Clinical Investigation Plan

Study title:

PArtial REbreathing for Migraine with Aura 1 (PAREMA1):

A prospective, multi-centre, randomized, double-blind, sham-controlled, parallel-group, group-sequential study to investigate safety and effectiveness of the Rehaler partial rebreathing device, in adults suffering from migraine with aura

Study identifier: **RH-001**

EUDAMED Number of clinical investigation: **CIV-23-04-042876**

Sponsor:

RE:HALER™

Absalonsgade 26

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Contact: Troels Johansen (Chief Technology Officer)

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Confidentiality statement:

The following confidential information is the property of Rehaler. As long as the information contained in this protocol has not been published it may only be used when permission has been obtained from Rehaler in writing. It is not permitted to reproduce all or sections of this protocol.

Table of contents:

1 Change log

Version	Issue Date	Changes
1.0	17 Aug 2022	Initial version
1.1	19 Sep 2022	• Addition of Figure 8 showing that the sham device does not impact CO2 and oxygen levels • Addition of the DASS-21 questionnaire to the protocol, administered at Site Visit 1 and 2 • Addition of an exploratory endpoint comparing DASS-21 score at Site Visit 1 and 2 • More detailed description of procedures during Site Visit 1 and the Instruction Session • Clarification of the study flow chart that patient instruction mainly takes place during Instruction Session • Minor edit in the Attack Checklist
2.0	07 Oct 2022	• Addition of global coordinating investigator to study • Inclusion of DASS-21 questionnarie • Minor clarification of unblinding procedures • Safety evaluation by Medical advisor • Update of risk section including salivation as a known side effect.
3.0	28 Nov 2022	• Change of method for measuring the anaemia exclusion criterion (cf. section 8.8), specifically using the more accurate parameter of total hemoglobin instead of ferritin concentration. • Clarification that trial sites are allowed to identify and contact potential study participants from among their relevant patients • Administrative updates
3.1	13 Feb 2023	• Clarification of methods for calculating statistical significance and confidence interval for primary endpoint (section 16.1) • Info on Intention-to-Treat analysis set to be used as population analysis set included (section 16.1) • Info on Multiple imputation for handling missing data included (section 16.9)
3.2	14 Feb 2023	• Added exclusion criterion: Sickle Cell Disease
3.3	14 Feb 2023	• Corrected maximum duration of treatment in consistency with IB and IFU (section 13.4) • Added clarifying sentences • Administrative updates
3.4	15 Jun 2023	• Clarified that the indication for use of the marketed device from 2018 to 2020 was also migraine with aura • Clarification that the minimum number of participants in the study will be 174

3.5	20 Sep 2023	Added section on the device materials and their contact with the user's body Added the clinical investigation's EUDAMED number
3.6	05 Feb 2024	Removal of mentions of electronic signature of the informed consent form that were left by mistake. Minor changes in study procedures and eligibility criteria to clarify the text interpretation. Updates in the number of participating sites and study timeline.

2 Sponsor statement

The sponsor of this study, Rehaler A/S, manufacturer of the Rehaler migraine treatment device, states the following:

a) This study will be conducted in compliance with the protocol (after being approved by the local Institutional Review Board (IRB)/Research Ethics Committee (REC) and, if required, by the relevant competent authorities), US 21 CFR Parts 50, 54, 56 and 812, 45 CFR Part 46, national laws and regulation concerning clinical trials, the Good Clinical Practices (GCP) set forth in ISO 14155 (2020) standard and the ethical principles that have their origin in the Declaration of Helsinki. The most stringent requirements, guidelines or regulations will also be followed.

b) The Clinical Investigation Plan, Informed Consent Form (ICF), patient's information material, and advertising material will be submitted and approved by the local IRB/REC and, if required, by the relevant competent authorities, and any request by the IRB/REC or regulatory agencies will be complied with. Approval will be obtained prior to enrolment of any patients.

c) Adequate insurance policy will be held valid for the entire study duration as well as for the discovery period required per local regulation.

Prepared by:

Date:

30-Apr-2024

_____

Troels Johansen
CEO
Rehaler

Approved by:	Date:	Signature:
Global Coordinating Investigator Charly Gaul, PhD Dr. Med		
Miranda Kunz, Biostatistician, Technomics Research		

3 Principal Investigator statement

As Principal Investigator I hereby confirm that:

- I will ensure that the study is conducted in accordance with the Clinical Investigation Plan (CIP) documents, its amendments, the clinical trial agreement and the applicable regulatory requirements, including the Declaration of Helsinki, *International Conference on Harmonisation Good Clinical Practice* (ICH E6(R2) GCP) and the International Organization for Standardization (ISO) 14155:2020 *Clinical investigation of medical devices for human subjects – Good Clinical Practice*.
- Written Institutional Review Board (IRB) or Research Ethics Committee (REC) and (if applicable) Competent Authority approval will be obtained prior to commencing with participant enrolment.
- I have read the entire CIP and I understand what my tasks are as a study investigator.
- I will conduct this study following this protocol and will make a reasonable effort to complete the study in the time frame agreed with Sponsor.
- I will provide copies of this CIP and all pertinent information to the study personnel under my supervision and ensure they are fully informed regarding the conduct of the Study.
- I understand that the study may be terminated or enrolment suspended at any time by the Sponsor, with or without cause, or by me if it becomes necessary to protect the interests of the study participants.
- I understand that the CIP documents contain information that is confidential and proprietary to the Sponsor, and I will ensure that my study staff keep the CIP documents confidential.

_____ _____

Principal Investigator's signature Date

_____

Principal Investigator's printed name

_____ _____

Site name Site number

4 Overview of Sponsor representatives

Clinical Project Manager	Lene Lyngsie, Qmed Consulting A/S Koebmagergade 53, 1. DK-1150 Copenhagen K. Denmark
Biostatistician	Miranda Kunz Technomics Research, LLC 1815 Medina Rd Suite 101 Long Lake, MN 55356, USA
Contract Research Organisation (CRO)	Qmed Consulting A/S Koebmagergade 53, 1. DK-1150 Copenhagen K. Denmark
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5 Study synopsis

STUDY TITLE	PArtial REbreathing for Migraine with Aura 1 (PAREMA1): A randomized, controlled, double-blind, parallel-group, group-sequential clinical trial to investigate safety and effectiveness of the Rehaler partial rebreathing device, in adults suffering from migraine with aura.
Sponsor	Rehaler A/S Absalonsgade 26 8000 Aarhus C Denmark
INVESTIGATION PLAN ACRONYM	PAREMA1
STUDY DESIGN	PAREMA1 is a randomized, controlled, double-blind, parallel-group, group-sequential clinical trial and will randomize minimum 174 and maximum 220 participants with migraine with aura (MA), ages 18 to 65. In Stage 1 of the trial the participants will treat up to four attacks each, with one of two devices: either an active device which delivers a moderate inspired CO_2 content (1.5 to 2.5%) or a sham device which delivers a very low inspired CO_2 content. After four treated attacks, participants will end study Stage 1 and start Stage 2 which is an open-label extension in which the participants are provided with active devices and each month are automatically prompted to report data about their migraine attacks and treatment effectiveness. After 60 participants have provided per-protocol data from two attacks in Stage 1, an interim analysis will be conducted by Technomics Research. At that point there will be an a priori determined decision point about how many additional participants to recruit, based on the interim data.
NUMBER OF SITES (Estimate)	Europe: 6 USA: 7
INVESTIGATIONAL DEVICE	The active device (Rehaler) is a single-use medical device for home use for the treatment of acute migraine attacks with aura in participants of ages 18+. The mechanism of the Rehaler device is partial rebreathing.
Intended Use	The intended use application of the Rehaler is early treatment of acute attacks of migraine with aura in patients of ages 18+ to stop or alleviate migraine with aura attacks. The user breathes through the mouthpiece from the beginning of the aura symptoms until they disappear.

EXPECTED STUDY DURATION	Randomized controlled Stage (Stage 1): Estimated 15 months from Study Initiation to end of Stage 1. Open-label extension (Stage 2): 12 months.
OBJECTIVES	Collect clinical data on Rehaler's effectiveness and safety for the early treatment of migraine with aura, in adults.
PRIMARY EFFECTIVENESS ENDPOINT	Absence of Moderate or Severe pain at 2 hours (AMSP2)
MOST IMPORTANT SECONDARY ENDPOINT	Pain Freedom at 2 hours (PF2)
SAFETY PARAMETERS	1. Incidence of device- or treatment-related adverse events. 2. Incidence of device- or treatment-related serious adverse events (SAEs) and/or serious adverse device effects (SADE).
SAMPLE SIZE	Minimum 174, maximum 220 (randomized participants)
STUDY POPULATION	Adult migraine with aura patients, ages 18-65
KEY INCLUSION CRITERIA	• Participant has migraine with typical aura (ICHD3 classification 1.2.1) with the additional criterion that a moderate or severe headache historically follows the aura in more than 75% of cases of aura, beginning between 10 and 60 minutes after aura onset. • 3 or more migraine-with-aura attacks over the last six months.
KEY EXCLUSION CRITERIA	• 15 or more headache days per month • History of chronic pulmonary disease or severe cardiovascular disease • Anaemia
TREATMENT	Treatment with the study device starting as early as possible after onset of aura, and continuing until the aura symptoms have disappeared
STUDY PROCEDURES	1. Site Visit 1 (screening/inclusion visit at trial site): - General and headache-specific anamnesis - Pulse oximeter spot check and anaemia test - Randomization 2. Teleconference instruction in use of study device and study diary app 3. Home treatment (by participant) of up to four migraine-with-aura attacks. Symptom intensities are reported at the time points 0 (treatment start), 60 minutes, 120 minutes, 24 hours and 48 hours. Symptoms are: Headache Pain, Nausea/Vomiting, Photophobia, Phonophobia and Functional Disability, all rated on a 4-point scale (0 = none, 1 = mild, 2 = moderate, 3 = severe) 4. Follow-up calls via telephone

	5. Site Visit 2 (End-of-Stage 1 visit + Stage 2 instruction): 6. End-of-study follow-up via telephone

6 Study Flowchart

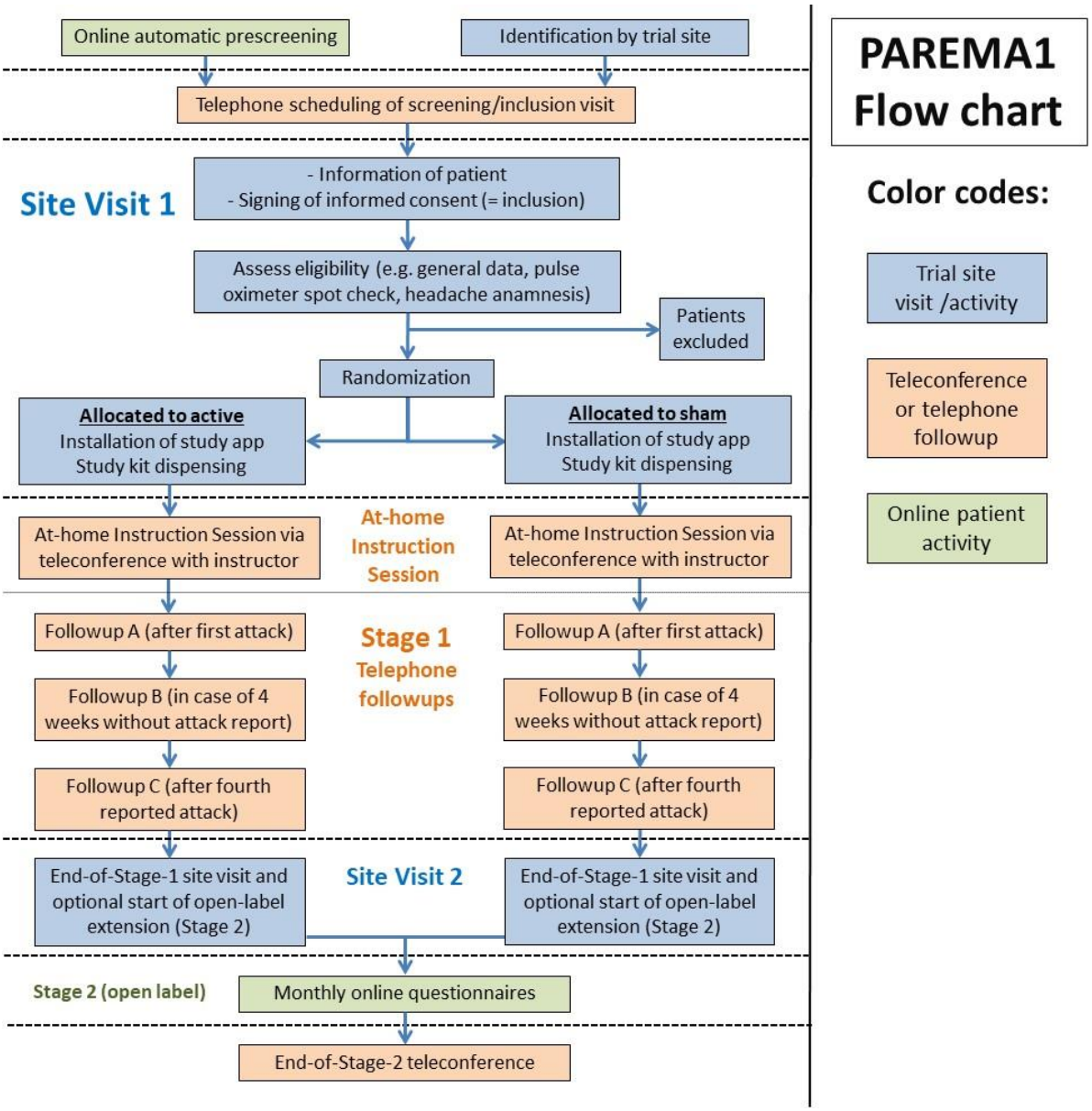


Figure 1: Participant contact flow chart, PAREMA1 clinical study

7 Abbreviations

ACT	Assessment of Current
AE	Adverse Event
ADE	Adverse Device Effect
AMSP2	Absence of moderate or severe pain at 2 hours (AMSP2) evaluated at the time point two hours after start of treatment, i.e. the percentage of participants who did not report a moderate (grade 2) or strong (grade 3) headache within two hours of starting the treatment.
C_{aO2}	Arterial oxygen content
CBF	Cerebral Blood Flow
CFR	Code of Federal Regulations
CGRP	Calcitonin Gene-Related Peptide
CHF	Congestive heart failure
CIP	Clinical Investigation Plan
CNS	Central Nervous System
COPD	Chronic Obstructive Pulmonary Disease
CRO	Contract Research Organization
CSD	Cortical Spreading Depression
DD	Device Deficiency, i.e. inadequacy of a medical device with respect to its identity, quality, durability, reliability, safety or performance
EDC	Electronic Data Capture
ECF	Extracellular Fluid
eCRF	Electronic Case Report Form
ePRO	electronic Patient Reported Outcome
ETCO2	End Tidal CO_2 tension, a widely used non-invasive proxy for arterial CO2 tension (P_{aCO2}), having high accuracy and precision in the patient group in question (individuals without pulmonary disease).
EU	European Union
FDS2	Functional Disability Score two hours after treatment initiation
FICO2	Fraction of CO_2 in inspired air (%)
FR48	Freedom from Relapse at 48 hours
GABA	Gamma-aminobutyric acid
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation (EU)
HS2	Headache Score two hours after treatment initiation
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ICHD-3	International Classification of Headache Disorders 3, classification system of the International Headache Society, available online at https://ichd-3.org/

IDA	Iron Deficiency Anaemia
IEC	Independent Ethics Committee
IFU	Instructions for Use
IHS	International Headache Society
IMD	Investigational Medical Device
IRB	Institutional Review Board
ISO	International Organization for Standardization
kPa	Kilo Pascal
KRI	Key Risk Indicator
LPIS1	Last patient In Stage 1
LPOS1	Last Patient Out Stage 1
LPOS2	Last Patient Out Stage 2
LSS2	Light Sensitivity score two hours after treatment initiation
MA	Migraine with aura
MBS	Most Bothersome Symptom other than pain, chosen among nausea, phonophobia and photophobia.
mmHg	Millimetres of mercury
MDCG	Medical Device Coordination Group
NH	Normoxic Hypercapnia, i.e. an arterial CO_2 tension above the normal range (35 - 45mmHg), combined with an arterial oxygen saturation within the normal range (90-100%)
NMDA	N-methyl-D-aspartate
NS2	Nausea Score two hours after treatment initiation
P_{aCO2}	Arterial partial pressure of CO_2
PAREMA1	PArtial REbreathing for Migraine with Aura 1
PF2	Pain Freedom, 2 hours after starting treatment, i.e. the percentage of participants with grade 0 on the 4-point headache scale, two hours after treatment start.
PI	Principal Investigator
PS48	Participant Satisfaction evaluated at 48 hours after treatment initiation
RBM	Risk-based Monitoring
RCT	Randomized Controlled Trial
REC	Research Ethics Committee
RES24	Percentage of attacks with use of rescue medication from the 2 hours' time point until 24 hours
SADE	Serious adverse device effect
SAE	Serious adverse event
SC	Study Coordinator
SKIC	Study Kit Identifying Code
SOP	Standard Operating Procedure
SPF24	Sustained Pain freedom at 24 hours

S_{pO2}	Arterial oxygen saturation percentage measured with pulse oximeter
SSS2	Sound Sensitivity score two hours after treatment initiation
USADE	Unanticipated Serious Device Effect
VARS	Visual Aura Rating Scale

8 Background

8.1 Disease State and Current Treatments

Migraine affects 14.7% of the world's population and is the most common neurological disease, characterized by recurrent debilitating attacks that can last from a few hours to several days. The primary symptoms are severe headache, nausea, vomiting and light/sound sensitivity. In some cases, attacks are triggered by hunger, stress, sleep disturbances, exercise, or by certain foods, drinks or drugs. For women, migraine attacks often occur in conjunction with their menstrual cycles.

Migraine with aura (MA) is a subset of migraine also known as classical migraine, comprising about a third of migraine patients (Launer et al., 1999; Russell et al., 1996). MA attacks are preceded or accompanied by so-called aura symptoms, most commonly visual disturbances, somatosensory disturbances and/or speech-related problems. Manifestations of visual auras can be numerous, e.g. zigzag lines. Somatosensory symptoms can be manifested as tingling or numbness.

Aura symptoms are thought to be caused by the phenomenon Cortical Spreading Depression (CSD).

Current acute treatments for MA include the so-called triptans (serotonin receptor agonists), over-the-counter pain-killers, opioids, anti-nausea medications, the recently approved so-called gepants (calcitonin gene related peptide (CGRP) receptor antagonists) and others. However, these medications are only moderately effective and often poorly tolerated, causing significant side effects and restricting use in many sub-groups (adolescents and children, pregnant and breast-feeding women, heart disease patients and more). Furthermore, too frequent use of migraine medications is a significant problem which often leads to a vicious cycle of medication overuse headache with headaches most days.

In a survey of more than 15,000 US migraine patients, 74% reported that they were not fully satisfied with their current acute treatment (Lipton et al., 2019).

A number of neurostimulation devices have been marketed for migraine but are not widely adopted or reimbursed, due to high prices, and (for many patients) side effects and limited effectiveness.

Consequently, there is a need for new, effective and affordable non-pharmaceutical migraine treatments.

8.2 Investigational Device and Usage

The active device in the PAREMA study is the Rehaler device, a single-use Class I medical device produced by the medical technology company Rehaler A/S (Denmark).

The intended use of the device is early treatment of acute attacks of migraine with aura in patients of ages 18+, in order to stop or alleviate attacks of migraine with aura.

The device was previously CE-marked under the Medical Device Directive (European Union Directive 93/42/EEC), after a randomized, controlled clinical study at Aarhus University Hospital (Denmark) had shown a favorable risk/benefit ratio. The device was marketed on a limited scale from October 2018 to April 2020 in Germany, Denmark and Sweden, a total of 1450 patients having bought and used the device in this time period. The indication for use of the device was migraine with aura. The post-market surveillance data collected in this period confirmed the low risk profile and beneficial treatment effect of the device.

The device has not yet been re-certified under the Medical Device Regulation (Regulation 2017/745 of the European parliament) which went into effect in 2021, and the device is therefore not currently being marketed.

The Rehaler device works through accurately balanced partial rebreathing, meaning that part of the expired air is captured and subsequently rebreathed together with a controlled amount of atmospheric air. The physiological effect of the Rehaler device is to induce steady state moderate hypercapnia with normoxia (NH), i.e. a moderate increase in arterial CO_2 tension combined with an arterial oxygen saturation within the normal range, no matter how long the device is used. *Moderate* hypercapnia is here defined as an increase in the CO_2 content of the user's body which is low enough to not cause discomfort and which is not detrimental to the health of the user. Though CO_2 comfort levels vary, this will generally mean that the increase in the arterial partial pressure of CO_2 (P_{aCO2}) should be less than 30%.

The user breathes through the mouthpiece from the beginning of the aura symptoms until they disappear.

The Rehaler device is shown in Figure 2 to Figure 5. Figure 3 shows the device in use.

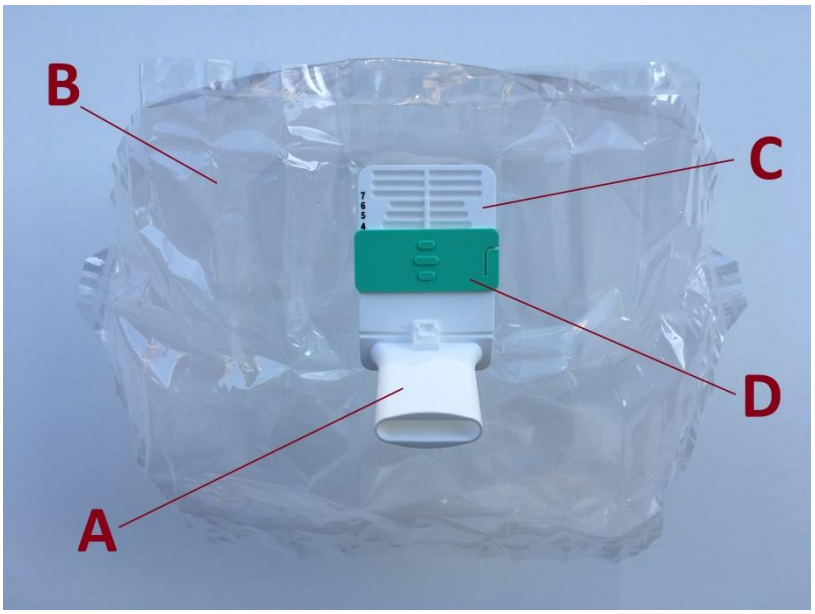


Figure 2: The Rehaler device, showing the mouthpiece (A), rebreathing chamber (B) and valve unit (C) with regulator (D).

When using the device, the user breathes through the mouthpiece (A) while wearing a nose clip. Part of his/her expired air is captured in the rebreathing chamber (B), while the rest of the expired air exits the rebreathing chamber through the slits in the valve unit (C). When the user subsequently inspires, the air collected in the chamber is re-inspired along with an amount of fresh air entering from the atmosphere through the slits in the valve unit.

Figure 3: The Rehaler device in use

The concentration of CO_2 in the inspired air can be regulated by means of the green regulator (D) mounted on the valve unit, from a minimum of 1.5% to a maximum of 2.5%. This will in turn increase the user's End Tidal CO_2 tension[1] (ETCO2) by between 10 and 30% above the normal level.

The inflow of fresh air through the slits in the valve unit can by design never be completely blocked, meaning that there is an absolute upper limit to the induced CO_2 concentration in the inspired air, and that the user at all times retains normal oxygen saturation no matter the duration of the treatment. As shown in Figure 5, this is primarily accomplished by the regulator's design preventing it from moving into a position in which it would cover the always-open slits.

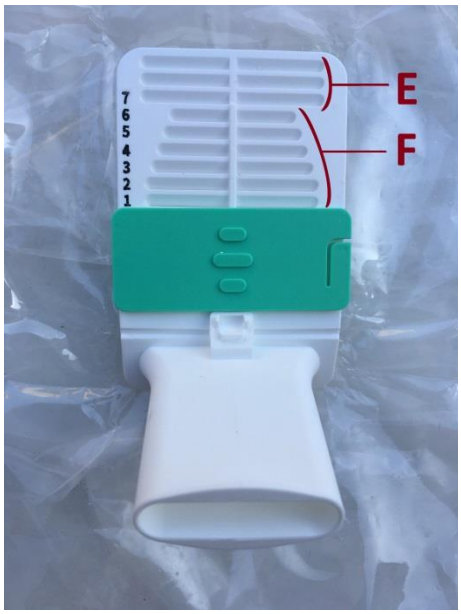


Figure 4: The maximum CO_2 level is attained by setting the regulator at level 7, in which case all the adjustable slits (F) are closed but air can still flow through the always-open slits (E) at the top.

If using excessive force it is theoretically possible that a user could push the regulator above the protrusion G (see Figure 5). In that case between one and three of the always-open slits (E) would become blocked, but moving the regulator in this way would simultaneously un-block the same number of adjustable slits (F). As a consequence, at least three slits will always remain open, even in cases of user error.

[1] End Tidal CO2 (ETCO2) is a widely used non-invasive proxy for arterial CO2 tension (P_{aCO2}), having high accuracy and precision in the patient group in question (individuals without pulmonary disease).

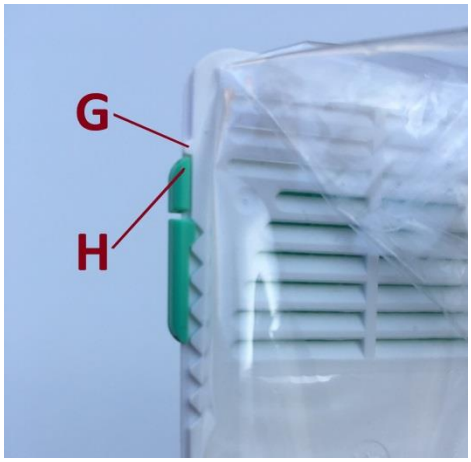


Figure 5: Valve unit seen from the rebreathing chamber side (i.e. the side facing away from the user), showing how the regulator's top corner (H) is prevented from moving above level 7 by a protrusion (G) on the edge of the valve unit.

By these design measures, the Rehaler has been provided with an in-built safety measure which prevents it from erroneously being used at a setting leading to oxygen desaturation or excessive CO_2 increases.

There are some specific cases of incorrect device use in which the user's oxygen saturation could conceivably drop below the normal level while using the device. These are specifically cases where a user doesn't follow the instructions for use as it pertains to A) that he/she should not suffer from the contraindication pulmonary disease, and B) that the device should not be used at atmospheric pressures below 80 kPa (reached at altitudes above 2000 meters and in most pressurized aircraft flying above that altitude). To ensure user safety the following precautions are taken:

1. Study participants' oxygen saturation at baseline is measured at Site Visit 1, with a high quality pulse oximeter (cf. section 8.7). Participants who have a baseline oxygen saturation level below 95% will not be included in the study.
2. Participant instructions (verbal and written), Instructions for Use and study kit label clearly state the conditions for use, and instruct the user to decrease the regulator setting if they experience any problematic discomfort or side effects (see the document ***PAREMA1 Instructions for Use***).

8.2.1 Device materials and contact with user

Table 1 lists the device components in contact with the patient, the material they are constructed from, and the degree of patient contact.

Parts	Material	Patient contact and duration
Mouth piece and valve unit	Polypropylene granulate (PP mb 4% white 0A900355MA)	Direct mucosal contact (up to two hours)
Rebreathing chamber	Transparent cast polypropylene (CPP) film	Indirect contact
Regulator	Thermoplastic polymer Acrylonitrile Butadiene Styrene (ABS)	Direct contact with intact skin (few seconds)

Nose clip component 1: Inner aluminium core	Aluminium	Indirect contact
Nose clip component 2: Thermoplastic casing	Mediprene type 500200M-02	Direct contact with intact skin (up to two hours)

Table 1

The device materials have been chosen specifically for their well-documented biocompatibility, and full biocompatibility evaluation has been carried out according to ISO 10993 and ISO 18562 (results available in Investigator's Brochure)

The device is indicated for use up to two hours continuously. Reapplication of the device is allowed, though limited in the PAREMA1 study (cf. the study IfU) to 12 hours in total, and a break of 30 minutes is indicated if the device has been used for two hours continuously.

The device components and the finished device are non-sterile, but produced in a controlled environment.

8.3 Intended Use and Population

The intended use application of the Rehaler is early treatment of acute attacks of migraine with aura in patients of ages 18+ to stop or alleviate migraine with aura attacks. The user breathes through the mouthpiece from the beginning of the aura symptoms until they disappear.

Migraine with aura is defined by the International Classification of Headache Disorders 3 (ICHD-3), category 1.2, according to the diagnostic criteria A to D below:

A. At least two attacks fulfilling criteria B and C
B. One or more of the following fully reversible aura symptoms:
    - visual
    - sensory
    - speech and/or language
    - motor
    - brainstem
    - retinal
C. At least three of the following six characteristics:
    - at least one aura symptom spreads gradually over ≥5 minutes
    - two or more aura symptoms occur in succession
    - each individual aura symptom lasts 5-60 minutes
    - at least one aura symptom is unilateral
    - at least one aura symptom is positive
    - the aura is accompanied, or followed within 60 minutes, by headache
D. Not better accounted for by another ICHD-3 diagnosis.

The study will include individuals aged 18 to 65, and exclude individuals with chronic pulmonary disease (e.g. COPD or pulmonary fibrosis), severe cardiovascular disease, cerebrovascular disease, anaemia, brain abnormalities, as well as pregnant women (see section 12).

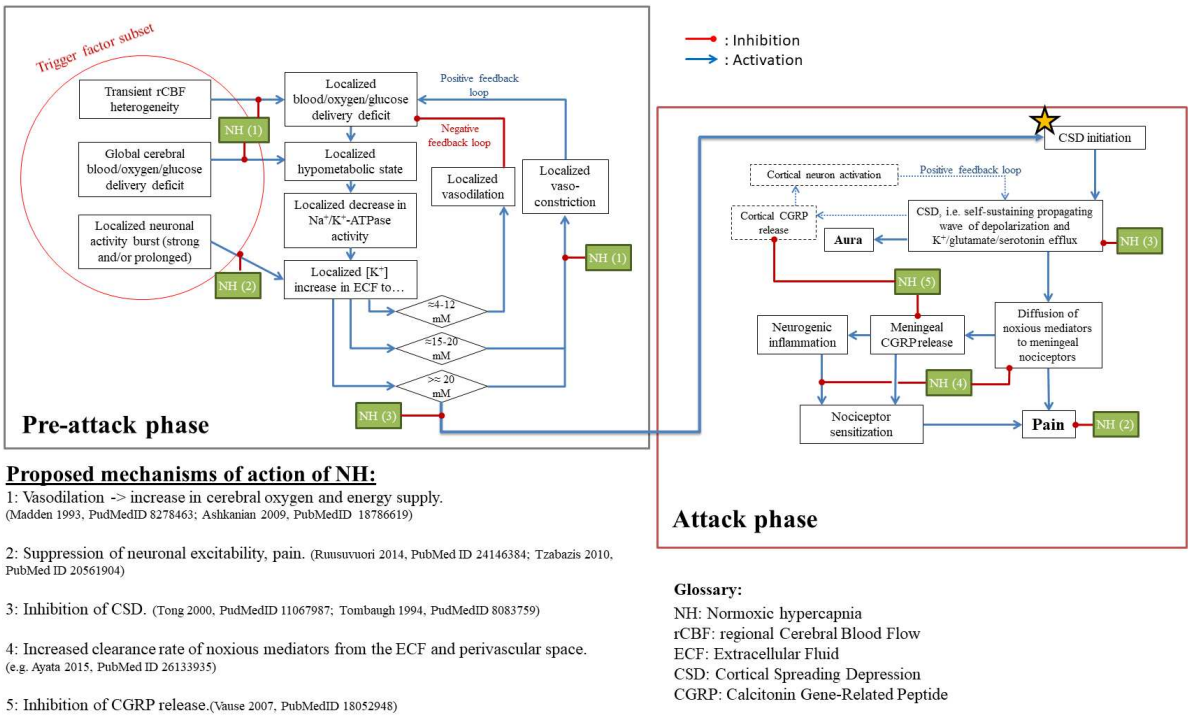
8.4 Scientific rationale for the investigational treatment

CO_2 inhalation has been known to be an effective abortive migraine treatment since a seminal 1950 paper by Wolff, and this has been confirmed since (Dexter, 1982; Marcussen and Wolff, 1950; Spierings, 2005), but until the Rehaler no CO_2-delivering device has existed that was at the same time safe (i.e. no risk of hypoxia or severe acidosis), compact (i.e. small enough to fit in a pocket) and practical (i.e. needing no power supply or gas refilling, and not requiring the user to breathe in any particular way).

Clinical, animal, brain slice and cell studies point to a number of interlinked physiological mechanisms that likely play a role for the observed migraine treatment effectiveness of normoxic hypercapnia (NH), as induced by the Rehaler:

1. Introducing moderate NH allows the cerebral oxygen/glucose delivery to be increased by up to 50% over baseline by increasing cerebral blood flow (Ashkanian et al., 2008; Fuglsang et al., 2018; Johansen, 2017); this has particular relevance in migraine with aura (MA) because imaging studies show that cerebral hypoperfusion is the norm before and long into MA attacks (Olesen et al., 1990), implicating hypoperfusion and resulting local hypoxia as a migraine trigger. This hypothesis is supported by the facts that A) hypoxemia reliably triggers attacks with and without aura in migraine patients (Arngrim et al., 2016), B) imaging studies show impaired regulation of cerebral oxygen/energy homeostasis in migraine patients (Lisicki et al., 2018) and C) local tissue hypoxia is known to induce and perpetuate Cortical Spreading Depression (CSD) (Ayata and Lauritzen, 2015; von Bornstädt et al., 2015) – the migraine trigger phenomenon implicated in MA. As one of the most effective cerebral vasodilators, and by far the fastest-acting, CO_2 elicits a marked increase in cerebral blood flow (CBF) within ten seconds (Claassen et al., 2007; Madden, 1993), counteracting local brain tissue hypoperfusion and increasing global cerebral oxygen/energy supply markedly (Ashkanian et al., 2009, 2008; Fuglsang et al., 2018; Johansen, 2017).
2. Whether or not CSD is, in a given case, elicited by local hypoxia/ischemia, NH has been shown to markedly inhibit CSD triggering and propagation (Tombaugh, 1994; Tong and Chesler, 2000). This inhibition likely stems partly from the capacity of NH to protect against spikes in the extracellular potassium level (a main factor in generation and propagation of the CSD wave (Pietrobon and Moskowitz, 2013)), by increasing the highly oxygen/glucose-reliant activity of Na^+/K^+-ATPase (Balestrino et al., 1999; Chang et al., 2013) as well as by raising the CBF-mediated clearance rate of potassium from the extracellular fluid (ECF) (Ayata and Lauritzen, 2015). Such NH-mediated increases in advective clearance from the ECF also accelerate the removal of other mediators released during CSD (ibid.) and implicated in down-stream activation of meningeal nociceptors, e.g. glutamate, nitric oxide and serotonin (Pietrobon and Moskowitz, 2013).
3. In addition, hypercapnia and the resulting acidemia have been shown to reduce the excitability of neurons, by a number of mechanisms (Ruusuvuori E. and Kaila K., 2014; Somjen and Tombaugh, 1998; Vause et al., 2007) including increase of resting membrane potential, increase of firing threshold, decrease of impulse conduction velocity, inhibition of $GABA_A$ and NMDA receptors, voltage-gated and acid-sensitive ion channels, and inhibition of Calcitonin Gene-Related Peptide (CGRP) release (Vause et al., 2007).

Figure 6 is a schematic overview of the proposed mechanisms of action responsible for NH's effects on migraine-with-aura attacks:



Proposed mechanisms of action of NH:

1: Vasodilation -> increase in cerebral oxygen and energy supply. (Madden 1993, PudMedID 8278463; Ashkanian 2009, PubMedID 18786619)

2: Suppression of neuronal excitability, pain. (Ruusuvuori 2014, PubMed ID 24146384; Tzabazis 2010, PubMed ID 20561904)

3: Inhibition of CSD. (Tong 2000, PudMedID 11067987; Tombaugh 1994, PudMedID 8083759)

4: Increased clearance rate of noxious mediators from the ECF and perivascular space. (e.g. Ayata 2015, PubMed ID 26133935)

5: Inhibition of CGRP release.(Vause 2007, PubMedID 18052948)

Glossary:

NH: Normoxic hypercapnia
rCBF: regional Cerebral Blood Flow
ECF: Extracellular Fluid
CSD: Cortical Spreading Depression
CGRP: Calcitonin Gene-Related Peptide

Figure 6 Physiological mechanisms of action proposed to be involved in the effect of normoxic hypercapnia (NH) on migraine-with-aura attacks

8.5 Report of Prior Investigations

8.5.1 Data on CO2 treatment from the scientific literature

A large number of clinical studies have in the past investigated the safety and physiological effects of increasing CO2 in the inspired air, including the following categories of studies:

- Investigating the increase in breathing depth and frequency elicited by increasing inspired CO2, e.g. (Schaefer, 1958).
- Investigating cerebrovascular reactivity, i.e. the increase of cerebral blood flow elicited by increasing CO2 in the inspired air, e.g. (Madden, 1993). CO2 inhalation is often used for this purpose (Liu et al., 2019).
- Use in combination with radiotherapy, to increase the effect of the latter, e.g. (Kaanders et al., 1998).
- Investigating physiological effects of an increased inspired CO2 administered over hours or days, e.g. (Elliott et al., 1998)

The effects of increasing inspired CO2 are thus well-studied and has been shown to be highly safe in the concentration range elicited by the Rehaler device (1.5 to 2.5%), as shown in e.g. (Guillerm and Radziszewski, 1979; Schaefer, 1958).

8.5.2 Device performance data relating to the intended physiological effect

As mentioned, the intended physiological effect is partial rebreathing inducing steady-state moderate hypercapnia without incurring hypoxemia.

The device has in previous, functionally equivalent, designs been tested on patients as part of the pilot studies CapnoMigra (migraine patients, (Fuglsang et al., 2018)) HVMASKE (patients with chronic hyperventilation, (Johansen et al., 2013)) and EpiCapno (patients with a history of petit mal epilepsy),

in which it was confirmed that the bodily CO_2 level increases into the desired therapeutic range, with negligible effects on arterial oxygen saturation and no SADE's (Fuglsang et al., 2018; Johansen, 2017; Johansen et al., 2013).

In the study HVMASKE, six participants used a functionally equivalent device continuously for two hours at home, each day for four weeks. No SADE's occurred.

In the study EpiCapno a functionally equivalent earlier device version was used in ten participants who were monitored during 1) a baseline period of 45 minutes without the device, 2) a period of 45 minutes using the device, and 3) a control period of 15 minutes without the device. No SADE's occurred.

Figure 7 shows average traces of ETCO2 and pulse oximeter saturation (S_{pO2}) for all ten participants in the EpiCapno study, demonstrating that ETCO2 increased to a new stable level after the participants started using the device, and fell back to the baseline level after it was discontinued.

The lowest oxygen saturation measured while using the device was 96%, and the highest increase in ETCO2 was 8.5 mmHg (1.13 kPa), corresponding to a 22% increase from baseline.

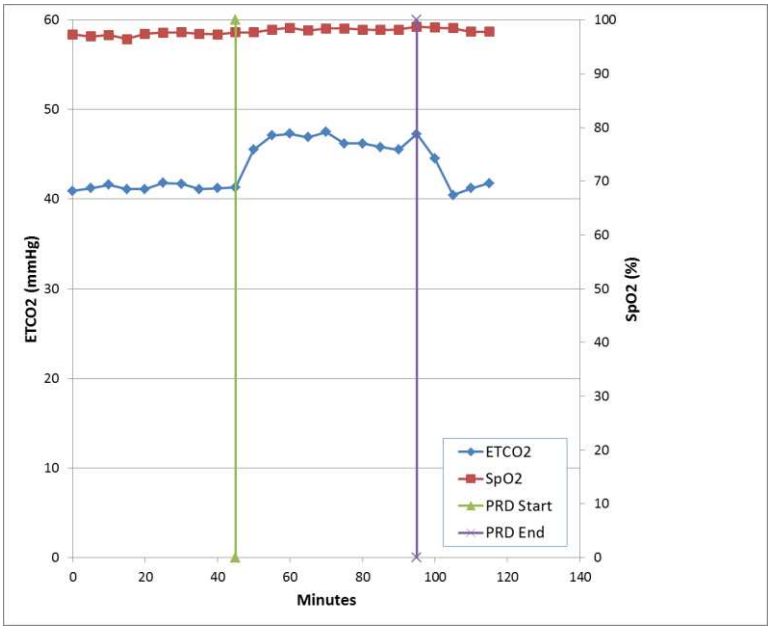


Figure 7: Average traces for ten participants of End Tidal CO2 and oxygen saturation, before, during and after use of a functionally equivalent PRD

In a study on one 34 year old male test subject, an indwelling arterial cannula was inserted, and arterial blood gases obtained before and during use of a functionally equivalent device. The arterial blood gas samples showed an increase in P_{aCO2} from 5.4 to 6.7 kPa (an increase of 24%), with only a very small drop in arterial oxygen content (C_{aO2}) from 20.6 ml/dl to 20.3 ml/dl, confirming that hypoxemia is unlikely to occur in use, provided that the user does not suffer from serious pulmonary disease or anaemia.

In the pilot study CapnoMigra (Fuglsang et al., 2018), 18 patients used the device at the hospital for between 15 and 25 minutes while being monitored with capnography and pulse oximeter. 11 patients subsequently used the device at home in two treatments, each with a duration of 20 minutes. In the hospital tests, the mean baseline ETCO2 was 36.8mmHg (range 26–41 mmHg). Using the device,

ETCO2 increased to a mean of 45.8mmHg (range 39 to 51); that is, a mean increase of 9.0mmHg (equal to a 24% increase compared to baseline).

The average oxygen saturation at baseline was 98.7% (range from 95% to 100%), compared to 97.3% during use of the device (likewise range 95 to 100%). There was thus no indication that the device leads to clinically relevant desaturation.

After the completion of the Capnomigra study, the device design was modified in the following ways:

- Reduction of weight from 60 to 10 grams, allowing hands-free use
- Device constructed in one piece, thereby removing the need to assemble the device before use – in turn reducing the likelihood of user error
- Reduction of the maximum rebreathing level which the device can be set at

Technical and clinical tests of the Rehaler device have shown that the final Rehaler device is functionally equivalent to the earlier version tested in the Capnomigra study, apart from a reduction in the maximum attainable concentration of inspired CO_2 from 2.9% to 2.5% (see Investigator's Brochure, section 11).

Overall, these clinical and technical data confirm that the Rehaler's physiological effect matches the intended effect, and that the device is safe for use by the patient population in the study.

8.5.3 Device performance data relating to the indication for use (migraine with aura)

Past clinical studies have shown that increasing inspired CO_2 (in turn inducing moderate hypercapnia) is effective in aborting a high proportion of migraine attacks (Dexter, 1982; Marcussen and Wolff, 1950; Spierings, 2005) and post-spinal headaches (Sikh and Agarwal, 1974). The studies by Marcussen and Dexter found that the effect was particularly effective in migraine with aura, and particularly when starting treatment already in the aura phase.

In contrast to the CO_2-delivering devices used to treat migraine in the studies cited above, the Rehaler instead operates by means of the partial rebreathing principle, yielding a number of advantages:

- Safe, since it does not incur hypoxia – no matter how long the device is used. In addition, the CO_2 level achieved is moderate, adjustable and stable, allowing the user to be in the correct treatment window for as long as needed.
- Practical: because it does not use gas bottles, the Rehaler is very lightweight (10 grams) and compact (can easily fit in a pocket), and does not require refilling.
- Ease of use: the user does not need to control his/her breathing in any particular way while using the device, but can relax and breathe normally.

The Rehaler treatment for migraine was from 2016-2017 tested in the aforementioned randomized, controlled, double-blind cross-over pilot study that included 11 participants with migraine with aura, treating at home for 20 minutes at the onset of aura. The study was conducted with a prototype of the Rehaler with the same effect on inspired CO_2 and oxygen as the current mass-produced device, compared against a non-rebreathing sham comparator device. The study was conducted at the Headache Clinic at Aarhus University Hospital (Denmark), and the results were in August 2018 published in the peer-reviewed headache journal Cephalalgia (Fuglsang et al., 2018).

The study found the following results:

- Absence of moderate or severe pain at two hours was statistically and clinically superior to sham ($p < 0.05$), and notably increased with each use of the active device (first attack: 45%, second attack: 78%).

- User satisfaction was superior to sham at the 5% significance level.
- The partial rebreathing device increased the mean body CO_2 level by 24%, while retaining mean oxygen saturation above 97% (no S_{pO2} level lower than 95% was recorded).
- No serious adverse events or oxygen desaturations were seen.

In addition, the study identified a number of potential improvements that were subsequently implemented in the device used in this clinical study, including:

- Reduction in size and weight of the device, making it possible to use the device hands-free.
- Integrating all device parts in one device, removing the need to assemble the device before use.
- A better effectiveness is achieved by using the device throughout the aura phase, and not (as in the pilot study) stopping after 20 minutes even if the aura is still present.

8.5.4 Post-market data

The Rehaler device was CE approved (Class I) in 2018 and put on the market on a limited scale from October 2018 until April 2020, in Denmark, Sweden and Germany. During this period, data and feedback was collected from users in order to ensure safety as well as improve the device, instructions for use and other aspects of the treatment.

If the present clinical study is successful, we intend to CE certify the device according to the new EU regulations (MDR), followed by resuming marketing of the device.

As detailed in the Investigator's Brochure, out of a total of 1450 patients that bought a Rehaler Starter Kit from 2018 to 2020, only two serious incidents occurred, the first with a patient who had the indication for use but also had intracranial hypertension, and the second with a patient who did not have the indication for use and who had previously undergone stenting of an unruptured brain aneurysm. In both incidents, the adverse event reported was headache.

Subsequently, the list of contraindications was expanded in order to prevent the occurrence of such incidents again in the future. Specifically, the following contraindications were added to the labelling and IfU:

- Previous aneurysm and/or brain surgery, including stenting
- Intracranial hypertension

Neither of the incidents qualified as Serious Adverse Events as defined in the MDR regulation (article 2, paragraph 58), since they did not incur a risk of death or permanent impairment, or led to hospitalization or a need for an acute medical or surgical intervention.

8.6 Sham device

NB: The details below concerning the sham device must not be communicated to the study participants.

The sham device (device to the left in Figure 9 and Figure 10) only has a minimal effect on P_{aCO2} because the air flow is redirected by means of a one-way check valve. During exhalation, the expired breath is allowed by the check valve to enter the rebreathing chamber, wherefrom it is expelled to the atmosphere over approximately two seconds, through the valve unit's slits. During inhalation, the inspired air is drawn in through three holes in the underside of the mouthpiece (see Figure 10). In this way the design of the sham device ensures that it looks very similar to the active device (also when in

use), but that minimal rebreathing occurs. A series of technical tests with the sham device found that the inspired CO_2 percentage (FICO2) with the sham device was less than 0.1% (cf. Figure 8) and no clinically or statistically significant changes in ETCO2 occur during use.

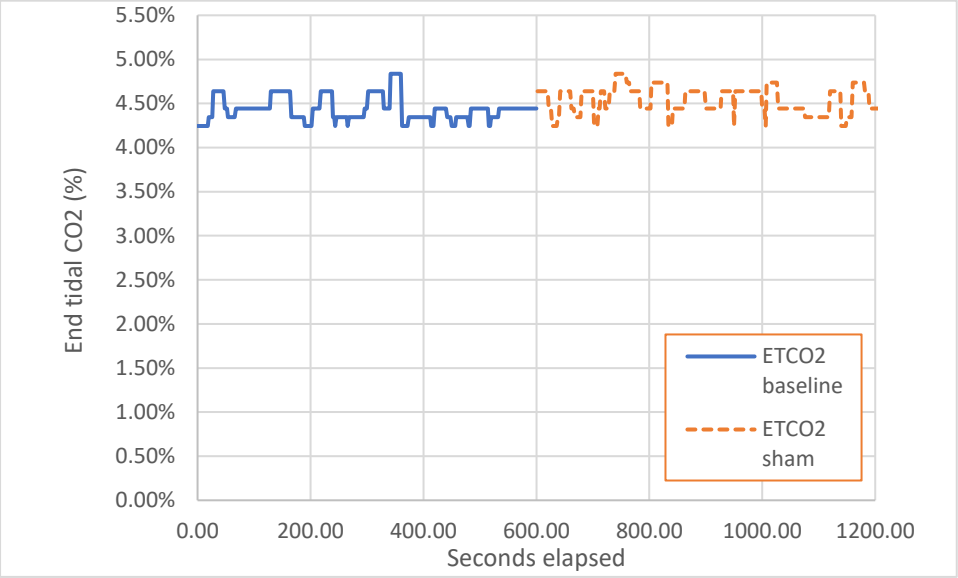


Figure 8: ETCO2 at baseline (10 minutes) followed by the sham device (10 minute). Average ETCO2 levels: 4.44% (baseline) vs. 4.55% (sham), Average FICO2 levels: 0.00% (baseline) vs 0.07% (sham).

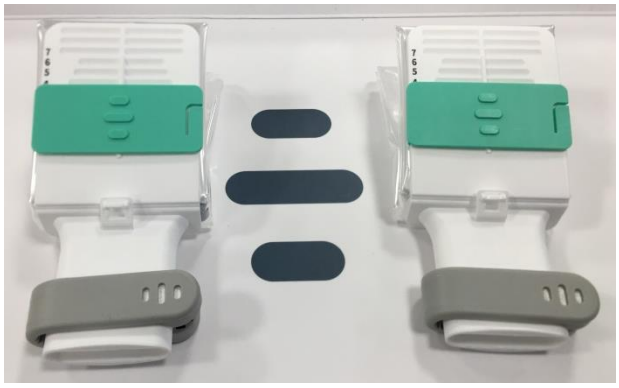


Figure 9: Active device (right side) and sham (left side)

Figure 10: Active device (right side) and sham (left side), devices facing upside-down.

8.7 Pulse Oximeter

The pulse oximeter used at Site Visit 1 is the CE and FDA approved Nonin PalmSAT® 2500. This has been proven in clinical studies to have superior accuracy to other models and provide accurate measurement irrespective of skin color or pulse strength (Feiner et al., 2007).

This pulse oximeter is not susceptible to calibration drift and so does not require continual calibration during the study.

8.8 Anaemia analyser

The anemia test device is the CE certified and FDA cleared DiaSpect Tm hemoglobin analyzer (Diaspect Medical GmbH, Barleben, Germany) which measures the total hemoglobin level (grams/deciliter) in capillary blood.

The cut-off point for exclusion is a hemoglobin level below 11.0 g/dL, in accordance with WHO guidelines (WHO, 2011).

8.9 Study diary smartphone app

In the study, the electronic Patient Reported Outcome (ePRO) smartphone app software TrialKit will be used for capturing patient symptom data (cf. section 17 and the Data Management Plan for the study). The ePRO app is linked directly to the study's Electronic Data Capture (EDC) system.

9 Justification for the design of the clinical investigation

Bench tests, pre-clinical data, clinical data and post market data are available for previous versions of the Rehaler device and support the design concept, performance claim and safety claim. The purpose of the proposed clinical pivotal study is to collect further safety and performance data using the current design of the device (Rehaler device) in patients undergoing migraine attacks with aura.

10 Objective and Hypotheses

10.1 Objective

The primary objective of the study is to assess the effectiveness and safety of the Rehaler device as treatment of migraine with aura when used during the migraine aura phase, compared to a sham device.

Effectiveness will be assessed by measuring the percentage of attacks in which moderate or severe headache was not present two hours after treatment initiation, with respectively the active and sham device.

10.2 Hypothesis

It is the primary hypothesis of the study that early treatment with the Rehaler device increases the likelihood of having no or only mild headache two hours after starting the treatment, compared with a sham device. "Early treatment" is here defined as treatment that is initiated during the aura phase, within ten minutes of aura symptoms onset, and before the start of moderate or severe migraine

headache. The primary endpoint is the absence of moderate or severe pain at 2 hours (AMSP2) after early treatment.

The primary effectiveness hypotheses are:

H_0: $p_{Rehaler} = p_{sham}$ (null hypothesis)

H_a: $p_{Rehaler} \neq p_{sham}$ (alternative hypothesis)

Where:

$p_{Rehaler}$ = proportion of attacks in the Rehaler group with AMSP2

p_{sham} = proportion of attacks in the sham group with AMSP2

The primary effectiveness hypothesis will be tested using a two-sided adjusted χ^2 test for clustered binary data developed by Donner and Banting (Donner and Banting, 1988), with a 5% level of significance.

10.3 Study Design Description

The study has two consecutive stages:

Stage 1 is a prospective, multi-centre, randomized, double-blind, sham-controlled, parallel-group, group-sequential study. The RCT design has been chosen to obtain high quality clinical data.

Stage 2 is an open-label extension with the active device (i.e. irrespective of the type of device (active/sham) used in Stage 1). At the start of Stage 2 the participant receives five devices for home use. This stage is included to provide more data on the consistency of the treatment response.

Each study participant will have two site visits:

- Site Visit 1 which is the screening/inclusion visit at which the participant starts their participation in Stage 1 (the randomized, controlled part of the study)
- Site Visit 2 which is the end of Stage 1 and start of Stage 2 (open-label extension)

10.3.1 Number of subjects and duration of study

A minimum of 174 participants and a maximum of 220 participants will be randomized in this clinical study at up to 15 sites in the EU and the United States. The enrolment of participants is intended to start in Q1 of 2023 and continue until the target number has been enrolled (“*Last Patient In Stage 1”* (LPIS1)), which is estimated to occur in Q2 of 2025. From the point of LPIS1, there will be a period of six months until conclusion of Stage 1 for all participants *(“Last Patient Out Stage 1”* (LPOS1)), estimated to occur in Q4 of 2025.

Individual participants will participate in Stage 1 until either of the following conditions are met:

1. The participant has treated and reported four attacks
2. The overall conclusion of Stage 1 (six months after LPIS1)
3. The participant no longer wishes to participate or is lost to follow up.

Participants meeting condition 1 or 2 will after concluding Stage 1 have the option of continuing in Stage 2 (open-label extension) which has a maximum duration of 12 months. Individual participants will participate in Stage 2 until either of the following conditions are met:

1. The participant has used all five devices received when starting Stage 2
2. 12 months have passed since the participant’s inclusion in Stage 2.
3. The participant no longer wishes to participate or is lost to follow up.

The study is concluded when all participants have concluded Stage 2 (*"Last Patient Out Stage 2"* (LPOS2)).

The maximum estimated duration of total participation for an individual participant (Stage 1 + Stage 2) is 27 months.

10.3.2 Measures to minimize bias

Design Attribute	Justification
Prospective	A prospective study design will eliminate the bias associated with case selection in a retrospective review and will ensure that identical procedures are followed for data capture and review.
Multi-centre	Inclusion of multiple study centres will reduce the impact of bias associated with any one investigator, clinic population, or geography.
Randomized	Randomization will minimize selection bias and minimize differences in demographic variables.
Double-blinded	The participants will not be informed about whether they have received the active or sham device, until the conclusion of Stage 1. The blinding of the investigators is preserved by having the training of the patient be carried out by an instructor who is not a study investigator (the training process may unblind the instructor because he/she may be able to discern which type of device the participant is using). Blinding the participants and investigators in this way will minimize bias that might impact participant selection and participant outcome measures. In order to minimize bias during data analysis, the data analysis will be conducted by a biostatistician who is blinded with respect to which of the two treatment groups received respectively the active or sham device.
Sham-controlled	Use of a sham device will allow to control for the placebo effect, thereby increasing the accuracy of the evaluation of the treatment's effectiveness.

10.3.3 Blinding

To ensure optimal blinding of participants, the active and sham devices have been designed to look and feel as similar as possible (see section 8.6).

11 Endpoints

For this study to be considered successful, all primary performance and safety endpoints must be achieved. Data for all endpoints will be collected through electronic Patient Reported Outcome (ePRO). All end points have been selected with the aim of measuring effects and factors that are relevant and valuable for migraine patients.

All primary and secondary effectiveness endpoints are calculated based on data from Stage 1 of the study.

11.1 Primary effectiveness endpoint

Absence of moderate or severe pain at 2 hours (AMSP2), i.e. the proportion of attacks in which the participant reported absence of headache of moderate or severe intensity at 2 hours post-treatment initiation.

Headache is measured on a 4-point Scale (0 = None, 1 = Mild, 2 = Moderate, 3 = Severe).

The choice of ASMP2 as primary endpoint is in line with earlier studies on migraine treatment starting at the onset of aura (Bates D. et al., 1994; Olesen J. et al., 2004).

In studies treating before the onset of pain, AMSP2 is the equivalent to the endpoint *Pain Relief at 2 hours* (PR2) in studies treating after pain onset. PR2 has been used as the primary end point in other recent acute migraine treatment device studies (see (Yarnitsky et al., 2019) and ClinicalTrials.gov study NCT03631550).

11.2 Primary safety endpoint

- Incidence of device- or treatment-related adverse events
- Incidence of device- or treatment-related serious adverse events (SAEs) and/or serious adverse device effects (SADE)

11.3 Secondary effectiveness endpoints

1. **Pain Freedom at 2 hours (PF2)**, i.e. the proportion of attacks with absence of headache pain two hours after treatment initiation (pain score 0 on the 4-point scale)
2. **Freedom from Most Bothersome Symptom at 2 hours (MBSF2)**, i.e. the proportion of attacks with absence of MBS two hours after treatment initiation (symptom score 0 on the 4-point scale)
3. **Sustained Pain Freedom at 24 hours (SPF24)**, i.e. the proportion of attacks having no headache pain at 2 hours after dose, with no use of rescue medication and no relapse of headache pain within 24 hours (pain score 0 on the 4-point scale)
4. **Headache Score at 2 hours (HS2)**, i.e. headache score two hours after treatment initiation (headache measured on 4-point scale)
5. **Most Bothersome Symptom Score at 2 hours (MBS2)**, i.e. MBS score two hours after treatment initiation (symptom score measured on 4-point scale)
6. **Functional Disability Score at 2 hours (FDS2)**, i.e. Functional Disability Score two hours after treatment initiation (disability measured on 4-point scale)
7. **Proportion of attacks with use of rescue medication from the 2 hours' time point until 24 hours (Res24)**
8. **Participant Satisfaction at 48 hours (PS48)**, i.e. participant's global impression of acute treatment effect on attack, evaluated at 48 hours after treatment initiation (5 point scale: 5 = Satisfied, 4 = Partially Satisfied, 3 = Neutral, 2 = Partially Unsatisfied, 1 = Unsatisfied)
9. **Light Sensitivity Score at 2 hours (LSS2)**, i.e. photophobia score two hours after treatment initiation (photophobia measured on 4-point scale)
10. **Nausea Score at 2 hours (NS2)**, i.e. Nausea Score two hours after treatment initiation (nausea measured on 4-point scale)
11. **Sound Sensitivity Score at 2 hours (SSS2)**, i.e. phonophobia score two hours after treatment initiation (phonophobia measured on 4-point scale)

12. **Freedom from Relapse at 48 hours (FR48)**, i.e. the proportion of attacks having no headache pain at 2 hours after dose, with no use of rescue medication and no relapse of headache pain within 48 hours (pain score 0 on the 4-point scale)

11.4 Explorative endpoints

11.4.1 Stage 1 exploratory endpoints

1. Impact of the migraine attack on participant's work/school attendance and ability to do household chores, during the first 48 hours since the beginning of the attack (impact measured on 4-point scale)
2. Impact of the migraine attack on participant's social life and leisure time, during the first 48 hours since the beginning of the attack (impact measured on 4-point scale)
3. Absence of moderate or severe pain at 1, 24 and 48 hours (% of reported attacks)
4. Pain Freedom at 1, 24 and 48 hours (% of reported attacks)
5. Freedom from MBS at 1, 24 and 48 hours (% of reported attacks)
6. MBS Score at 1, 24 and 48 Hours (4-point scale)
7. Functional Disability Score at 1, 24 and 48 Hours (4-point scale)
8. Nausea Score at 1, 24 and 48 Hours (4-point scale)
9. Light Sensitivity Score at 1, 24 and 48 Hours (4-point scale)
10. Sound Sensitivity Score at 1, 24 and 48 Hours (4-point scale)
11. Patient-estimated aura duration (minutes)
12. Overall participant satisfaction at end of stage 1, i.e. participant's global impression of study device's treatment effect averaged over all study attacks (5 point scale: 5 = Satisfied, 4 = Partially Satisfied, 3 = Neutral, 2 = Partially Unsatisfied, 1 = Unsatisfied)
13. Difference in DASS-21 score from Site Visit 1 to Site Visit 2
14. Correlation between AMSP2 and the following parameters: Gender, age, body weight, ethnicity, nationality, pain level at treatment start, treatment delay at attack (i.e. from first aura symptoms to treatment start), regulator setting during attack, Visual Aura Rating Scale (VARS) score, baseline MA attack frequency, baseline headache days per month, baseline Migraine-ACT score, baseline DASS-21 score, in prophylactic migraine treatment or not
15. Correlation between PF2 and the following parameters: Gender, age, body weight, ethnicity, nationality, pain level at treatment start, treatment delay at attack (i.e. from first aura symptoms to treatment start), regulator setting during attack, Visual Aura Rating Scale (VARS) score, baseline MA attack frequency, baseline headache days per month, baseline Migraine-ACT score, baseline DASS-21 score, in prophylactic migraine treatment or not
16. Consistency of response among participants who treated three attacks during Stage 1
17. Use of rescue medication from time 0 until the 2 hours' time point
18. Bang Blinding Index

11.4.2 Stage 2 exploratory endpoints

1. Number of MA attacks during past month of Stage 2 and how many of these attacks were treated with the study device.

2. Overall satisfaction with study device treatment effect in attacks treated in past month of Stage 2 (satisfaction measured on 5 point scale: 5 = Satisfied, 4 = Partially Satisfied, 3 = Neutral, 2 = Partially Unsatisfied, 1 = Unsatisfied)
3. How many times in the past month of Stage 2 has the participant taken respectively a prescription acute migraine drug, an over-the-counter acute analgesic or treated with a neurostimulator device, with the intention to treat migraine. Follow-up question: What was the type and dose of drug taken?
4. Proportion of participants who used an opioid drug to treat migraine during past month of Stage 2.
5. Proportion of participants who have been hospitalized for migraine during past month of Stage 2.
6. Consistency of response among participants who treated three attacks or more during Stage 2.

12 Study population

Participants who meet all the inclusion criteria and none of the exclusion criteria and who are willing and able to provide written informed consent, may be enrolled in the study. Participants may be recruited from patients followed at the study sites for their migraine, or from the general population by advertisements online and in newspapers, as well as informational brochures and posters.

12.1 Eligibility

12.1.1 Inclusion criteria

Participants must meet all of the following inclusion criteria:

1. Participant has migraine with typical aura (ICHD3 classification 1.2.1)[2] with the additional criterion that historically in more than 75% of cases of aura a **moderate or severe** headache begins between 10 and 60 minutes after aura onset.
2. Participant has had 3 or more migraine-with-aura attacks over the last six months.
3. Participant is 18 to 65 years of age.
4. Participant's age at onset of migraine with aura was less than 50 years.
5. If participant is taking migraine prophylactic drugs, the dose must have been stable for three months or more.
6. Participant agrees to withhold usual acute migraine medications until at least two hours after treatment with the study device.
7. Participant does not plan to initiate new (and/or change existing) migraine prophylaxis medication for the duration of Stage 1 of the study.
8. For female participants: is willing to use adequate contraception during study participation
9. Participant owns a smartphone compatible with the ePRO study diary app.
10. Participant agrees to use the study device as intended, comply with all study requirements including treatment, follow-up visits, and recording required study data in the ePRO app.
11. Participant is willing and able to provide written informed consent.

[2]Migraine without Aura (MO) is NOT an exclusion criterion. Patients with Aura (MA) very often also have MO.

12.1.2 Exclusion criteria

Participants must meet none of the following criteria:

1. Participant has a history of chronic pulmonary disease (e.g. Chronic Obstructive Pulmonary Disease (COPD) or pulmonary fibrosis).
2. Participant has a history of severe cardiovascular disease (e.g. symptomatic coronary artery disease, prior myocardial infarction, congestive heart failure) or cerebrovascular disease (e.g. prior stroke or transient ischemic attack, symptomatic carotid artery disease, prior carotid endarterectomy or other vascular neck surgery).
3. Participant has a history of intracranial hyper/hypo-tension.
4. Participant has a history of cerebral aneurysm.
5. Participant has had previous brain surgery, including stenting.
6. Anaemia, defined as a hemoglobin concentration in capillary blood lower than 11 g/dL
7. Participant has a baseline S_{PO2} level which is lower than 95%
8. Participant has 15 or more headache days per month
9. Participant has medication-overuse headache (ICHD3 classification 8.2).
10. Participant has a known history or suspicion of recurring secondary headache which in the opinion of the investigator may interfere with the study.
11. Hemiplegic migraine
12. Participant has other significant and relevant pain problem (e.g. cancer pain, fibromyalgia or other head or facial pain disorders)
13. Participant has a known history or suspicion of substance abuse or addiction (within the last 5 years) that in the opinion of the investigator may confound the study assessments.
14. Participant has a history of psychiatric or cognitive disorder and/or behavioural problems which in the opinion of the investigator may interfere with the study.
15. Participant belongs to a vulnerable population or has any condition such that his or her ability to provide informed consent, comply with the follow-up requirements, or provide self-assessments is compromised (e.g. homeless, developmentally disabled, prisoner).
16. For female participants: is pregnant or actively trying to become pregnant.
17. Participant is participating in any other clinical investigation or has participated in an interventional clinical trial in the preceding 30 days.
18. Participant has any condition that according to the investigator may pose the participant at risk or provide confounding data.
19. Participant is unable, as perceived by study personnel, to correctly understand and follow the instructions for use of the device and ePRO app.
20. Sickle Cell Disease
21. Participant is being treated with nerve blocks or injections for migraine prophylaxis on a regularly scheduled basis (including Botox, Aimovig/Erenumab, Ajovy/Fremanezumab, Emgality/Galcanezumab or Vyepti/Eptinezumab).

13 Study procedures

Figure 11 below shows the flow chart for the study and Table 2 lists the schedule of events.

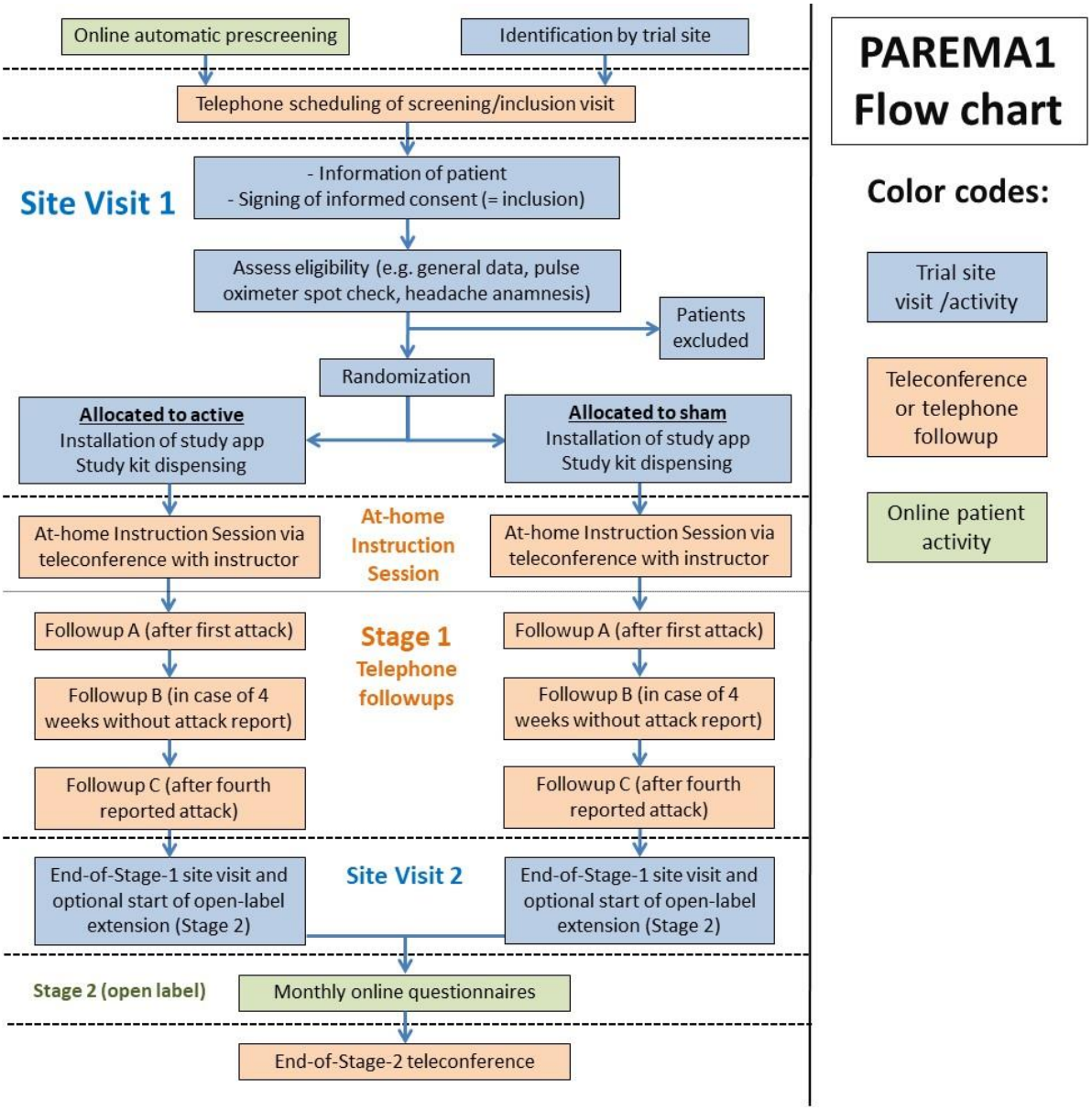

PAREMA1
Flow chart
Color codes:
Trial site visit /activity
Teleconference or telephone followup
Online patient activity
Online automatic prescreening
Identification by trial site
Telephone scheduling of screening/inclusion visit
Site Visit 1
- Information of patient
- Signing of informed consent (= inclusion)
Assess eligibility (e.g. general data, pulse oximeter spot check, headache anamnesis)
Patients excluded
Randomization
Allocated to active
Installation of study app
Study kit dispensing
Allocated to sham
Installation of study app
Study kit dispensing
At-home Instruction Session via teleconference with instructor
At-home Instruction Session
At-home Instruction Session via teleconference with instructor
Stage 1
Telephone followups
Followup A (after first attack)
Followup B (in case of 4 weeks without attack report)
Followup C (after fourth reported attack)
Followup A (after first attack)
Followup B (in case of 4 weeks without attack report)
Followup C (after fourth reported attack)
Site Visit 2
End-of-Stage-1 site visit and optional start of open-label extension (Stage 2)
End-of-Stage-1 site visit and optional start of open-label extension (Stage 2)
Stage 2 (open label)
Monthly online questionnaires
End-of-Stage-2 teleconference

Figure **11**

	Site Visit 1 (screening / inclusion)	At-home training session	Follow-up A	Follow-up B	Follow-up C	Site Visit 2	End-of-Stage-2 follow-up
Type of participant contact	Site visit	Tele-conference with instructor	Phone or tele-conference	Phone or tele-conference	Phone or tele-conference	Site visit	Phone or tele-conference
Time point	As soon as possible after successful online pre-screening or site identification	Maximally one week after randomization	Maximally one week after first reported attack	Within one week if the participant has not registered an attack in the past eight weeks	Maximally one week after 4 reported attacks	Maximally two weeks after Follow-up C	Maximally two weeks after participant has used all Stage 2 devices or 12 months after Site Visit 2
Procedures:							
Informed Consent	X						
Medical History	X						
Pulse oximeter test	X						
Anaemia test	X						
Pregnancy test (if applicable)	X						
Migraine ACT questionnaire (part of CRF)	X						
DASS-21 questionnaire (part of CRF)	X					X	
Medical history and migraine characteristics	X						
Eligibility evaluation	X						
Randomization	X						
ePRO/safety and DD training	X		X	X	X	X	
Device and ePRO training		X					
Device dispensing	X					X	
Device return						X	X (by mail)
Reimbursement of participant's costs						X	
AE/SAE/DD reporting			X	X	X	X	X
Deviation reporting	X		X	X	X	X	X

Table 2: Schedule of events and procedures during each contact

13.1 Identification of potential study participants

Study participants can be identified by either of two methods:

- Identification by the trial site personnel, from among the site's MA patients
- Online pre-screening

13.1.1 Identification by trial sites

Trial sites will be allowed to contact its MA patients, informing them about the study and inquiring about their interest in participating. The trial site personnel are allowed to contact such individuals via letter, telephone or in the clinic.

The sponsor will supply trial sites with informational brochures and/or posters about the study, for placing in relevant locations at the site (e.g. in waiting rooms), cf. the documents ***PAREMA1 information flyer*** and ***PAREMA1 advertisement***.

13.1.2 Online automatic pre-screening

This recruitment will be organized by the sponsor and will focus on areas near study sites.

Online and physical advertisements of the study will include a link to a pre-screening website with an online eligibility questionnaire to be completed by the potential participant before being invited to the study site for the screening/inclusion visit (Site Visit 1), cf. the document ***PAREMA1 Case Report Form content***. This pre-screening step aims to reduce the screen failure rate of the on-site visits. Before completing the pre-screening survey, the potential participant will be provided with a privacy statement and required to provide consent to being contacted by trial site personnel.

If the individual's answers in the online pre-screening conform to the in/exclusion criteria, they will receive the Informed Consent Form for their perusal, as well as the telephone number to the study site, and they will be asked to call in order to book a time for the Site Visit 1. The potential participant will in this process be required to provide their telephone number and email so that the study site has the option of contacting the patient.

The pre-screening website will be hosted by the sponsor on a secure server, and the telephone numbers and emails will be handled confidentially and in accordance with local and national data protection rules.

When the visit has been booked and entered in the study site's calendar, the potential participant will receive an email with information about the study and Site Visit 1. A text message and email reminder will also be sent to the patient 24 hours prior to the site visit.

13.2 Site Visit 1

13.2.1 Informed Consent

The potential participant is briefed by the PI or delegated study personnel (hereafter designated PI) about the study, including the study rationale, study plan, randomization to either study device or sham device, study design and possible side effects of the treatment. It is clearly explained to the patient that they may be assigned to either a functional (active) or non-functional (sham) device in Stage 1, that it is believed that the active device has the best treatment effect and that the participant is guaranteed to be assigned to this in Stage 2.

If the participant wishes, he/she can have up to a week to consider before deciding whether or not to participate (in that case, the visit will be stopped after Step 1 and resumed when the participant has decided).

A potential study participant considered for this study must be listed on a screening log to be maintained at the site. The PI must also enter in the participant's medical records that he/she is participating in the study. If the participant does not have a medical record at the study site, a new record must be started for him/her.

PI will instruct female participants of childbearing potential to practice effective contraception during their participation in the study.

13.2.2 Information to be recorded:

The following information is obtained and documented in the eCRF during Site Visit 1 (cf. document ***PAREMA1 Case Report Form content***):

- General eligibility questions (e.g. age, headache frequency, current participation in other clinical investigations)
- Eligibility questions related to migraine with aura diagnosis (presence of aura symptoms, frequency of MA attacks, age at MA onset)
- Eligibility questions related to headache drug use and overuse
- Eligibility questions related to comorbidities (e.g. pulmonary, cardiovascular and cerebrovascular disease)
- Eligibility questions related to secondary headaches (e.g. caused by head/neck trauma or infection)
- Pulse oximeter test result (see section 13.2.3)
- Capillary blood test for anaemia
- For female patients:
    - Whether the patient has gone through menopause
    - If patient has not gone through menopause: urine pregnancy test
- Other eligibility questions, including participant's willingness to abide by study instructions, and ability and willingness to provide informed consent.
- Migraine without aura (MO) diagnosis and frequency
- Miscellaneous baseline information (e.g. self reported height and weight, ethnicity, Most Bothersome Symptom during MA attacks, currently and previously used migraine drugs, questions regarding efficacy of current acute treatment, evaluation of depression, anxiety and stress via the DASS-21 (Depression Anxiety Stress Scales 21 item questionnaire))

13.2.3 Pulse oximeter test

The oxygen saturation (S_{pO2}) of the participant is measured with the pulse oximeter (see section 8.7). Initially, the study personnel should check whether the participant is wearing nail polish and/or false nails. In that case, the pulse oximeter finger probe must be placed on the finger at a 90 degree angle to avoid measurement inaccuracies.

The S_{pO2} measurement should be performed at baseline, after the participant has relaxed in a chair for one minute. The study personnel must enter the S_{pO2} reading at the one minute time point into the eCRF. The study personnel should during the one minute conduct small talk with the participant in order to make them relax and breathe normally, in order to avoid as much as possible that white coat

syndrome biases the reading. If the baseline S_{pO2} reading is lower than 95%, the patient is ineligible for the study.

13.2.4 Point of enrolment

A participant is considered finally enrolled at the time the informed consent form (ICF) is signed and all eligibility criteria are met. The PI is responsible for the selection and screening process at the site and will document eligibility in electronic CRF (eCRF). No study related tests or procedures will take place before informed consent is obtained. All participants that are successfully enrolled will be documented on the enrolment log. Participants not meeting eligibility criteria after having signed the ICF are considered screen failures and documented on the screening log. The reason for screen failure must also be documented. Excluded participants will at this point be informed about other treatment options. Screen failures are not included in the intended sample size. Screen failures may be enrolled at a later stage, if by then eligibility can be met. Participants can only be enrolled and participate in the study once.

13.2.5 General data capture

The participant will receive an email via the EDC system, with a link to download the ePRO app (iOS or Android), and with their unique Participant Identification Number (PIN) which is generated by the EDC system and is used as access code when entering data in the ePRO app.
At this point the PI must help the participant install the ePRO app on their smartphone. This includes:

- Setting up automatic login to the ePRO app via either thumb or face recognition (according to the participant's preference) – provided that this type of login is possible on the user's specific smartphone
- Turning on notifications for the Trialkit app, with all available types of notifications turned on (sounds, banners, popup messages and others).

13.2.6 Finalization of Site Visit 1

The participant receives their study device kit, which contains:

- Five study/sham devices. The Attack Checklist (cf. section 13.4.1) is printed on each device's packaging.
- PAREMA1 Instructions for Use (active or sham type as appropriate)
- Label on the study device kit's lid including the Study Kit Identifying Code (cf. Investigator's Brochure)

The participant's PIN is at this point written on the designated space on the packaging of all study devices, as well as on the study device kit's label.

The PI and participant then schedule the participant's at-home Instruction Session that takes place at home via teleconference between an instructor from the CRO and the participant (see section 13.3 below). The Instruction Session must be scheduled as soon as possible after Site Visit 1 (maximum one week later).

13.2.7 Summary of tasks during Site Visit 1:

Table 3: Tasks during Site Visit 1

TASKS, Site Visit 1:
1. Inform the participant about the study, including the study rationale, study plan and possible side effects of the treatment.
2. Participant signs informed consent form
3. General eligibility questions
4. Pulse oximeter test
5. Anaemia test
6. For female participants: Pregnancy test
7. Decision on eligibility for study according to checklist and investigator's evaluation
8. Randomization
9. Install ePRO study app on subject's smartphone
10. Patient receives study device kit and Instruction Session is scheduled

13.3 Instruction Session

In order to preserve the blinding of the investigator, the participant training and device configuration are performed via teleconference by a Contract Research Organization instructor. This Instruction Session takes place via teleconference and covers configuring and using the study device, reporting data via the ePRO app, and safety reporting.

The instruction session has five parts (for details refer to the document ***PAREMA1 Case Report Form Content***)

1. **Initial briefing of the participant**. This entails explaining the aims of the instruction session and what will happen during it. The participant is encouraged to ask any questions they have.
2. **Briefing on essential steps when an aura begins**. The instructor explains the steps the participant must follow when an aura begins, as described in Part 3 of the Instructions for Use and summarized in the Attack Checklist printed on the device packaging.
3. **Using the study device for the first time**. The participant follows the steps described in Part 1 of the IfU, in order to learn how to use the device and to find the optimal regulator setting. The instructor corrects any errors. Afterwards, the participant is instructed to write their optimal regulator setting on each of the attack checklists on the device packages.
4. **Learning how to report symptoms in the Trialkit app**. The participant follows the steps described in Part 2 of the IfU, in order to learn how to correctly report symptoms in the app. The instructor checks that the attack is correctly reported and registered in the EDC, and corrects any errors.
5. **Final instructions and reminders**. The participant describes the essential attack instructions in their own words to the instructor, and any errors are corrected. In addition, a number of general instructions and reminders are given to the participant (cf. ***PAREMA1 Case Report Form Content***)

13.4 Stage 1

In Stage 1 of the study, the participants will treat up to four attacks each, all of which with either the active or sham device. The treatment is started at the onset of aura symptoms and continues until the aura symptoms have disappeared, or until 120 minutes have passed, whichever occurs first. At that point, the participant turns the slider to position 1 and breathes through the device for five more minutes[3]. Due to the nature and normal duration of aura, and the in/exclusion criteria related to aura, the great majority of participants will use the device for less than an hour. As an added control mechanism, the eCRF study app asks participants if they are still using the device at the 2-hour time point; if this is confirmed the participant is directed to stop using the device at that point.

The first treated attack is designated a *training attack* and will not be used in the statistical analysis. The subsequent treated attacks are designated *study attacks* and will be used in the statistical analysis.

After four treated attacks or overall conclusion of Stage 1 (whichever comes first), participants will end study Stage 1 and have the option to start Stage 2 which is a follow-up open-label period during which the participant will treat up to five attacks with the active device type.

13.4.1 Stage 1 At-Home Activities

Participants are instructed to have a study device and the ePRO app at hand at all times, and at the onset of aura symptoms follow the Attack Checklist (see section 13.4.1.1) to start the treatment and the logging of the attack in the ePRO app.

In the following cases, the participant should NOT treat the attack with the study device or record it in the study diary app:

1) If there are no aura symptoms (these are pre-specified in the Instructions for Use and ICF)
2) If there is moderate or severe headache at the time the aura symptoms start
3) If they are not able to start treatment within 10 minutes of beginning of the aura symptoms

The start of treatment is counted as time point 0.

Data are collected via the ePRO app at specified time points (0, 1, 2, 24 and 48 hours). If the app is not available (e.g. internet connection issues), the patient should report the data in paper forms provided during Site Visit 1.

13.4.1.1 Attack Checklist

The following is the Attack Checklist:

Do this as soon as the aura symptoms start:

1. Unpack a study device

2. Move green slider to this setting: ☐

3. Unfold the device's bag and pull the black plastic strip

4. Click the top of the device into place at an angle

5. Put on the nose clip (not tight)

6. Inflate the bag with 2 to 3 breaths

[3]This prevents post-treatment hyperventilation symptoms (mainly paresthesias and lightheadedness) which otherwise may occur in approx. 10-15% of users.

7. Start breathing through the device while sitting comfortably

8. IMPORTANT: Open the study app with Touch ID or PIN: □ , then go to My Forms and press "Attack Start".

9. Answer all questions in the app (scroll all the way down), then press "Save"

10. Breathe through the device until the aura symptoms are gone (minimum 10 min.)

11. Turn the slider to setting 1 and breathe through it for five minutes

12. Set a timer to remember to report symptoms in app 1, 2, 24 & 48h after "Attack Start"

NB: The □ symbol in step 2 and 8 is an empty text box. This is filled out during the Instruction Session.

13.4.2 Stage 1 Telephone/Teleconference Follow-up Contacts

13.4.2.1 Follow-up A (obligatory)

When the participant has reported his/her training attack in the ePRO app, the PI must within one week check whether the data was entered correctly, and then contact the participant via phone or teleconference in order to correct any data entry method errors observed, verify that the participant understands the use of the device and ePRO app, and answer any questions the participant may have.

13.4.2.2 Follow-up B (conditional)

If the participant has not registered an attack in the pasteight weeks, the PI must within one week contact the participant via phone or teleconference, in order to verify that the participant understands the use of the device and ePRO app and answer any questions the participant may have.

13.4.2.3 Follow-up C (obligatory)

When participants have treated and reported four attacks, the PI must within one week contact the participant in order to schedule his/her Site Visit 2.

Participants who at the overall conclusion of Stage 1 have not reported at least one study attack will be concluded from Stage 1 and invited to participate in Stage 2.

13.5 Site Visit 2

At Site Visit 2, the participant answers additional general questions about Stage 1, and it is disclosed to the participant whether they have received the active or sham device in Stage 1. Participant is asked to re-consent to further participation in Stage 2. This will be documented in the eCRF form for Stage 2.

If the participant does not wish to continue, they at this point give back all study materials and their participation in the study is concluded. Devices are accounted for in accountability logs and filed in Investigational Site File and Trial Master File.

If the participant wishes, they at this point continue into Stage 2 in which case they receive a Stage 2 study kit including with five active devices and the Instructions for Use for the active device. PI trains the participant in study procedures for this stage, including use of the study device, as well as data and safety reporting.

Table 4: Tasks during Site Visit 2

TASKS, Site Visit 2:
- End-of-Stage-1 interview (subjective satisfaction, side effects, non-reported adverse events, DASS-21 questions, participant's belief about which device type they were assigned in Stage 1 (active/sham))
- Disclosure to patient of study device type used in Stage 1
- Return of unused devices from Stage 1
- Information and training about Stage 2 (open-label extension). Participant receives Stage 2 study kit if he/she re-consents to participating in Stage 2.

13.6 Stage 2

During Stage 2, participants can use the study devices to treat migraine-with-aura attacks.

Once each month during Stage 2, the participant is prompted (by notification via the study diary app) to answer the Stage 2 questions regarding use of the study device as well as use of prescription acute migraine drug or an over-the-counter acute analgesic, with the intention to treat migraine (cf. ***PAREMA1 Case Report Form content***). The study diary questionnaire includes an option for reporting problems and/or adverse events.

13.6.1 End-of-Stage-2 Follow-up

The End-of-Stage-2 Follow-up marks the end of the study participation and is a telephone/teleconference follow-up which must be performed by the PI at the latest two weeks after occurrence of one of the following:

1. The participant has reported a total of five treated attacks in Stage 2, implying that all the devices received at Site Visit 2 have been used.
2. 12 months have passed since the participant's inclusion in Stage 2.
3. The participant no longer wishes to participate or is lost to follow up.

The PI must during the telephone/teleconference follow-up interview collect and record in the eCRF the following information: subjective satisfaction, side effects, non-reported adverse events, any other comments the participant has about the treatment and study participation, as well as the DASS-21 questions (cf. document ***PAREMA1 Case Report Form content***). The patient should return unused Stage 2 devices by mail or at the site.

13.7 Randomization

The participants will be randomized in a 1:1 ratio to use either the active or sham device in Stage 1.

Participants and investigators will be blinded with respect to which device (active or sham) each participant will use or has used. The randomization scheme will be generated by study biostatisticians using permuted block randomization prior to the start of the clinical trial and implemented through the randomization module in the EDC. Randomization will be stratified by site, and each site will have a separate randomization scheme using random predetermined block sizes.

13.8 Treatment Unblinding

Blinding of individual study participants can be broken in circumstances of medical emergency where knowledge of blinded treatment is necessary. In such cases, the randomization codes will be broken and the PI must be unblinded with regards to the participant in question. Upon unblinding of a study participant, Stage 1 will be prematurely ended for this participant and site will call in for Site Visit 2 and participant will be transferred to Stage 2, if agreed to by the participant. If necessary to break the blind, the involved competent authority (if applicable) and EC/IRB will be informed. The procedure of unblinding is further described in Safety Management Plan for the study. In addition to emergency unblinding, the code for the individual participant will also be broken at conclusion of Stage 1 and study participant will at site visit 2 be informed of their study allocation during Stage 1.

13.9 Concomitant Medications

For this study, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Relevant medications to be reported in the eCRF are concomitant prescription medications and over-the-counter medications.

Medication usage will be documented for medications a participant is currently taking at the time of the screening visit, and any medication stopped within the previous thirty (30) days, and for medication changes (started, stopped, or change of dose/frequency) through to study exit.

Participants should, if possible, refrain from using rescue medication for at least the first two hours after the initiation of treatment with the study device during Stage 1. "Rescue medication" is here defined as the following:

- Prescription or OTC pharmaceuticals that have migraine as an indication for use
- OTC painkillers/analgesics, including acetaminophen/paracetamol, aspirin, ibuprofen and naproxen
- Neurostimulators for pain treatment

Any use of rescue medications within 48 hours from initiation of treatment will be documented via the ePRO app.

Additionally, any medication that is determined by the investigator to be a potential safety issue or concern, and/or any medication taken in connection with treatment for an adverse event (AE) will be documented.

13.10 Patient reported outcomes

Below is given short description of patient reported outcomes. Please refer to the document ***PAREMA1 Case Report Form content*** for full details of collected information.

13.10.1 Adverse events and device deficiencies

Participants are instructed to report adverse events experienced during the course of the study. The events can be encountered in relation to a treatment session or any time thereafter. Safety reporting will create an alert and site will call the participant without undue delay (within 1 business day) to follow up on the report.

13.10.2 Migraine Headache Pain Scale

Participants will be asked to report their migraine-associated headache pain via electronic Patient Reported Outcome (ePRO) app using a four-point Likert Scale. Participants will be prompted for this symptom rating at the initial report of aura symptoms and at the time points one, two, twenty-four and forty-eight hours after the initiation of treatment.

13.10.3 Other Migraine Symptoms

Participants will be asked to report their migraine symptoms via ePRO using a four-point Likert Scale, including the nausea, phonophobia, photophobia and functional disability. Participants will be prompted for this symptom rating at the initial report of aura symptoms and at the time points one, two, twenty-four and forty-eight hours after the initiation of treatment

13.10.4 Delay until treatment initiation

At the 0 time point, the participant is prompted to estimate how long time went by from the beginning of aura symptoms until the beginning of treatment.

13.10.5 Aura duration and symptoms

At the time point when the participant reports that the aura has stopped, he/she is prompted to estimate the duration of the aura phase (minutes), as well as indicate the type of aura symptoms experienced.

13.10.6 Use of rescue medication

The participants will via ePRO be prompted to answer whether they have used rescue medication, at the time points two, twenty-four and forty-eight hours after the initiation of treatment.

13.10.7 Impact on life and work

At the 48-hour time point, participants will via ePRO be prompted to rate:

- Impact of the migraine attack on participant's work/school attendance and ability to do household chores, during the first 48 hours since the beginning of the attack (4-point Likert scale)
- Impact of the migraine attack on participant's social life and leisure time, during the first 48 hours since the beginning of the attack (4-point Likert scale)

13.10.8 Patient satisfaction (Stage 1)

48 hours after each attack start, participants will be asked to rate their subjective satisfaction with the device treatment's effect (satisfaction measured on 5 point scale: 5 = Satisfied, 4 = Partially Satisfied, 3 = Neutral, 2 = Partially Unsatisfied, 1 = Unsatisfied)

13.10.9 Assessment of blinding

At Site Visit 2, patients will be asked whether they believed they received the active or placebo device during Stage 1. This data will be used to compute the Bang Blinding Index (Bang et al., 2004)

13.10.10 Long term patient satisfaction (Stage 2)

Once each month during Stage 2, participants will be prompted to report how many MA attacks they have had during the past month, how many of these they treated with the study device, as well as their overall subjective satisfaction with study device treatment overall during the last month (satisfaction measured on 5 point scale: 5 = Satisfied, 4 = Partially Satisfied, 3 = Neutral, 2 = Partially Unsatisfied, 1 = Unsatisfied). The question also has the option of answering that the participant did not have any attacks during the past month.

13.10.11 Long term migraine drug and neurostimulator use

Each month during Stage 2, participants will be asked how many times in the past month they have taken respectively a prescription acute migraine drug, an over-the-counter acute analgesic or a neurostimulation device, with the intention to treat migraine. They will also be asked about the type and dose.

13.10.12 Migraine-related hospitalizations

Each month during Stage 2, participants will be asked whether they have been hospitalized for migraine over the last month, and if yes, how many days in total.

14 Risks and Benefits

This section gives an overview of the risks and benefits of the study. For a more detailed risk analysis please refer to the Investigator's Brochure.

14.1 Known Potential Risks

14.1.1 Risks related to the active device

By increasing CO_2 in the inspired air, the device induces an increase in bodily CO_2 as well as a fall in pH, which are the intended physiological effects. With the device, the inspired CO_2 fraction is between 1.5% and 2.5% leading to an increase in P_{aCO2} of approximately 4 - 12 mmHg. This range has been in the previous experimental tests been found to be well-tolerated, safe and effective (Fuglsang et al., 2018; Johansen, 2017; Johansen et al., 2013); in the clinical studies undertaken using the device (in different but functionally equivalent forms), no serious adverse events occurred.

After having used the active device for five minutes, participants *may* start to experience harmless side effects which according to a benefit-risk ratio are judged as acceptable, and which for the purpose of the clinical investigation are categorized as '*expected side effects'*.

The expected side effects are (cf. (Fuglsang et al., 2018)):

- Deeper and/or faster breathing (hyperpnea due to the increase in P_{aCO2})
- Feeling slightly warmer or colder than normal
- Increase in salivation
- Increase in perspiration

Additional device effects have in the clinical pre-market data and in the post market surveillance been reported in a small percentage of patients using the device, including:

- Dyspnea (probability less than 10%)

- Mild nausea (probability less than 10%)
- Dizziness (probability less than 10%)
- Mild claustrophobia (probability less than 5%)
- Anxiety (probability less than 5%)
- Headache (probability less than 0.1%)
- Any other symptoms causing moderate to severe discomfort

The side effects listed above should be counteracted by decreasing the CO_2 setting of the Rehaler, in practice by moving the regulator (see Figure 2) to a lower setting. Study participants will receive very clear instructions about how to recognize and counteract such effects should they occur: respectively during the Training Session and in the Informed Consent Form and Instructions for Use (see these documents).

Apart from CO_2 effects, the risk assessment must also address the issue of possible hypoxemia. Any partial rebreathing device without an external source of compressed oxygen gas will inevitably lead to a decrease in the inspired oxygen level. However, individuals conforming to the in/exclusion criteria (i.e. without pulmonary or cardiovascular disease or anaemia) have a significant extra-capacity for oxygen uptake, meaning that decreases in the inspired oxygen pressure from the normal sea level value of 21 kPa (dry basis) down to 16 kPa are unproblematic and do not lead to hypoxemia (West, 2008). This corresponds to the situation which arises when a person travels from sea level to a high altitude. Signs of oxygen inadequacies upon exertion typically do not manifest themselves below altitudes of approx. 2200 meters, where the oxygen partial pressure is 16 kPa. In the present case, where the device users will be sitting at rest while using the device, the potential for hypoxemia is even smaller. This is supported by the arterial blood data which showed a drop of C_{aO2} of less than 2%, as well as the pulse oximetry data showing that the $S_{pO2\%}$ range was the same (95%-100%) with the study device as at baseline (see section 8.5.1).

Use of the Rehaler is contraindicated for the following:

- Chronic pulmonary disease (e.g. Chronic Obstructive Pulmonary Disease (COPD) or pulmonary fibrosis)
- Severe cardiovascular disease (e.g. symptomatic coronary artery disease, prior myocardial infarction, congestive heart failure (CHF)
- Cerebrovascular disease (e.g. prior stroke or transient ischemic attack, symptomatic carotid artery disease, prior carotid endarterectomy or other vascular neck surgery)
- Anaemia
- Sickle Cell Disease
- Uncontrolled intracranial hyper/hypo-tension
- Past or present cerebral aneurysm
- Individuals with previous brain surgery, including stenting
- Use in airplanes or at altitude over 2000 meters (6600 feet) above sea level
- Pregnant or breastfeeding women
- Children under the age of 18

Rehaler has potential interactions when used in conjunction with the following pharmaceutical agents:

1. Anesthetic agents: interaction can give rise to cardiac dysrhythmias.

2. Neuromuscular blocking agents and hypotensive agents: the change in pH induced by Rehaler can influence uptake, distribution and action of drugs (including neuromuscular blocking agents, and hypotensive anesthetic agents).
3. Adrenaline: carbon dioxide increases the uptake rate of adrenergic substances such as adrenaline.

14.1.2 Risks related to the sham device

The sham device has been altered so that participants breathe very low CO_2 concentrations when using it (cf. section 8.6 and Investigator's Brochure).

A series of technical tests with the sham device found that the inspired CO_2 percentage with the sham device was less than 0.1% and no clinically or statistically significant changes in ETCO2 occur during use.

There are therefore no known or suspected risks with the sham device.

14.2 Known Potential Benefits

Based on the migraine treatment results presented in section 8.5.3, we anticipate that the investigational (active) device will increase the likelihood of avoiding moderate or severe pain during migraine with aura attacks.

14.3 Benefit-Risk Analysis

Section 14.1 describes possible adverse device effects and risks pertaining to the clinical investigation. Based on that analysis, we believe that it is unlikely that the use of the investigational device or sham device will lead to serious adverse device effects.

As detailed in the Risk Management file and summarized in the Investigator's Brochure, relevant measures have been taken to mitigate residual risks.

Based on the data from the scientific literature, prior clinical studies of functionally equivalent versions of the device (the studies CapnoMigra, HVMASKE and EpiCapno), the extensive post-market safety data, and the mitigations applied to residual risks, we believe it will be safe to use the investigational device and the sham device in the clinical investigation, in the population defined by the in/exclusion criteria.

The study is intended to examine in a large-scale, randomized, controlled study whether it is possible to treat and prevent migraine with aura by using partial rebreathing. Such a study has not previously been reported in the literature and will provide valuable new knowledge about migraine and its treatment. In the light of the risk evaluation performed, it is our assessment that those potential benefits of the study outweigh the risks and possible discomforts that participants may experience during the study, and that the benefit-risk ratio of the clinical investigation is therefore acceptable.

Our group (sponsor and investigators) possesses the required expertise, experience, equipment and facilities to carry out the proposed study, as well as subsequently analyze and communicate the results in a peer-reviewed journal.

15 Adverse events and device deficiencies

15.1 Definitions

Device Deficiency

Inadequacy of a medical device with respect to its identity, quality, durability, reliability, usability, safety or performance. Device deficiencies include malfunctions, use errors and inadequacy in the information supplied by the manufacturer, including labelling.

This definition includes device deficiencies related to the investigational medical device or the comparator.

Adverse Event (AE)

An AE is any untoward medical occurrence, unintended disease or injury, or untoward clinical signs (including abnormal laboratory findings) in subjects, users or other persons, whether or not related to the investigational medical device and whether anticipated or unanticipated.

Adverse Device Effect (ADE)

Any AE related to the use of the investigational medical device.
This definition includes adverse events resulting from insufficient or inadequate instructions for use, deployment, implantation, installation, or operation, or any malfunction of the investigational medical device.
This definition includes any event resulting from use error or from intentional misuse of the investigational medical device.
This includes “comparator” if the comparator is a medical device.

Serious Adverse Event (SAE)

An AE that led to any of the following:

a) Death,
b) Serious deterioration in the health of the subject, users or other persons as defined by one or more of the following:
    1) a life-threatening illness or injury, or
    2) a permanent impairment of a body structure or a body function including chronic diseases, or
    3) in-patient or prolonged hospitalization, or
    4) medical or surgical intervention to prevent life-threatening illness or injury, or permanent impairment to a body structure or a body function,
c) Foetal distress, foetal death, a congenital abnormality, or birth defect including physical or mental impairment

Planned hospitalization for a pre-existing condition, or a procedure required by the CIP, without serious deterioration in health, is not considered a serious adverse event.

Serious adverse device effect (SADE)

Adverse device effect that has resulted in any of the consequences characteristic of a serious adverse event.

Unanticipated Serious Device Effect (USADE)

Serious adverse device effect which by its nature, incidence, severity or outcome has not been identified in the current risk assessment.

Anticipated serious adverse device effects (ASADE) is an effect which by its nature, incidence, severity or outcome has been identified in the risk assessment.

15.2 Classification of an Adverse Event

15.2.1 Severity of Event

For all AEs, the following guidelines will be used to describe severity:

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious".

15.2.2 Causality assessment

All AEs must have their relationship to both the study device and study procedure assessed and categorized by the Principal Investigator who examines and evaluates the participant based on the temporal and/or biological relationship and his/her clinical judgment. During assessment, relevant core documents (e.g. CIP, IB and RM report) must be consulted. The Sponsor and the investigators will use the following definitions to assess the relationship of the serious adverse event to the investigational device, sham or the investigation procedure.

Not related: Relationship to the device, sham or procedures can be excluded when:

- the event has no temporal relationship with the use of the investigational device, or the procedures related to application of the investigational device;
- the serious adverse event does not follow a known response pattern to the medical device (if the response pattern is previously known) and is biologically implausible;
- the discontinuation of medical device application or the reduction of the level of activation/exposure - when clinically feasible - and reintroduction of its use (or increase of the level of activation/exposure), do not impact on the serious adverse event;
- the event involves a body-site or an organ that cannot be affected by the device or procedure;
- the serious adverse event can be attributed to another cause (e.g. an underlying or concurrent illness/ clinical condition, an effect of another device, drug, treatment or other risk factors);
- the event does not depend on a false result given by the investigational device used for diagnosis, when applicable;

Possible: The relationship with the use of the investigational device or comparator, or the relationship with procedures, is weak but cannot be ruled out completely.

Alternative causes are also possible (e.g. an underlying or concurrent illness/ clinical condition or/and an effect of another device, drug or treatment). Cases where relatedness cannot be assessed, or no information has been obtained should also be classified as possible.

Probable: The relationship with the use of the investigational device or comparator, or the relationship with procedures, seems relevant and/or the event cannot be reasonably explained by another cause.

Causal relationship: The serious adverse event is associated with the investigational device, comparator or with procedures beyond reasonable doubt when:

- the event is a known side effect of the product category the device belongs to or of similar devices and procedures;
- the event has a temporal relationship with investigational device use/application or procedures;
- the event involves a body-site or organ that
  - the investigational device or procedures are applied to;
  - the investigational device or procedures have an effect on;
- the serious adverse event follows a known response pattern to the medical device (if the response pattern is previously known);
- the discontinuation of medical device application (or reduction of the level of activation/exposure) and reintroduction of its use (or increase of the level of activation/exposure), impact on the serious adverse event (when clinically feasible);
- other possible causes (e.g. an underlying or concurrent illness/ clinical condition or/and an effect of another device, drug or treatment) have been adequately ruled out;
- harm to the subject is due to error in use;
- the event depends on a false result given by the investigational device used for diagnosis, when applicable;

15.2.3 Expectedness

Expected events have been identified from vigilance data, available clinical data and literature searches. Investigator will use available technical documentation to determine if the event is anticipated or not. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention.

15.3 Adverse event recording

The Investigator will monitor the occurrence of adverse events for each subject during the course of the clinical study. Adverse events (AEs) may be reported by the participant, identified by the PI or documented in the medical record. All AEs regardless of source, must be recorded in the EDC system. Throughout the study, participants must immediately report any adverse events and device deficiencies experienced through the ePRO, which will be recorded by investigators in the electronic Case Report Form (eCRF). The Investigator must provide information on symptoms, date and time of onset, duration, severity, seriousness rating, causality, actions taken, outcome and any other pertinent information. All AEs occurring while in study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution. Any medical condition that is present

at the time of screening will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it may be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed.

A hard copy of the electronic AE section will be available to allow the Investigator to report SAEs and device deficiencies in the event the EDC is not available. The AE report must then be sent to: safety@qmed-consulting.com

Detailed instruction on reporting and completion of the AE section will be provided to the sites as part of the Site Initiation Visit (SIV).

15.4 Adverse Event Reporting by Investigators

If any event is considered (possibly) related to the device, event is considered serious or event is device deficiency with SAE potential, it is the responsibility of the Investigator to report this event within **24 hours** of awareness. If event is not serious or not related to the device, the Investigator must complete recording in EDC within 7 days of having become aware of the adverse event.

Overview of all (S)AEs will be provided to involved ECs in annual reports.

15.5 Adverse Event Reporting by Sponsor

The safety monitoring and documentation by Sponsor as well as reporting of events and device deficiencies to involved regulatory authorities is described in the Safety Management Plan for the study. Sponsor's Medical Advisor for study will review clinical events that occur during the study to adjudicate the relationship to the study device and safety, seriousness, severity and expectedness. Sponsor will together with Medical Advisor be responsible for monitoring the safety of study participants throughout the study on an on-going basis. Based on safety data, the Medical Advisor may recommend the Sponsor to modify or stop the clinical investigation. All relevant documentation for safety review will be filed in the TMF.

The Medical Advisor for study has no involvement in the design of the clinical study and no financial interest in the investigational study product and is not involved in the conduct of the study except for his/her role as Medical Advisor and for being part of Sponsor's advisory board.

No Data Monitoring Committee will be established for this study.

This plan will be updated throughout the duration of the study if new information and/or guidance become available. Adverse events and device deficiencies will be collected and documented and reported according to applicable regulation (EU, MDCG 2020-10; US: §812.150(b)(1). In addition to the reporting requirements outlined above, national requirements, will be followed. Once Eudamed becomes available and fully functional, the Safety plan will be updated to reflect the new web-based reporting structure.

15.6 Monitoring and Follow up

Study site will record all events and will monitor those throughout the study until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation.

16 Methods for analyzing data

The ***PAREMA1 Statistical Analysis Plan*** (SAP) describes the statistical method and analysis in detail.

Following is a summary of the intended analyses and sample size justification.

16.1 Primary Effectiveness Analysis

The primary endpoint is Absence of Moderate or Severe pain at 2 hours (AMSP2), i.e. the percentage of study attacks in which the participant reported absence of headache of moderate/severe intensity at 2 hours post-treatment initiation. "Study attacks" are all attacks treated and reported during Stage 1, apart from the first attack which is a training attack and is not included in the analysis. The intention-to-treat (ITT) analysis set will be used for the primary effectiveness hypothesis test. The primary effectiveness hypothesis is:

H_0: $p_{Rehaler} = p_{sham}$

H_a: $p_{Rehaler} \neq p_{sham}$

Where:

$p_{Rehaler}$ = proportion of attacks in the Rehaler group with AMSP2

p_{sham} = proportion of attacks in the sham group with AMSP2

The primary effectiveness hypothesis will be tested using a two-sided adjusted χ^2 test for clustered binary data (Donner and Banting, 1988), at a 5% level of significance. If Pearson's chi-squared test statistic is written as $\chi^2 = \sum_{i=1}^{G} \chi_i^2$ with G = the number of treatment groups, then the adjusted χ^2 approximately follows a chi-squared distribution with G-1 degrees of freedom and is calculated as follows (Donner and Banting, 1988):

$$\chi_A^2 = \sum_{i=1}^{G} \frac{\chi_i^2}{\overline{C_i}}$$

Where:

$$\overline{C_i} = \sum_{j=1}^{n_i} \frac{m_{ij} C_{ij}}{\sum m_{ij}}$$

$$C_{ij} = 1 + (m_{ij} - 1)\rho$$

G is the number of treatment groups

n_i is the number of individuals in group j

ρ is the correlation coefficient between any two responses in the same individual

m_{ij} denotes the number of observations for individual j in group i

The corresponding 95% confidence interval for the difference in proportion of attacks with AMSP2 between the Rehaler and sham groups will be calculated as follows (Donner and Klar, 1993):

$$95\% \text{ Confidence Interval: } (\hat{p}_{Rehaler} - \hat{p}_{sham}) \pm 1.96 * \widehat{SE}(\hat{p}_{Rehaler} - \hat{p}_{sham})$$

Where:

$\hat{p}_{Rehaler}$ = observed proportion of attacks in the Rehaler group with AMSP2

$\hat{p}_{sham}$ = observed proportion of attacks in the sham group with AMSP2

$$\widehat{SE}(\hat{p}_{Rehaler} - \hat{p}_{sham}) = \left(\frac{\overline{C_1}\hat{p}_{Rehaler}(1-\hat{p}_{Rehaler})}{n_1} + \frac{\overline{C_2}\hat{p}_{sham}(1-\hat{p}_{sham})}{n_2}\right)^{1/2}$$

$$\overline{C_i} = \sum_{j=1}^{n_i} \frac{m_{ij}C_{ij}}{\sum m_{ij}}$$

$$C_{ij} = 1 + (m_{ij} - 1)\rho$$

n_i is the number of individuals in group i

ρ is the correlation coefficient between any two responses in the same individual

m_{ij} denotes the number of observations for individual j in group i

16.2 Sample Size and Statistical Power

The study's key secondary endpoint is Pain Freedom at 2 hours (PF2). Recognizing that this is the ideal outcome for migraine patients, the study has been designed with a moderately high statistical power (80%) for PF2. The estimated study size of 174 included participants has been determined on the basis of data from the pilot study and post-market data collected in Denmark, Sweden and Germany from 2018 to 2020, indicating that the PF2 rate will be 30% in the active device group and 12% in the sham group, that each patient will contribute data from two attacks while in Stage 1, and that the total percentage of included patients not yielding analyzable data will be 30%. These data were utilized in a sample size analysis, conducted by simulation and using the χ^2 significance test adjusted for clustered data. Under the given assumptions and including 174 patients, the estimated power for PF2 is 80%. Pilot study and post-market data indicated an AMSP2 rate of 61% in the active device group and 26% in the sham group. Under the given assumptions, including 174 patients in the study will result in 95% power for AMSP2. Therefore, the clinical investigation will randomize at minimum 174 participants.

16.3 Interim analysis

After 60 subjects have provided data from at least one study attack in Stage 1, an interim analysis (IA) will be conducted to re-estimate the study sample size. Based on the data from the IA it will be decided how many additional patients to recruit into the study. The IA will include two hypothesis tests on the interim data set, respectively of PF2 and AMSP2. These analyses will use the O'Brien Fleming Spending function in which the significance thresholds of the p-value are respectively 0.0052 at the IA and 0.048 in the full analysis. The IA will estimate the power for PF2 in the final analysis based on the observed PF2 rates in the trial thus far and re-estimate the study sample size accordingly. Regardless of the results of the IA, enrolment will continue to the originally estimated 174 subjects, and the total number of included participants will be capped at 220 since this is the largest study that will be feasible to conduct. The minimum number of participants in the study will be 174.

16.4 Analysis of Secondary Effectiveness Endpoints

To support potential device labelling claims, key secondary end points will be analyzed according to a fixed-sequence step-down procedure. The ITT analysis set will be used for hypothesis tests for the secondary endpoints.

The end point analysis will be performed by an independent evaluator who is blinded to the type of intervention (active/sham) assigned to each of the two Stage 1 treatment groups.

16.5 Analysis of Explorative Endpoints

Explorative endpoints in Stage 1 and Stage 2 will be analyzed to gather additional effectiveness information.

16.6 Analysis of Safety Endpoints

The incidence of device- or treatment-related adverse events (AEs) and device- or treatment-related serious adverse events (SAEs) and/or serious adverse device effects (SADE) will be tabulated. The as-treated analysis set will be used for the assessment of safety.

16.7 Pass/fail criterion to be applied to the results of the clinical investigation

The pass/fail criterion for the clinical investigation is whether using the active device per protocol results in a higher percentage of absence of moderate or severe pain at two hours compared to the sham device.

16.8 Analysis sets

16.8.1 Intention-to-treat (ITT) analysis set

The ITT includes all study attacks from all participants who underwent randomization and who reported headache score at the two-hour time point in at least one study attack. The ITT analysis set will not exclude attacks with protocol deviations or use of rescue medication before the two-hour time point. The ITT data set will be used for analysis of the primary and secondary effectiveness end points.

If any subjects in the ITT population have a missing headache score at the two-hour time point, multiple imputation will be used to impute their headache score for the analysis of primary and secondary endpoints, as described in the Statistical Analysis Plan.

16.8.2 As-treated analysis set

The as-treated analysis set includes all participants who received a study device, whether or not it was the actual randomized device. Subjects will be grouped by the device they actually used. This analysis set will be used for assessment of safety.

16.8.3 Per-protocol analysis set

The per-protocol analysis set includes all study attacks with a reported headache score at the two-hour time point, in which:

- The participant started using the study device within five minutes of the beginning of aura and before development of moderate or severe pain
- The participant did not use rescue drugs before the two-hour assessment point
- There were no changes in concomitant preventive treatment during the study that might potentially affect response.

- There were no other major protocol deviations

When comparing the ITT and Per Protocol analysis sets, the impact of protocol deviations and user errors can be investigated with the aim of identifying potential improvements to the investigational device and instructions for use. The ITT analysis set will also be used to analyse the impact of protocol deviations on the outcome parameters (e.g. use of rescue medication during the first two hours and starting the use of the device after the onset of headache).

16.9 Handling of Missing Data

All attempts will be made to minimize missing data. However, if any subjects in the ITT population have missing primary endpoints, five (5) multiple imputations will be used for the analyses. This consists of imputing values for each missing value as a set, analyzing the results for each set, and then pooling the results. The primary effectiveness endpoint is binary, so the imputation method will be a multiple logistic regression.

17 Data capture and management

17.1 EDC system

The study will utilize the TrialKit electronic data capture (EDC) and ePRO platform (Crucial Data Solutions, 18124 Wedge Parkway, Suite 139, Reno, NV 89511, USA) for data collection, an EU GDPR compliant system. The TrialKit EDC may be accessed securely via either the web or the mobile app (smartphone or tablet). The data are hosted on a secure server located in the EU (for European study sites) or USA (for US trial sites).

At Site Visit 1, the TrialKit app will be downloaded to the participant's smartphone (the TrialKit ePRO app is compatible with all major smartphone types).

The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data may be entered directly into the database or from the source documents.

Study personnel will be able to access the EDC system via either web browser or mobile app.

The EDC database system will be designed based on the protocol requirements, the approved eCRF layouts and specifications (cf. document ***PAREMA1 Case Report Form content***), and in accordance with all local data protection regulations. The eCRF layouts and specifications define and identify the applicable source data that will be collected and captured into the EDC database system. The applicable source data will be electronically transcribed by the site designee onto the eCRF (data entry screens) in the EDC database system or entered directly into the eCRF without further written documentation available. The relevant sections of the eCRF must be completed without undue delay. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported. The investigator shall provide justification for any missing data and must document this as deviation. The Investigator can delegate EDC entries to a study team member, this delegation must be documented in Site Delegation Log. The Investigator is ultimately responsible for the accuracy of the data collected in the eCRF.

All study data will be entered into the eCRF. The information in the eCRF will not identify the participant by name, only by his/her study Participant Identification Number and initials. The Principal Investigator maintains (and has continual access to) the site's Participant List in the secure EDC system. This list includes the site's participants' name, Participant Identification Number, device kit number, cell phone and landline phone number(s), if applicable, email address, and physical address. This section of the EDC will be blinded to Sponsor and CRO.

Participants will only be identified by their study Participant Identification Number (not name), in the data analysis as well as in all communications and publications of results.

The CRO will be responsible for the data management for the study. Full details about the data management are described in the Data Management Plan for the study. This plan will include specifications for consistency and plausibility checks on data and also include data handling rules. Queries resulting from automated checks will be available for sites during data entry. Obvious data errors will be treated according to the established rules for the study and queries for unresolved issues will be created in the EDC system. All missing or spurious will be subjected to review. All data will be considered for analysis as specified in the Statistical Analysis Plan.

17.2 Source data

Source data includes all information in original records and certified copies or original records of clinical findings, observations, or other activities in a clinical investigation used to reconstruct and evaluate the study. Source documents include hospital records, laboratory findings, completed scales, images and reports etc. The source documents will be retained at the site but the Investigator/Institution will permit direct access to source data/documents for study related monitoring, Sponsor audits, representatives from the Ethics Committee and regulatory inspections. Site will ensure copies of patient data for distribution is redacted of patient identifiers.

The legal basis for accessing the medical records, including monitoring, audits and inspections will be the consent for participation provided by study participants.

17.3 Database lock and Record retention

At the conclusion of the study, the database will be locked once all subjects have a final status, queries have been resolved and any protocol deviations have been addressed. All edit/write access to the database will be removed and changed to view-only. Each participating site will receive a file of their site's data, including raw and final eCRF data and audit trail. Study documents and data should be retained for a minimum of 15 years after the close-out of the study and until there are no pending or contemplated marketing applications. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

18 Device accountability

When individual sites are activated within the EDC system, the Sponsor and site are alerted which device study kits they should expect to receive along with pertinent information of devices, based on a predefined site level ceiling. The Sponsor ships device study kits to site and site will then log the

receival in the system, thereby making the device study kits available to the study participants. When the number of device study kits at a site reaches a predefined minimum quantity of inventory, the EDC system will generate a re-order and new stock will be shipped from the Sponsor. Automated status reports can be generated from the system at all times. Such status reports may include inventory at a given site, audit history on a per item basis or an audit history of e-mail notifications sent out by the system.

19 Supporting Documentation and Operational Considerations

19.1 Study Oversight

The sponsor will be responsible for the following tasks and activities, performed either by the sponsor or a Contract Research Organization (CRO) employed by the sponsor:

- Overall project management to ensure adherence to the clinical investigation plan, and timely conclusion of the study.
- Training investigators in study procedures, study devices and the EDC system.
- Ensuring that all sites receive all test devices, study equipment and documents.
- Performing study site activations and monitoring.
- Supporting the study sites with patient recruitment (including producing and publishing advertisements of the study).
- Collaborating with the investigators on handling, reporting and following up on SAE's.

19.2 Quality Management

19.2.1 Site Selection

Principal Investigators and investigational sites will be selected by Sponsor based on their experience, training, qualifications and availability of resources for study conduct. PIs must be legally entitled to perform clinical research and to participate in clinical investigations of medical devices. Sites will be selected after screening procedures and upon review of a site assessment and PI qualification. A site qualification visit (onsite or remote) will be performed to ensure site facilities are adequate and site staff and other resources are available for study conduct.

19.2.2 Training

All Principal Investigators and site staff are required to attend training on the investigational medical device (IMD) and study procedures. Training will include, but is not limited to, the protocol, IMD usage, EDC completion, applicable legislation and PI responsibilities. All training will be documented in a Training Log.

The Principal Investigators acknowledge that the medical devices are investigational and must be treated accordingly. The Principal Investigators have the overall responsibility for the administration and accountability of the IMDs and the Sham Devices at their respective sites. This responsibility can be delegated to another study staff member. Delegation must be documented in a Delegation Log.

19.2.3 Monitoring

The study will be monitored throughout study duration according to the Monitoring Plan.

Study monitors are designated to oversee the progress of the study. The monitors are appropriately trained and qualified and monitoring will be carried out according to ISO 14155:2020 and applicable SOPs and Working Instructions.

Data monitoring and management will be performed in the EDC database system by the study clinical monitor.

The study monitor will conduct remote monitoring at a minimum of the following time points:

1. As part of the site's activation
2. At a time point maximum two business days after the first participant has been included at the site
3. At the close out of the site

The study monitor must confirm that the procedures described in the clinical investigation plan are adhered to, including:

1. Electronic Case Report Forms will be reviewed to ensure accurate and complete collection of data
2. Confirmation of timeliness and quality of data entry
3. Review of query resolution
4. Review of EDC to check for protocol compliance
5. Review of site's completion of outstanding action items
6. Assessment of site's subject recruitment end enrolment
7. Training of site staff
8. Confirmation that a medical record exists for each participant

The study monitor is also responsible for verifying that the appropriate measures are adhered to regarding any adverse events. The study monitor must perform full verification of the presence of signed informed consent forms.

This study will incorporate risk-based monitoring (RBM) practices to ensure data integrity for the study. Risk based monitoring allows for targeted review of critical data by assessing Site and Study level Key Risk Indicators (KRIs) throughout the study and determining where monitoring efforts are best employed. Data metrics from the EDC system, organized by site, will be reviewed during regular RBM meetings. This process will be detailed in the Monitoring Plan. Key Risk Indicators include: informed consent form (ICF) signed, inclusion/exclusion criteria, enrolment rate per month, number of protocol deviations, visits performed with protocol windows, adverse event reporting and query management. During the RBM meetings, the sites will be categorized into High, Medium or Low Risk, based on the site evaluations performed. The frequency of data review and percentage of participants checked will be adjusted based on the incidence rate of deviations and the enrolment rate.

The site investigators and sponsor will allow direct access to source data and other study documents at auditing and/or inspection from Sponsor and its representatives, Ethical Committees/Institutional Review Board and Competent Authorities.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical study is conducted and data are generated, documented (recorded), and reported in compliance with the protocol, *International Conference on Harmonisation Good Clinical Practice (ICH GCP)*, the

International Organization for Standardization (ISO) 14155:2020 Clinical investigation of medical devices for human subjects – Good Clinical Practice and applicable regulatory requirements.

19.3 Protocol deviations

A protocol deviation is any noncompliance with the Clinical Investigation Plan. The non-compliance may be either on the part of the participant, the investigator, or the study site staff.

The Principal Investigator is not allowed to deviate from the CIP, except to protect the patient's rights, safety and well-being. Such deviations will be documented and reported to the Sponsor and REC/IRBs as per national/local requirements.

A deviation may be identified by the site itself, the monitor, data manager, or Clinical Project Manager. Deviations must be reported to Principal Investigator and/or Sponsor within five (5) working days. Sponsor is responsible for the assessment of the deviation and its impact on the participant(s) or the scientific validity of the study. Reportable deviations will be reported to involved authorities according to local requirements. Sponsor will determine the need for corrective and preventive actions. The Principal Investigator shall be informed in writing of the violation and the actions to be taken. Sponsor will assess all CIP deviations periodically for trends, this will be performed for both systematically reoccurring deviations across sites and for site(s) that are consistently associated with the CIP deviations.

Investigators will be evaluated based on any CIP deviations affecting the integrity of the data and/or participant safety. Such evaluation may result in suspension of further enrolment at their site(s) or disqualification. Criteria for suspension are:

- CIP deviations specifically related to eligibility criteria, device accountability or participant safety
- Corrective actions pertaining to CIP deviations not implemented by site

19.4 Confidentiality and Privacy

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, the Contract Research Organization, and the sponsor, to the extent provided by applicable laws and regulations. This confidentiality is extended to cover the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the IRB/REC and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

Study participant research data intended for statistical analysis and scientific reporting will be captured in the EDC system. After the conclusion of the study, the data will be transferred to a secure database maintained by the sponsor. The transferred data will not include the participants' contact details or identifying information. Rather, individual participants and their research data will be identified by their unique Participation Identification Number. The study data entry and study

management systems used by clinical sites and by sponsor research staff will be secured and password protected.

19.5 Participant Discontinuation or Withdrawal from the Study

Participants leaving the study will not be replaced by new participants.

All participants have the right to withdraw consent and discontinue participation without prejudice at any time during the study.

An investigator may discontinue or withdraw a participant from the study for the following reasons:

- Significant study non-compliance.
- If any clinical AE, laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study is judged not to be in the best interest of the participant
- Lost to follow up
- Serious or repeated deviation detected by Sponsor at site audits

For participants wishing to leave the study, the study personnel must ask their permission to contact them regarding any side effects or possible adverse events that may have contributed to their decision.

The PI must inform the Sponsor of the discontinuation of a participant by completing the relevant section of the eCRF. No new data is collected from the participant after withdrawal from the study. In order to preserve the scientific integrity of the study, the study data collected up until the point when the participant leaves the study will not be deleted.

In case of a serious adverse event occurring, the participant is asked to consider not exiting the study before resolution of the event. However, participants are made fully aware that participation is voluntary at all times throughout the study.

19.6 Study Discontinuation or Suspension

The sponsor may choose to temporarily suspend or prematurely terminate the study or a study site with or without cause. Written notification, documenting the reason for study suspension or termination, will be provided by the sponsor, as applicable, to the investigator, regulatory authorities and REC/IRB. If the study is prematurely terminated or suspended, the Principal Investigator (PI) at each clinical site will promptly inform, as applicable, study participants, the REC/IRB, and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of any changes to the study.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of effectiveness that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study and/or study site may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IEC and/or relevant regulatory body.

19.7 Lost to Follow-up

A participant will be considered lost to follow-up if he or she fails to return for at least one scheduled visit and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, three telephone calls and, if necessary, a certified letter to the participant's last known mailing address, or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.

Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with the primary reason of *lost to follow-up*.

19.8 End of Study Definition

A participant is considered to have completed Stage 1 of the study if the participant has completed Site Visit 2. A participant is considered to have completed Stage 2 of the study if the participant has completed the End-of-Stage-2 follow-up. The end of the study is defined as the completion of the last participant's End-of-Stage-2 follow-up, or withdrawal from the study of the last participant. The End of Study Form in the eCRF must be completed by the PI.

After completion of the study, an integrated comprehensive Clinical Study Report will be prepared by Sponsor.

20 Ethical Considerations

20.1 Compliance statement

This study will be conducted in accordance with the Declaration of Helsinki, this Clinical Investigation Plan and applicable national and local laws and regulations. In writing the clinical investigation plan, we have adhered to the standard ISO-14155 and US regulation 21 CFR regarding clinical studies of medical devices according to Good Clinical Practice. This compliance includes the protection of the rights, safety and well-being of human subjects, controls to ensure the scientific conduct, credibility and data integrity of the clinical investigation as well as the clear definitions of responsibilities of the Sponsor and Principal Investigators.

The Sponsor will avoid improper influence on or inducement of the subject, monitor, any investigators, or other parties participating in, or contributing to the clinical investigation.

The Principal Investigators are not allowed to deviate from the CIP, unless there is a need to maintain the participant's rights, safety and well-being or the scientific integrity of the investigation. The principles of the Declaration of Helsinki are implemented in this study by the use of the informed consent process, ethical review, study training, registration of study in publicity available database, publication policy and risk benefit assessment.

The investigation will not begin until the required regulatory and ethical approvals are obtained. In addition, any additional requirements of the individual study site, local REC/IRB and any regulatory body will also be followed.

The study will be registered prior to first enrolment, in the database https://clinicaltrials.gov/

The study will adhere to all regional and local legislation concerning the collection and processing and safekeeping of personal data. Information about the participants is handled according to the appropriate regional and local data protection laws. All participants enrolled or screened in the study will be anonymous vis-a-vis the public and will not be identifiable from any published report or article on the study's findings.

The study data analysis only uses data collected as part of the study and does not use information obtained from patient files.

20.2 Modifications to the Clinical Investigation Plan

Sponsor or an individual Investigator can take initiative to modify the clinical investigation. Investigators/sites shall not make modifications to this protocol or any other study documentation without prior written approval from the Sponsor. All approved modifications must be described and duly justified. The modifications must be classified as either substantial or non-substantial. Substantial amendments will be submitted to the relevant authorities for approval. Substantial amendments with a substantial impact on the safety, health or rights of the participants or the robustness or reliability of the clinical data will not be implemented until approval by authorities. Non-substantial amendments are submitted for notification. The use of CIP waivers is strictly prohibited.

20.3 Responsibility of the Principal Investigator(s)

The individual Principal Investigator is responsible that the conduct of study at the study site is in compliance with the CIP, the Declaration of Helsinki and Good Clinical Practice. In addition, the PI is responsible for complying with any relevant local requirements at their study site. The Principal Investigator will ensure that all staff delegated to the study have the appropriate qualifications and that they have completed relevant study trainings and are GCP certified.

20.4 Informed Consent Process

Study activities for a given patient will not be initiated until informed consent has been obtained from them.

Before signing the informed consent, each patient will receive verbal and written information about the study and investigational device, study procedures, and risks. The study will be explained in lay language. The participant will also have the opportunity to ask questions they have about the study.

The Principal Investigator or delegated staff must obtain written consent from the eligible participants prior to any study procedures, using the most recent approved informed consent form. A verbal explanation of the informed consent form will be provided in terms suited to the participant's comprehension of the purposes, procedures, alternative treatments, and potential risks and benefits of the study and of their rights as research participants. Each participant will be provided the time necessary for them to read and understand the informed consent form and get the opportunity to ask questions they have about the study before signing.

The participants will have the opportunity to discuss the study with their family and/or think about it prior to agreeing to participate.

Participants will be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice and that they will in case of withdrawal not lose the right to any treatment which they were otherwise entitled to. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study or drop out at a later date. If any new information becomes available that may affect the participant's willingness to continue in the study, the Principal Investigator will inform the participant and ask if he/she wishes to continue in the study.

20.5 Insurance and compensation

The Sponsor has an insurance coverage for the treatment of participants in the event of injuries related to the clinical investigation, according to national regulations.

Participants will not receive payments for participation in the investigation. However, documented expenses such as travel and parking will be covered.

20.6 Financial Disclosure and Conflict of Interest

This study is initiated by and financed by Sponsor. Sites are reimbursed for all their expenses related to the study, including time and resources spent. Sponsor will provide all study devices and pay for all expenses related to the conduct of this study. Investigational sites and investigators with a financial interest in the outcome of the study will not be allowed to participate in the study as sites or investigators respectively.

21 Publication policy

Sponsor and Principal Investigators are committed to dissemination of study results. Results will not be withheld regardless of the findings.

The data and statistical analyses from Stage 1 and Stage 2 will be presented in scientific articles (one or more for each Stage) which will be submitted to a peer-reviewed journal in the field of headache, pain medicine or general neurology. Data and manuscripts will not be released before approval by Principal Investigators. All Principal Investigators will be listed as authors on abstracts and publications. All proposed publications and presentations resulting from or relating to the study (either from single sites or multiple sites) must be reviewed and approved by Sponsor before release.

The clinical investigation will be pre-registered in the database clinicaltrials.org.

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