PROTOCOL

PROTOCOL NUMBER: BHT-II-002

A PHASE 2 RANDOMISED, DOUBLE BLIND, PLACEBO-CONTROLLED, PARALLEL GROUP, MULTICENTRE STUDY TO EVALUATE THE SAFETY AND EFFICACY OF REPEATED ORAL DOSES OF Blautix™ IN ADULT SUBJECTS WITH IRRITABLE BOWEL SYNDROME (IBS) SUBTYPES IBS-C and IBS-D

Sponsor Name and Address:	4D Pharma Plc 9 Bond Court Leeds, U.K LS1 2JZ
Date and Version:	6 February 2018; Version 1.0 6 April 2018; Version 1.1 19 May 2018: Version 1.2 31 May 2018: Version 2.0 21 June 2018: Version 2.1 13 February 2019: Version 3.0 31 March 2020: Version 3.1
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Protocol Approval Signature Page

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Sponsor Name: 4D Pharma Plc

Date and Version: **31-Mar 2020, Version 3.1**

Protocol Approved by the following:

4D Pharma Plc:

PPD

PPD

PPD (Apr 6, 2020)

Signature

Apr 6, 2020

Date

4D Pharma Plc:

PPD

PPD

PPD (Apr 6, 2020)

Signature

Apr 6, 2020

Date

PPD

Co-Ordinating Investigator

PPD

Date

Protocol Acceptance Form

Protocol Number:	BHT-II-002
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Sponsor Name:	4D Pharma Plc
Date and Version:	**31-Mar 2020, Version 3.1**

I have carefully read this Protocol and agree to conduct this trial as outlined in the Protocol with reference to Good Clinical Practice (GCP), applicable regulatory requirements, and in accordance with the Declaration of Helsinki. Any modification to the protocol must be agreed upon by both the investigator and 4D Pharma Plc and documented in writing. By written agreement to this protocol, I agree to allow direct access to all documentation, including source data to authorised individuals representing 4D Pharma plc (including monitoring staff and auditors), to Institutional Review Boards/Independent Ethics Committees (IRBs/IECs) and to regulatory authorities.

I am aware of my responsibilities as an investigator and agree to conduct the study according to these responsibilities and to appropriately direct and assist the staff under my control, who will be involved in the study.

Principal Investigator: PPD

PPD

Name (pr

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1 SYNOPSIS

TITLE OF STUDY:	
A multicentre Phase 2, randomised, double blind, placebo-controlled, parallel group study to evaluate the safety and efficacy of repeated oral doses of Blautix™ in adult subjects with irritable bowel syndrome (IBS), subtypes IBS-C and IBS-D	
NAME OF SPONSOR: 4D Pharma Plc	
Protocol Number: BHT-II-002	
Clinical Phase:	Phase 2

Objectives and Endpoints:

The objectives of the study are:

Primary Objective

To assess the efficacy of repeated twice daily doses of Blautix™ >1x10^{10}MPN for 8 weeks in adult subjects with either IBS-C or IBS-D

Secondary Objectives

To assess the safety of repeated twice daily doses of Blautix™ >1x10^{10}MPN for 8 weeks in adult subjects with either IBS-C or IBS-D

Exploratory Objective

CCI [redacted]

The endpoints of the study are:

Primary Efficacy Endpoint

The primary efficacy endpoint is whether or not the subject is an overall responder.

A subject is an 'overall responder' if they have reported an improvement in their weekly (Cohort specific) symptoms (abdominal pain intensity and stool frequency or consistency) for ≥ 50% of the treatment period (in this case 4 out of 8 weeks).

Secondary Efficacy Endpoints

Subject global assessment of relief

Stool consistency (Cohort C) /Stool frequency (Cohort D)

IBS-QOL

IBS-SSS

HADS **Exploratory Endpoints** CCI [redacted] [redacted] [redacted] **Safety Endpoints** Incidence, nature, severity, relatedness, seriousness, expectedness and outcome of adverse events Haematology and blood chemistry assessments Vital signs
Study Design: A randomised, double-blind, placebo controlled parallel group study.
Planned Number of Sites: Approximately 20 sites in North America, UK and Ireland
Study Population: 500 subjects with a diagnosis of IBS as defined by the Rome IV criteria. Subjects will be classified into Cohorts according to the Rome IV classification of IBS subtypes: Cohort C: subjects diagnosed with IBS- C Cohort D: subjects diagnosed with IBS-D Within each cohort, 250 subjects will randomly receive either Blautix™ or matching placebo in a 1:1 ratio overall of treated to control subjects.
Test Product, Dose and Route of Administration: Capsules of *Blautia hydrogenotrophica* (Blautix™) administered orally. Each capsule contains ≥5x 10^9MPN *Blautia hydrogenotrophica* /capsule and subjects will receive two capsules twice daily approximately 30 minutes before food for 8 weeks.
Reference Therapy, Dose and Route of Administration: Matching placebo, administered orally. Each placebo capsule will be comparable in size, weight and appearance to the test formulation and subjects will receive two capsules twice daily approximately 30 minutes before food for 8 weeks.
Duration of Study: **Subjects will be screened up to 4 weeks prior to commencement of dosing followed by an 8-week treatment period and a 4-week follow-up. In total, subjects will make 5 visits to the clinic including screening.**

Criteria for Evaluation

Efficacy:

Abdominal pain intensity using a numeric pain rating scale

Subject global assessment of relief

Stool consistency/frequency

IBS-QOL

IBS-SSS

HADS

Exploratory:

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[redacted]

[redacted]

Safety:

Safety evaluation will include occurrence of adverse events; clinical laboratory safety tests (serum biochemistry and haematology), physical examinations, vital signs (blood pressure, pulse rate, respiratory rate and oral temperature), 12-lead ECG parameters and BMI.

Statistical Analysis:

All baseline, compliance, disposition, efficacy and safety variables will be listed and summarised.

The standard summary statistics for continuous baseline and outcome variables are: N, mean, standard deviation, median, quartiles, minimum and maximum. The standard summary statistics for categorical baseline and outcome variables are: count and proportion (expressed as percentage).

The two Cohorts, Cohort C and Cohort D, will be analysed separately except where exploratory pooled or combined analyses are conducted across cohorts.

Primary Efficacy Analysis

The primary efficacy analysis is the comparison of the proportion of overall responders between the Blautix™ and placebo groups using Pearson's test with Yates's correction in the full analysis set within each Cohort.

All other analyses of the primary endpoint are secondary analyses.

Secondary Efficacy Analyses

Primary Endpoint:

Secondary Analyses of the Primary Endpoint will include the use of baseline characteristics as covariates and analyses using the Efficacy Evaluable Analysis Set.

Secondary Efficacy Endpoints:

Secondary efficacy endpoints will be compared between the two treatment groups within each Cohort using Pearson's test with Yates's correction, the Mann Whitney test or other standard statistical tests as appropriate.

Exploratory Efficacy Analyses

Exploratory efficacy analyses include but are not limited to:

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analysis of the impact of missing data on the outcome of the analyses.

Safety Analyses

Safety endpoints will be listed and summarised by Cohort and treatment group using standard summary statistics.

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

AE	Adverse event
BAM	Bile Acid Malabsorption
BHT	*Blautia hydrogenotrophica*
BSFS	Bristol stool form scale
BMI	Body mass index
CFU	Colony forming units
CH_4	Methane
CI	Confidence interval
CMC	Chemistry, manufacturing and controls
CRF	Case Report Form
CO_2	Carbon dioxide
CSBMs	Complete spontaneous bowel movements
DSMB	Data Safety Monitoring Board
EC	Ethics Committee
ECG	Electrocardiogram
eCOA	Electronic Clinical Outcomes Assessments
ELISA	Enzyme linked immunosorbent assay
FFQ	Food Frequency Questionnaire
FODMAP	Fermentable, Oligo-,Di-,Mono- Saccharide and Polyol
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
HADS	Hospital Anxiety and Depression Scale
HIV	Human Immunodeficiency Virus
H_2	Hydrogen
H_2S	Hydrogen sulphide
IBS	Irritable bowel syndrome
IBS-C	Constipation predominant IBS
IBS-D	Diarrhoea predominant IBS
IBS-HMA	IBS-human microbiota-associated

IBS-M	Mixed bowel pattern IBS
IBS-QOL	IBS Quality of Life
IBS-SSS	IBS-Symptom Severity Score
IBS-U	Unclassified IBS
ICF	Informed consent form
ICH	International Conference on Harmonization
IL	Interleukin
IMP	Investigational medicinal product
IRB	Institutional Review Board
ITT	Intent-to-treat
IV/WRS	Interactive Voice/Web recognition system
Kg	Kilogram
LBP	Live biotherapeutic product
L	Litre
MedDRA	Medical Dictionary for Regulatory Activities
MPN	Most probable number
NOEL	No observable effect level
NOAEL	No observable adverse effect level
OD	Optical density
PP	Per protocol
PCR	Polymerase Chain Reaction
PRO	Patient-reported outcome
QA	Quality Assurance
QC	Quality Control
QP	Qualified Person
QOL	Quality of life
qRT-PCR	Quantitative real-time polymerase chain reaction
SAP	Statistical analysis plan
NRS	Numeric Rating Scale
SAE	Serious adverse event

SRB	Sulphate-reducing bacteria
SUSAR	Suspected Unexpected Serious Adverse Reaction
TAMC	Total aerobic microbial count
TNF	Tumour necrosis factor
WHO	World Health Organisation

2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1.Background Information

Irritable bowel syndrome (IBS) is a chronic, debilitating, gastrointestinal functional disorder with an estimated population prevalence in the United States of approximately 12% (Chey, Kurlander, and Eswaran 2015) and in Europe between 10-15% (2). IBS is characterised by abdominal pain or discomfort in association with alteration in either stool form or frequency. IBS patients can be classified as either diarrhoea predominant (IBS-D), constipation predominant (IBS-C), or with a mixed bowel pattern (IBS- M). Furthermore, IBS is associated with several comorbidities, including somatic pain syndromes (fibromyalgia, chronic fatigue, and chronic pelvic pain), gastrointestinal disorders (gastroesophageal reflux and dyspepsia) and psychiatric disorders (i.e., depression, anxiety, and somatisation) (Chey, Kurlander, and Eswaran 2015). Therefore, IBS-related symptoms can be debilitating and lead to a significant reduction in quality of life.

While the complete pathophysiology of IBS has not been fully elucidated, a disruption of the function of the brain-gut axis and an altered intestinal microbiota and/or host immune response are thought to be important factors. The risk of developing IBS increases six-fold after acute gastrointestinal infection. Furthermore, antibiotics are known to affect the homeostasis of the gut microbiome and its usage appears to increase the risk of developing IBS. Several recent studies using high throughput sequencing methods have independently shown that the microbiota of IBS patients is distinct from that of healthy controls and is characterised by differentially abundant taxa and lower global diversity (Jeffery et al. 2012; Tap et al. 2017).

Studies have demonstrated that treatment with live bacteria may have beneficial effects on IBS symptoms by restoring homeostasis in the gut microbiota, normalisation of cytokine blood levels, normalising intestinal transit time, decreasing small intestine permeability, and eliminating small intestinal overgrowth of fermenting bacteria. This emerging field of research demonstrates the feasibility of therapeutic manipulation of the microbiome using live bacteria to improve symptoms of IBS. (Shanahan F and Quigley EMM. 2014).

2.2.Scientific Rationale Test

Blautix™ is a live biotherapeutic product (LBP) under development by 4D Pharma for the treatment of Irritable Bowel Syndrome. The active ingredient of Blautix™ is a strain of the bacterium *Blautia hydrogenotrophica* (*B. hydrogenotrophica*) (BHT), which is [redacted] for oral administration. *B. hydrogenotrophica* is a Gram-positive anaerobic, non-sporulating coccobacillus bacterium that is a sub-dominant commensal in the human gastrointestinal tract.

The Blautix™ strain of *B. hydrogenotrophica* is a hydrogen (H_2)/carbon dioxide (CO_2)-utilising acetogenic bacterium isolated from the faeces of a non-methane-excreting healthy human subject.

Irritable bowel syndrome (IBS) is characterised by altered bowel habits and abdominal pain in the absence of detectable organic causes (Enck et al. 2016). There are three clinical subgroups of IBS defined by constipation, diarrhoea, or alteration between the two. Over the medium term, (12– 48 months) there is significant transition between the subgroups, with more than 75% of individuals alternating from one subgroup to another (Drossman et al. 2005). It is not believed that IBS is an immune mediated disease as studies have shown that there is a minimal increase in inflammation either systemically or locally at the mucosal surface of the gastrointestinal tract; however, some studies have put forward a role for micro-inflammation. Although little is known about precise causes of IBS, there is compelling evidence for an increased occurrence of IBS after gastroenteritis and/or antibiotic treatment, both of which may alter the microbiome. Studies have shown that an altered microbiome is associated with IBS, alterations that cannot be explained by alterations in diet or oral-anal transit time (Distrutti et al. 2016). Tap and colleagues recently showed that the subjects with the largest microbiome alterations had the highest symptom severity scores and the lowest quality of life scores (Tap et al. 2017). This and other studies allow us to hypothesise that IBS either results from, or is exacerbated by, abnormal interactions between the gut microbiome and the epithelium (Collins 2014).

Several studies have characterised the microbiome of IBS individuals compared to healthy controls (Collins 2014). Overall there are reductions in the proportions of commensals that are known to be health associated and there are proportional increases in abundance of potentially harmful taxa such as *Escherichia coli*. Homeostasis of the gut is normally maintained through the action of the resident gut microbiota that metabolises indigestible dietary fibres and produces short chain fatty acids (SCFAs). SCFAs signal cells in the epithelium to produce a range of factors that include minimise oxygen levels in the gut by maximising epithelial oxygen consumption. Beneficial bacteria that break down fibre are obligate anaerobes and often require a narrow range of pH values and so thrive under normal bowel conditions. When homeostasis is interrupted, the increased diffusion of oxygen into the gut lumen and the dysregulation of SCFA production can allow enteric pathobionts such as *Escherichia coli* or members of the *Enterobacteriaceae* family, to outcompete the commensals, and thereby maintain a dysbiotic state.

This hypothesis for IBS aetiology is supported by literature showing that both the total production of SCFAs and relative concentrations of the various SCFAs are altered in the gut of individuals with IBS. Both increased and decreased production of SCFAs have been reported. Changes in the ratio of butyrate to other SCFAs may be explained by changes in the microbiome in IBS such as

the loss of *Faecalibacterium* and an increase in the abundance of *Ruminococcaceae* and Clostridium cluster XIV taxa.

Excess H_2 and H_2S may be generated by the increased fermentation typical of IBS, and these gases may damage the intestinal barrier and contribute to T-cell activation and inflammation, while butyrate and other short-chain fatty acids are protective against inflammation. Thus, it is conceivable that an imbalance in the butyrate/acetate ratio and the production of excess H_2S may be an intrinsic property of severe IBS.

B. hydrogenotrophica (Blautix™) is being developed for the treatment of IBS and may possess three properties that could ameliorate IBS symptoms by counteracting IBS pathophysiology. CCI

[redacted]

2.2.1 Non-clinical Studies

2.2.1.1 Non-clinical Pharmacology

CCI

[redacted]

CCI

[redacted]

2.2.1.2 Toxicology

CCI

[redacted]

2.2.2 Clinical Studies

CCI

[redacted]

2.3.Potential Risks and Benefits

2.3.1 Potential Risks

The risk of administering Blautix™ is considered small as *B. hydrogenotrophica* is a bacterium that is a sub-dominant commensal in the human gastrointestinal tract. CCI

2.3.2 Known Potential Benefits

Although there was no statistically significant clinically relevant effect on symptoms of IBS observed in the Phase I study, treatment with Blautix™ demonstrated a trend towards reduction of abdominal pain/discomfort/cramps, bloating and diarrhoea by more than 33%. Blautix™ has also been shown to exert a positive impact on the microbiota in both pre-clinical models and the phase I clinical study.

2.3.3 Risk-Benefit Assessment

In this study, there may be some potential benefit of an 8-week treatment of Blautix™ $>1x10^{10}$ MPN given twice daily to IBS patients in the alleviation of IBS-related symptoms and associated quality of life.

The risk benefit assessment is considered appropriate to continue clinical development and initialise the Phase 2 study in the population described.

3 STUDY OBJECTIVES AND ENDPOINTS

3.1 Objectives

The objectives of the study are:

Primary Objective

To assess the efficacy of repeated twice daily doses of Blautix™ >1x10^{10}MPN for 8 weeks in adult subjects with either IBS-C or IBS-D

Secondary Objectives

To assess the safety of repeated twice daily doses of Blautix™ >1x10^{10}MPN for 8 weeks in adult subjects with either IBS-C or IBS-D

Exploratory Objective

CCI

[redacted]

3.2 Endpoints

Primary Efficacy Endpoint

The primary efficacy endpoint is whether the subject is an overall responder.

A subject is an 'overall responder' if they have reported an improvement in their weekly (Cohort specific) symptoms for $\geq$ 50% of the treatment period (4 out of 8 weeks).

	IBS-C	IBS-D
Primary Endpoint Responder Symptom Definition	**Abdominal Pain Intensity** Decrease in weekly average of worst abdominal pain in the past 24 hours score of at least 30% compared with baseline And **Stool Frequency** Increase of 1 or more Complete spontaneous bowel movements (CSBM) per week compared with baseline.	**Abdominal Pain Intensity** Decrease in weekly average of worst abdominal pain in past 24 hours score of at least 30% compared with baseline and **Stool Consistency (BSS)** Decrease at least 50% in the proportion of days per week with at least one stool that has a consistency of Type 6 or 7 compared with baseline.

Secondary Efficacy Endpoints

- Subject global assessment of relief
- Stool consistency/frequency
- IBS-QOL
- IBS-SSS
- HADS

Exploratory Endpoints

CCI [redacted]

Safety Endpoints

- Incidence, nature, severity, relatedness, seriousness, expectedness and outcome of adverse events
- Haematology and blood chemistry assessments
- Vital signs

4 STUDY RATIONALE AND DESIGN

4.1 Rationale

4.1.1 Rationale for the Study Design

This is a randomised, placebo-controlled, parallel group, repeat dose multicentre study in adult male and female subjects of 18 to 70 years of age with a confirmed diagnosis of either IBS- C or IBS-D.

An 8-week treatment period of Blautix™ or placebo is considered sufficient to assess any changes in the subject assessment of IBS symptoms and the analysis of pharmacodynamic changes on the microbiota. Subject assessment of symptoms by use of rating scales/scores and a quality of life questionnaire are considered appropriate.

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Studies of microbiota composition in IBS have indicated that those patients with a normal microbiota displayed higher scores for anxiety and depression, whereas IBS patients with an abnormal microbiota had on average normal scores for anxiety and depression (ref). The Hospital Anxiety and Depression score (HADS) will be used to determine levels of anxiety and depression during the study.

Use of periodic dietary recall by means of the Food Frequency Questionnaire (FFQ) may be used to determine if dietary habits influence exploratory study outcomes.

4.1.2 Rationale for the Dosage

Blautia hydrogenotrophica is a commensal bacterium that is naturally present in the human body. The bacterium was isolated and purified from human faeces and pre-clinical toxicology did not detect any clinical, morphological or laboratory deviations of clinical relevance. As *Blautia hydrogenotrophica* is a bacterium that naturally occurs in man, it is expected that it does not have any genotoxic, carcinogen, or mutagenic potential.

The dose of *B. hydrogentotrophica* administered in the study will be two capsules twice a day. Each capsule will contain a minimum of 5 x 10^9 MPN. Hence the daily dose will be 10^{10} to 10^{11} MPN. There is no established methodology for calculating human equivalent dose for live biotherapeutic products. CCI In order to calculate the equivalent human dose, body surface area calculations were used. It is known that the ratio of intestinal surface area (possibly the most relevant aspect for live biotherapeutic dosing) to total body surface area in rodents is similar to man (Casteleyn et al. 2010). In order to calculate body surface area, MPN/kg was first calculated, then multiplied by published factors (Nair and Jacob, 2016) to convert to MPN/m^2.

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[redacted]

4.2 Study Design

4.2.1 Overall Study Design

Subjects who are potentially suitable for the study will be identified from current patient lists at appropriate gastroenterology clinics and or by advertisement. Subjects will be approached to determine if they are interested and willing to participate. The investigative sites will provide interested subjects with a 'Research Study Information and Informed Consent Form' and they will be booked in for the initial screening visit when consent will be obtained, and the Subjects will be asked to sign the Consent Form

Screening Visit (Up to 4 weeks before dosing starts Day -28 to Day -1)

Following completion of the study consent form, subjects will be screened against the protocol requirements, and a medical history and concomitant medications will be documented, a full physical examination will be performed, and bloods will be drawn for haematology, serum biochemistry and viral serology. Vital signs (pulse, blood pressure, respiratory rate and body temperature) will be assessed and recorded, and a 12 lead ECG will be taken. Women of child bearing potential will have a serum pregnancy test. Height and weight will be measured, and if subjects fulfil all the eligibility criteria at this part of the Screening process they will be provided with a Smartphone containing an 'Electronic Clinical Outcomes Assessment' (eCOA) App and will be provided with training and instructions on how to use the device. At this Visit they will complete the IBS-Symptom Severity Score (SSS) on a tablet device during the clinic visit.

The subject will be asked to take the smart phone home and complete the following assessments daily over a 7-day period. These assessments will need to be completed prior to attending the clinic for their Baseline Visit 1

- An abdominal pain intensity rating scale daily
- Bowel movements, Stool consistency/frequency daily and laxative use
    - If no bowel movements, this should be recorded as zero

The subject will need to ensure they complete these two assessments at least one day prior to the day next clinic visit. As soon as the subjects have completed their assessments and click submit on the smartphone, their data will be sent to the hub for review by the investigator and study team. The same smartphone will be used throughout the study for all patient reported outcome assessments.

Subjects will be provided with a kit with instructions for the collection of a stool sample. They will be asked to provide a stool sample from the night before or on the morning of the next clinic visit. If necessary, a stool sample may be provided up to 2 days after the visit provided it is from the night before or from the day it is delivered to the clinic.

The next clinic visit will be booked with the Subjects prior to leaving the clinic.

Reasons for screen failure will be documented in the subject's notes. Subjects may be re-screened once, after the initial screening visit if considered to be appropriate by the Investigator. A waiting period of at least 2 weeks is required between screening visits.

Baseline: Visit 1 (Day 1)

Subjects will return to the clinical unit on Day 1 (Visit 1; within 28 days of screening visit or re-screening visit if applicable). The study doctor will review the subject screening data obtained from the smartphone eCOA app and the subject will be classified into either IBS-C or IBS-D subtypes.

A brief physical examination, assessment of vital signs and a urine pregnancy test for female subjects of child bearing potential, weight and BMI will be conducted and any changes in medication and adverse events will be checked. Blood samples will be taken for measurement of inflammatory markers (cytokine levels). A fresh urine sample will be taken to analyse the metabolites.

Final confirmation of eligibility for the study will be documented, signed by the Investigator and filed in the subject notes.

Classification into IBS subtype will be confirmed, and randomisation will take place. Subjects will receive either Blautix™ or placebo capsules, with instructions for two (2) capsules to be taken at approximately 12 hourly intervals; morning and evening, approximately 30 minutes before meals, giving a total of four (4) capsules per day. Subjects will receive instructions about storing the capsule packs in the fridge and be asked not to open the capsules. Subjects will be provided with enough medication to last until the next clinic visit.

Subjects will use their smartphone issued at the Screening visit to capture their study related data. The IBS-SSS and FFQ questionnaires will be performed at the clinic on a tablet device.

- Information on Adverse Events
- Review of Medication log completed daily
- Abdominal Pain Score completed daily
- Stool consistency, frequency, spontaneity and laxative use, completed daily
- Subject Global Assessment of Relief completed weekly
- IBS Quality of Life questionnaire (IBS QOL) on the Day of Clinic Visit or within 24 hours prior to the visit
- IBS Symptom Severity Score (IBS-SSS) on the Day of each Clinic visit

- Hospital Anxiety and Depression score (HADS) on Day of each Clinic visit or within 24 hours prior to the visit
- Food Frequency Questionnaire (FFQ) on the Day of each Clinic visit
- Review of concomitant medications

Subjects will be provided with a contact card containing an appropriate contact telephone number for the study team and hospital, should they need medical advice.

Subjects will be provided with a kit with instructions for the collection of a stool sample. They will be asked to provide a stool sample from the night before or on the morning of the next clinic visit.

Visit 2 (Week 4)

Subjects will return after 4 weeks (± 7 days) for a safety check including adverse events and to record any changes in concomitant medication. A urine pregnancy test for female subjects of childbearing potential. Blood samples will be taken for measurement of inflammatory markers (cytokine levels). A fresh urine sample will be taken to analyse the metabolites.

Subjects will continue to use their smartphones to capture all the patient related outcome data, except for IBS-SSS and FFQ questionnaires which will be completed during the clinic visit on a tablet device. The data will be reviewed on an ongoing basis as required by the study team and sponsor. At this visit the following information will be reviewed with the subject;

- Information on Adverse Events
- Review of Medication log completed daily
- Recording times of taking their medication completed daily
- Abdominal Pain Score completed daily
- Stools consistency/frequency and use of laxatives daily
- Subject Global Assessment of Relief completed weekly
- IBS Quality of Life questionnaire (IBS QOL) on the Day of Clinic Visit or within 24 hours prior to the visit
- IBS Symptom Severity Score (IBS-SSS) on the Day of Clinic visit
- Hospital Anxiety and Depression score (HADS) on Day of each Clinic visit or within 24 hours prior to the visit
- Food Frequency Questionnaire (FFQ) on the Day of Clinic visit
- Review of concomitant medications

Subjects will receive further study medication to last until the next clinic visit. Subjects will be reminded to take their medication; two (2) capsules to be taken at 12 hourly intervals approximately 30 minutes before meals. Subjects will receive a total of four (4) capsules per day and will be reminded to store the capsules in the fridge and not to open the capsules.

Subjects will be provided with a kit with instructions for the collection of a stool sample. They will be asked to provide a stool sample from the night before or on the morning of the next clinic visit

End of Treatment (Week 8)

Subjects will return to the clinic at the end of the 8-week (or up to -7 days) treatment period. Subjects will be assessed (vital signs, concomitant medication and adverse events) and a physical examination, including weight and BMI and an ECG will be conducted. A urine pregnancy test for female subjects of child bearing potential. Bloods for haematology, serum biochemistry and inflammatory markers will be taken. A fresh urine sample will be taken to analyse the metabolites.

Subjects will continue to use their smartphones to capture all the patient related outcome data, except for IBS-SSS and FFQ questionnaires which will be completed during the clinic visit on a table device. The data will be reviewed on an ongoing basis as required by the study team and sponsor. At this visit the following information will be reviewed with the subject;

- Information on Adverse Events
- Review of Medication log completed daily
- Abdominal Pain Score completed daily
- Stool consistency/frequency and laxative use daily
- Subject Global Assessment of Relief completed weekly
- IBS Quality of Life questionnaire (IBS QOL) on the Day of Clinic Visit or within 24 hours prior to the visit
- IBS Symptom Severity Score (IBS-SSS) on the Day of Clinic visit
- Hospital Anxiety and Depression score (HADS) on Day of Clinic visit or within 24 hours prior to the visit
- Food Frequency Questionnaire (FFQ) on the Day of Clinic visit
- Review of concomitant medications

Subjects will be provided with a kit with instructions for the collection of a stool sample. They will be asked to provide a stool sample from the night before or on the morning of the next clinic visit

Follow-Up (Week 12-14)

Subjects will return 4-6 weeks after the last dose of study medication for a safety check and clinical update. All female subjects of child bearing potential will have a urine pregnancy test. A physical examination, weight and vital signs will be measured and recorded, changes in concomitant medication and adverse events will be recorded. Bloods will be drawn for haematology, serum biochemistry CCI [redacted]

Subjects will use their smartphone issued to capture their study related data, except for IBS-SSS and FFQ questionnaires which will be completed during the clinic visit on a tablet device.

- Information on Adverse Events
- Abdominal Pain Score completed daily
- Stool consistency/frequency and laxative use, daily
- Subject Global Assessment of Relief completed weekly
- IBS Quality of Life questionnaire (IBS QOL) on the Day of Clinic Visit or within 24 hours prior to the visit
- IBS Symptom Severity Score (IBS-SSS) on the Day of Clinic visit
- Hospital Anxiety and Depression score (HADS) on Day of Clinic visit or within 24 hours prior to the visit
- Food Frequency Questionnaire (FFQ) on the Day of Clinic visit
- Review of concomitant medications

At this visit the smartphones will be returned from the subjects and given back to the study site team.

Summary of Assessments

Details of the study assessments and other procedures to be performed are presented in Table 1 and in Section 7, Study Assessments.

TABLE 2: SCHEDULE OF ASSESSMENTS AND PROCEDURES

	Screening Visit (Day-28 to Day-1)	Baseline Visit 1 (Day 1) Week 1	Visit 2: Week 4 (±7 days)	End of Treatment Week 8 (-7 days)	Follow-Up: Week 12-14
General Assessments					
Informed Consent (prior to any screening procedures)	x				
Inclusion/Exclusion	x	x			
Medical history/demographics	x				
Physical examination	x	x		x	x
Vital signs	x	x		x	x
Weight/Height/BMI	x	x[e]		x[e]	x[e]
ECG	x			x	
Concomitant medication	x	x	x	x	x
Adverse Event Recording [a]	x	x	x	x	x
Randomisation (8 Week Treatment Period)					
Study Drug		x	x		
Dosing Diary[b]		x	x		
Laboratory Assessments					
Blood samples (haematology and clinical chemistry)	x			x	x
Blood sample (viral serology)	x				
CCI [redacted]		x	x	x	x
Stool sample		x	x	x	x
CCI [redacted]		x	x	x	x

Pregnancy test [c]	x	x	x	x	x
Clinical Outcome Assessments - eCOA					
Abdominal Pain score[d]	x	x	x	x	x
Stool frequency and consistency score[d]	x	x	x	x	x
Food Frequency Questionnaire		x	x	x	x
Subject Global Assessment of Relief		x	x	x	x
IBS Quality of Life (QOL)		x	x	x	x
IBS-Symptom Severity Score (SSS)	x	x	x	x	x
Hospital Anxiety and Depression score (HADS)		x	x	x	x

[a] **Screening Visit:** Any AE's occurring prior to randomisation will be recorded as medical history; any SAEs or study related AEs that occur after signing the ICF but before randomisation will be recorded

[b] **Dosing Diary**: Patients will be provided with a smartphone at screening to capture Clinical Outcome Assessments (eCOA) and study treatment dosing data.

[c] **Pregnancy Test :** Serum at screening and Urine Dipstick test for all other visits. Female patients of childbearing potential are to continue using highly effective (refer to section 4.2.1) contraception for two (2) menstrual cycles after the last dose of study medication. Male subjects, who are not vasectomised must use a barrier method of birth control from randomisation until the follow-up visit.

[d] **Abdominal Pain Score and Stool Frequency and Consistency score:** These assessments will be recorded daily from randomisation and over a 7-day timeframe prior to randomisation to allow the investigator to classify into IBS-D or IBS-C subtypes

[e] **Weight** only to be measured and BMI calculated, after the screening visit

4.2.2 Stopping Criteria

4.2.2.1 Individual Subjects

Subjects will be withdrawn from the study on safety grounds to be established by the DSMB and as per section 5.4.1.

4.2.2.2 Study as a Whole

The DSMB will establish criteria for stopping the study. If the criteria are met, a substantial amendment will be filed, and the study will be stopped temporarily, and the events investigated to determine if they are related to the investigational medicinal product (IMP). If it is determined that the events are related to the IMP, the study will be stopped, and the Health Authorities and ethics committees informed.

4.2.3 Independent Data and Safety Monitoring Board (DSMB)

An independent DSMB comprising experts in drug safety, biostatistics and other relevant fields will be established by the sponsor to assess the progress of the trial, the safety data and efficacy endpoints. Stopping criteria for individual subjects and the study will be established.

The composition, responsibilities and procedures of the Board will be documented prior to the commencement of the study and randomisation of the first subject. Recommendations of the Board and actions taken by the sponsor will be documented.

5 POPULATION

500 subjects diagnosed with IBS will be enrolled into the study. Diagnosis of IBS will be defined by the Rome IV criteria and must be met for the last 3 months with symptom onset at least 6 months prior to diagnosis.

IBS patients will be screened based on the inclusion and exclusion criteria described in 5.1 below, and will be classified into one of the following Cohorts according to the Rome IV classification of IBS subtypes.

Cohort C: 250 subjects diagnosed with IBS-C

Cohort D: 250 subjects diagnosed with IBS-D

5.1 Inclusion Criteria

All subjects must meet all the following inclusion criteria:

1. Written consent on an Institutional Review Board (IRB)/ Independent Ethics Committee (IEC) approved ICF before any study specific evaluation
2. Males and Females between 18 and 70 years of age
3. Body Mass Index (BMI): 18-39 kg/m^2
4. Having IBS-C or IBS-D as defined by Rome IV, including Subtype Classification as defined per Table 2

   Recurrent abdominal pain on average, at least 1 day/week in the last 3 months associated with two or more of the following criteria:

- Related to defecation

- Associated with a change in frequency of stool
- Associated with a change in form (appearance) of stool

The above criteria must be met for the last 3 months with symptom onset at least 6 months prior to diagnosis.

IBS-C and IBS-D subtypes will be further classified as follows:

Table 3:

IBS-C	IBS-D
□ Abdominal Pain Intensity: weekly average of worst daily (in past 24 hours) abdominal pain score of > 3.0 on a 0 to 10-point scale And □ Stool Frequency: more than 25% of bowel movements with a consistency of Type 1 or Type 2 Bristol stool chart and less than 25% of bowel movements with Bristol stool form Type 6 or Type 7. Subject must have fewer than 3 CSBMs[1] within a one week period (7 days)	□ Abdominal Pain Intensity: weekly average of worst daily (in past 24 hours) abdominal pain score of > 3.0 on a 0 to 10-point scale And □ Stool Consistency: more than 25% of bowel movements with a consistency of Type 6 or Type 7 Bristol stool chart and less than 25% of bowel movements with Bristol stool form Type 1 or Type 2. Subjects must have at least one Type 6 or Type 7 bowel movements on at least four days within a one week period (7 days).

*Stool types based on Bristol Stool Chart; Hard stools -Type 1 and 2; Soft stools – Type 6 and 7 (see Appendix 3).
Source: (https://irritablebowelsyndrome.net/clinical/new-rome-iv-diagnostic-criteria/) (see Appendix 6)
[1] CSBM defined as a bowel movement that is both complete and spontaneous. Bowel movements where laxative use is recorded in the concomitant medication questions in a 24 hour period, by IBS-C subjects will not be counted as CSBMs when subtyping for randomisation.

5. Have a moderate or severe IBS symptom severity score ; ≥175 at the screening visit as defined by IBS-SSS. A tolerance of -10% (≥ an IBS-SSS score of 157.5) will be allowable at the Baseline (Visit 1).

5.2 Exclusion Criteria

Any of the following criteria will **exclude** the Subject from study participation:

1. Males or females <18 and >70 years of age
2. Have an IBS symptom severity score < 175 as defined by IBS-SSS
3. BMI: <18 or >39 kg/m^2
4. Have a significant acute or chronic coexisting illness (cardiovascular, gastrointestinal, endocrine, immunological, metabolic or any condition which contraindicates, in the investigators' judgment, entry to the study)
5. Confirmed clinical diagnosis of bile acid malabsorption and / or on medication for bile acid malabsorption

6. Individuals who, in the opinion of the investigator, are poor attendees or unlikely for any reason to be able to comply with the study requirements
7. Patient is currently enrolled in or has not yet completed at least 30 days since ending other investigational device or drug study(s), or patient is receiving other investigational agent(s)
8. Have an active or recent (within 3 years) malignant disease or any concomitant end-stage organ disease. A non-melanoma skin cancer that has been adequately treated with no recurrence within 3 months of screening is not excluded.
9. Females who are pregnant or breast feeding
10. Refusal to use acceptable methods of birth control (true abstinence, sterilisation, birth control pills, injections or contraceptive implants) for women of child bearing potential while on treatment and following completion of 2 menstrual cycles/ months after the last dose of study treatment. For Males, a barrier method of birth control from randomisation until the Follow-Up visit, unless vasectomised
11. Use of antibiotics within 30 days of screening
12. Use of systemic steroids within 30 days of screening
13. Change in dose or introduction of an antipsychotic within the last month
14. Have suffered from an uncontrolled or current major psychiatric disorder
15. Clinically diagnosed Lactose intolerance
16. Clinically diagnosed Coeliac disease
17. Change of diet e.g. FODMAP, gluten-free within last 3 months
18. Those > 50 will be excluded if their diagnosis of IBS is recent (<12 months) and if they have not had a sigmoidoscopy or colonoscopy within previous 5 years.
19. Any GI related abdominal surgery other than hernia repair or appendectomy. Cholecystectomy more than 6 months previously is not an exclusion
20. Subjects taking prucalopride
21. Known HIV infection, or hepatitis A, B, or C active infection
22. Subjects with abnormal laboratory values at screening deemed by the investigator to be clinically significant
23. Subjects who have taken commercially available probiotics within the last month (30 days prior to randomisation). See Appendix 7
24. Subjects with known or suspected hereditary fructose intolerance, glucose-galactose malabsorption or sucrase-isomaltase insufficiency
25. Subjects taking guanylate cyclase agonists; such as linaclotide and lubiprostone

5.3 Treatment assignment procedures

5.3.1 Randomisation

This is a double-blind study and subjects will randomly receive either Blautix™ or placebo in a 1:1 ratio respectively. At Baseline (Visit 1) , subjects will be classified into either the IBS- C (Cohort C) or the IBS-D (Cohort D) cohort of subjects and 250 subjects will be enrolled into each cohort. Within each cohort, 125 subjects will randomly receive Blautix™ and 125 subjects will randomly receive matching placebo. Allocation of randomisation numbers will be performed centrally using an interactive voice/web response allocation system (IV/WRS).

Subjects, 4D pharma Plc, designated CRO and clinical site staff will be blinded to the investigational product allocation for each subject. Randomisation data will be kept strictly confidential, filed securely by the Sponsor (or designee) and accessible only to authorised persons of the Sponsor (or designee), and accessible only to authorised per Sponsor (or designee)'s standard operating procedures (SOPs) until the time of unbinding.

5.3.2 Blinding Procedures

The blinding will be maintained using placebo capsules comparable in appearance to the Blautix™ capsules.

5.3.3 Subject Numbering

The investigators must maintain a screening log of all screened subjects and those randomised into the study.

Each subject will be given a unique identification subject number, to facilitate anonymous identification within the study. Once assigned to a subject, a subject number will not be re-used.

5.3.4 Emergency Unblinding Procedures

The IV/WRS will be used for emergency unblinding of the treatment codes. The treatment codes should not be broken except in medical emergencies when the management of the subject requires knowledge of the treatment randomisation. The investigator must sign and date the code break form supplied and document the reason for breaking the code. The Sponsor Medical Monitor should be informed as soon as possible and within 24 hours of the event.

In addition, each subject will be given a card and asked to carry it with them at all times in case of an emergency. The card will include the study number, subject number, Investigational product and the name and 24-hour telephone number of the investigative site. Subjects should be asked to return this card at the end of the study.

The Sponsor's pharmacovigilance provider may break the code for serious adverse events that are unexpected and are suspected to be causally related to the investigational product and potentially require expedited reporting to the regulatory authorities.

5.4 Subject withdrawal

5.4.1 Reasons for Withdrawal

Subjects have the right to withdraw from the study at any time and for any reason without prejudice to future medical care by the investigator or at the institution.

Reasons for removal from the Protocol-required investigational product(s) or procedural assessments might include:

- Withdrawal of informed consent
- Pregnancy
- Investigator's opinion that it is in the best interests of the subject

- Safety concern (due to an AE, Failure to follow contraception, and/or protocol requirements)

If a subject withdraws from the trial prematurely, the Investigator must determine the primary reason for this and record it in the CRF. If possible, subjects who withdraw from the study after the start of dosing and before completion should be seen by an Investigator and undergo the assessments and procedures scheduled for the follow-up visit. For subjects who are lost to follow-up, the investigator should show due diligence by documenting in the source documents all steps taken to contact the subject.

5.4.2 Replacement of Subjects

Subjects who are withdrawn from the study by the Investigator due to adverse events will not be replaced. Subjects who withdraw for any reason before the first dose or for reasons other than adverse events after the start of dosing may be replaced.

6 INVESTIGATIONAL PRODUCTS

6.1 Formulation

Blautix™ is formulated as CCI [redacted] for oral administration CCI [redacted]

CCI [redacted]

6.2 Dosage, Frequency and duration of Dosing

Subjects will be randomly assigned at Baseline (Visit 1, Day 1) to 1 of 2 treatment groups, as follows:

Blautix™ or placebo capsules will be administered twice daily at approximately 12 hourly intervals; morning and evening; approximately 30 minutes before food for a duration of 8 weeks. CCI [redacted] Subjects will be supplied with enough treatment supply at the Baseline Visit to last until the next scheduled Clinic Visit and then at subsequent visits.

The Pharmacy manual will include further detail regarding treatment storage and dosing guidelines.

6.3 Packaging and Labelling

Packaging and labelling will be in accordance with Good Manufacturing Practice Annexe 13 requirements and released by a Qualified Person (QP) in accordance with the Clinical Trials Directive 2001/20/EC and GMP Directive 2003/94/EC for Investigational Medicinal Products.

6.4 Storage and Expiry

CCI [redacted] Shelf life will be assigned in accordance with emerging stability data.

Arrangements will be made for subjects to transport their capsules in cool boxes between the study site and their home. CCI [redacted]

6.5 Treatment Compliance

Subjects will be asked to take their study medication (2 capsules twice daily) at approximately 12 hourly intervals from their first dose, approximately 30 minutes before food. They will record the time that they took their medication in their smartphone which will be supplied to them at Screening. Doses will be taken in the morning two (2) capsules approximately 30 minutes before breakfast and in the evening two (2) capsules approximately 30 minutes before the evening meal. Subjects will be asked not to open the capsules, CCI [redacted] and to return any unused capsules.

6.6 Accountability

An accountability record must be maintained at the Investigator site to record the date and number of capsules, received, dispensed to subjects, returned from subjects and not used.

At the end of the study, a full reconciliation must be documented and checked by the Monitor from the Sponsor. Supplies must be retained at the Investigator site until instructions for return and destruction are provided by the Sponsor.

6.7 Permitted Dose Adjustments and Interruptions

No dose adjustments or interruptions will be permitted. Treatment should be withdrawn in subjects who are unable to tolerate it.

6.8 Concomitant Treatment

Any concomitant medication taken within 30 days prior to the first dose of study medication and up to the follow-up visit should be recorded in the case report forms.

6.9 Prohibited Medication

Probiotics such as yogurts, yogurt-type drinks and other soft drinks e.g. fruit juices may be taken during the study. Probiotic supplements formulated as capsules or sachets are prohibited. Acceptable probiotics taken during the month before screening should be continued through the study period and to follow-up to ensure consistency.

Prucalopride is a prohibited medication and should not be taken during the study.

IBS-C subjects should not take guanylate cyclase agonists (such as linaclotide) and lubiprostone. IBS-D subjects should not take bile acid sequestrants (such as cholestyramine, colestipol, colesevelam), 5-hydroxytryptamine (serotonin) 3 receptor antagonists (such as alosetron), rifaximin and eluxadoline (see Appendix 7).

Subjects are not eligible for the study if they have received antibiotics or systemic corticosteroids within 30 days prior to the screening visit. Subjects on inhaled steroids for asthma may be included but oral or infused steroids for asthma are not permitted. If these medications are required during the study, the subject does not need to be withdrawn but details will be recorded in the concomitant medication case report forms.

7 STUDY ASSESMENTS

7.1 Clinical Assessments

Medical History

A medical history including prior and current medication will be obtained during screening. In addition, demographic data will be collected, including gender, race, and ethnic origin.

Physical Examination

A full physical examination will be performed by the Investigator at screening and at study visits as indicated in Table 1. Body weight in kilograms (kg) and height in metres (m) will be measured and the BMI will be calculated.

Vital Signs

Blood pressure, heart rate, respiratory rate and temperature will be taken as indicated in Table 1. Blood pressure and heart rate will be measured in the supine position using an automated blood pressure monitor.

ECG

A 12-lead ECG will be taken as indicated in Table 1. Subjects will be in a supine position prior to recording an ECG. The ECG traces will be reviewed and commented on by the Investigator and the traces signed and dated.

Subject Assessments:

Refer to Table 1: Schedule of Assessments and Procedures. The assessments will be completed by the subject either at home using the smartphone provided or during the clinic visit.

<u>Abdominal Pain Score</u>

Subjects will be asked to rate their worst abdominal pain over the past 24 hours using an 11-point Numeric Rating Scale (NRS) and a weekly average will be recorded. This data will be recorded daily for a 7-day period up to Baseline (Visit 1) to allow Subject to be classified into Cohorts of IBS-C or IBS-D sub-types. Once subjects are randomised they will be asked to record their score daily on their smartphone.

Subjects will respond to the following question: ‘How much abdominal pain have you felt today’ on a scale from 0 (none) to 10 (worst possible pain)” using an 11-point NRS ranging from 0 (none) to 10 (worst possible pain)?

Subject Global Assessment of Relief

A weekly subject response to the question Please consider how you felt this past week in regard to your IBS, in particular your overall wellbeing and symptoms of abdominal discomfort, pain and altered bowel habit. Compared to the way you usually felt before entering the study, how would you rate your relief of symptoms during the past week?.

Using a 5-point Likert scale with the following response options:

Completely relieved; considerably relieved; somewhat relieved; unchanged; worse

Subjects will be asked to record their assessment weekly on their smartphone.

IBS Quality of Life (QOL) (Appendix 1)

Subjects will be asked to complete a quality of life questionnaire consisting of 34 questions with a five-point response. The individual responses to the 34 items are summed and averaged for a total score and then transformed to a 0-100 scale for ease of interpretation with higher scores indicating better IBS specific quality of life. Once subjects are randomised they will be asked to record their score on their smartphone at each clinic visit or within 24hrs prior to the Clinic Visit.

Hospital Anxiety and Depression Score (HADS) (Appendix 2)

Subjects will be asked to complete HADS which is a 14-item scale that generates ordinal data. Seven of the items relate to anxiety and 7 relate to depression. Each item is scored from 0-3 so a subject can score 0 to 21 for either anxiety or depression. Once subjects are randomised they will be asked to complete this questionnaire on their smartphone at each clinic visit or within 24hrs prior to the Clinic Visit.

Bristol Stool Chart

Subjects will be asked to record stool frequency and consistency. Stool types will be based on the Bristol Stool Chart (Appendix 3). This data will be recorded daily for a 7-day period up to Baseline (Visit 1) to allow Subject to be classified into Cohorts of IBS sub-types. Once subjects are randomised they will be asked to record their stool frequency daily up to 10 bowel movements per day and score each stool type on their smartphone. No bowel movements in any given day will also be recorded.

Stool frequency will be defined as a sum of weekly CSBMs. Stool frequency will be recorded using the following question: "How many times did you open your bowels during the past 24 hours? If you have not had any bowel movements today, please enter 0." Subjects will be reminded to rate all of their bowel movements in the Bristol Stool Chart before answering the question. CSBMs will be determined by absence of laxative use recorded in concomitant medication questions for the same 24 hour period.

IBS Symptom Severity Score (SSS) (Appendix 4)

Subjects will be asked to complete a questionnaire on severity of abdominal distension, severity of abdominal pain, frequency of abdominal pain, dissatisfaction with bowel habits, and interference of IBS symptoms with daily life. A score ranging from 0 (no symptoms) to 500 (maximum severity) can be achieved as mild (74-174), moderate (175-299) or severe (>300) IBS symptoms.

The initial data from this questionnaire will be collected during the Screening visit on a tablet device during the clinic visit. This data will be used to classify into Cohorts of IBS sub-types prior to randomisation. Once subjects are randomised, they will be asked to record their responses to the questionnaire on a tablet device during each clinic visit.

Food Frequency Questionnaire (FFQ) (Appendix 5)

Subjects will be asked to complete a questionnaire to obtain frequency information about food and beverage consumption over a specified period of time (day, week and month). FFQ completion at baseline will be in relation to food (s) consumed over the prior 6 months. At each subsequent visit the FFQ shall capture responses to food (s) consumed since last subject visit. Subjects will be asked to complete this questionnaire on a tablet device during each Clinic Visit.

- Concomitant Medications:

Subjects will be asked to record name, dose and time of administration of any medication, other than IMP, taken during the study. This will be reviewed at each visit.

Dosing Diary:

Subjects will be asked to confirm their daily study medication intake by indicating "yes" or "no" to the following questions on the smartphone provided: "Did you take two of your study medication capsules this morning? " in the morning and "Did you take two of your study medication capsules this evening?" in the evening. Subjects will be prompted with reminders via this device.

Diet and Study Restrictions

Dosing should be twice daily at twelve hourly intervals (approximately 30 minutes before breakfast and 30 minutes before evening meal). Subjects with lactose intolerance and Coeliac disease and those with a change of diet within the 3 months before screening are excluded. Subjects taking commercially available supplements (probiotics formulated as capsules or sachets as in Appendix 7) are excluded 1 month (30 days) prior to randomisation and throughout the study to follow-up. Probiotic yogurts, yogurt-type drinks and soft drinks e.g. fruit juices are acceptable if taken during the month before screening and continued to follow-up.

7.2 Laboratory Assessments

A Laboratory Manual will be prepared to document the address and contact numbers for all laboratories involved in the study and the collection, processing, shipment, storage and reporting procedures for all samples.

7.2.1 Safety Assessments:

Refer to Table 1: Schedule of Assessments and Procedures for sample collection timepoints.

Blood Samples:

Haematology:

Haemoglobin (HGB), haematocrit (HCT), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), red blood cells (RBC), C-reactive Protein (CRP), white cell count (WCC) and platelets (PLT). Differential white cell count will include

neutrophils, lymphocytes, eosinophils, basophils and monocytes as absolute values and percentages.

Clinical Chemistry:

Total protein (TP), albumin (ALB), total bilirubin (BIL-T), alanine transaminase (ALT), aspartate transaminase (AST), gamma-glutamyl transferase (GGT), glucose (GLU), sodium (NA), potassium (K), bicarbonate (HCO_3), creatinine (CREA), urea, calcium (Ca), phosphate (PO_4), globulin.

Serology:

At screening the analyses will also include HIV-1 & HIV-2, Hepatitis B surface antigen (HBsAg), a Hepatitis B surface antibody (Anti-HBs),a Hepatitis core antibody test (anti-HBc) and Hepatitis C.

Pregnancy test:

A serum pregnancy test will be conducted for all female subjects at the screening visit.

Urine Samples:

A fresh urine sample will be collected for a pregnancy test in all female subjects at Baseline (Visit 1), Visit 2, End of Treatment and Follow-up (dipstick testing).

7.2.2 Exploratory Assessments

CCI

7.2.3 Total Blood Volumes

The total amount of blood taken from each subject during the study will be approximately 150 ml

7.2.4 Laboratory Manual

A Laboratory Manual will be prepared to document the address and contact numbers for all laboratories involved in the study and the collection, processing, shipment, storage and reporting procedures for all samples.

8 SAFETY DATA COLLECTION, RECORDING AND REPORTING

8.1 Responsibilities

The Safety and Medical Management Plan details the roles and responsibilities, Serious Adverse event processing, reporting of safety data, SAE reconciliation and Medical monitoring for this study.

8.2 Definitions

8.2.1 Adverse Events

An adverse event is any untoward medical occurrence in a subject administered study medication and which does not necessarily have to have a causal relationship with this treatment. Abnormal laboratory values or test results constitute adverse events only if they induce clinical signs or symptoms, are considered clinically significant, or require therapy.

The occurrence of adverse events should be sought by non-direct questioning of the subject at baseline (pre-dose) Adverse events will be checked at every clinic visit. Adverse events may be detected when they are volunteered by the subject or through physical examination, laboratory test, or other assessments. All adverse events must be recorded in the CRF with the following information:

1. Diagnosis: diagnosis if known, otherwise sign or symptom;
2. Seriousness: whether it constitutes a serious adverse event (SAE) and therefore requires reporting immediately (see Section 8.3)
3. Criteria for Adverse Events:
   Mild: Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated

   Moderate: Moderate; minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental ADL (Activities of Daily Living)

   Severe: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of existing hospitalization indicated; limiting self-care ADL (Activities of Daily Living)
4. Relatedness: relationship to the study medication will be assessed as reasonable possibility of being related or no reasonable possibility of being related;
5. Duration: start and end dates or, if continuing, at final examination;
6. Action: any action taken to manage it (none, dosing interrupted, dosing stopped, other, medication);

7. Outcome: the outcome (completely recovered, recovered with sequelae, condition improving, condition still present and unchanged, condition deteriorated, death).

8.2.2 Serious Adverse Events

A serious adverse event is defined as an event which:

- results in death
- is life-threatening
- requires hospitalisation or prolongation of hospitalisation
- results in persistent or significant disability or incapacity
- consists of a congenital anomaly or birth defect
- is a medically important event

All adverse events should be treated appropriately. Treatment may include one or more of the following: no action taken (i.e., further observation only); study medication discontinued due to this adverse event; concomitant medication given; non-drug therapy given. The action taken to treat the adverse event or resolve the adverse incident should be recorded on the CRF.

Once an adverse event is detected, it should be followed until its resolution. The suspected relationship to the study medication, the interventions required to treat it and the outcome must be recorded in the CRF.

8.3 Serious Adverse Event Reporting

A Serious Adverse Event (SAE) Form must be completed for every SAE, regardless of suspected causality, occurring after protocol-specified procedures begin after signing the consent form until 4 weeks after the End of Treatment Visit and must be reported within 24 hours of the investigative site becoming aware of the event and ideally within 24 hours of its occurrence. The Safety and Medical Management Plan will provide full details.

Any SAEs experienced after this 4-week period should only be reported to if the Investigator suspects a causal relationship to the study medication.

Recurrent episodes, complications, or progression of the initial SAE must be reported as follow-up to the original episode, regardless of when the event occurs. This report must be submitted within 24 hours of the investigator receiving the follow-up information. An SAE that is considered completely unrelated to a previously reported one should be reported separately as a new event. If recurrence after resolution of the previous episode occurs if will be reported as a new SAE.

SAEs should be followed to resolution. Follow-up information should describe whether the event has resolved or continues, if and how it was treated, and whether the subject continued or withdrew from study participation.

If the SAE is not previously documented in the investigator's brochure and is thought to be related to the investigational study medication, then this is defined as a serious unexpected suspected adverse reaction (SUSAR). It is the responsibility of the Sponsor to determine whether a reported SAE fits the classification of a SUSAR and to inform the Investigator of their decision as soon as

possible. It is the responsibility of the Sponsor to determine whether an event requires expedited reporting and to notify the Investigators of their decision as soon as possible.

4D Pharma, or its designee, reports SAE's and/or SUSARS as required to regulatory authorities, investigators/institutions, and IRBs/IECs in compliance with all reporting requirements according to local regulations and good clinical practice.

The investigator is to notify the appropriate IRB/IEC of SAEs occurring at the site and other AE reports received from 4D Pharma or it's designee, in accordance with local procedures and statutes.

8.3.1 Pregnancies

There is no reason to believe that Blautix™ would be harmful to a mother or baby during pregnancy. If a patient becomes pregnant during the study the investigator is to stop dosing with study medication immediately.

A pregnancy is not considered to be an AE or SAE; however, it must be reported to the sponsor using the Pregnancy report forms within the same timelines as an SAE. This applies to female patients as well as female partners of male patients. A pregnancy should be followed through to outcome, wherever possible.

Subjects who are women and who may be able to have children will be given a serum pregnancy test at screening and a urine dipstick test at all subsequent study visits. If the result is positive at screening, the subject will not be able to enter the study. Sexually-active women of child bearing potential must use an acceptable form of birth control throughout the study until completion of two (2) periods after the last dose to avoid pregnancy for at least one complete menstrual cycle. The following methods of birth control are considered acceptable for this study; true abstinence, sterilisation, birth control pills, vasectomised partner, injections or contraceptive implants. The following birth control pills are recommended:

- Combined (oestrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation:
    - Oral
    - Intravaginal
    - Transdermal
- Progestogen-only hormonal contraception associated with inhibition of ovulation:
    - Oral
    - Injectable
    - Implantable

Female Subjects must not breast feed an infant during the study.

Male subjects, able to father children, and sexually active with a female partner who is able to bear children, must use a barrier method of birth control (male condom with or without spermicide) from the time of randomisation until the Follow-up visit. The study doctor will discuss methods of

birth control with the subjects if needed. If a subject becomes pregnant or think they may be pregnant during the study, they will be told to stop using the study medication and contact the study doctor's office immediately. They will be asked to withdraw from the study. For Male Subjects with a partner who becomes pregnant or thinks she may be pregnant during the study, the subjects will be asked to contact the study doctor's office immediately.

Refer to the Safety Management and Medical Monitoring Plan for further details.

8.4 Clinical Management of Events

If a subject chooses to withdraw or is withdrawn, the Investigator must ensure the safety and well-being of the subject prior to final discharge from the study. All adverse events will be evaluated, treated and followed up. Repeated and unscheduled examinations and laboratory assessments should be conducted as needed until appropriate and satisfactory resolution of any abnormalities.

Every effort should be made for all randomised subjects to undergo final follow-up. In the event that a subject fails to return for the study visits and follow-up assessments, every effort should be made to determine the reason(s).

Any pregnancy will be followed up by the investigator to determine outcome for the mother and baby.

9 DATA REVIEW, DATABASE MANAGEMENT AND STUDY DOCUMENTATION

9.1 Site Monitoring

On behalf of 4D Pharma Plc contract research organisation (CRO) representative will manage the monitoring aspects of this study. Before study initiation, at a site initiation visit, a sponsor representative will review the protocol and CRFs with the investigator(s) and their staff. During the study, a sponsor representative (monitor) will visit the site regularly to check the completeness of subject records, the accuracy of entries on the CRFs, the adherence to the protocol and to Good Clinical Practice, the progress of enrolment, and to ensure that study medication is being stored, dispensed, and accounted for according to specifications. Key study personnel must be available to assist during these visits. In addition, 4D Pharma Plc staff may also attend site initiation, monitoring and close out visits with CRO personnel.

The investigator must maintain source documents for each subject in the study and must provide the monitor access to all relevant source documents for confirmation of their consistency with the CRF entries. Monitoring will require full verification for the presence of informed consent, adherence to the inclusion/exclusion criteria, documentation of serious adverse events, and the recording of data that will be used for all primary and safety variables.

Additional checks of the consistency of the source data with the CRFs may be performed according to the study-specific monitoring plan.

9.2 Source Documents

Source documents are original documents, data (e COA Smartphone data) and records and may include medical records, laboratory data, electrocardiograms, pharmacy records, subjects' diaries and the results of any other tests or assessments. Information on CRFs must be traceable to these

source documents in the subject's file. Data not requiring a source document record will be defined before study start and will be recorded directly on the CRFs.

The Investigator and clinical site will permit trial-related monitoring, audits, EC review, and regulatory inspections as requested, and must give direct access to source data/documents, CRFs and the Investigator site file.

Clinical Investigators and authorized study personnel will review and access the electronic eCOA data; collected on smartphones and tablets, according to patient activity on site (following screening and scheduled clinic visits as indicated in the Protocol, e.g. Screening Visit, Baseline Visit 1, Visit 2, End of Treatment and Follow-Up. Discrepancies will be resolved using data queries and site response, with the oversight of the monitor.

9.3 Data Collection

Data collected from the study must be recorded in case report forms by delegated personnel at the Investigator sites. Diary data will be collected using an app-based electronic Clinical Outcome Assessments (eCOA) solution for smartphone. Patients will receive daily reminders through the app to go and complete their diaries. As soon as they have completed their diary and click submit, their data will be sent to the hub. Patients will use the same devices to complete their other patient-reported outcome questionnaires when they come into sites. Only personnel delegated the duty from the site's PI, with documentation on the signature delegation log, will collect and enter data.

CRFs will be reviewed for completeness and accuracy by the monitor and site personnel may be asked to make any required corrections or additions, as applicable. The nature and location of source documents for the study will be documented in a specific document.

Externally collected laboratory data will be received and processed to present a complete dataset.

9.4 Database Management and Quality Control

The database includes password protection and encryption and the data management process includes quality checks, to identify data that appear inconsistent, incomplete, or inaccurate. Data will be reviewed, and queries will be raised by data management in accordance with the study Data Management Plan.

Errors or omissions will be detailed as Data Queries by Data Management and sent to the Investigational site for resolution. Quality control of all data in the database will be made prior to locking the database and the data set will then be transferred to the statistical programming team for creation of Tables, Figures, and Listing for review in accordance with the Statistical Analysis Plan.

Access to the eCOA data will be controlled via the vendor Support Helpdesk. Users must have successfully completed the vendor training module, with a pass rate of >80%. Full log of query and resolution steps will be maintained within the system, with full audit trail

Concomitant medications entered into the database will be coded using the WHO Drug Reference List, which employs the Anatomical Therapeutic Chemical classification system. Current medical conditions and adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) terminology.

9.5 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol or GCP. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

Exemptions or eligibility criteria waivers for enrolment are not permissible. Any subject enrolled who does not meet eligibility criteria will be considered an enrolment deviation and must be reported as such. All protocol deviations, as defined above, must be addressed in study subject source documents.

9.6 Study Records Retention

The study site will maintain a study file, which should contain, at minimum, the Investigator's brochure, the Protocol and any Amendments, drug accountability records, correspondence with the IEC/IRB, 4D Pharma plc and assigned CRO and other study related documents.

The investigator agrees to keep records and those documents that include (but are not limited to) the identification of all participating patients, medical records, study specific source documents, source worksheets, all original signed and dated ICFs, copies of all eCRFs, query responses and detailed records of drug disposition to enable evaluations or audits from regulatory authorities and 4D Pharma or its designees.

Study documents should be retained for a minimum of 25 years after the end of the clinical study. These documents should be retained for a longer period, however, if required by local regulations. Medical files of subjects should be archived in accordance with national requirements. No records will be destroyed without the written consent of the Sponsor, if applicable. It is the responsibility of the Sponsor to inform the Investigator when these documents no longer need to be retained

The Investigator and study staff are responsible for maintaining an Investigator site file containing all study related Essential documents as defined by ICH GCP Section 8. This must be available at any time during the study for review by the sponsor monitor and for inspection by the regulatory authority.

10 STATISTICAL CONSIDERATIONS

10.1 Populations for Analysis

The *Safety* Analysis Set includes all subjects randomised into the study who received at least one dose of Blautix™ or Placebo. Subjects will be allocated to the treatment group corresponding to the treatment actually received.

The Safety Analysis Set is the primary analysis set for the safety analyses.

The *Full* Analysis Set includes all members of the Safety Analysis Set who were appropriately randomised into the Study. Subjects will be allocated to the treatment group corresponding to the treatment actually received.

The Full Analysis Set is the primary analysis set for the efficacy analyses.

The *Efficacy Evaluable* Analysis Set includes all members of the Full Analysis Set who completed the 8-week treatment and assessment period without major protocol violations. Subjects will be allocated to the treatment group corresponding to the treatment actually received.

The final determination of the membership of analysis sets will be made at a blinded data review meeting convened by the Sponsor.

10.2 Sample Size Calculation

Within each Cohort, 125 subjects per treatment group provide at least 80% power to demonstrate a statistically significant difference between the treatment groups at a one-sided alpha of 10% using Pearson's test with Yates's correction when the true response rate in Placebo group is 40% and the true response rate in the Blautix™ group is 55%.

The total planned study size is therefore 500 subjects.

10.3 Interim Analysis

An informal interim analysis will be conducted once all recruited patients have completed the primary efficacy analysis 8-week treatment period. This interim analysis is planned to enable 4D pharma to expedite the clinical development strategy based on the outcome of the interim analysis. Planning for pivotal trials and the EU Paediatric Investigation Plan and the US Paediatric Study Plan can be initiated in preparation for Agency meetings and further clinical development. There will be no other consequences of the interim analysis, there is no need to adjust the value of alpha.

10.4 Statistical Analysis Plan

A detailed and comprehensive statistical analysis plan (SAP) describing the planned final analysis in detail with tables, figures and listings templates will be developed and finalised before the study is unblinded.

Minor changes to the analyses specified in this section need not trigger a protocol amendment but must documented in the Clinical Study Report.

10.5 Statistical Analysis

All baseline, compliance, disposition, efficacy and safety variables will be listed and summarised.

The standard summary statistics for continuous baseline and outcome variables are: N, mean, standard deviation, median, quartiles, minimum and maximum. The standard summary statistics for categorical baseline and outcome variables are: count and proportion (expressed as percentage).

There are 2 cohorts in the study:

Cohort C: 250 subjects diagnosed with IBS-C

Cohort D: 250 subjects diagnosed with IBS-D

The two Cohorts will be analysed separately except there may be exploratory pooled or combined analyses.

10.5.1 Primary Efficacy Analysis

The primary efficacy analysis is the comparison of the proportion of overall responders between the Blautix™ and placebo groups using Pearson's test with Yates's correction in the full analysis set within each Cohort.

All other analyses of the primary endpoint are secondary analyses.

10.5.2 Secondary Efficacy Analysis

Primary Endpoint:

Secondary Analyses of the Primary Endpoint will include the use of baseline characteristics as covariates and analyses using the Efficacy Evaluable Analysis Set.

Secondary Efficacy Endpoints:

Secondary efficacy endpoints will be compared between the two treatment groups within each Cohort using Pearson's test with Yates's correction, the Mann Whitney test or other standard statistical tests as appropriate.

10.5.3 Exploratory Efficacy Analysis

The exploratory efficacy analyses include but are not limited to:

- CCI [redacted]

10.5.4 Safety Analysis

Safety endpoints will be listing and summarised by Cohort and treatment group using the standard summary statistics.

10.6 Concomitant Medications

Concomitant medications will be coded by using the World Health Organization (WHO) Drug Dictionary and will be summarised with the number and percentage of subjects receiving concomitant medication by drug class and preferred drug name.

10.7 Handling of Missing Data

Subjects who do not provide enough data to enable assessment of whether that subject is a responder will be deemed a non-responder.

An 'evaluable week' is one in which there are at least four twenty-four hour (4 day) records of abdominal pain score and at least four daily records of the number of complete spontaneous bowel movements and Bristol stool chart.

If a subject has not provided four evaluable weeks of data, then they are deemed to be a non-responder.

If a subject has provided at least four evaluable weeks of data then they are deemed to be a responder if they have reported an improvement in their weekly symptoms for ≥ 60% of the

evaluable weeks (if the total number of evaluable weeks is four, five or six) or for ≥ 50% of the evaluable weeks (if the total number of evaluable weeks is seven or eight).

10.8 Multiplicity

As this is a Phase II study, there will be no formal adjustments for multiplicity.

11 QUALITY CONTROL AND QUALITY ASSURANCE

By signing this protocol, the Sponsor and Investigator agree to be responsible for implementing and maintaining QC and QA systems where applicable to ensure that the study is conducted, and the data is generated, documented and reported in compliance with the protocol, GCP and applicable regulations.

The study may be audited by the Sponsor or its designee. If such an audit occurs, the Investigator must allow access to required subject records and study related documentation.

Should the Investigator or designee be notified of a regulatory inspection involving this study, they should notify the Sponsor immediately.

12 ETHICS/REGULATORY

This study will be conducted in compliance with the Protocol: Good Clinical Practices (GCPs) including International Conference on Harmonization (ICH) Technical Requirements for Registration of Pharmaceuticals for Human Use Guidelines; FDA regulatory requirements; and in accordance with the ethical principles of the Declaration of Helsinki.

The sponsor or designee will submit the study protocol plus all relevant study documents to concerned regulatory agencies for approval before the study start. No patient will be admitted to the study until appropriate approval of the study protocol has been received.

Each investigator must complete a Form FDA 1572 or equivalent and provide the completed form according to written instructions to the sponsor (or designee). Each investigator must submit to the sponsor (or designee) financial disclosure information according to national law and/or local requirements.

US-generated data will be handled in accordance with the Health Information Portability and Accountability Act (HIPAA). The trial will be registered at www.clinicaltrials.gov using the Protocol Registration system.

12.1 Institutional Review Board (IRB)/ Ethics Committee (EC)

Prior to initiation of the study, the Investigator will submit the study protocol, participant information sheet, consent form and any other documents that pertain to subject information, to the IRB/EC. The Investigator must also submit any other information that may be requested to the IRB/EC for review and approval. The Investigator will request that the IRB/EC provide written favourable opinion of the study identifying all documents reviewed by name and version. The site will keep on file records of all documents and correspondence pertaining to this study. A letter confirming the favourable opinion must be forwarded to the Sponsor prior to initiation of this study.

The Investigator must submit and, where necessary, obtain favourable opinion from the IRB/EC for all substantial protocol amendments and changes to the ICF. The Investigator should notify the IRB/EC of serious breaches, urgent safety measures or SUSARs occurring at the site, in accordance with local procedures. The Investigator will be responsible for submission of periodic progress reports at intervals not to exceed 1 year, or as otherwise specified by the IRB/EC.

The Investigator will notify the IRB/EC within 90 days of the end of the study or within 15 days of early termination. A reason for early termination will be provided.

12.2 Regulatory

The Sponsor will obtain approval of the appropriate regulatory body prior to study initiation. The Sponsor will also ensure that the implementation of substantial amendments to the protocol occurs only after approval of the relevant regulatory body. The Sponsor should notify the regulatory body of serious breaches, urgent safety measures or SUSARs occurring at the site.

The Sponsor will notify the regulatory body within 90 days of the end of the study or within 15 days of early termination. A reason for early termination will be provided.

12.3 Serious Breaches

A serious breach of GCP is defined as a breach which is likely to effect, to a significant degree, the safety or mental integrity of the subjects or the scientific value of the study. Serious breaches must be reported by the Investigator to the Sponsor. The Sponsor must notify the MHRA of any serious breach within 7 days of becoming aware of the breach. The Investigator should report a serious breach to the IRB/EC.

12.4 Informed Consent

Informed consent must be obtained in writing for all subjects before any study specific procedure is performed. Subjects may be approached to discuss the study to determine if they are interested in participating in the study. Information sheets will be provided, and consent taken when they return for the initial study assessment. The information sheet will clearly explain what the study will involve and subjects must have adequate opportunity to ask questions.

The physician or designee who conducts the informed consent discussion must also sign and date the consent form. A copy of the signed consent document must be given to the subject. The original signed consent document will be retained by the Investigator.

The information sheet and consent forms must contain the minimum elements required by ICH GCP and must be submitted for favourable opinion from the IRB/EC.

If an amendment to the protocol changes the subject participation schedule in scope or activity, or if important new information becomes available that may be relevant to the subject's consent, the ICF must be revised with a new version number and submitted to the IRB/EC for review and favourable opinion. The revised ICF must be used to obtain consent from a subject currently enrolled in the study if he or she is affected by the amendment. The revised ICF must be used to obtain consent from any new subjects who are enrolled into the study after the date of the favourable opinion of the protocol amendment.

12.5 Future use of stored samples

Samples will not be labelled with information that directly identifies the subject but will be coded with the identification number for the subject.

Collected samples will be transferred for analysis to the Sponsor, or to other laboratories working for the Sponsor.

Biological samples will be stored for the time established by regulatory requirements or destroyed after the final clinical study report has been finalized if storage is not required. There might be a new request for these samples to be used for purposes related to the QA of the laboratory tests described in this protocol, in which case they will be used for this purpose. This may include the assessment of the quality of current tests, the maintenance or improvement of these tests, the development of new test methods for the markers described in this protocol, as well as making sure that new tests are comparable to previous methods and work reliably.

If study results suggest that further investigations using stored biological samples are warranted, these tests might be carried out on an exploratory basis. In addition, biological samples may be used by the Sponsor or their research partners for further research that is not related to the disease or the product under study. This testing will be done on pseudonymized samples (meaning that subjects will not be identifiable from their biological samples as their identity will have been removed and they will be assigned a unique clinical study identification number). Subjects will be asked to sign an additional, separate consent form for this optional testing and refusal of consent will not affect their possibility of participating in the study.

During the conduct of the study, an individual subject can choose to withdraw consent to have biological specimens stored for future research.

12.6 Subject Confidentiality

In order to maintain subject privacy, all CRFs, diary cards, study drug accountability records, study reports, and communications will identify the subject only by the assigned subject number. The Investigator will grant monitors and auditors from the Sponsor or its designee, and regulatory authorities, access to the subject's original medical records for verification purposes.

12.7 Insurance

The Sponsor has established an insurance policy for the anticipated duration of the study, covering the subjects with respect to the risks involved in taking part in this study in accordance with this protocol. In the case of injury or disability deriving from participation in the study, subjects are requested to inform the Investigator or his/her staff responsible for the study at the institution without delay

13 PUBLICATION POLICY

The Sponsor and Investigator will mutually manage the publication and presentation process. All manuscripts, abstracts, or other modes of presentation arising from the results of the study must be reviewed and approved in writing by the Sponsor in advance of submission in accordance with the

clinical site agreement. The review is aimed at protecting the Sponsor's proprietary information existing either at the date of the commencement of the study or generated during the study.

The Sponsor will register the study on a public registry and results database of clinical trials.

The Sponsor will publish the summary study results in EudraCT within six months following the end of the trial.

The Sponsor and the Investigator will disclose the study results, in the form of a clinical study synopsis to the Health Authorities and the EC / IRB respectively, within 1 year of the end of the study. The format of this synopsis and that of the clinical study report will comply with the ICH3 guidelines for the structure and content of a clinical study report.

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APPENDIX 1: IBS-QOL

PARTICIPANT ID:

PLEASE WRITE IN

TODAY'S DATE:

_____ _____ _____

Month Day Year

PLEASE READ THIS CAREFULLY

ON THE FOLLOWING PAGES YOU WILL FIND STATEMENTS CONCERNING BOWEL PROBLEMS (IRRITABLE BOWEL SYNDROME) AND HOW THEY AFFECT YOU.

FOR EACH STATEMENT, PLEASE CHOOSE THE RESPONSE THAT APPLIES BEST TO YOU AND **CIRCLE** THE NUMBER OF YOUR RESPONSE.

IF YOU ARE UNSURE ABOUT HOW TO RESPOND TO A STATEMENT, PLEASE GIVE THE BEST RESPONSE YOU CAN. **THERE ARE NO RIGHT OR WRONG RESPONSES.**

YOUR RESPONSES WILL BE KEPT STRICTLY CONFIDENTIAL.

IF YOU HAVE ANY QUESTIONS, PLEASE CONTACT:

SITE ADDRESS AND PHONE NUMBER TO BE PLACED HERE

PPD

About how you feel

Please think about your life over the **past month (last 30 days)** and look at the statements below. Each statement has five different responses. For each statement, please circle the response that best describes your feelings.

Q1. I feel helpless because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY

Q2. I am embarrassed by the smell caused by my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY

Q3. I am bothered by how much time I spend on the toilet. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

Q4. I feel vulnerable to other illnesses because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY

Q5. I feel fat/bloated because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

Q6. I feel like I'm losing control of my life because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

Q7. I feel my life is less enjoyable because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

Q8. I feel uncomfortable when I talk about my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY

Q9. I feel depressed about my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT

5 EXTREMELY

Q10. I feel isolated from others because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY

Q11. I have to watch the amount of food I eat because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

Q12. Because of my bowel problems, sexual activity is difficult for me. *(Please circle one number)*

(If not applicable, please circle "NOT AT ALL")

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY

Q13. I feel angry that I have bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY

Q14. I feel like I irritate others because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

Q15. I worry that my bowel problems will get worse. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

Q16. I feel irritable because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY

Q17. I worry that people think I exaggerate my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

Q18. I feel I get less done because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT

5 A GREAT DEAL

Q19. I have to avoid stressful situations because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

Q20. My bowel problems reduce my sexual desire. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

Q21. My bowel problems limit what I can wear. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

Q22. I have to avoid strenuous activity because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

Q23. I have to watch the kind of food I eat because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

Q24. Because of my bowel problems, I have difficulty being around people I do not know well. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

Q25. I feel sluggish because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY

Q26. I feel unclean because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY

Q27. Long trips are difficult for me because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY

Q28. I feel frustrated that I cannot eat when I want because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY

Q29. It is important to be near a toilet because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY

Q30. My life revolves around my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

Q31. I worry about losing control of my bowels. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

Q32. I fear that I won't be able to have a bowel movement. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

Q33. My bowel problems are affecting my closest relationships. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

Q34. I feel that no one understands my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY

APPENDIX 2: HOSPITAL ANXIETY AND DEPRESSION SCALE (HADS)

Instructions: Doctors are aware that emotions play an important part in most illnesses. If your doctor knows about these feelings he or she will be able to help you more. This questionnaire is designed to help your doctor know how you feel. Read each item and circle the reply which comes closest to how you have been feeling in the past week. Don't take too long over your replies: your immediate reaction to each item will probably be more accurate than a long thought out response.

I feel tense or 'wound up':	**A**
Most of the time	3
A lot of the time	2
Time to time, occasionally	1
Not at all	0

I feel as if I am slowed down:	**D**
Nearly all of the time	3
Very often	2
Sometimes	1
Not at all	0

I still enjoy the things I used to enjoy:	**D**
Definitely as much	0
Not quite so much	1
Only a little	2
Not at all	3

I get a sort of frightened feeling like 'butterflies in the stomach':	**A**
Not at all	0
Occasionally	1
Quite often	2
Very often	3

I get a sort of frightened feeling like something awful is about to happen:	**A**
Very definitely and quite badly	3
Yes, but not too badly	2
A little, but it doesn't worry me	1
Not at all	0

I have lost interest in my appearance:	**D**
Definitely	3
I don't take as much care as I should	2
I may not take quite as much care	1
I take just as much care as ever	0

I can laugh and see the funny side of things:	**D**
As much as I always could	0
Not quite so much now	1
Definitely not so much now	2
Not at all	3

I feel restless as if I have to be on the move:	**A**
Very much indeed	3
Quite a lot	2
Not very much	1
Not at all	0

Worrying thoughts go through my mind:	**A**
A great deal of the time	3
A lot of the time	2
From time to time but not too often	1
Only occasionally	0

I look forward with enjoyment to things:	**D**
A much as I ever did	0
Rather less than I used to	1
Definitely less than I used to	3
Hardly at all	2

I feel cheerful:	**D**
Not at all	3
Not often	2
Sometimes	1
Most of the time	0

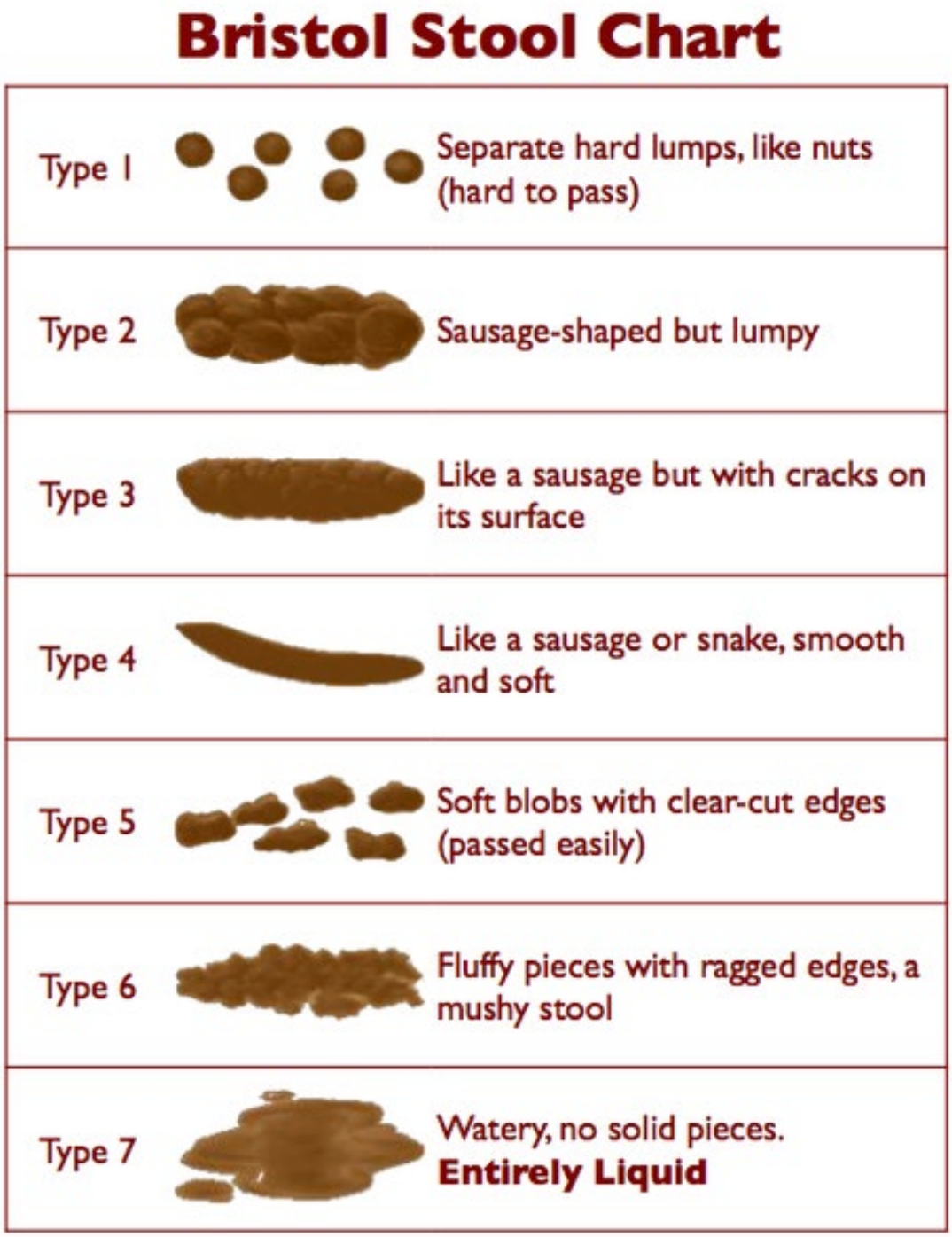
I get sudden feelings of panic:	**A**
Very often indeed	3
Quite often	2
Not very often	1
Not at all	0

I can sit at ease and feel relaxed:	**A**
Definitely	0
Usually	1
Not often	2
Not at all	3

I can enjoy a good book or radio or TV programme:	**D**
Often	0
Sometimes	1
Not often	2
Very seldom	3

Questions relating to anxiety are indicated by an 'A' while those relating to depression are shown by a 'D'. Scores of 0-7 in respective subscales are considered normal, with 8-10 borderline and 11 or over indicating clinical 'caseness'

APPENDIX 3: BRISTOL STOOL CHART



APPENDIX 4: IBS-SSS

IBS QUESTIONNAIRE

Name:_____ **G.P. Name:**_____
Address:_____ **Address:**_____
_____ _____
_____ _____
_____ _____
Telephone:_____ **Telephone:**_____
Date of birth:_____
Marital status: Single / Married / Divorced / Widowed / Co-Habit
Occupation:_____ **Sex:** **M** **F**

INSTRUCTIONS

This form is designed to enable us to record and monitor the severity of your IBS. It is to be expected that your symptoms might vary over time, so please try and answer the questions based on how you currently feel (ie over the last ten days or so). All information will be kept in **strict** confidence.

1. For questions where a number of different responses are a possibility please circle the response appropriate to you.

2. Some questions will require you to write in an appropriate response.

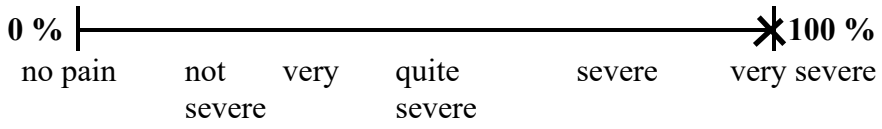
3. Some questions require you to put a cross on a line which enables us to judge the severity of a particular problem.

For example:

How severe was your pain?

Please place your cross (✗) anywhere on the line between 0-100% in order to indicate as accurately as possible the severity of your symptom.

This example shows a severity of approximately 90%



1

PART 1: SEVERITY SCORE

1. a) ***Do you currently suffer from abdominal (tummy) pain?*** **YES** **NO**

Circle appropriate box

For office use only

b) If yes, how severe is your abdominal (tummy) pain?

SCORE

0 % ——— **100 %**

no pain | not severe | very severe | quite severe | severe | very severe

c) Please enter the number of days that you get the pain in every ten days.
For example if you enter 4 it means that you get pain 4 out of 10 days. If you get pain every day enter 10

Number of days with pain [ ] x 10 [ ]

2. a) ***Do you currently suffer from abdominal distension* (bloating, swollen or tight tummy)*** **YES** **NO**

(women, please ignore distension related to your periods)*

Circle appropriate box

b) If yes, how severe is your abdominal distension/tightness

0 % ——— **100 %** [ ]

no distension | not severe | very severe | quite severe | severe | very severe

3. ***How satisfied are you with your bowel habit?***

0 % ——— **100 %** [ ]

very happy | quite happy | unhappy | very unhappy

4. Please indicate with a cross on the line below how much your Irritable Bowel Syndrome is affecting or interfering with your life in general.

0 % ——————————— **100 %** ☐

not at all not much quite a lot completely

IBS SEVERITY SCORE: ☐

2

PART 2: OTHER IBS DATA

BOWEL HABIT

5. a) What is the most number of times you open your bowels per day/week/month?

Number of times ☐ per day / week / month *(Circle appropriate)*

Note: For some people the answer to part a and b could be the same

b) What is the least number of times you open your bowels per day/week/month?

Number of times ☐ per day / week / month *(Circle appropriate)*

6. In the following questions you may circle more than one answer:

Are your motions ever:

a) normal	often / occasionally / never	*(Circle appropriate)*
b) hard	often / occasionally / never	*(Circle appropriate)*
c) very thin (like string)	often / occasionally / never	*(Circle appropriate)*
d) in small pieces (like rabbit pellets)	often / occasionally / never	*(Circle appropriate)*
e) mushy (like porridge)	often / occasionally / never	*(Circle appropriate)*
f) watery	often / occasionally / never	*(Circle appropriate)*

7. In the following questions you may circle more than one answer:

Do you ever:

	Circle appropriate box	
a) pass mucus (or slime or jelly) with your motions	**YES**	**NO**

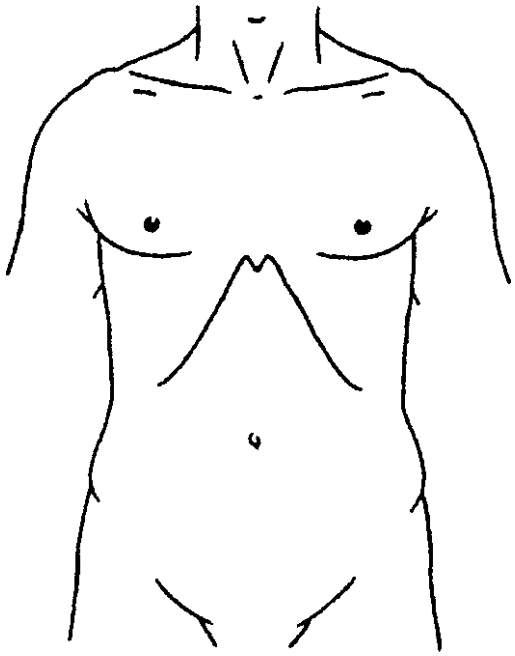
b) pass blood with your motions	**YES**	**NO**
c) have to hurry/rush to the toilet to open your bowels	**YES**	**NO**
d) strain to open your bowels	**YES**	**NO**
e) feel you haven't emptied your bowel completely after you have passed a motion	**YES**	**NO**

3

PART 2: Continued

SITE OF PAIN

Please mark with a cross (x) on the diagram below where you get your pain
(use more than one x if necessary)



8.Do you ever:

a) notice your stools are more frequent or loose when you get pain **YES** **NO**
Circle appropriate box

b) notice whether the pain is frequently eased by opening your bowels **YES** **NO**
Circle appropriate box

9.In the last year on approximately how many weeks were you:

i) absent from work due to IBS ----------
(enter 52 if you have given up completely work because of IBS)

ii) at work suffering from IBS ----------

APPENDIX 5: MODIFIED FFQ

(US/UK Terminology)

NOTE: *FFQ completion at baseline will be in relation to food (s) consumed over the prior 6 months. At each subsequent visit the FFQ shall capture responses to food (s) consumed since last subject visit.*

Food Name	Never or < than once a month	once a month	2-3 times a month	once a week	2-4 times a week	5 times a week	once a day	2-4 times a day	5 times a day	> 5 times a day	Comment
1. Beef roast (roast/steak)											
2. Beef: steak											
3. Beef: mince, ground beef											
4. Beef: stew											
5. Beef burger/meat loaf (1 burger)											
6. Pork: roast/chops											
7. Pork: chops											
8. Pork: slices/escalopes											
9. Lamb: roast											
10. Lamb: chops											
11. Lamb: stew											
12. Chicken portion or other poultry e.g. turkey: roast											
13. Breaded chicken, chicken nuggets, fried chicken, chicken burger											
14. Bacon											
15. Ham											
16. Corned beef, Spam, Luncheon meats, processed meats/deli meats											
17. Sausages, hot dog, chorizo, Frankfurters (1 sausage)											
18. Savoury pies (e.g. meat pie, pork pie, steak & kidney pie, sausage rolls)											
19. Liver, heart, kidney, tongue											
20. Liver paté											
21. Fish fried in batter), shrimps e.g. fish and chips											
22. Fish fried in breadcrumbs/cornmeal (catfish)											

Food Name	Never or < than once a month	once a month	2-3 times a month	once a week	2-4 times a week	5 times a week	once a day	2-4 times a day	5 times a day	> 5 times a day	Comment
23. Oven baked/grilled fish (in breadcrumbs or batter)											
24. Fish fingers/fish cakes/fish sticks/fish sandwich/fish taco											
25. Other white fish, fresh or frozen (e.g. cod, haddock, plaice, sole, snapper, trout, tilapia, halibut, coli)											
26. Oily fish, fresh or canned (e.g. mackerel, kippers, tuna, salmon, sardines, herring)											
27. Shellfish (e.g. crab, prawns, mussels)											
28. White bread and rolls (including ciabatta, panini bread & gluten free)											
29. Brown bread/rolls, whole wheat , gluten free											
30. Whole meal, rye, wholegrain bread/ rolls											
31. Cream crackers, crackers, cheese biscuits, Ritz, saltines											
32. Tortilla, (corn or flour)											
33. Crisp bread, crisp rye bread, e.g. Ryvita											
34. Pancakes, muffins, oatcakes, waffles, French toast											
35. Hot cereal (Porridge, Ready Brek, cream of wheat, oatmeal, grits)											
36. All Bran, High fibre, Raisin Bran, whole grain, grape nuts, bran cereal, Weetabix, Shredded Wheat, bran buds											
37. Bran flakes, Bran Buds											
38. Cornflakes, Rice Krispies, Cheerios											
39. Muesli (e.g. Country Store, Alpen, sugar coated)											
40. Sugar Coated Cereals (e.g. Frosties, Crunchy Nut Cornflakes, Crunchy Sugar Coated Muesli, fruit loops, lucky charms, fruit pebbles)											
41. Boiled, instant or jacket potatoes											
42. Mashed potatoes											

Food Name	Never or < than once a month	once a month	2-3 times a month	once a week	2-4 times a week	5 times a week	once a day	2-4 times a day	5 times a day	> 5 times a day	Comment
43. Chips (fries, French fries)											
44. Roast potatoes											
45. Potato Salad											
46. White Rice											
47. Brown Rice											
48. White/yellow or green pastas (e.g. spaghetti, macaroni, noodles, penne) without meat sauce											
49. Whole meal/gluten free pasta											
50. Lasagne (meat based)											
51. Lasagne (vegetarian)											
52. Moussaka, Burritos, Tacos, quesadillas											
53. Pizza											
54. Macaroni Cheese											
55. Cream (tablespoon)											
56. Full-fat (whole milk) yoghurt or Greek- style Yoghurt (125g carton)											
57. Dairy desserts (ice cream - 125g carton)											
58. Cheddar cheese, Monterey jack, Havarti, Munster, Swiss (medium serving)											
59. Low-fat cheddar cheese (medium serving)											
60. Cottage Cheese											
61. Eggs as boiled, fried, scrambled, poached (one)											
62. Quiche (medium serving)											
63. Omelette											
64. Light salad cream/dressing or light mayonnaise (tablespoon)											
65. Salad cream, regular mayonnaise/ salad dressing (tablespoon)											
66. French dressing (tablespoon)											
67. Other salad dressing e.g. ranch/blue cheese Other - specify(tablespoon)											

Food Name	Never or < than once a month	once a month	2-3 times a month	once a week	2-4 times a week	5 times a week	once a day	2-4 times a day	5 times a day	> 5 times a day	Comment
68. Butter (teaspoon)											
69. Lite Butter (reduce fat) e.g. Dawn Lite, Connacht Gold (teaspoon)											
70. Margarine (soft tub) e.g. Sunflower margarine, Flora (teaspoon)											
71. Low-fat/reduced fat margarine e.g. low- low (teaspoon)											
72.Lard or bacon grease											
73. Cholesterol Lowering Spreads e.g. Flora Pro Active, Dairy Gold Heart (teaspoon)											
74. Cream & Vegetable Oil spread e.g. Golden Pasture, Kerrymaid, Dairy Gold – teaspoon											
75. Olive oil spread/butter with olive oil e.g. Golden Olive (teaspoon)											
76. Apples											
77. Pears											
78. Oranges, satsumas, mandarins											
79. Grapefruit											
80. Bananas											
81. Grapes											
82. Melon, water melon, cantaloupe											
83. Peaches, plums											
84. Apricots											
85. Strawberries, raspberries, kiwi fruit, blue berries											
86. Tinned/canned fruit, fruit cocktails (specify)											
87. Dried fruit e.g. raisins											
88. Frozen fruit											
89. Carrots											
90. Spinach											
91. Broccoli, spring greens, kale, chard, spinach, collard greens											
92. Brussel sprouts											

Food Name	Never or < than once a month	once a month	2-3 times a month	once a week	2-4 times a week	5 times a week	once a day	2-4 times a day	5 times a day	> 5 times a day	Comment
93. Cabbage											
94. Peas											
95. Green beans, broad beans, runner beans, lima beans, fava beans											
96. Courgettes, zucchini, yellow squash											
97. Cauliflower											
98. Parsnips, turnips											
99. Leeks											
100. Onions											
101. Garlic											
102. Mushrooms											
103. Sweet peppers											
104. Beansprouts											
105. Green salad, lettuce											
106. Cucumber, celery											
107. Tomatoes											
108. Sweetcorn, corn, kernels on the cob											
109. Beetroot, beet											
110. Coleslaw											
111. Baked beans											
112. Dried lentils, beans, peas, pinto, black, red, peas											
113. Tofu, soya meat, TVP											
114. Chocolate coated sweet biscuits e.g. digestive (one)											
115. Plain sweet biscuits e.g. Marietta, digestives, rich tea (one)											
116. Cakes e.g. fruit, sponge											
117. Buns, pastries e.g. croissants, doughnuts											
118. Fruit pies, tarts, crumbles, cobblers											
119. Sponge puddings											
120. Milk puddings e.g. rice, custard, trifle, flan											

Food Name	Never or < than once a month	once a month	2-3 times a month	once a week	2-4 times a week	5 times a week	once a day	2-4 times a day	5 times a day	> 5 times a day	Comment
121. Ice cream, choc ices, Frozen desserts, ice cream sandwiches, frozen popsicles											
122. Chocolates, singles or squares											
123. Sweets, candy, toffees, mints											
124. Sugar added to tea coffee, cereal (teaspoon)											
125. Sugar substitute e.g. Canderel added to tea coffee, cereal (teaspoon)											
126. Crisps, potato chips, tortilla, Pretzel, popcorn or other packet snacks											
127. Peanuts											
128. Nuts (other than peanuts)											
129. Vegetable soups: homemade/fresh (1 bowl)											
130. Vegetable soups: tinned/packet (1 bowl)											
131. Meat soups: homemade/fresh (1 Bowl)											
132. Meat/cream soups: tinned/canned/packet (1 bowl)											
133.Chicken and noodle soup (hand/homemade) 1 bowl											
134. Sauces e.g. white sauce, cheese sauce, gravy (1 tablespoon)											
135. Tomato based sauces e.g. pasta sauces											
136.Tomato sauce with meat											
137. Miso, wonton soup											
138. Curry-type sauces											
139. Pickles, chutney, ketchup, mustard (tablespoon)											
140. Marmite, Bovril (tablespoon)											
141. Jam, marmalade, honey, syrup (teaspoon)											
142. Peanut butter (teaspoon)											
143. Tea (cup)											

Food Name	Never or < than once a month	once a month	2-3 times a month	once a week	2-4 times a week	5 times a week	once a day	2-4 times a day	5 times a day	> 5 times a day	Comment
144. Herbal tea (cup)											
145. Coffee instant (cup)											
146. Coffee ground (cup)											
147. Coffee, decaffeinated (cup)											
148. Coffee whitener e.g. coffee-mate, coffee creamer milk (teaspoon)											
149. Cocoa, Hot Chocolate (cup)											
150. Horlicks, Ovaltine, malt drinks (cup)											
151. Wine (glass)											
152. Beer, Larger or Cider (half pint)											
153. Alcopops (low alcoholic/alcohol free drinks e.g. Bacardi Breezer											
154. Whole milk (cup) – cow or goat (including milk used in tea, cereal etc)											
155. Milk substitute (almond, rice, cashew, soya) specify											
156. Semi-skimmed milk/half milk (cup) (including milk used in tea, cereal etc)											
157. Port, Sherry, Vermouth, liqueurs (glass)											
158. Spirits e.g. Gin, Whiskey (single measure)											
159. Low calorie or diet soft fizzy (glass)											
160. Fizzy Soft drinks e.g. Cocoa Cola (glass)											
161. Pure fruit drinks/fruit juice e.g. orange juice (small glass)											
162. Fruit squash/cordial (small glass)											
163. Probiotic drinks/yoghurt e.g. Actimel, Yakult, kefir											
164. Vitamin supplements (if so please include details below)											

APPENDIX 6: ROME IV CLASSIFICATION FOR IBS

IBS-C	IBS-D
□ Abdominal Pain Intensity: weekly average of worst daily (in past 24 hours) abdominal pain score of > 3.0 on a 0 to 10-point scale And □ Stool Frequency: more than 25% of bowel movements with a consistency of Type 1 or Type 2 Bristol stool chart and less than 25% of bowel movements with Bristol stool form Type 6 or Type 7. Subject must have fewer than 3 CSBMs within a one-week period (7 days)	□ Abdominal Pain Intensity: weekly average of worst daily (in past 24 hours) abdominal pain score of > 3.0 on a 0 to 10-point scale And □ Stool Consistency: more than 25% of bowel movements with a consistency of Type 6 or Type 7 Bristol stool chart and less than 25% of bowel movements with Bristol stool form Type 1 or Type 2. Subjects must have at least one Type 6 or Type 7 bowel movements on at least four days within a one-week period (7 days).

APPENDIX 7: PROHIBITED MEDICATIONS

IBS-C Patients:

Guanylate cyclase agonists -

Linaclotide

Lubiprostone

IBS-D Patients:

5-hydroxytryptamine (serotonin) 3 receptor antagonists

Alosetron

Bile Acid sequestrants

Cholestyramine

Colesevelam

Colestipol

Eluxadoline

Rifaximin

Probiotics

Probiotics
Alflorex (EU)
Align (North America)
Align ® (B. longu m infantis 35 624) Capsules
Align Probiotic Supplement Capsules ((Lactobacillus GG)
BioGaia L.Reuteri ProTectis Probiotic (Lactobacillus) Capsules
Bioglan products
Bio-Kult
Culturelle Probiotic (Lactobacillus GG)Capsules
Digestive Care™ (L. plantarum 299v) Capsules
Human MicroFlora products by Genestra™
IbSium® (Saccharomyces cerevisiae I-3856) Capsules
Mutaflor ® (active ingredient Escherichia coli strain Nissle 1917)
Prescript-Assist (Broad Spectrum Probiotic Prebiotic) Capsules
ProBio 7 products
Proxiflor ® (L. rhamnosus R0011 L.helveticus R0052)Capsules
TuZen® (L. plantar um 299v) Capsules
Udos probiotics

Ultra Probiotic Complex by GNC-
UltraFlora Intensive Care (L. planta rum 299v) Capsules
Vitabiotic products
Yakult (Lactobacillus casei Shirota) packet

APPENDIX 8: SUMMARY OF PROTOCOL CHANGES VERSION 1.1

PROTOCOL

PROTOCOL NUMBER: BHT-II-002

A PHASE 2 RANDOMISED, DOUBLE BLIND, PLACEBO-CONTROLLED, PARALLEL GROUP, MULTICENTRE STUDY TO EVALUATE THE SAFETY AND EFFICACY OF REPEATED ORAL DOSES OF Blautix™ IN ADULT SUBJECTS WITH IRRITABLE BOWEL SYNDROME (IBS) SUBTYPES IBS-C and IBS-D

Original Version:	Version 1
Version Date:	6 February 2018
Updated Version:	**Version 1.1**
Version Date:	**6 April 2018**

Sponsor Name and Address:

4D Pharma Plc

9 Bond Court

Leeds, U.K

LS1 2JZ

A6.1 Section: <u>Change to the header information on all Protocol pages</u>

Old Text:

Blautix™, *Blautia hydrogenotrophica*
[redacted]
February 2018

New Text:

Blautix™, *Blautia hydrogenotrophica*
Irritable Bowel Syndrome
Clinical Study Protocol No: BHT-II-002 Version 1.1
Date: 3rd April 2018

***Reason*:**

To amend the Protocol header information from the IND submission format and make clear reference to the therapy area and Protocol version and date. This change has been made at the request of the Investigators.

A6.2 Section: <u>Page 8 Synopsis</u>

Old Text:

Reference Therapy, Dose and Route of Administration:

Matching placebo, administered orally.

Each placebo capsule will be comparable in size, weight and appearance to the test formulation and subjects will receive **one capsule** twice daily before food for 8 weeks.

New Text:

Reference Therapy, Dose and Route of Administration:

Matching placebo, administered orally.

Each placebo capsule will be comparable in size, weight and appearance to the test formulation and subjects will receive **two capsules** twice daily **approximately 30 minutes** before food for 8 weeks.

Reason:

To amend an administrative error and correct the reference to 'one capsule twice daily' the daily dose is 'two capsules twice daily' and amend the timing of the dosing to be consistent with other sections of the Protocol. This administrative change has been made to ensure correctness and consistency throughout the protocol.

A6.3 Section: Page 8 Synopsis

Old Text:

Test Product, Dose and Route of Administration:

Capsules of *Blautia hydrogenotrophica* (Blautix™) administered orally.

Each capsule contains ≥5x 10^9MPN *Blautia hydrogenotrophica* /capsule and subjects will receive two capsules twice daily before food for 8 weeks.

New Text:

Test Product, Dose and Route of Administration:

Capsules of *Blautia hydrogenotrophica* (Blautix™) administered orally.

Each capsule contains ≥5x 10^9MPN *Blautia hydrogenotrophica* /capsule and subjects will receive two capsules twice daily **approximately 30 minutes** before food for 8 weeks

Reason:

To amend an administrative error regarding the timing of the dosing to be consistent with other sections of the Protocol. This administrative change has been made to ensure correctness and consistency throughout the protocol.

A6.4 Section: Overall Study Design Section 4.2.1

Old Text:

Baseline Visit 1 (Day 1)

Subjects will use their smartphone issued at the Screening visit to capture their study related data;

- Information on Adverse Events
- Review of Medication log completed daily
- Abdominal Pain Intensity Numeric Rating Scale (NRS) completed daily
- Stools consistency/frequency completed daily
- Subject Global Assessment of Relief completed weekly
- IBS Quality of Life questionnaire (IBS QOL) completed on the Day of each Clinic Visit
- IBS Symptom Severity Score (IBS-SSS) on the Day of each Clinic visit or within 24 hrs prior to the visit
- Hospital Anxiety and Depression score (HADS) on Day of each Clinic visit or within 24 hours prior to the visit
- Food Frequency Questionnaire (FFQ) on the Day of each Clinic visit or within 24hours prior to the visit

New Text:

Subjects will use their smartphone issued at the Screening visit to capture their study related data. **The IBS-SSS and FFQ questionnaires will be performed at the clinic on a tablet device.**

- Information on Adverse Events
- Review of Medication log completed daily
- Abdominal Pain Intensity Numeric Rating Scale (NRS) completed daily
- Stools consistency/frequency completed daily
- Subject Global Assessment of Relief completed weekly
- IBS Quality of Life questionnaire (IBS QOL) completed on the Day of each Clinic Visit
- **IBS Symptom Severity Score (IBS-SSS) on the Day of each Clinic visit**
- Hospital Anxiety and Depression score (HADS) on Day of each Clinic visit or within 24 hours prior to the visit
- **Food Frequency Questionnaire (FFQ) on the Day of each Clinic visit**

Reason:

Modification of data capture of IBS-SSS and FFQ questionnaires to a tablet device during the clinic visit as the smartphone functionality cannot accommodate these assessments due to the both the length and complexity. This change has been made at the request of the Investigators.

A6.5 Section: <u>Overall Study Design Section 4.2.1</u>

Old Text:

Visit 2 (Week 4-5)

Subjects will continue to use their smartphones to capture all the patient related outcome data. The data will be reviewed on an ongoing basis as required by the study team and sponsor. At this visit the following information will be reviewed with the subject;

- Information on Adverse Events
- Review of Medication log completed daily
- Recording times of taking their medication completed daily
- Abdominal Pain Intensity NRS completed daily
- Stools consistency/frequency daily
- Subject Global Assessment of Relief completed weekly
- IBS Quality of Life questionnaire (IBS QOL) on the Day of Clinic Visit

- IBS Symptom Severity Score (IBS-SSS) on the Day of Clinic visit or within 24 hrs prior to the visit
- Hospital Anxiety and Depression score (HADS) on Day of each Clinic visit or within 24 hours prior to the visit
- Food Frequency Questionnaire (FFQ) on the Day of Clinic visit or within 24hours prior to the visit

New Text:

Subjects will continue to use their smartphones to capture all the study related outcome data, **except for IBS-SSS and FFQ questionnaires which will be completed during the clinic visit.** The data will be reviewed on an ongoing basis as required by the study team and sponsor. At this visit the following information will be reviewed with the subject;

- Information on Adverse Events
- Review of Medication log completed daily
- Recording times of taking their medication completed daily
- Abdominal Pain Intensity NRS completed daily
- Stools consistency/frequency daily
- Subject Global Assessment of Relief completed weekly
- IBS Quality of Life questionnaire (IBS QOL) on the Day of Clinic Visit
- **IBS Symptom Severity Score (IBS-SSS) on the Day of Clinic visit**
- Hospital Anxiety and Depression score (HADS) on Day of each Clinic visit or within 24 hours prior to the visit
- **Food Frequency Questionnaire (FFQ) on the Day of Clinic visit**

Reason:

Modification of data capture of IBS-SSS and FFQ questionnaires to a tablet device during the clinic visit as the smartphone functionality cannot accommodate these assessments due to the both the length and complexity. This change has been made at the request of the Investigators.

A6.6 Section: <u>Overall Study Design Section 4.2.1</u>

Old Text:

End of Treatment (Week 8-9)

Subjects will continue to use their smartphones to capture all the study related outcome data. The data will be reviewed on an ongoing basis as required by the study team and sponsor. At this visit the following information will be reviewed with the subject;

- Information on Adverse Events
- Review of Medication log completed daily

- Recording times of taking their medication completed daily
- Abdominal Pain Intensity NRS completed daily
- Stools consistency/frequency daily
- Subject Global Assessment of Relief completed weekly
- IBS Quality of Life questionnaire (IBS QOL) on the Day of Clinic Visit
- IBS Symptom Severity Score (IBS-SSS) on the Day of Clinic visit or within 24 hrs prior to the visit
- Hospital Anxiety and Depression score (HADS) on Day of each Clinic visit or within 24 hours prior to the visit
- Food Frequency Questionnaire (FFQ) on the Day of Clinic visit or within 24hours prior to the visit

New Text:

Subjects will continue to use their smartphones to capture all the patient related outcome data, **except for IBS-SSS and FFQ questionnaires which will be completed during the clinic visit.** The data will be reviewed on an ongoing basis as required by the study team and sponsor. At this visit the following information will be reviewed with the subject;

- Information on Adverse Events
- Review of Medication log completed daily
- Recording times of taking their medication completed daily
- Abdominal Pain Intensity NRS completed daily
- Stools consistency/frequency daily
- Subject Global Assessment of Relief completed weekly
- IBS Quality of Life questionnaire (IBS QOL) on the Day of Clinic Visit
- **IBS Symptom Severity Score (IBS-SSS) on the Day of Clinic visit**
- Hospital Anxiety and Depression score (HADS) on Day of each Clinic visit or within 24 hours prior to the visit
- **Food Frequency Questionnaire (FFQ) on the Day of Clinic visit**

Reason:

Modification of data capture of IBS-SSS and FFQ questionnaires to a tablet device during the clinic visit as the smartphone functionality cannot accommodate these assessments due to the both the length and complexity. This change has been made at the request of the Investigators.

A6.7 Section: Overall Study Design Section 4.2.1

Old Text:

Follow-Up (Week 12-14)

Subjects will continue to use their smartphones to capture all the study related outcome data. The data will be reviewed on an ongoing basis as required by the study team and sponsor. At this visit the following information will be reviewed with the subject;

- Information on Adverse Events
- Review of Medication log completed daily
- Recording times of taking their medication completed daily
- Abdominal Pain Intensity NRS completed daily
- Stools consistency/frequency daily
- Subject Global Assessment of Relief completed weekly
- IBS Quality of Life questionnaire (IBS QOL) on the Day of Clinic Visit
- IBS Symptom Severity Score (IBS-SSS) on the Day of Clinic visit or within 24 hrs prior to the visit
- Hospital Anxiety and Depression score (HADS) on Day of each Clinic visit or within 24 hours prior to the visit
- Food Frequency Questionnaire (FFQ) on the Day of Clinic visit or within 24hours prior to the visit

New Text:

Subjects will continue to use their smartphones to capture all the study related outcome data, **except for IBS-SSS and FFQ questionnaires which will be completed during the clinic visit.** The data will be reviewed on an ongoing basis as required by the study team and sponsor. At this visit the following information will be reviewed with the subject;

- Information on Adverse Events
- Review of Medication log completed daily
- Recording times of taking their medication completed daily
- Abdominal Pain Intensity NRS completed daily
- Stools consistency/frequency daily
- Subject Global Assessment of Relief completed weekly
- IBS Quality of Life questionnaire (IBS QOL) on the Day of Clinic Visit
- **IBS Symptom Severity Score (IBS-SSS) on the Day of Clinic visit**
- Hospital Anxiety and Depression score (HADS) on Day of each Clinic visit or within 24 hours prior to the visit

- **Food Frequency Questionnaire (FFQ) on the Day of Clinic visit**

Reason:

Modification of data capture of IBS-SSS and FFQ questionnaires to a tablet device during the clinic visit as the smartphone functionality cannot accommodate these assessments due to the both the length and complexity. This administrative change has been made to ensure correctness and consistency throughout the protocol.

A6.8 Section: INVESTIGATIONAL PRODUCTS: Formulation Section 6.1

Old Text:

CCI [redacted]

New Text:

CCI [redacted]

Reason:

CCI [redacted]

A6.9 Section: Dosage, Frequency and Duration of dosing Section 1.2

Old Text:

Blautix™ or placebo capsules will be administered twice daily at 12 hourly intervals before food for a duration of 8 weeks. CCI [redacted] Subjects will be supplied with enough treatment supply at the Baseline Visit to last until the next scheduled Clinic Visit and then at subsequent visits.

New Text:

Blautix™ or placebo capsules will be administered twice daily at 12 hourly intervals **approximately 30 minutes** before food for a duration of 8 weeks. CCI [redacted] Subjects will be supplied with enough treatment supply at the Baseline Visit to last until the next scheduled Clinic Visit and then at subsequent visits.

[redacted]

Reason:

Correction of an administrative error and consistency with other sections of the Protocol. This administrative change has been made to ensure correctness and consistency throughout the protocol.

A6.10 Section: Clinical Assessments Section 7.1

Old Text:

Food Frequency Questionnaire (FFQ) (Appendix 5)

Subjects will be asked to complete a questionnaire to obtain frequency information about food and beverage consumption over a specified period of time (day, week and month). Once subjects are randomised they will be asked to complete this questionnaire on their smartphone at each clinic visit or within 24hrs prior to the Clinic Visit.

New Text:

Food Frequency Questionnaire (FFQ) (Appendix 5)

Subjects will be asked to complete a questionnaire to obtain frequency information about food and beverage consumption over a specified period of time (day, week and month). Once subjects are randomised they will be asked to complete **this questionnaire on a tablet device during their clinic visit.**

Reason:

Modification of data capture of FFQ to a tablet device during the clinic visit as the smartphone functionality cannot accommodate this assessment. This change has been made at the request of the Investigators.

A6.11 Section: Clinical Assessments Section 7.1

Old Text:

IBS Symptom Severity Score (SSS) (Appendix 4)

Subjects will be asked to complete a questionnaire on severity of abdominal distension, severity of abdominal pain, frequency of abdominal pain, dissatisfaction with bowel habits, and interference of IBS symptoms with daily life. A score ranging from 0 (no symptoms) to 500 (maximum severity) can be achieved as mild (75-175), moderate (176-300) or severe (>300) IBS symptoms.

The initial data from this score will be collected during the Screening visit on the smartphone provided to the subject. This data will be used to classify into Cohorts of IBS sub-types prior to randomisation. Once subjects are randomised they will be asked to record their score on their smart phone at each clinic visit or within 24hrs prior to the Clinic Visit.

New Text:

IBS Symptom Severity Score (SSS) (Appendix 4)

Subjects will be asked to complete a questionnaire on severity of abdominal distension, severity of abdominal pain, frequency of abdominal pain, dissatisfaction with bowel habits, and interference of IBS symptoms with daily life. A score ranging from 0 (no symptoms) to 500 (maximum severity) can be achieved as mild (75-175), moderate (176-300) or severe (>300) IBS symptoms.

The initial data from this score will be collected during the Screening visit **on a tablet device during the clinic visit.** This data will be used to classify into Cohorts of IBS sub-types prior to randomisation. Once subjects are randomised they will be asked to record **their score during each clinic visit on a tablet device.**

***Reason*:**

Modification of data capture of IBS-SSS to a tablet device during the clinic visit as the smartphone functionality cannot accommodate this assessment. This change has been made at the request of the Investigators.

A6.12 Section: Safety Assessments Section 7.2.1

Old Text:

Haematology:

Haemoglobin (HGB), haematocrit (HCT), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), red blood cells (RBC), **erythrocyte sedimentation rate (ESR)**, white cell count (WCC) and platelets (PLT). Differential white cell count will include neutrophils, lymphocytes, eosinophils, basophils and monocytes as absolute values and percentages.

New Text:

Haematology:

Haemoglobin (HGB), haematocrit (HCT), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), red blood cells (RBC), **C-reactive Protein (CRP),** white cell count (WCC) and platelets (PLT). Differential white cell count will include neutrophils, lymphocytes, eosinophils, basophils and monocytes as absolute values and percentages.

***Reason*:**

Removal of ESR and replacement with CRP test within the Hematology panel as this is a more accurate measurement for investigator review. In addition, CRP can be analyzed at the central lab, ESR would need to be measurement locally. The Protocol does not allow for local laboratory assessment, all safety assessments are to be carried out by the central laboratory. This change has been made at the request of the Investigators.

A6.13 Section: Safety Assessments Section 7.2.1

Old Text:

Serology: At screening the analyses will also include HIV-1 & HIV-2, hepatitis B and hepatitis C.

New Text:

Serology: At screening the analyses will also include HIV-1 & HIV-2, **Hepatitis B surface antigen (HBsAg), a Hepatitis B surface antibody (Anti-HBs) and a Hepatitis core antibody test (anti-HBc)**

***Reason*:**

Additional testing for Hepatitis B surface antigen, a Hepatitis B surface antibody and a Hepatitis core antibody test has been added for all subject at screening. This should avoid inclusion of subjects with previous or ongoing HBV infection, a positive result will exclude a subject unless the Hepatitis B surface antibody is the sole positive result (indicating subject's immunization). This change has been made at the request of the Investigators.

A6.14 Section: Safety Assessments Section 7.2.1

Old Text:

Fasting serum will be required for bile acid analysis where 7a-hydroxy-4-cholesterol-3-one (C4) will be assayed using LC-MS/MS as an alternative to the SeHCAT test where it is unavailable.

New Text:

Removal of this test from the planned per Protocol assessments.

***Reason*:**

Fasting serum is required for this test and it is not feasible for sites to obtain a fasting sample from patients enrolled in the study. In addition, this assessment is for an Exploratory Endpoint assessment and the decision was made to remove the test from the Protocol. This change has been made at the request of the Investigators.

A6.15 Section: Serious Adverse Event Reporting Section 8.3

Old Text:

It is the responsibility of the Sponsor to report fatal or life-threatening SUSARs to the MHRA as soon as possible but no later than 7 calendar days after they first become aware of the reaction. The Investigator is required to notify the ethics committee (EC) of any fatal or life-threatening SUSARs as soon as possible but no later than 7 calendar days after they first become aware of the reaction. Any additional relevant information should be sent within 8 days of the report.

The Sponsor is responsible for reporting other (non-fatal, non-life-threatening) SUSARS to the Health Authorities as soon as possible but no later than 15 calendar days after they first become

aware of the reaction. The Investigator is required to notify the EC of any non-fatal or non-life-threatening SUSARs as soon as possible but no later than 15 calendar days after they first become aware of the reaction.

The Sponsor is required to inform the Health Authorities, the Investigator and EC within 3 calendar days of an urgent safety issue.

New Text:

4D Pharma, or it's designee, reports SAE's and/or SUSARS as required to regulatory authorities, investigators/institutions, and IRBs/IECs in compliance with all reporting requirements according to local regulations and good clinical practice.

The investigator is to notify the appropriate IRB/IEC of SAEs occurring at the site and other AE reports received from 4D Pharma or it's designee, in accordance with local procedures and statutes.

***Reason*:**

The original text referred to the MHRA specifically and as this is a global study, the change was made to apply to a generic statement to comply with all agency and local requirements in all countries conducting the trial. This administrative change has been made to ensure correctness and consistency throughout the protocol.

APPENDIX 9: SUMMARY OF PROTOCOL CHANGES VERSION 1.2

PROTOCOL

PROTOCOL NUMBER: BHT-II-002

A PHASE 2 RANDOMISED, DOUBLE BLIND, PLACEBO-CONTROLLED, PARALLEL GROUP, MULTICENTRE STUDY TO EVALUATE THE SAFETY AND EFFICACY OF REPEATED ORAL DOSES OF Blautix™ IN ADULT SUBJECTS WITH IRRITABLE BOWEL SYNDROME (IBS) SUBTYPES IBS-C and IBS-D

Original Version:	Version 1
Version Date:	6 February 2018
Updated Version:	Version 1.1
Version Date:	6 April 2018
Updated Version as per MHRA response:	**Version 1.2**
	19 May 2018

Sponsor Name and Address:

4D Pharma Plc

9 Bond Court

Leeds, U.K

LS1 2JZ

Section: <u>Study Design Section 1.2</u>

Old Text:

Screening Visit

Sexually-active men or women must use an accepted form of birth control throughout the study until completion of two (2) periods after the last dose to avoid pregnancy for at least one complete menstrual cycle). The following methods of birth control are considered highly effective and acceptable for this study; true abstinence, sterilization, birth control pills, vasectomised partner, injections or contraceptive implants. For male subjects, able to father children, and sexually active with a female partner who is able to bear children, must use a barrier method of birth control (male condom with or without spermicide). Subjects should not donate sperm or have unprotected intercourse with a female who is pregnant or is breast-feeding from the time of informed consent until the Follow-up visit.

New Text:

Remove text relating to birth control from screening section as the text is repeated in Section 8.3.1 Pregnancies.

Section 8.3.1 Pregnancies

Old Text:

Subjects who are women and who may be able to have children, will be given a pregnancy test at screening and all subsequent study visits. If the result is positive at screening, the subject will not be able to enter the study. Sexually-active men and women must use an accepted form of birth control throughout the study until completion of two (2) periods after the last dose to avoid pregnancy for at least one complete menstrual cycle). The following methods of birth control are considered highly effective and acceptable for this study; true abstinence, sterilization, birth control pills, vasectomised partner, injections or contraceptive implants. Male subjects, able to father children, and sexually active with a female partner who is able to bear children, must use a barrier method of birth control (male condom with or without spermicide) together with a highly effective method of birth control used by your female partners. Subjects should not donate sperm or have unprotected intercourse with a female who is pregnant or is breast-feeding from the time of informed consent until the Follow-up visit. Female Subjects must not be breast feed an infant during the study.

New Text:

Subjects who are women and who may be able to have children will be given a serum pregnancy test at screening and a urine dipstick test at all subsequent study visits. If the result is positive at screening, the subject will not be able to enter the study. Sexually-active women must use an acceptable form of birth control throughout the study until completion of two (2) periods after the last dose to avoid pregnancy for at least one complete menstrual cycle. The following methods of birth control are considered acceptable for this study; true abstinence, sterilization, birth control pills, vasectomised partner, injections or contraceptive implants. Female Subjects must not be breast feed an infant during the study.

Male subjects, able to father children, and sexually active with a female partner who is able to bear children, must use a barrier method of birth control (male condom with or without spermicide) from the time of randomisation until the Follow-up visit.

Reason:

Revisions made in response to MHRA requests during review of the clinical trial application.

APPENDIX 10: SUMMARY OF PROTOCOL CHANGES VERSION 2.0

Summary of Protocol Changes

PROTOCOL

PROTOCOL NUMBER: BHT-II-002

A PHASE 2 RANDOMISED, DOUBLE BLIND, PLACEBO-CONTROLLED, PARALLEL GROUP, MULTICENTRE STUDY TO EVALUATE THE SAFETY AND EFFICACY OF REPEATED ORAL DOSES OF Blautix™ IN ADULT SUBJECTS WITH IRRITABLE BOWEL SYNDROME (IBS) SUBTYPES IBS-C and IBS-D

Previous Date and Version: 6 February 2018; Version 1.0
6 April 2018; Version 1.1
19 May 2018: Version 1.2

Updated Current Version: 31 May 2018: Version 2.0

Sponsor Name and Address:
4D Pharma Plc
9 Bond Court
Leeds, U.K
LS1 2JZ

Summary of Protocol Changes

Section: 3.2 Endpoints – Primary Efficacy Endpoint

Old Text:

IBS-D
Abdominal Pain Intensity Decrease in weekly average of worst abdominal pain in past 24 hours score of at least 30% compared with baseline and **Stool Consistency (BSS)** Decrease at least 50% in the number of days per week with at least one stool that has a consistency of Type 6 or 7 compared with baseline.

New Text:

IBS-D
Abdominal Pain Intensity Decrease in weekly average of worst abdominal pain in past 24 hours score of at least 30% compared with baseline and **Stool Consistency (BSS)** Decrease at least 50% in the proportion of days per week with at least one stool that has a consistency of Type 6 or 7 compared with baseline.

***Reason**:* A subject may only have recorded the worst stool on four, five or six days of the week (see Section 10.7: Handling of Missing Data). A reduction in the number of days with Type 6 or 7 consistency recorded may simply be due to data being available for fewer days. This difficulty is avoided by expressing the decrease as a proportion. This change has been made at the request of the Investigators.

Section: 4.2 Study Design

Screening Visit

Old Text:

At this visit they will complete the IBS-Symptom Severity Score on this device.

New Text:

At this visit they will complete the IBS-Symptom Severity Score on this **on a tablet device during the clinic visit.**

Reason:

Modification of data capture of IBS-SSS questionnaire to a tablet device during the clinic visit as the smartphone functionality cannot accommodate these assessments due to the both the length and complexity. This change has been made at the request of the Investigators.

Screening/Baseline (Visit 1)/ Visit 2 (Week 4-5)/ End of Treatment (Week 8-9)

Old Text:

Subjects will be provided with a stool collection kit to take home. They will be provided with instructions and asked to provide a stool sample within 48 hours prior to or on the morning of Baseline (Visit 1)

New Text:

Subjects will be provided with a kit with instructions for the collection of a stool sample. They will be asked to provide a stool sample from the night before or on the morning of the next clinic visit

***Reason*:**

Stool samples will need to be frozen at -80 degrees as soon as is possible to avoid significant degradation we are requesting that subjects provide samples as close to the timing of their clinic visit as possible. This change has been made at the request of the Investigators.

Baseline (Visit 1)/ Visit 2 (Week 4-5)/ End of Treatment (Week 8-9)/Follow-Up week 12-14)

Old Text:

IBS Quality of Life questionnaire (IBS QOL) on the Day of Clinic Visit

New Text:

IBS Quality of Life questionnaire (IBS QOL) on the Day of Clinic Visit **or within 24 hours prior to the visit**

Reason:

Consistency throughout the Protocol and provide more flexibility for completion of this assessment. This change has been made at the request of the Investigators.

Section: 10.1: Population for Analysis

Old Text:

The *Safety* Analysis Set includes all subjects randomised into the study who received at least one dose of Blautix™ or Placebo.

The Safety Analysis Set is the primary analysis set for the safety analyses.

The *Full* Analysis Set includes all members of the Safety Analysis Set who were appropriately randomised into the Study.

The Full Analysis Set is the primary analysis set for the efficacy analyses.

The *Efficacy Evaluable* Analysis Set includes all members of the Full Analysis Set who completed the 8-week treatment and assessment period without major protocol violations.

New Text:

The *Safety* Analysis Set includes all subjects randomised into the study who received at least one dose of Blautix™ or Placebo. ***Subjects will be allocated to the treatment group corresponding to the treatment actually received.***

The Safety Analysis Set is the primary analysis set for the safety analyses.

The *Full* Analysis Set includes all members of the Safety Analysis Set who were appropriately randomised into the Study. ***Subjects will be allocated to the treatment group corresponding to the treatment actually received.***

The Full Analysis Set is the primary analysis set for the efficacy analyses.

The *Efficacy Evaluable* Analysis Set includes all members of the Full Analysis Set who completed the 8-week treatment and assessment period without major protocol violations. ***Subjects will be allocated to the treatment group corresponding to the treatment actually received.***

Reason*:*

Text has been added to clarify that subjects will be analysed according to the treatment actually received, as is appropriate for a Phase II randomised study. Change made at the request of FDA.

Section: 10.7 Handling of Missing Data

Old Text:

Subjects who do not provide enough data to enable assessment of whether or not that subject is a responder will be deemed a non-responder.

New Text:

Subjects who do not provide enough data to enable assessment of whether or not that subject is a responder will be deemed a non-responder.

> *An 'evaluable week' is one in which there are at least four twenty-four hour (4 day) records of abdominal pain score and at least four daily records of number of complete spontaneous bowel movements (IBS-C subjects) or of highest Bristol stool chart (IBS-D subjects).*
>
> *If a subject has not provided four evaluable weeks of data, then they are deemed to be a non-responder.*
>
> *If a subject has provided at least four evaluable weeks of data then they are deemed to be a responder if they have reported an improvement in their weekly symptoms for ≥ 60% of the evaluable weeks (if the total number of evaluable weeks is four, five or six) or for ≥ 50% of the evaluable weeks (if the total number of evaluable weeks is seven or eight).*

***Reason**:*

Text has been added to set out the rules (i) for determining whether sufficient data is available to confirm a response and (ii) for determining whether or not a response has occurred if there is incomplete but sufficient data. Change made at the request of FDA.

Section: <u>5.2 Exclusion Criteria</u>

Exclusion Old Text:

Number 10.

> Refusal to use highly effective methods of birth control (true abstinence, sterilisation, birth control pills, injections or contraceptive implants) for fertile patients (females and males) while on treatment and following completion of 2 periods months after the last dose of study treatment

Exclusion New Text:

Number 10

> Refusal to use acceptable methods of birth control (true abstinence, sterilisation, birth control pills, injections or contraceptive implants) for fertile patients (females) while on treatment and following completion of 2 menstrual cycles/months after the last dose of study treatment. For Males, a barrier method of birth control from randomisation until the Follow-Up visit

Reason: Changes have been made to the classification of birth control during this study following MHRA requested changes. Further changes have been made to the Exclusion criteria to align with these revisions in Section 8.3.1 Pregnancies.

Exclusion Old Text:

Number 15:

Lactose intolerance

Number 16:

Coeliac disease

Exclusion New Text

Number 15. Clinically diagnosed Lactose intolerance

Number 16. Clinically diagnosed Coeliac disease

Reason:

To provide further guidance to the investigators that these exclusion points are referring to a clinical diagnosis and therefore clear medical records to confirm. This change has been made at the request of the Investigators.

Old Text

Exclusion Number 18

Those > 55 will be excluded if their diagnosis of IBS is recent (<12 months) and if they have not had a sigmoidoscopy or colonoscopy within previous 5 years.

New Text:

Exclusion Number 18

Number 18: Those > 50 will be excluded if their diagnosis of IBS is recent (<12 months) and if they have not had a sigmoidoscopy or colonoscopy within previous 5 years.

***Reason*:**

Reduction to the upper age limit from >55 to >50 to comply with the American standard as opposed to the European standard. This change has been made at the request of the Investigators.

<u>New Exclusion Criteria</u>

Old Text:

Addition of new exclusion criteria. No old text.

New Text:

Exclusion Number 22

22. Subjects with abnormal laboratory values at screening deemed by the investigator to be clinically significant

Reason:

Addition of a new Exclusion following a recommendation from the FDA.

New Exclusion Criteria

Old Text:

Addition of new exclusion criterion. No old text.

New Text:

Exclusion Number 23

23. Subjects who have taken commercially available probiotics within the last month (30 days prior to randomisation)

Reason:

Following FDA recommendation to revise the protocol and the eligibility criteria such that subjects are not consuming over the counter probiotics from 1 month (30 days) before administration of the investigational product through the end of the study. There is a concern may impact the biological action.

New Text:

Exclusion Number 24

24. Subjects with known or suspected hereditary fructose intolerance, glucose-galactose malabsorption or sucrase-isomaltose insufficiency

Reason:

CCI

[redacted]

Section: 7.1 Clinical Assessments

Old Text:

Food Frequency Questionnaire (FFQ) (Appendix 5)

Subjects will be asked to complete a questionnaire to obtain frequency information about food and beverage consumption over a specified period of time (day, week and month). Once subjects are randomised they will be asked to complete this questionnaire on a tablet device during their Clinic Visit.

New Text:

Food Frequency Questionnaire (FFQ) (Appendix 5)

Subjects will be asked to complete a questionnaire to obtain frequency information about food and beverage consumption over a specified period of time (day, week and month). FFQ completion at baseline will be in relation to food (s) consumed over the prior 6 months. At each subsequent visit the FFQ shall capture responses to food (s) consumed since last subject visit. Subjects will be asked to complete this questionnaire on a tablet device during each Clinic Visit.

***Reason*:**

Further clarification has been added to the Protocol regarding the length of time the subject will need to review their food frequency and consistency throughout the Protocol regarding completion of this questionnaire via a tablet device. This change has been made at the request of the Investigators.

Old Text:

Diet and Study Restrictions

Dosing should be twice daily at twelve hourly intervals (approximately 30 minutes before breakfast and 30 minutes before evening meal). Subjects with lactose intolerance and Coeliac disease and those with a change of diet within the 3 months before screening are excluded. Supplements (pre-, pro-biotics) are allowed provided they are recorded by the subject in the FFQ at Visit 1 and subsequent visits.

New Text:

Diet and Study Restrictions

Dosing should be twice daily at twelve hourly intervals (approximately 30 minutes before breakfast and 30 minutes before evening meal). Subjects with lactose intolerance and Coeliac disease and those with a change of diet within the 3 months before screening are excluded. **Subject taking commercially available supplements (probiotics) are excluded 1 month (30 days) prior to randomisation and throughout the study to follow-up.**

Reason:

Following FDA recommendation to revise the protocol and the eligibility criteria such that subjects are not consuming over the counter probiotics from 1 month (30 days) before administration of the investigational product through the end of the study. There is a concern may impact the biological action.

Section: 7.2.1 Safety Assessments

Old Text:

Serology:

At screening the analyses will also include HIV-1 & HIV-2, Hepatitis B surface antigen (HBsAg), a Hepatitis B surface antibody (Anti-HBs) and a Hepatitis core antibody test (anti-HBc)

New Text:

Serology:

At screening the analyses will also include HIV-1 & HIV-2, Hepatitis B surface antigen (HBsAg), a Hepatitis B surface antibody (Anti-HBs) and a Hepatitis core antibody test (anti-HBc) and Hepatitis C

Reason:

Hepatitis C was removed in error during the changes to the Version 1.1. This administrative change has been made to ensure correctness and consistency throughout the protocol.

Section: 8.2.1 Adverse Events

Old Text:

3. Severity; severity grade (mild, Moderate, Severe)

New Text:

3.

Mild
Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated

Moderate
Moderate; minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental ADL (Activities of Daily Living)

Severe
Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of existing hospitalization indicated; limiting self-care ADL (Activities of Daily Living)

Reason:

The FDA requested definition of the terms; mild, moderate and severe within the Protocol. The revised terminology which is in accordance with CTCAE (Common Terminology Criteria for Adverse Events):

Section: 7.1 Clinical Assessments

Old Text:

Stool Frequency and Consistency Score

Subjects will be asked to record stool frequency and consistency Stool types will be based on the Bristol Stool Chart (Appendix 3). This data will be recorded for a 7-day period up to Baseline (Visit 1) to allow Subject to be classified into Cohorts of IBS sub-types. Once subjects are randomised they will be asked to record their score daily on their smartphone.

New Text:

Bristol Stool Frequency and Consistency Score

Subjects will be asked to record stool frequency and consistency. Stool types will be based on the Bristol Stool Chart (Appendix 3). This data will be recorded daily for a 7-day period up to Baseline (Visit 1) to allow Subject to be classified into Cohorts of IBS sub-types. Once subjects are randomised they will be asked to record their stool frequency daily up to 10 bowel movements per day and score each stool type on their smartphone. No bowel movements in any given day will also be recorded.

Reason:

To ensure data required to meet the primary endpoint is sufficient we will require subject to record up to 10 bowel movements per day. This change has been made at the request of the Investigators.

Section: 13. PUBLICATION POLICY

Old Text:

PUBLICATION POLICY

The Sponsor and Investigator will mutually manage the publication and presentation process. All manuscripts, abstracts, or other modes of presentation arising from the results of the study must be reviewed and approved in writing by the Sponsor in advance of submission. The review is aimed at protecting the Sponsor's proprietary information existing either at the date of the commencement of the study or generated during the study.

New Text:

PUBLICATION POLICY

The Sponsor and Investigator will mutually manage the publication and presentation process. All manuscripts, abstracts, or other modes of presentation arising from the results of the study must be reviewed and approved in writing by the Sponsor in advance of submission in **accordance with the clinical site agreement.** The review is aimed at protecting the Sponsor's proprietary information existing either at the date of the commencement of the study or generated during the study.

Reason:

To ensure consistency with the terms of the Clinical Site Agreement. This change has been made at the request of the Investigators.

Appendix 5 Modified FFQ

Updates have been made to the questionnaire relating to certain food groups. US and UK terminology have been added to form a single column for ease of use and consistency for all countries in this global clinical trial.

Reason:

This administrative change has been made to ensure correctness and consistency throughout the protocol.

APPENDIX 11: SUMMARY OF PROTOCOL CHANGES VERSION 2.1

Summary of Protocol Changes

PROTOCOL

PROTOCOL NUMBER: BHT-II-002

A PHASE 2 RANDOMISED, DOUBLE BLIND, PLACEBO-CONTROLLED, PARALLEL GROUP, MULTICENTRE STUDY TO EVALUATE THE SAFETY AND EFFICACY OF REPEATED ORAL DOSES OF Blautix™ IN ADULT SUBJECTS WITH IRRITABLE BOWEL SYNDROME (IBS) SUBTYPES IBS-C and IBS-D

Previous Date and Version: 6 February 2018; Version 1.0
6 April 2018; Version 1.1
19 May 2018: Version 1.2
31 May 2018: Version 2.0

Updated Current Version: 21 June 2018: Version 2.1

Sponsor Name and Address:
4D Pharma Plc
9 Bond Court
Leeds, U.K
LS1 2JZ

Summary of Protocol Changes

Section: 8.3.1 Pregnancy

Old text:

Subjects who are women and who may be able to have children will be given a serum pregnancy test at screening and a urine dipstick test at all subsequent study visits. If the result is positive at screening, the subject will not be able to enter the study. Sexually-active women must use an acceptable form of birth control throughout the study until completion of two (2) periods after the last dose to avoid pregnancy for at least one complete menstrual cycle. The following methods of birth control are considered acceptable for this study; true abstinence, sterilisation, birth control pills, vasectomised partner, injections or contraceptive implants. Female Subjects must not be breast feed an infant during the study.

New Text:

Subjects who are women and who may be able to have children will be given a serum pregnancy test at screening and a urine dipstick test at all subsequent study visits. If the result is positive at screening, the subject will not be able to enter the study. Sexually-active women must use an acceptable form of birth control throughout the study until completion of two (2) periods after the last dose to avoid pregnancy for at least one complete menstrual cycle. The following methods of birth control are considered acceptable for this study; true abstinence, sterilisation, birth control pills, vasectomised partner, injections or contraceptive implants. **The following birth control pills are recommended:**

- **Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation:**
  - **Oral**
  - **Intravaginal**
  - **Transdermal**
- **Progestogen-only hormonal contraception associated with inhibition of ovulation:**
  - **Oral**
  - **Injectable**
  - **Implantable**

Female Subjects must not be breast feed an infant during the study.

Reason:

Information on the specific types of birth control pills has been added at the request of the UK Medicines and Healthcare products Regulatory Agency (MHRA) during review of the clinical trial application.

Section: 5.1 Inclusion Criteria

Old Text:

Inclusion Number 4:

Having IBS-C or IBS-D as defined by Rome IV*

New Text:

Inclusion Number 4:

Having IBS-C or IBS-D as defined by Rome IV* including Subtype Classification as defined per Table 2

Old Text:

IBS-C	IBS-D
□ Abdominal Pain Intensity: weekly average of worst daily (in past 24 hours) abdominal pain score of > 3.0 on a 0 to 10-point scale And □ Stool Frequency: more than 25% of bowel movements with a consistency of Type 1 or Type 2 Bristol stool chart and less than 25% of bowel movements with Bristol stool form Type 6 or Type 7	□ Abdominal Pain Intensity: weekly average of worst daily (in past 24 hours) abdominal pain score of > 3.0 on a 0 to 10-point scale And □ Stool Consistency: more than 25% of bowel movements with a consistency of Type 6 or Type 7 Bristol stool chart and less than 25% of bowel movements with Bristol stool form Type 1 or Type 2

New Text:

Table 2

IBS-C	IBS-D
□ Abdominal Pain Intensity: weekly average of worst daily (in past 24 hours) abdominal pain score of > 3.0 on a 0 to 10-point scale And □ Stool Frequency: more than 25% of bowel movements with a consistency of Type 1 or Type 2 Bristol stool chart and less than 25% of bowel movements with Bristol stool form Type	□ Abdominal Pain Intensity: weekly average of worst daily (in past 24 hours) abdominal pain score of > 3.0 on a 0 to 10-point scale And □ Stool Consistency: more than 25% of bowel movements with a consistency of Type 6 or Type 7 Bristol stool chart and less than 25% of bowel movements with Bristol stool form Type 1 or Type 2. Subjects must have at least one

6 or Type 7. Subject must have fewer than 3 CSBMs within a one week period (7 days)	Type 6 or Type 7 bowel movements on at least four days within a one week period (7 days).

Reason:

Inclusion Number 4 and Subtype Classifications (Table 2) have been updated in response to FDA recommendations to further pre-define the sub-type population groups

APPENDIX 12: SUMMARY OF PROTOCOL CHANGES VERSION 3.0

Summary of Protocol Changes

PROTOCOL

PROTOCOL NUMBER: BHT-II-002

A PHASE 2 RANDOMISED, DOUBLE BLIND, PLACEBO-CONTROLLED, PARALLEL GROUP, MULTICENTRE STUDY TO EVALUATE THE SAFETY AND EFFICACY OF REPEATED ORAL DOSES OF Blautix™ IN ADULT SUBJECTS WITH IRRITABLE BOWEL SYNDROME (IBS) SUBTYPES IBS-C and IBS-D

Previous Date and Version: 6 February 2018; Version 1.0
6 April 2018; Version 1.1
19 May 2018: Version 1.2
31 May 2018: Version 2.0
21 June 2018: Version 2.1

Updated Current Version: 13 February 2019: Version 3.0

Sponsor Name and Address:
4D Pharma Plc
9 Bond Court
Leeds, U.K
LS1 2JZ

Section: Synopsis

Objectives and Endpoints and Criteria for Evaluation

Old Text:

Secondary Efficacy Endpoints

CCI [redacted]

[redacted]

New Text:

Exploratory Endpoints

CCI [redacted]

[redacted]

Cytokine analysis

Exploratory Efficacy Analyses

CCI [redacted]

[redacted]

[redacted]

[redacted]

Section: 2.3.1 Potential Risks

Old Text:

CCI [redacted]

New Text:

New Text:

CCI [redacted].

Reason:

Ease of reading

Section 3.1: Objectives

Old Text:

Exploratory Objective

CCI [redacted].

New Text:

Exploratory Objective

CCI [redacted]

CCI [redacted]

Section 3.2: Endpoints

Old Text:

Primary Efficacy Endpoint

The primary efficacy endpoint is whether or not the subject is an overall responder.

New Text:

The primary efficacy endpoint is whether the subject is an overall responder.

Reason:

For clarity.

Old Text:

Secondary Efficacy Endpoints

CCI [redacted]

Metabolomics

New Text:

Exploratory Endpoints

CCI [redacted]

Reason:

CCI [redacted]

Section 4.2.1 Overall Study Design

Screening Visit

Old Text:

Women of childbearing potential will have a fresh urine pregnancy test.

New Text:

Women of childbearing potential will have a **serum** pregnancy test.

Reason:

To correct an error and ensure consistency throughout the protocol.

Old Text:

The subject will be asked to take the smart phone home and complete the following assessments daily over a 7-day period. These assessments will need to be completed prior to attending the clinic for their Baseline Visit 1

- An abdominal pain intensity rating scale daily
- Bowel movements and Stool consistency/frequency daily

The subject will need to ensure they complete these two assessments allowing one clear day prior to the next clinic visit.

New Text:

The subject will be asked to take the smart phone home and complete the following assessments daily over a 7-day period

- An abdominal pain intensity rating scale daily
- Bowel movements and Stool consistency/frequency daily **and laxative use**

  If no bowel movements, this should be recorded as zero

The subject will need to ensure they complete these two assessments **at least** one day prior to the next clinic visit.

Reason:

For clarity and to include laxative use. This change has been made at the request of the Investigators.

Old Text:

Subjects will be provided with a kit with instructions for the collection of a stool sample. They will be asked to provide a stool sample from the night before or on the morning of the next clinic visit.

New Text:

Subjects will be provided with a kit with instructions for the collection of a stool sample. They will be asked to provide a stool sample from the night before or on the morning of the next clinic visit. **If necessary, a stool sample may be provided up to 2 days after the visit provided it is from the night before or from the day it is delivered to the clinic.**

Reason:

To provide additional time for subjects to provide a stool sample. This change has been made at the request of the Investigators.

Old Text:

N/A

New Text:

Reasons for screen failure will be documented in the subject's notes. Subjects may be re-screened once, after the initial screening visit if considered to be appropriate by the Investigator. A waiting period of at least 2 weeks is required between screening visits.

Reason:

To provide guidance for screen failure and re-screening. This change has been made at the request of the Investigators.

<u>Baseline: Visit 1 (Day 1)</u>

Old Text:

Subjects will return to the clinical unit on Day 1 (Visit 1; within 28 days of screening visit)

New Text:

Subjects will return to the clinical unit on Day 1 (Visit 1; within 28 days of screening visit **or re-screening visit if applicable))**

Reason:

To confirm time interval between a re-screen and Visit 1. This change has been made at the request of the Investigators.

Old Text:

A brief physical examination, assessment of vital signs and a urine pregnancy test for female subjects, **height**, weight and BMI ……

New Text:

A brief physical examination, assessment of vital signs and a urine pregnancy test for female subjects **of child bearing potential**, weight and BMI ……

Reason:

To clarify that a pregnancy test ifs required only in female subjects of child bearing potential and to remove measurement of height as this will be measured at the screening visit only. **These changes have also been made to the Follow-up Visit.**

Old Text:

N/A

New Text:

Final confirmation of eligibility for the study will be documented, signed by the Investigator and filed in the subject notes.

Reason:

To confirm the procedure for final confirmation of eligibility at baseline prior to randomisation. This change has been made at the request of the Investigators.

Old Text:

…two (2) capsules to be taken at 12 hourly intervals, approximately 30 minutes before meals. Total of four (4) capsules per day.

New Text:

…two (2) capsules to be taken at **approximately** 12 hourly intervals, **morning and evening** approximately 30 minutes before meals **giving a t**otal of four (4) capsules per day.

Reason:

To clarify dosage instructions. This change has been made at the request of the Investigators.

Old Text:

Subjects will use their smartphone issued at the screening visit to capture their study related data

- Abdominal Pain completed daily
- Stools consistency, frequency completed daily

New Text:

Subjects will use their smartphone issued at the screening visit to capture their **patient** related data

- Abdominal Pain **Score** completed daily
- Stools consistency/frequency, **spontaneity and laxative use** completed daily
- **Review of concomitant medications**

Reason:

For consistency throughout protocol and addition of concomitant medication review and laxative use. **These changes have also been made for Visit 2, End of treatment and Follow-up.**

<u>Visit 2 (Week 4-5)</u>

Old Text:

Visit 2 (Week 4-5)

Subjects will return after 4-5 weeks

New Text:

Visit 2 (Week **4**)

Subjects will return after **4** weeks **(± 7 days)**

Reason:

To provide a window around the visit date. This change has been made at the request of the Investigators.

Old Text:

Four (4) capsules total per day and to store in the fridge and not to open the capsules.

New Text:

Subjects will receive a total of four (4) capsules per day and **will be reminded** to store **the capsules** in the fridge and not to open the capsules.

Reason:

To correct grammatical errors for clarity.

End of Treatment (Week 8-9)

Old Text:

End of Treatment (Week 8-9)

Subjects will return to the clinic at the end of the 8 week treatment period.

New Text:

End of Treatment (Week **8**)

Subjects will return to the clinic at the end of the 8 week **(or up to ± 7 days)** treatment period.

Reason:

To provide a window around the visit date. This change has been made at the request of the Investigators.

Follow-Up (Week 12-14)

Old Text:

Subjects will return 4-6 weeks after the last dose of study medication

New Text:

Subjects will return after 4-6 weeks **(± 7 days)** after the last dose of study medication.

Reason:

To provide a window around the visit date. This change has been made at the request of the Investigators.

Table 1: Schedule of Assessments

Old Text:

Visit 2: Week 4-5

New Text:

Visit 2: Week **4 (±7 days)**

Reason:

This change has been made for clarity at the request of the Investigators

Old Text:

End of Treatment Week 8-9

New Text:

End of Treatment Week **8 (±7 days)**

Reason:

This change has been made for clarity at the request of the Investigators

Old Text:

Abdominal Pain Intensity NRS score

New Text:

Abdominal Pain score

Reason:

For consistency throughout protocol

Old Text:

Weight/Height/BMI

New Text:

Weight/Height/BMI

Footer: [e] Weight only to be measured and BMI to be calculated, after the screening visit

Reason:

To clarify that height is measured only at screening

Footer Pregnancy test[c]

Old Text:

[c] Pregnancy Test **(Urine) :** Serum at screening and Urine Dipstick test for all other visits. **Fertile male** patients and female patients of childbearing potential are to continue using highly effective (refer to section 4.2.1) contraception **for two (2) periods after the last dose of study medication to avoid pregnancy for one complete menstrual cycle.**

New Text:

[c] **Pregnancy Test :** Serum at screening and Urine Dipstick test for all other visits. Female patients of childbearing potential are to continue using acceptable (refer to section 4.2.1) contraception **for two (2) menstrual cycles after the last dose of study medication. Male subjects who are not vasectomised must use a barrier method of birth control from randomisation until the follow-up visit.**

Reason:

To provide consistency with other sections in the protocol. This change has been made at the request of the Investigators. Changes have also been made to section 7.2.1 below.

Section 5.1 Inclusion Criteria

Footer Table 2

Old Text:

N/A

New Text:

[1] CSBM defined as a bowel movement that is both complete and spontaneous. Bowel movements where laxative use is recorded in the concomitant medication questions on the same calendar day by IBS-C subjects will not be counted as CSBMs when subtyping for randomisation.

Reason:

To provide a definition and clarification for CSBMs. This change was made at the request of the Investigators.

Inclusion Criteria 4

Old Text:

Have a moderate or severe IBS symptom severity score as defined by IBS-SSS.

New Text:

Have a moderate or severe IBS symptom severity score: **>175 at the screening visit** as defined by IBS-SSS. **A tolerance of -10% (≥ an IBS-SSS score of 157.5) will be allowable at the Baseline (Visit 1).**

Reason:

This change was made for clarity at the request of the Investigators.

Section 5.2 Exclusion Criteria

Exclusion 8

Old Text:

Have a malignant disease or any concomitant end-stage organ disease.

New Text:

8. Have a**n active or recent (within 3 years)** malignant disease or any concomitant end-stage organ disease. **A non-melanoma skin cancer that has been adequately treated with no recurrence within 3 months of screening is not excluded.**

Reason:

To provide clarification concerning malignant disease. This change has been made at the request of the Investigators.

Exclusion 10

Old Text:

Refusal to use acceptable methods of birth control (true abstinence, sterilisation, birth control pills, injections or contraceptive implants) for fertile patients while on treatment and following completion of 2 menstrual cycles/months after the last dose of study treatment. For Males, a barrier method of birth control from randomisation until the Follow-Up visit

New Text:

Refusal to use acceptable methods of birth control (true abstinence, sterilisation, birth control pills, injections or contraceptive implants) for **women of childbearing potential** while on treatment and following completion of 2 menstrual cycles/months after the last dose of study treatment. For Males, a barrier method of birth control from randomisation until the Follow-Up visit, **unless vasectomised.**

Reason:

To provide consistency with other sections in the protocol. This change has been made at the request of the Investigators.

Exclusion 11

Old Text:

Use of antibiotics within 1 month of screening

New Text:

Use of antibiotics **within 30 days** of screening

Reason:

To provide consistency with other sections in the protocol. This change has been made at the request of the Investigators.

Exclusion 12

Old Text:

Use of systemic steroids within the last month

New Text:

Use of systemic steroids within **30 days of screening**

Reason:

To provide consistency with other sections in the protocol. This change has been made at the request of the Investigators.

Exclusion 14

Old Text:

Have suffered from a major psychiatric disorder.

New Text:

Have suffered from a**n uncontrolled or current** major psychiatric disorder.

Reason:

To provide clarification. This change has been made at the request of the Investigators.

Exclusion 19

Old Text:

Any abdominal surgery other than hernia repair or appendectomy

New Text:

Any **GI related** abdominal surgery other than hernia repair or appendectomy.
Cholecystectomy more than 6 months previously is not an exclusion.

Reason:

To provide clarification. This change has been made at the request of the Investigators

Exclusion 20

Old Text:

Other investigational procedures while participating in this study are excluded.

New Text:

N/A Text deleted.

Reason:

This text has been deleted to avoid ambiguity and repetition. This change has been made at the request of the Investigators.

Exclusion 20

New Text:

The deleted exclusion has been replaced with:

Subjects taking prucalopride

Exclusion 23

Old Text:

Subjects who have taken commercially available probiotics within the last month (30 days prior to randomization.

New Text:

Subjects who have taken commercially available probiotics within the last month (30 days prior to randomization). **See Appendix 7**

Reason:

To reference the Appendix

Exclusion 24

Old Text:

Subjects with sucrose-isomaltose insufficiency

New Text:

Subjects with sucrose-isomalt**a**se insufficiency

Reason:

To correct a spelling error

Exclusion 25

Old Text:

N/A

New Text:

Subjects taking guanylate cyclase agonists: such as linaclotide and lubiprostone

Reason:

To add a new exclusion criterion

Section 5 Randomisation

Old Text:

Subjects, 4D Pharma Plc, designated CRO and **other** Clinical site staff

New Text:

Subjects, 4D **pharma** PLC, designated CRO and clinical site staff

Reason:

For clarification

Section 6 Investigational Products

6.1 Formulation

Old Text:

CCI

[redacted]

New Text:

CCI

[redacted]

Reason:

This change has been made for consistency with the formulation used in this study.

6.2 Dosage, Frequency and duration of dosing

Old Text:

capsules will be administered twice daily at 12 hourly intervals

New Text:

capsules will be administered twice daily at **approximately** 12 hourly intervals **morning and evening**

Reason:

This change has been made for consistency throughout the protocol.

Section 6.8 Concomitant Treatment

Old Text:

Any concomitant medication taken within 30 days prior to the first dose of study medication should be recorded in the case report forms.

New Text:

Any concomitant medication taken within 30 days prior to the first dose of study medication **and up to the follow-up visit** should be recorded in the case report forms.

Reason:

To clarify the period for recording of concomitant medication.

Section 6.9 Prohibited Medication

Old Text:

N/A

New Text:

Probiotics such as yogurts, yogurt-type drinks and other soft drinks e.g. fruit juices may be taken during the study. Probiotic supplements formulated as capsules or sachets are prohibited (see Appendix 7). Acceptable probiotics taken during the month before screening should be continued through the study period and to follow-up to ensure consistency.

Prucalopride is a prohibited medication and should not be taken during the study.

IBS-C subjects should not take guanylate cyclase agonists (such as linaclotide) and lubiprostone. IBS-D subjects should not take bile acid sequestrants (such as cholestyramine, colestipol, colesevelam), 5-hydroxytryptamine (serotonin) 3 receptor antagonists (such as alosetron), rifaximin and eluxadoline (see Appendix 7).

Subjects are not eligible for the study if they have received antibiotics or systemic corticosteroids within 30 days prior to the screening visit date. Subjects on inhaled steroids for asthma may be included but oral or infused steroids for asthma are not permitted. If these medications are required during the study, the subject does not need to be withdrawn but details will be recorded in the concomitant medication case report forms.

Reason:

A section on prohibited medication has been added for clarity. This change has been made at the request of the Investigators

Section 7.1 Clinical Assessments

Abdominal Pain Score

Old Text:

Abdominal Pain **Intensity NRS** Score

Subjects will be asked to rate their worst abdominal pain over the past 24 hours using an 11-point (NRS) and a weekly average will be recorded.

New Text:

Abdominal Pain Score

Subjects will be asked to rate their worst abdominal pain over the past 24 hours using an 11-point **Numeric Rating Scale** (NRS) and a weekly average will be recorded.

Reason

This change has been made for clarity at the request of the Investigators.

Subject Global Assessment of Relief

Old Text:

A weekly subject response to the question 'How have you felt during the past week in regard to your IBS, particularly overall well being and symptoms of abdominal comfort, pain and altered bowel habit'.

New Text:

A weekly response to the question **'Please consider how you felt this past week in regard to your IBS, in particular your overall wellbeing and symptoms of abdominal discomfort, pain and altered bowel habit. Compared to the way you usually felt before entering the study, how would you rate your relief of symptoms during the past week?'**

Reason:

This change has been made at the request of the Investigators.

IBS Quality of Life

Old Text:

N/A

New Text:

The individual responses to the 34 items are summed and averaged for a total score and then transformed to a 0-100 scale for ease of interpretation with higher scores indicating better IBS specific quality of life

Reason

This change has been made at the request of the Investigators.

Bristol Stool Chart

Old Text:

Bristol Stool **Frequency and Consistency** Score

New Text:

Bristol Stool Chart

Stool frequency will be defined as a sum of weekly CSBMs. Stool frequency will be recorded using the following question: "How many times did you open your bowels during the past 24 hours? If you have not had any bowel movements today, please enter 0." Subjects will be reminded to rate all of their bowel movements in the Bristol Stool Chart before answering the question. CSBMs will be determined by absence of laxative use recorded in concomitant medication questions for the same calendar day.

Reason

This change has been made at the request of the Investigators.

IBS Symptom Severity Score

Old Text:

A score ranging from 0 (no symptoms) to 500 (maximum severity) can be achieved as mild, moderate or severe (>300) IBS symptoms.

The initial data from this score will be collected during the Screening visit on a tablet device during the clinic visit. This data will be used to classify into Cohorts of IBS sub-types prior to randomisation. Once subjects are randomised, they will be asked to record their score during each visit on a tablet device.

New Text:

A score ranging from 0 (no symptoms) to 500 (maximum severity) can be achieved as mild (**74-174)**, moderate **(175-299)** or severe (>300) IBS symptoms.

The initial data from this **questionnaire** will be collected during the Screening visit on a tablet device during the clinic visit. This data will be used to classify into Cohorts of IBS sub-types prior to randomisation. Once subjects are randomised, they will be asked to record their **responses to the questionnaire on a tablet device** during each clinic visit.

Reason

This change has been made at the request of the Investigators.

Food Frequency Questionnaire

Old Text:

N/A

New Text:

- **Concomitant Medications**

Subjects will be asked to record name, dose and time of administration of any medication, other than IMP, taken during the study. This will be reviewed at each visit.

Reason

This change has been made at the request of the Investigators.

Dosing Diary

Old Text:

Subjects will be asked to complete times that the medication was taken on their smartphone and will be prompted with reminders via this device.

New Text:

Subject will be asked to **confirm their daily study medication intake by indicating "yes" or "no" to the following questions on the smartphone provided: "Did you take two of your study medication capsules this morning?" in the morning and "Did you take two of your study medication capsules this evening?" in the evening**. **Subjects will be prompted with reminders via this device.**

Reason

This change has been made for clarity at the request of the Investigators

Diet and Study Restrictions

Old Text:

Dosing should be twice daily at twelve hourly intervals (approximately 30 minutes before breakfast and 30 minutes before evening meal). Subjects with lactose intolerance and Coeliac disease and those with a change of diet within the 3 months before screening are excluded. Subject taking commercially available supplements (probiotics) are excluded 1 month (30 days) prior to randomisation and throughout the study to follow-up.

New Text:

Dosing should be twice daily at twelve hourly intervals (approximately 30 minutes before breakfast and 30 minutes before evening meal). Subjects with lactose intolerance and Coeliac disease and those with a change of diet within the 3 months before screening are excluded. Subject taking commercially available supplements **(probiotics formulated as capsules or sachets as in Appendix 7)** are excluded 1 month (30 days) prior to randomisation and throughout the study to follow-up. **Probiotic yogurts, yogurt-type drinks and soft drinks e.g.fruit juices are acceptable if taken during the month before screening and continued to follow-up.**

Reason:

Clarification of probiotic use and for consistency with section 6.9, prohibited medications. This change has been made at the request of the Investigators

Section 7.2.1 Safety Assessments

Blood Samples:

Old Text:

[redacted]

N/A

New Text:

Pregnancy test:

A serum pregnancy test will be conducted for all female subjects at the screening visit.

Reason:

For consistency with other sections in the protocol.. This change has been made at the request of the Investigators.

Urine samples:

Old Text:

A fresh urine sample will be collected for a pregnancy test in all female subjects at **Screening (serum test at screening only)** Baseline (Visit 1), Visit 2, End of Treatment and Follow-up (dipstick testing).

New Text:

A fresh urine sample will be collected for a pregnancy test in all female subjects at Baseline (Visit 1), Visit 2, End of Treatment and Follow-up (dipstick testing).

Reason:

For consistency with other sections in the protocol. This change has been made at the request of the Investigators.

Section 7.2.2 Exploratory Assessments

Old Text:

Blood Samples:

Blood samples will be collected at the times indicated in Table 1.

CCI [redacted].

New Text:

Blood Samples:

Blood samples will be collected at the times indicated in Table 1.

CCI [redacted]

Reason:

This change has been made for consistency with other sections of the protocol.

Section 8.3.1 Pregnancies

Old Text:

[redacted]

Sexually-active women must use an acceptable form of birth control throughout the study

New Text:

Sexually-active women **of child bearing potential** must use an acceptable form of birth control throughout the study

Reason:

This change has been made for consistency throughout the protocol.

Old Text:

Female Subjects must not **be** breast feed an infant during the study.

New Text:

Female Subjects must not breast feed an infant during the study.

Reason:

To correct a grammatical error. This change has been made at the request of the Investigators.

Section 9.2 Source Documents

Old Text:

N/A

New Text:

Clinical Investigators and authorized study personnel will review and access the electronic eCOA data; collected on smartphones and tablets, according to patient activity on site (following screening and scheduled clinic visits as scheduled in the Protocol, e.g.Screening Visit, Baseline Visit, Visit 2, End of Treatment, and Follow-Up. Discrepancies will be resolved using data queries and site response, with the oversight of the monitor.

Reason:

To add the procedure for review of eCOA data.

Section 9.4 Database Management and Quality Control

Old Text:

N/A

New Text :

Access to the eCOA data will be controlled via the vendor Support Helpdesk. Users must have successfully completed the vendor training module, with a pass-rate of >80%. Full log of query and resolution steps will be maintained within the system, with full audit trail.

Reason:

To add procedures relating to the eCOA data.

Section 9.6 Study Records Retention

Old Text:

The Investigator shall retain records required to be maintained for a period of 2 years following the date a marketing application in the ICH region is approved for the drug for the indication for which it is being investigated or, if no application is to be filed or if the application is not approved for such indication, until at least 15 years following the end of the study. However, these documents should be retained for a longer period if required by the applicable regulatory requirements(s) or if needed by 4D Pharma. In addition, the investigator must make provision for the patients' medical records to be kept for the same period of time.

New Text :

Study documents should be retained for a minimum of 25 years after the end of the clinical study. These documents should be retained for a longer period, however, if required by local regulations. Medical files of subjects should be archived in accordance with national requirements. No records will be destroyed without the written consent of the Sponsor, if applicable. It is the responsibility of the Sponsor to inform the Investigator when these documents no longer need to be retained.

Reason:

To update the requirements for study records retention.

Section 10.5.3 Exploratory Efficacy Anaylsis

CCI [redacted]

Reason:

To add an exploratory analysis.

Section 10.7 Handling of Missing Data

Old Text:

Subjects who do not provide enough data to enable assessment of whether **or not** that subject is a responder will be deemed a non-responder.

An 'evaluable week' is one in which there are at least four twenty-four hour (4 day) records of abdominal pain score and at least four daily records of number of complete spontaneous bowel movements (IBS C subjects)or of highest Bristol stool chart (IBS D subjects)

[redacted]

New Text :

Subjects who do not provide enough data to enable assessment of whether that subject is a responder will be deemed a non-responder.

An 'evaluable week' is one in which there are at least four twenty-four hour (4 day) records of abdominal pain score and at least four daily records of **the** number of complete spontaneous bowel movements **and** stool chart.

Reason:

This change has been made at the request of the Investigators

Old Text:

Partial dates will be imputed in a standard manner.

There will be no other imputation for missing data.

New Text :

N/A Old Text deleted

Reason:

This change has been made at the request of the Investigators

Section 12.4 Informed Consent

Old Text:

Subjects may be approached to discuss the study determine if they are interested in participating in the study.

New Text:

Subjects may be approached to discuss the study **to** determine if they are interested in participating in the study.

Reason:

To correct a grammatical error.

Old Text:

The physician who conducts the informed consent discussion must also sign and date the consent form.

New Text:

The physician **or designee** who conducts the informed consent discussion

Reason:

To allow the informed consent discussion to be delegated to a non- physician. This change has been made at the request of the Investigators.

Section 12.5 Future use of stored samples

Old Text:

N/A

New Text:

Samples will not be labelled with information that directly identifies the subject but will be coded with the identification number for the subject.

Collected samples will be transferred for analysis to the Sponsor, or to other laboratories working for the Sponsor.

Biological samples will be stored for the time established by regulatory requirements or destroyed after the final clinical study report has been finalized if storage is not required. There might be a new request for these samples to be used for purposes related to the QA of the laboratory tests described in this protocol, in which case they will be used for this purpose. This may include the assessment of the quality of current tests, the maintenance or improvement of these tests, the development of new test methods for the markers described in this protocol, as well as making sure that new tests are comparable to previous methods and work reliably.

If study results suggest that further investigations using stored biological samples are warranted, these tests might be carried out on an exploratory basis. In addition, biological samples may be used by the Sponsor or their research partners for further research that is not related to the disease or the product under study. This testing will be done on pseudonymized samples (meaning that subjects will not be identifiable from their biological samples as their identity will have been removed and they will be assigned a unique clinical study identification number). Subjects will be asked to sign an additional, separate consent form for this optional testing and refusal of consent will not affect their possibility of participating in the study.

During the conduct of the study, an individual subject can choose to withdraw consent to have biological specimens stored for future research.

Reason:

To provide procedures for the future use of stores samples.

Section 12.7 Insurance

Old Text:

N/A

New Text:

The Sponsor has established an insurance policy for the anticipated duration of the study, covering the subjects with respect to the risks involved in taking part in this study in accordance with this protocol. In the case of injury or disability deriving from

participation in the study, subjects are requested to inform the Investigator or his/her staff responsible for the study at the institution without delay

Reason:

To add insurance details.

Section 12.6 Study Discontinuation and Closure

Old Text:

Section deleted

New Text:

N/A Section deleted

Appendices

Appendix 6: Rome IV classification for IBS

Old Text:

N/A

New Text:

Appendix 6: Rome IV Classification for IBS has been added.

Reason:

This change has been made at the request of the Investigators.

Appendix 7 Prohibited Probiotics

Old Text:

N/A

New Text:

Appendix 7 Prohibited Probiotics has been added

Reason:

This change has been made at the request of the Investigators.

Appendices 6, 7, 8 and 9 have been re-numbered as Appendices 8, 9, 10 and 11 respectively.

APPENDIX 13: SUMMARY OF PROTOCOL CHANGES VERSION 3.1

Summary of Protocol Changes

PROTOCOL

PROTOCOL NUMBER: BHT-II-002

A PHASE 2 RANDOMISED, DOUBLE BLIND, PLACEBO-CONTROLLED, PARALLEL GROUP, MULTICENTRE STUDY TO EVALUATE THE SAFETY AND EFFICACY OF REPEATED ORAL DOSES OF Blautix™ IN ADULT SUBJECTS WITH IRRITABLE BOWEL SYNDROME (IBS) SUBTYPES IBS-C and IBS-D

Previous Date and Version: 6 February 2018; Version 1.0
6 April 2018; Version 1.1
19 May 2018: Version 1.2
31 May 2018: Version 2.0
21 June 2018: Version 2.1
13 February 2019: Version 3.0

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Section: 10.3 Interim Analysis

Old Text:

An informal interim analysis following randomisation of approximately 250 Subjects for futility will take place under the auspices of the DSMB. As there will be no provision of stopping the study for efficacy and as – other than stopping for futility – there will be no other consequences of the interim analysis, there is no need to adjust the value of alpha.

New Text:

An informal interim analysis will be conducted once all recruited patients have completed the primary efficacy analysis 8-week treatment period. This interim analysis is planned to enable 4D pharma to expedite the clinical development strategy based on the outcome of the interim analysis. Planning for pivotal trials and the EU Paediatric Investigation Plan and the US Paediatric Study Plan can be initiated in preparation for Agency meetings and further clinical development. There will be no other consequences of the interim analysis, there is no need to adjust the value of alpha.

Reason:

As there were no SUSAR submissions, it was deemed that there was no need to call a DSMB after the randomisation of 250 subjects and there was no need to stop the trial for futility. Although the DSMB was not called, this is not considered a withdrawal of the DSMB as no SUSARs have been raised during the study, to this point.

As all patients have now been randomised and have completed the assessments which will be used in the primary efficacy analysis, 4D is including an interim analysis for efficacy. This change in the interim analysis is not considered to be a change of study design which is likely to have a significant impact on primary or major secondary statistical analysis or the risk/benefit assessment.