CLINICAL TRIAL PROTOCOL

Study Title:	**TR**eatment of **I**rritable bowel syndrome with diarrhoea using **T**itrated **ON**dansetron
Short Study Title:	TRITON Trial
Grant Title	A randomised, placebo controlled trial to determine the efficacy and mode of action of ondansetron in the treatment of irritable bowel syndrome with diarrhoea
IRAS Number:	219133
EudraCT Number:	2017-000533-31
ISRCTN:	ISRCTN17508514
CTA Reference:	19162/0228/001-0001
REC Reference:	17/YH/0262
Product:	Ondansetron
Sponsor:	Nottingham University Hospital NHS Trust – 17GA001
Funding Source:	EME – NIHR (15/74/01)
Development Phase:	Phase III
Protocol Date:	V6.0 6th November 2018 amended 5th December 2018

SIGNATURE PAGES

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the trial in compliance with the approved protocol and will adhere to the principles outlined in the Medicines for Human Use (Clinical Trials) Regulations 2004 (SI 2004/1031), amended regulations (SI 2006/1928) and any subsequent amendments of the clinical trial regulations, GCP guidelines, the Sponsor's SOPs, and other regulatory requirements as amended.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of the Sponsor

I also confirm that I will make the findings of the study publically available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the study will be given; and that any discrepancies from the study as planned in this protocol will be explained.

For and on behalf of the Study Sponsor:	
Signature:	Date: ...../...../.....
Name (please print):	
Position:	

Chief Investigator:	
Signature:	Date: ...../...../.....
Name: (please print):	

Statistician:	
Signature:	Date: ...../...../.....
Name: (please print):	
Position:	

SIGNATURE PAGE (PRINCIPAL INVESTIGATOR)

I agree to perform this trial to the principles of Good Clinical Practice (GCP), as applicable under UK regulations, the NHS Research Governance Framework (and Scottish Executive Health Department Research Governance Framework for Health and Social Care 2006 for studies conducted in Scotland) and to comply with the Declaration of Helsinki 1996

I agree to work to procedures outlined in this current approved protocol, including reporting of adverse events, administration of trial treatment, handling of trial Investigational Medicinal Product(s) and emergency unblinding (including ensuring there is adequate local provision for out of hour unblinding.

I have received GCP training within the previous two years and have experience in taking informed consent in a clinical trial setting.

Study Title: **TR**eatment of **I**rritable bowel syndrome with diarrhoea using **T**itrated **On**dansetron

EudraCT Number: 2017-000533-31

Address of Institution: _____

_____

_____

Signed: _____

Print Name and Title: _____

Date: _____

CONTACT LIST

Study Enquiries	**Email: TRITON@leeds.ac.uk**	**Tel: 0113 343 9242**
Randomisation / Registration:	**Tel: 0113 343 2290 or https://lictr.leeds.ac.uk/webrand/**	
Emergency unblinding	**Tel: 0113 343 4930 Fax: 0113 343 6427**	
SAEs/SUSARs/protocol violations:	**Fax: 0113 343 0686**	

Chief Investigator:

Professor Robin Charles Spiller - *Professor of Gastroenterology*	
University of Nottingham Queens Medical Centre Campus, Derby Road, Nottingham. NG7 2UH	**Email:** robin.spiller@nottingham.ac.uk **Tel:** 0115 823 1090

Sponsor:

Research Project Manager	
Nottingham University Hospitals NHS Trust C Floor, South Block, Queens Medical Centre Campus, Derby Road, Nottingham. NG7 2UH	**Email:** researchsponsor@nuh.nhs.uk **Tel:** 0115 970 9049

Funder:

NIHR Efficacy and Mechanism Evaluation (EME) Programme	
Evaluation, Trials and Studies Coordinating Centre University of Southampton, Alpha House, Enterprise Road, Southampton SO16 7NS	**Email:** info@eme.ac.uk **Tel:** 023 8059 4303

Lead Methodologist:

Prof Amanda Farrin – *Professor of Clinical Trials & Evaluation of Complex Interventions*	
Clinical Trials Research Unit (CTRU) University of Leeds, Leeds. LS2 9JT	**Email:** A.J.Farrin@leeds.ac.uk **Tel:** 0113 343 8017

Supervising Statistician:

Mrs Ivana Holloway – *Senior Medical Statistician*	
Clinical Trials Research Unit (CTRU) University of Leeds, Leeds. LS2 9JT	**Email:** I.Holloway@leeds.ac.uk **Tel:** 0113 343 9241

Trial Statistician:

Dr Tom Morris – *Senior Medical Statistician*	
Clinical Trials Research Unit (CTRU) University of Leeds, Leeds. LS2 9JT	**Email:** T.Morris@leeds.ac.uk **Tel:** 0113 343 6049

Project Delivery Lead:

Ms Suzanne Hartley – *Head of Trial Management*	
Clinical Trials Research Unit (CTRU) University of Leeds, Leeds LS2 9JT	**Email** S.Hartley@leeds.ac.uk **Tel:** 0113 34 38041

Trial Manager / Data Manager:

Miss Rachael Kearns – *Trial Co-ordinator* /**Miss Lorna Barnard** – *Senior Trial Co-ordinator* /Ms **Catherine Olivier** - *Senior Trial Manager*	
Clinical Trials Research Unit (CTRU) University of Leeds, Leeds. LS2 9JT	**Email:** triton@leeds.ac.uk **Tel:** 0113 343 9242 / 0555 / 0504

Trial Pharmacist:

Sheila Hodgson - *Lead Pharmacist*	
Sheila Hodgson	**Email:** sheila.hodgson@nuh.nhs.uk **Tel:** 0115 924 9924 Ext 57698

Nottingham University Hospitals NHS Trust, QMC Nottingham NG7 2UH	

Collaborators:

Barnsley Hospital NHS Foundation Trust **Dr Kapil Kapur** *Consultant Gastroenterologist* kapil.kapur@nhs.net	**Barts Health NHS Trust, Royal London Hospital** **Dr Mark Scott** *Director GI Physiology Unit* m.scott@qmul.ac.uk
County Durham and Darlington NHS Trust **Professor Yan Yiannakou** *Professor of Neurogastroenterology* Yan.Yiannakou@cddft.nhs.uk	**Flinders University** **Professor Philip Dinning** *Senior Clinical Scientist* phil.dinning@flinders.edu.au
London North West Healthcare NHS Trust **Dr Ayesha Akbar** *Consultant Gastroenterologist* aakbar@doctors.org.uk	**Leeds Teaching Hospitals NHS Trust** **Professor Alexander Ford** *Honorary Consultant Gastroenterologist* A.C.Ford@leeds.ac.uk
Lothian Health Board **Dr Maria Eugenicos** *Senior Lecturer & Specialist in Gastroenterology* Maria.Eugenicos@nhslothian.scot.nhs.uk	**Queen Mary, University of London** **Professor Qasim Aziz** *Professor of Neurogastroenterology* q.aziz@qmul.ac.uk
Sandwell and West Birmingham Hospitals NHS Trust **Dr Nigel Trudgill** *Consultant Gastroenterologist* nigel.trudgill@nhs.net	**Sheffield Teaching Hospitals NHS Foundation Trust - Royal Hallamshire** **Professor David Sanders** *Consultant Gastroenterologist* David.Sanders@sth.nhs.uk
University College London Hospitals NHS Foundation Trust **Dr Anton Emmanuel** *Senior Lecturer and Consultant Gastroenterologist* a.emmanuel@ucl.ac.uk	**University Hospitals of North Midlands NHS Trust** **Dr Adam Farmer** *Consultant Gastroenterologist and Senior Lecturer* adam.farmer@uhns.nhs.uk
University Hospital of South Manchester NHS Foundation Trust **Professor Peter Whorwell** *Professor of Medicine & Gastroenterology* peter.whorwell@uhsm.nhs.uk	**The University of Manchester** **Professor John McLaughlin** *Consultant Gastroenterologist* John.Mclaughlin@manchester.ac.uk

TABLE OF CONTENTS

LIST OF TABLES

ABBREVIATIONS

Abbreviation	Explanation
5-HT	5-hydroxytryptamine
5HT3RA	5-HT3receptor antagonist
AE	Adverse event
AR	Adverse Reaction
ALT	Alanine aminotransferase
BNF	British National Formulary
BSFS	Bristol Stool Form score
CI	Chief investigator
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case report form
CRP	C-reactive protein
CTRU	Clinical Trial and Research Unit
DMEC	Data Monitoring and Ethics Committee
DNA	Deoxyribonucleic acid
DSUR	Development Safety Update Report
ECG	Electrocardiogram
EMA	European Medicines Agency
EME	Efficacy and Mechanism Evaluation
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GI	Gastro intestinal
GP	General Practitioner
HADS	Hospital Anxiety and Depression Scale
HAPCs	High amplitude propagated contractions
HPLC	High Performance Liquid Chromatography
IBS	Irritable Bowel Syndrome
IBS-D	Irritable Bowel Syndrome with Diarrhoea
IBS-QOL	IBS Quality Of Life Questionnaire
IBS-SSS	IBS Severity Scoring System
ICMJE	International Committee of Medical Journal Editors
ID	Identification
IMP	Investigational medicinal product
IV	Intravenous
MedDRA	Medical Dictionary for Regulatory Activities
mmHg	Millimeters mercury
mg	Milligrams
MHRA	Medicines and Healthcare products Regulatory Agency
ml	Millilitres
MRC	Medical Research Council
mRNA	Messenger RNA
NHS	National Health Service
NICE	National Institute for Health and Care Excellence.
NIHR	National Institute of Health Research
ng	Nanogram
nm	Nanometer
NUH	Nottingham University Hospital NHS Trust
PAR2	Protease activated receptor 2

PI	Principal Investigator
PICS	Patient Identification Centre
PHQ-12	Physical Symptoms Questionnaire
QMUL	Queen Mary University London
QP	Qualified Person
QT	Interval is the time from the start of the Q wave to the end of the T wave
QTc	Corrected QT
RCT	Randomised Control Trial
REC	Research Ethics Committee
RS	Responder Status
RSI	Reference Safety Information
SAE	Serious adverse event
SeHCAT	Selenium homocholic acid taurine
SF-LDQ	Short-form Leeds Dyspepsia Questionnaire
SmPC	Summary Product of Characteristics
SMS	Short Message Service
SAR	Serious Adverse Reaction
SSRI	Selective serotonin reuptake inhibitors
SUSAR	Suspected Unexpected Serious Adverse Reaction
t.d.s	Three times a day
TMG	Trial Management Group
ToR	Terms of Reference
TPH-1	Tryptophan hydroxylase 1
TSC	Trial Steering Committee
tTG	Tissue Transglutaminase
UCL	University College London
USMs	Urgent Safety Measures

TRIAL SUMMARY

Title:	**TRITON Trial: TR**eatment of **I**rritable bowel with diarrhoea syndrome using **T**itrated **ON**dansetron.		
Design:	This study is a phase III, parallel group randomised, double-blind, multi-centre, placebo-controlled trial, with embedded mechanistic studies within selected centres.		
Target Sample Size:	400 Patients with IBS-D.	**Sites:**	Approximately 24 UK Sites
Timelines:	First Patient Recruited: May 2018 Recruitment Duration: May 2018- October 2019 Last Patient Last Visit: Feb 2020		

	Clinical Objective	Endpoint
Primary:	Does 12 weeks of ondansetron increase the FDA defined responder rate (in relation to abnormal defaecation and abdominal pain) compared with placebo?	The primary endpoint will be measured at 12 weeks post randomisation and defined, as recommended by the FDA, as being a weekly responder for BOTH pain intensity AND stool consistency for at least 6 weeks in the 12 week treatment period. See section 3 for the definition of response in each case.
Secondary:	What is the estimated treatment effect of ondansetron in relation to stool frequency, consistency, urgency of defaecation , satisfactory relief of IBS symptoms, IBS-SSS, SF-LDQ, use of rescue medication and abdominal pain over 12 weeks of treatment?	**1.** Stool frequency - number of stools per day up to 12 weeks post randomisation. **2.** a. Stool consistency - number of days per week with at least 1 loose stool **2.** b. Average stool consistency over last month (weeks 9-12) **3.** Urgency of defaecation (on a scale 0-100) **4.** Satisfactory relief of IBS symptoms - for at least 6 out of 12 weeks **5.** Short-form Leeds Dyspepsia Questionnaire (SF-LDQ) **6.** IBS-Symptom Severity Scale (IBS-SSS) **7.** Use of rescue medication **8.** Abdominal pain score (on a scale 0-100)
	Does the treatment with Ondansetron improve patient's mood over 12 weeks of treatment?	**9.** Hospital Anxiety and Depression Scale (HADS) score **10.** IBS-QOL
	What is the longer term (one month) effect of ondansetron after 12 weeks of treatment?	**11.** Stool frequency **12.** Stool consistency **13.** Urgency of defaecation **14.** Abdominal pain These endpoints are collected from 13-16 weeks post randomisation and their definitions are the same as for secondary endpoints 1, 2, 3 and 8.
Mechanistic:	Does ondansetron slow colonic transit?	Ondansetron's impact on colonic transit will be assessed using radio-opaque markers and an abdominal X-ray at baseline and 12 weeks at all sites in n=400 patients.
	Does ondansetron increase cyclical retrograde propagated contractions in the sigmoid colon? Does this increase cause a slowing of left sided transit?	High resolution colonic manometry will be performed in 2 sites (QMUL, & The University of Nottingham), at baseline and 1 time point between 8-11 weeks, in n=40 patients. Effects of treatment will be assessed by: • Number of high amplitude propagated contractions (HAPCs) both fasting and postprandial • % time occupied by cyclical propagated contractions both retrograde and antegrade.

Mechansitic Cont:	Does ondansetron increase rectal compliance or pressure thresholds for pain/ urgency?	Barostat assessments in 4 sites (The University of Nottingham, QMUL, UCL & Leeds) at baseline and at 1 time point between 8-11 weeks, in n=80 patients. Effects of treatment will be assessed by: • Change in rectal compliance • Change in rectal sensitivity
	Does ondansetron thereby reduce urgency?	Change in rectal compliance will be correlated with change in urgency score from baseline to 12 weeks.
	Does ondansetron reduce total faecal bile acids? Does ondansetron reduce total tryptase?	Stool samples collected at all sites, at baseline and 12 weeks in n=400 patients will be analysed for faecal protease and bile acids. Effects of treatment will be assessed by: 1. Faecal Tryptase concentration 2. Total bile acid concentration
	Does the reduction in either / both correlate with changes in urgency?	Faecal tryptase and total bile acids will be correlated with faecal urgency data gathered from baseline to 12 weeks.
	Do polymorphisms in: • Tryptophan hydroxylase 1 (TPH-1) predict response to ondansetron and does this • Alter mucosal 5-HT content TPH1 mRNA	Blood samples taken at baseline will be used to assess genetic polymorphisms in the TPH-1 gene and correlate this to the final dose of ondansetron and responder status (RS) (n=400). Research biopsies will be used to measure mucosal 5-HT content and TPH-1 mRNA
Inclusion criteria:	Patients must fulfil all of the following criteria. 1. Written (signed and dated) informed consent. 2. Considered fit for study participation. 3. Meeting Rome IV criteria for IBS-D (see Appendices) 4. Aged ≥ 18 years 5. Undergone standardised workup to exclude the following alternative diagnoses a) Microscopic colitis (colonoscopy or flexible sigmoidoscopy) within previous 2 years b) Bile acid diarrhoea (SeHCAT results of > 10%, C4 results of <19 ng/ml or failed 1 week trial of a bile acid binding agent [colestyramine 4g t.d.s. , colesevelam 625mg t.d.s. or equivalent]) within previous 5 years ***Note:*** *Cholecystectomy will not be an exclusion criteria if bile acid diarrhoea has been excluded* *Patients with SeHCAT values of 5-10% will be eligible if they fail to respond to a 1 week trial of bile acid binding agent (see above)* c) Lactose malabsorption (In the opinion of the patient's clinician. Suggested but not mandated methods to exclude lactose malabsorption as an alternative diagnosis include hydrogen breath test/milk challenge or failure to respond to lactose free diet). d) Coeliac disease (tTG or duodenal biopsy) 6. Patients of child bearing potential or with partners of child bearing potential must agree to use methods of medically acceptable forms of contraception during the study and for 90 days after completion of study drug, (e.g. implants, injectable, combined oral contraceptives, barrier methods, true abstinence (when this is in line with the preferred and usual lifestyle of the patient) or vasectomised partners).	

	7. For women of child bearing potential, a negative pregnancy test should be performed within 72 hours of confirmation of eligibility. 8. Weekly average worst pain score ≥ 30 points on a 0 to 100 point scale. 9. Any stools with a consistency of 6 or 7 on the Bristol Stool Form score (BSFS) for 2 or more days per week.^ ^ ***Inclusion criteria 8 & 9*** *will be assessed after the patient has completed the 14 day pre-treatment daily stool and pain diary and returned the results at visit 2.*
Exclusion criteria:	Patients must not fulfil any of the following criteria: 1. Gastrectomy 2. Intestinal resection 3. Other known organic GI diseases (e.g. inflammatory bowel disease – Crohn's disease, ulcerative colitis). 4. Unable or unwilling to stop restricted medication including regular loperamide, antispasmodics (e.g. buscopan, mebeverine, peppermint oil, alverine citrate), eluxadoline, tricyclic antidepressant doses >30mg/day or other drugs likely in the opinion of the investigator to alter bowel habit. These medicines should be discontinued for a 7 day washout period prior to registration. ***Note:*** Intermittent loperamide will be permitted but only as rescue medication (see section 8.7) 5. QTc interval ≥450msec for men and ≥470msec for women. Assessed within the last 3 months by a 12-lead ECG. 6. Previous chronic use of ondansetron or contraindications to it (rare as per BNF) 7. Pulse, blood pressure, laboratory blood values outside the normal ranges according to the site's local definition of normal. Assessed within the last 3 months. ***Note:*** *Minor rises in ALT (<2 x upper limit of normal) will be acceptable but the patient's GP will be informed if they remain elevated at end of the study.* 8. Women who are pregnant or breastfeeding 9. Currently participating or who have been in an IMP trial in the previous three months where the use of the IMP may cause issues with the assessment of causality in this study. 10. Currently taking SSRIs or tricyclic antidepressants (unless at a stable dose for at least 3 months and with no plan to change the dose during the study). 11. Currently taking and unwilling or unable to stop any of the prohibited medications.* ***Prohibited medications*** **–** Apomorphine & tramadol which interact with ondansetron. ***Caution should be taken with patients on QT prolonging drugs and cardio toxic drugs.*** These patients should be reviewed by the PI to determine if they are suitable for the study (see section 8.5). 12. Stool consistency of only 7 on the Bristol Stool Form score (BSFS) for 7 days a week. ^^ ^^ ***Exclusion criterion 12*** *will be assessed after the patient has completed the 14 day pre-treatment daily stool and pain diary and returned at visit 2.*
All patients will receive 4-24mg of ondansetron or placebo for 12 weeks	

1. BACKGROUND

1.1. Irritable Bowel Syndrome

Irritable bowel syndrome (IBS), which affects around 10% of the population, accounts for 1.8 million consultations/year in primary care in England and Wales (0.6 million patients). Symptoms include frequent, loose, or watery stools with associated urgency, which can severely limit socialising, travelling, and eating out, with resulting marked reduction in quality of life and loss of work productivity. Around one third of patients meet criteria for IBS with diarrhoea (IBS-D). When patients are asked to rank symptoms in order of importance the erratic bowel habit is rated first, followed by abdominal pain and, for those with diarrhoea, urgency [1]. Despite its high prevalence, we have no satisfactory treatment for IBS-D at present. Loperamide reduces bowel frequency but does not improve abdominal pain [2] which is a key feature of IBS [3]. Dose titration is difficult with loperamide and patients often experience marked constipation and tend to only use the drug intermittently. Meta-analysis shows that 5HT3RAs are effective treatments for IBS-D [4], improving stool consistency and reducing frequency and urgency of defaecation. Their mode of action is unclear, but most likely involves effects on sensation, secretion and motility.

1.2. Role of 5HT3 receptor antagonists

Animal models suggest that 5HT3RAs act on enteric neurones [5], inhibiting the gastrointestinal motor [6] and secretory response [7] to feeding. They also slow transit in humans [8] and inhibit the colonic response to feeding [9], which often precipitates pain and defaecation in IBS-D. Despite its efficacy, alosetron was withdrawn after 6 months owing to adverse events, including the development of constipation in 25%, and ischaemic colitis in 1 in 700 [10] . This therapy has never been available in Europe and is only licensed for women. Another 5HT3RA, ondansetron, widely used as an anti-emetic, with an excellent safety record over 3 decades, is known to slow gastrointestinal transit, which led us to try it in IBS-D patients. A 5-week, randomised, placebo-controlled cross-over trial showed ondansetron was effective in improving diarrhoea and urgency [11].

However, we do not understand exactly how ondansetron works, nor can we predict the individual dose requirements for optimum effect, which varies widely. One key effect we found, also seen with other 5HT3RAs [12], is a marked reduction in urgency of defaecation, which plays such an important role in improving the quality of life for these patients [13].

1.3. Potential mechanisms of action of 5HT3 receptor antagonists

Our earlier study showed the decrease in urgency of defaecation correlated directly with the reduction in faecal protease [14], but whether this is important or just an epiphenomenon is unclear. Faecal proteases have been shown to be increased in IBS-D and, at least in animal models, act to cause hypersensitivity to rectal distension via their activation of protease activated receptor type 2 (PAR2) [15] . We have shown that most faecal proteases are endogenous [14], representing pancreatic enzymes that have escaped degradation by colonic bacteria.

We hypothesise that slowing transit reduces faecal protease, by allowing time for bacterial degradation, and that this may contribute to the beneficial effects of ondansetron. Bile acids have also

been shown to sensitise the rectum [16], and elevated faecal bile acids have been shown by several groups in IBS-D [17]. Slowing transit will increase the time for bile acid deconjugation by colonic bacteria and enhance absorption, but how important this is in reducing rectal sensitivity compared with the effects on faecal proteases is unclear. An alternative mechanism of action may be the pharmacological effect that 5HT3RAs have in terms of reducing rectal tone, as has been demonstrated with alosetron [18].

5HT3RAs also slow transit, an effect we found particularly marked in the left colon and rectosigmoid region, but the underlying mechanism was unclear [11]. Previous studies of the impact of 5HT3RAs on human colonic motility [19, 20] showed the 5HT3RAs alosetron and cilansetron increased periprandial frequency of colonic contractions, and mean amplitude of contractions in the left colon. Alosetron also increased high-amplitude propagated contraction (HAPC) frequency and mean propagation length. This finding is paradoxical since prokinetic drugs such as neostigmine [21, 22] and prucalopride [23], which increase HAPCs, are usually laxative in their effect. We hypothesise that 5HT3RAs increase retrograde sigmoid motility, a novel mode of action (see rationale).

1.4. 5HT3 receptor antagonist sensitivity

Our earlier study also showed that individuals vary widely in their responsiveness to ondansetron, explaining why trials using fixed doses of 5HT3RAs result in severe constipation in some patients. When patients were allowed to dose titrate we found the dose of ondansetron ranged from 4mg alternate days to 8mg t.d.s. This may be due to functionally significant polymorphisms in genes controlling 5HT synthesis. Serotonin availability in the rectal mucosa is determined by the activity of the rate-limiting synthetic enzyme tryptophan hydroxylase 1 (TPH-1), which produces serotonin in enterochromaffin cells.

1.5. Genetic influences on 5HT3RA responsiveness:

A recent study [24] showed TPH-1 mRNA levels in rectal mucosa, and so presumably serotonin synthesis rate, were approximately doubled in responders compared with non-responders, and that this was linked to the TPH-1 genotype. TPH-1 rs211105 minor allele G was found in 44% of non-responders but only 4% of responders, indicating that possessing the major allele increases responsiveness. It was also associated with worse diarrhoea, possibly because of the greater 5-HT synthesis.

1.6. Investigational Agent

Ondansetron is a potent, highly selective receptor antagonist (5HT3RA) which blocks 5HT3 receptors in the gastro-intestinal tract and in the CNS. Ondansetron is currently licenced for use in adults and children for the management of nausea and vomiting induced by cytotoxic chemotherapy and radiotherapy and for the prevention and treatment of post-operative nausea and vomiting.

1.7. Clinical Studies in IBS with diarrhoea

1.7.1. Pilot Study

120 patients were recruited to a randomised double-blind, placebo-controlled, cross-over trial of ondansetron treatment for IBS-D. Patients were randomised to receive ondansetron (4-24mg daily) followed by placebo or placebo followed by ondansetron (4-24mg daily).

Patients began on drug A for a period of 5 weeks, then underwent a washout period of 2-3 weeks and then began drug B for 5 weeks. The primary outcome measure for the study was the difference in average stool consistency in the last 2 weeks of treatment of ondansetron versus placebo.

Although our pilot study of ondansetron in IBS-D was encouraging, and our own clinical experience supports this, there were significant limitations of the study including the short duration and the cross-over design. Such a trial would not be accepted by regulatory authorities such as the FDA, EMA, and NICE, and hence will not change clinical practice. The main reason is that the duration of active treatment in the trial was just 5 weeks, which is too short for a chronic condition such as IBS, for which both FDA and EMA recommend treatment trials of at least 12 weeks duration. Furthermore cross-over studies tend to overestimate effectiveness because un-blinding occurs when those taking an effective medication switch over to placebo. Finally our primary endpoint was stool consistency, whereas most practitioners and regulatory agencies are more interested in improving the associated clinical features such as urgency, abdominal pain/ discomfort and increased stool frequency.

Not only do we wish to confirm effectiveness definitively, but we are also interested in understanding the mechanisms underlying any benefit, as this will allow design of better treatments in the future, especially as the therapeutic options in IBS-D are currently so limited.

2. RATIONALE

2.1. Study Aims

The overall aim of the study is to investigate the effectiveness and mechanism of action of ondansetron, a 5HT3RA, in patients with IBS-D, as assessed by stool frequency, consistency, urgency and abdominal pain.

The aims can be divided into CLINICAL and MECHANISTIC:

CLINICAL AIMS:

To determine the effectiveness and safety of the use ondansetron in patients with the symptoms of IBS-D including urgency, looseness of stool, frequency of defaecation and abdominal discomfort.

MECHANISTIC AIMS:

To further understand the mechanism of IBS-D through the use of ondansetron in this patient population. Looking specifically at the role of rectal sensitivity and compliance, faecal bile acids and proteases, postprandial sigmoid motility and genetic variation in serotonin synthesis in causing the symptoms of IBS-D, and in responsiveness to ondansetron. This will be achieved by performing mechanistic studies within the clinical trial to determine how the changes induced in these different biomarkers by ondansetron correlate with improvement in specific symptoms.

2.2. Risk – Benefit Assessment

2.2.1. Potential benefits

Current lack of effective treatment for IBS-D results in frequent referral to secondary care and such patients account for up to 20% of gastroenterology outpatients. This burden of multiple visits and treatments for the patients is also associated with a considerable cost to the NHS. A recent US meta-analysis suggests IBS patients have excess medical costs from $1,562-7,737 per year, with 17-50% being attributed to outpatient investigations [8], so assuming around 20% of the 0.2M IBS-D patients consulting their GP each year are referred, the annual cost for the UK would be between £40-120M.

If ondansetron is effective in our trial, it could easily be widely adopted since it is an inexpensive, safe, and generic drug. By providing an effective treatment, it could not only reduce patient symptoms, but also reduce costs of repeated referral and investigation.

2.2.2. Potential risks and their management

Ondansetron has been widely used for nearly 3 decades and, at the doses used in our pilot study (4-8mg, t.d.s.), has shown to be extremely safe. The pilot study found no adverse effects in 120 IBS-D patients, apart from constipation in 9%, which represents an over-effective action and could be alleviated by dose reduction. Only 2 out of 120 patients left the study because of this. When used for the prevention of chemotherapy-induced emesis, high dose (32mg) intravenous treatment has been associated with prolongation of the QT interval in a dose-dependent fashion with an average prolongation of 20msec for 32 mg intravenously (IV) and 6 msec for 8mg IV. Blood levels after 32mg IV boluses will be around 10 times that observed after oral ingestion of 4-8mg, so we do not anticipate such an effect in our trial. We will however be exercising caution with patients with a prolonged QT, or taking drugs known to cause this. We will also be keen to convincingly exclude the possibility of ischaemic colitis which has been associated with other 5HTRAs, and will recommend flexible sigmoidoscopy to anyone who develops rectal bleeding during the trial. Our previous experience suggests that this will almost always be due to bleeding from the anal canal associated with trauma induced by frequent defaecation.

3. OBJECTIVES AND ENDPOINTS

3.1. Primary objective and endpoint

Clinical Objective	Endpoint	Frequency of Data Collection	How is it being collected?
Does 12 weeks of ondansetron increase the FDA defined responder rate (in relation to abnormal defaecation and abdominal pain) compared with placebo?	The primary endpoint will be measured at 12 weeks post randomisation and defined, as recommended by the FDA, as patient being a weekly responder for BOTH pain intensity AND stool consistency for at least 6 weeks in the 12 week treatment period. Weekly responder status is defined as follows: **1.** Weekly responder for **Abdominal Pain Intensity**: • At least 30% decrease from baseline in weekly average of worst daily abdominal pain score (abdominal pain score measured on a 0 to 100 point scale in past 24 hours) **2.** Weekly responder **for Stool Consistency**: • Decrease of at least 50% in the number of days per week with at least one loose stool consistency (BSFS = 6 or 7) compared with baseline. To achieve success in the primary endpoint, a patient must be a weekly responder for both pain and for consistency during the same 6 weeks within the 12 week treatment period.	**1. Daily Weeks 0-12** **2. Daily Weeks 0-12**	**1. Abdominal pain** **Patient diary and SMS asking:** On a scale of 0-100, what was the worst abdominal pain you experienced today? (0=No pain, 100=Worst imaginable pain) **2. Stool consistency** **Patient diary and SMS asking:** Have you had a loose stool BSFS 6 or 7 today? (Y/N)

3.2. Secondary objectives and endpoints

Clinical Objective	Endpoint	Frequency of Data Collection/ When measured from randomisation	Data required & how is it being collected?
What is the estimated treatment effect of ondansetron in relation to stool frequency, consistency, urgency of defaecation , satisfactory relief of IBS symptoms, use of rescue medication and abdominal pain over 12 weeks of treatment?	**1. Stool frequency** will be defined as number of stools per day up to 12 weeks post randomisation using patient's diary. For the endpoint analysis, the mean number of stools per day over the last month (weeks 9-12) will be used. **2.** a. **Stool consistency** will be assessed from number of days per week with at least 1 loose stool b. **Average stool consistency** over the last month (weeks 9-12) Consistency will be assessed daily by Bristol Stool Form Score (BSFS) and a loose stool is defined as BSFS >5. For the endpoint analyses, the mean number of days per week with at least 1 loose stool over last month (weeks 9-12) and the mean daily stool consistency over the last month (weeks 9-12) will be used. **3. Urgency** of defaecation (on a scale 0-100) For the endpoint analyses, the mean daily urgency score over last month (weeks 9-12) will be used.	**1. Daily** **Weeks 0-12** **2. Daily** **Weeks 0-12** **3. Daily** **Weeks 0-12**	**1. Stool Frequency** **Patient Diary** Number of stools passed per day. **2. Stool consistency** **Patient Diary** What is the consistency of your stool according to the BSFS (1-7)? (Please record for each stool) **3. Urgency of defaecation** **Patient Diary** On a scale of 0-100, what was the worst bowel movement urgency you experienced today? (0=No urgency, 100=worst imaginable urgency)

	4. Satisfactory relief of IBS symptoms will be defined as satisfactory relief of IBS symptoms for at least 6 out of 12 weeks For the endpoint analyses, the proportion of patients with satisfactory relief of symptoms will be used.	**4. Weekly Weeks 0-12**	**4. Satisfactory relief of IBS symptoms** **Patient Diary** Overall, did you have satisfactory relief of your IBS symptoms during the last week? (Y/N)
	5. Score of **short-form Leeds dyspepsia questionnaire** (SF-LDQ) questionnaire for functional dyspepsia For the endpoint analyses, the functional dyspepsia score at 12 weeks will be used.	**5. At 0 and 12 weeks**	**5. SF-LDQ Score** **Clinic** Self-completed by patients during nurse visit at 0 &12 weeks.
	6. Score of **IBS Symptom Severity Scale** Questionnaire for IBS (IBS-SSS) For the endpoint analyses, the IBS-SSS score at 12 weeks will be used.	**6. At 0 and 12 weeks**	**6. IBS-SSS Severity Score** **Clinic** Self-completed by patients during nurse visit at 0 &12 weeks.
	7. Use of rescue medication will be defined as use of loperamide and assessed by total number of days having to take loperamide over 12 weeks.	**7. Daily Weeks 0-12**	**7. Use of loperamide** **Patient Diary** Have you taken loperamide (Imodium™ or equivalent) today? (Y/N)
	8. Abdominal pain score (on a scale 0-100) For the endpoint analyses, the mean daily pain score over last month (weeks 9-12) will be used.	**8. Daily Weeks 0-12**	**8. Abdominal pain** **Patient Diary:** How bad is your worst abdominal pain today? (between 0-100, 0=No pain, 100=worst imaginable pain)
Does the treatment with ondansetron improve	**9. Hospital Anxiety and Depression Scale (**HADS) scores	**9. At 0 and 12 weeks**	**9. HADS Questionnaire** **Clinic**

patient's mood over 12 weeks of treatment?	For the endpoint analyses, the HADS anxiety and depression scores at 12 weeks will be used. **10. IBS-QOL summary score** For the endpoint analyses, the IBS-QOL summary score at 12 weeks will be used.	 **10. At 0 and 12 weeks**	Self-completed by patients during nurse visit at 0 and 12 weeks. **10. IBS-QOL summary score** **Clinic** Self-completed by patients during nurse visit at 0 and 12 weeks.
What is longer term (one month) effect of ondansetron after 12 weeks of treatment (off-treatment)?	**11. Stool Frequency** For the endpoint analysis, the mean number of stools per day over the whole month (weeks 13-16) will be used. **12. Stool consistency** For the endpoint analyses, the mean number of days per week with at least 1 loose stool over the whole month (weeks 13-16) and the mean daily stool consistency over the whole month (weeks 13-16) will be used. **13. Urgency of defaecation** For the endpoint analyses, the mean daily urgency score over the whole month (weeks 13-16) will be used. **14. Abdominal pain** For the endpoint analyses, the mean daily pain score over the whole month (weeks 13-16) will be used.	**11. Daily Weeks 13-16** **12. Daily Weeks 13-16** **13. Daily Weeks 13-16** **14. Daily Weeks 13-16**	**11. Stool Frequency** **Patient Diary** Number of stools passed per day. **12. Stool consistency** **Patient Diary** What is the consistency of your stool according to the BSFS (1-7)? (Please record for each stool) **13. Urgency of defaecation** **Patient Diary** What was the worst bowel movement urgency you experienced today? (between 0-100, 0=No urgency, 100=worst imaginable urgency) **14. Abdominal pain** **Patient Diary:** How bad is your worst abdominal pain today? (between 0-100, 0=No pain, 100=worst imaginable pain)

	Endpoints 12-15 are collected from 13-16 weeks post randomisation and their clinical definitions are the same as definitions for secondary endpoints 1, 2, 3 and 8 respectively.		

3.3. Mechanistic objectives and endpoints

Mechanistic Objectives	Endpoint	Data Required	Frequency of Data Collection	How is it being collected?
1. Does ondansetron slow colonic transit?	Ondansetron's impact on colonic transit will be assessed using radio-opaque markers and an abdominal X-ray at baseline and 12 weeks in n=400 patients.	• Number of markers seen on the plain abdominal X-ray at each assessment.	Baseline & 12 weeks	Data will be collected at each local site on the colonic transit CRF. These CRFs will be completed by the local research team and posted to the CTRU. Data will entered into the MACRO database.
2. Does ondansetron increase cyclical retrograde propagated contractions in the sigmoid colon?	High resolution colonic manometry will be performed in 2 sites (Queens Mary University of London & The University of Nottingham), at baseline and weeks 8-11, in n=40 patients.	• Number of HAPCs – Fasting • Number of HAPCs – postprandial	Baseline & at one time point between 8-11 weeks	Colonic Manometry results will be collected by a registrar at the University of Nottingham & QMUL under the supervision of Dr Corsetti & Prof Aziz. The results will be link anonymised and sent to Flinders University for analysis.
3. Does an increase in cyclical retrograde propagated contractions cause a slowing of left sided transit?	Effects of treatment on change from baseline and 8-11 weeks in the following will be assessed: • Number of high amplitude propagated contractions (HAPCs) both fasting and postprandial • % time occupied by cyclical propagated contractions both retrograde and antegrade.	• % of time cyclical propagated retrograde contractions • % of time cyclical propagated antegrade contractions		They will then complete the results template spreadsheet (excel) and will return completed results to Nottingham University for Statistical Analysis.

4. Does ondansetron increase rectal compliance or pressure thresholds for pain/ urgency?	Barostat assessments in 4 sites (The University of Nottingham, QMUL, UCL & Leeds) at baseline & 8-11 weeks, in n=80 patients. Effects of treatment on change from baseline and 12 weeks in the following will be assessed: • Change in rectal compliance • Change in rectal sensitivity	• Change in rectal compliance ml/mmHg • Change in rectal sensitivity as measured by change in the threshold for pain mmHg	Baseline & at one time point between 8-11 weeks	Results from the barostat assessments will be completed on specified templates and sent directly to Nottingham University by the 4 specialist sites for statistical analysis.
5. Does ondansetron thereby reduce urgency?	Change in rectal compliance and sensitivity will be correlated with change in urgency score from baseline to 8-11 weeks.	• Change in rectal compliance ml/mmHg • Change in rectal sensitivity as measured by change in the threshold for pain mmHg • Change in mean daily urgency score at week 9-12.	Barostat Results - Baseline & at one time point between 8-11 Weeks Urgency - Daily	Change in rectal compliance and threshold for pain will be collected from the results of the barostat study. Change in mean daily score of urgency will be collected from the patient diary and calculated by the CTRU and sent through to Nottingham for this analysis.
6. Does ondansetron reduce total faecal bile acids? 7. Does ondansetron reduce total tryptase?	Stool samples collected at all sites, at baseline and 12 weeks in n=400 patients will be analysed for faecal protease and bile acids. Effects of treatment on change from baseline and 12 weeks in the following will be assessed: 1. Faecal Tryptase 2.Total bile acid concentration	• Faecal bile acid concentration • Faecal tryptase results	Sample collection - Baseline & 12 weeks	Stool samples will be collected at local sites at visit 3 and visit 5. They will be stored locally and batch transported to Nottingham university. Nottingham Laboratory will provide the sample results to Nottingham statistical team for analysis.

8. Does the reduction in faecal bile acid concentration and / or faecal tryptase correlate with changes in urgency?	Faecal tryptase and total bile acids will be correlated with faecal urgency data gathered from baseline to 12 weeks.	• Difference in faecal bile acids • Difference in tryptase levels • Change in mean daily urgency score at 9-12 weeks.	Sample Results - Baseline & 12 weeks Urgency - Daily	Change in bile acids and tryptase will be collected from the results of above sample analysis. Change in mean daily score of urgency will be collected from the patient diary and calculated by the CTRU and sent through to Nottingham for this analysis.
9. Do polymorphisms in: • Tryptophan hydroxylase 1 (TPH-1) predict response to ondansetron and does this alter mucosal 5-HT or TPH-1 mRNA	Blood samples taken at baseline will be used to assess genetic polymorphisms in the TPH-1 gene and correlate this to the final dose of ondansetron and responder status (RS) (n=400). Research biopsies will be used to measure mucosal 5-HT content and TPH-1 mRNA	• TPH-1 test • Mucosal TPH-1 mRNA concentration • Mucosal 5-HT concentration	Baseline & 12 weeks	Blood samples will be collected at local sites at visit 3 and visit 5. Research biopsies will be collected at visit 3. They will be stored locally and batch transported to Nottingham university. 4 biopsies stored in liquid nitrogen (2 spare in case of need for repeat analysis) and 2 biopsies in RNA later. Nottingham Laboratory will provide the sample results to Nottingham statistical team for analysis.

4. STUDY DESIGN

TRITON is a multi-site, parallel group, randomised, double-blinded, placebo controlled trial, with embedded mechanistic studies within selected sites. Our aim is to determine the superiority of ondansetron compared with placebo. 400 patients with IBS-D will be randomised on a 1:1 basis to receive either ondansetron or placebo, as shown in Table 1. Both treatments will be administered in oral doses of between 4-24mg daily for 12 weeks. Dose titration will be undertaken in the first two weeks of the study to avoid constipation, which at a standard dose occurs in one quarter of patients. The primary outcome of response will be assessed at 12 weeks post randomisation. Secondary and Safety outcomes will be measured up to 16 weeks following randomisation.

4.1. Treatment regimens

Treatment Arm	Number of Patients	Treatment	Period of Treatment
1	200	Ondansetron 4-24mg Daily	12 Weeks
2	200	Placebo	12 Weeks
Total number of patients	**400**		

Table 1: Summary of treatment arms

5. STUDY SETTING

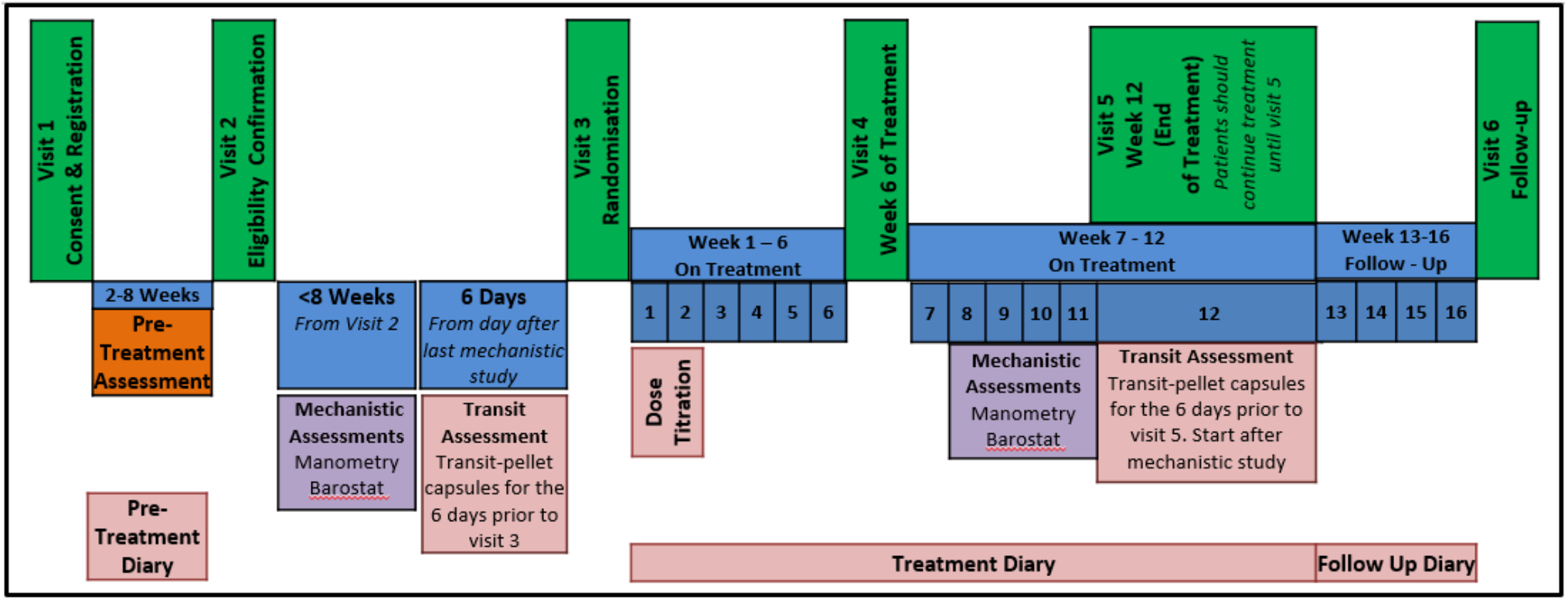
5.1. Participating Sites

This study will be performed at approximately 24 sites in the UK. Each site will be a NHS trust or NHS board. All sites will be required to recruit patients, perform all necessary protocol assessments and offer patients the opportunity to take part in the colonic transit assessment and the blood and stool collection.

In addition 4 of the sites will also act as mechanistic sites and will perform all mechanistic assessments for the study. Patients at all sites will be offered an opportunity to take part in the mechanistic study but will be required to travel to one of the specified sites for the assessments.

The patient pathway, including the timelines for the mechanistic studies can be seen below in the study timeline (section 5.2).

5.2. Study Timeline



6. ELIGIBILITY CRITERIA

Eligible patients who take part in the study must meet all of the listed inclusion criteria and none of the exclusion criteria. Eligibility waivers to inclusion/exclusion criteria are not permitted.

6.1. Inclusion Criteria

Patients must fulfil all of the following criteria.

1. Written (signed and dated) informed consent.
2. Considered fit for study participation.
3. Meeting Rome IV criteria for IBS-D (see Appendices)
4. Aged ≥ 18 years
5. Undergone standardised workup to exclude the following alternative diagnoses
   a) Microscopic colitis (colonoscopy or flexible sigmoidoscopy) within previous 2 years
   b) Bile acid diarrhoea (SeHCAT results of > 10%, C4 results of <19 ng/ml or failed 1 week trial of a bile acid binding agent [colestyramine 4g t.d.s. , colesevelam 625mg t.d.s. or equivalent]) within previous 5 years

   > ***Note:*** *Cholecystectomy will not be an exclusion criteria if bile acid diarrhoea has been excluded*
   > *Patients with SeHCAT values of 5-10% will be eligible if they fail to respond to a 1 week trial of bile acid binding agent (see above)*

   c) Lactose malabsorption (In the opinion of the patient's clinician. Suggested but not mandated methods to exclude lactose malabsorption as an alternative diagnosis include hydrogen breath test/milk challenge or failure to respond to lactose free diet).
   d) Coeliac disease (tTG or duodenal biopsy)
6. Patients of child bearing potential or with partners of child bearing potential must agree to use methods of medically acceptable forms of contraception during the study and for 90 days after completion of study drug, (e.g. implants, injectable, combined oral contraceptives, barrier methods, true abstinence (when this is in line with the preferred and usual lifestyle of the patient) or vasectomised partners).
7. For women of child bearing potential, a negative pregnancy test should be performed within 72 hours of confirmation of eligibility.
8. Weekly average worst pain score ≥ 30 points on a 0 to 100 point scale.
9. Any stools with a consistency of 6 or 7 on the Bristol Stool Form score (BSFS) for 2 or more days per week.^

> ^ ***Inclusion criteria 8 & 9*** *will be assessed after the patient has completed the 14 day pre-treatment daily stool and pain diary and returned the results at visit 2.*

6.2. Exclusion criteria

Patients must not fulfil any of the following criteria:

1. Gastrectomy
2. Intestinal resection
3. Other known organic GI diseases (e.g. inflammatory bowel disease – Crohn's disease, ulcerative colitis).

4. Unable or unwilling to stop restricted medication including regular loperamide, antispasmodics (e.g. buscopan, mebeverine, peppermint oil, alverine citrate), eluxadoline, tricyclic antidepressant doses >30mg/day or other drugs likely in the opinion of the investigator to alter bowel habit. These medicines should be discontinued for a 7 day washout period prior to registration.

   Note: Intermittent loperamide will be permitted but only as rescue medication (see section 8.7)

5. QTc interval ≥450msec for men and ≥470msec for women. Assessed within the last 3 months by a 12-lead ECG.
6. Previous chronic use of ondansetron or contraindications to it (rare as per BNF)
7. Pulse, blood pressure, laboratory blood values outside the normal ranges according to the site's local definition of normal. Assessed within the last 3 months.

   Note: *Minor rises in ALT (<2 x upper limit of normal) will be acceptable but the patient's GP will be informed if they remain elevated at end of the study.*

8. Women who are pregnant or breastfeeding
9. Currently participating or who have been in an IMP trial in the previous three months where the use of the IMP may cause issues with the assessment of causality in this study.
10. Currently taking SSRIs or tricyclic antidepressants (unless at a stable dose for at least 3 months and with no plan to change the dose during the study).
11. Currently taking and unwilling or unable to stop any of the prohibited medications.*

    Prohibited medications – Apomorphine & tramadol which interact with ondansetron.
    Caution should be taken with patients on QT prolonging drugs and cardio toxic drugs.
    These patients should be reviewed by the PI to determine if they are suitable for the study (see section 8.5).

12. Patients with only stools of consistency 7 on the Bristol Stool Form score (BSFS) for 7 days a week. ^^

    ^^ ***Exclusion criterion 12*** *will be assessed after the patient has completed the 14 day pre-treatment daily stool and pain diary and returned at visit 2.*

7. TRIAL PROCEDURES

Research sites have been identified based on track record to recruit to functional GI studies and will be required to have obtained local management approvals and undertake a site initiation meeting with the CTRU prior to the start of recruitment into the trial. The recruitment target is 400 patients.

7.1. Patient Identification and Screening process

7.1.1. Patient Identification

7.1.1.1. Secondary Care

Patients with IBS-D will be identified in the recruiting sites from outpatient clinics, a review of waiting lists, case records, referrals, hospital databases and lists of patients that have previously consented to contact for information on upcoming research studies by local investigators and research nurses. Patients with IBS-D can also be identified in other secondary healthcare trusts working as patient identification centres (PIC). PICs will be

responsible for the identification of potential patients for the trial and referral to the nearest recruiting site for formal study screening

7.1.1.2. Primary Care & Pharmacies

Potentially eligible patients with IBS-D will also be identified by primary care General Practices (GPs) and local pharmacies, working either as PICs or a source of Trial Advertising (see 7.1.1.3). PICs will be responsible for the identification of potential patients for the trial and referral to the nearest recruiting site for formal study screening. GP practices will retain responsibility for the healthcare of the patients outside the research and will be notified of a patient's participation.

7.1.1.3. Trial Advertising

The TRITON study will also be advertised using posters and leaflets in electronic and paper form. Promotional materials will be distributed to relevant locations including (but not limited to) gastroenterology departments, GP surgeries, community pharmacies and specialist IBS organisations such as the IBS Network to send out via their mailing list or advertise on their Website, Facebook & Twitter pages. Adverts placed in secondary care will contain details of how to contact the local research team in that research site for further information so that interested patients can arrange an appointment to further assess their eligibility, as well as a link to the trial website. Adverts that will be distributed outside of the secondary care setting will advise patients to visit the trial website for further information. They will then be directed to their nearest site if they complete the self-screening questionnaire.

7.1.1.4. TRITON Website

All Triton advertising material will direct potential patients to the TRITON website which will contain all the patient information documentation for the study. The website will also contain an ethically approved self-screening questionnaire that will ask a series of questions that will help the potential patient to assess their eligibility for the trial.

If the self-screening questionnaire indicates that the patients may be eligible they will be directed to a list of participating research centres open to recruitment and given key contact details for each site. They will be advised to assess whether there is a participating site that is convenient for them and read the patient information sheet before deciding whether they are interested in participating. Should they wish to take part they will be asked to contact the key contact at the site to discuss their potential participation in the study further.

7.1.2. Screening Process

Each participating research site will be required to complete screening information for all patients identified by any of the above methods. Documented reasons for ineligibility or declining participation should be recorded.

Documented reasons for ineligibility or declining participation will be closely monitored by the CTRU as part of a regular review of recruitment progress. Screening data should be returned to the CTRU either via the patients considered log or F01 Screening Form. Anonymised information will be collected including:

- Age
- Sex
- Method of identification
- Method of approach
- Whether the patient is registered onto the trial or not.

Screened patients who are not registered, either because they are ineligible or because they decline participation, will also have the following information recorded:

- The reason they are not eligible for study participation, OR
- The reason that they declined if eligible.

7.2. Patient Approach

Once screening has been completed all potentially eligible patients will be contacted by a member of the local research team. Contact will be made:

- **During clinic appointments** - Patients will be provided with verbal and written details about the trial (TRITON Patient Information Sheets/ Consent Forms) by a member of the attending clinical team. This will include detailed information about the rationale, design and personal implications of the study.
- **Via a telephone call** –Patients will be provided with verbal information about the study. If the patient is interested in taking part a copy of the Patient Information Sheets and Consent Forms will posted / emailed out to them to review. The research team will contact them once they have had time to read the information to determine if they wish to take part.
- **Via Written communication (Email / Letter)** - These patients will be sent a personalised letter / email offering them the opportunity to potentially take part if they are eligible. This letter / email will include a brief introduction to the study and contact details for the local research team to find out more information and to make an appointment to discuss the study further. If the patient expresses an interest in taking part a copy of the patient information sheet and consent form will be posted / emailed out to them to review. The research team will contact them once they have had time to read the information to determine if they wish to take part.

Following the provision of study information, patients will have as long as they need (at least 24 hours, unless the participant wishes to participate sooner) to consider participation and will be given the opportunity to discuss the study with their family and other healthcare professionals before they are asked whether they would be willing to take part. The Investigator or designee will have given the patient full and adequate oral and written information about the study including the background, purpose and risks/benefits of participation and ensure that each patient is given the opportunity to ask questions they have concerning study participation. The investigator will also confirm that they are free to withdraw from the study at any time without it affecting their future care.

7.3. TRITON Consent Forms

The TRITON study has 2 consent forms. The first is the ***main study consent form*** which includes consent to the main study as well as consent for the optional main study assessments. Such as:

- **Blood and stool collection** – This will be performed at all sites where this is available and will occur at visit 3 and visit 5 (see section 7.12.2).
- **Research biopsy collection** – This will be performed at all sites where this is available and will occur at visit 3 or during the mechanistic assessments see section 7.13.3).
- **Colonic transit assessments –** This will be performed at all sites and will occur at visit 3 and visit 5 (see section 7.12.1).

These assessments are optional and would not affect the patient's participation in the study.

The second is the ***specialised tests consent form*** (mechanistic assessments) which include:

- **Colonic Manometry** – This will be performed up to 8 weeks from visit 2 but prior to randomisation and between weeks 8-11 at The University of Nottingham or Queen Mary University London. Patients from all sites will be given the opportunity to take part, but will be required to travel to one of the named sites. We will aim to recruit 40 patients into this part of the study, and once this target has been reached this will no longer be offered as an option to potential patients (see section 7.13.1).
- **Barostat** - This will be performed up to 8 weeks from visit 2 but prior to randomisation and between weeks 8-11 at The University of Nottingham, Leeds, University College London or QMUL. Patients from all sites will be given the opportunity to take part, but will be required to travel to one of the above mentioned sites. We will aim to recruit 80 patients into this part of the study, once this target has been reached this will no longer be offered as an option to potential patients.
- **Research biopsies** – Patients undergoing the colonic manometry and / or barostat mechanistic assessments will be given the opportunity to consent to the collection of biopsies.

Participation in the mechanistic assessments is not mandatory and would not affect the patient's participation in the main trial.

7.3.1. Informed Consent

The Principal Investigator (PI) retains overall responsibility for the informed consent of patients at their site and must ensure that any person delegated responsibility to participate in the informed consent process is duly authorised, trained and competent to participate according to the ethically approved protocol, principles of Good Clinical Practice (GCP) and Declaration of Helsinki 1996.

Written Informed consent for the main study and specialised tests (signed & dated) will be collected from each patient on the study before they undergo procedures that are specifically for the purposes of the study. The right of a patient to refuse participation without giving reasons must be respected. The patient must remain free to withdraw at any time from the study without giving reasons and without prejudicing his/her further treatment. All patients will be provided with a contact point where he/she may obtain further information about the trial. Where a patient is required to re-consent or new information is required to be provided to a

patient, it is the responsibility of the PI to ensure this is done in a timely manner and according to any timelines requested by the CTRU.

A record of the consent process detailing the date of consent and those involved in the consent process will be detailed in the patient's hospital notes. The original copy of the signed, dated Informed Consent Form is stored in the Investigator site file, a copy is also filed in the medical records (as per local practice), one given to the patient and one returned to the CTRU.

Once consented, patients will be registered and given a unique trial ID that will be used throughout their duration in the study.

7.3.2. Loss of Capacity Following Informed Consent

Where valid informed consent is obtained from the patient, and the patient subsequently becomes unable to provide ongoing informed consent by virtue of physical or mental incapacity, the consent previously given when capable remains legally valid.

Patients who lose capacity after informed consent has been obtained will continue with protocol treatment and assessments in consultation with the Principal Investigator and patient's carer / family with the patient's best interests foremost in the decision making process. Ongoing collection of safety and follow-up data (where possible) will continue via the clinical care team for inclusion in the trial analysis in order to preserve the integrity of the trial's analysis and fulfil regulatory requirements specifically for pharmacovigilance purposes.

7.3.3. Patient Inconvenience allowance

Patients that consent to the main study will receive £175 inconvenience allowance. £75 will be paid once they have been randomised (Visit 3) and £100 at the completion of treatment and follow-up periods (Visit 6).

In addition if patients consent to the colonic manometry and barostat assessments they will receive the following allowance for inconvenience.

Colonic Manometry:

- Assessment and living within 20 miles of the site = £130 per visit
- Assessment and living within 20 - 30 miles of the site = £135 per visit
- Assessment and living within 30 - 40 miles of the site = £140 per visit
- Assessment and living within 40 - 50 miles of the site = £145 per visit
- Assessment and living within 50 - 60 miles of the site = £150 per visit
- Assessment and living within 60 - 70 miles of the site = £155 per visit
- Assessment and living within 70 - 80 miles of the site = £160 per visit
- Assessment and living within 80 - 90 miles of the site = £165 per visit
- Assessment and living within 90 - 100 miles of the site = £170 per visit

Barostat Assessment

- Assessment and living within 20 miles of the site = £60 per visit
- Assessment and living within 20 - 30 miles of the site = £65 per visit
- Assessment and living within 30 - 40 miles of the site = £70 per visit
- Assessment and living within 40 - 50 miles of the site = £75 per visit
- Assessment and living within 50 - 60 miles of the site = £80 per visit
- Assessment and living within 60 - 70 miles of the site = £85 per visit
- Assessment and living within 70 - 80 miles of the site = £90 per visit
- Assessment and living within 80 - 90 miles of the site = £95 per visit
- Assessment and living within 90 - 100 miles of the site = £100 per visit

Patients living over 100 miles or requiring an overnight stay must be approved by the CTRU prior to the patient consenting to take part in these assessments.

Patients will be given an expense form during the respective visit to claim this payment and be required to complete their bank details and return the form to the CTRU within 4 weeks of the visit. Payment will then be made directly into the patient's bank from the CTRU. All bank details will remain confidential and will be held securely at the CTRU until the end of the study. These payments are not classed as earnings by HMRC and therefore these payments do not need to be declared for tax purposes.

7.4. Registration

Following confirmation of written informed consent and confirmation of eligibility, patients will be registered into the trial by an authorised member of staff at the local research site. Registration will be performed centrally using the CTRU automated web based and 24-hour telephone registration and randomisation system. Authorisation codes, user names and PINs/passwords will be provided by the CTRU and will be required to access the registration and randomisation system.

The following information will be required at registration:

- Personal 5 digit authorisation code (for telephone) or user name (for web)
- Personal 4 digit PIN/password
- Name of study research site and site code
- Patient details, including initials, date of birth and mobile phone number
- Confirmation of eligibility
- Confirmation of written informed consent
- Confirmation of the diary having been issued
- Decision regarding consent to mechanistic assessments
    - Preference of centre
    - Whether research biopsies will take place at a mechanistic assessment

DIRECT LINE FOR 24-HOUR REGISTRATION

Online Access: https://lictr.leeds.ac.uk/webrand/

Telephone Access: 0113 343 2290

All patients will be allocated a unique trial ID after they have been registered.

In accordance with the trial inclusion criteria, patients with a weekly average worst pain score <30 and / or a stool consistency of 6 or 7 on the Bristol Stool Form score (BSFS) for less than 2 or for 7 days per week are not eligible for participation in the trial. As trial-specific data collection on these criteria is carried out over 2 weeks post registration, recruitment into the study is classed as a two-step process involving an initial registration of all potential patients, followed by randomisation for eligible patients. Randomisation of eligible patients will not take place until all inclusion and exclusion criteria have been confirmed.

7.4.1. Local Site Post Registration Activities

Once the patient has been registered they should be given the pre-treatment patient diary to record stool consistency, abdominal pain, urgency and use of loperamide over the next 2 weeks. The local site should advise the patients of the importance of this information and to bring the diary with them at their next visit.

If the patient has consented to:

- **Colonic Transit Assessment** –the local team should provisionally book an X-ray for the patient at their visit 3.
- **Text messages**- the patient should be provided with the Text Messaging Guide.
- **Colonic Manometry or Barostat assessments** - The CTRU trial team will notify the Clinical Research Fellow that a patient has consented to one or both of mechanistic assessments and provisional baseline and follow-up will be booked. If the patient is found to be eligible at visit 2 the patients will be advised of the appointment details. If the patient is found to be ineligible the local site should notify the clinical research fellow and CTRU so the appointment slots can be released.

7.5. Confirmation of Eligibility

Once the patient has completed the 2 week pre-treatment diary they should return to the clinic for their visit 2. At this point all the inclusion and exclusion criteria should be reviewed and the patient's eligibility confirmed.

If the patient is eligible the following actions should take place:

- If consented to:
    - Colonic transit assessment – patients should be given the pack of Transit-Pellet capsules containing the radiopaque markers, with written and verbal instructions on how and when they should be taken, and given confirmation of the date of their visit 3 and abdominal x-ray.
    - Colonic manometry and Barostat assessment – patients should be given the details of where and when these assessments will be performed.

- Stool collection – patients should be given equipment and instructions on how to collect their stool sample for visit 3.

If a patient has not consented to any of the above assessments then this visit will also be their visit 3 and they can be randomised straight away and begin treatment.

7.5.1. Ineligible patients

If the patient is ineligible, or no longer wishes to take part, the local research team will discuss other options of treatment available to them and they will not undergo any other study assessments. Reasons for non-randomisation will be documented where available.

If the patient is ineligible but they are still interested in taking part at a later date it may be possible to re-screen and re-register them if they become eligible. Guidance should be sought from the CTRU. Patients will be issued with a new screening number and unique trial ID and should be treated as a new patient, however details of the patient's previous screening number and trial ID will be required. A patient can be registered a maximum of two times.

7.6. Randomisation

Registered patients who have been confirmed as eligible will be randomised into the trial by an authorised member of the research team at the site; using either the automated secure 24-hour telephone randomisation service or via a web address based at the CTRU. The same authorisation codes, user names and PIN/passwords from the registration system will also give access to the randomisation system.

The following information will be required at randomisation:

- Personal 5 digit authorisation code (for telephone) or user name (for web)
- Personal 4 digit PIN/password
- Name of trial site and site code
- Patient details, including trial number and date of birth
- Confirmation of eligibility and completion of questionnaires and pre-treatment diary
- Confirmation of a negative pregnancy test if applicable
- Confirmation as to whether a colonic manomtery assessment has taken place (yes/no)
- Confirmation as to whether a barostat assessment has taken place (yes/no)

DIRECT LINE FOR 24-HOUR RANDOMISATION

Online Access: https://lictr.leeds.ac.uk/webrand/

Telephone Access: 0113 343 2290

Randomisation will be performed on a 1:1 basis to receive either ondansetron or placebo and each patient will be allocated 3 bottles of study medication each with a unique IMP kit code. Minimisation will be used to ensure treatment groups are well-balanced for the following characteristics, details of which will be required at randomisation:

- site
- colonic manometry assessment carried out

- barostat assessment carried out

7.6.1. Local Site Post Randomisation Activities

Once the patient has been randomised a notification of randomisation will be automatically sent to the PI, pharmacist, and research nurse. The following activities should also be completed.

Research Nurse / PI:

- **Patient Diary -** once the patient has been randomised they should be given a patient treatment diary to record:
    - Daily - stool consistency, abdominal pain, stool frequency, bowel movement urgency, trial medication & use of loperamide.
    - Weekly – satisfactory relief of IBS symptoms.

  The patient should be asked to complete the diary every day and to bring the diary with them at their next visit.
- **Questionnaires**: patients will be required to complete a questionnaire booklet containing the IBS-SSS, SF-LDQ, PHQ-12, HADS, and IBS-QOL questionnaire prior to randomisation. The completed form should be collected by the research nurse at the end of the visit.
- **Dose Titration** - explain to the patient that they will be contacted every two days to discuss their bowel habits. Provide the patient with guidance as to how to titrate their dose and provide them with the Participant Dose Adjustment Information leaflet.
- **Complete trial prescription** - with kit numbers taken from the notification of randomisation.
- **Trial ID card** - provide each patient with a trial ID card which they should carry with them and present to medical staff should they be admitted to hospital during their time on trial, or should they visit their GP
- **Notify the patient's GP** –the research site will inform the patient's General Practitioner (where applicable) by letter that the patient is taking part in the study provided the patient agrees to this, and information to this effect is included in the Patient Information Sheet and Informed Consent. A copy of the letter should be filed in the Investigator Site File.

Pharmacist:

- **Code break envelopes** - Add the Patient details to the relevant Code Break Envelopes
- **Dispense the trial medication** – On receipt of prescription dispense trial medication (relevant kit numbers specified on prescription and notification of randomisation) and record details in the Accountability Log in the Pharmacy Site File .

7.6.2. CTRU Post Randomisation Activities

The CTRU will receive a notification of patient randomisation to the TRITON email address. The CTRU will ensure that the PI, pharmacist and study nurse have received notification of the randomisation and are undertaking the appropriate actions.

7.7. Blinding

As the trial is double-blind, neither the patient nor those responsible for their care and evaluation (treating team and research team) will know the allocation or coding of the treatment allocation. This

will be achieved by identical packaging and labelling of both the ondansetron and placebo. Each bottle of ondansetron / placebo will be identified by a unique randomly generated kit code. Lists of the kit codes and their corresponding treatments will be generated by the CTRU and sent to the clinical supply company who will produce the kits and the code break envelopes.

Management of kit codes on the kit logistics application which is linked to the 24-hour randomisation system will be conducted by the CTRU Safety Statistician in addition to maintaining the back-up kit-code lists for each site.

Access to the opened code break envelopes will be restricted to the safety statistician and designated safety team. Code breaks will be permitted in emergency situations, where treatment allocation knowledge is needed to optimise treatment of the patient.

Any unblinded interim reports provided to the Data Monitoring and Ethics Committee (DMEC) will be provided by the CTRU Safety Statistician and the reports will be securely password-protected.

At the start of the study patients will be asked for consent to be contacted at the end of the study by the CTRU to confirm if they wish to know the results of the study and their treatment allocation.

7.8. Unblinding

Whilst the safety of patients in the trial must always take priority, maintenance of blinding is crucial to the integrity of the trial. Unblinding is strongly discouraged and Investigators should only break the blind when information about the patient's trial treatment is clearly necessary, and will alter the appropriate medical management of the patient.

Unblinding may be requested on the grounds of safety by the Chief Investigator, Local Principal Investigator or authorised delegate or treating physician. It is anticipated that requests for unblinding will most likely originate from a patient, carer (or friend/family member) or personal physician (e.g. GP) at the time of an adverse event or planned change in non-trial related drug therapy. In the event of a SAE, all patients should be treated as though they are receiving the active medication (i.e. ondansetron).

It is encouraged that requests for unblinding should be made directly to the CTRU, **During Office Hours** *(Monday to Friday 9am-5pm other than bank holidays, the period between Christmas Eve and New Year, Thursday afternoon before Good Friday and all Tuesdays following a bank holiday except for May Day and New Year's Day).* Investigators should telephone CTRU who will carry out the unblinding procedure.

It is encouraged that requests for unblinding should be made directly to CTRU wherever possible – Tel : 0113 343 4930**.**

The following information will be needed to perform an emergency unblinding:

- **Participant details, including trial ID number, initials and date of birth**
- **Name of trial research site and site code**
- **Name of person making the request for a code-break**
- **Reason for requesting a code-break**

- **Confirmation of whether the PI authorised the request**

If unblinding is performed at any stage during the trial, the decision as to whether the patient will continue trial drug is the responsibility of the Principal Investigator or delegate. These patients should continue to be followed up, and all data collected will be used in the final analysis.

Once all patients at a participating site have completed trial treatment, all code break envelopes will be returned to CTRU by the site pharmacy department. The CTRU will assess for evidence of tampering. Code break envelopes must not be opened by local pharmacy or the CTRU to reveal to patients their treatment allocation when they have completed trial therapy. Patients will be given the opportunity by the CTRU to be told their allocation at the end of the study.

7.9. Emergency Unblinding

Should the imminent safety of a patient be under question, the responsibility to break the allocation code resides solely with the treating physician / investigator responsible for the patients' medical care. In this situation they would be allowed to perform an emergency unblinding if no other alternative can be identified. It is encouraged that all requests for emergency unblinding are made through the CTRU if possible and if the event occurs within office hours.

For events occurring **Outside of Office Hours**, emergency unblinding may also be undertaken by contacting the local pharmacy department responsible for the unblinding at the site. The pharmacy team should then complete the unblinding CRF and return this to the CTRU within 1 working day. The decision as to whether the patient will continue trial drug remains the responsibility of the Principal Investigator or delegate. These patients should continue to be followed up, and all data collected will be used in the final analysis.

Further information on emergency unblinding can be found in the Emergency Unblinding Study Site Operating Procedure. The reason for emergency unblinding will be collected on the Emergency Unblinding Case Report Form.

7.10. Patient Pathway / Assessments / Data collection:

Data will be collected using paper case report forms (CRFs), templates of which will be provided by the CTRU. Data required at key follow-up visits may be obtained over the phone by the local site team or via return of Patient Diaries / Questionnaires direct to the site personnel for onward posting to the CTRU. Text messages will be used to collect key data items to feed into the primary outcome.

CRFs must only be completed by personnel authorised to do so by the Principal Investigator, as recorded on the trial-specific Authorised Personnel Log. The original 'wet ink' version of the completed CRFs should be returned to the CTRU at the address given in the Investigator Site File and a copy retained at site.

It is the responsibility of staff at participating sites to obliterate all personal identifiable data on any hospital reports, letters, etc., prior to sending to CTRU. Such records should only include Trial ID, initials and date of birth to identify the patient. The exception to this is the patient consent form, where the patient name and signature must not be obliterated. If signed copies of consent forms are

posted to CTRU, they must be sent in a separate envelope and not accompanied by any original CRFs or other documents containing clinical data.

Participating hospitals will be expected to maintain a file of essential trial documentation (Investigator Site File), which will be provided by the CTRU, and keep copies of all completed CRFs for the trial.

The CTRU will coordinate the shipment of samples from sites to the University of Nottingham for mechanistic assessment.

Please note that assessments performed specifically for the trial must not be performed until after consent has been received. Where a test has been performed as part of local care and is within the required time-frame it does not have to be repeated but cannot be used for trial purposes until the patient has given consent.

7.10.1. Visit 1: Consent and Registration Visit

At the consent and registration visit the following activities will take place:

- **Consent**
  - Both the main study and specialised test consent forms will be discussed with the patient, and the optional parts of the trial explained. Patients will then be asked to complete one or both forms, as appropriate.
- **Standard workup to exclude alternative diagnoses**

  Confirmation of the absence of microscopic colitis, lactose malabsorption, inflammatory bowel disease and coeliac disease will be collected on the eligibility checklist. ***If the patient requires a colonoscopy / SeHCAT scan or C4 Serum level test for the study and it is outside standard work up, please contact the CTRU for approval prior to conducting these assessments.***

  - **SeHCAT Scan, Bile Acid Binding Agent failure or C4 Serum Level**
    - To be performed prior to registration unless these have been done within 5 years of screening date. Confirmation that the SeHCAT level is >10%, the patient has failed a 1 week trial of bile acid binding agent or the C4 level is <19 ng/ml will be collected on the eligibility checklist. Patients with SeHCAT values of 5-10% will be eligible if they subsequently fail to respond to a 1 week trial of bile acid binding agent (see section 6.1). Patients with SeHCAT level <5% will be ineligible for the study.

  - **Colonoscopy with mucosal biopsies to exclude microscopic colitis**
    - To be performed prior to registration unless symptoms are stable and the test has been done within 2 years of screening date or within 5 years of screening date AND a faecal calprotectin is normal as defined below (Table 2).

Calprotectin Levels:

Score	Classification	Action	
Above 250	Abnormal	Colonoscopy required	
Between 50 & 250	Indeterminate	Repeat test.	If Normal - No further action needed.
			If Indeterminate - a colonoscopy would be required.
			If Abnormal - a colonoscopy would be required.
Below 50	Normal	No further action needed.	

Table 2: Calprotectin levels

- **Vital Signs**
  - The principal investigator or qualified designee will take vital signs (i.e. pulse, blood pressure) to confirm that these are within normal ranges, in accordance with sites local ranges. Results will be recorded on the eligibility checklist and the values will also be documented in the patient's notes.

- **Demographics**
  - Demographic data will be recorded on the relevant CRF and will include date of birth, sex, ethnicity and smoking history. NHS number will also be collected.

- **Electrocardiogram (ECG)**
  - A standard 12-lead ECG will be performed unless one has been performed as part of standard of care within the last 3 months. Confirmation that the QTc interval is <450msec for men and <470msec for women will be collected on the eligibility checklist.

- **Haematology & Biochemistry Tests**
  - Laboratory tests will be performed if not already done within the last 3 months. Samples will be analysed by the local study site laboratory using standard methods for routine tests. The following variables will be measured:

Haematology	Biochemistry	Other
• Haematocrit	• Albumin	• C-reactive Protein (CRP)
• Haemoglobin	• Alkaline phosphatase	
• Platelet count	• Alanine aminotransferase (ALT)	
• WBC & Differential	• Total protein	
• RBC	• Total bilirubin	
	• Sodium	

Haematology	Biochemistry	Other
	• Potassium	
	• Calcium	

Table 3: Laboratory tests

- The local site will be required to confirm all of the above haematology and biochemistry levels are within the site's local normal range on the eligibility checklist. If the patient's results of the tests named in Table 3 are not within the site's local normal ranges they will be excluded from the study. Minor rises in ALT (<2 x upper limit of normal) will be acceptable but the patient's GP will be informed if they remain elevated at end of the study.

- **Review of patient's current medication**
  - The investigator or qualified designee will confirm if the patient is taking any of the following medications in Table 4. This information will be recorded on the eligibility checklist. Any patients taking any of the medication listed below and who are unable to discontinue these drugs will be ineligible to continue into the study.

Apomorphine	Reported hypotension and loss of consciousness when ondansetron was administered with apomorphine hydrochloride, concomitant use with apomorphine is contraindicated.
Tramadol	Data from small studies indicate that ondansetron may reduce the analgesic effect of tramadol.
Medications likely to alter bowel habit (in the opinion of the investigator).	Including opiates, loperamide*, antispasmodics (eg buscopan, mebeverine, peppermint oil, alverine citrate), eluxadoline or tricyclic antidepressant doses >30mg/day
*see section 8.7	

Table 4: Prohibited medication

Caution should be taken *with patients on QT prolonging drugs and cardio toxic drugs, as ondansetron may increase the risk of QT prolongation and arrhythmias. Patients on these drugs should be reviewed by the PI for suitability for the trial.*

- **Book X-ray for Transit Assessment**
  - This should be booked for visit 3
    - Visit 3 should be 7 days after visit 2 or, if the patient has consented to the mechanistic studies, 7 days after their last mechanistic assessment.

- **If the patient has consented confirm mechanistic assessments with the CTRU**
  - As described in section 7.4.2

- **Patient Diary**
  Patients will be asked to complete a 2-week daily paper diary recording:
    - Stool frequency
    - Stool consistency for each stool
      - Using the Bristol stool form scale (BSFS);
    - Worst abdominal pain experienced that day
      - On a scale of 0-100, where 0 is no pain and 100 is worst imaginable pain.
    - Worst bowel movement urgency
      - On a scale of 0-100, where 0 is no urgency and 100 is worst imaginable urgency.
    - If they have used loperamide that day.

  The completed diaries will be collected at each visit by the local study team. This information will be used to confirm eligibility at the randomisation visit.

- **Text Messaging**
  In addition the CTRU will send each patient two text messages to the mobile phone number provided every day.

    - The first will ask if they have passed a stool which has had a consistency 6 or 7 on the BSFS and the patient will need to text back yes or no.
    - The second will ask what their worst abdominal pain score was that day, from a scale of 0-100 (where 0 is no pain and 100 is worst imaginable pain) and the patient will need to text back the score from 0-100.

  The study nurse will discuss this process with the patients and provide them with the guide. If it is highlighted that this will be a problem, the patient will complete only the paper diary. The mobile number provided will be verified prior to the text messages commencing. The patient may withdraw from receiving text messages upon request.

- **Patient Registration**
  - As described in section 7.4

7.10.2. Visit 2: Eligibility Confirmation

Confirmation of eligibility should occur as soon as possible after the patient has been registered and completed two weeks of their pre-treatment diary. It is recommended that visit 2 occurs no later than 8 weeks from the date of consent. Visit 2 can be used both to confirm eligibility and randomise the patient if they are eligible and no optional assessments are required.

Activities to take place at this visit include:

- **Review and Collection of completed pre-treatment patient diary*.**
  - These will be examined to confirm:
    - Stool consistency of 6 or 7 on Bristol Stool Form score (BSFS) for 2 or more days per week
    - Stool consistency must not be only 7 on the BSFS for 7 days of the week
    - And an average worst pain score ≥ 30 in both weeks. The worst pain scores in each day of the week should be added together and divided by the number of days the diary was completed that week. The diary must have been completed on at least 4 days in each week

  *** If the patient has forgotten the diary then the visit must be rearranged and the patient told to bring the diary with them.**

- **Pregnancy test for women of child-bearing potential.**
  - Female patients of childbearing potential should have a negative urine or serum pregnancy test within 72 hours prior to confirmation of eligibility. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required. Confirmation of a negative pregnancy test will be collected on the eligibility checklist.

- **Completion of the eligibility checklist**

- **If the patient is confirmed eligible and they have consented the site will:**
  - **Dispense Transit-Pellets & Instructions**
    - Transit-Pellet capsules should be started 6 days prior to visit 3
  - **Confirm Colonic Transit X-ray appointment**
  - **Dispense Equipment and Instructions for Stool Collection**
  - **Confirm mechanistic appointments.**

7.10.3. Visit 3: Randomisation

This visit should be conducted as soon as eligibility has been confirmed and once all applicable mechanistic assessments and 6 days of the colonic transit preparation has been completed. It is recommended that visit 3 occurs no later than 8 weeks from the confirmation of eligibility.

Activities to take place at this visit include:

- **Pregnancy test for women of child-bearing potential.**
  - Female patients of childbearing potential should have a negative urine or serum pregnancy test prior to randomisation. If the urine test is positive, or cannot be confirmed as negative, a serum pregnancy test will be required. Patients with a positive pregnancy test should not be randomised into the study.

- **Completion of the baseline questionnaire booklet that will include:**

- **IBS-SSS, SF-LDQ, PHQ-12, HADS, and IBS-QOL questionnaire**

- **Randomisation onto the trial (see section 7.6)**

- **Dispensing of trial treatment**

- **Dispensing of patient diary**
  Patients will be asked to continue to record the following information for the next 6 weeks:
    - Daily:
      - Stool frequency
      - Stool consistency for each stool - using the Bristol stool form scale (BSFS);
      - Worst abdominal pain experienced that day - on a scale of 0-100, where 0 is no pain and 100 is worst imaginable pain.
      - Worst bowel movement urgency - on a scale of 0-100, where 0 is no urgency and 100 is worst imaginable urgency.
      - Number of capsules taken.
      - If they have used loperamide that day.
    - Weekly:
      - If they feel they have had satisfactory relief from their symptoms that week – yes / no

  The completed patient diaries will be collected in at each visit by the local study team.

- **Text Messaging**
  Patients will again begin to receive two text messages to their mobile phone every day unless they have withdrawn from this aspect.
    - The first will ask if they have passed a stool which has had a consistency 6 or 7 on the BSFS and the patient will need to text back yes or no.
    - The second will ask what their worst abdominal pain score was that day, from a scale of 0-100 (where 0 is no pain and 100 is worst imaginable pain) and the patient will need to text back the score from 0-100.

  The study nurse will discuss this process with the patients and if it is highlighted that this will be a problem, the patient will complete only the paper diary.

- **If consented to:**
    - Colonic transit assessment X-ray
    - Research biopsies
    - Research blood collection
    - Stool collection

7.10.4. Dose Titration

During the first 2 weeks patients will be contacted every 2 days by the local site team to discuss symptoms. The dose will then be titrated as required. Additional guidance on dose titration will be given to the site in a study site operating procedure and the patient in a Dose Adjustment

Information leaflet. A check for SAEs will be performed during each telephone call. The steady dose to be taken forward for the remainder of the study will be confirmed in week 2 though this may be altered during the 12 weeks if required.

If after week 2 the patient has reached a steady dose of 4 or more capsules a day then the research nurse should request additional prescriptions for the patient to ensure they have enough capsules until their week 6 visit.

7.10.5. Visit 4: Week 6 Visit

To be performed within weeks 6 - 7.

Activities to take place at this visit include:

- **Pregnancy test for women of child-bearing potential.**
  - Female patients of childbearing potential should have a negative urine or serum pregnancy test. If the urine test is positive, or cannot be confirmed as negative, a serum pregnancy test will be required. Patients with a positive pregnancy test should discontinue study treatment and the pregnancy should be reported to the CTRU on the pregnancy CRF within 24 hours.

- **Review and collection of completed patient diary**

- **Confirm the occurrence of any reportable AEs**
  - The investigator or qualified designee will document on the relevant CRF whether any reportable AEs have occurred since the last visit.

- **Dispensing of trial treatment**
  - Patients should be advised that their last dose of treatment should be taken on the day of visit 5.

- **Dispensing of the patient diary**

  Patients will be asked to continue to record the following information for the next 6 weeks:
    - Daily:
      - Stool frequency
      - Stool consistency for each stool - using the Bristol stool form scale (BSFS);
      - Worst abdominal pain experienced that day - on a scale of 0-100, where 0 is no pain and 100 is worst imaginable pain.
      - Worst bowel movement urgency - on a scale of 0-100, where 0 is no urgency and 100 is worst imaginable urgency.
      - Number of capsules taken.
      - If they have used loperamide that day.
    - Weekly:
      - If they feel they have had satisfactory relief from their symptoms that week – yes / no

  The completed patient diaries will be collected in at each visit by the local study team.

- **Text Messaging**

  Consenting patients will continue to receive two text messages to their mobile phone every day unless they have withdrawn from this aspect.

  - The first will ask if they have passed a stool which has had a consistency 6 or 7 on the BSFS and the patient will need to text back yes or no.
  - The second will ask what their worst abdominal pain score was that day, from a scale of 0-100 (where 0 is no pain and 100 is worst imaginable pain) and the patient will need to text back the score from 0-100.

  The study nurse will discuss this process with the patients and, if it is highlighted that this will be a problem the patient will complete only the paper diary.

- **Review Concomitant Medication (see section 8.5)**

If the patient has consented the site will:

- **Dispense Transit–Pellet capsules & Instructions**
  - Transit-Pellet capsules should be started 6 days prior to visit 5.

- **Book X-ray to take place at visit 5**

- **Confirm mechanistic appointments (if applicable)**
  - These should be performed at one time point during week 8-11 of treatment

- **Dispense equipment and instructions for stool collection and remind patient to bring stool sample to visit 5.**

7.10.6. Visit 5: Week 12 Visit

To be performed within weeks 12 - 13.

Activities to take place at this visit include:

- **Vital Signs**
  - The principal investigator or qualified designee will take vital signs (i.e. pulse, blood pressure) to confirm that these are within normal ranges in accordance with sites local ranges. Results will be recorded on the Week 12 CRF and the values will also be documented in the patient's notes.

- **Pregnancy test for women of child-bearing potential.**
  - Female patients of childbearing potential should have a negative urine or serum pregnancy test. If the urine test is positive, or cannot be confirmed as negative, a serum pregnancy test will be required. The pregnancy should be reported to the CTRU on the pregnancy CRF within 24 hours.

- **Review and collection of completed patient diary**

- **Confirm the occurrence of any reportable AEs**
    - The investigator or qualified designee will document on the relevant CRF whether any reportable AEs have occurred since the last visit.

- **Completion of the 12 week questionnaire booklet that will include:**
    - IBS-SSS, SF-LDQ, HADS, and IBS-QOL questionnaire

- **Collection of unused medication**
- Patients must return all used medication and empty bottles to the site.

- **Dispensing of the patient diary**

  Patients will be asked to continue to record the following information until their next visit.
    - Daily:
        - Stool frequency
        - Stool consistency for each stool - using the Bristol stool form scale (BSFS);
        - Worst abdominal pain experienced that day - on a scale of 0-100, where 0 is no pain and 100 is worst imaginable pain.
        - Worst bowel movement urgency - on a scale of 0-100, where 0 is no urgency and 100 is worst imaginable urgency.
        - If they have used loperamide that day.

  The completed patient diaries will be collected at each visit by the local study team.

- **Text Messaging**

  Consenting patients will continue to receive two text messages to their mobile phone every day until the end of their follow up period unless they have withdrawn from this aspect.
    - The first will ask if they have passed a stool which has had a consistency 6 or 7 on the BSFS and the patient will need to text back yes or no.
    - The second will ask what their worst abdominal pain score was that day, from a scale of 0-100 (where 0 is no pain and 100 is worst imaginable pain) and the patient will need to text back the score from 0-100.

  The study nurse will discuss this process with the patients, and if it is highlighted that this will be a problem the patient will complete only the paper diary.

- **Review of Concomitant Medication (see section 8.5)**

- **Haematology & Biochemistry Tests**
    - Laboratory tests will be performed at this visit. Samples will be analysed by the local study site laboratory using standard methods for routine tests. The following variables will be measured:

Haematology	Biochemistry	Other
• Haematocrit	• Albumin	• C-reactive Protein (CRP)
• Haemoglobin	• Alkaline phosphatase	
• Platelet count	• Alanine aminotransferase (ALT)	
• WBC & Differential	• Total protein	
• RBC	• Total bilirubin	
	• Sodium	
	• Potassium	
	• Calcium	

Table 5: Laboratory tests

- The local site will be required to confirm all of the above haematology and biochemistry level is within the site's local normal range on the Week 12 CRF. Any abnormal findings should be followed up clinically for as long as medically indicated without further reporting on the CRFs. Minor rises in ALT (<2 x upper limit of normal) will be acceptable but the patient's GP will be informed if they remain elevated at end of the study.

- **If consented to:**
  - Research Blood Collection
  - Stool Collection
  - Colonic transit assessment X-ray

7.10.7. Visit 6: Follow-Up Visit

To be performed within week 16.

Activities to take place at this visit include:

- **Pregnancy test for women of child-bearing potential.**
  - Female patients of childbearing potential should have a negative urine or serum pregnancy test. If the urine test is positive, or cannot be confirmed as negative, a serum pregnancy test will be required. The pregnancy should be reported to the CTRU on the pregnancy CRF within 24 hours.
- **Review and collection of completed patient diary**
- **Confirm the occurrence of any reportable AEs**
  - The investigator or qualified designee will document on the relevant CRF whether any reportable AEs have occurred since the last visit.
- **Return of unused medication**
  - If not already returned at visit 5
  - Reconciled by local team
- **Patient / Research nurse completion of exit poll (F14 Exit Poll: Site Perspective and F15 Exit poll: Patient perspective).**

7.11. Study Schedule

The schedule of study assessments below summarises the trial procedures to be performed at each visit. It may be necessary to perform these procedures at unscheduled time points if deemed clinically necessary by the investigator.

Trial Period:	Screening	Visit 1 Registration	Pre-Treatment Assessments	Visit 2 Eligibility Confirmation	Mechanistic Assessments	Colonic Transit Preparation	Visit 3 Randomisation	Dose Titration via telephone contact [12]	Visit 4 6 Week Visit	Mechanistic Assessment	Colonic Transit Preparation	Visit 5 12 Week Visit	Visit 6 Follow-Up Visit
Scheduling Window (Days):			*14 days*	*Within 8 weeks of consent if possible*	*Within 8 weeks of visit 2 if possible*	*6 Days prior to Visit 3*	*Week 0*	*Every 2 days For first 2 weeks*	*6 Weeks from Week 0*	*Week 8-11*	*6 Days before visit 5*	*12 Weeks from Week 0*	*4 Weeks from last dose*
Clinical Assessments													
Screening Form F01	X												
Study Informed Consent		X											
Mechanistic Study Consent		X											
Demographics		X											
Vital Signs		X										X	
Review Concomitant medications		X							X			X	
Haematology & Biochemistry [1]		X										X	
SeHCAT Scan / C4 Serum Level [2]		X											
Colonoscopy with mucosal biopsies [3]		X											
12 Lead ECG [4]		X											
Pregnancy Test (if applicable)				X			X		X			X	X
Patient Diary Distribution & Collection		X		X			X		X			X	X
Confirmation of Eligibility				X									
Randomisation							X						
Drug Dispensing							X		X				
Unused Drug Collection												X	
Review Reportable AEs									X			X	X
Patient Reportable Data Collection													
Text Message Responses			X				X – Daily from randomisation until Visit 6						
Patient Diary			X				X – Daily from randomisation until Visit 6						
IBS-SSS, SF-LDQ, HADS, & IBS-QOL Questionnaire							X					X	
PHQ-12							X						
Ingestion of Transit–Pellet capsules						X					X		
Optional Main Study Assessments – if applicable													
Dispense Transit-Pellet capsules & Confirm X-Ray				X					X				
Colonic Transit Assessment [5]							X					X	

Trial Period:	Screening	Visit 1 Registration	Pre-Treatment Assessments	Visit 2 Eligibility Confirmation	Mechanistic Assessments	Colonic Transit Preparation	Visit 3 Randomisation	Dose Titration via telephone contact [12]	Visit 4 6 Week Visit	Mechanistic Assessment	Colonic Transit Preparation	Visit 5 12 Week Visit	Visit 6 Follow-Up Visit
Scheduling Window (Days):			*14 days*	*Within 8 weeks of consent if possible*	*Within 8 weeks of visit 2 if possible*	*6 Days prior to Visit 3*	*Week 0*	*Every 2 days For first 2 weeks*	*6 Weeks from Week 0*	*Week 8-11*	*6 Days before visit 5*	*12 Weeks from Week 0*	*4 Weeks from last dose*
Research biopsies [6]							X						
Research Sample - 5ml Whole Blood							X						
Research Sample - 5ml Serum							X					X	
Dispense equipment for stool collection				X					X				
Collect Research Sample - 4 Aliquots of Stool [7]							X					X	
Mechanistic Assessments – if applicable													
Confirm Mechanistic Assessments Appointments [8]		X							X				
High Resolution Colonic Manometry [9]					X					X			
Barostat study [10]					X					X			
Other Reported Data													
Exit Polls [11]													X

Table 6: Schedule of assessments

1. ***Haematology & Biochemistry includes - WBC, RBC, Haemoglobin, Haematocrit, Platelets, ALT, Total Bilirubin, ALP, Albumin, Total Protein, Sodium, Potassium, Calcium, CRP (At screening - if not performed within the last 3 months.)***
2. ***SeHCAT required if not performed within the last 5 years***
3. ***Colonoscopy with mucosal biopsies required if not performed within the last 2 years, OR last 5 years if faecal calprotectin is normal***
4. ***ECG required if not performed within the last 3 months***
5. ***Transit-Pellet capsules should be taken as follows – 1 Transit-Pellet capsule of 10 markers for 5 days, then 1 Transit-Pellet capsule of 5 markers at 8am and 1 Transit-Pellet capsule of 5 markers at 8pm on day 6 then x-ray on day 7.***
6. ***Research biopsies - 4 Snap Frozen in liquid nitrogen, 2 RNA later.***
7. ***Patients should collect the 4 aliquots of stool at home, stored in a freezer, no more than a few days prior to the visit.***
8. ***Appointments should be booked for baseline (between visit 2 and visit 3) and at a time point in 8-11 weeks of treatment.***

9. ***Manometry – (with biopsies at baseline if not already done) will be performed at University of Nottingham & QMUL only (n=40), patients will receive additional payment for participation.***
10. ***Barostat Study – (with biopsies at baseline if possible and not already done) will be performed at Nottingham/Leeds/QMUL/UCL only (n=80), patients will receive additional payment for participation.***
11. ***Exit poll completed by both site (F14 Exit Poll-Site Perspective) and patient (F15 Exit Poll-Patient Perspective). Do not give the patient F15 to complete, ask them the questions directly.***
12. ***Steady dose to be confirmed at the end of week 2***

7.12. Optional Main Study Assessments

Patients who consent will undergo one or both of these optional assessments at their local site:

7.12.1. Colonic Transit (up to n=400)

This assessment is a standard measurement of colonic transit using the radio-opaque marker technique from Tornblom [25]. Colonic transit assessments will take place at visit 3 and visit 5. Prior to the visit patients will ingest a Transit-Pellet capsule containing 10 markers per day on awakening for 5 days and a spilt dose of 2 Transit–Pellet capsules of 5 markers each at 8am and 5 markers at 8pm on day 6. They will then come in on the morning of day 7 (visit 3 & 5) to have a plain abdominal X-ray. The local Investigator or designee will review both x-rays and complete the Colonic Transit X-ray CRF with the number of markers observed. Transit is calculated in hours as 2.4 times the number of remaining markers.

7.12.2. Blood & Stool Sample Collection (up to n=400)

Sites will be required to collect, where possible, 5ml whole blood, 5ml serum and 4 aliquots of stool at baseline (visit 3) and 5ml serum and 4 aliquots of stool at week 12 (visit 5). The stool sample collection kits are given to the patients prior to visit 3 and visit 5 to allow the patient to collection the 4 aliquots of stool at home no more than a few days prior to the visit. The samples are to be stored at -70^{o}C until transfer to the University of Nottingham. The CTRU will arrange more regular shipments for those with access only to -20^{o}C freezers. Each sample will be given a specific sample number and will be link anonymised with the patient's trial ID number. Further details are given in the Sample Processing (Blood, Stool & Biopsy) Study Site Operating Procedures.

7.12.2.1. Faecal Tryptase & Bile Acids

Aliquoted stool samples will be analysed at the University of Nottingham to assess faecal tryptase and bile acids.

1. Tryptase will be measured using a previously established assay involving proteolysis of azocaesin and measurement of absorbance at 440nm [14]
2. Bile acids will be measured using high performance liquid chromatography (HPLC) and mass spectrometry.
3. Water content will be assessed by freeze drying

7.12.2.2. Genotyping & Mucosal Serotonin Measurements (up to n=400)

Whole blood samples will be analysed to extract DNA for genotyping to assess:

- Tryptophan hydroxylase 1 TPH-1 SNP 12517T>G (rs211105) [24]
- Research colonic biopsies (n=approx. 80) will be analysed for total serotonin (5-HT) and mRNA for TPH-1

7.13. Optional Mechanistic Assessments

Patients who consent will undergo some or all of the following mechanistic studies:

7.13.1. High Resolution Colonic Manometry (HRM) (n=40)

Colonic Manometry will be undertaken at 2 sites, the University of Nottingham and QMUL at baseline (between visit 2 & 3) and within weeks 8-11 on treatment. Patients from all sites will be offered the opportunity to take part, but would be required to travel to one of the 2 named sites for the assessment.

Patients will be required to fast overnight prior to the assessment. They will be asked to bring their usual dose of the study drug for the 8-11 week visit and we will leave 60 minutes after taking the dose before we make any measurements. They will then undergo enema preparation prior to flexible sigmoidoscopy to position a manometry catheter within the left colon. After a 1-hour wait to allow the motility to return to baseline, information on fasting motility will be collected for 2 hours. This will then be followed by a standard nutrient test meal and a further 2 hours of recording.

7.13.2. Rectal Compliance and Sensitivity (n=80)

The Barostat study will be undertaken at 4 sites, the University of Nottingham, Leeds, QMUL and UCL at baseline (between visit 2 & 3) and within weeks 8-11 on treatment. As before, all patients will be offered the opportunity to take part, but would be required to travel to one of the 4 named sites for the assessment.

Patients will be required to fast from midnight for morning slots, or from 8am for afternoon slots) prior to the assessment. They will be asked to bring their usual dose of the study drug for the 8-11 week visit and we will leave 60 minutes after taking the dose before we make any measurements. They will then undergo a tap water enema preparation prior to manual introduction of a catheter-mounted, infinitely compliant bag (max. volume 600 ml) into the rectum. Bag distension will be performed using the automated electromechanical barostat (Distender series: G&J Electronics Inc, Canada), with rectal pressure/volume relationships assessed during a phasic isobaric, ascending method of limits distension protocol (4 mmHg steps to maximum toleration; 1 min distension period, with 1 min rest period between distensions). This will be followed by a random phasic distension protocol distending to 8, 16, 24 and 36 mmHg with subjects rating sensation of gas, urgency, discomfort and pain on a 0-10 visual analogue scale. The maximum pressure used will be adjusted depending on established pain threshold to avoid excessive pain and pressure will immediately be released if patients report > 80mm of discomfort or pain on the VAS, and higher distensions will not subsequently be administered. Once the series has been completed the bag will then be deflated and removed.

7.13.3. Research Biopsies (n=approximately 80)

Research colonic biopsies from the upper rectum may be collected at 3 potential timepoints:

- As part of the colonic manometry assessment during placement of manometry catheter.
- At visit 3.
- As part of the barostat assessment after completing the procedure.

For consenting patients, 6 research biopsies will be taken. Four biopsies should be snap frozen in liquid nitrogen and 2 put in RNA later prior to storing at -70°C, where possible. The CTRU will arrange regular shipment for those with access only to -20°C freezers. Each sample be given a specific sample number and will be linked anonymised with the patient's trial ID number. Biopsies will be analysed using assays to assess TPH-1 mRNA and 5-HT. Further details are given in the Sample Processing (Blood, Stool & Biopsy) Study Site Operating Procedures.

7.14. Chain of Custody of Biological Samples

In all cases, patients will be consented for the collection and use of their biological samples and a full chain of custody will be maintained for all samples throughout their lifecycle. The Investigator at each site is responsible for maintaining a record of full traceability of biological samples collected from patients while these are in storage at the site, either until shipment or disposal. Anyone with custody of the samples e.g. sub-contracted service provider will have to keep full traceability of samples from receipt to further shipment or disposal (as appropriate).

The CTRU will keep overall oversight of the entire lifecycle through internal procedures and monitoring of study sites. Samples retained for further use will be registered with the Nottingham Biobank at the end of the study.

7.15. Total Blood Volumes

The total volume of blood that will be drawn from each trial patient for the assessments described in the sections above is shown below. Calculations are based on a registration visit, randomisation visit and 12 week visit.

	Registration Visit			Randomisation Visit			12 Week Visit		
	Sample volume (mL)	**Number of samples**	**Total volume (mL)**	**Sample volume (mL)**	**Number of samples**	**Total volume (mL)**	**Sample volume (mL)**	**Number of samples**	**Total volume (mL)**
Clinical chemistry [a]	6ml	1	6ml	n/a	0	0	6ml	1	6ml
Haematology [a]	8ml	1	8ml	n/a	0	0	8ml	1	8ml
Whole Blood Sample	n/a	0	0	5ml	1	5ml	n/a	0	0
Serum Sample	n/a	0	0	5ml	1	5ml	5ml	1	5ml
Total			**14ml**			**15ml**			**24ml**

[a] exact volume of blood for clinical chemistry and haematology may vary depending on local practice

Table 7: Volume of blood to be drawn from each patient

7.16. Discontinuation of Study Treatment & Withdrawal Criteria

In line with usual clinical care, cessation or alteration of regimens at any time will be at the discretion of attending clinicians or the patients themselves. All patients withdrawn from treatment or prescribed alternative treatment will still attend follow-up assessments unless unwilling to do so and CRFs will continue to be completed.

7.16.1. Discontinuation of Study Treatment

Patients who discontinue the study treatment would still be expected to complete their follow-up assessments, unless they withdraw from the trial. Reasons for study treatment discontinuation would include:

- Patient decision.
- Significant adverse events.
- Severe non-compliance to this protocol as judged by the Investigator.
- Safety, including allergic reaction to study medication.
- If the investigator considers that a patient's health will be compromised due to adverse events or concomitant illnesses that develop after entering the study.
- Receipt of prohibited concomitant medication after entering the study (please provide name of medication on the CRF).
- Pregnancy.
- Overdose >120mg.
- Drug intolerance (constipation on lowest possible dose).

The reason for discontinuation would be recorded on F18 Treatment Discontinuation & Patient Withdrawal Request.

Once study medication is permanently discontinued it cannot be restarted as part of the study. Patients should return all unused medication and packaging on cessation of trial treatment.

7.16.2. Withdrawal from Study

Patients who discontinue the study treatment and do not attend any further follow-up assessments will be deemed to have withdrawn from the study. Reasons for withdrawal from the follow-up assessments would include:

- Patient decision.
- Lost to follow-up.
- Death.

7.16.3. Procedures for withdrawal from study

The PI or delegate should ensure that the specific wishes of any patient who wishes to withdraw consent for further involvement in the trial, be that from further treatment and/or follow-up data collection, are defined and documented using the Withdrawal CRF within 24 hours of the research team becoming aware. This is in order that the correct processes are followed by the CTRU and site following the withdrawal of consent.

Where the patient has withdrawn due to an AE, the investigator should follow the procedures documented in section 9.0 in order to assess the safety of the study medication.

It should be made clear to any patient specifically withdrawing consent for further data collection in a CTIMP that data pertaining to safety will continue to be collected for regulatory reporting purposes and will be included in any safety analysis and the final analysis. In addition it is suggested that the patient is made aware of the fact that if any significant new information

becomes available in regard to the treatment they have received in the trial it may be necessary to contact them in the future.

If it is the decision of the attending clinician to withdraw the patient from further involvement in the trial then this should be documented on the relevant CRF.

7.17. Treatment after study

Following participation in the study, patient care will be decided by their local doctor according to usual practice.

8. STUDY MEDICATION

8.1. Investigational Medicinal Product

Within the trial, the following is classed as an Investigational Medicinal Products (IMPs):

8.1.1. Ondansetron

Ondansetron is a potent, highly selective receptor antagonist (5-HT3RA) that in adults is indicated for the management of nausea and vomiting induced by cytotoxic chemotherapy and radiotherapy and for the prevention and treatment of post-operative nausea and vomiting.

For this study ondansetron will be available for oral administration in a unit dose of 4 mg.

8.2. Supply, Storage & Dispensing.

8.2.1. IMP & Placebo Supply

A blinded, trial specific supply of ondansetron and placebo will be provided to all participating sites. Ondansetron tablets will be purchased directly from the wholesaler by the Nottingham University Hospital NHS Trust (NUH) Pharmacy and shipped to Sharp Clinical Services (UK) Ltd. who will over encapsulate the ondansetron tablets and produce a matching placebo capsule whose composition has been agreed with sponsor and approved by the MHRA. Both will be manufactured in accordance with good manufacturing practice.

Once the manufacture of the study medication has been completed they will be shipped in bulk to the NUH pharmacy who will package, label and QP release (carried out by the designated person) to provide identical treatment packs for ondansetron and placebo, each containing 50, identical, size 0 capsules for oral administration.

These packs will be labelled and delivered to the participating sites by Nottingham University Hospitals NHS Trust Pharmacy. In line with Directive 2001/20/EC and the Medicines for Human Use (Clinical Trials) Regulations 2004 (amended 2006). The site pharmacy will be responsible for completing individual patient details on each label as part of the dispensing process.

As a minimum the labels will include the following information:

a) name of the Sponsor;
b) kit allocation code, dose, quantity of dose units and route of administration;
c) batch number to identify the contents and packaging operation;
d) blank space for recording the trial ID;
e) directions for use;
f) CI name
g) trial EudraCT number;
h) storage conditions;
i) expiry date;
j) "for clinical trial use only";
k) "keep out of reach of children".

8.2.2. Kit Allocation

The Kit Allocation system operates as follows. Participating sites will be provided with a supply of bottles at time of authorisation. Each bottle will be allocated a unique kit code. Patients will be allocated 3 bottles with 3 unique kit codes at the time of randomisation and subsequent bottles will be replenished at the appropriate trial time. In addition, sites will also receive the associated code break envelopes for these bottles. These envelopes should be stored securely in the pharmacy and should only be opened if an out of office hours (Normal office hours are *Monday to Friday 9am-5pm other than bank holidays and the Tuesday following bank holiday Mondays)* emergency unblinding is required by the principal investigator or authorised delegate. Unblinding procedures are defined in section 7.8.

8.2.3. Storage at the hospital site

The investigational products must be stored below 30 °C in a secure area with restricted access to authorised site staff.

8.2.4. Dispensing

The investigational product should only be dispensed and administered as directed in the protocol, and only by site staff authorised to do so.

Receipt and dispensing of study medication must be recorded by an authorised person at the trial site. Only patients randomised in the trial may receive trial IMP.

After the randomisation of a new patient, the pharmacist will be notified of the patient's trial ID number and allocated kit numbers by CTRU. It is the pharmacist's responsibility to ensure the correct trial ID is added to the appropriate code break envelope and that the correct IMP bottles are dispensed to the allocated patient.

8.2.5. Stock Replenishment

The CTRU will oversee the kit allocation system and will monitor the supplies at each site. When the supplies at the participating site reach a pre-determined level then a re-order will be

triggered and a further supply of study medication is sent to the corresponding participating site.

Upon receipt of the study medication at sites, delivery details will be checked for accuracy and receipt acknowledged by signing or initialling and dating the documentation provided. The site will inform the CTRU that the study medication has arrived and the kit allocation system will be updated to allow the kits to be allocated to trial patients.

8.2.6. Replacing dispensed study medication

If the study medication bottle is lost or damaged between randomisation and the end of the patient's treatment period, the study medication should be replaced using the CTRU 24 hour system which will allocate a new bottle with a new kit code.

Site staff should complete the kit replacement Case Report Form prior to using the CTRU 24 hour system. A copy of which is available in the Investigator Site File along with details of kit replacement procedures.

Further details are given in the Procedures for Randomisation / Kit Replacement Study Site Operating Procedure.

8.3. Dosage and Duration

Patients will be allocated on randomisation 3 bottles of 50 capsules of either 4mg ondansetron or identical in appearance placebo, for their first 6 week treatment period.

Patients will be instructed to start their treatment with 1 capsule once a day. Depending on the response, patients will be asked to increase the dose in 4mg steps every 2 days to a maximum of 8mg three times a day. To facilitate this dose titration, during the first 2 weeks, the research nurse will attempt to contact the patient every 2 days to discuss dose adjustments. After the first 2 weeks the patient should continue the study on a steady dose of drug unless a further change is required to achieve normalisation of stool frequency as confirmed by discussion between the patient and the research team. A study site operating procedure will be provided to the local sites to guide research nurses through the dose titration period.

If after week 2 the patient has reached a steady dose of 4 or more capsules a day then the research nurse should request additional prescriptions for the patient to ensure they have enough capsules until their week 6 visit.

When the patient returns after 6 weeks they will have their dose and their medication levels checked, and be asked to return any empty bottles and remaining unused medication to the research nurse. They will then be given a new supply of capsules, this could be up to 252 capsules (6 bottles) to complete the 12 weeks supply.

8.4. Dosage Modification

The dose will be modified throughout the study to achieve normalisation of stool frequency and consistency. If stool consistency increases to a hard (stool form 1 or 2) or if bowel frequency drops below 1 per day patients will be instructed to reduce the dose to a minimum of one capsule taken every third day. Patients who remain constipated on such a low dose will be deemed intolerant of the drug and the treatment discontinued.

8.5. Concomitant treatments

The use of prohibited and restricted concomitant medications will be reviewed prior to confirmation of eligibility. These should be discontinued for a 7 day washout period prior to registration. Concomitant medications started prior to the study will be allowed to continue at a stable dose providing, in the opinion of the investigator they are not likely to alter bowel habit or are on the prohibited medication list (see Table 4). Antibiotics should be avoided if possible.

Patients that are unable to stop taking prohibited medication will be unable to enter the study. Concomitant reviews will also take place at visits 4 and 5, and confirmation that the patient is not taking any prohibited or restricted medication will be recorded on the CRF. It is the responsibility of the local PI (or delegate) to review any new concomitant medication at the study visit to confirm the patient's continuing suitability for the study.

Caution should be exercised when ondansetron is coadministered with drugs that prolong the QT interval and/or cause electrolyte abnormalities. Use of ondansetron with QT prolonging drugs may result in additional QT prolongation. Concomitant use of ondansetron with cardiotoxic drugs (e.g. anthracyclines (such as doxorubicin, daunorubicin) or trastuzumab), antibiotics (such as erythromycin), antifungals (such as ketoconazole), antiarrhythmics (such as amiodarone) and beta blockers (such as atenolol or timolol) may increase the risk of arrhythmias. Patients taking these drugs should be reviewed by the PI for their suitablility in the study.

There is no evidence that ondansetron either induces or inhibits the metabolism of other drugs commonly co-administered with it. Specific studies have shown that there are no pharmacokinetic interactions when ondansetron is administered with alcohol, temazepam, furosemide, alfentanil, morphine, lidocaine, thiopental, or propofol.

Ondansetron is metabolised by multiple hepatic cytochrome P-450 enzymes: CYP3A4, CYP2D6 and CYP1A2. Due to the multiplicity of metabolic enzymes capable of metabolising ondansetron, enzyme inhibition or reduced activity of one enzyme (e.g. CYP2D6 genetic deficiency) is normally compensated by other enzymes and should result in little or no significant change in overall ondansetron clearance or dose requirement.

8.6. Assessment of Compliance

A record of the number of capsules dispensed to, and returned by, each patient must be maintained and reconciled with the study medication and compliance records in the patient diary.

8.6.1. Accountability

In accordance with local regulatory requirements, the investigator / designated site staff will document the amount of study drug received, the amount dispensed to study patients, the amount returned by study patients, and the amount destroyed locally.

Product accountability records will be maintained throughout the course of the study and filed with delivery documentation. Destruction will be documented, as per local policy and undertaken only after approval for destruction is given from the CTRU.

Patients should return all unused study medication and empty packaging to the Investigator. Unused study medication must not be discarded or used for any other purpose than the present study. Study medication that has been dispensed to a patient must not be re-dispensed to a different patient. Unused study medication and empty bottles will be returned to the local pharmacy.

Residual numbers of capsules will be recorded and accountability completed before destruction.

8.7. Rescue Medication

Patients are not allowed to use regular loperamide whilst taking part in the study. However, if they experience excessive diarrhoea (>7 bowel movements per day) they may take loperamide as rescue medication. This must be kept to a minimum, documented in the patient diary. No more than one 2mg capsule on two separate days per week is allowed.

9. PHARMACOVIGILANCE

9.1. Definition of an Adverse Event (AE)

An Adverse Event (AE) is any untoward medical occurrence (including deterioration of a pre-existing medical condition) in a patient or clinical trial patient administered a medicinal product, and which does not necessarily have a causal relationship with this.

9.2. Definition of an Adverse Reaction (AR)

An 'Adverse Reaction' (AR) is all untoward and unintended responses to an investigational medicinal product related to any dose administered. This definition implies a reasonable possibility of a causal relationship which is supported by facts, evidence or arguments to suggest a causal relationship. This definition includes medication errors and uses outside what is foreseen in the protocol (i.e. if an AR occurs as a result of a medication error).

9.3. Definition of a Serious Adverse Event (SAE) / Serious Adverse Reaction (SAR)

A SAE is an AE occurring during any part of the study that meets one or more of the following criteria:

- Results in death.
- Is life-threatening*.
- Requires hospitalisation or prolongation of existing inpatient's hospitalisation**.

- Results in persistent or significant disability or incapacity.
- Is a congenital abnormality or birth defect.
- Other important medical event.

** "Life-threatening" in the definition of "serious" refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.*

*** "Hospitalisation" means any unexpected admission to a hospital department. It does not usually apply to scheduled admissions that were planned before study inclusion or visits to casualty (without admission).*

Medical judgement should be exercised in deciding whether an adverse event is serious. These characteristics must be considered at the time of the event and do not refer to an event which hypothetically may have caused one of the above.

Where an SAE is deemed to have been related to an IMP used within the trial the event is termed as a Serious Adverse Reaction (SAR). Any suspected transmission via a medicinal product of an infectious agent is also considered a SAR.

9.4. Definition of a Suspected Unexpected Serious Adverse Reactions (SUSARs)

A Suspected Unexpected Serious Adverse Reaction (SUSAR) is a serious adverse drug reaction which also demonstrates the characteristic of being unexpected, the nature, seriousness, severity or outcome of which is not consistent with the information about the medicinal product as set out in the Reference Safety Information (RSI), section 4.8 of the trial supplied SmPC.

9.5. Reportable Expected Adverse Events

The occurrence of reportable AEs will be recorded at visit 4, visit 5 and visit 6. At each visit the research nurse will complete the AE checklist to determine if the patient has suffered with any of the following expected AEs. Only the confirmation of occurrence and corresponding severity (mild-noticeable but not preventing normal activities, moderate-restricting some activities, severe- preventing any activities) will be recorded.

- Constipation.
- Abdominal Pain / Bloating (as part of the condition being treated).
- Headache.
- Nausea.
- Vomiting.
- Rectal Bleeding:
    - Should a patient suffer rectal bleeding, the PI or delegate will review the patient and, if clinically indicated, they will be offered investigation by flexible sigmoidoscopy/colonoscopy within 30 days of the event, as per local policy.
    - Should this lead to a diagnosis of ischaemic colitis this should be reported as an SAE to the CTRU as defined in section 9.7.

Any other adverse events meeting the criteria of a Serious Adverse Event will be recorded on the SAE form as described in section 9.7.

9.6. Recording & Reporting of SAEs / SUSARs

All SAEs / SARs / SUSARs will be collected from time of randomisation until the follow-up visit. After this period, investigators are still required to report any SARs or SUSARs that they become aware of. All SAEs / SUSARs should be reported to the CTRU within 24 hours of the Investigator or designee becoming aware of the event.

The following details will be collected for each SAE:

- Full details in medical terms with a diagnosis, if possible
- Duration (date of onset and date of resolution)
- Seriousness criteria
- Causality, in the opinion of the Investigator
- Local opinion on expectedness as defined by the trial supplied RSI
- Action taken with regard to study medication
- Outcome.

In addition to the above the following details will be collected for each SUSAR:

- Place SUSAR started
- Relevant Medical History
- Concomitant Medications
- Treatment for SUSAR
- Relevant Diagnostics Tests

To report an SAE/SUSAR please complete as much detail as you can regarding the event in the SAE /SUSAR case report form and ensure that the Investigator or designee has reviewed the event for causality, expectedness (Appendix 18.2) and has signed the form.

Once completed a copy of the SAE/SUSAR CRF should be faxed to the study team at CTRU on 0113 343 0686

All SAEs/SUSARs will be followed-up until resolution or a final outcome has been reached. All follow-up information should be faxed/emailed to the CTRU as soon as it is available.

Please retain the original SAE/SUSAR CRF until the CTRU confirm all information is complete and any resulting queries have been resolved. At this point the original should be posted to the CTRU in real time and a copy retained in the site file.

All SAEs/SUSARs will be reviewed by the NUH medical monitor, Chief Investigator or designated representative to confirm causality (section 18.1) and expectedness (section 18.2).

In the event of a SUSAR the CTRU will take responsibility of unblinding the patient prior to submission of the SUSAR to the MHRA and Ethics. Investigators will only receive information on the results of the unblinding if it is judged necessary for the safety of the patient. SUSARS will be reported to the MHRA & Ethics within the required expedited reporting timelines.

Copies of all completed SAEs and SUSARs will be sent monthly to the sponsor for their records.

9.6.1. Events Not Classified as an SAE

The following events will not be recorded as SAEs within this study:

Hospitalisation for:

- Routine treatment or monitoring of the studied indication not associated with any deterioration in condition.
- Treatment which was elective or pre-planned, for a pre-existing condition not associated with any deterioration in condition.
- Admission to hospital or other institution for general care, not associated with any deterioration in condition.
- Treatment on an emergency, outpatient basis for an event not fulfilling any of the definitions for serious as given above and not resulting in hospital admission.

9.7. Responsibilities

9.7.1. Principal Investigator (or delegate)

1. Checking for AEs and ARs when patients attend for treatment/follow-up.
2. Using medical judgement in reviewing/assigning seriousness, causality (see appendices) and giving a local opinion on expectedness using the Reference Safety Information approved for the trial.
3. Ensuring that all SAEs and SARs (including SUSARs) are recorded and reported to the CTRU within 24 hours of becoming aware of the event and providing further follow-up information as soon as available.
4. Ensuring that SAEs and SARs (including SUSARs) are chased with CTRU if a record of receipt is not received within 2 working days of initial reporting.
5. Ensuring that AEs and ARs are recorded and reported to the CTRU in line with the requirements of the protocol.

9.7.2. Chief Investigator (CI) (or nominated individual in CIs absence)

- Clinical oversight of the safety of patients participating in the trial, including an ongoing review of the risk/benefit.
- Using medical judgement in reviewing/assigning of seriousness, causality and expectedness of SAEs (where the NUH monitor is unavailable) (see appendices).
- Immediate review of all SUSARs (if medical monitor is unavailable). In the event of disagreement between local assessment and CI review with regards to SUSAR status, local assessment will not be overruled, but the CI may add comments prior to expedited reporting.

- Review of specific SAEs and SARs in accordance with the trial risk assessment and protocol as detailed in the Trial Monitoring Plan.
- Assigning Medical Dictionary for Regulatory Activities (MedDRA) or Body System coding to all reportable AEs and SAEs.
- Preparing the clinical sections and final sign off of the Development Safety Update Report (DSUR).

9.7.3. CTRU (as delegated by the sponsor)

- Central data collection and verification of AEs, ARs, SAEs, SARs and SUSARs according to the trial protocol onto a MACRO database.
- Record all SUSARs and ensure that recording, reviewing, assessment and notifications are completed within agreed timelines. Notification to:
    a. the competent authority (MHRA) and
    b. the research ethics committee (REC).

  In any event not later than 7 days (fatal or life threatening) or 15 days (non-fatal or life threatening) after the first awareness of the reaction. A report of additional information to be sent to the MHRA and REC within 8 days.
- Ensure that all other recruiting sites involved are promptly informed of SUSARs and any changes to research protocol in light of ongoing risk/benefit analyses of safety data.
- Reporting safety information to the CI, delegate or independent clinical reviewer for the ongoing assessment of the risk/benefit according to the Trial Monitoring Plan.
- Reporting safety information to the independent oversight committees identified for the trial (Data Monitoring and Ethics Committee (DMEC) and/or Trial Steering Committee (TSC)) according to the Trial Monitoring Plan.
- Notifying Investigators of SUSARs that occur within the trial.
- The unblinding of a patient for the purpose of expedited SUSAR reporting.
- Annually checking for and notifying PIs of updates to the Reference Safety Information for the trial.
- Preparing standard tables and other relevant information for the Development Safety Update Report (DSUR) in collaboration with the CI and ensuring timely submission to the MHRA and REC.

9.7.4. Nottingham University Hospitals NHS Trust - Sponsor

1. Maintain records of all serious adverse events reported to the Sponsor.
2. Record and review urgent safety measures and perform initial notification to:
    a. the competent authority (MHRA),
    b. the research ethics committee (REC).

   In any event not later than 3 days.
3. Ensure appropriate urgent safety measures are implemented in order to protect the patients of a clinical trial against any immediate hazards to their health or safety.
4. Provide a medical monitor who will review all SAEs and return a judgement of causality and expectedness within 1 working day.

9.7.5. NUH Medical Monitor

- Using medical judgement in assigning causality and expectedness (see appendices) of SAEs within 1 working day.
- Immediate review of all SUSARs.
    (a) In the event of disagreement between local assessment and Medical Monitor review with regards to SUSAR status, local assessment will not be overruled, but the Medical Monitor may add comments prior to expedited reporting.

9.7.6. Trial Steering Committee (TSC) duties

In accordance with the Trial Terms of Reference for the TSC, periodically reviewing safety data and liaising with the DMEC regarding safety issues.

9.7.7. Data Monitoring and Ethics Committee (DMEC) duties

In accordance with the Trial Terms of Reference for the DMEC, periodically reviewing unblinded overall safety data to determine patterns and trends of events, or to identify safety issues, which would not be apparent on an individual case basis.

9.8. Notification of Death

All deaths occurring from randomisation until the end of follow-up or withdrawal from the study, irrelevant of their relationship to the study medication should be reported to CTRU as an SAE.

In addition, if the investigator becomes aware of a patient death, outside this period, that appears to be study medication related, this should also be reported as a SUSAR.

9.9. Pregnancy Reporting

Ondansetron is listed by FDA as category B with no harm or adverse effects in laboratory animals but no well controlled studies in humans. Their recommendation is therefore that it should only be used in pregnancy if clearly needed. We are adopting a conservative approach and recommending that all women of childbearing age (defined as women who had any menstrual bleeding in the last 24 months and who have not had a hysterectomy) should be informed of the potential risks to the unborn child should they fall pregnant whilst receiving treatment. Any woman who is pregnant at the time of eligibility assessment or is unwilling to use medically approved contraception whilst receiving treatment will be refused entry to the study.

Pregnancies occurring in patients or patients partners during the study may therefore represent a safety issue. For this reason, all pregnancies should be reported to the CTRU within 24 hours of the PI or designee becoming aware of the event and a Notification of Pregnancy CRF should completed and returned by fax. The pregnancy should be followed for outcome and any adverse outcome of pregnancy assessed for causality to the treatment received.

9.10. Overdose Definition and Reporting

In the unlikely event of overdose (>120mg ingested), all study medication should be discontinued and the patient should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated.

If an adverse event(s) is associated with ("results from") the overdose, the adverse event(s) should be recorded as a serious adverse event, even if no other seriousness criteria are met.

9.11. Reporting Urgent Safety Measures

The Sponsor or Investigator may take appropriate urgent safety measures (USMs) in order to protect the patients of a clinical trial against any immediate hazard to their health or safety. USMs identified shall take immediate effect and the event will be notified to the REC and MHRA no later than 3 calendar days from the date the measures are taken.

10. STATISTICAL ANALYSES

10.1. Sample size and power considerations for the RCT

In the RCT we plan to recruit 400 patients from 13 sites across England and Scotland. This will provide 90% power at 5% significance to detect a 15% absolute clinically important difference between the randomised groups in the proportion of patients achieving the FDA recommended [26] endpoint of weekly responder for pain intensity & stool consistency for at least 6 weeks in the 12 week follow-up period. This assumes a placebo response rate of 17%, as recently reported using this endpoint [11] and allows for 15% attrition.

Sample size for mechanistic studies:

10.1.1. Colonic manometry

Previous studies with the closely related 5HT3RA Alosetron showed an increase in motility index compared with placebo, with a mean (standard deviation (SD)) of 1.0 (1.2)[18], indicating we would have a power of 80% to detect a standardised effect size of 1 with n=17. We will aim for n=20 on each treatment to allow for dropouts i.e. 40 each undergoing 2 studies, a total of 80 HRM studies.

10.1.2. Rectal compliance and sensitivity

Previous studies [18] with the closely related 5HT3RA Alosetron showed an increase in compliance from 5.9 (SD 1.3) to 9.8 (SD 1.2) ml/mmHg using n=22. We propose to study more patients to calculate correlations with symptoms, which typically require much larger numbers, so we will aim to study 40 patients on each treatment.

10.2. Data analysis

10.2.1. General considerations

Statistical analysis is the responsibility of the CTRU Statistician. The analysis plan outlined in this section will be reviewed and a final statistical analysis plan will be written before any analysis is undertaken. The analysis plan will be written in accordance with current CTRU standard operating procedures and will be finalised and agreed by the following people: the trial and supervising statistician, the Chief Investigator, the CTRU Lead Methodologist, Project Delivery Lead and Trial manager. Any changes to the final analysis plan and reasons for change will be

documented. Any deviation(s) from the final statistical plan in the final analysis will be described and justification given in the final report.

All hypothesis tests will be two-sided and use a 5% significance level. Methods to handle missing data are described for each analysis. Analysis and reporting will be in line with CONSORT. As TRITON is a double-blind study, the Trial Statistician will be blinded to treatment group allocation throughout the trial until the database has been locked and downloaded for final analysis. Only the Safety Statistician, Supervising Trial Statistician, back-up Safety Statistician and authorised unblinded Individual at the CTRU will have access to unblinded treatment group allocation prior to final analysis.

The number of patients withdrawing from study treatment will be summarised by treatment arm, along with reasons for withdrawal.

10.2.2. Frequency of analyses

Outcome data will be analysed once only, at final analysis, although statistical monitoring of safety data will be conducted throughout the trial and reported at agreed intervals to the Data Monitoring and Ethics Committee (DMEC).

Final analysis will take place 16 weeks post last patient randomisation.

10.2.3. Endpoint analysis

All analyses will be conducted on the intention-to-treat population defined as all patients randomised regardless of non-compliance with the intervention. A per-protocol analysis of the primary endpoint will be carried out to indicate whether results are sensitive to the exclusion of patients who violated the protocol (e.g. those patients randomised but subsequently found to be ineligible or took more than the allowed rescue medication). Outcome measures will be analysed by regression models appropriate to the data type. Such analyses will adjust for randomisation minimisation factors: site, completion of colonic manometry assessment, and barostat assessment, as well as the endpoint at baseline where applicable, age and sex.

Baseline characteristics will be summarised by randomised group.

10.2.4. Primary analysis

The primary analysis will compare the difference in the proportion of patients achieving the FDA recommended endpoint (as defined in section 3.1) at 12 weeks post randomisation between treatment groups using a logistic regression model adjusted for minimisation, age and sex. Any missing data will be assumed missing at random (MAR) and imputed for the primary analysis. Odds ratios and corresponding 95% confidence intervals will be presented.

Sensitivity analysis to assess the impact of missing data on the treatment effect will be performed. This will include complete case analysis and alternatives to multiple imputation (e.g. pattern mixture modelling) if missing patterns suggest data are missing not at random.

10.2.5. Secondary analyses

The difference in the proportions of patients with satisfactory relief of IBS symptoms between the treatment groups at 12 weeks post randomisation will be compared using logistic regression models, adjusting for minimisation, baseline values, age and sex. Odds ratios and corresponding 95% confidence intervals will be presented. Any missing data will be assumed MAR and imputed.

The differences between the two treatment groups for the continuous secondary endpoints at 12 weeks post randomisation will be compared using linear regression models, adjusted for the minimisation variables, the endpoint at baseline where applicable, age and sex. These endpoints are urgency of defaecation over the last month, stool frequency over the last month, number of days per week with at least 1 loose stool (BSFS>5) over the last month, average stool consistency, number of days rescue medications was used over 12 weeks, abdominal pain score, HADS depression and anxiety score, SF-LDQ score, IBS-QOL score and subscales, and IBS-SSS severity score. Treatment estimates and corresponding 95% confidence intervals will be reported. Any missing data will be assumed MAR.

The differences between the treatment groups post treatment over weeks 13-16 post randomisation in following endpoints: stool frequency, abdominal pain and urgency of defaecation will be compared using linear regression model adjusting for minimisation factors, baseline values and relevant baseline factors. Treatment estimates and corresponding 95% confidence intervals will be reported. Any missing data will be assumed MAR.

Exploratory analyses on the daily measurements (worst abdominal pain, loose stool, number of stools passed, consistency of stool, worst urgency, and use of loperamide) will be carried out, using repeated measures models which incorporate correlation between measurements from the same patient. SAS software version 9.4 will be used in the analyses of primary and secondary endpoints.

10.2.6. Safety analyses

All patients who receive at least one dose of study treatment will be included in the safety analysis set. The number of patients reporting a serious adverse event (up to 28 days after the last dose of treatment) and details of all serious adverse events will be reported for each treatment group.

All safety analyses performed prior to final analysis will be undertaken by the safety statistician (rather than the trial statistician), thus ensuring that the trial team remain blinded.

10.2.7. Subgroup analyses

No subgroup analyses are planned.

10.2.8. Mechanistic studies

Mechanistic studies will be analysed by the research fellow blinded to the intervention allocation under supervision of the CI and local supervising PIs. The differences between treatment groups for changes in gut transit times, colonic motility, rectal compliance, sensitivity, bile acids, and faecal tryptase will each be assessed by linear regression models. In

addition, exploratory mediator analyses will explore whether treatment effects in terms of changes in urgency or pain are mediated through changes in faecal bile acids or protease.

Exploratory subgroup analysis (tests of interactions) will be performed to investigate the effect of presence of each specified SNP allele on response to treatment using logistic regression with addition of an interaction term for the allele and treatment.

11. DATA HANDLING

11.1. Data

11.1.1. Data collection

In compliance with Good Clinical Practice (GCP), the medical records/medical notes should be clearly marked and allow easy identification of a patient's participation in the clinical trial. Labels will be provide by the CTRU to support easy identification.

11.1.2. Source data

All original records and certified copies of original records of clinical findings, observations, or other activities necessary for the reconstruction and evaluation of the trial are classified as source data. Source data are contained in source documents.

11.1.3. Source Documents

Original documents, data, and records e.g., completed patient diaries, hospital records, clinical and office charts, laboratory notes, memoranda, patients diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate copies, microfiches, photographic negatives, microfilm or magnetic media, x-rays, patient files, and records kept at the pharmacy, at the laboratories and at medico-technical departments involved in the clinical trial.

11.1.4. Case Report Forms

Paper Case Report Forms (CRF) will be used to collect individual patient data for the study. The Investigator (or delegated member of the site study team) must record all data relating to protocol procedures and study medication administration safety and efficacy data into the trial case report forms. The original 'wet ink' version of the completed CRFs should be returned to the CTRU at the address given in the Investigator Site File. Copies of the completed CRFs should be retained by the site and stored in the investigator site file (or a statement of their location).

11.1.5. Patient Reported Data

Patients will be required to complete a patient diary for the 14 days post registration, whilst on treatment and during follow-up. In addition during these periods patients will be asked to respond to 2 text messages each day asking them about their worst abdominal pain and if they have passed a stool of 6 or 7 on the BSFS. Patients who are unable or unwilling to respond to the text messages will complete the patient diary only.

In addition, patients will also be asked to complete:

- SF-LDQ questionnaire.

- HADS.
- PHQ-12 (baseline only).
- IBS- SSS questionnaire.
- IBS-QOL.

At baseline (week 0) and week 12 of treatment.

11.2. Archiving Trial data and documents held by CTRU

At the end of the trial, all data held by the CTRU and all trial data will then be securely archived in line with the Sponsor's procedures for a minimum of 15 years. Data held by the CTRU will be archived in the University of Leeds archive facility and site data and documents will be archived at the participating centres.

Following authorisation from the Sponsor, arrangements for confidential destruction will then be made. If a patient withdraws consent for their data to be used, it will be confidentially destroyed.

12. MONITORING, AUDIT & INSPECTION

12.1. Monitoring

Trial supervision will be established according to the principles of GCP and in-line with the NHS Research Governance Framework (RGF). This will include establishment of a core Project Team, Trial Management Group (TMG), an independent TSC and independent DMEC. A trial monitoring plan will be developed and will be informed by a trial risk assessment which will consider the safety or physical or mental integrity of the trial patients and the scientific value of the research. This trial monitoring plan will detail the timing and content of reports to monitor trial conduct, implementation, and adherence with the Consolidated Standards of Reporting Trials (CONSORT). Procedures will be in place to assess risk on an ongoing basis with adjustments made accordingly.

12.2. Data Monitoring

Data will be monitored for quality and completeness by the CTRU. Missing data will be chased until they are received, confirmed as not available or the trial is at analysis.

The CTRU/Sponsor will reserve the right to intermittently conduct source data verification exercises on a sample of patients, which will be carried out by staff from the CTRU/Sponsor. Source data verification will involve direct access to patient notes at the participating hospital sites and the ongoing central collection of copies of consent forms and other relevant investigation reports.

12.3. Clinical governance issues

To ensure responsibility and accountability for the overall quality of care received by patients during the study period, clinical governance issues pertaining to all aspects of routine management will be brought to the attention of the TSC and, where applicable, to individual NHS Trusts/Health Boards.

13. ETHICAL & REGULATORY CONSIDERATIONS

The trial will be performed in accordance with the recommendations guiding physicians in biomedical research involving human subjects adopted by the 18th World Medical Assembly, Helsinki, Finland, 1964, amended at the 48th World Medical Association General Assembly, Somerset West, Republic of South Africa [1996]. Informed written consent will be obtained from the patients at the registration visit, consenting patients will then be registered into the study at that visit. The right of a patient to refuse participation without giving reasons must be respected. The patient must remain free to withdraw at any time from the study without giving reasons and without prejudicing his/her further treatment.

13.1. Independent Ethics Committee/ Health Research Authority / Regulatory authority approval

13.1.1. Initial approval

The trial will be submitted to and approved by a Research Ethics Committee (Main REC), the Medicines & Healthcare products Regulatory Agency (MHRA), the Health Research Authority (HRA) and the appropriate local R&D department for each participating site prior to entering patients into the trial. The CTRU will provide a copy of the final protocol, patient information sheets, consent forms and all other relevant trial documentation that will be provided to the patients, any advertisements that will be used, and details of any patient compensation.

13.1.2. Approval of amendments

Proposed amendments to the protocol and aforementioned documents must be submitted for ethical, HRA and, where relevant, regulatory approval by the CTRU once Sponsor and funder review has been obtained. Amendments requiring approval may be implemented only after a copy of the approval letter has been obtained.

Urgent safety measures which result in a substantial trial amendment may be implemented prior to receiving Sponsor or ethical/regulatory approval. However, in this case, approval will be obtained as soon as possible after implementation.

13.1.3. SUSAR Reports, Annual safety reports and end of trial notification

The main REC/MHRA will be sent all SUSAR reports and annual safety updates in order to facilitate their continuing review of the study and will also be informed about the end of the trial, within the required timelines.

13.2. Radiation exposure

Patients will undergo a single plain abdominal x-ray during visits 3 and 5 to measure whole gut transit time. The estimated procedure dose – 0.6 mSv giving a total study exposure =1.2mSv

The abdominal X-ray is necessary in this study to visualise the position of the radio-opaque markers to calculate whole gut transit time. This is standard clinical measurement for which there is currently no alternative technique which involves less or no radiation.

In addition, if the patients have consented they will undergo a single plain abdominal x-ray between visit 2 and visit 3 and at 8-11 weeks of treatment to confirm the position of the catheter in the colonic

manometry assessment. The estimated procedure dose – 0.6 mSv giving a total study exposure =1.2mSv

If required to confirm eligibility patients will also undergo a SeHCAT test at baseline to exclude alternative diagnosis. The estimated procedure dose – 0.3mSv.

The mean overall survival time is assumed to be that of an average 40 year old leading to a lifetime risk of fatal cancer induction of approximately 1 in 10,000. This is classified by PHE as a 'very low risk' and of course adds a negligible amount to the natural lifetime risk of about 1 in 3. The amount of radiation received by participants is approximately equal to 1 years radiation dose for the average UK person. The doses involved are too small to cause any direct radiation tissue effects.

13.3. Peer Review

This study has been peer reviewed by 6 experts and the MRC EME panel in competition with many other applications as part of their call for IBS studies and funded which implies a rating of "excellent / international standing"

13.4. Protocol Compliance

Protocol compliance will be assessed throughout the study.

Protocol deviations, unplanned non-compliance, or breaches are considered departures from the approved protocol.

Frequently re-occurring deviations are not acceptable and could potentially be classified as a serious breach. Prospective, planned deviations or waivers to the protocol are not allowed under the UK regulations on clinical trials.

Protocol violations should be reported immediately to the CTRU using the Protocol Violations CRF.

Protocol violations that need to be reported include:

- Breaches of the eligibility criteria.
- Drug administration errors related to the study drugs which lead to an SAE.
- Overdose.

If the protocol violation is also associated with an event which meets the criteria of an SAE or SUSAR this should also be reported in accordance with section 9 of the protocol.

14. QUALITY ASSURANCE

14.1. Quality assurance

The trial will be conducted in accordance with the principles of Good Clinical Practice in clinical trials, as applicable under UK regulations, the NHS Research Governance Framework (and Scottish Executive Health Department Research Governance Framework for Health and Social Care 2006 for studies conducted in Scotland), and through adherence to CTRU Standard Operating Procedures (SOPs).

14.1.1. Serious breaches of GCP or trial protocol

CTRU and the Sponsor have systems in place to ensure that serious breaches of GCP or the trial protocol are picked up and reported. Investigators will promptly notify the CTRU of a serious breach (as defined in Regulation 29A of the Medicines for Human Use (Clinical Trials) Regulations 2004 [Statutory Instrument 2004/1031], as amended by Statutory Instrument 2006/1928) that they become aware of. A "serious breach" is a breach which is likely to effect to a significant degree:

(a) the safety or physical or mental integrity of the subjects of the trial; or

(b) the scientific value of the trial".

Sites should contact the CTRU trial co-ordinators for further information.

14.2. Data Protection & Patient Confidentiality

Precautions will be taken to ensure that patient confidentiality is preserved at all times. The Patient Consent form will identify those individuals who will require access to patient data and identifiable details and obtain appropriate permission from the consenting patient.

The trial staff at participating sites at the participating site will be responsible for ensuring that any data/documentation sent to the CTRU is appropriately anonymised as per instructions given by CTRU in accordance with the trial procedures to conform to the 1998 Data Protection Act.

All information collected during the course of the main trial will be kept strictly confidential. Information will be held securely on paper at the CTRU. In addition, the CTRU will hold electronic information on all trial patients. The CTRU will have access to the entire database for monitoring, co-ordinating, and analysis purposes.

The CTRU will comply with all aspects of the UK 1998 Data Protection Act. Operationally this will include:

- Explicit written consent from patients to record personal details including name, date of birth, NHS number.
- Appropriate storage, restricted access and disposal arrangements for patients personal and clinical details.
- Consent from patients for access to their medical records by responsible individuals from the research staff, or from regulatory authorities, where it is relevant to trial participation.
- Consent from patients for the data collected for the trial to be used to evaluate safety and develop new research.
- Copies of patients consent forms, which will include patient's names, will be collected when a patient is randomised into the trial by the CTRU. In addition patient name and address will be collected for questionnaire posting. All other data collection forms that are transferred to or from the CTRU will be coded with a unique patient trial number and will include two patient identifiers, usually the patient's initials and date of birth.

- Where central monitoring of source documents by CTRU (or copies of source documents) is required (such as scans or local blood results), the patients name must be obliterated by site before sending.

- Where anonymisation of documentation is required, research sites are responsible for ensuring only the instructed identifiers are present before sending to CTRU.

If a patient withdraws consent from further trial treatment and/or further collection of data, their data will remain on file and will be included in the final trial analysis.

14.3. Conflicts of Interest

Chief Investigator Professor Robin Spiller has received research funding from Lesaffre and Ironwood. He has also acted on advisory boards for Allergan, Commonwealth Diagnostics International, Danone, Ipsen, and Yuhan, and received speakers' fees from Menarini.

14.4. Insurance and indemnity

Insurance and indemnity for trial patients and trial staff is covered within the NHS Indemnity Arrangements for clinical negligence claims in the NHS, issued under cover of HSG (96)48. There are no special compensation arrangements, but trial patients may have recourse through the NHS complaints procedures.

14.5. End of trial

The end of the trial is defined as the date of the last patient's last data item.

15. TRIAL ORGANISATIONAL STRUCTURE

15.1. Responsibilities

Chief Investigator - The Chief Investigator will have responsibility for the design and set-up of the trial, the investigational medicinal product supply, pharmacovigilance, and oversight of mechanistic work within the trial.

Clinical Trials Research Unit – The CTRU will have responsibility for conduct of the trial in accordance with relevant GCP standards and CTRU SOPs. The CTRU will also be responsible for the site and patient payments.

Sponsor: Nottingham University Hospitals NHS Trust - The Sponsor or delegate is responsible for trial initiation management and to ensure the costs are covered and appropriate financial management is in place. The sponsor will also be responsible for contracting with a Clinical Supplies Company to procure bulk supply of over-encapsulated ondansetron and placebo and providing a medical monitor for the review of SAEs.

Nottingham University Hospital NHS Trust Pharmacy – responsible for packaging, labelling, and study medication distribution to hospital sites

The University of Nottingham – will be responsible for holding the grant. They will also be responsible the storage and analysis of the transferred biological samples collected at sites. All samples will be

used as described in the protocol and at the end of the study samples will be retained in the university biobank for all patients that have given consent for their use in future research.

QMUL / UCL / University of Leeds / University of Nottingham - Dedicated teams at these institutions will be responsible for performing all mechanistic assessments for the study. It is the responsibility of the named site to ensure the results of these assessments are transferred to the University of Nottingham for analysis.

15.2. Operational structure

Chief Investigator (CI) – The CI is involved in the design, conduct, co-ordination, and management of the trial. The CI will have overall responsibility for the design and set-up of the trial, the investigational drug supply, and pharmacovigilance within the trial.

Trial Sponsor (Nottingham University Hospitals NHS Trust) – The Sponsor is responsible for trial initiation management, and financing of the trial as defined by Directive 2001/20/EC. These responsibilities are delegated to the CTRU as detailed in the Delegation of Duties.

Clinical Trials Research Unit (CTRU): The CTRU at the University of Leeds will have responsibility for the conduct of the trial in accordance with the NHS Research Governance Framework (RGF) and CTRU and Sponsor SOPs as per the Delegation of Duties. The CTRU will provide set-up and monitoring of trial conduct to CTRU SOPs including randomisation design and service, database development and provision, protocol development, CRF design, trial design, source data verification, ongoing management including training, monitoring reports and trial promotion, monitoring schedule, and statistical analysis for the trial. In addition, the CTRU will support ethical approval submissions, any other site-specific approvals, and clinical set-up. The CTRU will be responsible for the overall day-to-day running of the trial including trial administration, database administrative functions, data management, safety reporting, and all statistical analyses. At the end of the trial, CTRU will be responsible for archiving all data and trial data held by the CTRU in line with the Sponsor's procedures for a minimum of 15 years.

15.3. Trial Oversight/monitoring groups

15.3.1. Trial Management Group (TMG):

A Trial Management Group (TMG) will be convened including the Chief Investigator, co-investigators and identified key collaborators, the trial statistician, and trial manager. Principal Investigators and key study personnel may be invited to join the TMG as appropriate to ensure representation from a range of sites and professional groups and, where possible, membership will also include a lay/consumer representative. Notwithstanding the legal obligations of the Sponsor and Chief Investigator, the TMG will have operational responsibility for the conduct of the trial.

The TMG Terms of Reference (ToR) will define the membership, roles, and responsibilities of the TMG, each member of the committee will be required to confirm participation on the committee under the ToR. The TMG will meet quarterly as a minimum. Specifically the TMG will be responsible for:

- Protocol completion.
- CRF development.
- Obtaining approval from the HRA, UK REC and supporting applications local approvals.
- Submitting a CTA application and obtaining approval from the MHRA.
- Nominating members and facilitating the TSC and DMEC.
- Monitoring of screening, recruitment, treatment and follow-up procedures.
- Monitoring of consent procedures, data collection, trial end-point validation.
- Oversight of the mechanistic assessments / results and analysis.

15.3.2. Data Monitoring Committee

A Data Monitoring Committee (DMEC) will be convened to monitor data collected during the study, and make recommendations to the TSC on whether there are any ethical or safety reasons as to why the trial should not continue. It will consist of an independent Chair, an independent statistician, and an independent clinician.

The DMEC Terms of Reference will define the membership, roles, and responsibilities of the DMEC, each member of the committee will be required to confirm participation on the committee under the ToR. The Committee will meet annually as a minimum.

15.3.3. Trial Steering Committee

A Trial Steering Committee (TSC) will be convened with an independent majority. Participation will include as a minimum an independent Chair, an independent statistician, independent clinician, PPI representative, the Chief Investigator, sponsor representative and other members of the TMG as required to update on trial progress. The role of the TSC will be to provide overall supervision of the trial progress and, as necessary, advice to the Trial Management Group on operational issues. The Committee will meet annually as a minimum.

The TSC Terms of Reference will define the membership, roles, and responsibilities of the TSC, each member of the committee will be required to confirm participation on the committee under the ToR.

15.4. Public & Patient Involvement

The study will have PPI representation on the Trial Steering Committee. The aim of PPI at this level is to ensure that the patient perspective is included in all decisions related to the trial. Patients will input into patient leaflets, invitation letters, recruitment strategies and the protocol and will advise on how best to capture daily symptoms including the design and piloting of an online stool diary. PPI representatives can also act as advisors to patients contemplating taking part.

During recruitment, PPI perspective will be sought on emerging issues and the study will convene a larger data interpretation workshop at trial completion. The study team has also established an expert patient training module under the supervision of a dedicated PPI facilitator to ensure this is done appropriately. We will offer free places on the CTRU 'Introduction to Clinical Trials' training (in 2017). Financial reimbursement will be offered in line with INVOLVE guidance

16. FUNDING

This project is funded by the National Institute for Health Research. (NIHR) Efficacy and Mechanism Evaluation (EME) Programme (Grant Ref: 15/74/01)

17. PUBLICATION POLICY & DATA DISCLOSURE

The trial will be registered with an authorised registry, according to ICMJE Guidelines, prior to the start of recruitment.

The success of the trial depends upon the collaboration of all investigators. For this reason, credit for the main results will be given to all those who have collaborated in the trial, through authorship and contribution. Authorship decisions will be guided by standard requirements for authorship relating to submission of manuscripts to medical journals. These state that authorship credit should be based only on the following conditions being met (http://www.icmje.org):

- Substantial contribution to conception and design, or acquisition of data, or analysis and interpretation of data.
- Substantial contribution to drafting the article or revising it critically for important intellectual content.
- Substantial contribution to final approval of the version to be published.

In light of this, the CI, other grant co-applicants, and relevant senior CTRU staff will be named as authors in any publication, subject to journal authorship restrictions. In addition, all collaborators will be listed as contributors for the main trial publication, giving details of roles in planning, conducting and reporting the trial.

To maintain the scientific integrity of the trial, data will not be released prior to the first publication of the analysis of the primary endpoint, either for trial publication or oral presentation purposes, without the permission of the TSC. In addition, individual collaborators must not publish data concerning their patients which is directly relevant to the primary endpoint until the first publication of the analysis of the primary endpoint.

On completion of the research project a draft final report will be submitted to the EME programme (trial funder) by the CTRU, within 14 days. This will be peer reviewed and then published on the EME website. The CTRU is obliged to provide NIHR/EME with advanced notice of any publication relating to the trial. Copies of any materials intended for publication will be provided to NIHR/EME at least 28 days prior to submission for publication.

18. APPENDICES

18.1. Causality of Serious Adverse Events

The Investigator or authorised medical designee will assess the causal relationship between the event and the investigational medicinal product for each SAE. As this is a double blinded study Investigators should evaluate causality as though the patient is receiving ondansetron.

18.2. Expectedness of Serious Adverse Event

The Investigator authorised medical designee will also assess if the expectedness of the AE in relation to previously known information about the investigational medicinal product found in the reference safety information. As this is a double blinded study Investigators should evaluate expectedness as though the patient is receiving ondansetron.

Expected:	Event is known to be associated with the investigational medicinal product or condition under study.
Unexpected:	Nature or severity of the event is not consistent with information about the investigational medicinal product within the IB or SmPC or the condition under study.

18.3. Rome IV Diagnostic Criteria for Irritable Bowel Syndrome

Recurrent abdominal pain, on average, at least 1 day per week in the last 3 months, associated with 2 or more of the following criteria*:

1. Related to defaecation
2. Associated with a change in frequency of stool
3. Associated with a change in form (appearance) of stool

*Criteria fulfilled for the last 3 months with symptom onset at least 6 months before diagnosis.

19. REFERENCES

1. Tillisch, K., et al., *Characterization of the alternating bowel habit subtype in patients with irritable bowel syndrome.* Am J Gastroenterol, 2005. **100**(4): p. 896-904.
2. Lavo, B., M. Stenstam, and A.L. Nielsen, *Loperamide in treatment of irritable bowel syndrome--a double-blind placebo controlled study.* Scand J Gastroenterol Suppl, 1987. **130**: p. 77-80.
3. Mearin, F., et al., *Bowel Disorders.* Gastroenterology, 2016.
4. Andresen, V., et al., *Effects of 5-hydroxytryptamine (serotonin) type 3 antagonists on symptom relief and constipation in nonconstipated irritable bowel syndrome: a systematic review and meta-analysis of randomized controlled trials.* Clin Gastroenterol Hepatol, 2008. **6**(5): p. 545-55.
5. Michel, K., et al., *Serotonin excites neurons in the human submucous plexus via 5-HT3 receptors.* Gastroenterology, 2005. **128**(5): p. 1317-26.
6. Gershon, M.D., *Review article: serotonin receptors and transporters -- roles in normal and abnormal gastrointestinal motility.* Aliment Pharmacol Ther, 2004. **20 Suppl 7**: p. 3-14.
7. Li, Y., et al., *Intestinal serotonin acts as paracrine substance to mediate pancreatic secretion stimulated by luminal factors.* Am J Physiol Gastrointest Liver Physiol, 2001. **281**(4): p. G916-23.
8. Camilleri, M., et al., *Serotonin-transporter polymorphism pharmacogenetics in diarrhea-predominant irritable bowel syndrome.* Gastroenterology, 2002. **123**(2): p. 425-32.
9. Bjornsson, E.S., et al., *Differential 5-HT3 mediation of human gastrocolonic response and colonic peristaltic reflex.* Am J Physiol, 1998. **275**(3 Pt 1): p. G498-505.
10. Chang, L., et al., *Incidence of ischemic colitis and serious complications of constipation among patients using alosetron: systematic review of clinical trials and post-marketing surveillance data.* Am J Gastroenterol, 2006. **101**(5): p. 1069-79.
11. Garsed, K., et al., *A randomised trial of ondansetron for the treatment of irritable bowel syndrome with diarrhoea.* Gut, 2014. **63**(10): p. 1617-25.
12. Lembo, A.J., et al., *Effect of alosetron on bowel urgency and global symptoms in women with severe, diarrhea-predominant irritable bowel syndrome: analysis of two controlled trials.* Clin Gastroenterol Hepatol, 2004. **2**(8): p. 675-82.
13. Spiegel, B., et al., *Predictors of patient-assessed illness severity in irritable bowel syndrome.* Am J Gastroenterol, 2008. **103**(10): p. 2536-43.
14. Tooth, D., et al., *Characterisation of faecal protease activity in irritable bowel syndrome with diarrhoea: origin and effect of gut transit.* Gut, 2014. **63**(5): p. 753-60.
15. Gecse, K., et al., *Increased faecal serine protease activity in diarrhoeic IBS patients: a colonic lumenal factor impairing colonic permeability and sensitivity.* Gut, 2008. **57**(5): p. 591-9.
16. Edwards, C.A., et al., *Effect of bile acid on anorectal function in man.* Gut, 1989. **30**(3): p. 383-6.
17. Bajor, A., et al., *Increased colonic bile acid exposure: a relevant factor for symptoms and treatment in IBS.* Gut, 2015. **64**(1): p. 84-92.
18. Delvaux, M., et al., *Effect of alosetron on responses to colonic distension in patients with irritable bowel syndrome.* Aliment Pharmacol Ther, 1998. **12**(9): p. 849-55.

19. Clemens, C.H., et al., *Effect of alosetron on left colonic motility in non-constipated patients with irritable bowel syndrome and healthy volunteers.* Aliment Pharmacol Ther, 2002. **16**(5): p. 993-1002.
20. Stacher, G., et al., *Effects of the 5-HT3 antagonist cilansetron vs placebo on phasic sigmoid colonic motility in healthy man: a double-blind crossover trial.* Br J Clin Pharmacol, 2000. **49**(5): p. 429-36.
21. Bell, A.M., et al., *Variations in muscle tone of the human rectum: recordings with an electromechanical barostat.* Am J Physiol, 1991. **260**(1 Pt 1): p. G17-25.
22. Ponec, R.J., M.D. Saunders, and M.B. Kimmey, *Neostigmine for the treatment of acute colonic pseudo-obstruction.* N Engl J Med, 1999. **341**(3): p. 137-41.
23. De Schryver, A.M., et al., *The effects of the specific 5HT(4) receptor agonist, prucalopride, on colonic motility in healthy volunteers.* Aliment Pharmacol Ther, 2002. **16**(3): p. 603-12.
24. Shiotani, A., et al., *Pilot study of Biomarkers for predicting effectiveness of ramosetron in diarrhea-predominant irritable bowel syndrome: expression of S100A10 and polymorphisms of TPH1.* Neurogastroenterol Motil, 2015. **27**(1): p. 82-91.
25. Tornblom, H., et al., *Colonic transit time and IBS symptoms: what's the link?* Am J Gastroenterol, 2012. **107**(5): p. 754-60.
26. FDA. Available from: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM205269.pdf