CLINICAL TRIAL PROTOCOL

The effect of obeticholic acid on gut microbiota, gastric motility, accommodation, gastrointestinal peptide in healthy volunteers

OCARINA

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Sponsor

UZ Leuven

Herestraat 49, B-3000 Leuven

Coordinating Investigator

Prof. Dr. Jan Tack

LIST OF PARTICIPATING SITES

(as applicable)

List Of Participating Sites	Principal Investigator
UZ Leuven	Jan Tack

SIGNATURES

Title: The effect of obeticholic acid on gut microbiota, gastric motility, accommodation, gastrointestinal peptide in healthy volunteers

Protocol: OCARINA

The undersigned confirm that the above referenced protocol has been acknowledged and accepted, and agree to conduct the Trial in compliance with the approved protocol, and will adhere to: the principles outlined in the requirements for the conduct of clinical trials in the EU as provided for in Directive 2001/20/EC or the EU Clinical Trial Regulation 536/2014 (as soon as in effect) and any subsequent amendments thereto, the ICH guidelines, the most recent version of the Declaration of Helsinki, the Belgian law of May 7th 2004 regarding experiments on the human person (as amended) or the Belgian law of May 7th 2017 related to clinical trials on medicinal products for human use (as soon as in effect), the EU General Data Protection Regulation 2016/679 (GDPR), relevant Belgian laws implementing the GDPR, the Belgian Law of August 22nd 2002 on patient rights, and any other regulatory requirements and Standard Operating Procedures (SOPs), as applicable.

The undersigned agree not to disclose the confidential information contained in this document for any purpose other than the evaluation or conduct of the Trial, without prior written consent of the Sponsor.

The undersigned also commit to making the findings of the Trial publicly available through publication and/or other dissemination tools, in accordance with this protocol and applicable regulations, without any unnecessary delay and to provide an honest, accurate and transparent account of the Trial; and to explain any discrepancies or deviations from the approved Trial protocol.

Coordinating Investigator

Prof. Dr. Jan Tack		
JAN TACK		16/9/2021
Name & Title	Signature	Date

Principal Investigator (Participating Site) *(in case of monocentric Trial, the Principal Investigator is the same as the Coordinating Investigator)*

Prof. Dr. Jan Tack		
JAN TACK		16/9/2021
Name & Title	Signature	Date

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LIST OF ABBREVIATIONS

Abbreviation	Definition
(e)CRF	(electronic) Case Report Form
AE	Adverse Event
AESI	Adverse Event of Special Interest
APR	Annual Progress Report
ASR	Annual Safety Report
AR	Adverse Reaction
CA	Competent Authority
CI	Coordinating Investigator
CIOMS	Council for International Organizations of Medical Sciences
CM	Concomitant Medication
CSR	Clinical Study Report
CTP	Clinical Trial Protocol
DMP	Data Management Plan
DPA	Data Processing Annex
DTA	Data Transfer Agreement
DSMB	Data Safety Monitoring Board
DSUR	Development Safety Update Report
EC	Ethics Committee
EU	European Union
ECG	Electrocardiogram
EoT	End of Trial
FPFV	First Patient First Visit
GCP	Good Clinical Practice (latest version of ICH E6)
GDPR	General Data Protection Regulation
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IMP	Investigational Medicinal Product
ISF	Investigator Site File
JCI	Joint Commission International
LPLV	Last Patient Last Visit
MAH	Marketing Authorisation Holder
MP	Monitoring Plan
PI	Principal Investigator (Participating Site)
PRO	Patient Reported Outcome
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction

SOP	Standard Operating Procedure
SmPC	Summary of Product Characteristics
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMF	Trial Master File
GI	Gastro Intestinal
MMC	Migrating motor complex measurement
IGP	Intragastric pressure measurement
GLP-1	Glucagon-like peptide-1
GLP-2	Glucagon-like peptide-2
GIP	Gastric inhibitory polypeptide
FD	Functional dyspepsia
EPS	Epigastric pain syndrome
PDS	Postprandial distress syndrome
BA	Bile acids
FXR	Farnesoid X receptor
OCA	Obeticholic acid
PBC	Primary biliary cirrhosis
NASH	Non-alcoholic steatohepatitis
UDCA	Ursodeoxycholic acid
BMI	Body Mass Index

FUNDING AND SUPPORT

Funder	Type of Financial or Non-Financial Support
Methusalem funding	Financial Support

Reasonable time investment and travel expenses incurred by Trial participants and directly related to participation in the Trial, will be reimbursed by the Sponsor, as follows:

(i) At a flat rate of € 950
When the subject does not complete the study, but terminates the study earlier, the compensation will be calculated as follow:
Stop at the end of visit 2: € 225
At the end of visit 4: € 575
At the end of visit 6: € 950

(ii) If the subject require contraception to take part in this study, the subject will be reimbursed for the actual cost incurred.

A reimbursement payment log will be kept by the Investigator Site staff and made available for monitoring and verification purposes.

ROLES AND RESPONSIBILITIES

The Principle Investigator (PI) is responsible for the conduct of the Trial at his/her Participating Site, and for protecting the rights, safety and well-being of the Trial participants. As such the PI must ensure adequate supervision of the Trial conduct at the Participating Site. If any tasks are delegated, the PI will maintain a log of appropriately qualified persons to whom he/she has delegated specified Trial-related duties. The PI will ensure that adequate training is provided and documented for all Trial staff, prior to conducting assigned Trial-related activities.

It is the Coordinating Investigator's (CI's) responsibility to supervise the general conduct (e.g. Trial progress, communication, protocol training and support of the participating sites, annual reporting to the Ethics Committee (EC), end of Trial notification(s) and results reporting…) of the Trial. The CI fulfils both Investigator and Sponsor responsibilities, as outlined in International Conference on Harmonisation – Good Clinical Practice (ICH-GCP) E6(R2) and applicable regulations.

PI and CI shall each be referred to as «Investigator(s)».

TRIAL SYNOPSIS

Title of clinical Trial («Trial»)	The effect of obeticholic acid on gut microbiota, gastric motility, accommodation, gastrointestinal peptide in healthy volunteers
Protocol Short Title Acronym	OCARINA
Trial Phase (I, II, III, IV)	II
Sponsor name	UZ Leuven
Coordinating Investigator	Prof. Dr. Jan Tack
Contact Address CI	Herestraat 49, bus 701
Contact Email CI	jan.tack@kuleuven.be
Contact Phone CI	+32-16-344225
EudraCT number	2020-004180-13
Other public database nbr	
Principal Investigators and Participating Sites	Prof. Dr. Jan Tack/ KU Leuven
Medical condition or disease under investigation	Functional dyspepsia
Trial rationale	Randomized controlled
Primary objective	To detect changes in appetite/hunger after administration of obeticholic acid compared to placebo
Secondary objective(s)	(1) To detect changes in the release of gastrointestinal peptide after administration of obeticholic acid compared to placebo. (2) To detect changes in gastric motility, accommodation after administration of obeticholic acid compared to placebo. (3) To detect changes in gut microbiota after administration of obeticholic acid compared to placebo.
Trial Design	Phase II, placebo-controlled, randomized, single-blind, crossover study
Endpoints	Primary endpoint: - Appetite-related sensations compared between placebo and treatment with obeticholic acid Secondary endpoints: - Gastrointestinal hormone release compared between placebo and treatment with obeticholic acid - Gastric motility, accommodation compared between placebo and treatment with obeticholic acid changes in gut microbial diversity abundance of specific bacteria compared between placebo and treatment with obeticholic acid
Sample Size	12 healthy individuals

IMP, dosage and route of administration	One tablet containing obeticholic acid (10 mg) once per day oral administration
Active comparator product(s)	Obeticholic acid
Maximum duration of treatment and Follow Up of a Participant	21 days
Maximum duration of entire Trial	3 months
Date anticipated First Patient First Visit (FPFV)	01/10/2021
Date anticipated Last Patient Last Visit (LPLV)	31/12/2022
Third parties	None

TRIAL FLOWCHART

Schedule of Events – Trial specific Procedures / Assessments

Please indicate in the flowchart with different colors whether a procedure is performed as part of the standard of care or specifically for the Trial.
Describe the visits and applicable procedures/investigations (with a reference number or letter) in more detail in a footnote below the Trial flowchart

Procedures/ Assessment	**Screening**	**Rando-misation**	**Treatment Period**							
Visits / Contacts	Visit 0	Visit 0	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6		
Timing (weeks)		0	0	0	3	3	10	10		
Visit Window (days)	-30 Days to -1	-30 Days to -1	day -7 to 0	day -7 to 0	day 17 to 21	day 17 to 21	day 66 to 70	day 66 to 70		
Informed consent	X [1]									
Inclusion / Exclusion criteria	X									
Demographics	X									
Medical, Surgical history	X									
Physical examination	X									
Weight / Height	X									
Vital Signs	X									
Pregnancy Test	X									
Urinalysis										
Randomisation		X								
Study drug administration [2]					X	X	X	X		
Migrating motor complex measurement [3]			X		X		X			
Intragastric pressure measurement [4]			X		X		X			
Blood collection [5]			X		X		X			
Duodenal aspirates [6]			X		X		X			
Patient Reported Outcomes (PRO) 1 (questionnaire) [4,5]										
Endoscopy [7]				X		X		X		
Duodenal biopsy [7]				X		X		X		
Diary					X	X	X	X		
Stool specimens [8]				X		X		X		
(Serious) Adverse event (S)(AE) assessment					X	X	X	X		
Concomitant Medication (CM)	X	X	X	X	X	X	X	X		

I Enrollment and screening questionnaires: the following questionnaires will be used for screening: Patient Health Questionnaire, Dutch version of Rome IV criteria questionnaire for functional GI disorders. Enrollment and screening will be done via an online system (Qualtrics).

2 Study drug administration: assignment of treatment (placebo and obeticholic acid) will be randomized before study visit 1. Either placebo or obeticholic acid will be administered orally in a single-blind fashion for 21 days. After a washout period of 28 days, either placebo or obeticholic acid will be administered as appropriate for an additional 21 days.

3 Migrating motor complex measurement (MMC): Subjects will come to the clinic within 7 days prior to study start medication. On that day, subjects will come in the morning after an overnight fast of 12 hours. Prior to the start of the study, an intravenous catheter will be placed in an antecubital vein by a trained nurse for collecting blood samples continually. The catheter will be infused continuously with saline during the entire study visit.

A manometry probe, a small, flexible tube, will be passed through the nose into the stomach of the subject. The probe contains 36 channels that measure pressure. The manometry probe will be positioned in the duodenum. An aspiration catheter is advanced via the nostril into the second part of the duodenum using the manometry catheter as a guide, with a brief fluoroscopic check (2-3 seconds, never more than 15 seconds). After positioning, the manometry probe and the catheter will be fixed and subjects will be asked to take place in a bed in a comfortable sitting position with the trunk upright.

Migrating motor complex measurement (MMC) cycle is a pattern of electrical activity observed in the gastrointestinal tract in a regular cycle during fasting. To evaluate this MMC cycle, the duration of two consecutive phases III will be measured. Blood samples for motilin and ghrelin will be taken every 20 min between the two phases III (maximum 300 min). After 60 min to 120 min, blood samples will be taken every 10 min, because the rise of motilin levels and MMC phase III are known to be observed in time zone in most cases (11). The first sample will be taken 10 min after the end of the first phase III. The subject will be asked to fill in a visual analogue scale for hunger and satiation and 6 epigastric symptoms (fullness, nausea, belching of air, cramps in the abdomen, bloating and pain) at 10-minute intervals. Duodenal aspirates will be taken for analysis of the luminal microbiota and BA composition in the beginning of the test and at around MMC phase III. Duodenal fluids will be then centrifuged at 4°C for 5 min at maximum speed and stored at -20°C and -80°C in the TARGID lab and the Laboratory of Molecular Bacteriology (Rega Institute) until further analysis, respectively.

4 Intragastric pressure measurement (IGP): Gastric accommodation will be assessed subsequently after MMC measurement. The drinking test will start from phase II of MMC. For the drinking test, nutrient drink (Nutridrink, Nutricia 150 Kcal/100m) will be taken orally at a constant speed of 60 ml/min. The maximum of liquid nutrient drink will be total 600ml. The subject will be asked to fill in a visual analogue scale for hunger and satiation and 6 epigastric symptoms (fullness, nausea, belching of air, cramps in the abdomen, bloating and pain) at 5-minute intervals. In addition, the subject also will have to score his/her satiation at 1-minute intervals by using a graphic rating scale that combines verbal descriptors on a scale graded from 0–5 (1, threshold; 5, maximum satiety). Blood samples will be taken every 10 min. Orally feeding will be stopped as soon as the subject reached the maximum score of 5 on their satiation scale or when they score maximally on one of the epigastric symptoms. In that case, the time of stop drinking will be recorded. One hour hereafter the catheters will be disconnected and removed.

5 Blood collection: During MMC measurement, blood samples (4ml per collection for motilin, octa-ghrelin) will be taken every 20 min within the first 60 min of MMC measurement, then every 10 min until the end of MMC phase III. If over 120 min, then blood samples will be collected every 20 min until the end of MMC measurement (maximum 300 min). During IGP measurement, blood samples (12 ml per collection for motilin, octa-ghrelin, GLP-1, GLP-2, GIP, insulin) will be collected every 10 min within the first 60 min. Plasma C4 will be evaluated at the first collected sample. A maximum amount of collected blood will be 150 ml.

6 Duodenal aspirates: Duodenal aspirates will be taken at around MMC phase III. Bile acid analysis: Concentration of bile acids, including, glyco-cholic acid (GCA), tauro-cholic acid (TCA), glyco-chenodeoxycholic acid (GCDCA), and tauro-chenodeoxycholic acid (TCDCA), glyco-deoxycholic acid (GDCA), tauro-deoxycholic acid (TDCA), glyco-ursodeoxycholic acid (GUDCA), and tauro-ursodeoxycholic acid (TUDCA), for each participant will be measured with liquid chromatography-mass spectrometry (LC-MS) selected ion monitoring by the Pharmacotechnology and biopharmacy group of Prof. Patrick Augustijns (KU Leuven) according to a previously published protocol (2) . The pH of every duodenal fluid sample will be measured with pH-Meter 766 Calimaticon at room temperature.

7 Endoscopy: Endoscopy will be performed after a minimal fasting period of 8 hours. First, a sheathed and sealed biopsy forceps for aseptic mucosa sampling and outer diameter 1.8mm (MTW, Wesel, Germany) will be used for taking a maximum of 3 small biopsies (approximately 1 mm^3). Second, a single-use cytology brush will be used for superficial mucosal sampling. Finally, a Radial Jaw3 with needle forceps and outside diameter 2.2mm (Boston Scientific, Heredia, Costa Rica) will be used for 6 biopsies (approximately 2 mm^3) from the duodenum. All endoscopies will be performed by an experienced endoscopist. Before the procedure, local anesthetic xylocaine spray is applied in the throat with no sedation, unless demanded by subject in which case intravenous access is obtained before administration of IV Midazolam (maximum of 4mg). Aseptic biopsies (maximum 3) and brushings will be separately collected in sterile tubes, snap frozen in liquid nitrogen and stored at -80°C in the Laboratory of Molecular Bacteriology (Rega Institute) until microbial analysis. Of the conventional biopsies (n= 9 per subject per visit), 2 will be collected in RNAlater and stored at -80° in the Biobank until analysis of gene expression, 2 will be snap frozen in liquid nitrogen and stored at -80° in the Biobank until analysis of protein expression, 2 will be fixed in formalin or fixative with(-out) glutaraldehyde and stored at 4°C in the TARGID lab until later analysis of histology and 3 will be collected in ice-cold Hanks' Balanced Salt Solution (HBSS) buffer and analyzed immediately in the TARGID lab for permeability.

8 Stool samples will be collected before administration of placebo or obeticholic acid (day 0 for intervention 1, day 28 for intervention 2), after administration (day 2, 4, 7, 14, 21 for intervention 1, day 30, 32, 35, 42, 49 for intervention 2). Samples are temporarily stored by the participants in their home freezer. Within 2 weeks, samples will be brought back to UZ Leuven.

1 Background, Rationale and Risk Assessment

Functional dyspepsia (FD) is a common functional gastroduodenal disorder that has a great social and economic impact in patients. It is subdivided in two groups depending on the symptom pattern. Epigastric pain syndrome (EPS) is characterize by meal-unrelated symptoms such as epigastric pain and epigastric burning; and postprandial distress syndrome (PDS) characterize by meal-related symptoms such as postprandial fullness and early satiation (1). The pathophysiology of FD remains poorly understood and is most likely heterogeneous, but likely candidates are genetic factors, stress and duodenal luminal factors, including bile acids (BA) (2).

Bile acid and Gut microbiota

BA are essential molecules in fat absorption by acting as a surfactant that emulsifies them into micelles (3). It is well established that the majority of patients with FD report onset or worsening of symptoms after a meal, which coincides with the release of BA into the duodenum (4). Furthermore, we proved that patients with FD are characterized by a decreased duodenal BA concentration in fasted state compared to healthy volunteers (2).

Another important feature of bile acids is their strong antimicrobial activity. In fact, oral administration of bile acid regulates the composition of gut microbiota in rats (5). The gut microbiota maintains a symbiotic relationship with the host and regulates several important functions including host metabolism, immunity, and intestinal barrier function. Intestinal inflammation is commonly associated with dysbiosis of the gut microbiota (6). Duodenal inflammation has been observed in up to 40% of patients with FD and, hence, it is thought to be an important factor in the pathogenesis of FD (7). A decreased duodenal BA might play a role in duodenal inflammation via duodenal dysbiosis in the pathophysiology of FD, while it is not well proven the relationship between the pathogenesis of FD and BA (2).

Gastric sensorimotor function

Impaired gastric sensorimotor function, such as decreased gastric accommodation, delayed gastric emptying and gastric hypersensitivity, has been implicated in symptom generation in FD (8). These alterations may represent new targets for therapy (e.g. improving gastric accommodation). We have already established a new minimally invasive technique that measures the intragastric pressure (IGP) drop during a liquid meal challenge as a method to assess gastric accommodation in FD patient (9, 10).

The migrating motor complex (MMC) is a cyclic motor pattern in the gastrointestinal tract that occurs during the interdigestive state and is responsible for the rumbling experience when hungry (11). Its absence has been associated with FD, gastroparesis and small intestinal bacterial overgrowth (11). IGP and MMC techniques use the same catheter as in esophageal high-resolution manometry and, therefore, have the potential to gain a similar acceptance and feasibility level for clinical routine diagnostic test.

Gastrointestinal hormones

The gastrointestinal (GI) hormones constitute a group of hormones secreted by enteroendocrine cells in the stomach, pancreas, and small intestine that control various functions of the digestive organs, including gastric motility. Motilin and ghrelin are the GI hormones released in a fasting state to stimulate the GI motility of the MMC (12). Recent research has shown that both the MMC and motilin play a role in hunger signaling and changes in both occur in food intake disorders (13). Glucagon-like peptide-1 and 2 (GLP-1, GLP-2) inhibits gastric emptying with secondary effects on the release of insulin in fed state (14, 15). BA stimulate GLP-1, GLP-2 and insulin (16, 17).

Farnesoid X receptor (FXR) agonist

Farnesoid X receptor (FXR) is a bile acid receptor, which is mainly expressed in the liver and intestine. FXR regulate synthesis, secretion and transport of bile acids (18). It has been considered as a promising target for the treatment of cholestatic disorders, including primary biliary cirrhosis (PBC) (19). FXR is also important for the regulation of the lipid and glucose metabolism (18). The activation of FXR showed beneficial effects on various metabolic diseases, including fatty liver diseases, type 2 diabetes, dyslipidemia, and obesity (18). Obeticholic acid (OCA), a FXR agonist, is proven as an effective drug for PBC (19). In 2016, OCA was approved in U.S. and Europe for the treatment of PBC. OCA is used together with another

medicine, ursodeoxycholic acid (UDCA), in patients who do not respond sufficiently to UDCA alone, and on its own in patients who cannot take UDCA. Now, phase III study for NASH is ongoing. In healthy volunteers, patients with primary biliary cholangitis (PBC) and patients with non-alcoholic steatohepatitis (NASH), single and multiple doses of obeticholic acid were well tolerated (19, 20). The most frequently reported adverse events considered to be drug-related were pruritus and fatigue (19, 20). Some abdominal pain and discomfort were reported. Based on a multiple-center phase III study in PBC patients, the recommended starting dosage of OCA is 5 mg orally once daily for 3 months with titration to 10 mg once daily based upon tolerability and response(21). In conclusion, obeticholic acid was found to be safe.

Recently, Friedman ES et al showed alteration of gut microbiome by OCA. In this study, male C57BL/6 mice were gavaged daily with water, vehicle, or OCA (10 mg/kg) for 2 weeks. Small intestine luminal contents were collected by flushing with saline and fecal pellets were collected at baseline and day 14. Culture experiments were performed to determine taxonomic-specific effects of bile acids and OCA on bacterial growth. As a result, administration of OCA dramatically led lower endogenous bile acid levels and an increased proportion of Firmicutes, specifically in the small intestine but not in stool, compared to control in mice (22). Friedman ES et al also conducted healthy volunteers on OCA doses of 5, 10 or 25mg/d and analyzed their microbiome in stool (22), however it is still unclear how the intestinal microbiome changes in human.

OCA might alter the gut microbiota and duodenal inflammation. Moreover, gastric motility, accommodation and gastrointestinal peptide might be affected through the change of duodenal circumstance. Therefore, the aim of the current research protocol is to investigate the relationship between FXR agonist and gut microbiota, gastric motility, accommodation, gastrointestinal peptide in healthy volunteers.

2 Trial Objectives and Design

2.1 Trial objectives

The aim of this study is to investigate the effect of OCA (FXR agonist) on gut microbiota, gastric motility, gastrointestinal peptide in healthy individuals.

2.2 Primary Endpoints

The primary endpoint of this study is to compare appetite/hunger between placebo and treatment with OCA .

2.3 Secondary Endpoints

The secondary endpoint is to compare gastric motility, the distribution of gut microbiota and BA composition between placebo and treatment with OCA .

Gastric motility and the release of the hormones motilin, ghrelin, GLP-1, GLP-2, GIP and insulin will be compared between placebo and treatment with OCA .

2.4 Trial Design

This study is a single-blind, placebo-controlled, randomized, study in healthy individuals.

2.5 Expected Duration of the Trial

01/10/2021-31/12/2022

3 Trial Population / Eligibility Criteria

3.1 Inclusion criteria

Participants eligible for inclusion in this Trial must meet **all** of the following criteria:

1. Voluntary written informed consent of the participant or their legally authorized representative has been obtained prior to any screening procedures

2. Use of highly effective methods of birth control; defined as those that, alone or in combination, result in low failure rate (i.e., less than 1% per year) when used consistently and correctly; such as implants, injectables, combined oral contraceptives, some IUDs, true sexual abstinence (i.e. refraining from heterosexual intercourse during the entire period of risk associated with the Trial treatment(s)) or commitment to a vasectomised partner.
3. Subject is between 18 and 65 years of age
4. Subject has a BMI between 18 and 25 kg/m²

All participants that are considered for Trial participation, per the above criteria will be documented on the Screening Log, including Screen Failures.

3.2 Exclusion criteria

Participants eligible for this Trial must **not** meet any of the following criteria:

1. Participant has a history of gastrointestinal or other significant somatic or psychiatric diseases or drug allergies, diabetes, a significant heart, lung, liver or kidney disease, a neurological disorder, abdominal surgery (including gallbladder removal, but those having undergone a simple appendectomy more than 1 year prior to the screening visit may participate),
2. Any disorder, which in the Investigator's opinion might jeopardise the participant's safety or compliance with the protocol
3. Any prior or concomitant treatment(s) that might jeopardise the participant's safety or that would compromise the integrity of the Trial
4. Female who is pregnant, breast-feeding or intends to become pregnant or is of child-bearing potential and not using an adequate, highly effective contraceptive
5. Participation in an interventional Trial with an investigational medicinal product (IMP) or device
6. High caffeine intake (> 500 ml coffee daily or equivalent).
7. Subject consumes excessive amounts of alcohol, defined as >21 units per week for men, >14 units per week for women.
8. Subject is currently (defined as within approximately 1 year of the screening visit) a regular or irregular user (including "recreational use") of any illicit drugs (including marijuana) or has a history of drug (including alcohol) abuse. Further, patient is unwilling to refrain from the use of drugs during this study.
9. Inability or unwillingness to perform all of the study procedures, or the subject is considered unsuitable in any way by the principal investigator.
10. Recent participation (<30 days) or simultaneous participation in another clinical study.
11. Prior participation in a clinical trial of obeticholic acid

Participants who meet one or more of the above exclusion criteria **must not proceed** to be enrolled/randomized in the Trial and will be identified on the Screening Log as Screen Failure.

4 Trial Procedures

4.1 Participant consent and withdrawal of consent

The Trial will be conducted only on the basis of prior informed consent by the Trial participants and/or their legally authorized representative(s). As such, no Trial-related procedures will be conducted prior to obtaining written informed consent from potential Trial participants.

The process for obtaining and documenting initial and continued informed consent from potential Trial participants will be conducted in accordance with ICH-GCP E6(R2), applicable regulatory requirements and internal Standard Operating Procedures (SOPs).

All originally signed obtained Informed Consent Forms (ICFs) must be retained/archived in the Investigator Site File (ISF) at the Participating Site and must not be destroyed (even when a scanned copy is available) before expiration of the legal archiving term as defined in the protocol section entitled "Archiving".

Participants may voluntarily withdraw consent to participate in the Trial for any reason at any time. The participant's request to withdraw from the Trial must always be respected without prejudice or consequence to further treatment. Consent withdrawal will be documented in the participant's medical record.

Trial data and samples collected before withdrawal can be used in the trial. No new trial data or samples will be collected after withdrawal of the participant.

4.2 Selection of Participants / Recruitment

Potential subjects will be recruited by means of an existing volunteer database from the research group of the Sponsor-Investigator. Potential subject will be contacted by phone or per email with a short introduction on the study purpose and the proposal to make an appointment if they are interested in participating, through oral advertisement and through advertisement on the UZ Leuven intranet and on notice boards of the KU Leuven.

4.3 Randomization Procedure / Blinding (if applicable)

Randomization

To ensure the integrity of the Trial, the following randomization procedures have been established:

A randomization online software (http://www.randomization.com) will be used to generate randomization plan.

A randomization list will be prepared by the Sponsor's designated staff, not involved in recruiting Trial participants, medical care, drug administration or follow-up.

Blinding

To avoid bias, treatment arms will be blinded to the Drug provider, as follows:

The IMP will be manufactured, packaged and labelled in such a way that the visual appearance, nor the smell and/or touch of the IMP can be distinguished from the comparator treatment and/or placebo.

Furthermore, to maintain the blind, the IMP and any comparator treatment(s) and/or placebo will follow the same administration route and process.

4.4 Unblinding

After all participants have completed the Trial, the database will be locked and the collected Trial data will be unblinded to allow analysis of the Trial data.

In case of an emergency in a blinded Trial, the Investigator has the sole responsibility for determining if premature unblinding of a participant's treatment assignment is warranted. Participant safety must always be the first consideration in making such a determination. Every effort should be made to contact the Sponsor prior to unblinding a participant's treatment assignment, unless this would delay emergency treatment of the participant. The Sponsor must be notified as soon as possible and no later than within 24 hours after breaking the blind. The date and reason for unblinding must be recorded in the source documentation and electronic Case Report Form (eCRF), as applicable.

4.5 Premature discontinuation of Trial treatment

Participants may voluntarily discontinue from Trial treatment and/or prematurely end their participation in the Trial for any reason at any time. In such case the Investigator must make a reasonable effort to contact the participant (e.g. via telephone, e-mail, letter) in order to document the primary reason for this decision.

The Investigator may also decide at any time during the course of the Trial, to temporarily interrupt or permanently discontinue the Trial treatment if it is deemed that continuation would be detrimental to, or not in the best interest of the participant.

Similarly, de Sponsor, Ethics Committee or authorized regulatory authority can decide to halt or prematurely terminate the Trial when new information becomes available whereby the rights, safety and well-being of Trial participants can no longer be assured, when de integrity of the Trial has been compromised, or when the scientific value of the Trial becomes obsolete and/or unjustifiable.

Circumstances requiring premature treatment interruption or discontinuation of the Trial, include but are not limited to:

- Safety concerns related to IMP or unacceptable intolerability
- Trial participation while in violation of the inclusion and/or exclusion criteria
- Pregnancy
- Intention of becoming pregnant

In any such case of early Trial termination and/or treatment interruption/discontinuation, the Investigator will continue to closely monitor the participant's condition and ensure adequate medical care and follow-up. It is recommended that follow-up information will be collected as follows:

The Investigator will assess physical damage at the end of treatment and provide medical care and follow-up as necessary

For participants whose status is unclear because they fail to appear for Trial visits without stating an intention to discontinue or withdraw, the Investigator must make every effort to demonstrate "due diligence" by documenting in the source documents which steps have been taken to contact the participant to clarify their willingness and ability to continue their participation in the Trial (e.g. dates of telephone calls, registered letters, etc.).

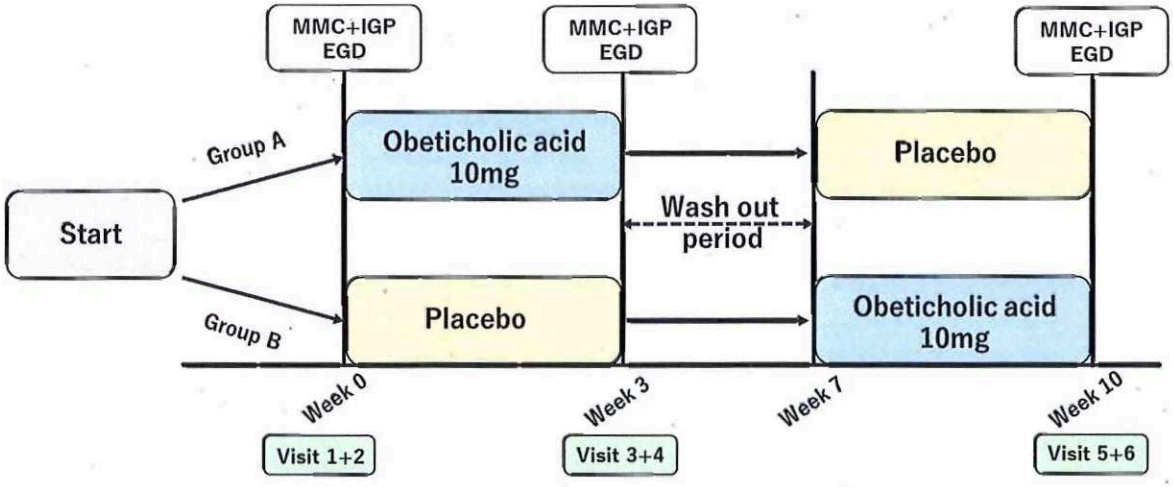
A participant should not be considered lost to follow-up until due diligence has been completed.

5 Trial Medication / Drug

Generic Drug Name (& company brand name)	IMP or non-IMP	Used within Indication? (Y or N)	Route of administration (po,sc,iv,...)	Dose/dosage and units
Obeticholic acid (OCALIVA®)	**IMP**	N	**po**	10 mg
Placebo	**IMP**	N	**po**	placebo

5.1 Investigational Medicinal Product and Dosing Regimen

In this study, participants will receive in total one dose of OCA (OCALIVA®, Intercept Pharmaceuticals, New York, United States) and one dose of placebo (Intercept Pharmaceuticals, New York, United States) in a phase II, single-blind, randomized crossover fashion.



One dose of study medication consists of 1 tablet (10mg) of placebo or OCA. OCA and placebo will be orally administered with water in the morning for 21 days after the examinations at preintervention.

OCALIVA® is a drug which is only available on prescription, it is indicated for the treatment of primary biliary cholangitis (ATC code A05AA04). OCALIVA ® tablets contain 10 mg obeticholic acid and 176 mg Microcrystalline cellulose, 12 mg Sodium starch glycolate, 2 mg Magnesium stearate, tablets are film coated and dissolve in the acidic environment of the stomach. One dose of study medication will contain 10 mg obeticholic acid.

Placebo tablets will be obtained from Intercept Pharmaceuticals, New York, United States and will contain 320 mg lactose monohydrate, 10 mg carmellose sodium, 70 mg corn starch, 55 mg cellulose microcrystalline, 20 mg silicium dioxide anhydrous and 13 mg magnesium stearate per tablet.

Administered medications will be properly registered in KWS system.

Participants will take each OCA and placebo for 21 days across washout period at home. Drug compliance will be monitored with a daily questionnaire and subjects will state the time of taking the medication. When participant forgets to take dose at regular time, participant can still take drug until 12 hours to next dose. The bottles with the tablets will be collected after the end of each treatment period and the number of tablets will be counted and registered. With regard to medication compliance, it is not considered a deviation if the rate of taking study medication is more than 80%.

5.2 Drug Accountability

Participants will take medication home. The investigators will prepare blinded containers which contain the study medication to assure single blinding. Therefore, each subject's medication kit will be labelled as following:

Seulement pour les études cliniques	Alleen voor klinische studies	Nur für klinische Studien
Numéro de protocole: OCARINA Numéro EudraCT: 2020-004180-13	Protocolnummer: OCARINA EudraCT-Nummer: 2020-004180-13	Protokollnummer: OCARINA EudraCT Nummer: 2020-004180-13
Contenu: Acide obéticholique 10 mg ou placebo Conditions de stockage: 20-25°C Posologie: 1 comprimé une fois par jour le matin Utilisation orale Numéro d'identification: XX Numéro de personnage: XXXXXXXX Date d'expiration: MM / DD/ YYYY	Inhoud: Obeticholzuur 10 mg of placebo Opslag condities: 20-25°C Dosering: 1 tablet 1 keer per dag in de ochtend Oral gebruik ID nummer: XX Chargenumber: XXXXXXXX Vervaldatum: MM / DD / YYYY	Inhalt: Obeticholsäure 10 mg oder Placebo Lagerbedingungen: 20-25°C Dosierung: 1 tablette einmal täglich morgens Oraler Gebrauch ID Nummer: XX Chargenumber: XXXXXXXX Ablaufdatum: MM / DD / YYYY
Docteur: Prof Dr Jan tack, University of Leuven, UZ Gasthuisberg, Belgium, tel: +32-16-344225	Dokter: Prof Dr Jan tack, University of Leuven, UZ Gasthuisberg, Belgium, tel: +32-16-344225	Doktor: Prof Dr Jan tack, University of Leuven, UZ Gasthuisberg, Belgium, tel: +32-16-344225
Tenir hors de la portée et de la vue des enfants	Buiten het bereik en zicht van kinderen houden	Darf nicht in die Hände von Kindern gelangen

5.3 Concomitant / Prohibited Medication / Treatment

The following drugs must not be used 2 weeks before, during and 1 week after the study

- Drugs that can affect gastrointestinal function, motility or sensitivity or gastric acidity.
- Centrally acting medication, including antidepressants, antipsychotics and/or benzodiazepines
- Warfarin
- Proton pump inhibitors

If these drugs are taken during study medication, IMP medication will be discontinued and the subject will be excluded from the study at that time. Study data up to the time of exclusion will be available.

If these drugs are taken during the washout period, a period of at least 2 weeks should be allowed before starting the second medication.

The following drugs must not be used 1 year before, during and 1 week after the study

- Centrally acting medication, including antidepressants, antipsychotics and/or benzodiazepines (in the last year before screening visit).

If these drugs are taken during study medication, IMP medication will be discontinued and the subject will be excluded from the study at that time. Study data up to the time of exclusion will be available.

5.4 Rescue Medication

None

6 Safety

Definitions[1,2]

6.1 Adverse Event (AE)

An AE is any untoward medical occurrence in a patient, clinical investigation participant or participant of the treated group during an experiment who had been administered a pharmaceutical product, and which does not necessarily have a causal relationship with this treatment.

An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of a medicinal (investigational) product, whether or not considered to be related to the product. Any worsening (i.e. any clinically significant adverse change) in the frequency or intensity of a pre-existing condition, should be considered an AE.

6.2 Adverse Reaction (AR)

An AR is any untoward and unintended response to an investigational medicinal product or to an experiment and, when an investigational product is concerned, related to any dose administered. This means that a causal relationship between a medicinal product and an adverse event is at least a reasonable possibility, i.e. the relationship cannot be ruled out.

6.3 Serious Adverse Event (SAE)

A SAE is any untoward medical occurrence that at any dose, results in any of the following:

- Death
- A life-threatening[a] experience
- In-patient hospitalisation or prolongation of existing hospitalisation
- A persistent or significant disability or incapacity
- A congenital anomaly or birth defect
- Important medical events that may be considered an SAE when - based on appropriate medical judgement - they could jeopardise the participant's safety and may require medical or surgical intervention to prevent one of the above outcomes[3]

[a] The term "life threatening" in the definition of SAE refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it were more severe.

6.4 Suspected Unexpected Serious Adverse Reaction (SUSAR)

A SUSAR is an AR, the nature or severity of which is not consistent with the information on the experiment, and, when a clinical Trial is concerned, with the applicable product information (e.g. Investigator's brochure (IB) for an unauthorised investigational product or the patient leaflet joined to the summary of product characteristics for an authorised product).

[1] Guideline for good clinical practice E6(R2) Step 5 EMA/CHMP/ICH/135/1995 dated 1 December 2016
2 Belgian law of May 7th 2004 regarding experiments on the human person (as amended) or the Belgian law of May 7th 2017 related to clinical trials on medicinal products for human use (as soon as in effect),

[3] ICH E2A Clinical Safety Data Management: Definitions and Standards for Expedited Reporting CPMP/ICH/377/95 dated Jun 1995

6.5 Adverse Events of Special Interest (AESI)

The following events should always be reported within the same timelines as SAEs:

- Overdose
- Misuse/abuse
- Medication error

6.6 Safety Events that do not require reporting

In general, the following should not be reported as AEs:

- Pre-existing conditions, including those found as a result of screening (these should be reported as medical history or concomitant illness), unless the condition worsens during Trial treatment

The following AEs are commonly observed and expected, and/or are part of the well-known safety profile of OCALIVA® as documented in the Summary of Product Characteristics (SmPC) when used within the approved indication, and are therefore not considered adverse events for the purpose of the Trial:

- stomach pain
- feeling tired
- thyroid hormone irregularity
- dizziness
- fast or irregular heart beat (palpitations)
- pain in the mouth and throat
- constipation
- itchy, dry and/or red skin (eczema)
- rash
- pain in your joints
- swelling in the hands and feet
- fever

Although these events should not be reported to the Sponsor, these should be recorded in the participant's medical notes according to routine practice.

6.7 Recording and Reporting of Safety Events

Investigators will seek information on the occurrence of safety events at each participant contact. All events, whether reported by the participant or noted by Trial staff, will be recorded in a timely manner in the participant's medical record and in the (e)CRF. If available, the *diagnosis* should be reported on the appropriate (S)AE page in the (e)CRF, rather than the individual signs or symptoms. If no diagnosis is available, the Investigator should record each sign and symptom as individual safety events.

The following minimum information should be recorded for each event:

- event description
- start and stop date of the event
- severity
- seriousness
- causality assessment to the IMP and/or Trial procedures
- outcome

6.7.1 Assessment

All safety events must be evaluated by an Investigator with regards to:

- **Seriousness:** determine whether the AE is an SAE. See above for the seriousness criteria.
- **Severity:**
  - Severity grading must be evaluated by the Investigator according to the current version of the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events *(CTCAE*, most recent version)
  - Severity must be evaluated by the Investigator according to the following definitions:

- *Mild* – no or transient symptoms, no interference with the participant's daily activities
- *Moderate* – marked symptoms, moderate interference with the participant's daily activities
- *Severe* – considerable interference with the participant's daily activities, unacceptable

- **Causality:**
  - *None* – The AE is not related to the IMP or participation in the experiment
  - *Unlikely* – It is unlikely that the AE is related to the IMP or participation in the experiment; an alternative explanation is more likely (e.g. concomitant medication(s), concomitant disease(s)), and/or the relationship in time suggests that a causal relationship is unlikely
  - *Possible* – The AE might be due to the use of the IMP or participation in the experiment. An alternative explanation is inconclusive. The relationship in time is reasonable; therefore the causal relationship cannot be ruled out
  - *Probable* - The AE might be due to the use of the IMP or participation in the experiment. The relationship in time is suggestive (e.g. confirmed by dechallenge). An alternative explanation is less likely
  - *Definitely* – The AE is listed as a possible adverse reaction and cannot be reasonably explained by an alternative explanation. The relationship in time is very suggestive (e.g. it is confirmed by dechallenge and rechallenge)

6.7.2 Timelines for reporting

After obtaining informed consent and prior to initiation of Trial treatment, only (serious) adverse events caused by a Trial specific procedure should be reported in the (e)CRF.

After initiation of Trial treatment, safety events will be reported as follows:

- All AEs, SAEs and AESIs occurring during Trial treatment or within 30 days after last Trial treatment administration, or last follow-up visit (whichever occurs first) will be reported.
- All SAEs and AESIs as defined in the protocol must be reported to the Sponsor within 24 hours of the Trial staff becoming aware of the event. The initial report shall be followed by detailed, written reports. Both the initial and follow-up reports shall identify participants only by their Trial-specific identification.
- SAE details will be reported by the Investigator to the Sponsor:
  - By completing the SAE form in the (e)CRF
- If an authorised Investigator from the reporting site is unavailable, initial reports without causality and expectedness assessment should be submitted to the Sponsor by a healthcare professional within 24 hours of becoming aware of the SAE, but must be followed-up by a medical assessment performed by an authorised Investigator, as soon as possible thereafter.

6.7.3 Follow-up

The Investigator must record follow-up information by updating the participant's medical records and the appropriate form(s) in the (e)CRF.

SAE follow-up information should only include new information (e.g. corrections or additions) and must be reported within 24 hours of the Investigator's first awareness of the information. This is also the case for previously non-serious AEs which subsequently become SAEs.

- All **SAEs** must be followed until the outcome of the event is 'recovered', 'recovered with sequelae', 'not recovered' (in case of death due to another cause than the SAE) and until all related queries have been resolved, or until the end of the Trial (whichever occurs first)
- **Non-serious AEs** must be followed until the participant's last Trial visit, and until all related queries have been resolved

SAEs after the end of the Trial: If the Investigator or Trial team becomes aware of an SAE with suspected causal relationship to the IMP or experiment, after the participant has ended the Trial, than this SAE must be reported within the same timelines as for SAEs occurring during the Trial.

6.7.4 Pregnancy

Female participants must be instructed to notify the Investigator immediately if they become pregnant during the Trial. The Investigator must report to the Sponsor, any pregnancy in participants who have received Trial product(s).

6.7.5 Technical Complaints

Technical complaints with the IMP or comparator treatment should be reported to the Sponsor and to the Marketing Authorisation Holder (MAH) (e.g. change in colour, unequal sizes, broken vials/pills, sedimentation, inconsistent packaging or labelling etc.). If the technical issue puts the safety of Trial participants at risks and/or jeopardises the integrity of the Trial (e.g. when the issue causes unblinding), than the Ethics Committee must be notified immediately and no later than 24 hours of the Trial team becoming aware.

6.7.6 Death

All deaths (except those defined under section 6.6) will be reported without delay to the Sponsor (irrespective of whether the death is related to disease progression, the IMP, participation in the experiment or an unrelated event). The Sponsor will notify all deaths as soon as possible after becoming aware to the central EC and the local EC of the concerned research site (via the contact person of the local research site, as applicable) and provide additional information if requested.

6.8 Reporting requirements to Ethics Committees (ECs) and Competent Authorities (CAs)

The Investigator is responsible for ensuring that all safety events are recorded in the (e)CRF and reported to the Sponsor in accordance with instructions provided in the protocol.

The Sponsor will promptly evaluate all SAEs and AESIs against medical experience to identify and expeditiously communicate possible new safety findings to Investigators, EC(s) and applicable CA(s) and participants as appropriate, in accordance with applicable legislation.

6.8.1 Sponsor's reporting of Suspected Unexpected Serious Adverse Reactions (SUSARs)

After receiving the SAE report form from the Investigator, the Sponsor must perform a causality (relationship) assessment. The term Serious Adverse Drug Reaction (SADR) is to be used whenever either the Investigator or the Sponsor deems the SAE is possibly or probably related to the IMP.

The Sponsor must evaluate (and document the evaluation of) the expectedness for each SADR against the Reference Safety Information, e.g. the Investigator's Brochure or applicable product information. In case the event is Unexpected (i.e. a SUSAR) it must be reported by the Sponsor to the EC(s), CA(s) (through the EudraVigilance database or other local process) and other participating Investigators using the Council for International Organizations of Medical Sciences (CIOMS) form within the following timelines:

- **7 calendar days** if the event is fatal or life-threatening (follow-up information to be provided within an additional 8 calendar days)
- **15 calendar days** if non-fatal or non-life-threatening event (follow-up information be provided as soon as possible)

6.8.2 Annual reporting

It is the Sponsor's legal obligation to, at least once a year and for as long as the clinical Trial runs (or ad hoc on request), submit a progress report to the EC(s) and CA(s) containing an overview of all Serious Adverse Reactions (SARs) that occurred during the reporting period and taking into account all new available safety information received during the reporting period. This information can be communicated as part of the Annual Progress Report (APR) or as a separate Development Safety Update Report (DSUR, cfr. ICH E2F) or an Annual Safety Report (ASR)

6.8.3 Overview reporting requirements

WHO	WHAT	HOW	TO	TIMELINES

Investigator	AE	AE form	Sponsor	as defined in protocol
	SAE	SAE form	Sponsor	Initial & follow-up information: Immediately (within 24 hours of becoming aware of the event) Exceptions: as defined in protocol
	Death	SAE form	Sponsor	asap
Sponsor	SUSAR	CIOMS form: - EC-reporting: according to EC process - Reporting to CA(s): through EudraVigilance or local CA process	**Unblinded report:** - EC(s) - CA(s) - MAH (if applicable) **Blinded report to:** - PI's of participating sites	For fatal or life-threatening SUSAR: asap, but no later than: - 7 calendar days (initial report) - 8 calendar days (follow-up report) For non-fatal and non-life-threatening SUSAR: - 15 calendar days (initial report) - asap (follow-up report)
	Death	SAE form + narrative	- EC(s)	asap
	Annual Progress/ Safety Report	DSUR or APR/ASR template	- EC(s) - CA(s) participating countries	annually

6.9 Data Safety Monitoring Board (DSMB)

Not relevant for this study.

6.10 Dissemination update safety information

Safety updates need to be reported yearly in the updated Investigator Brochure and/or annual safety report and in case of substantial safety changes these need to be reflected in an updated informed consent form and approved by the EC, prior to providing to the Trial participants.

7 Statistics and Data Analysis

Statistical analysis will be performed in accordance with ICH E9; a detailed description of the analysis is provided in the Trial-specific Statistical Analysis Plan (SAP). ICH E3 and E8 will guide the structure and content of the clinical trial report.

Twelve healthy volunteers will be enrolled in this placebo-controlled cross-over study. The number of volunteers to be included is calculated with a two-tailed t-test comparing two dependent means. We specified the following parameters: an error probability of 5%, effect size of 1.0 and a power of 80%. Based on these parameters we need to include 10 volunteers in order to highlight the expected differences. Twelve volunteers will be included to anticipate a potential dropout. Data from all subjects who completed the study will be included for analysis. Statistical analysis of the different parameters will be performed in SPSS for Windows (SPSS Inc., Chicago, IL, USA). Curves of whole blood glucose, plasma levels of motilin and ghrelin GLP-1, GLP-2, GIP and insulin and hunger / appetite scores over time will be

analyzed using the mixed models procedure, taking into account the course of the curves over different time points. Gastric motility and BA composition will be compared with Two-tailed paired t tests or Wilcoxon signed-rank tests, depending on data distribution. Non-parametric test were applied to analyse microbiome data, with multiple testing correction whenever applicable (adjustment for false discovery rate (FDR)).

7.1 Sample Size Determination

Twelve healthy volunteers will be enrolled in this placebo-controlled cross-over study. The number of volunteers to be included is calculated with a two-tailed t-test comparing two dependent means. Power calculations were based on previous results on the effect of cholestyramine (a bile acid sequestrant) on hunger sensation in healthy volunteers (23). We assume a similar effect of obeticholic acid on hunger sensation and a similar within-participant variability. We specified the following parameters: an error probability of 5%, effect size of 1.0 and a power of 80%. Based on these parameters we need to include 10 volunteers in order to highlight the expected differences. Twelve volunteers will be included to anticipate a potential dropout. Power calculations were performed with GPower version 3.0.10.

Approximately 15 participants will be screened to achieve 6 randomly assigned to Trial treatment and 6 evaluable participants for an estimated total of 12 evaluable participants per treatment group.

A maximum of 12 participants will be randomly assigned to Trial treatment such that approximately 10 evaluable participants complete the Trial.

7.2 Statistical Analysis

Data from all subjects who completed the study will be included for analysis. Statistical analysis of the different parameters will be performed in SPSS for Windows (SPSS Inc., Chicago, IL, USA). Curves of whole blood glucose, plasma levels of motilin and ghrelin GLP-1, GLP-2, GIP and insulin and hunger / appetite scores over time will be analyzed using the mixed models procedure, taking into account the course of the curves over different time points. Gastric motility and BA composition will be compared with Two-tailed paired t tests or Wilcoxon signed-rank tests, depending on data distribution. Non-parametric test were applied to analyse microbiome data, with multiple testing correction whenever applicable (adjustment for false discovery rate (FDR)). Data obtained from endoscopy and gastric motility measurements prior to the start of treatment in the first part of the crossover study will be used as pre-treatment data in the second part. Results will be found significant at $p<0.05$ significance level.

7.2.1 Efficacy Analysis

Endpoint	Statistical Analysis Methods
Primary	mixed models procedure (hunger / appetite)
Secondary	mixed models procedure (whole blood glucose, plasma levels of motilin and ghrelin GLP-1, GLP-2, GIP and insulin), Two-tailed paired t tests or Wilcoxon signed-rank tests (Gastric motility and BA composition), Non-parametric test, multiple testing correction (microbiome data)
Exploratory	Two-tailed paired t tests or Wilcoxon signed-rank tests (Signaling pathway analysis for duodenal biopsies)

7.2.2 Other Analysis

For signaling pathway analysis using duodenal biopsies, Analysis of gene expression, protein expression, histology and permeability will be evaluated.

7.3 -Interim Analysis and Final Database Lock

No interim analysis is planned.

The Statistical Analysis Plan will describe the planned interim analysis/analyses in greater detail.

8 Data handling

Data handling and data flows for the Trial are summarized below and will be described in more detail in the Trial-specific Data Management Plan (DMP).

Data for this study will be captured via electronic CRFs (open clinica) with pseudonymisation. Time and date of entry, changes made and reasons for change will be recorded. Complete information for each patient visit, status, procedure, and test will be appropriately recorded. For dose administration, the date and exact time will be captured. For each patient randomized and treated, an eCRF must be completed. If a participant withdraws from the study, the reason must be noted on the eCRF. The Investigator will ensure the accuracy, completeness, and timeliness of the data reported in the eCRF. The participant's name or other identifiers will be stored separately from the Trial data and replaced with a unique code to create a new identity for the participant, for the purpose of the Trial. In such case data are encoded and solely the Investigator and his/her Trial staff shall be able to link the data to an identifiable person. The code list must be retained on site, in a secured place with restricted access and can under no circumstances leave the site or be accessed by unauthorized persons.

8.1 Data Collection Tools and Source Document Identification

8.1.1 Operational aspects

Data collection, handling, processing and transfer for the purpose of this Trial will be performed in compliance with applicable regulations, guidelines for clinical trials and internal procedures, as follows:

8.1.1.1 Data collection

Source Data will be collected and recorded in the Trial participant's files/medical records.

If applicable, worksheets may be used for capturing some specific data in order to facilitate completion of the (e)CRF. Any such worksheets will become part of the Trial participant's source documentation and will be filed together with or as part of the medical records (during but also following completion of the Trial).

It remains the responsibility of the Investigator to check that all data relating to the Trial, as specified in the Trial protocol, are entered into the (e)CRF in accordance with the instructions provided and that the forms are filled out accurately, completely and in a timely manner.

(e)CRFs are provided by the Sponsor for each participant. The Trial data will be transcribed from the source records (i.e. participant's medical file or Trial-specific source data worksheets) into an (e)CRF by Trial Staff. Transcription to the (e)CRF will be done as soon as possible after a participant visit and in a pseudonymized manner using a unique identifier assigned by the Sponsor.

The (e)CRFs will be available for review at the next scheduled monitoring visit (as applicable) and shall under no circumstances capture personal data such as but not limited to the participant or their relative(s) name, home address, contact details, full date of birth medical record number (e.g. UZ Leuven EAD number), social security number etc.

8.1.1.2 Data Validation

All data relating to the Trial must be prepared and validated by the Investigator. Any (e)CRF entries, corrections and alterations must be made by the Investigator or other authorized Trial staff.

Proper audit trails must be available to demonstrate the validity of the Trial data collected. This includes historical records of original data entries, by whom and when the data was entered, as well as detailed records of any corrections or additions made to the original data entry (i.e. who made the correction/addition, when and why), without obliterating the original data entry information.

8.1.1.3 Data Management

The Trial Data Manager will perform extensive consistency checks on the received data. Queries will be issued in case of inconsistencies in accordance with internal procedures. A Data Management Plan (DMP) will be developed to map data flows, data validation measures that will be taken, how (interim) database lock(s) will be managed and, as applicable, the role and responsibilities of the Data Safety Monitoring Committee (DSMB)

8.1.1.4 Data Transfer

Any participant records or datasets that are transferred to the Sponsor or any partners of the Sponsor will contain the Trial-specific participant identifier only; participant names or any information which would make the participant identifiable will not be transferred. All pseudonymized data relating to the Trial must be transmitted in a secure manner to the Sponsor or any partners of the Sponsor(see 8.1.2. legal requirements).

8.1.2 Legal requirements

All source data will be kept at a secured location with restricted access at all times. These data must be collected and processed with adequate precautions to ensure confidentiality and compliance with applicable data protection laws and regulations and more in particular the EU General Data Protection Regulation 2016/679 (GDPR) and relevant national laws implementing the GDPR. Appropriate technical and organizational measures to protect the data against unauthorized disclosure or access, accidental or unlawful destruction, or accidental loss or alteration must be established. Trial staff whose responsibilities require access to personal data agree to keep the data confidential.

The Investigator and the Participating Site(s) (as applicable) shall treat all information and data relating to the Trial disclosed to them as confidential and shall not disclose such information to any third parties or use such information for any purpose other than the objectives of the Trial as described in this protocol. The collection, processing and disclosure of personal data, such as participant health and medical information is subject to compliance with applicable laws and regulations regarding personal data protection and the processing of personal data.

The Investigator will maintain all source documents and completed (e)CRFs that support the data collected from each Trial participant, and will maintain a Trial Master File (TMF)/Investigator Site File (ISF) containing all Trial documents as specified in ICH-GCP E6(R2) Chapter 8 entitled "Essential Documents for the Conduct of a Clinical Trial", and as specified by applicable regulatory requirement(s). The Investigator will take appropriate measures to prevent accidental or premature destruction of these documents.

Transfer of the pseudonymized data will be performed via a secured method of transfer taking into account all applicable security arrangements and regulations (such as the European General Data Protection Regulation). The receiving party will be bound by contractual agreement to keep the transferred data confidential at all times and to only process the data for the purpose of the Trial. To this end, appropriate Data Transfer Agreements (DTAs) will be established.

8.2 Audits and Inspections

The Investigator will permit direct access to Trial data and documents for the purpose of monitoring, audits and/or inspections by authorized entities such as but not limited to: the Sponsor or its designees and competent regulatory or health authorities. As such (e)CRFs, source records and other Trial related documentation (e.g. Investigator Site File, the Trial Master File, pharmacy records, etc.) must be kept current, complete and accurate at all times.

8.3 Monitoring

In accordance with ICH-GCP E6(R2) the Sponsor is responsible for monitoring the Trial to ensure compliance with GCP and current legislation, and to verify, among other requirements, that proper written informed consent has been obtained and documented, that the Trial procedures have been followed as shown in the approved protocol, and that relevant Trial data have been collected and reported in a manner that assures data integrity. To this end Source Data will be compared with the data recorded in the (e)CRF. A risk-based approach will be applied to determine the extent of monitoring activities and monitoring of the Trial will be performed by qualified individuals (independent from the site Trial staff), as applicable.

At the time of enrollment of six patients, the status of consent acquisition, eligibility, randomization, data collection, and serious adverse events will be checked in a few sampled cases. Again, at the end of all cases, monitoring will be conducted in the same way.

The Sponsor and Investigator/Participating Site will permit direct access to the Trial data and corresponding Source Data, and to any other Trial related documents or materials to verify the accuracy and completeness of the data collected. More details about the monitoring strategy are described in the Trial specific Monitoring Plan (MP).

8.4 Archiving

As specified in ICH-GCP E6(R2) section 8.1 Addendum, the Sponsor and Investigator/Participating Site will maintain a record of the location(s) of all respective Essential Trial Documents (including but not limited to Source Documents, completed and final (e)CRF and ISF/TMF). The Sponsor should ensure that the Investigator has control of and continuous access to the (e)CRF data reported to the Sponsor during the Trial.

The Investigator/Participating Site should have control of all Essential Documents and records generated by the Investigator/Participating Site before, during and following termination of the Trial.

The Sponsor is responsible for archiving Trial specific documentation (such as but not limited to the Trial protocol, any amendments thereto, the final Clinical Study Report (CSR) and the Trial database) according to ICH-GCP E6(R2). Source data and site-specific Trial documents (such as but not limited to the original signed ICFs) will be archived by the participating site(s) according to local practice, and for at least 25 years following termination of the Trial. Archived data may be held on electronic record, provided that media back-up exists, hard copies can be obtained, if required and measures are taken to prevent accidental or premature loss or destruction of data. Destruction of Essential Documents prior to, during or upon completion of the required archival period, will require written authorisation from the Sponsor.

9 Ethical and Regulatory Considerations

9.1 Ethics Committee (EC) review & reports

Before the start of the Trial, this protocol and other related documents (e.g. ICF, advertisements, IB, etc.) will be submitted for review to the EC and to the relevant CA for Trial authorization. The Trial shall not commence until such approvals have been obtained.

It is the responsibility of the CI to produce the Annual Progress Report (APR) and submit to the EC/CA within 30 days of the anniversary date on which favourable opinion to start the Trial was given, and annually until the Trial is declared ended.

The CI shall notify the EC/CA of the end of the Trial. Should the Trial be temporarily suspended or, ended prematurely, the CI will notify the EC/CA and include the reasons for suspension/premature termination within 15 days of the decision. The CI will submit a final report with the results of the study, including any publications/abstracts, to the EC/CA within 1 year of trial termination or within 6 months for paediatric Trials.

9.2 Peer review

This Trial protocol was peer reviewed by 3 number independent experts. Peer review was conducted by expert referees to the professional and scientific standards expected for clinical studies.

9.3 Regulatory Compliance

The Trial will be conducted in compliance with the principles outlined in the requirements for the conduct of clinical Trials in the EU as provided for in Directive 2001/20/EC or EU Regulation 536/2014, as applicable, and any subsequent amendments, as well as in compliance with ICH-GCP E6(R2) guidelines, other GxP guidelines, the most recent version of the Declaration of Helsinki, the Belgian law of May 7th 2004 regarding experiments on the human person (as amended) or the Belgian law of May 7th 2017 on clinical Trials with medicinal products for human use, as applicable, and with the EU General Data Protection Regulation 2016/679 (GDPR), the relevant Belgian laws implementing the GDPR, the Belgian Law of August 22nd 2002 on patient rights and all other applicable legal and regulatory requirements.

9.4 Protocol / GCP compliance

The Trial must be performed in accordance with the protocol, current ICH and ICH-GCP guidelines, and applicable regulatory and country-specific requirements. ICH guidelines are an international ethical and scientific quality standard for designing, conducting, recording, and reporting studies that involve the participation of human participants. Compliance with this standard provides public assurance that the rights, safety, and well-being of Trial participants are protected, consistent with the principles that originated in the most recent version of the Declaration of Helsinki, and that the Trial data are credible, reliable and reproducible.

The Investigator and Trial team acknowledge and agree that prospective, planned deviations or waivers to the protocol are not permitted under applicable regulations on clinical studies. However, should there be an accidental protocol deviation, such deviation shall be adequately documented in the source documents and on the relevant forms and reported to the CI and Sponsor. Deviations should also be reported to the EC as part of the EC's continued review of the Trial (e.g. through the ASR, APR, etc.). Protocol deviations which are found to frequently recur, will require (immediate) action. The Investigator acknowledges that such recurring protocol deviations could potentially be classified as a serious violation of ICH and/or the protocol.

It is understood that "a serious violation" is likely to affect to a significant degree:

- the safety or physical or mental integrity of the Trial participants; or
- the scientific validity of the Trial

The Investigator is expected to take any immediate action required to protect the safety of any participant included in the Trial, even if this action represents a deviation from the protocol. In such cases, the Sponsor should be notified of this action and the EC at the Trial site should be informed according to local procedures and regulations.

9.5 Data protection and participant confidentiality

The Trial will be conducted in compliance with the requirements of the EU General Data Protection Regulation 2016/679 (GDPR), the relevant Belgian laws implementing the GDPR including the Belgian Privacy Act of 30 July 2018 on the protection of privacy in relation to the processing of personal data. Any collection, processing and disclosure of personal data, such as participant health and medical information is subject to compliance with the aforementioned personal data protection laws (cfr. Data Processing Annex (DPA) in Appendix). In case personal data is transferred outside the European Economic Area, safeguards will be taken to ensure that appropriate protection travels with the data in accordance with the GDPR. (https://ec.europa.eu/info/law/law-topic/data-protection/international-dimension-data-protection/rules-international-data-transfers_en#documents)

Any personal data shall be treated as confidential at all times including during collection, handling and use or processing, and the personal data (including in any electronic format) shall be stored securely at all times and with all technical and organizational security measures that would be necessary for compliance with EU and national data protection legislation (whichever is more stringent). The Sponsor shall take appropriate measures to ensure the security of all personal data and guard against unauthorized access thereto or disclosure thereof or loss or destruction while in its custody.

9.6 Insurance

The Participating Site, the Investigator and Sponsor shall have and maintain in full force and effect during the term of this Trial, and for a reasonable period following termination of the Trial, adequate insurance coverage for: (i) medical professional and/or medical malpractice liability, and (ii) general liability.

For Belgian Participating Sites

Art 29 of the Belgian Law relating to experiments on human persons dated May 7th, 2004 applies.
Prior to the start of the Trial, the Sponsor shall enter into an insurance contract in order to adequately cover Trial participants from Belgian sites in accordance with art. 29 of the said law.

For non-Belgian Participating Sites

The Participating Site shall have and maintain in full force and effect during the term of this Trial (and for a reasonable period following termination of the Trial, adequate insurance coverage for other possible damages resulting from the Trial at the Participating Site, as required by local law. Each such insurance coverage shall be in amounts appropriate to the conduct of the services of the Participating Site under this Trial. The Participating Site and Sponsor shall be solely responsible for any deductible or self-insured retention under any such policies.

9.7 Amendments

Unless for urgent reasons as specified in ICH-GCP E6(R2) section 4.5.4, amendments must not be implemented prior to EC and/or CA review and/or approval, as applicable.
In accordance with the Belgian law of May 7th 2004 regarding experiments on humans, the Sponsor may develop a non-substantial amendment at any time during the Trial. If a substantial amendment to the clinical Trial agreement or the documents that supported the original application for the clinical Trial authorisation is needed, the Sponsor must submit a valid substantial amendment to the Competent Authority (CA) for consideration, and to the EC for review and approval. The CA and/or EC will provide a response in accordance with timelines defined by applicable regulations. It is the Sponsor's responsibility to assess whether an amendment is substantial or non-substantial for the purpose of submission to the CA and/or EC.

Amendments to the Trial are regarded as 'substantial' when they are likely to have a significant impact on the safety or physical or mental integrity of the clinical Trial participants, or the scientific value of the Trial.

https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-10/2010_c82_01/2010_c82_01_en.pdf

9.8 Post-Trial activities

Not applicable as this is a clinical study in healthy subjects.

10 Research Registration, Dissemination of Results and Publication Policy

The Declaration of Helsinki (latest version) and European and Belgian regulations require that every research Trial involving human participants be registered in a publicly accessible database before recruitment of the first participant. The CI is responsible for registering the Trial.

In addition, the CI will fulfil their ethical obligation to disseminate and make the research results publicly available. As such the CI is accountable for the timeliness, completeness and accuracy of the reports. Researchers, authors, Sponsors, editors and publishers must adhere to accepted guidelines for ethical reporting. Negative and inconclusive, as well as positive results must be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest must be declared in publication.

Publications will be coordinated by the CI. Authorship to publications will be determined in accordance with the requirements published by the International Committee of Medical Journal Editors and in accordance with the requirements of the respective medical journal.

Any publication by a Participating Site must be submitted to the Sponsor for review at least thirty (30) calendar days prior to submission or disclosure. Sponsor shall have the right to delay the projected publication for a period of up to three (3) months from the date of first submission to the Sponsor in order to enable the Sponsor to take steps to protect its intellectual property rights and know-how.

11 Intellectual Property

Any know how, inventions, methods, developments, innovations, discoveries and therapies, whether patentable or not, arising from the Trial or made in the performance of the Trial protocol ("Inventions") shall vest in the Sponsor. The Participating Site, its employees and Investigator(s) shall promptly disclose to the Sponsor any such Inventions. Parties have expressly agreed that any and all Trial data as collected

and prepared in the performance of the Trial protocol shall be the sole property of Sponsor unless otherwise agreed in the clinical trial agreement.

12 Joint Commission International (JCI)

In order to ensure the same quality and safety standards in patient care for clinical research as commonly applied by the Sponsor in its regular activities, and in accordance with JCI standards, the Sponsor shall comply with the following obligations: (a) the Sponsor will use trained and qualified employees or contractors to manage and coordinate the Trial; (b) the Sponsor will ensure that multi-center Trial reporting is reliable and valid, statistically accurate, ethical, and unbiased. (c) the Sponsor will not grant incentives, other than standard compensations and reimbursement of costs, to Trial participants or to participating site's staff that would compromise the integrity of the research; (d) the Sponsor is responsible for monitoring and evaluating the quality, safety, and ethics of the Trial and will respect the participating site's policies and processes when performing such monitoring and evaluation activities; (e) the Sponsor will protect the privacy and confidentiality of the Trial participants in accordance with all applicable laws.

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APPENDICES

Possible side effects of obeticholic acid, Ocaliva®

(Ocaliva : EPAR - Product Information;https://www.ema.europa.eu/en/medicines/human/EPAR/ocaliva)

Like all medicines, this medicine can cause side effects, although not everybody gets them.

Tell your doctor or pharmacist if you experience itching of the skin (pruritus) or if the itch gets worse while on this medicine. In general itching of the skin is a very common (may affect more than 1 in 10 people) side effect that begins within the first month following the start of treatment with Ocaliva and usually becomes less severe over time.

Other possible side effects may be:

Very common side effects (may affect up to 1 in 10 to 100 people)

- stomach pain
- feeling tired

Common side effects (may affect up to 1 in 100 to 1000 people)

- thyroid hormone irregularity
- dizziness
- fast or irregular heart beat (palpitations)
- pain in the mouth and throat
- constipation
- itchy, dry and/or red skin (eczema)
- rash
- pain in your joints
- swelling in the hands and feet
- fever

Risks of upper gastrointestinal endoscopy

Rare incidents of upper gastrointestinal endoscopy include gastrointestinal bleeding and perforation. This may require hospitalisation or emergency treatment or surgery.

Risks associated with insertion of the manometry catheter and duodenal fluid suction catheter

- Risks of this test include:
- Slight nosebleed
- Sore throat
- Hole, or perforation, in the esophagus (this rarely happens)

14 Appendix 1: Clinical trial protocol history

Original CTP version:	1.0 dated 30/08/2021

Amendment #1:	dated
Modifications made / Reason for amendment:	

Amendment #2:	dated
Modifications made / Reason for amendment:	

15 Appendix 2: Data Processing Annex (DPA)

Definitions:

- "Protocol" means the document entitled The effect of obeticholic acid on gut microbiota, gastric motility, accommodation, gastrointestinal peptide in healthy volunteers containing the details of the academic Trial as developed by the Sponsor and approved by the relevant Ethics Committee.
- "Sponsor" means University Hospitals Leuven (UZ Leuven).
- Participating site acts as a data processor as defined under article 4, 8) of the Regulation (EU) 2016/679 ("Data Processor") for the Sponsor who acts as data controller as defined under article 4, 7) of the Regulation (EU) 2016/679 ("Data Controller").
- "Applicable Law" means any applicable data protection or privacy laws, including:
    a) the Regulation (EU) 2016/679 also referred as the General Data Protection Regulation ("GDPR");
    b) other applicable laws that are similar or equivalent to or that are intended to or implement the laws that are identified in (a) of this definition;
- "Personal Data" means any information relating to an identified or identifiable natural person ("Data Participant"), including without limitation pseudonymized information, as defined in Applicable Law and described in the Protocol.

Rights and obligations:

1. The Data Processor is instructed to process the Personal Data for the term of the Trial and only for the purposes of providing the data processing tasks set out in the Protocol. The Data Processor may not process or use Personal Data for any purpose other than a Data Participant's medical records, or other than provided in the instructions of the Trial protocol, including with regard to transfers of personal data to a third country or an international organization, unless the Data Processor is required to do so according to Union or Member State law.
2. Data Processor shall at all times maintain a record of processing of Personal Data in accordance with Applicable Law and if the Data Processor considers an instruction from the Data Controller to be in violation of the Applicable Law, the Data Processor shall promptly inform the Data Controller in writing about this.
3. The Data Processor must ensure that persons authorized to process the Personal Data have committed themselves to confidentiality or are under an appropriate statutory obligation of confidentiality.
4. The Data Processor shall implement appropriate technical and organizational measures to prevent that the Personal Data processed is:
    (i) accidentally or unlawfully destroyed, lost or altered,
    (ii) disclosed or made available without authorization, or
    (iii) otherwise processed in violation of Applicable Law.
5. The appropriate technical and organizational security measures must be determined with due regard for:
    (i) the current state of the art,
    (ii) the cost of their implementation, and
    (iii) the nature, scope, context and purposes of processing as well as the risk of varying likelihood and severity for the rights and freedoms of natural persons.
6. Taking into account the nature of the processing, the Data Processor shall assist the Data Controller, by means of appropriate technical and organizational measures, insofar as this is possible, in fulfilling its obligation to respond to requests from Data Participants pursuant to laws

and regulations in the area of privacy and data protection (such as, the right of access, the right to rectification, the right to erasure, the right to restrict the processing, the right to data portability and the right to object)

7. The Data Processor shall upon request provide the Data Controller with sufficient information to enable the Data Controller to ensure that the Data Processor's obligations under this DPA are complied with, including ensuring that the appropriate technical and organizational security measures have been implemented.

8. The Data Controller is entitled to appoint at its own cost an independent expert, reasonably acceptable to the Data Processor, who shall have access to the Data Processor's data processing facilities and receive the necessary information for the sole purpose of auditing whether the Data Processor has implemented and maintained said technical and organizational security measures. The expert shall upon the Data Processor's request sign a non-disclosure agreement provided by the Data Processor, and treat all information obtained or received from the Data Processor confidentially, and may only pass on, after conferral with the Data Processor, the findings as described under 10) (ii) below to the Data Controller.

9. The Data Processor must give authorities who by Union or Member State law have a right to enter the Data Controller's or the Data Controller's processors' facilities, or representatives of the authorities, access to the Data Processor's physical facilities against proper proof of identity and mandate, during normal business hours and upon reasonable prior written notice.

10. The Data Processor must without undue delay in writing notify the Data Controller about:

    (i) any request for disclosure of Personal Data processed under the Protocol by authorities, unless expressly prohibited under Union or Member State law,

    (ii) any finding of (a) breach of security that results in accidental or unlawful destruction, loss, alteration, unauthorized disclosure of, or access to, Personal Data transmitted, stored or otherwise processed by the Data Processor under the Protocol, or (b) other failure to comply with the Data Processor's obligations, or

    (iii) any request for access to the Personal Data (with the exception of medical records for which the Data Processor is considered data controller) received directly from the Data Participants or from third parties.

11. Such a notification from the Data Processor to the Data Controller with regard to a breach of security as meant in 10) (ii)(a) above will contain at least the following information:

    (i) the nature of the Personal Data breach, stating the categories and (by approximation) the number of Data Participants concerned, and stating the categories and (by approximation) the number of the personal data registers affected (datasets);

    (ii) the likely consequences of the Personal Data breach;

    (iii) a proposal for measures to be taken to address the Personal Data breach, including (where appropriate) measures to mitigate any possible adverse effects of such breach.

12. The Data Processor shall document (and shall keep such documentation available for the Data Controller) any Personal Data breaches, including the facts related to the Personal Data breach, its effects and the corrective measures taken. After consulting with the Data Controller, the Data Processor shall take any measures needed to limit the (possible) adverse effects of Personal Data breaches (unless such consultation cannot be awaited due to the nature of the Personal Data breach).

13. The Data Processor must promptly and reasonably assist the Data Controller (with the handling of (a) responses to any breach of security as described in 10) (ii) above and (b) any requests from Data Participants under Chapter III of the GDPR, including requests for access, rectification, blocking or deletion. The Data Processor must also reasonably assist the Data Controller by implementing appropriate technical and organizational measures for the fulfilment of the Data Controller's obligation to respond to such requests.

14. The Data Processor must reasonably assist the Data Controller with meeting the other obligations that may be incumbent on the Data Controller according to Union or Member State law where the assistance of the Data Processor is implied, and where the assistance of the Data Processor is necessary for the Data Controller to comply with its obligations. This includes, but is not limited to, at the request to provide the Data Controller with all necessary information about an incident under 10) (ii), and all necessary information for an impact assessment in accordance with Article 35 and Article 36 of the GDPR.

Subprocessor:

15. The Data Processor may only engage a subprocessor, with prior specific or general written consent from the Data Controller. The Data Processor undertakes to inform the Data Controller of any intended changes concerning the addition or replacement of a subprocessor by providing a reasonable prior written notice to the Data Controller. The Data Controller may reasonably and in a duly substantiated manner object to the use of a subprocessor. The Data Processor must inform the Data Controller in writing of the discontinued use of a subprocessor.
16. Prior to the engagement of a subprocessor, the Data Processor shall conclude a written agreement with the subprocessor, in which at least the same data protection obligations as set out in this DPA shall be imposed on the subprocessor, including obligations to implement appropriate technical and organizational measures and to ensure that the transfer of Personal Data is done in such a manner that the processing will meet the requirements of the Applicable Law.
17. The Data Controller has the right to receive a copy of the relevant provisions of Data Processor's agreement with the subprocessor related to data protection obligations. The Data Processor shall remain fully liable to the Data Controller for the performance of the subprocessor obligations under this DPA. The fact that the Data Controller has given consent to the Data Processor's use of a subprocessor is without prejudice for the Data Processor's duty to comply with this DPA.