CIP number: H2-MCI-R1-001-2025

Clinical Investigation Plan

Safety assessment of molecular hydrogen inhalation in patients with mild cognitive deficiency

Sponsor: H2 Medical Technologies s.r.o.
Muglinovská 154/73
Ostrava
712 00
Czech Republic

Medical device: H2-Air Blend Inhaler

CIP number: H2-MCI-R1-001-2025

CT registration number:

Version: 1.3.
Date: April, 17th, 2025

The confidential information contained in this Clinical Investigation Plan is provided to you in confidence, for review by you, your staff and an applicable regulatory authority or institutional review committee. It is understood that this information may not be disclosed to any other party, in any form, without prior written authorization from H2 Medical Technologies s.r.o.

Sponsor and Organization of the clinical investigation

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Company Signature and Approval Page

Safety assessment of molecular hydrogen inhalation in patients with mild cognitive impairment

We hereby declare that this Clinical Investigation Plan was prepared scientifically accurately and in full compliance with the applicable regulatory guidelines.

With our signatures, we agree to conduct this pilot investigation in accordance with the Clinical Investigation Plan, applicable local laws and regulatory requirements for conducting pilot investigations and clinical trials with medicinal products. Moreover, we will keep all information obtained in this pilot investigation confidential unless otherwise agreed in writing.

For the Sponsor this Clinical Investigation Plan has been approved by:

SPONSOR

17.4.2025

…… …….

Date Signature

Investigator Signature Page

Safety assessment of molecular hydrogen inhalation in patients with mild cognitive impairment

I hereby declare that I have read the Clinical Investigation Plan and I understand the information. With my signature, I agree to conduct this pilot investigation in accordance with the Clinical Investigation Plan, applicable local laws and regulatory requirements for conducting pilot investigations and clinical trials with medicinal products, and the ethical principles set forth in the Declaration of Helsinki. Moreover, I will keep all information obtained from participation in this pilot investigation confidential unless otherwise agreed in writing.

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2. Abbreviations

ADE	Adverse Device Effect
AE	Adverse Event
ASADEs	Anticipated Serious Adverse Device Effects
BP	Blood pressure
CE	Conformity mark
CIP	Clinical Investigational Plan
eCRF	Electronic Case Report Form
EC	Ethics Committee
VAS	Visual Analogue Scale

3. Synopsis

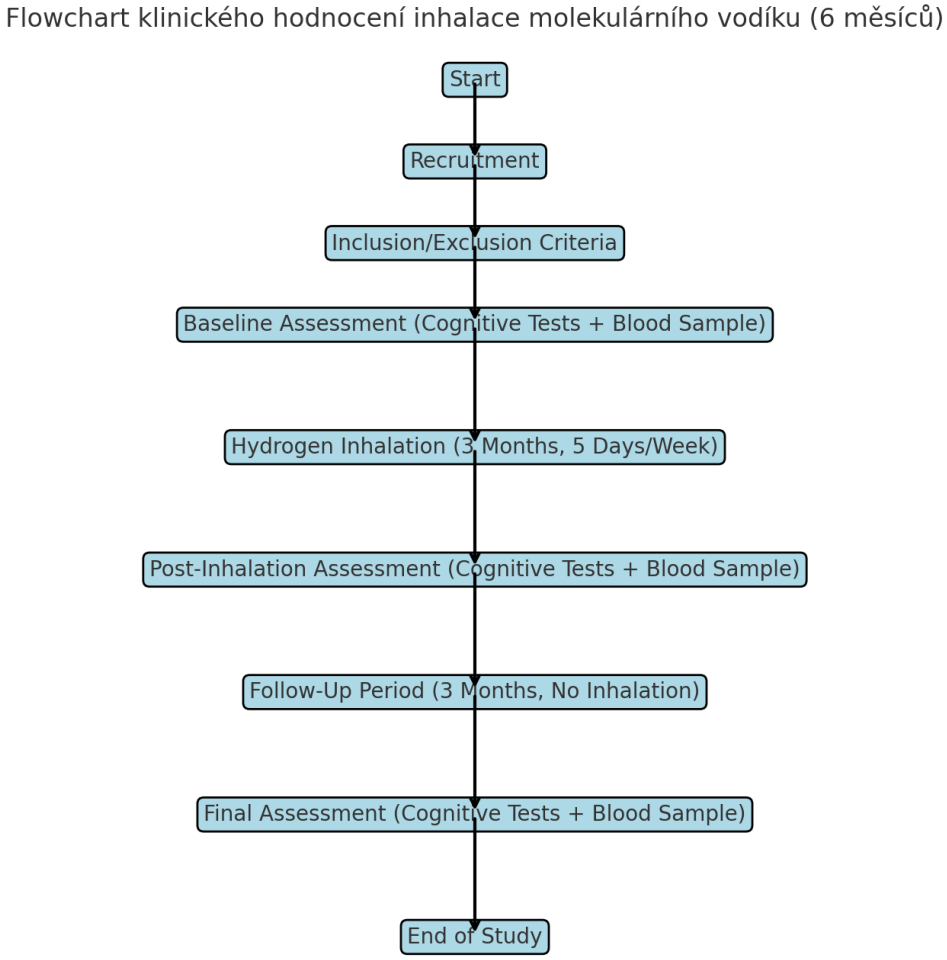
Study Title	**Safety assessment of molecular hydrogen inhalation in patients with mild cognitive impairment**
Version	V1.2.
Study Sponsor	H2 Medical Technologies s.r.o.
Study Phase	A pilot study
Number of sites	A single-site study
Number of subjects	30
Study design	Open-label, one-arm, single-center clinical study
Study timelines	3+3M
Study intervention type and intervention schedule	Experimental group. Daily dosage of H2 gas in a form of inhalation in duration for one hour. Dosage administered during morning hours every working day according to detailed instructions to study participants. Total duration of study: 6M (Intervention phase 3M, follow-up postinterventional phase 3M)
Study objectives	Primary objective: To verify the safety of hydrogen gas inhalation on a sample of patients with mild cognitive impairment (according to the objective opinion of the physician) and diagnosed mild to moderate form of dementia, as well as to verify cognitive abilities via tests from prof. MUDr. Aleš Bartoš, Ph.D. - ALBA (Amnesia Light and Brief Assessment) and POBAV ("Pojmenování obrázků a jejich vybavení" Naming of pictures and their equipment) Secondary objective: To assess the effect on specific health indicators (e.g. inflammation, antioxidant capacity, metabolism) by blood sampling as well as blood pressure/pulse measurement and BMI
Study endpoints	Primary endpoint: Inhalation Safety, Questionnaire - Subjective Assessment (ALBA and POBAV) Secondary endpoints: Biochemical indicators (e.g. inflammation, antioxidant status), blood analysis and BP/Pulse + BMI
Study population	50-80 y.o. in overall good health condition
Inclusion criteria	• patients diagnosed with mild cognitive impairment (MCI) with an overall good health condition • mild cognitive deficit or mild to moderate dementia confirmed by standardized neurocognitive assessments • generally good health, with the ability to perform daily

	activities independently (modified rankin score 0-2, barthel index minimum 70 points) • age between 50 and 80 years, ensuring inclusion of individuals within the typical risk range for cognitive decline • a reliable study partner or caregiver who can provide accurate information about the participant's cognitive and functional abilities throughout the study • stable medical condition, without major fluctuations in health status that could interfere with cognitive assessments • signed informed consent, ensuring that participants understand the study protocol and potential risks
Exclusion criteria	• pregnancy or lactation, as the safety of hydrogen inhalation therapy in these populations has not been established • use of medications or investigational treatments that could significantly affect cognitive function or interfere with study outcomes • history of severe psychiatric disorders such as major depression, bipolar disorder, or schizophrenia, which may independently influence cognitive performance • history of cerebrovascular events, including recent stroke or transient ischemic attack, which may confound cognitive outcomes • severe cardiovascular diseases, including uncontrolled hypertension, recent myocardial infarction, or heart failure, that could pose additional risks during the study • chronic kidney disease (stage 3 or higher), as impaired renal function may alter systemic metabolism and affect the study's findings • significant respiratory disorders, such as severe chronic obstructive pulmonary disease (COPD) or restrictive lung diseases, that could impact the safety of hydrogen inhalation • known hypersensitivity or allergic reactions to materials used in the hydrogen delivery system, such as nasal cannulas or facial masks • recent participation in another clinical trial within the past 90 days, to prevent potential interactions and ensure unbiased study results
Study procedures	Regular monitoring and blood samples testing
Discontinuation criteria	Allergic reaction, negative adverse effects, fails to follow the study instructions

Statistics	This pilot study is not designed for formal hypothesis testing; its purpose is to gather descriptive safety data and estimate effect sizes for future research. We will enroll 30 participants to gather enough information on safety, tolerability, and feasibility. The primary analysis will concentrate on the frequency and severity of adverse events (AEs/SAEs) and ALBA + POBAV scores to evaluate cognitive changes. Descriptive statistics will summarize adverse events, vital signs, and lab results. In the secondary analysis, we will assess variations in biochemical markers (inflammation, antioxidant capacity, metabolism) and physiological metrics (blood pressure, pulse, BMI) using paired t-tests or Wilcoxon signed-rank tests, depending on how the data is distributed. Any missing safety data will be conservatively imputed, and multiple imputation techniques will be utilized for efficacy measurements. While there is no formal interim analysis planned, continuous safety monitoring will be carried out. Results will be shared through summary statistics, tables, and appropriate graphical representations, offering preliminary insights into the feasibility and potential advantages of molecular hydrogen inhalation therapy.

4. Flowchart and schedule of assessments

4.1. Flowchart



4.2. Schedule of assessments

Clinical Study Assessment Schedule

	Timepoint	Cognitive Tests	Blood Sample	Vital Signs Monitoring	Adverse Event Monitor
1	Baseline (Week 0)	Yes	Yes	Yes	Yes
2	During Inhalation (Week 4)	No	No	Yes	Yes
3	During Inhalation (Week 8)	No	No	Yes	Yes
4	Post-Inhalation (Week 12)	Yes	Yes	Yes	Yes
5	Follow-Up (Week 24)	Yes	Yes	Yes	Yes

5. Introduction

Mild cognitive impairment (MCI) serves as a critical link between normal cognitive aging and dementia, impacting millions globally. This condition is marked by subtle yet measurable cognitive decline that does not significantly influence daily life functions. Importantly, those with MCI face a heightened risk of advancing to Alzheimer's disease and other neurodegenerative illnesses.

Currently, options for treating MCI are quite limited, with no proven methods available to halt or reverse cognitive deterioration. While pharmacological treatments offer only symptomatic relief, non-pharmacological methods like lifestyle changes demand long-term commitment and often produce inconsistent outcomes. Consequently, there is a pressing need for safe, well-tolerated, and easily implemented strategies to enhance current treatment modalities.

Molecular hydrogen (H_2) has garnered attention for its antioxidant and anti-inflammatory qualities, potentially aiding in neuroprotection and the preservation of cognitive functions. Preliminary studies indicate that hydrogen inhalation might alleviate oxidative stress and inflammation, both of which are known to contribute to neurodegenerative processes. Nonetheless, while these early results are encouraging, the safety of molecular hydrogen inhalation for patients with MCI remains insufficiently explored.

This clinical study aims to assess the safety and tolerability of molecular hydrogen inhalation via the H2-Air Blend Inhaler in patients diagnosed with MCI. The research will systematically evaluate any adverse effects while laying the groundwork for subsequent investigations into its clinical efficacy.

By filling these gaps, this study aspires to provide essential safety information necessary for the cautious clinical application of H_2 therapy and its prospective role in managing cognitive health.

6. Study rationale

1. Clinical and Scientific Problem Statement

Mild cognitive Impairment (MCI) is an increasingly recognized health concern, affecting millions worldwide and often serving as an early indicator of progressive neurodegenerative diseases such as Alzheimer's disease. It is characterized by subtle impairments in memory, attention, and executive function that interfere with daily activities but do not meet the threshold for dementia.

The pathophysiology of MCI is complex, involving oxidative stress, neuroinflammation, and mitochondrial dysfunction. These factors contribute to neuronal damage and accelerate cognitive decline. Current therapeutic options are primarily symptomatic and fail to address these underlying mechanisms. Pharmacological treatments, such as cholinesterase inhibitors and NMDA receptor antagonists, provide only temporary symptom relief and are associated with adverse effects. Some anti-beta amyloid antibodies were approved as a medical drug, but this antibody slowed down the progression of AD, does not show the improvement, and exhibits frequent adverse effects. Non-pharmacological strategies, including cognitive training, diet, and physical activity, require long-term adherence and offer limited neuroprotective benefits.

Given the increasing burden of MCI on individuals and healthcare systems, there is a pressing need for safe, effective, and easily implementable interventions that target the core

mechanisms driving cognitive decline. This study seeks to evaluate molecular hydrogen inhalation as a novel therapeutic approach with potential neuroprotective effects, addressing a significant gap in current treatment strategies.

2. Limitations of Existing Treatments and Unmet Needs

Currently available treatments exhibit significant limitations:

- **Pharmacological options (e.g., cholinesterase inhibitors, NMDA receptor antagonists)** provide only symptomatic relief and are associated with adverse effects.
- **Lifestyle interventions (e.g., diet, exercise, cognitive stimulation)** require high levels of adherence and individual motivation.
- **Antioxidant and anti-inflammatory therapies** lack sufficient clinical validation for sustained cognitive benefits.
- anti-beta amyloid antibody delays the progression of Alzhemer's disease, but frequent adverse effects cannot be neglected.

Given these limitations, a significant gap exists in effective early interventions that can slow or mitigate cognitive decline at an early stage. There is an increasing demand for safe, accessible, and evidence-based therapies that can complement existing treatment paradigms.

3. Justification for Molecular Hydrogen as a Potential Intervention

Molecular hydrogen (H_2) has emerged as a promising therapeutic agent due to its selective antioxidant and anti-inflammatory properties. Studies suggest that H_2 inhalation may provide neuroprotection by targeting key pathways implicated in cognitive decline.

Key scientific justifications include:

- **Oxidative stress reduction:** H_2 selectively neutralizes hydroxyl radicals and peroxynitrite, mitigating cellular damage in neural tissues.
- **Neuroinflammation modulation:** Preclinical studies indicate that H_2 reduces neuroinflammatory markers and cytokine expression, potentially delaying cognitive impairment progression.
- **Mitochondrial function improvement:** H_2 has been shown to enhance mitochondrial efficiency, supporting neuronal energy metabolism and function.

Despite promising preclinical data, the safety profile of H_2 inhalation in MCD patients remains insufficiently characterized. This study aims to establish safety and tolerability parameters, serving as a foundation for future efficacy trials.

4.Primary Objectives and Expected Contributions

This clinical investigation aims to:

1. **Assess the safety and tolerability** of molecular hydrogen inhalation in patients with MCD, ensuring that it meets regulatory standards for future clinical application.
2. **Provide robust scientific data** supporting the potential neuroprotective benefits of H_2 inhalation in early cognitive impairment.
3. **Establish standardized dosing protocols** to optimize treatment efficacy and guide subsequent clinical trials.

4. **Contribute to regulatory approvals**, paving the way for molecular hydrogen inhalation devices to be classified as medical interventions.

The results of this study will have significant implications for:

- **Scientific knowledge**, advancing the understanding of hydrogen-based neuroprotection.
- **Clinical practice**, providing healthcare professionals with a new tool for managing MCI.
- **Medical device regulation**, offering necessary safety data for potential integration into broader therapeutic frameworks.

5. Clinical and Regulatory Impact

This investigation is designed to:

1. Establish the **safety and tolerability** of molecular hydrogen inhalation in MCI patients.
2. Provide essential data for **regulatory submissions** to facilitate potential classification of H_2 inhalation devices as medical interventions.
3. Lay the groundwork for future **efficacy trials**, evaluating the role of H_2 in mitigating cognitive decline.

By bridging existing knowledge gaps, this study supports the advancement of molecular hydrogen as a potential MedTech/HealthTech innovation, contributing to the development of novel neuroprotective therapies.

7. Investigational device

7.1. Description of the device

H2-Air Blend Inhaler is a medical device designed to generate and deliver molecular hydrogen (H_2) gas for therapeutic inhalation. It is manufactured by H2 Medical Technologies s.r.o. and utilizes SPE/PEM electrolysis technology to ensure high-purity hydrogen production.

The device is intended for use in clinical and research settings, where it can provide controlled hydrogen inhalation therapy to patients. It is equipped with multiple safety features and operational modes, making it suitable for a variety of applications.

The device consists of several key components:

- main unit – the core hydrogen generation system, including the electrolysis chamber
- water reservoir – a compartment for purified water, essential for hydrogen production
- inhalation delivery system – includes nasal cannulas, facial masks, and additional adapters to facilitate patient-specific usage
- control panel and display – provides operational controls, status indicators, and timer settings
- power supply unit – an external power adapter ensuring stable operation

Each device is individually identified by a serial number (SN), which is displayed on the label and included in clinical documentation for tracking and regulatory purposes.

7.2 Intended use

The H2-Air Blend Inhaler is designed to produce molecular hydrogen gas for controlled inhalation therapy. It aims to serve as a potential neuroprotective treatment, especially for those with mild cognitive impairment (MCI).

This device is meant for clinical research settings, where it can be utilized under medical oversight. Studies are underway to evaluate the safety and tolerability of hydrogen inhalation among patients at risk of neurodegenerative disorders.

This device is not for self-use by untrained individuals and should be operated in accordance with the instructions for use (IFU).

7.3 Technical specifications

H2-Air Blend Inhaler is built with advanced hydrogen generation technology, incorporating multiple safety and performance features. The key specifications of the device are as follows:

Parameter	**Specification**
Model name	H2-Air Blend Inhaler
Technology	SPE/PEM electrolysis (DuPont membrane)
Hydrogen flow rate	up to 300 ml/min
Hydrogen purity	99,9% molecular hydrogen
Hydrogen mixture concentration	3,75% molecular hydrogen
Power supply	AC 230V/50Hz
Power consumption	300 W
Operating temperature	5°C to 30°C
Dimensions	210 x 300 x 310 mm
Weight	5.5 kg without purified water
Water type	purified, laboratory-grade water (H_2 READY®)
Max operating time	6 hours continuous use, 30 min cooling
Safety features	overpressure protection, automatic shut-off, real-time monitoring

To maintain proper functionality, the device must be regularly refilled with purified water, and operational safety procedures must be followed.

7.4 mode of operation

H2-Air Blend Inhaler is designed for simple and efficient operation, ensuring safe and controlled hydrogen delivery.

- **Preparing the device**

1. the user fills the water reservoir with purified, laboratory-grade water as specified in the instructions for use
2. the device is connected to a power source and switched on

3. the user selects the appropriate inhalation adapter, such as a nasal cannula or facial mask

- **Starting the inhalation session**

1. the device is activated via the control panel, where the user can set the desired inhalation duration and mode
2. hydrogen is generated through the SPE/PEM electrolysis system and delivered at a constant flow rate
3. the device maintains real-time monitoring, ensuring a consistent and safe inhalation experience

- **Ending the session and maintenance**

1. once the session ends, the device automatically shuts off to prevent overuse
2. the user drains excess water if necessary and cleans the inhalation accessories according to the instructions
3. the device must be stored in a well-ventilated area to prevent hydrogen accumulation

The maximum continuous usage time is 2 hours, after which the device requires a 30-minute cooling period. It should always be operated in a ventilated environment to ensure safety.

7.5 Regulatory status

H2-Air Blend Inhaler is a medical device currently undergoing clinical evaluation. Its regulatory classification is under assessment, and the device is being tested to ensure compliance with safety and performance standards.

The device:

- meets IEC 60601-1 standards for medical electrical equipment
- complies with ISO 13485 for medical device quality management
- is under evaluation for conformity with EU MDR (2017/745)

This clinical investigation will generate essential safety data, supporting potential regulatory submissions for CE certification.

7.6 Risk and safety considerations

H2-Air Blend Inhaler has been designed with multiple safety features, but potential risks must be considered.

Potential risks and adverse effects:

- mild respiratory irritation, particularly in individuals with pre-existing respiratory conditions such as asthma or COPD
- dizziness or lightheadedness, especially with prolonged inhalation
- dry throat or mild throat discomfort, which may occur during extended use
- allergic reactions, though rare, due to sensitivity to hydrogen exposure
- transient headaches, occasionally reported in previous studies

Safety measures and risk mitigation:

- the device is equipped with automatic shut-off and pressure control to prevent overuse
- only purified water must be used, preventing damage to the electrolysis membrane
- proper ventilation is required to minimize the risk of hydrogen accumulation
- vital signs must be monitored during clinical use to detect any adverse effects

Based on risk analysis according to ISO 14971, the device is classified as low risk, with appropriate mitigation strategies in place. All identified risks have been evaluated, and the benefits of hydrogen therapy outweigh the potential risks.

7.7. Indications, contraindications and population

Information related to indications, contraindications and relative contraindications are described in the approved IFU and the appendix described the handling the device concerning to the clinical investigation, see Appendix A and inclusion and exclusion criteria (see Section 10.3.)

7.8. Potential complications

Potential complications are described in the approved IFU, for reference see Appendix A.

7.9. Handling

Detailed handling conditions are described in the approved IFU and should be followed, for reference see Appendix A.

The device includes accessories:

The traceability will be done thanks to serial number “SN”, which is described in the User information and placed on the label of the device and on the accessories (except the neutral electrode, which is standard single use neutral electrode).

7.10. H2-Air Blend Inhaler handling procedures

Description of handling comes from IFU.

8. Risks and benefits of the investigational device and clinical investigation

Molecular hydrogen inhalation therapy is a new strategy aimed at reducing oxidative stress, diminishing neuroinflammation, and enhancing mitochondrial function. These factors are thought to significantly influence the development of neurodegenerative diseases, especially in those with mild cognitive impairment (MCI).

By targeting these harmful processes, hydrogen inhalation could potentially slow down cognitive decline, strengthen neuronal resilience, and boost overall cognitive capabilities. In contrast to traditional medications, which often come with considerable side effects and may only alleviate symptoms, hydrogen therapy provides a non-invasive, well-tolerated option that could be integrated into wider early intervention plans for neurodegenerative disease prevention.

H2-Air Blend Inhaler has been created to ensure the safe and controlled delivery of high-purity molecular hydrogen gas. Past studies indicate that hydrogen inhalation is typically well tolerated, with a low rate of adverse effects. Nevertheless, any new intervention must undergo thorough clinical assessments to evaluate both safety and therapeutic potential.

This study will methodically assess the advantages and risks of hydrogen inhalation therapy, ensuring that all possible safety issues are comprehensively documented and addressed. Ongoing monitoring during the research will allow for the detection of any unexpected adverse events, while also evaluating the device's capacity to deliver meaningful clinical improvements.

8.1. Anticipated adverse device effects

Description – Anticipated Adverse Device Effects

While molecular hydrogen inhalation is widely researched and deemed safe, the possibility of adverse effects remains. Drawing from earlier clinical studies, preclinical research, and expert evaluations, the following negative effects have been recognized as potential results of utilizing the H2-Air Blend Inhaler.

- mild respiratory irritation, particularly in individuals with pre-existing respiratory conditions such as asthma or chronic obstructive pulmonary disease (COPD). Inhalation of molecular hydrogen may cause temporary irritation of the airways, leading to mild coughing or throat discomfort
- dizziness or lightheadedness, which could result from prolonged inhalation sessions or individual sensitivity to hydrogen exposure. This effect is expected to be transient and to resolve upon cessation of inhalation
- dry throat or mild throat discomfort, which may occur during extended use due to the continuous flow of dry hydrogen gas
- potential allergic reactions, although rare, due to individual sensitivity to the hydrogen delivery mechanism or the materials used in the nasal cannulas and masks
- transient headache, which has occasionally been reported in clinical studies involving molecular hydrogen inhalation. This effect is expected to be mild and self-limiting

These effects are expected to be mild, temporary, and not life-threatening. To ensure participant safety, we will conduct ongoing monitoring throughout the study. Any adverse events reported will be recorded, evaluated, and managed following established safety protocols. Participants who have persistent symptoms will undergo proper medical evaluation, and if necessary, adjustments to the inhalation protocol will be made.

8.2. Residual risks associated with the investigational device

A thorough risk analysis of the H2-Air Blend Inhaler has been performed in accordance with ISO 14971, the standard for medical device risk management. This evaluation addressed every element of the device's design, manufacturing, and usage, ensuring that residual risks are reduced and accepted based on the expected clinical advantages. The risk assessment process evaluated the following critical factors:

- compliance with the established risk management plan, ensuring that all potential risks have been evaluated and controlled
- adherence to international medical device safety standards, including IEC 60601-1, which specifies general safety requirements for medical electrical equipment
- application of usability engineering principles in accordance with IEC 62366 to ensure that the device can be used safely and effectively by trained personnel

- implementation of quality control measures under ISO 13485, ensuring that appropriate methods are in place for detecting and addressing risks in both production and post-production phases
- confirmation that all identified risks have been assessed in relation to the current state of the art and available scientific knowledge

Residual risk refers to the risk level that persists after implementing all reasonable mitigation measures. The risk management evaluation has determined that all identified risks linked to the H2-Air Blend Inhaler fall within acceptable limits, resulting in a low overall risk profile.

However, despite thorough mitigation actions, some residual risks continue to exist, especially concerning human factors and device operation. These include:

- user error, such as failure to follow operating instructions, which could lead to inappropriate inhalation duration or incorrect device settings
- inadequate maintenance, which may result in contamination of the device's internal components if proper cleaning procedures are not followed
- improper use by untrained individuals, as the device is intended for supervised clinical use rather than self-administration. A lack of understanding of the correct inhalation settings or operational procedures may compromise safety and effectiveness

To reduce these risks, the device's usage guidelines provide explicit, step-by-step instructions for correct operation, maintenance, and troubleshooting. Furthermore, built-in safety features like automatic shut-off and real-time monitoring help mitigate user-related errors.

From a regulatory standpoint, the device's residual risk has been evaluated as acceptable, with its benefits surpassing any potential safety issues. Classified as a low-risk medical device when operated according to the specified clinical protocol, it is thus suitable for investigational use in this study.

The risk assessment process has not revealed any new potential hazards beyond those stated in the risk management plan. Nonetheless, maintaining vigilance is essential, and any unforeseen issues that arise during the study will be logged, analyzed, and handled with suitable risk mitigation strategies.

The main concern regarding residual risk involves the possible misuse of the device by untrained users or unauthorized individuals. This risk has been reduced by enforcing strict training requirements for users and ensuring that the device is operated solely in supervised clinical environments. Clear warnings in the usage instructions emphasize that only qualified medical professionals should handle the device.

In conclusion, the analysis of risk versus benefit supports ongoing research into molecular hydrogen inhalation therapy as a possible treatment for neurodegenerative diseases. The established risk management strategies guarantee that participant safety is prioritized while facilitating the gathering of valuable clinical data regarding the effectiveness and tolerability of the H2-Air Blend Inhaler.

8.3. Risks associated with participation in the clinical investigation

There is a very small risk associated with participation in this trial – the device will be used within its approved IFU and amendment.

8.4. Possible interactions with concomitant medical treatments

For the H2-Air Blend Inhaler there is no interaction with medical treatment.

8.5. Steps that will be taken to control or mitigate the risks

To guarantee patient safety throughout the hydrogen inhalation procedure in this clinical trial, a robust array of risk mitigation strategies will be employed. These strategies aim to reduce the chances of adverse events, ensure correct device operation, and allow for prompt intervention if unexpected reactions occur.

- **Pre-screening for contraindications** – All participants will undergo a thorough medical evaluation before enrollment to identify potential contraindications. Individuals with pre-existing respiratory conditions such as chronic obstructive pulmonary disease (COPD), severe asthma, or any other condition that could be exacerbated by inhalation therapy will either be excluded from the study or closely monitored to prevent complications.
- **Controlled administration environment** – All inhalation sessions will take place in a medically supervised setting to allow for continuous monitoring of participants and immediate intervention if any adverse effects occur. The controlled environment ensures that trained medical personnel are available at all times to manage any unexpected reactions, provide appropriate medical support, and document any safety concerns.
- **Real-time monitoring of vital signs** – To ensure participant safety during inhalation, continuous monitoring of key physiological parameters such as oxygen saturation (SpO_2), heart rate, blood pressure, and respiratory rate will be conducted. This allows for early detection of any physiological changes that could indicate a potential adverse effect, enabling immediate adjustments to the inhalation protocol if necessary.
- **Clear patient education and guidance** – Prior to participating in the study, all participants will receive comprehensive training on the correct use of the H2-Air Blend Inhaler. They will be instructed on proper inhalation techniques, device handling, and how to recognize early signs of discomfort or adverse reactions. Educational materials, including written guidelines and visual demonstrations, will be provided to reinforce safe device usage.
- **Post-inhalation observation periods** – After each inhalation session, participants will remain under medical supervision for a defined period to monitor for any delayed adverse effects. This precaution ensures that any potential issues are identified and addressed before participants leave the study site.

These steps are crucial for conducting molecular hydrogen inhalation therapy safely, minimizing risks to participants, and enabling the collection of high-quality clinical data.

8.6. Risk-to-benefit rationale

The assessment of risks and benefits regarding molecular hydrogen inhalation therapy for patients with mild cognitive impairment (MCI) strongly indicates its promise as a safe and effective treatment. The expected advantages of this therapy surpass the minimal risks linked to its use, highlighting it as a valuable option for tackling early cognitive decline.

Anticipated benefits

- **Neuroprotection and cognitive support** – Molecular hydrogen has demonstrated the ability to reduce oxidative stress and neuroinflammation, both of which are key contributors to neurodegenerative processes. By mitigating these harmful effects, hydrogen therapy may help preserve neuronal function and slow cognitive deterioration.

- **Non-invasive and well-tolerated treatment** – Unlike pharmacological interventions, which may cause systemic side effects, molecular hydrogen inhalation is a non-invasive therapy that is expected to be well tolerated by most patients. Existing studies have shown that hydrogen inhalation does not lead to significant adverse reactions, further supporting its safety profile.
- **Potential to slow disease progression** – By targeting mitochondrial function, synaptic plasticity, and neuronal resilience, molecular hydrogen may help delay the transition from mild cognitive impairment to more advanced neurodegenerative conditions such as Alzheimer's disease. This potential disease-modifying effect represents a significant advantage over current symptomatic treatments.
- **Improved quality of life** – Maintaining cognitive function and reducing the rate of cognitive decline can significantly impact patients' daily lives. A therapy that supports memory, executive function, and overall mental clarity could enhance independence and overall well-being, reducing the burden on caregivers and healthcare systems.

Risk considerations

The risks linked to molecular hydrogen inhalation therapy are minimal and mainly involve mild, temporary side effects. These include:

- mild respiratory irritation, particularly in individuals with pre-existing respiratory conditions
- dizziness or lightheadedness, potentially due to prolonged inhalation or individual sensitivity
- dry throat or mild throat discomfort resulting from continuous exposure to hydrogen gas
- transient headache, which has occasionally been reported in studies involving hydrogen therapy

These risks are effectively mitigated through several key measures:

- **Careful patient selection** – Excluding individuals with severe respiratory conditions ensures that participants do not have predisposing factors that could amplify minor adverse effects.
- **Monitored administration** – All inhalation sessions will be supervised by medical professionals, allowing for immediate response to any discomfort or physiological changes.

Considering the positive safety profile of molecular hydrogen and its promising potential in preventing neurodegenerative diseases, the advantages of this treatment far exceed the risks. This clinical study's organized and regulated design reduces risks while optimizing the chance to evaluate the therapeutic effects of hydrogen inhalation in those with mild cognitive impairment.

9. Study Objectives and Endpoints

9.1. Study Objectives

Primary objective: To verify the safety of hydrogen gas inhalation on a sample of patients with mild cognitive impairment (according to the objective opinion of the physician) and diagnosed mild to moderate form of dementia, as well as to verify cognitive abilities (ALBA and POBAV)

Secondary objective: To assess the effect on specific health indicators (e.g. inflammation, antioxidant capacity, metabolism) by blood sampling as well as blood pressure/pulse measurement and BMI

9.2. Study Endpoints

Primary endpoint: Inhalation Safety, Questionnaire - Subjective Assessment (ALBA and POBAV)

Secondary endpoints: Biochemical indicators (e.g. inflammation, antioxidant status), blood analysis and BP/Pulse + BMI

Safety assessments:

- Adverse events (AEs)
- Treatment-emergent adverse events (TRAEs)
- Serious adverse events (SAEs)
- Serious adverse device effects (SADEs) - both anticipated SADEs (ASADEs) and unanticipated SADEs (USADEs)
- Vital signs

10. Design of the extension investigation

10.1. General

Clinical safety testing includes thorough blood testing to catch any adverse effects of inhalation early, especially with regard to possible negative effects on organ function, metabolic parameters and inflammatory reactions. The following spectrum of parameters will be monitored for the 3-month clinical trial in patients with MCI/mild/advanced dementia:

1. Baseline blood count and differential leukocyte score:

- **Hemoglobin (Hb)**, **hematocrit (Hct)**, RBCs **(RBC)**: To ensure adequate oxygenation of tissues and to detect possible anemia.
- **Leukocytes (WBC)** and **differential leukocyte score**: To detect infection or inflammatory reaction.
- **Platelets (PLT)**: To evaluate the risk of bleeding or thrombosis.

2. Basic biochemical parameters:

- **Liver function**: **AST (aspartate aminotransferase)**, **ALT (alanine aminotransferase)**, **gamma-glutamyltransferase (GGT)**, **bilirubin**, **alkaline phosphatase (ALP)**. These parameters monitor liver function.
- **Renal function**: **Creatinine**, **urea (BUN)**, **estimated glomerular filtration rate (eGFR)**. To monitor kidney health.
- **Electrolytes**: **Sodium (Na+), Potassium (K+), Chloride (Cl-), Calcium (Ca2+), Phosphate**. To prevent and detect electrolyte balance disorders.
- **Glucose** (fasting): To assess the risk of sugar metabolism disorders.

3. Inflammatory and immunological markers:

- **C-reactive protein (CRP)**: To detect the influence of systemic inflammation.

4. Lipid measurements:

- **Total cholesterol**, **HDL cholesterol**, **LDL cholesterol**, **triglycerides**. This profile is particularly important for cardiovascular risk assessment.

5. Metabolic parameters:

- **Glycated haemoglobin (HbA1c)**: If long-term monitoring of blood sugar levels is necessary (e.g. in patients at risk of diabetes).
- **Uric acid**: To detect gout risk.
- **Albumin and total protein**: To assess nutritional status and liver function.

6. Hormonal and endocrine parameters:

- **Thyroid Stimulating Hormone (TSH)**: Thyroid can affect cognitive function, so checking its status is a good idea.
- **Free T4 (thyroxine) and T3** as the active hormone.

7. Coagulation parameters:

- **Prothrombin time (PT)**, **activated partial thromboplastin time (aPTT)**, **INR**: If there is a risk of coagulation disorders or the patient is on anticoagulation therapy.

8. **Measurement of blood pressure (systolic/diastolic) and pulse**

- Measurements are made according to WHO standards (3 measurements, the first is crossed off and the arithmetic mean of the 2 and 3 measurements is taken)

10.2. Subject selection

All subjects willing to participate, having signed an informed consent form and meeting all inclusion and none of the exclusion criteria listed in Sections 10.3.1 and 10.3.2 will be included into the pilot investigation.

10.3. Inclusion

10.3.1. Inclusion criteria

- patients diagnosed with mild cognitive impairment (MCI) with an overall good health condition
- mild cognitive deficit or mild to moderate dementia confirmed by standardized neurocognitive assessments
- generally good health, with the ability to perform daily activities independently (modified ranking score 0-2, barthel index minimum 70 points)
- age between 50 and 80 years, ensuring inclusion of individuals within the typical risk range for cognitive decline
- a reliable study partner or caregiver who can provide accurate information about the participant's cognitive and functional abilities throughout the study
- stable medical condition, without major fluctuations in health status that could interfere with cognitive assessments
- signed informed consent, ensuring that participants understand the study protocol and potential risks

10.3.2. Exclusion criteria

- pregnancy or lactation, as the safety of hydrogen inhalation therapy in these populations has not been established
- use of medications or investigational treatments that could significantly affect cognitive function or interfere with study outcomes
- history of severe psychiatric disorders such as major depression, bipolar disorder, or schizophrenia, which may independently influence cognitive performance
- history of cerebrovascular events, including recent stroke or transient ischemic attack, which may confound cognitive outcomes
- severe cardiovascular diseases, including uncontrolled hypertension, recent myocardial infarction, or heart failure, that could pose additional risks during the study
- chronic kidney disease (stage 3 or higher), as impaired renal function may alter systemic metabolism and affect the study's findings
- significant respiratory disorders, such as severe chronic obstructive pulmonary disease (COPD) or restrictive lung diseases, that could impact the safety of hydrogen inhalation
- known hypersensitivity or allergic reactions to materials used in the hydrogen delivery system, such as nasal cannulas or facial masks
- recent participation in another clinical trial within the past 90 days, to prevent potential interactions and ensure unbiased study results

10.3.3. Subject enrolment

All eligible subjects having given informed consent and fulfilling all eligibility criteria will be considered enrolled in the pilot investigation.

10.3.4. Subject completion

The subject has completed the entire pilot investigation when completing the inhalation schedule and the follow-up phase including the medical screening/markers.

10.3.5. Subject discontinuation

Participants can choose to stop their involvement in the study at any moment and for any reason, without facing any repercussions on their future medical treatment. Should a participant opt to withdraw, their reason for discontinuation will be recorded in both the subject's source documents and the electronic case report form (eCRF).

If withdrawal is due to an adverse event, the participant will be monitored until the event is fully resolved, or a satisfactory explanation for the observed changes is provided.

Moreover, a participant may be removed from the study if any of the following conditions occur and are properly documented:

- withdrawal of informed consent at the request of the participant
- medically significant adverse event(s) that require discontinuation
- pregnancy, as the safety of hydrogen inhalation therapy during pregnancy has not been established
- major protocol violation, such as lack of compliance with study requirements, failure to meet or maintain eligibility criteria, or other protocol deviations that may impact study validity
- investigator or sponsor decision due to safety concerns or any other relevant condition that may compromise the participant's well-being
- evidence of drug use or excessive alcohol consumption, defined as an intake of more than 100 mg of ethanol per day

All subject discontinuations will be thoroughly recorded in the source documents and eCRF. Participants withdrawn from the study will not have replacements. After discontinuation, the management of the participant's medical care will be under the investigator's discretion, ensuring suitable follow-up and support as necessary.

10.3.6. Lost-to Follow up

Subjects who do not return for any post-baseline visits as defined by visit window and cannot be contacted after repeated attempts may be considered lost to follow-up. Any lost to follow up must be also recorded in the eCRF. The investigator must take every effort to contact these subjects and to ask them to come for a visit. Such follow-up attempts need to be documented in source documentation.

10.4. Study method

10.4.1. Visit schedule

The visit schedule is in accordance of the protocol.

10.4.2. Unscheduled visit

Additional visits may be scheduled, as necessary, to ensure the safety and well-being of subjects.
All additional exams should be fully documented in the source documents and on unscheduled visit eCRF pages, as appropriate. Visits intended to fulfil scheduled visit requirements that fall outside the designated scheduled visit range are not unscheduled visits. In these cases, the visit data will be collected and transcribed to the appropriate scheduled visit eCRF page. If a subject is seen for multiple visits during a given visit timeframe, the data from the visit that is intended to meet the protocol requirements for the scheduled visit should be captured on the visit eCRF page. Where such determination cannot be made, the first visit within the scheduled visit interval will be used for completion of the protocol required scheduled visit eCRF page. Data

from any additional visits within a scheduled visit interval will be captured on an unscheduled visit eCRF page.

The following procedures will be performed (if applicable):

- Physical examination
- Measurement of vital signs
- Measurement of body weight
- Routine hematology, blood chemistry and urinalysis assessment
- Assessment of concomitant medications
- Assessments of AEs

10.4.3. Study procedures description

Demographics and baseline characteristics

The subject's date of birth, gender, race, height, weight will be recorded at baseline visit.
For body weight measurement, subjects must be undressed with the exception of underwear. For height measurement, subjects must be without shoes; subject's height will be measured by site staff by local standard.

Medical history

A detailed medical history for each subject will be obtained at baseline visit. All relevant past or present conditions including concomitant medication, as well as prior surgical interventions will be recorded. In addition, subject's history of tobacco, alcohol and caffeine use will be recorded.

MCI – Mild cognitive impairment

Mild cognitive impairment (MCI) is a prevalent condition characterized by cognitive decline that exceeds normal aging but does not meet the criteria for dementia.

Vital signs

Vital signs include blood pressure and pulse rate.

- Subject should avoid food immediately before the measurement
- Subject should go to the toilet before BP measurement. A full bladder can increase blood pressure slightly
- Subject should sit for ten minutes in a comfortable position with his/her legs and ankles uncrossed and his/her back supported against a chair before and during a measurement
- Subject should be asked to sit quietly before and during monitoring
- When subject is ready to take his/her blood pressure, subject should be calm and not think about stressful things and not to talk at all
- Subject's arm must be positioned properly. The same arm must be always used when taking subject's blood pressure. Subject should rest his/her arm, raised the arm to the level of his/her heart, and put the arm on a table, desk or chair arm. If needed, a pillow or cushion should be placed under subject's arm to elevate the arm high enough
- The cuff must be placed on bare skin, not over clothing. The appropriately sized cuff should be always used
- One measurement should be performed. If the monitor doesn't automatically log blood pressure readings or heart rates, the measurement should be repeated until a valid reading is, given that the procedure is accepted by the subject.

Physical examination:

This includes examination of the main body systems.

Neurological examination:

The physical examination includes detailed neurological examination so that the eligibility of patients for this study can be evaluated and confirmed.

Patient questionary
Description.

European Quality of Life 5 Dimensions questionnaire (EQ-5D-5L)
This tool represents a standardized measure of health-related quality of life. It comprises the following five dimensions, each describing a different aspect of health: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. Each dimension has five response levels: no problems, slight problems, moderate problems, severe problems, unable to/extreme problems. The result is represented by the EQ-5D-5L index, which is derived by applying a formula that attaches values (weights) to each of the levels in each dimension. The higher the EQ-5D-5L index, the better is health-related quality of life.
In addition, the EQ Visual Analog Scale (EQ-5D VAS) provides a quantitative measure of the patient's perception of their overall health status. EQ-5D VAS is numbered from 0 ("the worst health status you can imagine") to 100 ("the best health status you can imagine").[33]
In this study, EQ-5D-5L version 3.0 will be used - see Appendix.

10.4.4. Concomitant medication

All medications that are considered necessary for the subject's welfare may be used at the Investigator's discretion, however dose of any chronic medications, if applicable, must be stable for at least 90 days prior to study entry. Clinical trial reports will follow accurate and comprehensive documentation of concomitant medications to assess drug safety, efficacy, and potential interactions.

11. H2-Air Blend Inhaler treatment

11.1. H2-Air Blend Inhaler treatment description

The H2-Air Blend Inhaler is a medical-grade device crafted for the safe and controlled inhalation of molecular hydrogen (H_2) gas. It utilizes advanced solid polymer electrolyte (SPE)/proton exchange membrane (PEM) electrolysis technology, guaranteeing the generation of high-purity hydrogen gas with a stable and reliable output.

This device is specifically designed for clinical use and supervised home applications, offering a non-invasive method for cognitive support, neuroprotection, and reducing oxidative stress. Its compact, user-centric design, along with integrated safety features, ensures precision, reliability, and ease of use in both research and therapeutic settings.

Key features and functionalities

- high-purity hydrogen generation – utilizes SPE/PEM electrolysis technology to produce hydrogen with a purity of not exceeding 4 %, ensuring safe and efficient inhalation therapy
- fixed and controlled hydrogen flow rate – delivers a standardized concentration of hydrogen gas, ensuring consistency in therapeutic exposure while maintaining safety standards
- lightweight and portable design – compact and easy to handle, making it suitable for both clinical applications and supervised home-based therapy

- integrated safety mechanisms – features automatic shutoff, overpressure protection, and real-time hydrogen concentration monitoring, minimizing risks associated with prolonged or improper use
- interval-based inhalation mode – the device operates in predefined inhalation cycles, allowing for optimized exposure without risk of excessive hydrogen intake

Clinical application and expected benefits

H2-Air Blend Inhaler aims to act as a possible neuroprotective solution, especially for individuals experiencing mild cognitive impairment (MCI) and other issues related to oxidative stress and neuroinflammation. Expected advantages of molecular hydrogen inhalation therapy consist of:

- cognitive function support – by reducing oxidative stress and neuroinflammation, hydrogen inhalation may help preserve neuronal function and slow cognitive decline
- mitochondrial efficiency enhancement – hydrogen therapy has been shown to support mitochondrial function, improving energy metabolism in neuronal cells and enhancing synaptic plasticity
- non-invasive neuroprotection – unlike pharmaceutical treatments, hydrogen inhalation offers a safe and well-tolerated alternative that does not require systemic drug exposure
- improved overall well-being – as a complementary therapy, hydrogen inhalation may enhance quality of life by supporting mental clarity, executive function, and general cognitive resilience

This device represents an innovative approach to early intervention strategies in neurodegenerative disease prevention, offering a scientifically grounded, safe, and accessible therapeutic option for individuals at risk of progressive cognitive decline.

11.2. Responsibility for the use of the H2-Air Blend Inhaler during the clinical trial

Access to the investigational medical device must be tightly regulated, with its use limited solely to the clinical trial as detailed in the clinical investigation plan (CIP). It is prohibited to use the investigational device for any purposes outside the approved study framework.

The sponsor holds the responsibility for tracking, managing, and documenting the physical location and condition of all investigational medical devices from their dispatch to the trial site until they are either returned, disposed of, or otherwise properly accounted for as per regulatory standards. Clear procedures must be established by the sponsor for the storage, handling, and transportation of these devices to guarantee their integrity. When necessary, suitable packaging materials should be provided for the safe return or disposal of investigational medical devices, especially those that may present safety risks.

Each study site must ensure that the principal investigator or an assigned responsible person maintains precise records documenting specific information related to the investigational medical device.

- names of all personnel involved in the receipt, use, return, or disposal of the investigational device
- date of receipt, identification details, and quantity of each trial device, including batch number, production number, or unique device identifier (UDI)
- expiry date of the device, if applicable
- date or dates of use for each participant, recorded in the participant's study file

- date of return or explantation of the investigational device from the participant, if applicable
- date of return of unused, expired, or non-functional investigational devices, if applicable
- date and documentation of disposal of investigational devices according to sponsor instructions, regulatory guidelines, and environmental safety regulations

To guarantee accountability and compliance, it's essential that all aspects of medical device handling, usage, and disposal are well-defined in written procedures. These guidelines should outline the roles, responsibilities, and processes for distributing, storing, using, tracking, and returning or disposing of devices. The principal investigator is responsible for making sure that all study personnel involved in device handling receive training on these procedures. Regular audits and monitoring visits may be carried out to confirm adherence to these standards, ensuring that all investigational medical devices are utilized in line with the study protocol and relevant regulations.

12. Quality control and monitoring

12.1. Quality control and monitoring

Quality control encompasses the verification and monitoring of all activities performed before, during, and after the pilot investigation. These operational methods and procedures will align with relevant local laws, regulatory standards for clinical investigations involving medical devices, and the sponsor's standard operating procedures (SOPs). To guarantee the accuracy and reliability of the data, the following quality control measures will be put into action:

- selection of qualified investigators with relevant experience in clinical research and the investigational device
- careful selection of appropriate study sites with adequate infrastructure, equipment, and trained personnel
- comprehensive training of investigators and study personnel to ensure adherence to the clinical investigation plan (CIP) and regulatory standards
- review and standardization of all study procedures with investigators and site personnel before the initiation of the study
- ongoing monitoring visits by sponsor representatives to ensure compliance with the protocol, adherence to Good Clinical Practice (GCP), and data integrity

Data entered in the electronic case report form (eCRF) will be cross-checked with source documents, such as medical records, to ensure completeness and accuracy. This review will be carried out by trained monitors assigned by the sponsor. Any discrepancies found during the monitoring process will be swiftly addressed and resolved with the investigator or an appointed study team member.

12.2. Quality assurance

The sponsor's quality assurance (QA) department and regulatory authorities may review and audit the pilot investigation. Auditors and inspectors are authorized to examine study-related records and procedures anytime during or after the study to confirm adherence to relevant regulations and study requirements. This encompasses access to:

- study-related documents and source data

- medical records of study participants, provided that confidentiality requirements are met
- investigational sites and facilities where study activities are conducted

By joining this pilot study, investigators commit to undergoing regulatory inspections and sponsor audits, thereby promoting transparency and meeting quality and compliance standards.

12.3. Document retention

The sponsor and all investigational sites are required to keep study-related documents in line with relevant regulatory standards. Investigational sites need to adopt secure storage methods to avoid accidental loss, early destruction, or unauthorized access to study documents. The retention duration for crucial documents will adhere to both national and international regulations. Investigational sites should notify the sponsor before disposing of any records to ensure compliance with documentation requirements for the study. If an investigational site cannot retain records for the necessary time, it must arrange for the secure transfer of documents to the sponsor or a designated storage facility.

12.4. Confidentiality and data protection

Information gathered during the study will remain strictly confidential. Sharing participant details with unauthorized third parties is not allowed, unless mandated by regulatory bodies, ethics committees, or for safety monitoring reasons. The sponsor and its affiliates are committed to maintaining data privacy and protection by ensuring all study-related information is anonymized. Participants will be referenced solely by a unique subject number throughout the study and in all subsequent analyses. With a participant's consent, pertinent medical information may be disclosed to their general practitioner or other healthcare providers responsible for their care. Data collected in this study will be available to:

- the sponsor and its designated representatives
- regulatory authorities, including national and international health agencies
- ethics committees involved in study oversight

A subject identification log linking participant names to study IDs will be maintained and updated solely by the investigator to ensure confidentiality. Access to this document will be restricted to authorized study personnel.

13. Statistical considerations

This pilot study aims to evaluate the safety and tolerability of inhaling molecular hydrogen in individuals with mild cognitive impairment (MCI). As an exploratory study, it is not designed to detect statistically significant efficacy outcomes; instead, it seeks to gather preliminary data on the intervention's feasibility and its possible impacts on cognitive function. The results will offer important insights into the variability of responses among participants, which will inform the development of future large-scale clinical trials.

The study will include 30 participants. This size was selected to ensure enough data is collected to assess safety parameters, such as the incidence and severity of adverse events (AEs) and serious adverse events (SAEs). Although this sample size does not allow for definitive statistical comparisons regarding treatment efficacy, it is anticipated to provide valuable information on patient adherence, device usability, and the feasibility of long-term inhalation of hydrogen as a potential treatment for cognitive decline.

A detailed statistical analysis plan has been crafted to ensure that data collection and interpretation follow best practices in clinical research. The main focus of the study will be evaluating safety, specifically examining the frequency, type, and severity of adverse events (AEs) and serious adverse events (SAEs) that occur during the intervention period. Adverse events will be classified using standardized systems, such as the Common Terminology Criteria for Adverse Events (CTCAE), enabling a clear understanding of the risks linked to molecular hydrogen inhalation. Beyond safety monitoring, descriptive statistics will summarize key physiological parameters, including blood pressure, pulse rate, and other significant laboratory findings. These parameters will be scrutinized for possible trends or patterns that could reveal systemic effects resulting from hydrogen inhalation.

While the main focus of the study is on safety, exploratory analyses will also investigate potential signals of cognitive benefits. Changes in cognitive function will be evaluated with validated neuropsychological assessments, including the ALBA and POBAV tests. Quality of life will be evaluated through standardized patient-reported outcome measures, notably the European Quality of Life 5 Dimensions Questionnaire (EQ-5D-5L). Statistical comparisons between pre- and post-intervention scores will be performed using suitable methods based on data distribution. If the normality assumptions hold, paired t-tests will be employed; otherwise, non-parametric alternatives like the Wilcoxon signed-rank test will be utilized. Additionally, effect sizes will be calculated to estimate the magnitude of any changes observed, supporting a preliminary assessment of possible therapeutic benefits.

Managing missing data is crucial in any clinical research, as incomplete datasets can lead to bias and undermine the validity of conclusions. This study will utilize multiple imputation techniques to address missing data whenever possible, thereby maintaining the dataset's integrity. Additionally, sensitivity analyses will be conducted to evaluate the robustness of the findings and to assess the potential impact of missing data on study results.

All statistical analyses will be performed using trusted statistical software such as R or SPSS, ensuring transparency and reproducibility. These tools offer advanced statistical functions that meet regulatory standards, facilitating precise data handling and interpretation. Data collection, management, and analysis will adhere to Good Clinical Data Management Practices (GCDMP) to guarantee high-quality outcomes.

Given the study's design, no formal interim analyses are envisioned. Nonetheless, continuous safety monitoring will occur throughout the trial, with significant safety concerns reported promptly to the ethics committee and relevant regulatory authorities. The data collected will be summarized descriptively, illustrated graphically, and analyzed inferentially where applicable. Key outcomes will be summarized with mean values, standard deviations, and confidence intervals, accompanied by graphical tools like box plots and line charts to depict trends over time.

This pilot study aims to gather preliminary safety data and lay the groundwork for future research exploring the potential neuroprotective effects of hydrogen inhalation. Although the results won't be adequate to confirm efficacy, they will assist in refining study protocols, identifying feasibility issues, and shaping the design of larger, more statistically sound clinical trials.

13.1. Study Design and Objective

This clinical investigation is designed as a single-arm, open-label pilot study aimed primarily at assessing the safety and tolerability of inhaling molecular hydrogen in individuals diagnosed with mild cognitive impairment. Participants will be monitored throughout a specified intervention period, receiving regular hydrogen inhalation sessions under controlled conditions. Data will be collected at various time points to track adverse events, evaluate cognitive function, and measure overall adherence to the intervention.

At the study's outset, participants will complete a baseline assessment that encompasses a thorough medical history, cognitive testing, and quality-of-life evaluations. After this initial assessment, they will enter the intervention phase, inhaling molecular hydrogen according to a set schedule. This administration will occur under medical supervision to ensure safety, with participants being closely monitored for any signs of discomfort or adverse reactions.

Key safety measures throughout the study will involve monitoring vital signs, documenting any adverse events, and conducting regular follow-ups to evaluate overall well-being. Cognitive function will be assessed using standardized neuropsychological tests to explore potential therapeutic effects. Additionally, quality of life and patient-reported outcomes will be measured to provide further context regarding the intervention's impact on daily activities.

The primary objective of this study is to establish a safety profile for molecular hydrogen inhalation in individuals with mild cognitive impairment. Secondary objectives include gathering preliminary data on cognitive function and quality of life, which may shed light on the feasibility of future larger-scale trials. By systematically collecting and analyzing this data, the study aims to offer valuable insights into the potential role of hydrogen inhalation as a non-invasive neuroprotective intervention.

13.2. Endpoints

This study's primary and secondary endpoints focus on assessing the safety and cognitive potential of molecular hydrogen inhalation in individuals experiencing mild cognitive impairment. Given its exploratory nature, these endpoints aim to offer initial insights into tolerability, feasibility, and early signs of efficacy, which will inform the design of larger future trials.

The main objective is to evaluate the frequency and severity of adverse events (AEs) and serious adverse events (SAEs) throughout the intervention period. These events will be systematically documented and categorized based on the Common Terminology Criteria for Adverse Events (CTCAE) to ensure uniformity in reporting and analysis. This outcome is essential for determining the safety and tolerability of daily hydrogen inhalation in this group of patients.

Secondary outcomes will analyze changes in cognitive function and quality of life post-intervention. Cognitive performance will be evaluated using the ALBA and POBAV tests, a widely recognized tool for identifying cognitive impairment and monitoring cognitive changes over time. Quality of life will be assessed through the European Quality of Life 5 Dimensions questionnaire (EQ-5D-5L), which measures important areas of daily functioning, such as mobility, self-care, usual activities, pain/discomfort, and mental well-being. These outcomes will provide preliminary evidence regarding hydrogen inhalation's impact on cognitive function and quality of life for those with MCI.

Exploratory outcomes will look at potential mechanisms and long-term feasibility. If possible within the study's scope, biomarkers related to neurodegeneration, oxidative stress, or neuroinflammation may be evaluated to investigate biochemical changes associated with the intervention. Furthermore, participants will be invited to give self-reported evaluations of their overall well-being, cognitive clarity, and functional status to gather insights into subjective benefits that standardized assessments may overlook. Lastly, the study will monitor the long-term tolerability of repeated hydrogen inhalation by analyzing adherence, dropout rates, and any cumulative adverse effects.

13.3. Hypothesis

This research is grounded in the idea that inhaling molecular hydrogen could provide neuroprotective effects due to its antioxidative and anti-inflammatory properties, possibly

enhancing cognitive function and overall quality of life. The main hypothesis suggests that daily inhalation of molecular hydrogen for three months will be safe and well-tolerated while aiding cognitive improvement in individuals with mild cognitive impairment or mild to moderate dementia. The secondary hypothesis posits that inhaling molecular hydrogen will lower oxidative stress and neuroinflammation, resulting in better neuroprotection and a corresponding rise in quality of life. To evaluate these hypotheses, the study will investigate the following statistical assertions:

- the **null hypothesis (H_0)** assumes that molecular hydrogen inhalation has no significant effect on cognitive function, quality of life, or biochemical markers associated with neurodegeneration.
- the **alternative hypothesis (H_1)** suggests that molecular hydrogen inhalation positively influences cognitive function, enhances quality of life, and reduces biomarkers of oxidative stress and neuroinflammation in individuals with mild cognitive impairment.

13.4. Sample Size

13.4.1. Estimation

This study is an exploratory investigation in its early phase, focusing on collecting preliminary data regarding safety and feasibility rather than identifying statistically significant treatment effects. Due to these aims, no formal sample size calculation has been conducted. Instead, we selected a sample size of 30 participants in accordance with standard practices for pilot clinical studies. This number is deemed adequate to detect trends in safety, tolerability, and potential cognitive effects, while also allowing for variability estimation in outcome measures. The findings from this study will yield essential insights necessary for planning larger clinical trials that are more statistically robust. If significant trends emerge, they will guide the design of future studies with improved endpoints and stronger statistical frameworks.

13.5. Analysis Population

The study will define multiple analysis populations to ensure a structured and meaningful evaluation of the collected data.

- **full analysis set (FAS)**: This population will include all enrolled participants who have received at least one hydrogen inhalation session and have undergone at least one post-baseline assessment. The FAS will be used for descriptive safety and exploratory efficacy analyses to provide insights into potential treatment effects.
- **per-protocol population (PP)**: This population will consist of participants who complete the study without significant protocol deviations, such as non-compliance with treatment schedules or major data inconsistencies. The PP will be used for secondary and exploratory efficacy analyses.
- **safety evaluation set (SES)**: This group will include all participants who have received at least one hydrogen inhalation session. The SES will be the primary population for safety analyses, ensuring that all potential treatment-related adverse events are documented and analyzed.

13.6. Statistical Analysis

13.6.1. General Descriptive Analysis

Descriptive statistical methods suitable for each variable's nature will present all study variables. Unless noted otherwise, data will be summarized individually for each study visit and for changes noted throughout the treatment period.

13.6.1.1. Continuous Variables

Descriptive statistics for continuous variables will include at least number of non-missing observations, mean, standard deviation, minimum, maximum, median and quartiles per study arm.

13.6.1.2. Categorical Variables

In case of categorical items, at least counts and percentages are presented as well as the number of missing and of non-missing observations per study arm and site.

13.6.2. Demographic and Other Baseline Characteristics

Demographic information and baseline traits will be summarized descriptively to give an overview of the study group. Continuous variables like age, weight, height, and initial cognitive scores will be summarized in tables detailing central tendency and variability. Categorical variables, such as sex, race, and medical history, will be displayed in frequency tables.

13.6.3. Primary Analysis

The main analysis will examine the occurrence and seriousness of adverse events (AEs) and serious adverse events (SAEs) during the intervention period. Descriptive statistics will be used to analyze the distribution of adverse events, comparing event rates across relevant subgroups. Adverse event severity will be categorized based on the Common Terminology Criteria for Adverse Events (CTCAE), while trends in event frequencies over time will also be assessed. The analysis will encompass an evaluation of treatment-related adverse effects and any unforeseen safety issues.

13.6.4. Secondary Analysis

The secondary analysis will examine shifts in cognitive function and quality of life from baseline to post-treatment visits. Cognitive scores, evaluated through the ALBA and POBAV tests ,will be compared using paired t-tests or alternative non-parametric methods, based on the data distribution. Quality of life evaluations, assessed via the EQ-5D-5L, will be analyzed using comparable approaches.

Additionally, exploratory analyses may assess relationships between cognitive changes and other variables, including baseline health characteristics or adherence to the intervention.

13.6.5. Safety Analysis

Safety analyses will utilize the safety evaluation set (SES). Descriptive statistics will be presented for all treatment-emergent adverse events (TEAEs), covering both the frequency and severity of reported symptoms. The analysis will distinguish among events related to the investigational device, those with an uncertain relationship, and those assessed as unrelated.

Detailed reporting will cover serious adverse events (SAEs), serious adverse device effects (SADEs), anticipated serious adverse device effects (ASADEs), and unanticipated serious

adverse device effects (USADEs). The analysis will also evaluate adverse events that led to study discontinuation or were classified as life-threatening.

For continuous safety parameters like vital signs, changes from baseline will be assessed using summary statistics and visualized when applicable. If noteworthy trends in safety parameters arise, additional sensitivity analyses may be conducted to evaluate potential risk factors.

13.6.6. Efficacy analysis

The selection of the statistical test will be based on the scope and characteristics of the data to meet the relevant criteria and provide statistically significant results. Efficacy outcomes will be evaluated by comparing changes in cognitive function and quality of life scores from baseline to post-treatment visits. A paired t-test will be used for the primary comparison if the data distributions satisfy normality assumptions; otherwise, a Wilcoxon signed-rank test will be utilized. For categorical efficacy measures, such as cognitive status classification or subjective patient-reported outcomes, the differences between pre- and post-treatment assessments will be assessed using the chi-square test. If any of the criteria for the mentioned tests, such as data normality or sample size, are not met, an appropriate alternative test will be used, such as the Friedman test, the Kolmogorov-Smirnov test, or another suitable test. As this is a pilot study, formal testing of statistical significance will not be conducted, but probability values will be reported to reflect the strength of the observed effects.

13.7. Interim Analysis

There are no formal interim analyses planned because of the study's exploratory nature. Nevertheless, ongoing safety monitoring will occur during the study, and if any unexpected trends arise, adjustments to the study protocol may be made.

13.8. Handling of Missing Data/ Dropouts

Participants identified as ineligible during screening or who do not meet the inclusion criteria will be replaced to sustain the target sample size. However, those who withdraw from the study after the baseline assessment will not be substituted. In regard to reporting adverse events, any missing values will be filled in using conservative assumptions:

- if causality is missing, the adverse event will be assumed to be related to the investigational device
- if severity is missing, the adverse event will be classified at the highest intensity reported for similar events in the study

For missing efficacy data, multiple imputation methods will be applied where appropriate. Sensitivity analyses will be conducted to assess the potential impact of missing data on study conclusions.

14. Data management

14.1. Data collection and documentation

H2 Medical Technologies s.r.o. is responsible for clinical data management in this study. The Data Management Department will supply electronic case report forms (eCRFs) for data collection. All subject-related information will be documented in the eCRFs using a pseudonymized format, which means subjects will be identified solely by their assigned subject number. Each eCRF will feature a comprehensive electronic audit trail that records all changes and updates made to the documents.

To ensure data accuracy and completeness, eCRFs must be regularly maintained to reflect each subject's current status during every visit. Investigators and authorized study staff are accountable for guaranteeing that all data entries are made promptly, remain consistent, and are traceable.

Data entry and storage will adhere to relevant regulatory standards, including ICH-GCP (Good Clinical Practice) and GDPR (General Data Protection Regulation), to ensure secure management of data and protection of privacy.

14.2. User roles within clinical data management system

In accordance with 21 CFR Part 11, every user of the Electronic Data Capture (EDC) system will receive a unique username and password, enabling role-based access control. Users' permissions in the EDC system will be based on their assigned study roles, allowing them to access only the features necessary for their specific responsibilities in the study.

Access to the eCRF system will require a password, with login credentials provided exclusively to authorized study personnel. Anyone who enters or modifies study data will undergo documented training on how to use the system, ensuring adherence to study protocols and regulatory standards.

The system roles may include:

- investigators and sub-investigators, responsible for data entry, reviewing subject records, and approving final data entries
- study coordinators, assisting in data entry and verification
- study monitors, responsible for source data verification (SDV) and monitoring data integrity
- data managers, ensuring data consistency, resolving queries, and preparing the database for final analysis
- sponsor representatives and auditors, reviewing study data as per GCP and regulatory requirements

Each modification within the system will be time-stamped and documented in an audit trail, allowing full traceability of data entry and corrections.

14.3. Study monitoring, source data verification and query management

The investigator ensures that the data entered into the eCRF is accurate, complete, and authentic. Each investigator will electronically approve eCRF entries for every subject using a validated electronic signature, equivalent to a handwritten signature. To uphold data integrity, study monitors will conduct source data verification (SDV) at designated intervals. This verification entails comparing eCRF entries with source documents, like medical records, to ensure the accuracy and consistency of the recorded data. Any discrepancies or missing information found during this process will result in data queries, which must be addressed by the investigator or an authorized team member. The eCRF system will facilitate both automated and manual generation of queries.

- automated system-generated queries will be triggered when data inconsistencies or missing values are detected during data entry
- manual queries may be issued by study monitors or data management personnel during medical review, safety review, or routine data verification

Investigators must respond to all inquiries promptly, ensuring data resolution prior to the study's conclusion. The audit trail in the eCRF system will record all modifications, responses, and data corrections, ensuring complete transparency and adherence to ICH-GCP standards. After completing the eCRFs for each participant, study monitors will carry out a final review of the data before the database is prepared for locking.

14.4. Data Base Lock and Archiving

Once data collection is complete, all queries resolved, and quality control checks conducted, the sponsor will approve the database lock. This action finalizes the study dataset, preventing any further modifications.

Each investigational site will be provided with a copy of the finalized eCRF data for their records. Investigators are also required to securely store copies of key study documents, adhering to ICH-GCP and regulatory requirements.

The sponsor will securely archive all study-related documents, including pseudonymized subject data, monitoring reports, and regulatory submissions, for the legally mandated retention period. Steps will be implemented to safeguard against accidental deletion, modification, or unauthorized access to these files.

In rare instances, if a significant error is discovered post-database lock, a controlled unlocking process may be initiated. Unlocking will only be allowed if the error identified is:

- significantly affects the statistical outcomes related to efficacy assessments
- alters the safety profile of the investigational device
- has implications for regulatory reporting and study conclusions

All changes made after the lock will be thoroughly documented, justified, and provided to the appropriate regulatory agencies as needed. This methodical approach guarantees that the collection, monitoring, and archiving of data meet the highest standards of regulatory compliance, data security, and scientific integrity during the study.

15. Informed consent

Prior to study entry, the investigator must explain to potential subjects all study related procedures and their implications. Subjects must be explicitly informed that their participation is voluntary, and they may withdraw their consent at any time during the clinical investigation and that such refusal will not prejudice their future treatment. Finally, subjects have to be informed that their records may be accessed by competent authorities and/or Sponsor´s representatives in the extent permitted by applicable law(s) and regulations. By signing the Informed Consent Form the subject will authorize such access.
Subjects must get sufficient time to read the informed consent form and to ask additional questions. These questions shall be answered by the investigator. If the subject has no further questions, two copies of the document have to be dated and signed by the subject and investigator. One copy must be given to the subject and the process should be documented in subject`s medical files. One original signed consent form must remain in each subject’s study file and has to be available for monitor’s verification at any time.
In case the subject is unable to read, an impartial witness must attest the informed consent. Subjects who are unable to comprehend the information provided shall be not included.

If new safety information results in significant changes in the risk/benefit assessment, the consent form should be reviewed and updated if necessary. All subjects, including those already being implanted, have to be informed about all new facts and must give their consent to continue in the clinical investigation.

16. Safety reporting

Throughout the course of this study, all efforts will be made to remain alert to possible adverse events (AEs). If an AE occurs, the first concern will be the safety of the subject, and appropriate medical intervention will be made.
The collection of AEs begins at the time the subject is enrolled into the study (eligible subject with signed informed consent).
All AEs and all SAEs will be reported in the investigation and will be entered in the appropriate eCRFs.

16.1. Definitions

16.1.1. Adverse Event

An Adverse Event (AE) is any untoward medical occurrence, unintended disease or injury or any untoward clinical signs, including an abnormal laboratory finding, in subjects, users or other persons, in the context of a clinical investigation, whether or not related to the investigational device. (MDR Article 2 (57).
Adverse events include Adverse Device Effects (ADE).
Note:

a) This definition includes events that are anticipated as well as unanticipated events.
b) This definition includes events occurring in the context of a clinical investigation related to the investigational device, the comparator or the procedures involved.

16.1.2. Treatment-Emergent Adverse Event

Treatment-emergent adverse events (TRAEs) are all AEs that occur at or after the first day of treatment of a subject with the medical device.

16.1.3. Adverse Device Effect

Adverse Device Effects (ADEs) are any inadequacy in the identity, quality, durability, reliability, safety or performance of an investigational device, including malfunction, use errors or inadequacy in information supplied by the manufacturer. (MDR Article 2 (59).

This definition includes any event that is a result of a user error and any event that affects a user of the device (ie, caregiver, bystander, etc).

Device deficiency (Incident): Any malfunction or deterioration in the characteristics or performance of a device made available on the market, including use-error due to ergonomic features, as well as any inadequacy in the information supplied by the manufacturer and any undesirable side-effect. (MDR Article 2 (64).

Malfunction: Failure of an investigational medical device to perform in accordance with its intended purpose when used in accordance with the instructions for use or Clinical Investigation Plan.

User error: Act or omission of an act that results in a different medical device response than intended by the manufacturer or expected by the user.

All ADEs (expected and unexpected) must be reported on the AE eCRF page.

16.1.4. Serious Adverse Event

A Serious Adverse Event (SAE) is any adverse event that led to any of the following:
a) death,
b) serious deterioration in the health of the subject, that resulted in any of the following:
i. life-threatening illness or injury,
ii. permanent impairment of a body structure or a body function,
iii. hospitalization or prolongation of patient hospitalization,
iv. medical or surgical intervention to prevent life-threatening illness or injury or permanent impairment to a body structure or a body function,
v. chronic disease,
c) fetal distress, fetal death or a congenital physical or mental impairment or birth defect (MDR Article 2 (58).

- Results in death.
- Results in serious injury, defined as:
  - life-threatening,
  - permanent impairment of a body structure or function,
  - necessitates medical or surgical intervention to prevent permanent impairment to a body structure or a body function or,
  - results in a potentially sight-threatening condition.
- Is a malfunction that might cause or contribute to a serious injury or death if it were to recur.
- Requires in-subject hospitalization or prolongation of existing hospitalization.
- Leads to fetal distress, fetal death, a congenital abnormality or birth defect.

Note: Hospitalization is a criterion for assessment of seriousness. Hospitalization in the absence of a medical AE is not in itself an AE. For example, the following reports of hospitalization without a medical AE should not be considered either serious or an AE:

- Admission for treatment of a pre-existing condition not associated with the development of a new AE or with worsening of the pre-existing condition (eg, for work-up of persistent pretreatment lab abnormality).
- Social admission (eg, subject has no place to sleep).
- Administrative admission (eg, for yearly physical exam).
- Optional admission not associated with a worsening of a pre-existing condition (eg, elective cosmetic surgery or elective surgery for pre-existing repair of the Achilles tendon [which had not worsened while on study]).
- Hospitalization for admission without a medical AE.

16.1.5. Serious Adverse Device Effect

A Serious Adverse Device Effect (SADE) is defined as an ADE that has led to any of the outcomes that characterize an SAE (e.g. death, life-threatening illness, birth defect, etc.).
Anticipated serious adverse device effect (ASADE) is defined as a SADE which, to its nature, occurrence, severity or outcome, was identified during the risk assessment.
Unanticipated serious adverse device effect (USADE) is defined as a SADE which, to its nature, occurrence, severity or outcome, was not identified during the risk assessment.

16.1.6 Serious incident

Any incident that directly or indirectly led, might have led or might lead to any of the following:

a) The death of a patient, user or other person.
b) The temporary or permanent serious deterioration of a patient's, user's or other person's state of health.
c) A serious public health threat (MDR Article 2 (65).

16.2. Evaluation

When evaluating AEs, the Investigator must determine if the event is serious, assess the severity of symptoms, and evaluate the relationship of the event to the study device and application procedure or comparator, using the following guidelines:

Severity

- Mild: Subject awareness of a sign or symptom that is easily tolerated, requires no treatment, and does not interfere with the subject's daily activities
- Moderate: Subject awareness of a sign or symptom which may be a low level of concern to the subject and may interfere with daily activities, but can be relieved by simple therapeutic care
- Severe: A sign or symptom that interrupts the subject's daily activity and requires systemic therapy or other treatment

Relationship (Causality) to Study Device

- Not Related: AEs which are clearly and incontrovertibly due to causes other than the study device or study protocol (e.g., concomitant disease, etc.)
- Related: AEs which are felt with a reasonable degree of certainty to be related to the study device or study protocol
- Unknown: AEs for which a connection with the study device or study protocol cannot be ruled-out with certainty, or not enough information is available to assess the relationship

Serious Adverse Event vs. Serious Adverse Device Effect

SAEs and SADEs are both adverse events with serious consequences for the subject. The difference between these two AE classifications is what they communicate about the cause of the event. When an event is classified as an SAE, the implication is that the event was not related to the investigational medical device. But when an event is classified as a SADE, the implication is that the event was directly related or caused by the operation of the medical device.

16.3. Reporting requirements

16.3.1. Expedited Reports

The Investigator is obligated to report the following events within 24 hours of becoming aware of the event to ensure the safety of all participants in the study and to meet regulatory reporting requirements:

- all SAEs, regardless of relationship to study device/comparator or study protocol
- all non-serious AEs determined to be related to the study device (ADE)
- all device deficiencies that do not result in one of the above reportable events

When reporting these events to the Sponsor, the site should forward any supporting documents along with the appropriate reporting form and complete the corresponding eCRF. When eCRF is not available from any reasons the events described above must be reported on provided forms to the following contacts:

E-mail: milan.krajicek@h2global.group

Subsequent reporting to ECs and the corresponding regulatory authorities will be organized by the Sponsor or its designee or in accordance with all applicable legal requirements of the concerned country.

16.3.2. Recording of Adverse Events

At each visit/assessment/call after the signature of ICF, all adverse events, either observed by the investigator, or reported by the subject spontaneously, or reported in response to a direct question, must be evaluated by the investigator and recorded in subject´s source documentation and on the AE form of the eCRF.

Pre-existing Conditions
Pre-existing conditions or hospitalization for elective surgery or routine clinical procedures that are not the result of an adverse event need not be considered adverse events. Pre-existing condition should be recorded in subject´s source documentation and on the medical history form of the eCRF.

Overdose
Not applicable.

Pregnancy
Female subjects must be instructed to notify the investigator immediately if they become pregnant during the study. Pregnancies occurring during the study must be reported by the investigational staff within 24 hours of their knowledge. Study medications or use of the investigational medical device will be immediately discontinued and study medications will not be tapered. Follow-up information regarding the outcome of the pregnancy and any postnatal squeal in the infant will be required. Pregnancy will be recorded as an AE in all cases. It will be classified as an SAE only if it fulfils SAE criteria. eCRF will be completed and pregnant subjects will be follow-up until the outcome of the pregnancy has been determined

17. Suspension and premature termination

17.1. Study Completion

Sponsor or its designee will notify the responsible EC and/or regulatory authority when the study is complete.

17.2. Early Study Termination

Sponsor reserves the right to suspend or prematurely terminate either the investigation in an individual investigational site or the entire investigation. The reasons for such decision may include: Safety concerns, sufficient data suggesting device deficiency, or failure of the investigator to comply with the Clinical Investigation Plan or legal requirements.

Following every SAE, both device-related and unrelated, the designated representative of the Sponsor (medical monitor) and the Investigator will discuss each case and determine the most appropriate course of action for the subject and the study. From the standpoint of the study as whole the following courses of action may be put forward:

- Continue “as is”
- Continue with modifications
- Stop the study immediately

In each case the appropriate notifications will be made to the ethics committee and the regulatory authority per the applicable guidelines. There are currently no ASADEs related to this device. Should more than three (3) USADEs occur, the study will be stopped immediately and shall not be restarted until full review and a discussion with the EC is done and the course of action is decided.
Appropriate notification will be given to the Investigator(s) and EC, as applicable. Sponsor will instruct the Investigators to stop dispensing study materials/treatment, to assure appropriate therapy and follow-up for the subjects, and to arrange for study closeout at each site as appropriate.
An investigator, EC(s) or a regulatory authority may also suspend or prematurely terminate participation of an investigational site for which they are responsible. If suspension or premature termination occurs, the terminating party must justify its decision in writing and promptly inform the other parties. If for any reason, the Sponsor suspends or prematurely terminates the pilot investigation at a single investigational site or the entire pilot investigation, the responsible regulatory authorities and ECs must be notified.
In case of investigational site suspension or premature pilot investigation termination the concerned investigators are obliged to follow-up all subjects according to their routine practice. All SAEs shall be reported to the Sponsor 30 days after premature pilot investigation termination/or site suspension and at any time if the event is related to the implanted medical device.

18. Amendments to the clinical investigation plan

If it is found during the study that certain procedures cannot be performed as specified in the clinical investigation plan (CIP), study personnel must promptly inform the investigator. The investigator is then responsible for notifying the clinical monitor or project manager about the issue.

After this notification, it will be assessed whether a protocol amendment is necessary. If it is, an amendment will be drafted and submitted for review and approval by the Ethics Committee (EC) and the Regulatory Authority (SÚKL) before the study can proceed. Any amendment that receives approval from regulatory authorities will become part of the CIP and must be implemented accordingly.

The updated CIP will require formal approval and signatures from relevant personnel at H2 Medical Technologies s.r.o. and the investigator prior to implementation. Investigators must not enact any deviations or changes to the protocol without prior approval from H2 Medical Technologies s.r.o. and recorded approval from the EC and, if necessary, from the Regulatory Authority.

The only exception to this rule occurs when an immediate modification is needed to eliminate an urgent threat to study participants. Additionally, minor logistical or administrative changes that do not affect participant safety or data integrity may be approved internally by H2 Medical Technologies s.r.o. without requiring submission to the EC or regulatory authorities.

19. Deviations from clinical investigation plan

Investigators must strictly adhere to the CIP, as outlined in the Investigator's Agreement. Any failure to comply with the CIP or ISO 14155 requirements is classified as a protocol deviation, which must be documented and addressed appropriately.

If a deviation occurs, the clinical monitor and study site staff should quickly formulate and

execute corrective measures to avert future incidents.

All protocol deviations need to be:

- recorded by the clinical monitor and signed by the investigator in a timely manner
- documented in the study source records, with clear justification for the deviation
- reported to the sponsor, with appropriate corrective and preventive actions
- submitted to the local EC, if required by local guidelines

Investigators and study personnel must maintain continuous vigilance to identify and report any deviations as part of ongoing compliance monitoring.

20. Ethics and regulatory requirements

20.1. Regulatory requirements

This clinical investigation will be carried out in compliance with all relevant local laws and regulatory standards pertaining to medical device clinical trials in each participating country. The study will follow the ethical guidelines set forth in the Declaration of Helsinki and adhere to international standards, such as ISO 14155 and Good Clinical Practice (GCP).

The study sponsor and investigators will ensure that the investigation meets the national regulations for medical devices as well as any specific requirements set by the regulatory authorities in the countries where the study is conducted.

20.2. Independent Ethics Committee

The study will commence only after receiving a favorable opinion from an Independent Ethics Committee (IEC) and the appropriate Regulatory Authority (SÚKL) in each participating country. Approval must be documented, dated, and clearly reference:

- the clinical investigation plan (CIP), including its title and version number
- any CIP amendments requiring IEC review and approval
- the informed consent form (ICF) and any revisions
- all documentation related to the H2-Air Blend Inhaler
- recruitment materials used to enroll participants in the study

During the investigation, the following documents will be submitted to the IEC for review, as required:

- documentation related to the H2-Air Blend Inhaler, including any safety updates
- reports of serious adverse events (SAEs), especially those that are unlisted or associated with the investigational device
- any amendments to the CIP or informed consent form requiring prior IEC approval
- interim progress reports on the clinical investigation, as specified in IEC guidelines

If a protocol amendment raises the risk to subjects, the updated protocol and relevant informed consent forms must be submitted to the IEC for review and approval before they can be implemented. Moreover, if relevant, periodic safety and progress reports will be sent to the IEC at the intervals outlined in its guidelines. Once the study concludes, the IEC will receive formal notification of the investigation's closure, confirming that all regulatory requirements have been met.

20.3. Regulatory approval

The sponsor declares:

The clinical trial will be conducted:

- in accordance with the ethical principles originating in the Declaration of Helsinki
- in accordance with this clinical trial plan and all regional and national regulations

A clinical trial will not start until:

- EC approval will be obtained.
- Any additional requirements from the EC will be met.
- Regulatory Authority (SÚKL) approval will be obtained.

Financing of the clinical trial including insurance is provided.

21. Publication policy

Results of this pilot investigation will be reported in a single Clinical Investigation Report. This report will be prepared by H2 Medical Technologies s.r.o. according to the applicable local laws and regulatory requirements for conducting pilot investigations and applicable Standard Operating Procedures. It will contain all data from all participating sites and will be provided to all participating investigators.
Publication of results is not permitted prior obtaining of a written consent of the Sponsor. All manuscripts and abstracts must be sent to H2 Medical Technologies s.r.o. at least one month prior to their submission for publication or presentation. Timing and publication of results will be subject of mutual agreement between the investigators and/or hospitals and H2 Medical Technologies s.r.o.. The initial publication will be based on the total data set from all contributing sites.

22. Financing and insurance

This clinical study is entirely funded by H2 Medical Technologies s.r.o., which will cover all study-related expenses such as site fees, costs associated with participants, monitoring, regulatory filings, and insurance for those involved. Financial agreements and compensation structures will be set up in accordance with ethical and regulatory standards relevant to clinical trials of medical devices.

22.1. Compensation/payment to the subjects

Participants in the study will not be compensated financially for their involvement. However, they may receive reasonable reimbursement for travel expenses or other direct costs linked to attending study visits, ensuring that their participation does not create a financial burden. Reimbursements will follow national regulations, ethical guidelines, and institutional policies. Prior to enrollment, participants will be informed of any available reimbursements and must sign a consent form to confirm their understanding of the compensation policies.

22.2. Institution fee

A financial contract will be formed between the sponsor and each research site, detailing the duties, commitments, and financial terms for executing the study. The contract will outline:

- costs associated with study-related procedures, materials, and personnel

- reimbursement for services required to conduct the investigation according to the clinical investigation plan (CIP)
- obligations of the sponsor, investigator, and institution regarding financial reporting and compliance

Financial arrangements will comply with all relevant legal, ethical, and institutional requirements, ensuring transparency and adherence to financial disclosure regulations.

22.3. Insurance

All participants who are enrolled in the study and adhere to the clinical investigation plan (CIP) will be covered by the sponsor's insurance policy. This coverage extends to any injuries or adverse events possibly linked to the use of the H2-Air Blend Inhaler or the study-related procedures.

The insurance policy will meet all relevant national and international regulations for clinical investigations of medical devices. Investigators and study sites will receive comprehensive information about the insurance coverage, which includes:

- the scope of coverage and conditions under which compensation applies
- procedures for reporting adverse events and potential claims
- the process for participants to file claims if necessary

Before enrollment, participants will receive information about the insurance coverage and written documentation detailing their rights under the insurance policy will be part of the informed consent process. The sponsor will ensure the insurance stays valid and active for the study's duration, offering ongoing protection for participants.

23. References

History of changes, amendments

Date	Version	Created	Description of the change
20.1.2025	V1.1.		Initial draft and general information
13.3.2025	V1.2.		Finalization and formatting

24. Appendices

24.1. Appendix A: Instruction for use

Delivered as a separate document

24.7. Appendix G: European Quality of Life 5 Dimensions questionnaire (EQ-5D-5L)

TS005			
Patient ID:		**Date:**	
Visit 1	☐	**Visit 5**	☐
Visit 2	☐	**Visit 6**	☐
Visit 3	☐	**Visti 7 (End of study)**	☐
Visit 4	☐		

Under each heading, please tick the ONE box that best describes your health TODAY

MOBILITY

- I have no problems in walking about ☐
- I have slight problems in walking about ☐
- I have moderate problems in walking about ☐
- I have severe problems in walking about ☐
- I am unable to walk about ☐

SELF-CARE

- I have no problems washing or dressing myself ☐
- I have slight problems washing or dressing myself ☐
- I have moderate problems washing or dressing myself ☐
- I have severe problems washing or dressing myself ☐
- I am unable to wash or dress myself ☐

USUAL ACTIVITIES *(e.g. work, study, housework, family or leisure activities)*

- I have no problems doing my usual activities ☐
- I have slight problems doing my usual activities ☐
- I have moderate problems doing my usual activities ☐
- I have severe problems doing my usual activities ☐
- I am unable to do my usual activities ☐

PAIN / DISCOMFORT

- I have no pain or discomfort ☐
- I have slight pain or discomfort ☐
- I have moderate pain or discomfort ☐
- I have severe pain or discomfort ☐
- I have extreme pain or discomfort ☐

ANXIETY / DEPRESSION

- I am not anxious or depressed ☐
- I am slightly anxious or depressed ☐
- I am moderately anxious or depressed ☐
- I am severely anxious or depressed ☐
- I am extremely anxious or depressed ☐

- We would like to know how good or bad your health is TODAY.
- This scale is numbered from 0 to 100.
- 100 means the best health you can imagine.
  0 means the worst health you can imagine.
- Mark an X on the scale to indicate how your health is TODAY.
- Now, please write the number you marked on the scale in the box below.

YOUR HEALTH TODAY = ☐

