Clinical Trial Protocol

NCT04448847

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CLINICAL TRIAL PROTOCOL

Discovering the mechanisms of action for the *in-vivo* protection of Aspirin-induced enteropathy by *Bifidobacterium breve* Bif195 in man – a randomised, double-blinded, placebo-controlled, cross-over trial in healthy volunteers

Trial Acronym:
PIP-M

Trial ID number:
HND-GI-036

Version:
2.0 Final

Date:
18-Februar-2020

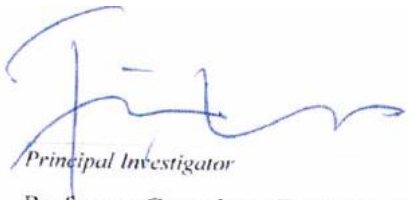
Sponsor:
Chr. Hansen A/S
Bøge Allé 10-12
DK-2970 Hørsholm

Principal Investigator:
Professor, MD Filip Knop

Signatures and Agreement with Protocol

We, the undersigned, acknowledge that we have read this protocol.

We hereby agree to conduct this trial in accordance with the trial protocol, the current version of the Declaration of Helsinki, the International Conference on Harmonisation E6 Good Clinical Practice (ICH-GCP) and with any additional local laws and regulations.



18-Feb-2020

Principal Investigator — *Date*

Professor, Consultant Endocrinologist and Head of Center for Clinical Metabolic Research

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Center for Clinical Metabolic Research, Gentofte Hospital, University of Copenhagen, Hellerup, Denmark

18. feb. 2020

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Synopsis

Title of Trial
Discovering the mechanisms of action for the *in-vivo* protection of Aspirin-induced enteropathy by *Bifidobacterium breve* Bif195 in man – a randomised, double-blinded, placebo-controlled, cross-over trial in healthy volunteers

Protocol Code
HND-GI-036

Sponsor
Chr. Hansen A/S, Bøge Alle 10-12, DK-2970 Hørsholm

Principal Investigator
Dr Filip K. Knop

Clinical Research site
Center for Clinical Metabolic Research at Gentofte Hospital, University of Copenhagen, Gentofte Hospitalsvej 7, 3. Sal, 2900 Hellerup, Denmark

Expected Trial Period
First subject, first visit: April 2020
Last subject, last visit: April 2021

Objective
To map the molecular mechanisms of action responsible for the *in-vivo* protection of Aspirin-induced enteropathy by the bacterial strain *Bifidobacterium breve* Bif195 in humans.

Methodology
This trial is a single-site, randomised, double-blinded, placebo-controlled, two-armed, cross-over trial in healthy volunteers.

The trial will investigate effects of daily intake of the bacterial strain Bif195 or placebo when co-administered to once-daily oral intake of 300 mg of Acetylsalicylic Acid (ASA).
The trial includes a run-in period of two weeks duration followed by a 4-week intervention period in which Bif195/placebo and ASA are co-administered. This period is followed by a 6-week wash-out period before a new 4-week period is performed with a cross-over Bif195/placebo intervention as well as ASA co-administration. Bif195 and placebo

interventions are performed double-blinded in randomised order in a cross-over fashion for each subject.
Subjects will participate in the trial for a total duration of approximately 17 weeks including the run-in phase. Besides the screening visit, the trial will consist of 4 visits.
After having given their written informed consent, subjects will complete the screening procedures to evaluate their eligibility for participation in the trial and complete a run-in period of two weeks duration to washout possible pre-trial probiotics and/or use of medication. On the morning of day 4 after baseline assessments at Visit 2, all subjects will start daily intake of 300 mg ASA in combination with Bif195 or placebo in a ratio of 1:1 according to the randomisation performed at Visit 2.

At visit 2 – 5, all subjects will be biopsied from the upper small intestine and the ventricle during a gastroscopy procedure. At each of these 4 visits, 6 biopsies will be taken from pre-specified locations in the duodenum and 2 biopsies will be taken from the ventricle (approximately 5 mg each). Luminal fluids will also be collected during the gastroscopy (approximately 2 ml per visit). One venous blood sample (of maximum 25 ml per visit) will also be collected at each of these visits.

The analysis on biopsies and luminal fluid samples will include a combination of transcriptomic, microbiome, proteomics and metabolomics analysis.

Planned Number of Subjects
We aim for 20 subjects to complete the trial, and assuming a drop-out rate around 20%, we aim to randomise 25 subjects. Subjects that drop out or are withdrawn from the study can be replaced. We expect for up to 50 subjects to be screened for participation.

Main Criteria for Inclusion

- Written informed consent
- Healthy and without any gastrointestinal discomfort/pain or other significant symptoms
- Age ≥ 18 and ≤ 40 years
- Willing to abstain from any other probiotic products and/or medication known to alter gastrointestinal function throughout the participation of the trial

ASA challenge product
The ASA product used during the ASA challenge periods will be the 300 mg Aspirin oral tablet from Alliance Pharmaceuticals, Ireland. Subjects will consume 300 mg ASA daily together with the trial product/placebo and breakfast.

Test Product
The investigational product is a vegetable capsule containing *Bifidobacterium breve* DSM 32356. Subjects will consume 1 capsule together with ASA and breakfast and 1 capsule in the evening, equivalent to a dose of minimum $100*10^9$ CFU daily. All product will be produced at the trial sponsor Chr. Hansen laboratories in Hørsholm, Denmark, which are certified for food production.

Reference Product (placebo)
The reference product (placebo) is the same vegetable capsule, identical in composition, taste and appearance, but without bacteria. Subjects will consume 1 capsule daily with ASA and breakfast and 1 capsule in the evening. Placebo capsules will be produced at Chr. Hansen laboratory in Hørsholm, Denmark, which is certified for food production.

Duration of ASA challenge: **Two times 4 weeks**
Duration of trial product/placebo intervention: **Two times 4 weeks**

Criteria for Evaluation

Endpoints, all exploratory:

- The effects of daily intake of Bif195 versus placebo on transcriptomics measured in ventricle and upper small intestinal mucosal biopsies obtained before and after a 4-week ASA challenge

- The effects of daily intake of Bif195 versus placebo on microbiota composition profile measured in ventricle and upper small intestinal mucosal biopsies obtained before and after a 4-week ASA challenge

- The effects of daily intake of Bif195 versus placebo on proteomics profile measured in ventricle and upper small intestinal mucosal biopsies obtained before and after a 4-week ASA challenge

- The effects of daily intake of Bif195 versus placebo on metabolomics profile measured in small intestinal luminal fluid samples obtained before and after a 4-week ASA challenge

- The effects of Bif195 versus placebo administration on the Lanza score of the ventricle obtained before and after a 4-week ASA challenge

- The effects of Bif195 versus placebo administration on the Lanza score of the duodenum obtained before and after a 4-week ASA challenge

3. Table of Contents

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3.1 Abbreviations and Definition of Terms

AE	Adverse event
ASA	acetylsalicylic acid
AUC	Area-under-the-curve
Bif195	Bifidobacterium breve DSM 32356
BMI	Body mass index
CE	Capsule endoscopy
CFU	colony forming units
CRF	Case report form
CVD	cardiovascular disease
EFSA	European Food Safety Authority
FSSG	Frequency Scale for the Symptoms of Gastroesophageal Reflux Disease
GI	Gastrointestinal
GSRS	Gastrointestinal symptoms rating score
ICH-GCP	International Conference on Harmonisation E6 Good Clinical Practice
IEC	Independent Ethics Committee
n	Sample size
NSAID	Non Steroidal Anti-Inflammatory Drugs
QPS	Qualified Presumption of Safety
SAE	Serious adverse event
VCE	Video Capsule Endoscopy

4. Ethics and Regulations

4.1 Independent Ethics Committee

The relevant Independent Ethics Committee (IEC) will be consulted about this trial and the trial will be initiated only after a favourable opinion has been obtained from IEC.

A report summarising the results of the trial will be sent to the IEC after the conduct of the trial, if applicable according to the local regulations.

4.2 Ethical Conduct of the Trial

The trial will be conducted in accordance with the ethical principles set forth in the current version of the Declaration of Helsinki, the International Conference on Harmonisation E6 Good Clinical Practice (ICH-GCP) and all applicable local regulatory requirements.

4.3 Subject Information and Informed Consent

The study will recruit the population of healthy volunteers from online advertisement (e.g. www.forsøgsperson.dk and www.facebook.com) and via posters. Potential subjects will be asked to make the initial contact to the study team via phone or email, based on the information given in the advertisement material.

The investigator or delegated trial staff will introduce each subject to the objectives, nature, significance, risks and implications of the trial over a phone call. This information will then be repeated in the written subject information sheet which is sent to the subject along with the brochure "*Forsøgspersoners rettigheder i et sundhedsvidenskabeligt forskningsprojekt*".

The Investigator or delegated staff will ensure that the study participant is adequately informed about the study and implications for participating. In particular, the subjects will be informed about the following:

- The possibility to withdraw their consent to participate in the study at any time and without having to give an explanation
- How personal and health-related data will be collected and used during the trial

The participant will then be invited for a pre-screening visit at the clinical site and given the opportunity to ask questions to the Investigator or delegated staff. The participant is encouraged to bring along a friend or family member to the visit and will be allowed time to consider his/her decision to participate subsequently. Should the participant need further time, a follow-up meeting will be scheduled.

If the participant wishes to take part in the study an informed consent form will be signed by both the participant and the Investigator or delegated staff at the visit. No study-related examinations will be conducted before the informed consent form has been signed but may be initiated on the same day, if the participant agrees to.

All subjects will receive a copy of the subject information sheet and the signed informed consent form. The original signed informed consent form will be retained by the Investigator.

4.4 Protocol Changes

Substantial amendments to this protocol may be implemented only after a favourable opinion of the IEC has been obtained. Amendments to the protocol are regarded as substantial if they have a significant impact on

- The safety, physical health and mental integrity of the trial subjects
- The scientific value of the trial
- The conduct or the management of the trial
- The quality or safety of the investigational product used in the trial

Any amendments to this protocol will be signed by the signatories included in section 1.

If an event occurs related to the conduct of the trial or the development of the test product which may affect the safety of the trial subjects, the Sponsor and the trial Investigator will take appropriate measures to protect the subjects against immediate hazards. The Sponsor or the Sponsor's representative will inform the applicable IEC of the new events and the measures taken as soon as possible.

4.5 Protocol Deviations

No systematic deviations from the protocol are allowed, and no protocol waivers will be given. All protocol deviations noted during the trial will be recorded and evaluated as major or minor by the Sponsor before the blinding is broken.

4.6 Insurance

All subjects will be covered by a general insurance against injury caused by their participation in the trial according to local legal requirements in the country where the trial takes place.

4.7 Subject compensation

Each subject will receive a compensation for participating in the trial of 10.000 DKK. The compensation will be evenly divided in four parts corresponding to completion of visits 2, 3, 4, and 5 (2.500 DKK for each visit). If a subject withdraws consent prior to completing the entire trial, the subject will receive compensation corresponding to the actual visits completed.

Travel expenses related to all visits at the clinical site will be reimbursed. Additionally, the subjects will be offered a free meal and a taxi to their home when the gastroscopy procedure is completed on visit days 2-5.

5. Introduction

5.1 Background

Non-steroidal anti-inflammatory drugs (NSAIDs) are used worldwide both as prescription and over-the-counter products for their analgesic, anti-inflammatory and cardiovascular-protective properties, and are among the most used pharmaceuticals today[1]. Chronic, low-dose use (commonly defined as 75-325 mg daily) of the NSAID acetylsalicylic acid (ASA) is widely recommended to reduce the risk for cardiovascular disease (CVD). More than 35 million of the US population aged above 40 are estimated to be on chronic, daily, low-dose ASA for primary CVD prevention alone[2]. However, use of NSAIDs, such as ASA, is associated with gastrointestinal (GI) injury, and the degree of damage depends on dose, duration of treatment, concomitant medication and patient risk profile. Accordingly, ASA has emerged as one of the most prominent causes of peptic ulcer bleeding in developed countries over the last two decades, and is associated with a 2 to 4-fold increased risk of upper GI bleeding and ulcers[3,4]. Guidance from the American College of Cardiology Foundation Task Force on Clinical Expert Consensus Documents state that low dose ASA use is associated with a 2 to 4-fold increase in GI events, increasing with concomitant medication use[5,6]. Generally, patients taking NSAIDs experience a relative risk of upper GI bleeding and perforations of up to 4.7 compared with non-users[7].
Strategies to prevent GI complications associated with ASA use include proton pump inhibitors and synthetic prostaglandins. However, drugs are generally associated with undesired side effects whereas live bacteria formulated as probiotics may offer a safe alternative to reduce the risk of negative side effects of ASA. Probiotic bacteria have been demonstrated to have possible beneficial effects against intestinal inflammation[8]. *Lactobacillus casei (L. casei*) has been shown to prevent the development of experimental colitis. Furthermore, *L. casei* has been demonstrated to exhibit a preventive effect on indomethacin-induced small bowel injury in an animal experiment. In a clinical trial, twenty-five patients, including 13 in the L. casei group and 12 in the control group, underwent full analysis of the small intestine (CE score) using wireless video capsule endoscopy[9]. Significant decreases in the number of mucosal breaks and the CE score were observed at the 3-month evaluation in the L. casei group as compared with the results in the control group.

More recently Suzuki et al. reported a trial in which 64 users of ASA who had received ASA for more than 1 month were enrolled. The patients received a yogurt containing *L. gasseri* or placebo twice daily for 6 weeks. Small bowel injuries were evaluated by video capsule endoscopy (VCE) before and after consuming the yogurt. The effect of *L. gasseri* on patient symptoms was also assessed using the Frequency Scale for the Symptoms of Gastroesophageal Reflux Disease (FSSG) and Gastrointestinal Symptom Rating Scale (GSRS) questionnaires before and after 6 weeks of treatment. In contrast with the placebo group, the *L. gasseri* group had significantly fewer small bowel mucosal breaks and reddened

lesions after 6 weeks. The FSSG and GSRS scores were also significantly improved in the *L. gasseri* group but not in the placebo group[10].

Based on the above literature and an in-house preclinical screening program, we recently conducted a clinical trial testing whether oral co-administration with a 50 billion colony forming units (CFU) daily dose of Bif195 could reduce the risk of ASA-induced intestinal ulceration in humans in a randomised, placebo-controlled, double-blinded trial using serial VCE as our main efficacy parameter[11] (ClinicalTrials.gov ID number NCT03228589). This trial indeed showed significant protection against ASA-induced enteropathy in healthy volunteers.

The main aim of the current clinical trial is to take a wide, exploratory approach to map the molecular mechanisms of action responsible for the *in-vivo* protection of ASA-induced enteropathy by Bif195 in humans. This trial will, together with other clinical data, form the basis of bringing a dietary supplement product to market that can protect against small-intestinal ulcerations induced by ASA.

5.2 Assessment of Anticipated Benefits and Risks

The ASA intervention planned for this trial is designed (in length and daily dose) to create only a mild degree of deterioration of small intestinal mucosa tissue in healthy volunteers in the form of villous edema and ulcers, as we have observed with similar ASA dosing in a previous trial[11]. This may create a mild degree of GI discomfort and symptoms for subjects during the trial. However, this is quickly reversed upon cessation of daily ASA use and the daily dose of ASA of 300 mg used in this trial is within the dose range recommended for chronic use of ASA for CVD protective reasons.

There are no known risks with administration of probiotics unless the trial population consists of severely ill or immunocompromised patients. In Europe, certain species of *Bifidobacteria* have been granted Qualified Presumption of Safety (QPS) status by the European Food Safety Authority (EFSA), including the *Bifidobacterium breve* to which the strain Bif195 belongs. This means that Bif195 is considered safe to use in food and as a dietary supplement (EFSA, 2013). Therefore, there are no known or expected risks or side effects for either the test or the placebo products used in this trial, but the risk of side effects cannot be excluded. Side effects, if any, are however expected to be minor and transient in nature. Side effects could typically be symptoms of digestive discomfort (abdominal discomfort, bloating, flatulence/passage of gas, borborygmi/rumbling stomach). With more than 4600 days of human exposure to the Bif195 strain in their clinical program, the sponsor Chr Hansen has not observed any adverse events assumed related to Bif195 intake.

A gastroscopy is a very safe and common procedure. Rare complications include bleeding, infections, perforation and reaction to sedation.

There are no direct benefits to the subjects for participation in this trial. The subjects, while being in the active arm with Bif195 co-administration might experience a reversal or dampening of the very mild GI symptoms induced by the ASA intervention described above.

Chronic, low-dose use (commonly defined as 75–325 mg daily) of ASA is widely recommended for both primary and secondary prevention of CVD. More than 30% of the US population aged above 40 years are estimated to be taking chronic, daily, low-dose ASA for that reason alone. However, chronic use of ASA is also associated with adverse effects, including small-intestinal mucosal lesions and ulcers, perforations, major hemorrhage, and, in rare instances, death. A recent review and meta-analysis addressing both the efficacy of ASA in prevention of CVD and also bleeding-related adverse effects concluded that a balanced, cautious approach should be taken in the case of primary CVD prevention because of these adverse effects, highlighting the unmet need to reduce the risk of adverse effects associated with chronic ASA use. The present trial is part of a larger clinical program by the sponsor aiming at bringing a dietary solution to the marked that can help chronic ASA users with these side-effects. As such, the investigators and sponsor believe that the potential benefits the trial results will bring outweigh the mild discomfort that the healthy volunteers enrolled in this trial may experience.

5.3 Additional Information

The strain of Bif195 included in this trial have been shown to be susceptible to the 8 antibiotics tested as recommended in the EFSA Guidance for assessment of bacterial susceptibility to antimicrobials of human and veterinary importance[12] (ampicillin, vancomycin, tetracycline, clindamycin, erythromycin, chloramphenicol, streptomycin, gentamicin) (Chr. Hansen, Internal Report). In support of these data, Bif195 has been sequenced and no antibiotic resistance genes was found in the genome.

6. Trial Objectives

6.1 Primary Objective

The objective of this trial is:
To map the molecular mechanisms of action responsible for the *in-vivo* protection of Aspirin-induced enteropathy by Bif195 in humans.

6.2 Trial Design

This trial is a single-site, randomised, double-blinded, placebo-controlled, two-armed, cross-over trial in 25 healthy volunteers.

The trial will investigate effects of daily intake of the *Bifidobacterium breve* Bif195 or placebo when co-administered with daily intake of 300 mg of ASA.
The trial includes a run-in period of two weeks duration followed by a 4-week intervention period where Bif195/placebo and ASA are co-administered. This period is followed by a 6-week wash-out period before a new 4-week period is performed with Bif195/placebo and

ASA co-administration. Bif195 and placebo interventions are performed double-blinded in randomised order for each subject in a cross-over design.
Subjects will participate in the trial for a total duration of approximately 17 weeks including the run-in phase. Besides the screening visit, the trial will consist of 4 visits at site and 4 phone visits.
After having given their written informed consent, subjects will complete the screening procedures to evaluate their eligibility for participation in the trial and complete a run-in period of two weeks duration to washout possible pre-trial probiotics and/or use of medication. Four days after baseline assessments at Visit 2, all subjects will start daily intake of 300 mg ASA and Bif195 or placebo in a ratio of 1:1 according to the randomisation performed at Visit 2.

At visit 2 – 5, all subjects will be biopsied from the upper small intestine and the ventricle during a gastroscopy procedure. At each of these 4 visits, 6 biopsies will be taken from pre-specified locations in the duodenum and 2 biopsies will be taken from the ventricle (approximately 5 mg each). Luminal fluids will also be collected during the gastroscopy (approximately 2 mL per visit) and one blood sample (of maximum 25mL per visit) will be collected. During the entire trial subjects will be instructed to maintain their habitual life style with regards to diet, physical activity level and sleep habits.

The analysis on biopsies will include a combination of transcriptomics analysis, microbiome analysis, proteomics profiling and metabolomics profiling. In addition, a clinical evaluation of the damage to the ventricle and the duodenum will be provided via the Lanza score. Also, a general safety monitoring, blood sampling and subject baseline phenotype will be obtained.

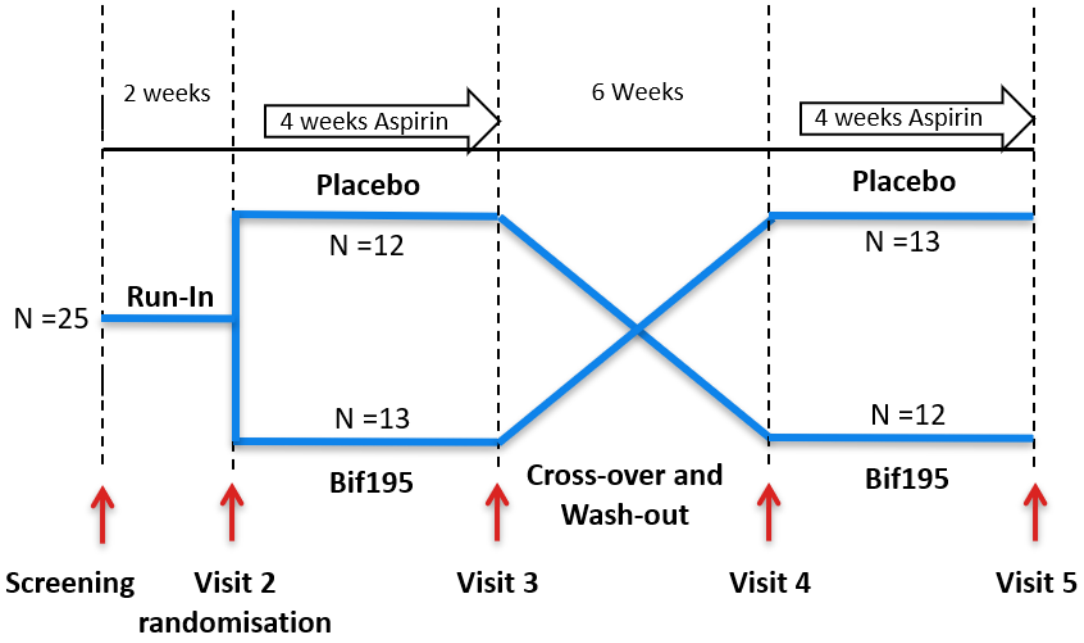

Figure 1 Visit overview

Table 1 Trial flow chart

Visit	**1**	**2**		**3**		**Wash-out**	**4**		**5**	
Phone visit			P1		P2			P3		P4
Day (visit window)	-14 (+5/-14)	0	4	31 (±1)	35 (±1)	6 weeks (+7)	0	4	31(±1)	35 (±1)
Phone call			X		X			X		X
Written informed consent	X									
Check of inclusion/exclusion criteria	X									
Urine pregnancy test (women only)	X									
Medical history	X									
Routine physical examination incl. blood pressure	X									
Demography	X									
Alcohol consumption	X									
Concomitant medication	X	X		X			X		X	
Height	X									
Body weight	X	X		X			X		X	
Randomisation		X								
Overnight fasting before visit		X		X			X		X	
Blood safety panel	X	X		X			X		X	
Other blood sampling		X		X			X		X	
Gastroscopy Incl. biopsies		X		X			X		X	
Hand-out of ASA		X					X			
Hand-out of trial product		X					X			
ASA accountability				X					X	
Trial product accountability				X					X	
Adverse events		X		X			X		X	

6.4 Discussion of trial design

We recently conducted a clinical trial showing that oral co-administration with Bif195 can reduce the risk of low-dose ASA-induced intestinal ulceration in humans using serial VCE as our main efficacy parameter[11] (ClinicalTrials.gov ID number: NCT03228589).

For the present trial, we will re-use this clinical challenge model that we have already established and tested. A mild deterioration of the upper small-intestinal mucosa will be induced by ASA, a chemical agent commonly used and with well-established injurious effects on the small intestine. Based on the dataset from our previous trial, we observed that 300 mg of ASA daily for 4-6 weeks represents a relevant challenge model, both in terms of daily dose and duration, and ability to induce a mild deterioration in the small intestine as observed with VCE.

A run-in period is included to wash out the potential impact of any pre-study probiotic products. The 6 weeks between visit 3 and 4 serve the purpose as wash-out for any Bif195 effects as well as recovery of the mucosal tissue between the ASA challenge periods. A cross-over design was chosen to obtain the statistical benefits of the subjects being their own controls.

6.4.1 Inclusion Criteria

For a subject to be eligible for the trial, all the below criteria must be checked with a "yes":

- Written informed consent
- Healthy and without any gastrointestinal discomfort/pain/symptoms
- Age ≥ 18 and ≤ 40 years at the day of screening
- Willing and able to abstain from any other probiotic products and/or medication known to alter gastrointestinal function throughout the participation of the trial

6.4.2 Exclusion Criteria

For a subject to be eligible for the trial, all the below criteria must be checked with a "no":

- Abdominal surgery which, as judged by the Investigator, might affect the GI function (except appendectomy and cholecystectomy)
- History of peptic ulcer disease
- Known history of *Helicobacter Pylori* infection
- Any known bleeding disorder
- Allergy to ASA
- Resting diastolic blood pressure ≥ 95 mmHg
- Resting systolic blood pressure ≥ 150 mmHg
- A current diagnosis of psychiatric disease

- Systemic use of antibiotics, steroids (except contraceptives) or antimicrobial medication in the last month
- Daily usage of non-steroidal anti-inflammatory drugs in the last 2 months or incidental use in the last 2 weeks prior to screening (ASA, Ibuprofen, Diclofenac, Naproxen, Celecoxib, Mefenamic acid, Etoricoxib, Indometacin)
- Usage of medications, except contraceptives, in the last 2 weeks prior to screening
- Regular use of probiotics in the last month
- Use of laxatives, anti-diarrheals, anti-cholinergics and proton pump inhibitors within last 2 months prior to screening
- Use of immunosuppressant drugs within last 4 weeks prior to screening
- Diagnosed inflammatory gastrointestinal disease and/or irritable bowel syndrome
- Any other disease that, by the Investigators discretion, could interfere with the intestinal barrier function of the subject
- Participation in other clinical trials in the past 2 months prior to screening
- Smoking and/or frequent use of other nicotine products
- Higher alcohol intake than what the board of Health recommends
- Desire and/or plans to change current diet and/or exercise regime during the participation of this trial
- For Women: Pregnancy or lactation

6.4.3 Subject Withdrawal Criteria

Participation in the trial is voluntary, and subjects have the right to withdraw from the trial at any time without providing a reason, and with no loss of benefits to which the subject is otherwise entitled. If a trial subject chooses to withdraw, the trial personnel must be informed immediately, and every effort should be made to complete the end-of-trial assessments and document the reason for discontinuation in the case report form (CRF). The Sponsor should be notified as soon as possible about any discontinued subjects.
Any subject who prematurely terminates participation and who has consumed the test or reference product at least once will be asked to undergo a final examination (end-of-trial visit/early termination visit). For subjects considered lost to follow-up, the CRF will be completed up to the last visit.
The Investigator has the right to terminate participation of any subject at any time if they deem it in the subject's best interest.
Examples of possible reasons for premature withdrawal of a trial subject include, but are not limited to:

- Subject withdraws consent for personal reasons
- Subject’s general condition and/or data from the visit 2 gastroscopy procedure contraindicates continuing the trial, as judged by the trial personnel and/or the principal Investigator

- Participation in other clinical trials during participation of this trial
- Significant non-compliance with trial protocol or general lack of cooperation
- Pregnancy
- A serious adverse event (SAE)
- Lost to follow-up
- Any other reason as determined by the principal Investigator

6.5 Investigational Products

6.5.1 Identification and Description of Investigational Product

Bifidobacterium is a genus of lactic acid- and acetic acid-producing, gram-positive, non-spore forming, non-motile, anaerobic bacteria. They are common constitutes of the microbiota in the human intestinal tract.
The investigational product is a vegetable capsule containing *Bifidobacterium breve* DSM 32356, name simplified to Bif195 throughout this document.
The reference product (placebo) is the same capsule, but without Bif195. All products will be produced at Chr. Hansen laboratory in Hørsholm, Denmark, which is certified for food production.
The CFU stability of the production batch of *Bifidobacterium breve* DSM 32356 that will be used in this trial will be monitored closely during the trial. The internal analyses at Chr. Hansen A/S indicate that the specific product stability loss is approximately 25% during the product shelf life, and we expect that the CFU per capsule will be minimum $50*10^9$ CFU per capsule at the time of trial intervention. The CFU stability of the investigational product batch will be analysed in parallel with performing this trial and these data will be available and included in the final clinical trial report.

Table 2 Composition of investigational product

	Placebo capsules	**Probiotic capsules Bifidobacterium breve**
Manufacturing	Chr. Hansen A/S, Denmark	Chr. Hansen A/S, Denmark
Brief description	Capsules with excipients only	Capsules containing *Bifidobacterium breve* DSM 32356 and excipients
Capsules	Size 1 HPMC capsules	Size 1 HPMC capsules
Capsules shell	73.6 mg Hypmellose 1.4 mg Titanium dioxide	73.6 mg Hypmellose 1.4 mg Titanium dioxide
Active Ingredients	None	*Bifidobacterium breve* DSM 32356
Excipients	Microcrystalline Cellulose 6 mg per capsule Magnesium Stearate 1.5 mg per capsule Maltodextrin 277.8 mg per capsule Sodium Ascorbate 14.7 mg per capsule	
Supplied as	CSP Activ-Vials containing 24 capsules in each vial	
Storage conditions	Store at +2-8 ºC	

6.5.2 Labelling

The trial products will be labelled according to the standard procedures of the clinical site in a manner so that every person with an active role in the conduct of the trial will remain blinded. The labels on the trial products will as minimum bear the following information:

Master label text
Trial code: HND-GI-036 Sponsor: Chr. Hansen A/S Principal Investigator: Dr. Filip Knop Capsule with or without probiotic 1 capsule with breakfast 1 capsule in the evening Randomisation number: XXXX Expiry date: April, 2021 To be stored at +2-8 ºC For clinical trial use only

6.5.3 ASA challenge product

The ASA product used during the ASA challenge periods will be the 300 mg Aspirin from Alliance Pharmaceuticals Ireland.

6.5.4 Blinding

Both active (Bif195) and reference (placebo) product will be similar in smell, taste and appearance. All trial products will be packaged in identical packs with identical labelling, except for the randomisation number.
Trial subjects, the clinical team and the sponsor will all be blinded during the entire trial until database lock is performed. Only one or two appointed Trial Supply Coordinators, not otherwise involved in the conduct of the trial, will be un-blinded as necessary to perform labelling of the product.
An emergency unblinding procedure will be established to allow the principal Investigator the option of disclosing the product assignment for an individual subject if clinical circumstances require such an unblinding. Unblinding will only be performed if medical decisions should be taken which mandate knowledge about the treatment received. The Sponsor will be informed immediately and before unblinding in case a subject will be unblinded by the Investigator. In the event, that emergency disclosure of product assignment is required, the subject will be withdrawn from further participation in the trial.

6.5.5 Method of Assigning Subjects to Treatment Groups

Prior to the start of the trial, the allocation of subject numbers to trial groups will be performed by randomisation lists organised by an un-blinded partner not otherwise involved in the trial conduct. .
Particularities of the randomisation – such as variable or fixed block length, or no block length at all – will be stated in the randomisation list itself. Neither the investigator site nor the sponsor will receive any information about randomisation blocks.
At screening, subjects will be assigned a screening number starting at 1, according to their chronological entry into the trial. As an example, the first subject will have screening number 1.
If the subjects are suitable for trial participation, they will receive their 4-digit randomisation number at Visit 2, starting with number 1001.
Access to the randomisation list is limited to the unblinded partner that will generate the list and to the appointed Trial Supply Coordinators, not otherwise involved in the conduct of the trial, who will label the products according to the randomisation list.

6.5.6 Selection of Dosages

The active test product will contain approximately $100*10^9$ CFU of Bif195 per day in the active treatment part of the trial. In a previous trial run by Chr Hansen A/S with a 6-week ASA challenge regime, a daily dose of $50*10^9$ CFU of Bif195 per day was used.

6.5.7 Administration of Trial Treatment for Each Subject

The subjects will consume one tablet of 300 mg ASA daily for 4 weeks, 28 days, in each trial period together with breakfast. If a subject forgets to consume a dose of ASA, it can be taken later on the same day, with or without a meal, but an additional dose should not be taken on the following day to compensate for a missing dose on the day before.

The subjects will consume 2 capsules of trial product (Bif195/active or placebo) daily during the 4 weeks, 28 days, treatment period. One of the capsules should be taken with the subject's breakfast, but preferably 10 minutes before intake of the ASA. The other capsule should be taken in the evening, with or without a meal.

In order to allow 3 days of healing of the mucosal wall after biopsies, the first administration of ASA and trial product will take place at breakfast on the 4^{th} day after Visit 2 and again on the 4^{th} day after visit 4.

6.5.8 Compliance

Subjects will return all unused ASA and trial product at Visits 3 and 5 for accountability as dictated in the flow chart. Subjects consuming ≥ 80% of expected ASA tablets between Visit

2 and Visit 5 and ≥ 80% of trial product between Visit 2 and Visit 5 will be considered compliant with this protocol.

6.5.9 Accountability and Disposal

The site will keep proper accountability records at a subject level and at a site level.
Subjects must return unused vials and capsules/tablets to the site for accountability.
The trial personnel will keep accurate records of the trial products received from the Sponsor, stored, dispensed to and returned from subjects, and destroyed using appropriate forms. The trial products will be stored at the site at +2-8ºC in a place with restricted access.
If the Sponsor decides to, all trial product that is returned by subjects will be destructed at the trial site after written approval from the Sponsor, and destruction documented accordingly.

6.5.10 Prior and Concomitant Therapy

Any concomitant medication should be avoided during the entire duration of the trial, except contraceptives. All rescue medications taken during the trial period as well as dietary and herbal supplements will be registered on the appropriate page in the CRF. Use of concomitant medication and supplements during the last year and use of rescue medication from screening visit until end of trial should be documented.
The last 2 months prior to screening, or incidental use in the last 2 weeks prior to screening , and until final examination (Visit 5); all medications (including over-the-counter products and large doses of vitamins and/or mineral) that might have an effect on gut microbiota, gut regularity or gut symptoms, in particular NSAID (e.g. ibuprofen, ASA), laxative and intake of probiotics products are prohibited.
Use of systemic immunosuppressant drugs is not allowed for 4 weeks prior to the screening visit.
Antibiotics and steroids (except contraceptives) are prohibited from 2 months prior to the screening visit until the end of the trial. Subjects taking antibiotics during the trial will be discontinued from the trial.

6.5.11 Other Restrictions and Instructions for Trial Subjects

The subjects will be instructed to avoid changes in their general life style, such as diet, exercise and sleep habits, during the trial.
Intake of probiotic products as well as food and dietary supplements containing probiotics are not allowed from the screening visit and until the end of the final intervention period. Subjects will not be withdrawn from the trial due to single violations, but violations will be recorded as protocol deviations.
Any use of illicit drugs (euphorics or stimulants, such as cannabis and opium) is prohibited during the trial.
During the trial, subjects will be asked to:

- Refrain from consuming any food products that may contain live microbes (i.e. fermented milk products)
- Refrain from taking any non-steroidal anti-inflammatory drugs (NSAIDs)
- Refrain from participating in other clinical trials

For the two days prior to Visit 2, 3, 4 and 5, subjects will also be asked to:

- Refrain from alcohol and any nicotine products
- Refrain from consuming spicy food
- Refrain from consuming caffeine
- Avoid strenuous exercise

Subjects will also be asked to fast for 6 hours before attending Visit 2,3,4 and 5.

6.6 Assessments and Schedule of Measurements

6.6.1 Gastroscopy procedure incl. clinical scoring

A gastroscopy is a procedure where a thin, flexible tube called an endoscope is used to look inside the oesophagus, stomach and first part of the small intestine (duodenum). The gastroscopy procedure is expected to take less than 30 minutes including clinical scoring, sampling of biopsies and collecting luminal fluid.

During the procedure, a clinical evaluation of both the ventricle and the duodenum will be performed using the 5-point Lanza scale described previously[13]. In short, the gastroenterologist will enumerate mucosal hemorrhages, erosions, and ulcers for the ventricle and for the duodenum separately using the following 5-point Lanza scale:
0=normal stomach or duodenum
1=mucosal hemorrhages only
2=1 or 2 erosions observed
3=numerous (3–10) areas of erosion
4=large number of erosions (>10) and/or ulcer(s).

Erosion will be defined as a lesion producing a definite discontinuance in the mucosa but having no depth. Ulcer will be defined as any lesion of unequivocal depth at least 3 mm in diameter. The same gastroenterologist will perform all gastroscopies and perform all the clinical scoring, while remaining blinded for treatment arm.

6.6.2 Assessment of Safety

In Europe, strains of Bifidobacterium belonging to the specie *breve* have been granted QPS status by EFSA. This means that the strain used in this trial is considered safe to use in food and as a dietary supplement (EFSA, 2013). Bif195 has also been used in 3 previous (plus one ongoing) Chr Hansen-sponsored human clinical trials without any Adverse events (AEs) associated to trial product being reported.

The ASA challenge period of 4 weeks and the ASA dose of 300 mg can lead to some degree of discomfort for the subjects, but this is within the dose range clinically recommended and considered safe for chronic, daily use of ASA.

Subjects can always withdraw from the trial. Symptoms arising from ASA use is usually normalised within a few days. Subjects will also have access to call a medical doctor dedicated to this trial at any time, also in between trial visits. Also, the below safety assessments will be applicable for this trial:

Adverse Events
AEs will be recorded from Visit 2 until the end of the trial at Visit 5. Subjects will be asked during all visits to site to report any AEs experienced throughout the trial. AEs will be recorded and assessed as described in detail in protocol Section 7.10.

Pregnancy test
Women of childbearing potential will have a urinary pregnancy test performed using instant urine stick test at the screening visit (Visit 1).

Medical history
Relevant medical history such as chronic conditions and diseases or major surgery will be recorded at Visit 1. Smoking history and habits will also be recorded.

Physical examination
A short physical examination including auscultation of heart, lungs and blood pressure will be performed by a medical doctor at Visit 1.

Concomitant medication
Use of concomitant medication one year prior to the screening visit as well as herbal and dietary supplements will be registered on the appropriate page in the CRF until the end of the trial. Trial personnel will ask subjects at all trial visits about medication used.

Phone visits

On the fourth day after each gastroscopy (Visit 2,3,4 and 5), a phone visit will be conducted where trial staff will contact the subject and ensure the well-being of the subjects and at the same time remind the subject to start intake of ASA and trial product (the latter for phone visit 1 and 3 only).

Blood sampling

A non-fasting blood sample will be collected from all participants at all 5 visits for safety reasons and in order to detect any unknown condition that may compromise a participant's eligibility for the study. The parameters include creatinine, potassium, sodium, haemoglobin, haematocrit, red blood cells, HbA1c, alanine aminotransferase, aspartate transaminase, INR, leucocytes, thrombocytes and CRP.

6.6.3 Other Assessments

Baseline demographics
At the screening visit, information will be collected for date of birth, gender, ethnicity, height and weight and average weekly alcohol consumption.

6.6.4 Description of Visits and Trial Periods

Visit 1, Screening Visit
At visit 1 the following procedures and assessments will be performed:

- Subjects will receive oral and written information about the trial and be allowed to ask any questions they may have
- Subjects will sign the informed consent document
- Inclusion and exclusion criteria will be checked
- Relevant medical history will be recorded
- Concomitant medication will be recorded
- Demographic data will be recorded
- A physical examination including auscultation of heart, blood pressure and lungs will be performed by a medical doctor and safety blood samples will be collected.
- A urine pregnancy test will be performed in all women of child-bearing potential.
- Height and weight will be recorded, and BMI calculated as kg/m^2. Weight will be recorded with the subject wearing only light clothing and without shoes
- Subjects will receive instruction on exclusion of specified probiotic products as well as food and dietary supplements containing probiotics for the duration of the run-in and intervention period and receive a list with prohibited products
- Subjects will be instructed not to change their habitual diet and life style (physical activity level, sleep habits) during the entire course of the trial
- Subjects will be instructed to fast for 6 hours before visit 2

Run-in Period
During the run-in period (2 weeks) and in between all visits, subjects will:

- Exclude probiotic products as well as food and dietary supplements containing probiotics from their diet
- Maintain their habitual diet and life style (physical activity level, sleep habits)
- Refrain from taking any NSAIDs or other medication

Before Visit 2-5, subjects will:

- Refrain from consuming alcohol, caffeine and spicy food for 48 hours prior to visit
- Avoid strenuous exercise 48 hours prior to visit
- Fast for 6 hours before attending the visit

Visit 2-5:

- Any adverse events will be recorded
- Concomitant medication will be recorded
- Body weight will be measured with subject in light clothing and without shoes

- Collection of blood samples will be performed
- Gastroscopy procedure including clinical scoring, mucosal biopsies and obtaining luminal fluid will be performed as previously described
- Subjects will be re-instructed on exclusion of prohibited food products and supplements that apply in the period leading up to the next visit
- The subject will be randomised (Visit 2 only)
- Dispensing of ASA and trial product (Visit 2 only)
- ASA and trial product accountability will be performed as described in the trial flow chart
- Subjects will receive date and time for the next visit (Visit 2-4 only)
- The termination form in the CRF will be completed (Visit 5 only)

6.7 Endpoints

Endpoints, all exploratory:

- The effects of daily intake of Bif195 versus placebo on transcriptomics measured in ventricle and upper small intestinal mucosal biopsies obtained before and after a 4-week ASA challenge

- The effects of daily intake of Bif195 versus placebo on microbiota composition profile measured in ventricle and upper small intestinal mucosal biopsies obtained before and after a 4-week ASA challenge

- The effects of daily intake of Bif195 versus placebo on proteomics profile measured in ventricle and upper small intestinal mucosal biopsies obtained before and after a 4-week ASA challenge

- The effects of daily intake of Bif195 versus placebo on metabolomics profile measured in small intestinal luminal fluid samples obtained before and after a 4-week ASA challenge

- The effects of Bif195 versus placebo administration on the Lanza score of the ventricle obtained before and after a 4-week ASA challenge

- The effects of Bif195 versus placebo administration on the Lanza score of the duodenum obtained before and after a 4-week ASA challenge

6.8 Laboratory Analyses

6.8.1 Laboratory

All laboratory analyses will be performed at selected laboratories with respect to specific analysis. The safety blood panel is analysed in-house on the day of collection while the remaining samples will be prepared for storage by the clinical site and shipped to the appointed laboratory for analysis. The laboratory manual for the trial will provide all details on sample preparation requirements, storage requirements etc.

6.8.2 Sample Handling

Blood samples will be collected at all 5 visits. At each visit, a maximum of 25 mL whole blood will be drawn.

During the gastroscopy procedure, 6 biopsies will be taken from duodenum and 2 biopsies will be taken from the ventricle (approximately 5 mg each) at each visit.

Up to 2 ml of luminal fluid will be taken from the Duodenum during the gastroscopy procedure.

Details about the collection and preparation of blood and mucosal biopsies will be provided in the specific laboratory manual for this trial.

6.8.3 Storage of Samples at site

All blood samples, mucosal biopsies and luminal fluid samples will be listed on trial specific sample logs and stored at the clinical site during the trial.
Storage condition for blood samples is -80°C.
Storage condition for mucosal biopsies is -80°C.
Storage condition for luminal fluid samples is -80°C.
Freezers will be temperature monitored and connected to an alarm.

6.8.3 Storage of Samples at sponsor

All blood samples, mucosal biopsies and luminal fluid samples will be listed on trial specific sample logs and stored at the sponsor site in Hørsholm after the trial, while sample analysis at various labs are planned and executed.
Storage condition for blood samples is -80°C.
Storage condition for mucosal biopsies is -80°C.
Storage condition for luminal fluid samples is -80°C.
Freezers will be temperature monitored and connected to an alarm.

6.8.4 Shipment of Samples

Shipment of samples will be performed in accordance to the laboratory manual. The laboratory manual for the trial will provide details on shipment materials, shipment address, courier details etc.

6.8.5 Analysis of Samples

All samples will be analysed using validated assays and methods. Particulars about the methods used will be provided in the analytical report from the appointed laboratories and provided in the laboratory manual.

6.8.6 Research biobank and Destruction of Samples

A research biobank will be established in order to perform all planned analyses on mucosal biopsies, luminal fluid and blood samples described in this protocol. The described lab analyses are planned to take place in 2020 and 2021. Samples that are left after all planned analyses are performed, and all needed re-analyses are performed, will be discarded by the sponsor no later than ultimo 2022. No sample analysis will be performed that is not described in this protocol. No biological material and/or data from the trial will be shared with third-parties from this trial.

6.9 Data Management, Statistics and Quality Control

6.9.1 Data Collection and Processing

All clinical data will be collected using a CRF provided by the Sponsor.
The Investigator and authorised staff at the clinical site can add data to the CRF and must keep the CRF current to reflect subject status during the course of the trial. The CRFs for any subject leaving the trial should be completed at the time of the final visit or shortly thereafter.
A screening and randomisation number and the date of birth will identify the subjects on the CRF. The trial personnel must make a separate confidential record of personalised details (name and initials) on the subject identification and enrolment log which is kept separately from the CRF. Once the CRF for a subject is completed, the Investigator will approve the data and confirm the accuracy of the data recorded.
The CRFs for any subject leaving the trial should be completed at the time of the final visit or shortly thereafter. Paper source data will be shipped to Sponsor after trial closure and archived for 15 years.

6.9.2 Confidentiality

In order to maintain anonymity, subjects will only be identified by their date of birth and the assigned screening and randomisation numbers for all documents submitted to the Sponsor. Documents that will not be submitted to the Sponsor and that identify the subject (such as the signed informed consent document) must be maintained in strict confidence by the Investigator, except to the extent necessary to allow auditing and/or monitoring by the appropriate regulatory authority, the Research Associate, or Sponsor representatives.
All information obtained during the trial will be handled according to local regulations and the European Directive 95/46/CE (Directive on protection of individuals with regard to the processing of personal data and on the free movement of such data).

6.9.3 Data Quality Assurance

Trial specific training of trial staff at the clinical site will be performed prior to initiation of subject recruitment by the Sponsor. The training will include as a minimum a review of all procedures to be performed at the clinical site, CRF training, blood sampling and procedures for handling mucosal biopsies and luminal fluid samples as well as accountability of trial products.

6.9.4 Audits and Inspections

The Investigators will permit auditing by the Sponsor and inspection by applicable regulatory authorities. The Investigators agree to allow the auditors/inspectors to have direct access to all trial records for review, on the understanding that the auditors are bound by professional secrecy and will respect the confidentiality of the data verified and the anonymity of the subjects.

As soon as the Investigator is notified of a future inspection by the authorities, he/she will inform the Sponsor and authorise the Sponsor to participate in this inspection.

6.9.7 Termination of Trial or Trial Site

The Sponsor reserves the right to terminate the trial at any time. Conditions that may warrant termination of the trial may include, but are not limited to, the following:

- Failure of the trial site to conduct the trial in accordance with the protocol or any other local regulations.
- Failure of the trial site to enrol subjects at an acceptable rate.
- Failure of the trial site to ensure the quality of the data collected.
- New information on the trial product, at any moment during the trial, causing doubt about the benefit/risk ratio of the trial conduct.
- A decision at the discretion of the Sponsor to discontinue the trial for any reason.

Trial termination and follow-up will be performed in accordance with the conditions set forth in ICH-GCP and local regulatory requirements.

6.9.8 Statistics and power calculation

Data will be entered into a database and analysed using statistical software. The effect of the intervention on the endpoints will be assessed using paired statistical analysis and the level of significance will be set to 0.05. Pathway analysis will also be performed using a more explorative approach.
Previous studies using the same endoscopy procedure with biopsies being analysed for arrays of gene expression have showed an approximate negative binomial distribution and a coefficient of variation of 0.344. Assuming a negative binomial distribution of the data in the present study, we have calculated that a sample size of 20 will result in a power of 0.95 for identifying genes that are down or up-regulated more than 1.5-fold as a consequence of Bif195 intervention at a significance level of 0.05. In order to achieve this statistical power, we aim to have 20 subjects completing the intervention. Assuming a drop-out rate of around 20%, we aim to randomise 25 subjects.

6.10 Procedures for Handling Adverse Events

Adverse events (AEs) will be collected from Visit 2, the day before start of intervention with ASA and the trial product and through the end of the trial (Visit 5).

6.10.1 Definition of Adverse Events

An adverse event (AE) is any untoward medical occurrence in a trial subject administered a test product, whether considered related to the trial treatment or not. An AE can therefore be any new unfavourable and unintended sign, including abnormal laboratory findings, symptoms, or disease, or worsening of existing symptoms, which is temporarily associated with the use of a product under investigation.

6.10.2 Eliciting, Recording and Follow-up of Adverse Events

The subject will be asked if he/she has noticed any unfavourable effects when taking the investigational product. If the subject has noticed any unfavourable effects, an AE form in the CRF will be filled in for each separate AE. The form will cover onset, end, intensity, causality, action taken and outcome of the AE.
The subject must be followed-up by any clinical or biological examination considered as necessary by the medical judgment of the Investigator, until the abnormal condition is resolved, or the Investigator deems further observations or examinations to be no longer medically indicated.

6.10.3 Assessment of Intensity

The Investigator should for each AE reported by a trial subject rate the maximum intensity of the AE. The intensity grades are defined as follows:

- MILD The AE does not interfere with the subject's usual function
- MODERATE The AE interferes to some extent with the subject's usual function
- SEVERE The AE interferes significantly with the subject's usual function

Note the distinction between the seriousness and the intensity of an AE. Severe is a measure of intensity; and a severe reaction will not be classified as serious unless it meets one of the criteria for serious events listed below.

6.10.4 Assessment of Causality

The Investigator should for each AE reported by a trial subject rate the causality of the AE. The causality grades are defined as described in
Table 3.

Table 3 Assessment of causality of adverse events
For causality classification, all criteria of a category have to be met.

Definite	An AE occurring in a plausible time relationship to test product administration, and which cannot be explained by concurrent disease or concomitant drugs or chemicals. The response to withdrawal of the product (de-challenge) should be clinically plausible, and the AE should recur on re-challenge.
Probable	An AE with a reasonable time sequence to administration of the test product, unlikely to be attributed to concurrent disease or concurrent drugs or chemicals, and which follows a clinically reasonable response on withdrawal (de-challenge). Re-challenge information is not required to fulfill this definition.
Possible	An AE with a reasonable time sequence to administration of the test product, but which could also be explained by concurrent disease or concomitant drugs or chemicals. Information on drug withdrawal may be lacking or unclear.
Unlikely	An AE with a temporal relationship to administration of test product which makes a causal relationship improbable, and in which concomitant drugs, chemicals or underlying disease provide plausible explanations.
Unrelated	An event which has clearly and incontrovertibly no temporal relationship to the test product or is due to underlying/concurrent illness or effect of a concomitant drug.

6.10.5 Definition of Serious Adverse Events

A serious adverse event (SAE) is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening at the time of the event
- Requires inpatient hospitalisation
- Results in persistent or significant disability or incapacity
- Is another important medical event

6.10.6 Reporting of Serious Adverse Events

If an SAE occurs, trial personnel must fill in a “Serious Adverse Event reporting form” and notify the Investigator immediately. The Investigator should report all SAEs (whether or not considered related to trial treatment) to the Sponsor within 24 h after awareness of the SAE. The reporting by the Investigator to the Sponsor should cover the seriousness criteria, intensity and initial causality assessment.
The initial report of a SAE should as far as possible be supplemented by detailed information on diagnosis/symptoms, the relationship with the start of treatment and the latest dose of trial treatment taken and any further relevant data. A follow-up report of the SAE will be written as applicable and sent to the Sponsor.

6.10.7 Follow-up of Adverse Events and Serious Adverse Events

After an AE or SAE, the subject should be followed by any clinical or biological examination, considered as necessary by the medical judgment of the Investigator, until the AE/SAE has resolved, stabilised, the Investigator deems further observations or examinations to be no longer medically indicated or until the subject is under professional medical care. Follow-up should always be performed until a potential causality between the trial treatment and the AE has been assessed.

6.10.8 Procedures in Case of Medical Emergency

The medical expert for the trial can advise on trial-related medical questions and problems. The medical expert for the trial is:

Professor Filip K. Knop
Head of Center for Clinical Metabolic Research at Gentofte Hospital, University of Copenhagen, Gentofte Hospitalsvej 7, 3. Sal, 2900 Hellerup, Denmark;
e-mail: filip.krag.knop.01@regionh.dk; phone: +45 38 67 42 66

If an emergency occurs, the Sponsor representative below should also always be notified within 24 hours:

Brynjulf Mortensen, Senior Clinical Development Scientist
Chr. Hansen A/S
Kogle Alle 6
DK-2970 Hørsholm
Denmark
Phone: +45 51 80 54 88
E-mail: dkbrmo@chr-hansen.com

7. Trial Documentation

7.1 Trial Files

The following documents will be present at the clinical site before trial start can take place:

- Signed confidentiality and clinical trial agreement between the site and sponsor
- IEC approval of the protocol and informed consent document
- Signed protocol page
- Blank copy of the IEC-approved informed consent document and any other documents to the subjects
- Blank copy of the CRF
- Laboratory manual
- Signed Investigator / site agreement
- Insurance certificate from Sponsor
- CVs of Investigator and sub-investigators
- Site signature and responsibility log

Copies of these documents as well as supplemental information, such as the Investigational Product Information and final protocol should be kept on-site in a dedicated investigator's trial file, which should be kept strictly confidential. This file should also contain subject accountability records (screening and randomisation logs), investigational product accountability records (receipt/dispensing), Sponsor/Investigator correspondence, IEC correspondence, protocol deviations, biological samples records, and SAE/Safety reports.
The Investigator will keep a list of the subjects, identifying the names (with addresses and/or medical dossier numbers), their respective code number and the dates of start and end of the trial, in order to verify the concordance between the data contained in the CRFs and that in the source documents (European Directive 91/507/EEC).
All source documentation (i.e. medical notes etc.) should be available in print at the site.

7.2 Retention of Records

No trial documents will be destroyed or moved to a new location without prior written approval from the Sponsor. If the Investigator relocates, retires, or withdraws from the clinical trial for any reason, all records required to be maintained for the trial should be transferred to an agreed-upon designee, such as another Investigator at the institution where the trial was conducted.

7.3 Publication of Results

After completion of the trial, the results will be tabulated, evaluated and issued as a complete final clinical trial report. A summary of the report will be sent to the IEC, if applicable according to the local regulations.

All data generated by this clinical trial is the sole property of the Sponsor who will use the information for communications, regulatory purposes and/or publications as appropriate according to the clinical development plan for the trial product. All information from the trial is strictly confidential and should not be published or disclosed in any form without prior written agreement of the Sponsor.
The sponsor aims to make the data from the trial publicly available via scientific papers in appropriate scientific journals. The Principle Investigator and sub investigators will be included in scientific publications based on the trial. Authorship to other trial personnel can only be given to persons who fulfil the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" from the International Committee of Medical Journal Editors. Persons, who do not meet these criteria will be acknowledged in a publication, if they wish to.

8 Trial Administrative Structure

8.1 Responsibilities

- Chr. Hansen A/S, Hørsholm, Denmark is the Sponsor of the trial
- Filip K. Knop is the principal Investigator of the trial
- Anne Haaber is co-Investigator of the trial
- Center for Clinical Metabolic Research at Gentofte Hospital, University of Copenhagen, and its staff will organise the operational conduct of the trial. The responsibility split between Sponsor, Principal Investigator and Center for Clinical Metabolic Research will be specified in detail in the Clinical Trial Agreement.
- Signifikans ApS will be responsible for all making the randomisation list for the trial

8.2 Funding and duality of interest

The initiative to this study is taken by Brynjulf Mortensen, Senior Clinical Development Scientist and PhD representing the sponsor Chr Hansen A/S. The trial will take place at Center for Clinical Metabolic Research at Gentofte Hospital, University of Copenhagen. The Principal Investigator and physician responsible for the trial is Filip K. Knop. The study is funded entirely by the sponsor Chr Hansen A/S under a contract agreement between Chr Hansen A/S and Center for Clinical Metabolic Research at Gentofte Hospital in the amount of 2.900.000 DKK covering trial conduct. This reimbursement does not cover any lab analyses planned related to biopsies and luminal fluid obtained in the stomach and gut. These expenses will be covered by the sponsor Chr Hansen A/S under separate contract agreements with specialised labs agreed upon after conduct of the study. The Investigators do not have any financial interest in Chr Hansen A/S and will not receive any extra personal financial reimbursement from conducting the study.

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