Strictly Confidential

CLINICAL INVESTIGATION PLAN

Assessment of sino-nasal microbial communities changes in adult patients with Chronic Rhinosinusitis by 16S rRNA gene amplicon sequencing before and after 1-month treatment duration with Healsea® Chronic: an exploratory study

Study Number: LPH-2102

Short Title : ISONAM

PMCF investigation for MD CE marked and used in its intended purpose with invasive and/or burdensome procedure (MDR Art. 74(1))

ID-RCB number: 2021-A01458-33

Version 1.0; 14 June 2021

This Clinical Investigation is being sponsored by:

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HISTORY OF CLINICAL INVESTIGATION PLAN'S UPDATES

Version	Date	Purpose of Update
Version 1.0	14-06-2021	Creation

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APPROVAL FORM
STUDY: LPH-2102
CLINICAL INVESTIGATION PLAN
Version 1.0, 14-06-2021

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Signature:

Principal Investigator:

Pr Guillaume de Bonecaze

Date: 14 June 2021
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Date: 18 June 2021
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Date: 21 June 2021
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1. Statement of compliance

PRINCIPAL INVESTIGATOR STUDY APPROVAL PAGE
STUDY: LPH-2102
CLINICAL INVESTIGATION PLAN
Version 1.0, 14-06-2021

By signing the hereinafter form, I hereby confirm that I agree:

- To conduct the trial described in the Clinical Investigation Plan (LPH-2102) dated 14 June 2021, in accordance with the ethical principles that have their origin in the Declaration of Helsinki, in compliance with GCP, with applicable regulatory requirements and with the Clinical Investigation Plan agreed upon by the sponsor and given approval/favourable opinion by the Ethics Committee and Competent Authority;
- To document the delegation of significant study related duties and to notify the sponsor of changes in site personnel involved in the study;
- To comply with procedures for data recording and reporting;
- To permit monitoring, auditing and inspection;
- To retain the trial-related essential documents until the sponsor informs these documents are no longer needed.

Furthermore, I hereby confirm that I will have and will use the availability of adequate resources, personnel and facilities for the conduct of this trial.

Principal Investigator's Name: Guillaume de Bonnecaze

Principal Investigator's Title: Professor

Principal Investigator's Address: Department of Otorhinolaryngology and Cervico-Facial Surgery
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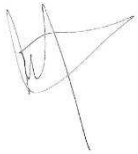
24 Chemin de Pouvourville, 31400 Toulouse, France

Principal Investigator's Signature: 

Date of signature: 14 June 2021

LIST OF ABBREVIATIONS

Abbreviation	Definition
AE	**A**dverse **E**vent
ANSM	**A**gence **N**ationale de **S**écurité du **M**édicament et des produits de santé
BoV	**B**ag-**o**n-**V**alve
CA	**C**ompetent **A**uthority
CIP	**C**linical **I**nvestigation **P**lan
CNIL	**C**omission **N**ationale de l'**I**nformatique et des **L**ibertés
CRA	**C**linical **R**esearch **A**ssociate
CRF	**C**ase **R**eport **F**orm
CRO	**C**linical **R**esearch **O**rganization
CRS	**C**hronic **R**hino**S**inusitis
DNA	**D**esoxyribo **N**ucleic **A**cid
EC	**E**thics **C**ommittee
EPOS	**E**uropean **P**osition Paper on Rhinosinusitis and nasal polyps
FSCA	**F**ield **S**afety **C**orrective **A**ction
GCP	**G**ood **C**linical **P**ractice
GDPR	**G**eneral **D**ata **P**rotection **R**egulation
IFU	**I**nstruction **F**or **U**se
LPLV	**L**ast **P**atient **L**ast **V**isit
MDCG	**M**edical **D**evice **C**oordination **G**roup
MDR	**M**edical **D**evice **R**egulation
MIR	**M**anufacturer **I**ncident **R**eport
MR	**M**éthodologie de **R**éférence
OTU	**O**perational **T**axonomic **U**nit
PCR	**P**olymerase **C**hain **R**eaction
PI	**P**rincipal **I**nvestigator
PNF	**P**rimary **N**otification **F**orm
RIPH	**R**echerche **I**nterventionnelle chez la **P**ersonne **H**umaine

RNA	**R**ibo **N**ucleic **A**cid
SAE	**S**erious **A**dverse **E**vent
SNOT-22	**S**ino **N**asal **O**utcome **T**est-22

2. Synopsis

Sponsor:	LALLEMAND PHARMA AG
Principal Investigator	Pr. G de Bonnecaze, Department of Otorhinolaryngology and Cervico-Facial Surgery, University Hospital of Toulouse (Larrey), France
Title:	**Assessment of sino-nasal microbial communities changes in adult patients with Chronic Rhinosinusitis by 16S rRNA gene amplicon sequencing before and after 1-month treatment duration with Healsea® Chronic: an exploratory study**
Short Title	ISONAM (**I**mpact of **S**ymbiofilm® **O**n **NA**sal **M**icrobiota)
N° ID-RCB	2021-A01458-33
CIP version:	1.0
Rationale:	Chronic rhinosinusitis (CRS) is an inflammation of the nose and paranasal sinuses and is characterized by two or more symptoms, one of which should be either nasal blockage/obstruction/congestion or nasal discharge (anterior/posterior nasal drip), lasting for 12 weeks or longer. In addition, facial pain or pressure and a reduction in the sense of smell can occur [1]. The condition can occur with or without nasal polyps. Chronic rhinosinusitis (CRS) is a significant health problem and affects 5-12% of the general population [1]. The physiopathology of CRS is poorly understood with multiple environmental, host and microbial factors being implicated. Putative pathological factors include changes in the microbiota, imbalance of the local or systemic immune system, allergens, toxins and genetic predisposition [2]. A dysbiosis mechanism has been proposed as modulating inflammation in diseased sinuses. This hypothesis suggests that externally influenced changes in the nasal microbiome can result in dysbiosis, i.e. a shift from a “normal” or “healthy” microbial community structure and that this shift may be responsible for the initiation or maintenance of CRS. For example, the disruption of the commensal biofilm during a viral upper respiratory tract infection can create a niche for pathogenic species to grow [3]. Despite many contradictory statements in the different studies some common trends emerge. Less diversity in the microbial community rather than an increased overall bacterial load seems to characterize CRS compared to the healthy state with no consensus about specific genera indicative of disease [4], [5], [6]. However, anaerobes and *S. aureus* are found to be significantly more

	prevalent and abundant in CRS versus healthy controls [4]. Bacterial biofilm is detected on the sinus mucosa in up to 80% of CRS patients and its presence does not imply that it is causing mucosal inflammation. However, in the context of CRS, there are several possible mechanisms by which biofilms may be pro-inflammatory including the release of planktonic organisms and the release of superantigens, which can cause ciliary dysfunction and inhibition of ciliary clearance. Bacterial biofilms are likely a key modulator of the refractory nature of CRS [7]. Although clinical evidence from well-designed trials is scarce, European Guidelines for chronic rhinosinusitis recommend daily nasal saline irrigation for reduction of the severity of symptoms of CRS [1]. A recent Cochrane analysis has concluded that daily nasal irrigation with hypertonic saline solution is more effective than placebo to improve patient symptoms [8]. The exact mechanisms by which nasal irrigation works are not known. However, most of the experts agree that it is primarily a mechanical intervention leading to direct cleansing of the nasal mucosa. Nevertheless, the efficacy of such solution remains moderate. Healsea® Chronic is a CE marked medical device indicated in adults for the treatment of nasal symptoms of chronic rhinosinusitis. This is a seawater-based nasal spray supplemented with a natural Symbiofilm® extract (0.02%) isolated from marine bacteria. The nasal solution is hypertonic (NaCl 2.2%). Symbiofilm® is a marine postbiotic comprising active exopolysacharides with emulsifying properties and *in vitro* antibiofilm activity. Antibiofilm properties have been demonstrated with the colorimetric microtiter plate assay [9]. In this model, a significant inhibition of biofilm formation by *Staphylococcus aureus* and *Haemophilus influenzae* are observed. Detachment properties from human nasal epithelial cells of *Staphylococcus aureus* and *Pseudomonas aeruginosa* has also been demonstrated *in vitro*, suggesting an inhibition of biofilm formation at early stage in this model. Symbiofilm® has no bacteriostatic nor bactericidal activities. To date, properly designed study to evaluate the effect of topical therapies on microbiome are scarce so no definite conclusion can be made. In one study, use of saline irrigation with or without budesonide[DCI] used was not associated with significantly distinct microbiota composition among either controls or post-operative CRS with polyp patients [10]. We design this exploratory study aiming at determining if the antibiofilm properties of Symbiofilm® may modify sino-nasal microbiota, impacting α and/ or β diversities. To this end middle meatus swab specimen will be taken from CRS patients before and after treatment with Healsea® Chronic. Bacteria colonization will be assessed using quantitative PCR and 16S rRNA gene sequencing.

Objectives:	**Primary Objective:** - To characterize the change of sino-nasal microbial communities from adult CRS patients via 16S rRNA gene high throughput sequencing before and after 1-month treatment duration with Healsea® Chronic. **Secondary objectives:** -To assess the efficacy of Healsea® Chronic in improving health-related quality of life of CRS adult patients. -To assess the global satisfaction of patients with the use of Healsea® Chronic for CRS. - Safety: to assess systemic and local tolerance of Healsea® Chronic over the study period.
Endpoints:	**Primary endpoint:** **Modifications in microbial load and/or profile** To determine the impact of Healsea® Chronic on the nasal microbiome, the microbial load will be evaluated by quantitative PCR and microbiome taxonomic profiling by 16S rRNA gene sequencing. Data collected in patients before and after 1 month-treatment with Healsea® Chronic will be compared using statistical analyses to assess changes in microbial species abundance and diversity. Furthermore, special attention will be given to typical sino-nasal pathogens (*e.g. Staphylococcus aureus*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*) at genus level, and if reachable at species level. **Secondary Endpoints:** -To measure the clinical performance of Healsea® Chronic, the quality of life of CRS patients will be assessed with the Sino-Nasal Outcome Test score-22 (SNOT-22) [11]. This questionnaire is a disease-specific, quality-of-life-related measure of sinonasal function. Patients should rate sino-nasal symptoms and their impact from 0 to 5 where 0 means that there is no complaint and 5 means that there are serious complaints. - Patient satisfaction questionnaire assessing medical device usability, sensory perception and efficacy in using Healsea® Chronic for CRS. - **Safety**: Assessment of adverse events/incidents/expected side effects throughout the study.
Indication:	Treatment of nasal symptoms of chronic rhinosinusitis
Investigation Design:	PMCF investigation for MD CE marked and used in its intended purpose with invasive and/or burdensome procedure (MDR Art. 74(1)). Non randomized, open and uncontrolled interventional study.

	Visit 1 (V1) – (Day 1): Screening/Inclusion Information and consent, demographic data and medical history, ongoing medication, rhinologic examination, inclusion/non-inclusion criteria, collection of middle meatus swab specimens, SNOT-22, reporting of adverse events, dispensation of Healsea® Chronic Nasal Spray. **Telephone call 1 (TC1) – (Day 15±3)** Investigator/nurse telephone call to review patient status and study progress, adverse events/incidents/expected undesirable side effects and concomitant treatments. **Visit 2 (V2) – (Day 30±5): end of treatment** End of Healsea® Chronic nasal spray treatment, physical and clinical examination, reporting of adverse events/incidents/expected side effects, collection of middle meatus swab specimens, SNOT-22, subject's satisfaction questionnaire.
Number of Subjects:	An arbitrary sample of **20** subjects
Target Population:	Adults with chronic rhinosinusitis according to EPOS criteria [1].
Permitted and prohibited concomitant medication	**Permitted concomitant medication:** Nasal corticosteroids **Not permitted concomitant medication:** Other nasal irrigations Need for systemic corticosteroids Need for nasal application of antibiotics and systemic antibiotics
Inclusion/Non inclusion criteria:	**Inclusion criteria** In order to be eligible to participate in this study, an individual must meet all of the following criteria: 1. Male or female, aged 18 – 70 years 2. Provision of signed and dated informed consent form 3. Stated willingness to comply with all study procedures and availability for the duration of the study 4. Diagnosed with CRS based on the diagnostic criteria of the EPOS guideline 5. Registered with a social security scheme or covered by such a regime **Non inclusion criteria** An individual who meets any of the following criteria will be excluded from participation in this study: 1. Antibiotics or oral corticosteroids intake in the month prior to the study 2. Endoscopic sinus surgery in the past 6 months 3. Cystic fibrosis 4. Wegener's granulomatosis 5. Immunodeficiency

	6. Defective access to middle meatus 7. Lidocaine allergy 8. Known hypersensitivity/allergy to any component of the test device 9. Pregnant/Lactating female or absence of efficient contraception 10. Under tutorship or guardianship
Number of sites:	1
Test Device:	Healsea® Chronic nasal spray will be administered twice a day (1 puff, 1-2 sec) in each nostril during 30 days.
Comparator Device:	Not applicable
Duration of investigation:	Duration of inclusion period: 4 months Duration of patient's participation: 30 days Total study duration: 5 months
Study Start Date:	September 2021
Statistical Analysis:	Patients and disease characteristics will be described at baseline. **16S rRNA Gene Sequence Analysis:** The normal distribution of the values is verified thanks to normality tests (e.g. Shapiro-Wilk test). However, it is most likely that the group size will be insufficient to apply parametric statistical tests. Significant variations in total number of bacterial 16S rRNA gene copies or in alpha diversity are assessed using the Kruskal-Wallis test or the Wilcoxon rank-sum test. Multidimensional scaling analyses (MDS) are performed on beta diversity distances matrices and differences between groups were assessed using PERMANOVA and PERMDISP analyses (2000 permutations). LEfSe (Linear discriminant analysis Effect Size) analyses, based on non-parametric tests are used to determine significant variations in taxa relative abundance [17]. **Secondary endpoints:** Values and changes from baseline to D30/end of study of the SNOT-22 total score will be described. Patients satisfaction will be described at D30/end of study. **Safety:** All adverse events/incidents/expected side effects of the device will be listed, but only treatment-emergent adverse events/incidents (non-serious + serious) and expected side effects will be summarized by the number and percentage of patients, classified by System Organ Class and Preferred Term as defined by MedDRA dictionary. Frequency and percentage of patients with at least one reported adverse event/incident/expected side effect will be tabulated by System Organ Class.

3. Flow Chart

Visit name	Screening/Inclusion	At home	Telephone call	At home	End of Treatment
Visit Number	**V1**		**TC1**		**V2**
Days/Weeks	D1	D1 – D15± 3	D15±3	D15± 3 – D30± 5	D30 ± 5
Screening and General Assessments					
Patient information and Consent collection	X				
Eligibility criteria	X				
Demography and Medical history	X				
Physical and clinical examination	X				X
Ongoing medication	X				
Treatment					
Healsea® Chronic		X		X	
Assessments					
SNOT 22	X				X
Collection of middle meatus swab specimens	X				X
Adverse Events/incidents and concomitant medication reporting	X		X		X
Satisfaction questionnaire					X
Compliance to study product					X

4. Identification and description of the investigational Device

4.1 Summary description of the investigational device

Healsea® Chronic is a CE marked nasal spray composed with an hypertonic seawater solution (2.2% NaCl) and Symbiofilm® (0.02%). Symbiofilm® is a Lallemand proprietary marine postbiotic composition including an exopolysaccharide mainly composed of N-acetyl hexosamines, acidic and neutral monosaccharides.

Healsea® Chronic is a nasal spray indicated in adults over 18 years in the treatment of nasal symptoms of chronic sino-nasal conditions : allergic rhinitis, chronic sinusitis, and/or sensitive nose. It can be used also as post-operative nasal care after nasal surgery.

According to direction of use, one puff (1-2 sec) twice a day in each nostril is to be administered during 30 days or as recommended by the physician or the pharmacist.

The technical performances are summarized below:

Ingredient	**Function(s)**
Hypertonic Seawater solution 2.2%	- Improve nasal mucosa function - Clean the nasal cavity and eliminate allergens and infectious agents
Symbiofilm®	- Enhancing the cleansing efficacy - Reducing biofilm formation

Intended clinical performances in the intended destination are:

- Treatment of symptoms of chronic sino-nasal disorders: allergic rhinitis, chronic sinusitis and/or sensitive nose..
- It can be used also as post-operative nasal care after nasal surgery

4.2 Manufacturer

Healsea® Chronic is a class IIa Medical Device manufactured by Lallemand Pharma. CE marking has been obtained on 30 March 2021. The subcontractor for the manufactoring is AURENA, Fjarrvikvagen 22, ES-653 50 Karlstad, Sweden.

4.3 Name or number of the model to permit full identification

The test device is named Healsea® Chronic. It is a nasal spray (150ml presentation), hypertonic seawater (2.2%) supplemented with Symbiofilm® (0.02%) identified by the code article: 75012DMMG

4.4 Traceability

Tracability of the Device will be achieved through the lot number (YYMMXXX, YY for year, MM for Month and XXX internal number of the manufacturer).

4.5 Intented purpose of the investigational device in the clinical investigation.

Healsea® Chronic will be used within its intended use in adult patients with CRS according to EPOS criteria [1].

4.6 Population and indications for which the device is intented

Healsea® Chronic will be used in the intended population (adults over 18 years) and within its intended indication (CRS) and according to the IFU.

4.7 Device Description

Healsea® Chronic is a nasal spray (available in 50 or 150mL) using the Bag-on-Valve (BoV) technology.

The BoV technology consists of an aerosol valve with a welded bag. The product is placed inside the bag while the propellant is filled in the space between bag and can. The product is dispensed by the propellant simply squeezing the bag when the spray button is pressed, the product is squeezed out of the bag by the compressed air/nitrogen, which creates the dispensing as a spray. Therefore, the product keeps its integrity, remaining separated from the propellant at all times. Compared to traditional aerosol spray technology, BoV has several benefits, for manufacturers, consumers and the environment. Packaging description of Healsea® Chronic is reported in Figure 1.

***The Spray Pin:** Packaging element ensuring the delivery of the product in the patient's nose.

***The Actuator:** Pacifica Spray actuator with a flow rate of 8ml/10s pressed enables the Propellant to squeeze the bag to dispense the product through the Spray Pin.

***The Valve assembly:** Packaging element ensuring the link between the bag and the actuator.

The Can: Standard aerosol aluminum can containing the bag and the propellant.

The Product: Hypertonic Seawater solution (2.2%) supplemented with Symbiofilm® (0.02%)

***The Bag:** FDA-approved laminated bag containing the product.

The Propellant: Because of the separation between product and propellant, BoV technology is used with compressed nitrogen filled between the can and the bag.

****Packaging element in contact with the product***

Figure 1- Packaging description of Healsea® Chronic

As summarized below, Healsea® Chronic is intended to be used on nasal mucosa. The duration of use is 30 days.

Type of contact	Device in contact with a surface
Type of tissues	Mucous membranes
Total duration treatment	It can be used for 30 days

4.8 Investigation Device Training/Experience

There is no need for specific training in using Healsea® Chronic.

An usability engineering process test according to the standard EN ISO 62366-1: 2015 was conducted with another product using BoV technology from the Healsea® range, Healsea® Children. Ten parents were recruited to ensure that the leaflet and the label were well understood and that the medical device was used correctly to ensure its safe understanding and use.

The analysis of the results demonstrates that:

- The information provided by the user interface is readable and understandable by participants: the information tested was easy to understand, without interpretation.
- The observations made during the simulations of use of the device have confirmed that all safety-related handling steps are properly followed, in accordance with the instructions given in the instruction for use.

Briefly, the adult to place the nozzle into the nostril while keeping head straight. Once the nozzle is placed, to press on the nozzle for 1-2 seconds in each nostril. To let flow the excess of solution, and wipe. To clean the nozzle with tissue, soapy water rinse and dry after use.

4.9 Reference to the IFU

The IFU of Healsea® Chronic is provided in Appendix A.

5. Justification for the design of the clinical investigation

Chronic rhinosinusitis (CRS) is an inflammation of the nose and paranasal sinuses and is characterized by two or more symptoms, one of which should be either nasal blockage/obstruction/congestion or nasal discharge (anterior/posterior nasal drip), lasting for 12 weeks or longer. In addition, facial pain or pressure and a reduction in the sense of smell can occur [1]. The condition can occur with or without nasal polyps.

The overall prevalence of symptom-based CRS in the population has been found to be between 5% and 12% [1].

The physiopathology of CRS is poorly understood with multiple environmental, host and microbial factors being implicated. Putative pathological factors include changes in the microbiota, imbalance of the local or systemic immune system, allergens, toxins and genetic predisposition [2].

A dysbiosis mechanism has been proposed as modulating inflammation in diseased sinuses. This hypothesis suggests that externally influenced changes in the nasal microbiome can result in dysbiosis, i.e. a shift from a "normal" or "healthy" microbial community structure and that this shift may be responsible for the initiation or maintenance of CRS. For example, the disruption of the commensal biofilm during a viral upper respiratory tract infection can create a niche for pathogenic species to grow [3]. Despite many contradictory statements in the different studies some common trends emerge. Less diversity in the microbial community rather than an increased overall bacterial load seems to characterize CRS compared to the healthy state with no consensus about specific genera indicative of disease [4], [5], [6]. However, anaerobes and *S. aureus* are found to be significantly more prevalent and abundant in CRS versus healthy controls [4]. Bacterial biofilm is detected on the sinus mucosa in up to 80% of CRS patients and its presence does not imply that it is causing mucosal inflammation. However, in the context of CRS, there are several possible mechanisms by which biofilms may be pro-inflammatory including the release of planktonic organisms and the release of superantigens, which can cause ciliary dysfunction and inhibition of ciliary clearance. Bacterial biofilms are likely a key modulator of the refractory nature of CRS [7].

Although clinical evidence from well-designed trials is scarce, European guidelines for chronic rhinosinusitis recommend daily nasal saline irrigation for reduction of the severity of symptoms of CRS [1]. A recent Cochrane analysis has concluded that daily nasal irrigation with hypertonic saline solution is more effective than placebo to improve patient symptoms [8]. The exact mechanisms by which nasal irrigation works are not known. However, most of the experts agree that it is primarily a mechanical intervention leading to direct cleansing of the nasal mucosa. Nevertheless, the efficacy of such solution remains moderate.

Healsea® Chronic is a CE marked medical device indicated in adults for the treatment of nasal symptoms of chronic rhinosinusitis. This is a seawater-based nasal spray supplemented with a natural Symbiofilm® extract (0.02%) isolated from marine bacteria. The nasal solution is hypertonic (NaCl 2.2%). Symbiofilm® is a marine postbiotic comprising active exopolysacharides with emulsifying properties and *in vitro* antibiofilm activity. Antibiofilm properties have been demonstrated with the colorimetric microtiter plate assay [9]. In this model, a significant inhibition of *Staphylococcus aureus* and *Haemophilus influenzae* biofilms are observed. Detachment properties from human nasal epithelial cells of *Staphylococcus aureus* and *Pseudomonas aeruginosa* has also been demonstrated *in vitro*, suggesting an inhibition of biofilm formation at early stage in this model. Symbiofilm® has no bacteriostatic nor bactericidal activities.

To date, properly designed study to evaluate for the effect of topical therapies on microbiome are scarce so no definite conclusion can be made. In one study, use of saline irrigation with or without budesonide[DCI] used was not associated with significantly distinct microbiota composition among either controls or post-operative CRS with polyp patients [10].

We design this exploratory study aiming at determining if the antibiofilm properties of Symbiofilm may modify sino-nasal microbiota, impacting α and/or β diversities. To this end middle meatus swab specimen will be taken from CRS patients before and after treatment with Healsea ® Chronic. Bacteria colonization will be assessed using quantitative PCR and 16S rRNA gene sequencing.

6. Risks and Benefits of the Investigational Device and Clinical Investigation

6.1 Anticipated clinical benefit

Healsea® Chronic is a CE marked class IIa medical device indicated in the treatment of nasal symptoms of sino-nasal conditions of which CRS. The clinical benefit of nasal irrigation with saline solution used during several weeks to improve CRS symptomatology is well known. Due to its technical performances, Symbiofilm® may enhance the cleansing activity of the device.

6.2 Anticipated adverse device effects

The only anticipated adverse device effect is the feeling of itching and irritation while initiating the treatment with the medical device Healsea® Chronic. In case of such adverse reaction, the participant will be instructed to contact the investigator if wishing to stop the nasal spray use.

Furthermore, as mentionned in the IFU, patients with known hypersensitivity/allergy to any component of the test device will not be enrolled in the study.

6.3 Risks associated with participation in the clinical investigation

The collection of middle meatus specimen will be performed by the Principal Investigator or co-investigator who are familiar with this medical procedure. This additional procedure is invasive (MDCG 2021-6-Regulation (EU) 2017/745) but no expected risk is associated with this procedure. This procedure is definitely comparable to nasopharyngeal swab which is routinely performed for Sars-CoV-2 detections without safety concern. Furthermore, nasopharyngeal swabs were mentioned in the « Arrêté du 12 avril 2018 fixant la liste des recherches mentionnées au 2° de l'article L. 1121-1 du code de la santé publique » previously applicable for RIPH-2 with medical device. Additionally, in order to avoid any inconvenience, patients with defective access to the middle meatus, e.g. with deviated nasal septum, will not be enrolled (non-inclusion criterion) and a topical anesthesia with a lidocaine spray will be proposed to the eligible patients.

6.4 Possible interactions with concomitant medical treatments as considered under the risk analysis

No interaction is expected. Nevertheless, patient will be instructed to respect a 60-minute minimum interval after administration of the investigational medical device before administration of another nasal spray e.g. decongestant or mucolytics if prescribed.

6.5 Steps that will taken to control and mitigate the risks

In the line with *NF EN ISO 14971:2019* standard (Medical Devices – Application of risk management to medical devices), a risk analysis has been conducted to estimate the risks associated with each step of the device lifecycle. Most of the risk has been mitigated through product design and manufacturing.

The residual risk to patients who are administered this intervention is low. A list of potential risks associated with the device, procedures undertaken to minimise them and methods used for their management is described in the device Risk Management Process File.

Considering:

- The risk analysis (ISO 14971 activities) that demonstrates:
  - There is no longer High-level risk,
  - That, the 41 Medium risk level residual risks have an acceptable benefit-risk balance.
  - And all of the other residual risks (85) are at Low risk
- The fact that the device Healsea® Chronic can be considered as safe and effective according to the Clinical Evaluation Report,

the overall residual risk is acceptable and the benefit/risk ratio is considered satisfactory.

The verification of the implementation of risk control measures during life cycle phases of Healsea® Chronic is mainly performed by LALLEMAND PHARMA AG in compliance with the operating procedures currently used.

No specific risk for subject in participating in the study is identified, provided that the eligibility criteria are fulfilled. Thus the establishment of risk acceptability thresholds is assumed to be not necessary although it is a requirement from the ISO 14155:2020. Nevertheless, the safety and well being of subjects will be monitored throughout the study. Should a potential unanticipated risk be detected, the clinical investigation will be suspended, the risk assessment will be updated to adapt the risk control measures and the benefit-risk analysis.

6.6 Rationale for the benefit-risk ratio

Chronic rhinosinusitis is a painful disease that impacts the quality of life. Orbital and intracaranial complications are rare but potentially serious.

Nasal saline irrigation are recommended by European and American guidelines as adjuvant treatment for CRS. The addition of Symbiofilm® to nasal saline irrigation may improve the clinical performances of the device. Furthermore, apart from feeling of itching and irritation while initiating the treatment, no adverse effect is anticipated.

The analysis of middle meatus swabs is expected to provide first insights on the *in vivo* mechanism of action of Healsea® Chronic. If an improvement of nasal microbiota is observed in the present study, this could justify to further study the impact of Symbiofilm® in a larger population of patients with CRS but also in other diseases with reported sino-nasal dysbiosis, e.g. asthma, especially in the paediatric population [12].

7. Objectives and hypothesis of the clinical investigation

7.1 Hypothesis

Our hypothesis is that a one month-course of Healsea® Chronic nasal spray could modify the sino-nasal microbiota diversity, impacting α and/or β diversities. Although performed on a small cohort of CRS patients, this clinical investigation is expected to provide first insights into the mechanism of action of Healsea® Chronic nasal spray *in vivo*.

7.2 Primary Objective

To characterize the change of sino-nasal microbial communities from adult CRS patients via 16S rRNA gene high throughput sequencing before and after 1-month treatment duration with Healsea® Chronic.

7.3 Secondary Objectives

-To assess the efficacy of Healsea® Chronic in improving health-related quality of life of CRS adult patients.

-To assess the global satisfaction of patients in using Healsea® Chronic nasal spray for CRS.

-Safety: to assess systemic and local tolerance of Healsea® Chronic over the study period.

7.4 Scientific justification and clinical relevance for effect sizes, non inferiority margins or equivalence

Not applicable. This is an exploratory clinical investigation.

7.5 Risks and anticipated adverse effects that are to be assessed

No specific risk for subject in participating in the study is identified, provided that the eligibility criteria are fulfilled. Expected and foreseeable side effects are feeling of itching and irritation while initiating the treatment. Nevertheless, the safety according to post market safety regulations (see paragraph 16) and well being of subjects will be monitored throughout the study.

8. Design of the clinical investigation

8.1 General

8.1.1 Design type of clinical investigation

This is an exploratory study, open label in one site, in France. No control group is deemed necessary at this stage.

8.1.2 Measures to be taken to minimize or avoid bias

Not applicable.

8.1.3 Primary Endpoint

Modifications in microbial load and/or profile

To determine the impact of Healsea® Chronic on the nasal microbiome, the microbial load will be evaluated by quantitative PCR and the microbiome taxonomic profiling by 16S rRNA gene sequencing. Data collected in patients before and after 1 month-treatment with Healsea® Chronic will be compared using statistical analyses to assess changes in microbial species abundance and diversity. Furthermore, special attention will be given to typical sino-nasal pathogens (*e.g. Staphylococcus aureus*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*) at genus level, and if reachable at species level.

8.1.4 Secondary Endpoints

To measure the clinical performance of Healsea® Chronic, the quality of life of CRS patients will be assessed with the Sino-Nasal Outcome Test 22 score (SNOT-22) [13]. This questionnaire is a disease-specific, quality-of-life-related measure of sinonasal function. Patients should rate sino-nasal symptoms and their impact from 0 to 5 where 0 means that there is no complaint and 5 means that there are serious complaints (Appendix B).

Patient satisfaction questionnaire assessing medical device usability, sensory perception and efficacy in using Healsea® Chronic for CRS (Appendix C).

Safety: Assessment of adverse events/incidents/expected side effects throughout the study.

8.1.5 Methods and timing for assessing, recording and analysing variables

8.1.5.1 Middle meatus swab specimen

Collection of middle meatus swab specimens will be performed at V1 and V2. A sterile stick swab (reference MW940; Medical Wire) will be introduced deeply within the middle meatus rotated at least 5 turns until visibly saturated. Immediately after collection, the swab will be placed in a dry tube (reference 62 554502; Sarstedt) and immediately stored in a freezer at -20°C.

8.1.5.2 SNOT-22

SNOT-22 (Appendix B) will be self-evaluated by the patient at V1 and V2 during the visit.

8.1.5.3 Assessment of sino-nasal microbial communities by 16S rRNA gene amplicon sequencing

DNA extraction

Genomic DNA (gDNA) is extracted using an optimized tissue-specific technique as previously described [14], [15]. This technique was carefully designed to maximize the recovery of bacterial DNA and to minimize any risk of contamination from the environment and the reagents. The quality and quantity of extracted gDNA are monitored by gel electrophoresis (1% w/w agarose in 0.5× TBE buffer) and NanoDrop 2000 UV spectrophotometer (ThermoFisher Scientific). All gDNA samples are stored at −20 °C until further processing.

Bacterial quantification by quantitative PCR (qPCR)

Total number of bacterial 16S rRNA gene copies is evaluated by real-time PCR amplification targeting the hypervariable regions V3-V4 with the primers: EUBF 5′-TCCTACGGGAGGCAGCAGT-3′ and EUBR 5′-GGACTACCAGGGTATCTAATCCTGTT-3′. The qPCR is realized in triplicate on a VIIA 7 PCR system (Life Technologies) using SYBR Green technology and the specificity of all qPCR products is assessed by systematic analysis of a post-PCR dissociation curve performed between 60°C and 95°C. The absolute number of copies of the 16S rRNA gene is determined by comparison with a quantitative standard curve generated by serial dilution of linearized plasmid standards.

Bacterial 16S rRNA gene sequencing

The V3-V4 hypervariable regions of the 16S rRNA gene are amplified by PCR using universal primer Vaiomer 1F (CTTTCCCTACACGACGCTCTTCCGATCT–TCCTACGGGAGGCAGCAGT, partial P5 adapter–primer) and universal primer Vaiomer 1R (GGAGTTCAGACGTGTGCTCTTCCGATCT–GGACTACCAGGGTATCTAATCCTGTT, partial P7 adapter–primer), which are fusion primers based on the qPCR primers with sequencing adapters. The first PCR reaction is carried out on a Veriti Thermal Cycler (Life Technologies) as follows: an initial denaturation step (94°C for 10 min), 35 cycles of amplification (94°C for 1 min, 68°C for 1 min and 72°C for 1 min) and a final elongation step at 72°C for 10 min. Amplicons (467 bp on the Escherichia coli reference genome) are then purified using the magnetic beads CleanNGS for DNA clean-up (CleanNA). A second PCR reaction for sample multiplexing is performed using tailor-made 6-bp unique index sequences with the forward primer Vaiomer 2F (AATGATACGGCGACCACCGAGATCTACACT–CTTTCCCTACACGAC, partial P5 adapter–primer targeting primer 1F) and reverse primer Vaiomer 2R (CAAGCAGAAGACGGCATACGAGAT–index–GTGACT–GGAGTTCAGACGTGT, partial P7 adapter including index–primer targeting primer 1R). This second PCR step is run as follows: an initial denaturation step (94°C for 10 min), 12 cycles of amplification (94°C for 1 min, 65°C for 1 min and 72°C for 1 min) and a final elongation step at 72°C for 10 min. Amplicons are purified as described for the first PCR round. All libraries are pooled in the same quantity to generate an equivalent number of raw reads and are sequenced on a MiSeq Illumina platform (2 x 300 bp paired-end MiSeq kit v3, Illumina).

16S rRNA Gene Sequence Analysis

The targeted metagenomic sequences are analyzed using a bioinformatics pipeline based on ‘find, rapidly, OTUs with Galaxy solution’ (FROGS) guidelines [16]. In brief, after demultiplexing of barcoded Illumina paired reads, single-read sequences were cleaned and paired into longer fragments. OTUs are produced with single-linkage clustering. The taxonomic assignment is performed by BLAST against SILVA database to determine bacterial profiles from phylum to genus, and when reachable to species level. The following filters are applied: amplicons with a length of <350 nt or a length of

>490 nt are removed and OTUs with abundance lower than 0.005% and that appear less than twice in the entire dataset are removed. Alpha and beta diversity analyses are conducted on the OTU table.

8.1.5.4 Safety

Adverse events, incidents and expected side effects of the medical device will be reported in the CRF at each visit/telephone call.

8.1.6 Arrangements for the monitoring maintenance and calibration of equipment used for genomic analysis

Annual maintenance by the manufacturer is performed on the Freedom EVO 150 (Tecan) and pipetting volumes are regularly controlled. The ViiA 7 Real-Time PCR System (ThermoFisher Scientific) is monthly controlled by Vaiomer staff and annually calibrated by the manufacturer. All handling, measurement and safety equipment (*e.g.* pipettes, scale, Captair Biocap PCR Workstations, spectrophotometers, safety and fume hoods) is annually controlled by specialized providers.

8.1.7 Any procedures for the replacement of subjects

No replacement of subjects is planned.

8.1.8 Investigation sites: number, location

The clinical investigation will be undertaken in 1 site in France. The principal investigator, Pr G de Bonnecaze is Otorhinolaryngologist and familiar with study procedures.

8.1.9 Definition of the completion of the study

This is defined as the date of last patient last visit (LPLV).

8.2 Investigational device and comparator

The investigational device which is Healsea® Chronic (nasal spray; 150 mL presentation) is a CE marked Medical Device. As this is an interventional study, the device will be provided to the patient. No specific labelling is required as the device will be used within its intended use in this post-market clinical investigation.

No comparator is used in this study.

8.3 Subjects

The target population is adult patients patients over 18 years diagnosed with CRS according to EPOS criteria [1]. According to these criteria, chronic rhinosinusitis (with or without nasal polyps) in adults is defined as presence of two or more symptoms one of which should be either nasal blockage/obstruction/ congestion or nasal discharge (anterior/posterior nasal drip), ± Facial pain/pressure ± reduction or loss of smell, lasting for 12 weeks or longer.

The subjects will be recruited by the site among outpatients coming for routine consultation because of CRS.

8.3.1 Eligibility criteria

8.3.1.1 Inclusion criteria

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

1. Male or female, aged ≥18 – ≤70 years
2. Provision of signed and dated informed consent form
3. Stated willingness to comply with all study procedures and availability for the duration of the study
4. Diagnosed with CRS based on the diagnostic criteria of the EPOS guideline
5. Registered with a social security scheme or covered by such a regime

8.3.1.2 Non-inclusion Criteria

An individual who meets any of the following criteria will be excluded from participation in this study:

1. Antibiotics or oral corticosteroids intake in the month prior to the study
2. Endoscopic sinus surgery in the 6 past months
3. Cystic fibrosis
4. Wegener's granulomatosis
5. Immunodeficiency
6. Defective access to the middle meatus
7. Lidocaine allergy
8. Known hypersensitivity/allergy to any component of the test device
9. Pregnant/Lactating female or absence of efficient contraception
10. Under tutorship or guardianship

8.3.2 Criteria and procedures for subject withdrawal or lost to follow-up

8.3.2.1 Withdrawal Criteria and procedures

The reasons for a subject's premature withdrawal from the study may be the following:

- A subject can withdraw his/her consent from the study for any reason at any time but he/she must inform the investigator. In all cases, whenever possible, the investigator should attempt to contact the patient as soon as possible for a final assessment in order to:
    - Obtain the reason(s) for withdrawal and report it/them in the Case Report Form,
    - Evaluate the patient's clinical condition,
    - If necessary, take appropriate therapeutic measures: management of an adverse effect or concomitant disease, prescription of another treatment.

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

- Significant study product non-compliance,
- If any clinical adverse event (AE)/incident, laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant,

- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation. In the context of COVID-19 pandemic, the diagnosis of COVID-19 during the study will justify the withdrawal of the patient from the study.

A premature end of study visit will be scheduled. Available data will be retained for the safety/efficacy analysis.

8.3.2.2 Subjects lost to follow-up

If a patient cannot be contacted to collect data at the end of the treatment period after repeated unsuccessful attempts including sending through a registered letter, he/she will be considered “lost to follow-up”. But before declaring that a patient is "lost to follow-up", the investigator (or his/her team) must do his/her best effort to contact the patient and attempts should be made via all available routes. A registered letter should be sent to the permanent address on file.

The methods used to attempt to contact the patient should be noted in the patient medical file.

8.3.2.3 Subject replacement

No subject replacement is planned.

8.3.3 Point of enrolment

The subjects will be recruited by the site among outpatients coming for routine consultation because of CRS.

In order to facilate the enrolment, a study nurse or a CRA will contact patients before the consultation in order to explain the study aim and design. This will allow the patient to have a period for though before the consultation with the investigator.

When coming for the consultation, the patient will be informed again about the study by the investigator. All questions relative to the study will be answered by the investigator. If the patient agrees study participation, he/she will sign/date the Informed Consent Form before any study procedure.

8.3.4 Total expected duration of the clinical investigation

The total study duration is planned to be 5 months.

8.3.5 Expected duration of each subject’s participation

The duration of each patient’s participation is 30 days.

8.3.6 Number of subjects required to be included in the clinical investigation

An arbitrary sample of 20 patients completing the investigation without major protocol deviation has been chosen.

8.3.7 Estimated time needed for the enrolment

It is expected to enrol 20 patients within 4 months.

8.3.8 Relationship of investigation population to target population

Patients with CRS have two or more symptoms, one of which should be either nasal blockage/obstruction/congestion or nasal discharge (anterior/posterior nasal drip), lasting for 12 weeks or longer. Healsea® Chronic is indicated in adults over 18 years in the treatment of nasal symptoms of sinonasal conditions. The investigation population is thus a target population of Healsea® Chronic.

8.3.9 Vulnerable, pregnant and breastfeeding population

Patient under tutorship or guardianship are not eligible.

Pregnant and breastfeeding women are not eligible too.

8.3.10 Compensation

A compensation of 200 euros for costs resulting from participation in the clinical investigation (transportation, absence of work..) will be paid to each patient who complete the study.

8.4 Procedures

8.4.1 Study procedures

The study will comprise 2 visits and 1 telephone call.

Visit 1 (V1) – (Day 1): Screening/Inclusion
The investigator will give oral information relative to the study to the participant and answer to all the questions raised by the patient. If he/she agrees to participate in the study, he/she will be asked to sign and date a consent form.

Each screened subject will be assigned a subject identifier number during screening. The subject identifier number will contain the site number (01) and the subject number assigned in numerical order at the screening visit based on chronological order of screening dates (e.g., 01-10 for the 10th subject screened at the Site #01).

The investigator or his/her delegated designee will:

- Record demographic data and medical history and ongoing medication,
- Perform a rhinologic examination,
- Verify that eligibility criteria are fulfilled,
- Proceed with the middle meatus swab collection,
- Detail the questions of the SNOT-22 and record the patient's score,
- Record adverse events if applicable,
- Explain how to collect adverse events/incidents/expected side effects and concomitant medications for reporting at the telephone call/visit,
- Deliver Healsea® Chronic and explain to the patient how to use it.

The patient will be ask to bring back the container at the end of the treatment period.

D1-D30 (at home):
The patient will be instructed to self administer Healsea® Chronic nasal spray twice a day (1 puff, 1-2 sec) in each nostril during 30 days.

D15±3 (at home): Telephone call
The Investigator or his/her delegated designee will call the patient to review patient status and study progress, adverse events/incidents/expected side effects and concomitant treatments.

Visit 2 (V2) – (Day 30 ±5): end of treatment
The investigator or his/her delegated designee will:

- Perform a physical and clinical examination,
- Record the SNOT 22 score in the CRF,
- Proceed with the middle meatus swab collection,
- Report the adverse events/incidents/expected side effects and concomitant medications in the CRF,
- Ask the patient to complete the satisfaction questionnaire and report the result in the CRF,
- Measure compliance to Healsea® Chronic intake.

8.4.2 Permitted and forbidden concomitant medications

All concomitant medications are authorized during the investigation, except nasal irrigation, systemic corticosteroids, topical (nasal mucosa) and systemic antibiotics.

8.4.3 Exclusion period

The patients will be asked to not participate in another study during their study participation.

8.4.4 Activities performed by sponsor representatives (excluding monitoring)

The sponsor will implement and write clinical quality procedures to ensure that the clinical investigation is conducted and monitored and that data are generated, documented, recorded, evaluated and reported in compliance with the ISO 14155:2020, the CIP, any subsequent amendment(s) and any other applicable standards and in accordance with the regulatory requirements. The sponsor will ensure ongoing risk management throughout the clinical investigation and will take all measures to protect rights, safety and weel-being of children who participate in the study.

The sponsor is required to report noncompliance trends and safety issues to the CA and investigators.

The sponsor shall report on the progress and status of the clinical investigation to the CA.

The sponsor will determine the frequency and type of audit to be performed. An audit can be used:

i) As a routine part of the sponsor's quality assurance,
ii) To assess the effectiveness of the monitoring activity,
iii) Whenever there are serious or repeated CIP deviations or suspicion of fraud,
iv) To bring an investigation site into “inspection readiness” (i.e. to prepare the investigation site for a potential regulatory inspection),

v) When requested or suggested by a regulatory authority.

8.4.5 Any known/foreseeable factors that can compromise the outcome of the clinical investigation and methods for addressing these factors

No known or foreseeable factors that can compromise the outcome of the clinical investigation is anticipated.

8.4.6 Follow-up and medical care after completion of the study

Neither follow-up nor specific medical care for patients are foreseen after the completion of the study. Nevertheless, routine care for CRS will be provided to the patients.

8.4.7 Potential use of samples obtained from subjects

Patients' samples will be used to assess sino-nasal microbiota through 16S RNA gene amplicon sequencing only as per approved protoocol.

8.5 Monitoring plan

A risk-based monitoring plan included in a separate document will describe in detail who will conduct the monitoring, at what frequency monitoring will be done, at which level of detail monitoring will be performed, and the distribution of monitoring reports. Results of the risk assessment will be used to develop a risk-based monitoring plan and a supporting rationale.

8.5.1 Initiation visit

The study will only start at a site after:

- The Ethics Committee has granted approval for the conduct of the study,
- ANSM approval,
- Essential documents are in place, such as CVs of the Investigators and site staff, and the Clinical Trial Agreement signed.

In agreeing to participate, the investigator undertakes to strictly comply with the study protocol, Good Clinical Practice, the French regulation and to MDR. The investigator also guarantees the authenticity of the data collected in the context of the study and agrees to the legal provisions for study sponsor quality control.

8.5.2 Monitoring

Study monitoring under responsibility of Lallemand Pharma by qualified staff will be performed at various stages of the study. Monitoring will include on-site visits and centralized data review to assure that the investigation is conducted according to the CIP and comply with applicable regulations and deadlines. On-site review of Case Report Form (paper CRFs) will include the review of forms for completeness, clarity and consistency with source documents available for each subject. In case of queries, the investigator should respond within agreed timelines.

The investigator must permit study-related monitoring visits, audits review by the Ethic Committee and ANSM, and allow direct access to source data and source documents provided that subject confidentiality is protected. In case of an audit appointed by Lallemand Pharma, the investigator will receive written notification in advance.

9. Statistical design and analysis

Patients and disease characteristics will be described at baseline.

16S rRNA Gene Sequence Analysis

The normal distribution of the values is verified thanks to normality tests (e.g. Shapiro-Wilk test). However, it is most likely that the group size will be insufficient to apply parametric statistical tests. Significant variations in total number of bacterial 16S rRNA gene copies or in alpha diversity are assessed using the Kruskal-Wallis test or the Wilcoxon rank-sum test. Multidimensional scaling analyses (MDS) are performed on beta diversity distances matrices and differences between groups were assessed using PERMANOVA and PERMDISP analyses (2000 permutations). LEfSe (Linear discriminant analysis Effect Size) analyses, based on non-parametric tests are used to determine significant variations in taxa relative abundance [17].

Secondary endpoints

Values and changes from baseline to D30/end of study of the SNOT-22 total score will be described.

Patients satisfaction will be described at D30/end of study.

Safety

All adverse events/incidents/expected side effects will be listed, but only treatment-emergent adverse events/incidents (non-serious + serious) and expected side effects will be summarized by the number and percentage of patients, classified by System Organ Class and Preferred Term as defined by MedDRA dictionary.

Frequency and percentage of patients with at least one reported adverse event/incident/expected side effect will be tabulated by System Organ Class.

10. Data collection

10.1 CRF entry

All the data provided in the protocol and collected by the investigator are to be reported in the case report forms (CRF).

The case report forms will consist of triplicate self-copying sheets (Carbonless copy paper):

- The white sheet (original) is intended for the data entry,
- The blue sheet (median copy) is a working document for reference purpose,
- The yellow sheet (bottom copy) is retained by the investigator.

The CRFs will be completed and signed by the investigator. Each correction will also be initialled and dated by the investigator or his designee.

10.2 Source documents

The investigator is asked to report in the patient's medical record:

- Mention of the subject's participation in the study
- Demographic data (date of birth, sex, name)
- Past medical and surgical history
- Recent past treatments
- Concomitant treatments
- Clinical assessment
- Date of each study visit
- Change in any of the concomitant treatment
- Any adverse event/incident

and all relevant data according to the investigator's opinion.

In case of computerized medical file, a printout will be edited, dated and signed by the investigator.

Source data are documented on the "source data agreement form".

11. Data management

11.1 Methods for data entry and collection

Clinical data related to the study will be collected and saved into a computerized database by the Data Management Department of AXIODIS using the EXAGIS e-CRF software. In order to ensure maximum data quality, the paper CRF will be transcribed into the electronic database using double data entry.

11.2 Data review

After double data entry, a computerized data review will be performed by AXIODIS according to the Data Validation Plan.

Results of this data review will be sent to the Study CRA and/or Study Manager. Whenever required, queries issued from this review will be submitted to the investigator for resolution and signature and then tracked until corrections are entered and validated.

11.3 Data coding

Adverse events, concomitant diseases, medical/surgical histories will be coded by AXIODIS using the MedDRA dictionary (last version in use).

Concomitant medication will be coded using the WHO-DD dictionary (last version in use).

The study manager and/or other relevant sponsor representative will validate the coding.

11.4 Procedures for verification, validation and securing of electronic clinical data system

The EXAGIS software is installed on a Linux server (secure Data Center) hosted at the OVH company that guarantees the security, maintenance, as well as regular antivirus and firewall updates of this dedicated server. Any modification to the data is tracked, i.e. creation and changes are timestamped and authors are recorded. Software access is restricted to authorized users using individual encrypted passwords. Data processing via SAS software (SAS Institute, Cary, NC, USA) is carried out on a Windows 2012 R2 production server at OVH, using direct access by SAS or MySQL Workbench.

11.5 Procedures to maintain and protect subject privacy

Data privacy is ensured through restricted access to the anonymized data located in a dedicated separate database.

11.6 Methods for database locking at the start of analysis and storage upon completion of the clinical investigation

The validated database will be locked upon request of the Data Manager following the completion of all steps required, i.e. resolution of all queries, validation of the coding, data review meeting.

The review process prepared by the clinical research assistant, data manager, study monitor, medical manager and statistician will be concluded during a meeting (Validation Committee) to be attended by at least the following:

- Coordinating investigator,
- Study monitor,
- Sponsor Vigilance representative,
- Study Coordinator,
- Data manager,
- Statistician.

The review is also required for defining the analysis populations and validating the statistical analysis plan.

The meeting will be documented by written and signed minutes that will act as the basis for data processing by the Biometrics Department.

All decisions on the evaluability of the data from each individual subject for the statistical analysis must be made and documented before locking the database.

11.7 Procedure for data retention

Once the Clinical Investigation Report is signed and validated, the data are archived on a dedicated hard disk file with limited access rights. This specific file is mounted on AXIODIS's OVH server with a

back-up every two months as well as copied to an external disk that will be stored under seal within the premises of AXIODIS. The data are conserved for at least 10 years.

12. Amendments to the CIP

Neither the investigator nor the sponsor may alter the protocol without the authorisation of the other party.

All changes to the protocol are subject to an amendment which must be dated and signed by both parties and must appear as an amendment to the protocol.

Substantial amendments are submitted to the EC and ANSM for approval.

13. Deviation from clinical investigation plan

All protocol deviations will be managed as per the Standard Operating Procedures of the CRO in charge of the study coordination.

A deviation log shall be maintained by the study site. All deviations will be included, as required in the final study report.

Any major deviation from the protocol that has not been previously approved by the sponsor must be reported to the sponsor within 2 working days of the deviation occurrence. Any deviations from the clinical investigation plan that are identified during routine monitoring visits will be reported to the sponsor within 24 hours of being identified.

Corrective and preventive actions and principal investigator disqualification criteria will be described in the monitoring plan.

Major protocol deviations are defined as deviations liable to prevent or change the interpretation of the results of the study. The following deviations are considered major:

- Non-compliance with the inclusion or exclusion criteria
- Non-compliance with treatment
- No collection of middle meatus swab sample at any visit
- Intake of forbidden medication

All other cases will, a priori, be considered as minor deviations.

14. Device accountability

Not applicable for this post market interventional study.

15. Ethical consideration

15.1 Informed consent process

According to Regulation UE 2017/745, ISO 14155:2020 and articles L1122-1 and L1122-2 of Code de la Santé Publique, the Princial Investigator or his/her authorized designee must given all pertinent information relative to all aspects of the research before any study procedure. Participants will have the opportunity to carefully review the information document and ask questions prior to accepting or not to participate in the study. As this investigation is interventional, the consent shall be obtained in writing and the study participation must be documented in the patient medical file.

15.2 Subject privacy

This research falls within the scope of the reference methodology MR-001 relating to the processing of personal health data carried out in the context of a research involving the subject's consent collection. The sponsor has signed a commitment to comply with the provisions of MR-001 (CNIL receipt number: 2222437v0 dated from 21st May 2021).

All records identifying directly and/or indirectly the subject will be kept confidential according to the French law n°78-17 *relative to data processing, files and liberties* and the European Regulation 2016/679 effective as of 25 May 2018 (hereafter the "GDPR") and will not be made publicly available.

The present study will be conducted under Regulation 2016/679/EU of the European Parliament and of the Council of 27 April 2016 on the protection of natural persons with regard to the personal data and free movement of such data (GDPR).

In accordance with GDPR, the Sponsor is the "Controller" and the participating sites, monitors, data managers and statisticians are "Processors". Lallemand Pharma, sponsor of the study, is responsible for the processing of the study data. The Data Protection Officer appointed by Lallemand Pharma: Ms Yannick Hervy, yhervy@lallemand.com.

Confidentiality of data shall be observed by all parties involved throughout the clinical investigation. All data will be secured against unauthorized access. Subject names will not be sent to the sponsor. Only the patient number will be recorded in the CRF, and if the patient's name appears on any document, it must be obliterated before a copy of the document is supplied to the sponsor. All subject data that appear in reports and publications will be anonymised such that the privacy and confidentiality of each subject is maintained.

To characterize the nasal microbiome by quantitative PCR and 16S rRNA gene sequencing, VAIOMER will receive patient biological samples identified by anonymized sample codes. To ensure that personal data cannot be attributed to specific samples, the decoding index will not be communicated to Vaiomer at any time. Moreover, to protect the integrity and the confidentiality of the data generated by Vaiomer, below are the relevant actions in place:

- **Physical location**

Vaiomer will produce data derived from the biological samples which will be stored on Vaiomer local servers (no cloud storage). As Vaiomer does not own the sequencing machines (MiSeq), the sequencing step itself is performed on a public genomic platform (INRAE) in our close environment. This is realized by Vaiomer staff who is validated beforehand by the platform. Sequencing data is

transferred by encrypted download links to Vaiomer server after data acquisition and purged from the data provider disk after 3 months.

- **Data backup**

Vaiomer storage server is backed up externally daily to a secured data center fully owned by our subcontractor.

- **Access control**

Access to these data is restricted to Vaiomer staff via a password-protected personal account. The computer and storage servers are located in a restricted area through badge access control.

No data processing will be performed outside of the European Union but the data base will be transferred to the sponsor at Massagno in Switzerland. Although outside EU, this country is recognized by the EU as offering an adequate data privacy protection.

To fullfil the requirements of source data verification, the PI will be required to obtain consent from each subject stating that they agree for their medical records to be accessed (this will form part of the consent process). The information document and the consent form provided to patient will include a specific paragraphe relative to data privacy explaining the lawfulness of personal data processing conditions and patients rights.

The patients's rights are the followings:

- The right to request access to, rectification, deletion or restriction of processing concerning personal data collected during the study,
- The right to get back all data linked to the research and to forward them to another data processing manager (portability right),
- The right to withdraw consent to data collection at any time and to request a restriction of processing concerning personal data as mentioned in GDPR's article 18. However, the data processing manager keeps the ability to reject such request should this right likely make impossible or seriously compromise the achievement of the research objectives.

These rights can be exercised with the investigator or his designated representative or by contacting the Data Protection Officer appointed by Lallemand Pharma: Ms Yannick Hervy, yhervy@lallemand.com

15.3 Insurance policy

In accordance with the provisions of the French law *n° 2012-300 du 5 mars 2012 et son décret d'application n°2016-1537 du 16 novembre 2016*, Lallemand Pharma, has an insurance policy intended to guarantee against possible damage resulting from the investigation (appendix D).

It is advisable to underline that non-compliance with the Research Legal Conditions is a cause for guarantee exclusion.

16. Vigilance

Healsea® Chronic will be used within the intended use covered by the CE-mark.

Pursuant to (EU) 2017/745 Medical Device Regulation, for a post market clinical follow-up investigation of a medical device used within the intended use covered by the CE-mark, requirement of MDR articles 87 to 90 apply (materiovigilance).

This additional procedure “collection of middle meatus swab specimens “ is invasive (MDCG 2021-6 Regulation (EU) 2017/745) but no expected risk is associated with this procedure. However, should a serious adverse event related to this additional procedure occur, MDR article 80 shall apply.

16.1 Definitions

Incident means 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 2017/745, article 2 (64)).

Serious incident means 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 2017/745, article 2(65)).

Serious public health threat means an event which could result in imminent risk of death, serious deterioration in a person's state of health, or serious illness, that may require prompt remedial action, and that may cause significant morbidity or mortality in humans, or that is unusual or unexpected for the given place and time (MDR 2017/745, article 2(66)).

Expected and foreseeable side effects (MEDDEV 2 12-1 rev. 8)
They meet all the following criteria:

- Clearly identified in the manufacturer's labelling;
- Clinically well known as being foreseeable and having a certain qualitative and quantitative predictability when the device is used and performs as intended;
- Documented in the device master record, with an appropriate risk assessment, prior to the occurrence of the incident and
- Clinically acceptable in terms of the individual patient benefit.

Adverse event means 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 related or not related to the investigational device (MDR 2017/745, article 2(57)).

Adverse or intercurrent events are graded as follows:

- Mild: Awareness of signs or symptoms but easily tolerated.
- Moderate: Uncomfortable enough to cause interference with usual activity.
- Severe: Incapacity with inability to work or do usual activity.

Serious Adverse Event means 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) hospitalisation or prolongation of patient hospitalisation,
(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) foetal distress, foetal death or a congenital physical or mental impairment or birth defect (MDR 2017/745 Article 2(58)).

16.2 Reporting in the CRF

All serious and non serious incidents/serious and non serious risk of incidents/expected side effects/adverse events (except pre-existing condition but CRS) occurring during the clinical investigation will be reported in the CRF.

16.3 Investigator's responsibilities and processing timelines

16.3.1 Materiovigilance (art 87 to 90)

All incidents and expected side effects will be reported to Lallemand Pharma vigilance manager according to MDR, MEDDEV 2.12 rev 8 modalities, national requirements, ISO 14155:20 and Lallemand Pharma procedures which are described in a safety management plan. This document will be signed by the Principal Investigator, representants of the Clinical Research Organization (CRO) before study start.

As soon as the investigator will be informed of the event, he will complete a Primary Notification Form template (PNF, appendix E) and send it to LP vigilant manager (officelp@lallemand.com) and to the clinical CRO with any relevant supportive documentation within the same day (<24h) for serious (risk)incident and within two calendar days (<48h) for non serious incidents and expected side effects.

16.3.2 Vigilance according to article 80 for the invasive procedure

Any serious adverse event that has a causal relationship with the additional procedure or a causal relationship reasonably possible, will be reported by the investigator to the sponsor (officelp@lallemand.com) and to the CRO (etudes@bgclinicals.com) immediately, but not later than 3 calendar days after investigation site study personnel's awareness of the event. To this end, the investigator will complete the SAE form (appendix F).

16.4 Sponsor responsibilities and processing time lines

16.4.1 Materiovigilance (art 87 to 90)

The vigilance manager of Lallemand Pharma is responsible for the reporting to the French Health Authority (ANSM).

Briefly, for serious incidents or risk of serious incident, the vigilance manager will complete a Manufacturer Incident Report (MIR). The result of the investigation conducted by the sponsor may lead to the implementation of a safety corrective action and completion of a Field Safety Correction Action (FSCA) and of a Field Safety Notice. These documents will be transmitted to the French Health Authority in accordance with the timelines provided in the table below:

Serious Public Health Threat	Report immediately but not more than 2 days
Death or UNANTICIPATED Serious Deterioration in State of Health	Report immediately but not more than 10 days
Others (could have led to death or serious deteriation in health)	Report immediately but not more than 15 days
Every FSCA	Immediately before the measure is implemented except in case of emergency, when the vigilance manager of the sponsor has to take immediate safety corrective action

Increase rate or severity of expected undesirable side effects and non serious incidents will be reported to the ANSM in Trend reports as per regulation requirements.

16.4.2 Vigilance according to article 80 for the invasive procedure

Any serious adverse event that has a causal relationship with the additional procedure or a causal relationship reasonably possible, will be reported to the ANSM by the sponsor as follows:

Imminent risk of death, serious injury, or serious illness and that requires prompt remedial action for patients/subjects or a new finding to it	Immediately, but not later than 2 calendar days after awareness by sponsor of a new reportable event or of new information in relation with an already reported event.
Other reportable events or a new finding/update to it	Immediately, but not later than 7 calendar days following the date of awareness by the sponsor of the new reportable event or of new information in relation with an already reported event.

In the absence of a fully functional electronic system referred to in Article 73, the sponsor will complete the « Investigation summary safety report form » (MDCG-2020-10/2):

https://ec.europa.eu/health/sites/health/files/md_sector/docs/md_mdcg_2020-10-2_guidance_safety_report_form_en.xlsx.

Where an investigator assessment is not available and/or the sponsor remains uncertain about classifying the serious adverse event, the sponsor should not exclude the relatedness; the event should be classified as “possible” and the reporting not be delayed.

17. Suspension, premature termination and routine close out

17.1 Suspension or Premature Termination of the Clinical Investigation

Lallemand Pharma may suspend or prematurely terminate the clinical investigation. The reasons will be documented. Reasons for suspension or premature termination may include occurrences where monitoring or auditing identifies serious or repeated deviations done by an investigator. Lallemand Pharma will notify the ANSM as appropriate and ensure that the EC is notified of any suspension or early termination of the clinical investigation.

A principal investigator, EC, or ANSM may suspend or prematurely terminate participation in the clinical investigation at the investigation site.

If suspicion of an unacceptable risk to subjects arises during the clinical investigation, or when so instructed by the EC or ANSM, Lallemand Pharma will suspend the clinical investigation while the risk is assessed. The sponsor will terminate the clinical investigation if an unacceptable risk is confirmed. Should the risk not be confirmed Lallemand Pharma will, in accordance with regulations, provide relevant persons with justification and data supporting the decision to resume the clinical investigation.

17.2 Routine close out

Routine close-out procedures will be conducted ensuring that the PIs records are complete, all documents needed for the sponsor files are retrieved, remaining clinical investigation materials are returned to th sponsor, previously identified issues have been resolved.

The Principal Investigator will retain all copies of the records for a period of 10 years from the completion of the clinical investigation. In any circumstances, the PI must contact the sponsor prior to disposing of any records related to the clinical investigation. Should the data be no longer required for regulatory purposes then the confidential destruction of said documents will be approved. Should the data be maintained for longer Lallemand Pharma will make this information available to all appropriate bodies in the same way. A list of essential documents to be maintained will be provided to each site at initiation.

Should the PI have to move/retire, or otherwise leaves his(her) position, he(she) will provide Lallemand Pharma with the name and address of the person assuming responsibility for records relating to this clinical investigation.

18. Publication policy

The information and data collected during the conduct of this clinical study are considered confidential and are used by the sponsor in connection with the development of the study treatment. This information will be disclosed by the sponsor depending on current regulations.

To allow use of the information derived from this clinical study and to ensure compliance with current regulations, the investigator must provide the sponsor with complete test results and all data collected during the study.

Only the sponsor may make study information available to physicians and to Regulatory Agencies, except as required by current regulations.

All the results of this study including data and reports are the property of the sponsor.

In the event that the sponsor chooses to publish study data, the manuscript must be provided to the author(s) at least 30 days prior to the expected date of submission to the intended publisher.

The investigator(s) can reserve the right to publish or present study data; if so, the manuscript or abstract must be provided to the sponsor for review at least 30 days prior to the expected date of submission to the intended publisher or of planned presentation.

In addition, if necessary, (the) investigator(s) shall withhold publication for an additional 60 days, to allow the filing of a patent application, or to allow the sponsor to take any measures he deems appropriate to establish and preserve his proprietary rights.

It is agreed that publication of study results by each site shall be made only as part of a publication of the study results obtained by all sites performing the protocol, once the study is completed and finalised.

A description of the clinical investigation is registered in *clinicaltrials.gov*. The content will be updated throughout the conduct of the clinical investigation and the results entered at completion of the clinical investigation and made publicly available.

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20. List of Appendix

Appendix A: Instruction for Use

Appendix B: SNOT-22

Appendix C: Satisfaction questionnaire

Appendix D : Insurance certificate

Appendix E: Primary Notification Form (PNF)

Appendix F: SAE form

Annexe A: Instructions for use

English version:

Healsea®
CHRONIC
NASAL CARE

Discover Healsea®!
Healsea® is a range of medical devices in nasal spray form with seawater from the Swedish Gullmarn Fjords ensuring a high level of purity.
It contains an innovative extract, Symbiofilm®, isolated from the marine biosphere of the deep seas of Panarea islands in Sicily. Symbiofilm® was discovered in a protected ecosystem ensuring a unique marine biodiversity.
Healsea® is a natural care preservative free allowing to clean the fragile nasal mucosa.

Healsea® CHRONIC is the ideal solution for those who suffer from allergic rhinitis, chronic sinusitis and/or sensitive nose.

Symbiofilm® is a marine postbiotic, which contains exopolymeric substances, an important natural component, enhancing the cleansing efficacy of **Healsea® CHRONIC.** Symbiofilm® reduces the development of biofilm from respiratory pathogenic microorganisms by mechanical action.

Healsea® CHRONIC is a nasal spray ensuring an efficient cleansing and decongestion of the nasal mucosa by osmotic action.

INDICATION

Healsea® CHRONIC is a nasal spray indicated in adults over 18 years in the treatment of nasal symptoms of chronic sino-nasal conditions: allergic rhinitis, chronic sinusitis and/or sensitive nose.
It can be used also as post-operative nasal care after nasal surgery.

DIRECTIONS FOR USE

One puff (1-2 sec) twice a day in each nostril for 30 days or as recommended by your doctor or pharmacist.

COMPOSITION

Hypertonic seawater solution (salinity 2.2%), Symbiofilm®.

CONTRAINDICATIONS

Do not use in children and adolescents under 18 years of age.
Do not use in case of hypersensitivity or allergy to one or several components.

STORAGE AND WASTE RECOMMENDATIONS

Store at room temperature and not above 30°C.
See the expiry date on the box or the spray bottle.
Ask your pharmacist to throw out the spray bottle after use. These measures contribute to protect the environment.

PRECAUTIONS OF USE

- Keep out of reach of children.
- If symptoms persist, ask for advices to your doctor or pharmacist.
- The bottle shall be used by only one person for hygiene reason and to avoid the transmission of pathogenic agents that could be in contact with the nozzle.
- Keep away from heat, hot surfaces, sparks, flames or sunlight.
- As the bottle is pressurised, do not pierce or burn even after use.
- Do not smoke while using the spray.

Lallemand Pharma AG - Via Selva 2 - 6900 Massagno
SWITZERLAND

Authorized representative :
ASPE CONSEIL -21, chemin de la Favasse -31140 Aucamville – France

UNDESIRABLE EFFECTS

Feeling of itching and irritation can occur while initiating the treatment. In case of undesirable effects contact your doctor.

BAG ON VALVE TECHNOLOGY

The Bag on Valve technology warrants a perfect airtight of the bag containing the seawater-based solution. This technology permits to dispense a preservative free solution.

How to use Healsea® CHRONIC?

1. Place the nozzle smoothly into the nostril while keeping head straight.
2. Once the nozzle is placed, press on the nozzle for 1-2 sec in each nostril. Let flow the excess of solution and wipe.
3. Clean the nozzle with tissue, soapy water, rinse and dry after every use.

Symbiofilm® has been scientifically documented in vitro for its physical properties helping to reduce the biofilm formation by pathogens. Learn more about biofilms by flashing this QR code!

First authorization date of CE marking: 2021/03/30

CE 0459

Publication date : 2021/06/10_R1

LALLEMAND
LALLEMAND PHARMA

French version:

Healsea®
CHRONIC
SOIN NASAL

Découvrez Healsea® !
Healsea® est une gamme de dispositifs médicaux sous forme de spray nasal à base d'eau de mer provenant des Fjords Suédois de Gullmarn, ce qui assure un excellent niveau de pureté.
Il contient un extrait innovant, Symbiofilm®, isolé de la biosphère marine au large des îles Panarea en Sicile. Symbiofilm® provient d'un écosystème préservé garantissant une biodiversité marine unique.
Healsea® est un soin naturel sans conservateurs permettant de nettoyer la muqueuse nasale des plus fragiles.

Healsea® CHRONIC est la solution idéale pour ceux qui souffrent de rhinite allergique, sinusite chronique et/ou nez sensible.

Symbiofilm® est un postbiotique d'origine marine, composé d'exopolymères naturels, renforçant l'efficacité de nettoyage unique de notre soin **Healsea® CHRONIC**. Symbiofilm® permet de réduire le développement du biofilm de micro-organismes pathogènes respiratoires par action mécanique.

Healsea® CHRONIC est un soin nasal assurant un nettoyage efficace et une décongestion de la muqueuse nasale par action osmotique.

INDICATION
Healsea® CHRONIC est un spray nasal indiqué chez les adultes à partir de 18 ans pour le traitement des symptômes nasaux des affections sino-nasales chroniques: rhinite allergique, sinusite chronique et/ou nez sensible.
Il peut également être utilisé comme soin post-opératoire après une chirurgie nasale.

CONSEILS D'UTILISATION
Une pulvérisation (1-2 sec) deux fois par jour dans chaque narine pendant 30 jours ou selon les recommandations de votre médecin ou pharmacien.

COMPOSITION
Solution d'eau de mer hypertonique (salinité 2,2%), Symbiofilm®.

CONTRE-INDICATIONS
Ne pas utiliser chez les enfants et adolescents de moins de 18 ans.
Ne pas utiliser en cas d'hypersensibilité ou d'allergie à l'un ou plusieurs des composants.

CONDITIONS DE CONSERVATION ET D'ÉLIMINATION
Conserver à température ambiante et ne pas exposer à une température supérieure à 30°C.
Voir la date d'expiration indiquée sur la boîte ou le flacon du spray.
Demander à votre pharmacien d'éliminer le flacon après usage. Ces mesures contribueront à protéger l'environnement.

PRÉCAUTIONS D'EMPLOI
-Tenir hors de portée des enfants.
- Si les symptômes persistent, demander conseils à votre médecin ou pharmacien.
- Utiliser le flacon pour une personne seulement pour des raisons d'hygiène et ce afin d'éviter le risque de transmission d'agents pathogènes qui pourraient être en contact avec l'embout.
- Garder le flacon loin de toutes sources de chaleur, des surfaces chaudes, des étincelles, des flammes ou des rayonnements solaires.
- Le flacon étant pressurisé, ne pas percer ou brûler après utilisation.
- Ne pas fumer pendant l'utilisation du spray.

Lallemand Pharma AG - Via Selva 2 - 6900 Massagno
SUISSE

Mandataire : ASPE CONSEIL -21, chemin de la Favasse -31140 Aucamville – France

EFFETS INDÉSIRABLES
Des sensations de picotement et d'irritation peuvent survenir au début du traitement. En cas d'apparition d'effets indésirables, contacter votre médecin.

TECHNOLOGIE BAG ON VALVE
La technologie Bag on Valve garantit une totale étanchéité de la poche contenant la solution d'eau de mer. Cette technologie permet de dispenser une solution sans conservateurs.

Comment utiliser Healsea® CHRONIC ?
1. Mettre délicatement l'embout dans la narine en maintenant la tête droite.
2. Une fois le flacon placé, effectuer une pression sur l'embout pendant 1 à 2 secondes par narine. Laisser s'écouler l'excédent de solution puis essuyer.
3. Nettoyer l'embout avec un tissu propre et de l'eau savonneuse, rincer et sécher après chaque utilisation.

Symbiofilm® a été documenté scientifiquement *in vitro* pour ses propriétés physiques permettant de réduire la formation du biofilm pathogène. Vous souhaitez en apprendre plus sur les biofilms ? Flashez ce QR code !

Date de la première autorisation de marquage CE: 2021/03/30
CE
0459
Date de publication : 2021/06/10_R1

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Appendix B: SNOT-22

Sino-Nasal Outcome Test-22 Questionnaire v4

Below you will find a list of symptoms and social/emotional consequences of your nasal disorder. We would like to know more about these problems and would appreciate you answering the following question to the best of your ability. There are no right or wrong answers, and only you can provide us with this information. Please rate your problems, as they have been over the past two weeks. Thank you for your participation.

Considering how severe the problem is when you experience it and how frequently it happens, please rate each item below on how 'bad' it is by circling the number that corresponds with how you feel using this scale →	No problem	Very mild problem	Mild or slight problem	Moderate problem	Severe problem	Problem as bad as it can be
1. Need to blow nose	0	1	2	3	4	5
2. Sneezing	0	1	2	3	4	5
3. Runny nose	0	1	2	3	4	5
4. Cough	0	1	2	3	4	5
5. Post nasal discharge (dripping at the back of your nose)	0	1	2	3	4	5
6. Thick nasal discharge	0	1	2	3	4	5
7. Ear fullness	0	1	2	3	4	5
8. Dizziness	0	1	2	3	4	5
9. Ear pain/pressure	0	1	2	3	4	5
10. Facial pain/pressure	0	1	2	3	4	5
11. Difficulty falling asleep	0	1	2	3	4	5
12. Waking up at night	0	1	2	3	4	5
13. Lack of a good night's sleep	0	1	2	3	4	5
14. Waking up tired	0	1	2	3	4	5
15. Fatigue during the day	0	1	2	3	4	5
16. Reduced productivity	0	1	2	3	4	5
17. Reduced concentration	0	1	2	3	4	5
18. Frustrated/restless/irritable	0	1	2	3	4	5
19. Sad	0	1	2	3	4	5
20. Embarrassed	0	1	2	3	4	5
21. Sense of taste/smell	0	1	2	3	4	5
22. Blockage/congestion of nose	0	1	2	3	4	5
TOTAL:	____	____	____	____	____	____

Appendix C: Satisfaction questionnaire

Please score from 0 to 3 your satisfaction regarding the following points:	**0**	**1**	**2**	**3**
How would you characterize the use of Healsea® Chronic nasal spray? *(0 : not easy; 1 : pretty easy ; 2 : easy ; 3 : very easy)*				
How would you characterize the residual taste after spraying Healsea® Chronic? *(0 : not pleasant ; 1 : neutral ; 2 : pleasant ; 3 : very pleasant*				
How would you characterize the cleansing and moistening of nasal mucosa with Healsea® Chronic ? *(0 : no improvement ; 1 : slight improvement ; 2 : moderate improvement; 3 : very clear improvement)*				
Total :				

Appendix D: Insurance certificate

HDI Global SE
Tour Opus 12 – Défense 9
77 Esplanade du Général de Gaulle
92914 PARIS LA DEFENSE CEDEX
478 913 882 RCS Nanterre
N° SIRET : 478 913 882 000 54

ATTESTATION D'ASSURANCE RESPONSABILITE CIVILE PROMOTEUR DE RECHERCHE IMPLIQUANT LA PERSONNE HUMAINE

Nous soussignés **HDI Global SE** - TOUR OPUS 12 – LA DEFENSE 9 - 77, Esplanade du Général de Gaulle F.92914 PARIS LA DEFENSE CEDEX, certifions que la société :

LALLEMAND PHARMA AG
VIA SELVA 2
6900 MASSAGNO - SUISSE

a souscrit un contrat de Responsabilité Civile Promoteur de Recherche impliquant la personne humaine sous le numéro **01005345-14058 210068** conforme aux dispositions légales et réglementaires françaises sur les recherches impliquant la personne humaine et notamment de la loi n° 88.1138 du 20.12.1988, modifiée par les textes subséquents notamment la loi n° 2012-300 du 5 mars 2012 et son décret d'application n° 2016-1537 du 16 novembre 2016, ainsi que le règlement européen (UE) 2017/745 sur le dispositif médical du 26 Mai 2021 pour la recherche impliquant la personne humaine dénommée ci-après :

Nom du promoteur :	**LALLEMAND PHARMA AG**
Numéro d'enregistrement : (EUDRACT ou n° fourni par l'ANSM)	**2021-A01458-33**
Titre de la recherche :	**Assessment of sino-nasal microbial communities changes in adult patients with Chronic Rhinosinusitis by 16S rRNA gene amplicon sequencing before and after 1-month treatment duration with Healsea® Chronic: an exploratory study (Study ISONAM)** **Protocole LPH-2102**
Nombre de patients :	**20**
Début et fin prévisionnels de la recherche :	DU **01/09/2021** AU **31/01/2022**

La garantie est conforme à l'obligation d'assurance instituée par les textes de loi précités ainsi que l'article L1121-10 du code de la santé publique et à la charge du promoteur, tant pour sa responsabilité que pour celle des intervenants au titre de la recherche impliquant la personne humaine.

La garantie prévue au contrat restera acquise à l'Assuré en cas de modification affectant la date de prise d'effet de la recherche.

La présente attestation est valable pour la durée de la recherche assurée et sa présentation vaut présomption de garantie à la charge de l'Assureur. Elle est délivrée, sous réserve du paiement de la prime, pour servir et valoir ce que de droit et ne peut en aucun cas engager l'Assureur au-delà des clauses et conditions du contrat auquel elle se réfère.

Fait à Paris, le 17 juin 2021
Pour l'Assureur

HDI Global SE
RCS Nanterre 478 913 882
TOUR OPUS 12 - LA DEFENSE 9
77, Esplanade du Général de Gaulle
F 92914 PARIS LA DEFENSE CEDEX
Tél. : +33 1 44 05 56 00 – Fax : +33 1 44 05 56 66

Appendix E: Primary Notification Form

Date of the report: ……
Type of report: initial/Follow up
Recorded by (name and position) : …….
Reference number:
Information on reporter:

Contact name :				
Health professional :		YES		NO
Address :				
Country :				
Phone :				
Fax :				
Email :				

Support : Mail / Phone / Fax / Email / Other
(1) Delete as appropriate

Product concerned by notification :

Product Name :	
Country :	
Batch number :	
Expiry date :	
Classification :	
Quantity concerned :	

Consumer information :
Age :
Gender :
Remedial action :
Outcome :

Detailed description the incident :

Date of the incident occurred:	
Seriousness criteria : Incident description narrative:	
Usage of the medical Device/frequency of use :	

INVESTIGATION

- Date : ……..
- Manager : ……

Elements to control during investigation :	
Risks identification :	
Recurrence study /	

Impact on other batches ?	

Field Safety Corrective Action (FSCA), preventive actions, corrections, and corrective actions

FSCA

- Date : ……..
- Manager : ……

Nature of the FSCA :	
Control of the FSCA implemantation :	
Control of the FSCA effectiveness:	

Preventive actions

- Date : ……..
- Manager : ……

Nature of preventives actions :	

Control of the preventive actions' implementation :	
Control of the preventives actions' effectiveness :	

Corrective actions

- Date : ……..
- Manager : ……

Nature of corrective actions :	
Control of the corrective actions' execution :	
Control of the corrective actions' implementation :	

Corrections :

- Date : ……..
- Manager : ……

Nature of corrections :	

Closure

- Date of investigation conclusion : ……..
- Manager : ……
- Signature :

Appendix F: SAE form

Serious Adverse Event (SAE) notification form **Post market clinical investigation with invasive/burdensome additional procedure(s) (art 74 MDR)**	Box reserved for sponsor, internal reference.

This form is to be used when a causal relationship between the serious adverse event and the additional investigational procedure is suspected (art 80.5 and 80.6).

This form is to be completed immediately and no later than 3 days after the investigation site study personnel have been made aware of the event and sent immediately by e-mail to the vigilance manager of the sponsor (e-mail: officelp@lallemand.com) and to the CRO (e-mail: etudes@bgclinicals.com)

Study title:	**Assessment of sino-nasal microbial communities changes in adult patients with Chronic Rhinosinusitis by 16S rRNA gene amplicon sequencing before and after 1-month treatment duration with Healsea® Chronic: an exploratory study.**
Study number:	**LPH-2102**

Report

1. Type of report: ☐ Initial report ☐ Follow-up report ☐ Final report

2. Date of report

3. Date of initial notification to sponsor

Investigator site

4. Investigator's name:

5. Investigator's address:

6. Phone number:

7. Fax number:

8. Email:

Subject

9. Patient ID:

10. Age:

11. Gender:

12. Consent signature date:

13. Relevant medical history:

Serious Adverse Event

14. Date of occurrence:

15. Place (e.g., hospital):

16. Event description:

17. Relation with invasive/burdensome procedures:

☐ 1. Not related ☐ 2. Possible ☐ 3. Probable ☐ 4. Causal relationship

18. SAE: 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. Hospitalisation or prolongation of patient hospitalisation

☐ 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 congenital physical or mental or birth defect

19. Action/treatment/outcome:

20. Investigational arm:

☐ Test group

☐ Comparison group

☐ Blinded

☐ Not applicable

21. Event status:

☐ Resolved

☐ Resolved with sequelae

☐ Ongoing

☐ Death

22. Date and time of resolution (if applicable):

23. Date and time of death:

24. Had subject been excluded from the study because of the event? ☐ Yes ☐ No

Contact and Signatures

Please give any necessary contact(s) if complementary information should be needed:

25. Person to contact:	
26. Phone number:	
27. Email address:	

....

Signature (person who completed the form) Name (capital letter), First Name

Date: ..... (JJ/MMM/YYYY)

....

Signature of investigator if he/she does not complete the form Name (capital letter), First Name

Date: ..... (JJ/MMM/YYYY)