3), Prevention Treatment

Week 4 (V4), Prevention Treatment Week 8 (V5), Prevention Treatment Week 12 (V6), Follow-Up Week 16 (End of Study; V7), and at Unanticipated/Early Termination Visit should one occur. The physical examination includes an assessment of general appearance and a review of systems (dermatologic, head, eyes, ears, nose, mouth/throat/neck, thyroid, lymph nodes, respiratory, cardiovascular, gastrointestinal, extremities, musculoskeletal, neurologic systems).

11.3 Neurological Examinations

As part of the physical examination at each visit, a neurological examination will be performed for assessment of sensory neuron and motor responses, especially reflexes, to determine whether the nervous system is impaired.

11.4 Blood Collection for Biomarkers

In addition to the blood drawn for the safety laboratory assessments, additional whole blood will be collected for the biomarker analysis at Randomization (end of 4 week baseline period; V3), Prevention Treatment Week 4 (V4), Prevention Treatment Week 8 (V5), Prevention Treatment Week 12 (V6), and Follow-Up Week 16 (End of Study; V7) according to Appendix A. Samples will also be collected in the event of an Unanticipated/Early Termination Visit.

Approximately 15 ml of whole blood will be drawn for biomarkers at each visit. Patients will be asked to fast for at least 8 hrs before blood for biomarkers is drawn.

Samples will be processed to serum and plasma on site and stored at -80 ℃ until shipment to a designated central lab for biostorage and may be evaluated for select biomarkers that may include several of the following as well as others:

1. Inflammatory cytokines/chemokines/immune cell markers/neuropeptides: IL-1beta, IL-2, IL-4, IL-5, IL-6, IL-8, IL-9, IL-10, IL-12p40, IL-12p70, IL-13, IL-15, IL-16, IL-17, IL-18, TNF-alpha, GM-CSF, MIP-1 alpha, MIP-1 beta, MCP-1, Eotaxin-1, MDC, PGD2, beta-tryptase, histamine, C3a/C4a/C5a, LTC4, MMP9, CGRP

11.5 12-Lead ECG

12-lead ECG will be taken at Screening (V1), Randomization (end of 4 week baseline period; V3), and Prevention Treatment Week 12 (V6). Standard 12-lead ECGs will be performed according to the site SOPs. ECGs should be performed after the subject has been resting supine for ≥5 minutes. The ECG will include all 12 standard leads and a Lead II rhythm strip on the bottom of the tracing. The ECG will be recorded at a paper speed of 25 mm/sec. The following ECG parameters will be collected: PR interval, QRS interval, QT interval, and QTc interval (QTcB; Bazett"s correction). All ECGs must be presence of abnormalities.

11.6 Nasal Inspection

Nasal inspection will include clinical examination of the nasal mucosa, sinuses, and upper airway using an otoscope. Nasal inspection will be performed at the following visits: Screening

(V1), Baseline (beginning of 4 week baseline period; V2), Randomization (end of 4 week baseline period; V3), Prevention Treatment Week 4 (V4), Prevention Treatment Week 8 (V5), Prevention Treatment Week 12 (V6), Follow-Up Week 16 (End of Study; V7), and at Unanticipated/Early Termination Visit should one occur.

11.7 Laboratory Assessments

Blood sample will be taken for hematology and clinical chemistry, including standard metabolic panel, complete blood count (CBC), clinical chemistry, lipid panel, and liver function test. Approximately 15 ml of whole blood will be drawn. Urinalysis will be performed as part of safety labs. Laboratory assessments will be made at Screening (V1), Randomization (end of 4 week baseline period; V3), Prevention Treatment Week 12 (V6), and at Unanticipated/Early Termination Visit should one occur.

Blood samples should be taken using standard venipuncture techniques. Blood sampling will be performed according to the site SOPs.

The following laboratory variables will be determined: Fasting glucose, Uric acid, BUN (blood urea nitrogen), Creatinine, BUN/creatinine ratio, eGFR (estimated glomerular filtration rate), Sodium, Potassium, Chloride, Calcium, Total protein, Bicarbonate, Albumin, Bilirubin, Alkaline phosphatase, AST (aspartate aminotransferase), ALT (alanine transaminase), total cholesterol, HDL, LDL, triglycerides, CBC (standard panel including RBC, hemoglobin, hematocrit, platelets, and WBC, including neutrophils, lymphocytes, monocytes, eosinophils, basophils), and proteinurea.

Any value outside the normal range (except those affected by not fasting) will be flagged for the attention of the Investigator or designee at the site. The Investigator or designee will indicate whether or not the value is of clinical significance. If the result of the clinical chemistry test from the samples taken during the screening phase is indicated as clinically significant, the study subject will NOT be allowed into the study.

11.8 Urine Pregnancy Test

Standard urine pregnancy testing will be performed on WOCBP as part of screening.

11.9 Nasal Fluid Samples for Biomarkers

Nasosorption with synthetic absorptive matrices (SAM) have been used to sample nasal mucosal lining fluid [16]. This technique can be considered as “precision mucosal sampling”, since it samples directly from the respiratory mucosa, and is free from the salivary contamination that occurs in breath and sputum sampling. Nasosorption sampling involves manipulating the synthetic absorptive matrix (SAM) up the lumen of the nasal cavity, and then holding it in position against the mucosa by external firm finger pressure. This is more comfortable and less invasive than using a conventional swab, where rotation against the mucosal surface is generally required.

Nasosorption will be performed by placing strips of a hydrophilic polyester absorptive matrix

(Mucosal Diagnostics, Hunt Developments Ltd., Midhurst, UK: available as a CE-marked device) measuring 7 × 35 mm into each nostril for 1 min. When the SAM strip is removed, it may be placed back in to its container and frozen at −80°C until shipment to a designated central lab for biostorage, elution, and testing. After shipment to a designated lab, SAM strips will be washed in PBS buffer pH 7.4 (100 µl) containing BSA cup of a spin filter insert (Costar® Spin-X®). Mucosal lining fluid will be then eluted from the SAM by spin filter centrifugation (5 min at 16,000G at 4 °C), left and right nostril samples combined, and aliquots tested for biomarkers to evaluate the immune response.

Nasal fluid for biomarkers will be collected at Randomization (end of 4 week baseline period; V3), Prevention Treatment Week 4 (V4), Prevention Treatment Week 8 (V5), Prevention Treatment Week 12 (V6), and Follow-Up Week 16 (End of Study; V7) according to Appendix A. Samples will also be collected in the event of an Unanticipated/Early Termination Visit.

Samples may be evaluated for select biomarkers that may include several of the following as well as others:

1. Inflammatory cytokines/chemokines/immune cell markers/neuropeptides: IL-1beta, IL-2, IL-4, IL-5, IL-6, IL-8, IL-9, IL-10, IL-12p40, IL-12p70, IL-13, IL-15, IL-16, IL-17, IL-18, TNF-alpha, GM-CSF, MIP-1 alpha, MIP-1 beta, MCP-1, Eotaxin-1, MDC, PGD2, beta-tryptase, histamine, C3a/C4a/C5a, LTC4, MMP9, CGRP

Optionally, samples may be evaluated for nasal microbiome (e.g., PCR) to confirm presence of B244.

11.10 Study Diary

Subjects will be asked to fill out a daily migraine-history diary from screening to treatment with study drug and a migraine-treatment diary from treatment through the remainder of the follow-up period. Study diary will be electronic using an electronic patient reported outcomes (ePRO) device.

Study diary entries will be daily and will include recording of migraine frequency, duration, and severity that includes information on migraine days (experiencing migraine with or without aura in a given day), headache days (experiencing headache in a given day), migraine intensity, migraine duration, headache intensity, and headache duration. Additional patient reported outcomes may include recording of nausea, vomiting, photophobia, and sonophobia, as well as information about the headache in the trial (i.e., headache intensity, presence/absence of associated symptoms, unilateral/bilateral location, aggravation by exercise, throbbing/non-throbbing) to verify migraine headache symptoms. Subjects will also record their use of rescue (acute migraine specific) medications and dosing administration. The diary will ask the subjects about their headache which will allow the patients' responses to determine whether that headache is migraine or not.

The following definitions will be used for determination of migraine, migraine days, and other inclusion/exclusion criteria:

Episodic migraine:

- No more than 14 headache days per month and for this study at least 4 of those days must be migraine days.

Migraine without aura (per International Headache Society [IHS] 1.1 criteria):

- Headache attacks lasting 4-72 hours
- At least 2 of the following:
  - unilateral location
  - pulsating quality
  - moderate or severe pain intensity
  - aggravation by or causing avoidance of routine physical activity
- At least one of the following
  - nausea and/or vomiting
  - photophobia and sonophobia

Migraine with aura (IHS 1.2):

- Migraine associated with one or more of the following fully reversible aura symptoms: visual, sensory, speech and/or language, motor, brainstem, retinal.
- At least 3 of the following:
  - At least one aura symptoms spreads gradually over >5 min
  - 2 or more aura symptoms occur in succession
  - Each individual aura symptoms lasts 5-60 min
  - At least one aura symptom is unilateral
  - At least one aura symptom in positive
  - The aura is accompanied or followed within 60 min by HA

Migraine day:

- Migraine day is defined as any calendar day in which the subject experiences a qualified migraine headache (onset, continuation, or recurrence of the migraine headache). It is a self-reported headache which meets the IHS criteria for migraine with or without aura or probable migraine. A probable migraine is defined as a headache that meets only 2 of the 3 criteria (listed in the migraine without aura definition).
- If the patient uses a migraine specific medication such as a triptan, that will be counted as a migraine day no matter how short the headache attack is as long as it's at least 30 minutes.
- Migraine day will be determined from a 24 hour period from 12 am to 11:59 pm each day.

Headache day:

- A non-migraine headache day.

Migraine attack:

- An episode of any qualified migraine headache. To distinguish an attack of long duration from two attacks or to distinguish between attacks and relapses:

- A migraine attack that is interrupted by sleep, or temporarily remits, and then recurs within 48 hours (i.e., <48 hours between the start of the migraine attack to the time of the recurrence) will be considered as one attack and not two.
- An attack treated successfully with medication but with relapse within 48 hours (i.e., <48 hours between the start of the migraine attack to the time of recurrence) will be considered as one attack.

Subjects will be advised to record their diary in the evening (after the night time dose) as a 24 hour reflective diary entry. For missed diary entries, subjects will be allowed to record up to 48 hours retrospective data. Subjects will be encouraged to record at least 80% of the daily diary days (80% diary compliance) during the study (from baseline to end of follow-up period) to remain on the study.

11.11 Migraine Pain Intensity Score

International Headache Society guidelines for the conduct of controlled trials of headache treatments recommend the use of the 4-point verbal rating scale (VRS) to measure pain intensity.

Migraine pain intensity score will be recorded daily in the study diary at the times indicated in the Schedule of Events Table (Appendix A) beginning at Screening (V1) and daily until Follow-Up Week 16 (End of Study; V7). More specifically, daily assessments of migraine severity will be recorded in the study diary during screening, the baseline period (Day -28 to 0), prevention treatment period (Day 0 to 84), and follow up observation period (Day 84 to 112).

4-point VRS scale for pain intensity is as follows:
0 = no pain
1 = mild pain (bothersome but not interfering with normal function)
2 = moderate pain (interfering with normal function)
3 = severe pain (requiring bedrest and unable to function)

11.12 MIDAS

Migraine Disability Assessment (MIDAS) questionnaire will be administered at the times indicated in the Schedule of Events Table (Appendix A): Baseline (beginning of 4 week baseline period; V2), Randomization (end of 4 week baseline period; V3), Prevention Treatment Week 12 (V6), and at Unanticipated/Early Termination Visit should one occur. The participant will answer a questionnaire examining the relationship between impact of migraine and quality of life.

MIDAS questionnaire can be found in Appendix B.

11.13 HIT-6

Headache Impact Test-6 (HIT-6) questionnaire will be administered at the times indicated in the Schedule of Events Table (Appendix A): Baseline (beginning of 4 week baseline period; V2), Randomization (end of 4 week baseline period; V3), Prevention Treatment Week 4 (V4),

Prevention Treatment Week 8 (V5), Prevention Treatment Week 12 (V6), Follow-Up Week 16 (End of Study; V7), and at Unanticipated/Early Termination Visit should one occur. The participant will answer a questionnaire examining the relationship between impact of migraine and quality of life.

HIT-6 questionnaire can be found in Appendix C.

11.14 MSQL

Migraine Specific Quality of Life (MSQL) questionnaire will be administered at the times indicated in the Schedule of Events Table (Appendix A): Baseline (beginning of 4 week baseline period; V2), Randomization (end of 4 week baseline period; V3), Prevention Treatment Week 4 (V4), Prevention Treatment Week 8 (V5), Prevention Treatment Week 12 (V6), Follow-Up Week 16 (End of Study; V7), and at Unanticipated/Early Termination Visit should one occur. The participant will answer a questionnaire examining the relationship between impact of migraine and quality of life.

MSQL questionnaire can be found in Appendix D.

11.15 CGI

Clinical Global Impression (CGI) scale is an investigator assessment that will be administered at the times indicated in the Schedule of Events Table (Appendix A): Baseline (beginning of 4 week baseline period; V2), Randomization (end of 4 week baseline period; V3), Prevention Treatment Week 4 (V4), Prevention Treatment Week 8 (V5), Prevention Treatment Week 12 (V6), Follow-Up Week 16 (End of Study; V7), and at Unanticipated/Early Termination Visit should one occur. The clinician will assess measures of symptom severity, treatment response and the efficacy of treatments in patients.

CGI scale can be found in Appendix E.

11.16 Sample Shipment

All laboratory testing samples are to be shipped overnight in designated temperature conditions to the central laboratory where samples will be analyzed.

All frozen serum/plasma and nasal fluid for biomarkers are to be shipped monthly on dry ice to the central laboratory. Shipments should be made only on Mondays and Tuesdays to ensure receipt of the specimens by Friday.

12 SAFETY ASSESSMENTS

12.1 Compliance

At Randomization visit (V3), study personnel will be asked to take out all study medication bottles from the carton, weigh the bottles and record the weight. At each subsequent visit, study personnel will need to weigh the bottle without the carton and record weight. In addition, weight

measurements will be performed with the protection cap and safety clip from each bottle removed. This procedure should be followed every time study medication returned and weighed.

Participants will be asked to bring study medication with them to selected scheduled visits (V4, V5, V6, and Unanticipated Visit if necessary) after delivery of IP at V3. Each site participating in the trial will be instructed to assess subject's compliance by weighing the investigational product and obtaining the weight of the bottle in grams pre and post application during preventive treatment (all 3 unused bottles at visit V3 before the on-site first dose, after priming of first bottle at V3, after 4 weeks dosing of first bottle at V4, after 4 weeks dosing of second bottle at V5, and after 4 weeks dosing of third bottle at V6). Sites will be provided scales, which will be calibrated prior to each use. Study personnel will be instructed to record measurements into the eCRF.

12.2 Safety Monitoring

Local and systemic adverse events (AEs) related to intranasal route of administration during prevention treatment and follow-up will be recorded for the duration of the study from screening to end of follow-up.

Safety monitoring will include infectious complications related to B244 (by symptoms and/or testing; pulmonary, nasal, neurological), local and systemic AEs related to intranasal route during treatment and at follow-up. Local AEs include runny nose, nasal congestion, sneezing, nasal itching, palate itching, anosmia, nasal ulceration, nasal bleeding, sore throat, cough, Bell's palsy, other neurologic complications, fevers, chills, headache, muscle aches, decreased appetite, nausea, vomiting, and rash.

Sponsor will distinguish between immediate (onset of reaction is during the first 30 minutes after administration) and delayed (onset of action is after the first 30 minutes of administration) effects for safety reporting of adverse events.

12.3 Pregnancy Reporting

Any pregnancy will be reported by study participants during their study participation. Participants who report pregnancy or lactation during the review of inclusion/exclusion criteria prior to randomization will not be enrolled in the trial. In case of pregnancy, Investigational Product should be discontinued and the Sponsor informed. Follow-up of the pregnancy will be mandatory until the outcome is available.

12.4 Study Completion

A completed participant is one who has completed all study visits. Day 112 (Week 16) study visit is defined as the participant's last visit (V7).

12.5 Subject Withdrawal Criteria

A subject may withdraw from the study at any time at his/her own request, or may be withdrawn at any time at the discretion of the Investigator for safety, behavioral, or administrative reasons.

Reasons for withdrawal (subjects who refuse to return for any remaining study visits) or discontinuation (subjects who prematurely stop the application) at any time during the study may include, but are not limited, to the following:

- For safety reasons, either at the discretion of the Investigator or at the subject's request
- For protocol violations at the discretion of AOBiome
- Due to concomitant therapy that could interfere with the results of the study (the Investigator will report all such information on the CRFs and decide, in accordance with AOBiome, whether the subject is to be withdrawn).

All premature discontinuations and their causes must be carefully documented by the Investigator on the CRF or if needed on the AE form.

If, for any reason, a subject is withdrawn before completing the final visit, the reason for termination will be entered on the CRF. All data gathered on the subject prior to termination will be made available to AOBiome. Subjects not completing the entire study should be fully evaluated when possible. The appropriate CRFs should be completed.

If the subject chooses to withdraw before completing the study, the subject should notify the study coordinator who will instruct the subject on completion of assessments for Unanticipated/Early Termination visit (Appendix A). For subjects who refuse to complete the assessments for their early termination, every attempt must be made to check on their status, using any mode of communication such as telephone, email, fax, or text.

13 EFFICACY ASSESSMENTS

Efficacy endpoints will be descriptively summarized and will include the number of observations, mean, median, standard deviation, minimum, and maximum of scores/values at all applicable time points and for all treatments in the ITT and PP Population.

For preventive treatment, the following secondary efficacy assessments will be obtained:

- Mean change in monthly migraine days from baseline to 12 weeks of treatment.
- Proportion of subjects experiencing a ≥50%, ≥75%, and 100% reduction in monthly migraine days from baseline to 12 weeks of treatment.
- Mean change in monthly migraine attacks from baseline to 12 weeks of treatment.
- Mean change in monthly acute migraine specific medication days from baseline to 12 weeks of treatment.
- Mean change in monthly moderate and severe headache days (migraine pain-intensity score) from baseline to 12 weeks of treatment.
- Mean change in monthly headache days from baseline to 12 weeks of treatment.
- Mean change in monthly headache hours from baseline to 12 weeks of treatment.

- Mean change from baseline to 12 weeks of treatment in disability, as measured by the Migraine Disability Assessment (MIDAS) questionnaire.
- Mean change from baseline to 12 weeks of treatment in monthly Headache Impact Test-6 (HIT-6) questionnaire.
- Mean change from baseline to 12 weeks of treatment in monthly Migraine Specific Quality of Life questionnaire (MSQL) questionnaire.
- Mean change in baseline Clinical Global Impression (CGI) score to end of treatment period.

For preventive treatment, the following exploratory efficacy assessments will be obtained:

- Changes in cytokine concentration in nasal fluid and blood from baseline to 12 weeks of treatment.
- Mean change in migraine days from end of treatment (last month of 12 weeks of treatment) to follow-up.
- Mean change in migraine associated symptoms: nausea/vomiting, photophobia and sonophobia from baseline to 12 weeks of treatment.

For the continuous endpoints of the change in migraine days, the change in migraine attacks, the change in days using rescue medication, the change in headache days, the change in headache hours, and the change in MIDAS, HIT-6, MSQL, and CGI scores, a one-sided two-sample equal-variance z-test will be used to compare treatment group means. Fisher's Exact test will be used to make treatment group comparisons on efficacy outcomes including the following binary outcomes:

- Proportion of subjects experiencing a ≥50%, ≥75%, and 100% reduction in monthly migraine days from baseline to 12 weeks of treatment.

Exploratory endpoints will be analyzed using the same method as for continuous efficacy endpoints. The analyses will be performed on the ITT and PP population.

14 STATISTICAL CONSIDERATIONS

14.1 Sample Size

Approximately 303 subjects will be enrolled in order to achieve a target sample size of 264 (i.e., 88 subjects per arm), allowing for a 10-15% drop-out rate.

Group sample sizes of 88 per arm achieve 80% power to reject the null hypothesis of equal means when the population mean difference is -3.8 - (-2.3) = -1.5, with $\mu_1 = -3.8$ is the mean reduction in migraine headache days for an active treatment arm and $\mu_2 = -2.3$ is the mean reduction in migraine headache days for placebo. The sample size calculation assumes a standard deviation for both groups of 4.0 and a Bonferroni adjusted significance level (alpha) of 0.05 using a one-sided two-sample equal-variance z-test. Testing each active treatment arm versus vehicle at an adjusted significance level of 0.05 maintains an overall error rate of 0.10.

14.2 Populations for Analysis

Male and female subjects, 18 to 65 years of age, with a history of episodic migraine are eligible for enrollment.

ITT: includes all randomized participants who receive at least 1 dose of study medication.

Safety: includes all subjects who received at least 1 dose of study medication.

Per Protocol: subjects who administered at least 50 % of IP (based on the weight of IP used), have completed their Week 12 visit, and did not have any major protocol violations.

14.3 Data Analysis

The analyses will be conducted on all participant data when the trial ends. Data will be presented by treatment group and overall.

Categorical variables will be summarized by frequency distributions (number and percentages of subjects) and continuous variables will be summarized by descriptive statistics (mean, standard deviation etc).

Adverse events will be summarized by treatment using the Medical Dictionary for Regulatory Activities (MedDRA) system organ class and preferred term. Separate tabulations will be produced for all treatment-emergent AEs (TEAEs), treatment-related AEs (those considered by the Investigator as at least possibly drug related), SAEs, discontinuations due to AEs, and AEs ≥ Grade 3 - subject listings will be provided for any deaths, SAEs, and AEs leading to discontinuation.

Descriptive statistics will be provided for clinical laboratory values (e.g., hematology, serum chemistry, and urinalysis) and vital signs data, presented as both actual values and changes from baseline relative to each on-study evaluation. Abnormal clinical laboratory values will be listed.

All data will be provided in by-subject listings.

14.3.1 Disposition

A tabulation of the disposition of subjects will be presented, including the number enrolled, the number randomized, the number treated, and the reasons for study discontinuation will be reported. Summaries of the number in each analysis set will be summarized. Entry criteria and protocol deviations will be listed.

14.3.2 Demographic and Baseline

Demographic and baseline characteristic data summarization will be performed in order to descriptively assess the comparability of the treatments. Data to be tabulated will include age, race, ethnicity, height, weight, and BMI, as well as baseline characteristics related to medical history.

14.4 Safety Analyses

The primary endpoint is safety, as assessed by incidence and severity of AEs, physical examination, neurological examination, and vital signs (blood pressure, heart rate, respiratory rate). A secondary safety endpoint is safety assessments after 28 day follow-up period.

The analysis of safety will be based on the safety population, which is defined as all subjects receiving at least 1 dose of B244 or Vehicle. Adverse events (AEs) will be coded using the Medical Dictionary for Regulatory Activities (MedDRA®) for purposes of summarization. All AEs occurring during the study will be included in by-subject data listings and tabulated. Events leading to death, serious AEs, and events resulting in study discontinuation will be summarized using standard descriptive statistics, and presented by treatment arm. Exposure to study drug and reasons for discontinuation of study treatment will be tabulated. Shift tables relative to the normal range will be provided for laboratory parameters. Additional analyses will be performed, if warranted, upon review of the data.

14.4.1 Definitions

All adverse events recorded during the study will be coded according to Medical Dictionary for Regulatory Activities.

The primary analysis of safety will be based on the safety population, which is defined as all subjects receiving at least 1 dose of B244 or placebo. Adverse events (AEs) will be coded using the Medical Dictionary for Regulatory Activities (MedDRA®) for purposes of summarization. All AEs occurring during the study will be included in by-subject data listings and tabulated. Events leading to death, SAEs, and events resulting in study discontinuation will be summarized using standard descriptive statistics, and presented by treatment arm. Exposure to study drug and reasons for discontinuation of study treatment will be tabulated. Shift tables relative to the normal range will be provided for laboratory parameters. Additional analyses will be performed, if warranted, upon review of the data.

14.4.2 Adverse Events

All adverse events (AEs) recorded during the study through the date of randomization through 28 days after the last dose of study drug will analyzed.

All AE's will be coded according to Medical Dictionary for Regulatory Activities and summarized using System Organ Class (SOC) and Preferred Term (PT).

AE's will be summarized using incidence rates. Therefore, each subject will only contribute once for a given adverse event SOC or PT.

A summary of the incidence of any adverse event, SAE, and adverse events leading to discontinuation will be presented. Summaries will display, by treatment, the incidence of patients with events, the frequency of patients with events within each primary system organ class and by preferred terms. For each preferred term and each system organ class a patient

will be counted only once. For summaries on severe or drug-related AE, for a given patient, the highest severity or relationship for a specific preferred term will be considered.

14.4.3 Deaths and Serious Adverse Events

Serious adverse events and events leading to death will be summarized overall and by primary system organ class and preferred term.

14.4.4 Adverse Events Leading to Treatment Discontinuation

Adverse events leading to treatment discontinuation will be summarized overall and by primary system organ class and preferred term.

14.5 Efficacy Analyses

Efficacy analyses will be performed on the intent-to-treat (ITT) population, which consists of all randomized subjects who receive at least 1 dose of study medication, and on the per protocol (PP) population, which consists of subjects who complete their Week 12 visit and have percent compliance (based on the weight of IP used). For the continuous endpoints of the change in migraine days, the change in migraine attacks, the change in days using rescue medication, the change in headache days, the change in headache hours, and the change in MIDAS, HIT-6, MSQL, and CGI scores, a one-sided two-sample equal-variance z-test will be used to compare treatment group means. Fisher's Exact test will be used to make treatment group comparisons for the following binary outcomes:

- Proportion of subjects experiencing a ≥50%, ≥75%, and 100% reduction in monthly migraine days from baseline to 12 weeks of treatment.

Exploratory endpoints will be analyzed using the same method as for continuous efficacy endpoints. The analyses will be performed on the ITT and PP population.

14.5.1 Handling of Dropouts or Missing Data

Missing data will not be imputed for analysis.

Subjects who dropout after enrollment but prior to randomization will be replaced.

14.6 Clinical Trial Protocol Deviations

All the following deviations will be summarized on the all randomized patient population:

- Inclusion or exclusion criteria not satisfied.
- Deviations related to the Investigational Product administration
- Not permitted concomitant medications.

15 ADVERSE EVENTS (AE) AND SERIOUS ADVERSE EVENTS (SAE)

The Investigator and study staff are responsible for detecting and recording AEs and SAEs during scheduled safety evaluations and whenever such information is brought to their attention. This section of the protocol provides definitions and detailed procedures to be followed.

15.1 Definition of an AE

An AE is any untoward medical occurrence in a study participant which is temporally associated with the use of a medicinal product, regardless of its potential relationship to the medicinal product. An AE, therefore, can be any unfavorable or unintended sign, including an abnormal symptom, or disease (new or exacerbated), whether or not related to the investigational product (IP).

Examples of an AE include:

- Exacerbation of a pre-existing condition including either an increase in frequency and/or intensity of the condition.
- New conditions detected or diagnosed after investigational product administration even though it may have been present prior to the start of the study.
- Signs, symptoms of a drug interaction.
- Signs, symptoms of a suspected overdose of either investigational product or a concurrent medication (overdose per se should not be reported as an AE/SAE).

AEs may include pre- or post-treatment events that occur as a result of protocol-mandated procedures (i.e., modification of participant's previous therapeutic regimen).

15.2 Definition of a SAE

A serious adverse event is any untoward medical occurrence that, at any dose:

(a) results in death.

(b) is life-threatening.

NOTE: The term 'life-threatening' in the definition of 'serious' refers to an event in which the participant 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.

(c) requires hospitalization or prolongation of existing hospitalization.

NOTE: In general, hospitalization signifies that the participant has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or out -patient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious. Routine hospitalizations or elective surgeries are generally not regarded as SAEs.

(d) results in disability/incapacity, or

NOTE: The term disability means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g. sprained ankle) which may interfere or prevent everyday life functions but do not constitute a substantial disruption.

(e) is a congenital anomaly/birth defect

(f) 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 participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These should also be considered serious. Examples of such events are invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

15.3 Time Period, Frequency, and Method of Detecting AEs and SAEs

All AEs occurring after administration of the first dose of study medication and on or before the final assessment must be reported as AEs. All AEs must be recorded irrespective of whether they are considered drug-related.

At each assessment in the period defined above, AEs will be evaluated by the Investigator and recorded.

Any AEs already documented at a previous assessment and designated as ongoing, should be reviewed at subsequent assessments as necessary. If these have resolved, this should be documented. Changes in intensity or frequency of AEs should be recorded as separate events (i.e., a new record started).

The recording of AEs and SAEs are described in Section 15.4 ("Recording of AEs and SAEs").

15.4 Recording of AEs and SAEs

All clinical events, including either observed or volunteered problems, complaints or symptoms are to be recorded on the Adverse Events page(s) of the CRF. The need to capture this information is not dependent upon whether the clinical event is associated with study treatment. Adverse clinical events resulting from concurrent illnesses or reactions to concurrent medications are also to be recorded. In order to avoid vague, ambiguous, or colloquial expressions, the AE should be recorded in standard medical terminology rather than the participant's own words.

Each adverse clinical event is to be evaluated for duration, intensity, and whether the event may be associated with the investigational product (IP) or other causes. Start and stop dates,

relationship to investigational product (IP), medical management, and alternative causality of event must be recorded in the Adverse Events section of the CRF. AEs believed to be possibly related to investigational product (IP) must be followed until resolution.

15.5 Evaluating AEs and SAEs

15.5.1 Severity Rating

The severity of an adverse event (AE and SAE) is to be scored according to the following scale:

Mild	Awareness of sign or symptom, but easily tolerated
Moderate	Discomfort enough to cause interference with usual activity
Severe	Incapacitating with inability to work or perform usual activity
Potentially Life Threatening	Life threatening consequences; urgent intervention indicated
Fatal	Death related to AE

An AE that is assessed as severe should not be confused with a SAE. An event is defined as 'serious' when it meets one of the pre-defined outcomes as described in Section 15.2 "Definition of a SAE".

15.5.2 Relationship to Investigational product (IP)

SAEs will be classified as "**definitely not related**", "**unlikely related**", "**possibly related**", "**probably related**", or "**definitely related**" (including unknown).

For AEs, the relationship to study treatment is to be assessed according to the following definitions:

Definitely not related: The AE is definitely not related to the drug. This designation should be reserved for those events which occur prior to study treatment or for those events which cannot be even remotely related to study participation (e.g., injuries sustained in an automobile accident).
Unlikely related: There is no reasonable association between the study treatment and the suspected event and the event could have been produced by the participant 's clinical state or other modes of therapy administered to the participant.

Possibly related: The suspected adverse event may or may not follow a reasonable temporal sequence from study treatment administration but seems to be the type of reaction that cannot be dismissed as unlikely. The event could have been produced or mimicked by the participant 's clinical state or by other modes of therapy concomitantly administered to the participant.
Probably related: The suspected adverse event follows a reasonable temporal sequence from study treatment administration, abates upon discontinuation of the treatment, and cannot be reasonably explained by the known characteristics of the participant 's clinical state.
Definitely related: This designation should be reserved for those events which have no uncertainty in their relationship to treatment administration.

15.6 Pregnancy

Any pregnancy that occurs in a female participating in the study must be reported to the Sponsor within 3 working days of learning of the pregnancy. Follow-up must occur to determine the outcome of the pregnancy (including premature termination) and the status of mother and child. Pregnancy complications and elective terminations for medical reasons must be reported as AEs or SAEs. Spontaneous abortions must be reported as an SAE.

Any SAE occurring in association with a pregnancy and considered by the Investigator as possibly related or related to the investigational product must be promptly reported to the Sponsor, even if the event occurred after the participant completed the study.

The Investigator must attempt to collect pregnancy information on any female partners of male participants who become pregnant while the male participant is enrolled in the study. Pregnancy information must be reported to the Sponsor as described above.

15.7 Prompt Reporting of SAEs to the Sponsor

In the case of a Serious Adverse Event the Investigator must immediately:

- **SEND** (within 1 working day, by fax) the signed and dated corresponding page(s) in the Case Report Form to the representative of the Monitoring Team whose name, address and fax number appear on the Clinical Trial Protocol (Larry Weiss, MD, safety@aobiome.com; 617-475-1605), or to a designated Safety contact provided by the Monitoring Team, as well as to the Central Database number;
- **ATTACH** a photocopy of all examinations carried out and the dates on which these examinations were performed. Care should be taken to ensure that the patient's identity is protected and the patient's identifiers in the Clinical Trial are properly documented on all copies of source documents provided to the Sponsor. For laboratory results, include the laboratory normal ranges;
- **Follow-up of any Serious Adverse Event** that is fatal or life threatening should be provided within one additional calendar week. The treatment code will be unblinded for reporting of Serious Adverse Events that are unexpected and reasonably associated with the use of the Investigational Product.

Follow-up

- The Investigator should take all appropriate measures to ensure the safety of the patients, including referral to a specialist if indicated. Notably he/she should follow up the outcome of any adverse events (clinical signs, laboratory values or other, etc) until the return to normal or stabilization of the patient's condition;
- In the case of any serious adverse event, the patient must be followed up until clinical recovery is complete and laboratory results have returned to normal, or until progression has been stabilized. This implies that follow-up may continue after the patient has left the Clinical Trial and that additional investigations may be requested by the Monitoring Team;
- In case of any serious adverse event brought to the attention of the Investigator at any time after cessation of Investigational Product and considered by him/her to be caused by the Investigational Product with a reasonable possibility, this should be reported to the Monitoring Team.

Primary Contact
AOBiome Reportable Events Hotline
24 Hour Phone: 617-475-1605
Email: safety@aobiome.com
Call medical monitor to email a scanned report

16 ETHICAL AND REGULATORY STANDARDS

16.1 Ethical Conduct of Study

This clinical trial was designed and shall be implemented, executed, and reported in accordance with the ICH Harmonized Tripartite Guidelines for Good Clinical Practice, with applicable local regulations (including US Code of Federal Regulations Title 21), and with the ethical principles laid down in the Declaration of Helsinki.

The Investigator(s) should conduct the study in accordance with this protocol, the Declaration of Helsinki and ICH GCP guidelines and FDA regulations. The Investigator(s) and the Sponsor will sign the protocol and study contract, to confirm agreement. The Investigator(s) will not implement any amendment (deviation or changes of the protocol) without agreement by the Sponsor and IRB approval, except where necessary to eliminate immediate hazard(s) to study participants, or when change(s) involve only logistical or administrative aspects of the study.

Records that may reveal the identities of participants must be well protected, with consideration given to confidentiality and the right to privacy of participants.

16.2 Laws and Regulations

This Clinical Trial will be conducted in compliance with all international laws and regulations, and national laws and regulations of the countries in which the clinical trial is performed, as well as any applicable guidelines. The trial will be registered and updated on www.clintrials.gov and on other sites, as deemed appropriate.

16.3 Informed Consent

Each participant must be provided with a statement that the investigation involves research and that the IRB has approved solicitation of participants to participate; a fair explanation of the procedures to be followed and their purposes, including identification of any procedures which are experimental; a description in lay language of any possible side effects; a description of any attendant discomforts and risks reasonably to be expected; a description of any benefits reasonably to be expected; a disclosure of any appropriate alternative procedures that might be advantageous for the participant; an offer to answer any inquiries concerning the procedures, and instruction that the person is free to withdraw consent and discontinue participation in the project or activity at any time without prejudice to the participant. Payment to research participants for taking part in the study is based on time and inconvenience. All information concerning payment, including the schedule of payments, must be set forth in the informed consent, including a disclosure that the Investigator is being paid to perform the stated research.

A participant must give consent to take part in the study. Participants below the age of majority in the municipality must give written assent to participate in this study. This consent must be dated and retained by the Principal Investigator as part of the study records. A downloadable digital copy shall be given to the person signing the form. The informed consent process must be documented in the participant's source documents.

The Investigator agrees that the Sponsor, its employees or agents will have the right from time to time during the course of this study to audit and review pertinent medical records relating to this clinical trial. A statement will be obtained from each person participating in the study permitting the release of his/her medical records as necessary for inspection by authorized personnel of the Sponsor, FDA, and the staff managing the clinical study.

The release of medical records and the review of the contents will be in compliance with the Health Insurance Portability and Accountability Act of 1996 (HIPAA).

16.4 Institutional Review Board/Independent Ethics Committee (IRB/EC)

The protocol and informed consent form and the electronic version of the consent for this study must be approved by the IRB. A copy of the Letter of Approval from the Board, which contains specific identification of the documents approved, must be received by the Sponsor prior to shipment of drug supplies to the Principal Investigator.

All changes to the protocol, as well as a change of Investigator, must also be approved by the IRB and documentation of this approval provided to the study monitor. Records of the IRB's review and approval of all documents pertaining to the study must be kept on file by the Principal Investigator and are subject to FDA inspection at any time. IRB renewal for approval is required each year. The Investigator is to notify AOBiome, in writing, of the approval to continue the study.

16.5 Clinical Monitoring/Record Keeping

There shall be no alterations in the protocol design without the written consent and approval of the Sponsor and the approval of the IRB, except in the case that participants are at immediate risk without immediate implementation of such alterations. In the aforementioned situation, the site should notify the Sponsor and IRB of the deviation as soon as possible, and should seek the written consent and approval of the Sponsor and the approval of the IRB.

All results of this trial must be recorded on eCRFs. Each participant who has been randomized must have a completed eCRF. Reasons for termination must be stated in the early termination section. Study participants are not to be identified by name on eCRFs, but rather by coded identifiers and participant initials.

The study monitor will verify the accuracy of the data by reviewing pertinent source documents such as office records or hospital charts of the participants.

Study records include eCRFs, signed FDA Form 1572, original reports of test results, and digitally signed electronic informed consent forms. IRB approval letters and other documents pertaining to the conduct of the study are to be kept on file by the Investigator. If the study files are assigned to someone else or removed to another location, the Investigator is to notify the study monitor or Sponsor in writing of the change. All study records are subject to FDA inspection at any time.

All information supplied to the Investigator by the Sponsor before, during, and after the study is confidential. Such information is to be used solely in connection with the clinical study. The study protocol, IB, and any other pertinent study related materials or records provided are to be maintained in a confidential manner, reviewed carefully with attention to admonitions and returned to the Sponsor upon request. No part of these materials may be reproduced or transmitted in any form without prior written permission from the Sponsor.

17 ADMINISTRATIVE RULES

17.1 Curriculum Vitae

An updated, signed, and dated copy of the curriculum vitae limited to the experience, qualification and training for each Investigator and/or Sub-Investigator(s) will be provided to the Sponsor prior to the beginning of the Clinical Trial.

17.2 Archiving of Study Documentation

The Investigator must maintain confidential all study documentation, and take measures to prevent accidental or premature destruction of these documents.

It is recommended that the Investigator retain the study documents at least fifteen (15) years after the completion or discontinuation of the Clinical Trial. However, applicable regulatory requirements should be taken into account in the event that a longer period is required.

The Investigator must notify the Sponsor prior to destroying any study essential documents within the fifteen (15) year period following the Clinical Trial completion or discontinuation.

If the Investigator's personal situation is such that archiving can no longer be ensured by him/her, the Investigator shall inform the Sponsor and the relevant records shall be transferred to a mutually agreed upon designee.

17.3 Internal Safety Review Committee

An internal safety review committee may be set up as needed to protect the ethical and safety interests of participants and to protect the scientific validity of the study. Ad-hoc safety interim analyses might be performed by an independent statistician if the safety review committee identifies potential safety signals during its routine blinded safety review. The details for the analysis plan will be documented in the trial's Statistical Analysis Plan.

18 STUDY MONITORING

18.1 Responsibilities of the Investigator(s)

The Investigator(s) undertake(s) to perform the Clinical Trial in accordance with this Clinical Trial Protocol, ICH guidelines for Good Clinical Practice and the applicable regulatory requirements.

The Investigator is required to ensure compliance with all procedures required by the Clinical Trial Protocol and by study procedures provided by the Sponsor (including security rules). The Investigator agrees to provide reliable data and all information requested by the Clinical Trial Protocol (with the help of the Case Report Form [CRF], Discrepancy Resolution Form [DRF] or other appropriate instrument) in an accurate and legible manner according to the instructions provided and to ensure direct access to source documents to Sponsor representatives. If any particular circuits have to be defined (e.g., e-CRF, Fax), particular attention should be paid to the confidentiality of the patient's data to be transferred. The Investigator may appoint such other individuals as he/she may deem appropriate as Sub-Investigators to assist in the conduct of the Clinical Trial in accordance with the Clinical Trial Protocol. All Sub-Investigators shall be timely appointed and listed. The Sub-Investigators will be supervised by and under the responsibility of the Investigator. The Investigator will provide them with a Clinical Trial Protocol and all necessary information.

18.2 Responsibilities of the Sponsor

The Sponsor of this Clinical Trial is responsible to Health Authorities for taking all reasonable steps to ensure the proper conduct of the Clinical Trial Protocol with regard to ethics, Clinical Trial Protocol compliance, integrity and validity of the data recorded on the Case Report Forms. Thus, the main duty of the Monitoring Team is to help the Investigator and the Sponsor maintain a high level of ethical, scientific, technical and regulatory quality in all aspects of the Clinical Trial. At regular intervals during the Clinical Trial, the site will be contacted, through monitoring visits, letters or telephone calls, by a representative of the Monitoring Team to review study progress, Investigator and patient compliance with Clinical Trial Protocol requirements, and any emergent problems. During these monitoring visits, the following but not exhaustive list of points will be scrutinized with the Investigator: patient informed consent, patient recruitment and follow-up, Serious Adverse Event documentation and reporting, outcome events documentation and reporting, Investigational Product allocation, patient compliance with the Investigational Product regimen, Investigational Product accountability, concomitant therapy use and quality of data.

18.3 Source Document Requirements

According to the ICH guidelines for Good Clinical Practice, the Monitoring Team must check the Case Report Form entries against the source documents, except for the preidentified source data directly recorded in the Case Report Form. The Informed Consent Form will include a statement by which the patient allows the Sponsor's duly authorized personnel, the Ethics Committee (IRB/EC), and the regulatory authorities to have direct access to source data which supports the data on the Case Report Forms (e.g., patient's medical file, appointment books, original laboratory records, etc.). These personnel, bound by professional secrecy, must keep confidential all personal identity or personal medical information (according to confidentiality rules).

18.4 Use and Completion of Case Report Forms (CRFs) and Additional Requests

The sponsor or CRO will be responsible for activities associated with the data management of this study. This will include setting up a relevant database and data transfer mechanisms, along with appropriate validation of data and resolution of queries. Data generated within this clinical study will be handled according to the relevant SOPs of the data management and biostatistics departments of sponsor or CRO.

For Electronic Data Capture (EDC):
Study sites will enter data directly into an EDC system by completing the eCRF via a secure internet connection. Data entered into the eCRF must be verifiable against source documents at the study site. Any changes to the data entered into the EDC system will be recorded in an automated, secure audit trail and is Food and Drug Administration (FDA) Code of Federal Regulations (CFR) Title 21 Part 11 compliant.

Data entered into the eCRF will be validated as defined in the Data Validation Specifications (DVS). Validation includes, but is not limited to, validity checks (for example, missing data, range checks) and consistency checks (logical checks between variables) to ensure that study data are accurately reported. Additionally, CRO Data Management will perform aggregate data review as defined in the DVS to ensure that the data are complete, consistent and reasonable. The

electronic edit checks will run continually throughout the course of the study and queries reviewed by CRO personnel to assure validity as compared to source records. Manual queries may also be entered into EDC by Monitoring or Data Management personnel to address identified discrepancies.

Medical conditions/procedures will be coded using MedDRA and prior and concomitant medications will be coded using WHODrug.

At the conclusion of the study, each site will be provided with their subject CRFs in Portable Document Format (PDF) for archival. The CRF PDFs will contain subject data, audit trail information, queries including responses, and comments.

19 PUBLICATIONS

All data generated from this study are the property of AOBiome LLC and shall be held in strict confidence along with all information furnished by AOBiome. Independent analyses and/or publication of these data by the Investigator or any member of his/her staff is not permitted without prior written consent of AOBiome.

Any formal presentation or publication of data from this trial will be considered as a joint publication by the Investigator(s) and appropriate Sponsor personnel. Authorship will be determined by mutual agreement. Written permission to the Investigator will be contingent on the review by the Sponsor of the methodology and statistical analysis and any publication or presentation will provide for nondisclosure of AOBiome confidential or proprietary information. In all cases, the parties agree to submit all manuscripts or abstracts to all other parties at least 30 days prior to submission. This will enable all parties to protect proprietary information and to provide comments based on information that may not yet be available to other parties.

20 PROTOCOL ADHERENCE

This protocol defines the study objectives, the study procedures and the data to be collected on study participants. Additional assessments required to ensure safety of participants should be administered as deemed necessary on a case by case basis. Under no circumstances is an Investigator allowed to collect additional data or conduct any additional procedures for any research related purpose involving any investigational drugs under the protocol.

Investigators ascertain they will apply due diligence to avoid protocol deviations. If an Investigator feels a protocol deviation would improve the conduct of the study this must be considered a protocol amendment, and unless such an amendment is agreed upon by the sponsor and approved by the IRB/IEC and health authorities, where required, it cannot be implemented.

21 CONFIDENTIALITY

All information disclosed or provided by the Sponsor (or any company/institution acting on their behalf), or produced during the Clinical Trial, including, but not limited to, the Clinical Trial Protocol, the CRFs, the Investigator's Brochure and the results obtained during the course of the Clinical Trial, is confidential. The Investigator or any person under his/her authority agrees to

undertake to keep confidential and not to disclose the information to any third party without the prior written approval of the Sponsor. However, the submission of this Clinical Trial Protocol and other necessary documentation to the Ethics Committee (IRB/EC) is expressly permitted, the IRB/EC members having the same obligation of confidentiality. The Sub-Investigators shall be bound by the same obligation as the Investigator. The Investigator shall inform the Sub-Investigators of the confidential nature of the Clinical Trial. The Investigator and the Sub-Investigators shall use the information solely for the purposes of the Clinical Trial, to the exclusion of any use for their own or for a third party's account.

22 PROPERTY RIGHTS

All information, documents and Investigational Product provided by the Sponsor or its designee are and remain the sole property of the Sponsor. The Investigator shall not mention any information or the Product in any application for a patent or for any other intellectual property rights. All the results, data, documents and inventions, which arise directly or indirectly from the Clinical Trial in any form, shall be the immediate and exclusive property of the Sponsor. The complete verified database will be shared with the Operations Committee, which shall have full access to all data. The Sponsor may use or exploit all the results at its own discretion, without any limitation to its property right (territory, field, continuance). The Sponsor shall be under no obligation to patent, develop, market or otherwise use the results of the Clinical Trial. As the case may be, the Investigator and/or the Sub-Investigators shall provide all assistance required by the Sponsor, at the Sponsor's expense, for obtaining and defending any patent, including signature of legal documents.

23 DATA PROTECTION

The patient's personal data and Investigator's personal data which may be included in the Sponsor database shall be treated in compliance with all applicable laws and regulations;

When archiving or processing personal data pertaining to the Investigator and/or to the patients, the Sponsor shall take all appropriate measures to safeguard and prevent access to this data by any unauthorized third party.

24 SPONSOR AUDITS AND INSPECTIONS BY REGULATORY AGENCIES

For the purpose of ensuring compliance with the Clinical Trial Protocol, Good Clinical Practice and applicable regulatory requirements, the Investigator should permit auditing by or on the behalf of the Sponsor and inspection by applicable regulatory authorities.

The Investigator agrees to allow the auditors/inspectors to have direct access to his/her study records for review, being understood that this personnel is bound by professional secrecy, and as such will not disclose any personal identity or personal medical information.

The Investigator will make every effort to help with the performance of the audits and inspections, giving access to all necessary facilities, data, and documents. As soon as the Investigator is notified of a future inspection by the authorities, he will inform the Sponsor and authorize the Sponsor to participate in this inspection.

The confidentiality of the data verified and the protection of the patients should be respected during these inspections.

Any result and information arising from the inspections by the regulatory authorities will be immediately communicated by the Investigator to the Sponsor.

The Investigator shall take appropriate measures required by the Sponsor to take corrective actions for all problems found during the audit or inspections.

25 PREMATURE DISCONTINUATION OF THE STUDY OR PREMATURE CLOSE-OUT OF A SITE

25.1 Decided by the Sponsor in the Following Cases:

1. In the event the results of the Clinical Trial do not appear to be scientifically convincing to the Sponsor;
2. If the aim of the Clinical Trial has become outdated or is no longer of interest;
3. If the information on the product leads to doubt as to the benefit/risk ratio;
4. If the Investigator has received from the Sponsor all Investigational Product, means and information necessary to perform the Clinical Trial and has not included any patient after a reasonable period of time mutually agreed upon;
5. In the event of breach by the Investigator of a fundamental obligation under this agreement, including but not limited to breach of the Clinical Trial Protocol, breach of the applicable laws and regulations or breach of the ICH guidelines for GCP;
6. If the total number of patients are included earlier than expected; In any case the Sponsor will notify the Investigator of its decision by written notice.

25.2 Decided by the Investigator

The Investigator must notify (30 days' prior notice) the Sponsor of his/her decision and give the reason in writing. In all cases (decided by the sponsor or by the Investigator), the appropriate Ethics Committee(s) (IRB/EC) and Health Authorities should be informed.

26 CLINICAL TRIAL PROTOCOL AMENDMENTS

Any protocol amendments will be added as stand-alone documents. In addition, any and all revisions dictated by the amendments will be made in the protocol. Each time a protocol is amended, a new amended version date will be added to the cover page.

All appendices attached hereto and referred to herein are made part of this Clinical Trial Protocol. The Investigator should not implement any deviation from, or changes of the Clinical Trial Protocol without agreement by the sponsor and prior review and documented approval/favorable opinion from the IRB/EC of an amendment, except where necessary to eliminate an immediate hazard(s) to clinical trial patients, or when the change(s) involves only logistical or administrative aspects of the trial. Any change agreed upon will be recorded in

writing, the written amendment will be signed by the Investigator and by the Sponsor and the signed amendment will be filed with this Clinical Trial Protocol. Any amendment to the Clinical Trial Protocol requires written approval/favorable opinion by the IRB/EC prior to its implementation, unless there are overriding safety reasons. In some instances, an amendment may require a change to the Informed Consent Form. The Investigator must receive an IRB/EC approval/favorable opinion concerning the revised Informed Consent Form prior to implementation of the change.

27 APPENDIX A-SCHEDULE OF EVENTS

Visit name (Day)	Screening	Baseline (Beginning of 4 week baseline period)	Randomization (End of 4 week baseline period)	Prevention Treatment Week 4	Prevention Treatment Week 8	Prevention Treatment Week 12	Follow-Up (End of Study) Week 16	Unanticipated/Early Termination Visit
Visit	V1 (Day -29)	V2 (Day -28)	V3 (Day 0)	V4 (Day 28)	V5 (Day 56)	V6 (Day 84)	V7 (Day 112)	
Visit Window, in Days	-42 to -28	-31 to -27	±3	±3	±3	±3	±3	
Informed Consent[1]	X							
Inclusion/Exclusion Criteria[2]	X	X	X[17]					
Demographics	X							
Medical history	X							
Smoking status	X							
Concomitant medications	X	X	X	X	X	X	X	X
Vital signs (HR, BP, respiratory rate)	X	X	X	X	X	X	X	X
Brief Physical exam	X	X	X	X	X	X	X	X

Neurological exam	X	X	X	X	X	X	X	X
Body weight	X	X	X	X	X	X	X	X
Height	X							
Blood collection[3]			X	X	X	X	X	X
12-lead ECG	X		X			X		
Randomization[4]			X					
Nasal inspection[5]	X	X	X	X	X	X	X	X
Delivery of Investigational Product			X					
Investigational Product application[6]			X	X	X	X		
Investigational Product compliance[7]			X	X	X	X		X
Counseling[8]			X	X	X			X
Laboratory testing[9]	X		X			X		X
Urinary pregnancy test[10]	X							
Nasal fluid[11]			X	X	X	X	X	X
Safety AEs	X	X	X	X	X	X	X	X

Study diary[12]	X	X	X	X	X	X	X	X
Migraine pain intensity score	X	X	X	X	X	X	X	X
MIDAS[13]		X	X			X		X
HIT-6[14]		X	X	X	X	X	X	X
MSQL[15]		X	X	X	X	X	X	X
CGI[16]		X	X	X	X	X	X	X
Subject Satisfaction Survey							X	X[18]

1. Informed consent can be obtained up to 14 days prior to the baseline visit (V2).
2. Inclusion and exclusion criteria will be reviewed at Screening (V1), Baseline (V2), and Randomization (V3) visits to make sure nothing changed.
3. Blood samples for biomarkers will be collected and processed on site. Serum/plasma samples will be frozen onsite and shipped to the Central lab for storage.
4. Subject's demographic data and medical history are blinded to the statistician for randomization and treatment assignment. Only subjects with 4-14 migraine headache days per month during baseline period (28 days between V2 and V3) will be randomized.
5. Nasal inspection will include clinical examination of the nasal mucosa, sinuses, and upper airway using an otoscope.
6. Subjects will be trained for in-home treatment/assessment on the use of the nasal spray, and have their first dose of treatment on site (V3). Subjects will remain onsite for observation for 1 hour post dosing. Subjects will then be dispensed study medication for 12 weeks of at home dosing.
7. Weight of individual IP bottles will be obtained at visits V3 (before and after first dose), V4, V5, V6, and unanticipated/early termination visit.
8. Subjects will be counseled on the application of the nasal spray, study diary, and answer any questions.
9. Fasting blood sample for a comprehensive metabolic panel, complete blood count, chemistry, lipid panel, and liver function test-15 ml. Also includes urinalysis. Patients should fast for at least 8 hours before the test. Blood will be shipped to the central lab for processing.
10. Urine pregnancy test to be done on WOCBP.
11. Intranasal strip will be used to collect nasal fluid for inflammatory cytokines. Optionally, nasal fluid may be evaluated for nasal microbiome (e.g., PCR) to confirm presence of B244.
12. Subjects will be asked to fill out a daily migraine-history diary from screening to treatment with study drug and a migraine-treatment diary from treatment through the remainder of the follow-up period. Baseline period, prevention treatment, and follow up observation period for study assessments will be daily and will include dosing, migraine days, headache days, migraine intensity, headache intensity, rescue meds (acute migraine specific meds), nausea, vomiting, photophobia, and sonophobia. Collect sufficient information about the headache in the trial (i.e., headache intensity, presence/absence of associated symptoms, unilateral/bilateral location, aggravation by exercise, throbbing/non-throbbing) to verify the headache is a migraine.
13. Migraine Disability Assessment questionnaire.
14. Headache Impact Test-6 questionnaire.
15. Migraine Specific Quality of Life questionnaire.
16. Clinical Global Impression scale
17. Subjects are randomized upon confirming the inclusion/exclusion criteria and the entry criteria of 4-14 migraine headache days during the baseline period.
18. Only for Early Termination Visit due to study discontinuation or withdrawal.

28 APPENDIX B- MIGRAINE DISABILITY ASSESSMENT (MIDAS) QUESTIONNAIRE

The Migraine Disability Assessment Test

The **MIDAS** (Migraine Disability Assessment) questionnaire was put together to help you measure the impact your headaches have on your life. The information on this questionnaire is also helpful for your primary care provider to determine the level of pain and disability caused by your headaches and to find the best treatment for you.

INSTRUCTIONS

Please answer the following questions about ALL of the headaches you have had over the last 3 months. Select your answer in the box next to each question. Select zero if you did not have the activity in the last 3 months. Please take the completed form to your healthcare professional.

1. On how many days in the last 3 months did you miss work or school because of your headaches?

2. How many days in the last 3 months was your productivity at work or school reduced by half or more because of your headaches? (Do not include days you counted in question 1 where you missed work or school.)

3. On how many days in the last 3 months did you not do household work (such as housework, home repairs and maintenance, shopping, caring for children and relatives) because of your headaches?

4. How many days in the last 3 months was your productivity in household work reduced by half of more because of your headaches? (Do not include days you counted in question 3 where you did not do household work.)

5. On how many days in the last 3 months did you miss family, social or leisure activities because of your headaches?

Total (Questions 1-5)

What your Physician will need to know about your headache:

- On how many days in the last 3 months did you have a headache? (If a headache lasted more than 1 day, count each day.)

- On a scale of 0 - 10, on average how painful were these headaches? (where 0=no pain at all, and 10= pain as bad as it can be.)

Scoring: After you have filled out this questionnaire, add the total number of days from questions 1-5 (ignore A and B).

MIDAS Grade	Definition	MIDAS Score
I	Little or No Disability	0-5
II	Mild Disability	6-10
III	Moderate Disability	11-20
IV	Severe Disability	21+

If Your MIDAS Score is 6 or more, please discuss this with your doctor.

29 APPENDIX C- HEADACHE IMPACT TEST-6 (HIT-6) QUESTIONNAIRE

HIT-6 Questionnaire (Evaluation of headache disability) This questionnaire was designed to help you describe and communicate the way you feel and what you cannot do because of headaches.

INSTRUCTIONS : To complete, please circle one answer for each question.

1. When you have headaches, how often is the pain severe?

Never Rarely Sometimes Very often Always

2. How often do headaches limit your ability to do usual daily activities including household work, work, school, or social activities?

Never Rarely Sometimes Very often Always

3. When you have a headache, how often do you wish you could lie down?

Never Rarely Sometimes Very often Always

4. In the past 4 weeks, how often have you felt too tired to do work or daily activities because of your headaches?

Never Rarely Sometimes Very often Always

5. In the past 4 weeks, how often have you felt fed up or irritated because of your headaches?

Never Rarely Sometimes Very often Always

6. In the past 4 weeks, how often did headaches limit your ability to concentrate on work or daily activities?

Never Rarely Sometimes Very often Always

Column 1 Column 2 Column 3 Column 4 Column 5

Column 1 (Never): 6 points each _____

Column 2 (Rarely): 8 points each _____

Column 3 (Sometimes): 10 points each _____

Column 4 (Very often): 11 points each _____

Column 5 (Always): 13 points each _____

To score, add points for answers in each column.

Total Score: _____

Class I: 36-49, **Class II**: 50-55, **Class III**: 56-59, **Class IV**: 60 and more.

It is suggested to talk to your physician for class II and more.

30 APPENDIX D- MIGRAINE SPECIFIC QUALITY OF LIFE (MSQL) QUESTIONNAIRE

MIGRAINE SPECIFIC QUALITY OF LIFE QUESTIONNAIRE (VERSION 2.1)

PATIENT INSTRUCTIONS:

Please fill out this questionnaire. It will help us understand the effects of migraine headache on your daily activities.

The questionnaire has been designed so that it can be completed quickly and easily. Please check only one answer for each question. You should answer every question.

Thank you for your time.

While answering the following questions, please think about ***all migraine attacks*** you may have had ***in the past 4 weeks***.

1. In the past 4 weeks, how often have migraines **interfered** with how well you dealt with family, friends and others who are close to you? (Select only **one** response.) 1 None of the time 2 A little bit of the time 3 Some of the time 4 A good bit of the time 5 Most of the time 6 All of the time

2. In the past 4 weeks, how often have migraines **interfered** with your leisure time activities, such as reading or exercising? (Select only **one** response.) 1 None of the time 2 A little bit of the time 3 Some of the time 4 A good bit of the time 5 Most of the time 6 All of the time

3. In the past 4 weeks, how often have you had **difficulty** in performing work or daily activities because of migraine symptoms? (Select only **one** response.) 1 None of the time 2 A little bit of the time 3 Some of the time 4 A good bit of the time 5 Most of the time 6 All of the time

4. In the past 4 weeks, how often did migraines **keep you** from getting as much done at work or at home? (Select only **one** response.) 1 None of the time 2 A little bit of the time 3 Some of the time 4 A good bit of the time 5 Most of the time 6 All of the time

5. In the past 4 weeks, how often did migraines **limit** your ability to concentrate on work or daily activities? (Select only **one** response.) 1 None of the time 2 A little bit of the time 3 Some of the time 4 A good bit of the time 5 Most of the time 6 All of the time

6. In the past 4 weeks, how often have migraines **left you too tired** to do work or daily activities? (Select only **one** response.) 1 None of the time 2 A little bit of the time 3 Some of the time 4 A good bit of the time 5 Most of the time 6 All of the time

7. In the past 4 weeks, how often have migraines **limited** the number of days you have felt energetic? (Select only **one** response.) 1 None of the time 2 A little bit of the time 3 Some of the time 4 A good bit of the time 5 Most of the time 6 All of the time

8. In the past 4 weeks, how often have you had to **cancel** work or daily activities because you had a migraine? (Select only **one** response.) 1 None of the time 2 A little bit of the time 3 Some of the time 4 A good bit of the time 5 Most of the time 6 All of the time

9. In the past 4 weeks, how often did you **need help** in handling routine tasks such as every day household chores, doing necessary business, shopping, or caring for others, when you had a migraine? (Select only **one** response.) 1 None of the time 2 A little bit of the time 3 Some of the time 4 A good bit of the time 5 Most of the time 6 All of the time

10. In the past 4 weeks, how often did you have to **stop** work or daily activities to deal with migraine symptoms? (Select only **one** response.) 1 None of the time 2 A little bit of the time 3 Some of the time 4 A good bit of the time 5 Most of the time 6 All of the time

11. In the past 4 weeks, how often were you **not able to go** to social activities such as parties, dinner with friends, because you had a migraine? (Select only **one** response.) 1 None of the time 2 A little bit of the time 3 Some of the time 4 A good bit of the time 5 Most of the time 6 All of the time

12. In the past 4 weeks, how often have you **felt** fed up or frustrated because of your

migraines? (Select only **one** response.) 1 None of the time 2 A little bit of the time 3 Some of the time 4 A good bit of the time 5 Most of the time 6 All of the time

13. In the past 4 weeks, how often have you **felt** like you were a burden on others because of your migraines? (Select only **one** response.) 1 None of the time 2 A little bit of the time 3 Some of the time 4 A good bit of the time 5 Most of the time 6 All of the time

14. In the past 4 weeks, how often have you been **afraid** of letting others down because of your migraines? (Select only **one** response.) 1 None of the time 2 A little bit of the time 3 Some of the time 4 A good bit of the time 5 Most of the time 6 All of the time

31 APPENDIX E- CLINICAL GLOBAL IMPRESSION (CGI) SCALE

CLINICAL GLOBAL IMPRESSION

1. Severity of illness

Considering your total clinical experience with this particular population, how mentally ill is the patient at this time?

0 = Not assessed 1 = Normal, not at all ill 2 = Borderline mentally ill 3 = Mildly ill

4 = Moderately ill 5 = Markedly ill 6 = Severely ill 7 = Among the most extremely ill patients

2. Global improvement: Rate total improvement whether or not, in your judgement, it is due entirely to drug treatment. Compared to his condition at admission to the project, how much has he changed?

0 = Not assessed 1 = Very much improved 2 = Much improved 3 = Minimally improved

4 = No change 5 = Minimally worse 6 = Much worse 7 = Very much worse

3. Efficacy index: Rate this item on the basis of **drug effect only**. Select the terms which best describe the degrees of therapeutic effect and side effects and record the number in the box where the two items intersect. EXAMPLE: Therapeutic effect is rated as 'Moderate' and side effects are judged 'Do not significantly interfere with patient's functioning'.

Therapeutic effect	**Side effects**			
	None	*Do not significantly interfere with patient's functioning*	*Significantly interferes with patient's functioning*	*Outweighs therapeutic effect*
Marked Vast improvement. Complete or nearly complete remission of all symptoms	01	02	03	04
Moderate Decided improvement.	05	06	07	08

Partial remission of symptoms				
Minimal Slight improvement which doesn't alter status of care of patient	09	10	11	12
Unchanged or worse	13	14	15	16

Not assessed = 00

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