1 Protocol details

1.1 PROTOCOL TITLE:

Biomarkers of Relapse In ulcerative colitis patients after Tofacitinib dose reduction (BRITE)

1.2 Names (titles), roles and contact details of:

Sponsor
Name of Sponsoring Organisation/s: Guy's and St Thomas's NHS Foundation Trust
Name of Sponsor Representative: Rachel Fay
Address: Guy's and St Thomas's NHS Foundation Trust, R&D Department, 16th Floor, Tower Wing Great Maze Pond
Telephone: 02071889811
Fax: 02071881295
Email: R&D@gstt.nhs.uk

Co-Sponsor
Name of Sponsoring Organisation/s: King's College London
Name of Sponsor Representative: Professor Reza Razavi, Vice President & Vice Principal (Research), King's College London
Address: Room 5.31, James Clerk Maxwell Building, 57 Waterloo Road, London SE1 8WA
Telephone: +44 (0)207 8483224
Email: reza.razavi@kcl.ac.uk

Chief Investigator
Name: Dr Peter Irving, Consultant Gastroenterologist/Honorary Reader in Gastroenterology
Address: St Thomas' Hospital, Department of Gastroenterology, 1st Floor College House, South Wing, Westminster Bridge Road, London, SE1 7EH
Telephone: 02071882499
Fax: 02071882484
Email: peter.irving@gstt.nhs.uk

Name and address of Investigator(s)
Name: Dr Sailish Honap
Address: Department of Gastroenterology, Guy's and St Thomas' Hospital, Westminster Bridge Rd, Lambeth, London SE1 7EH
Telephone: 02071882499
Fax: 02071882484
Email: Sailish.Honap@gstt.nhs.uk

Name: Dr Mark Samaan
Address: Department of Gastroenterology, Guy's and St Thomas' Hospital, Westminster Bridge Rd, Lambeth, London SE1 7EH
Telephone: 02071882499
Fax: 02071882484
Email: mark.samaan@gstt.nhs.uk

Name: Dr Raphael Luber
Address: Department of Gastroenterology, Guy's and St Thomas' Hospital, Westminster Bridge Rd, Lambeth, London SE1 7EH
Telephone: 02071882499
Fax: 02071882484
Email: raphael.luber@gstt.nhs.uk

Name: Dr Susan John
Address: Department of Immunobiology, Floor 2, Borough Wing, Guy's Hospital, St Thomas' Street, London, SE1 9RT
Telephone: 02071889614
Email: susan.john@kcl.ac.uk

Name: Dr Robin Dart
Address: Peter Gorer Dept of Immunobiology, DIIID, Floor 2, Borough Wing, Kings College London, Guy's Hospital, St Thomas Street, London, SE1 9RT
Telephone: 02071882499
Fax: 02071882484
Email: robin.dart@kcl.ac.uk

Statistician
Name: Dr Sailish Honap
Address: Department of Gastroenterology, Guy's and St Thomas' Hospital, Westminster Bridge Rd, Lambeth, London SE1 7EH
Telephone: 02071882499
Fax: 02071882484
Email: sailish.honap@gstt.nhs.uk

Laboratories
Name: Dr Susan John
Address: Department of Immunobiology, Floor 2, Borough Wing, Guy's Hospital, St Thomas' Street, London, SE1 9RT
Telephone: 02071889614
Email: susan.john@kcl.ac.uk

1.3 Protocol details

Version number 1.0
Final/draft 07.06.21
Date 07/06/2021

3 Contents Page

List of Abbreviations and Definitions

AE	Adverse Event
AR	Adverse Reaction
ASR	Annual Safety Report
CA	Competent Authority
CI	Chief Investigator
CRF	Case Report Form
CRO	Contract Research Organisation
DMC	Data Monitoring Committee
EC	European Commission
GAfREC	Governance Arrangements for NHS Research Ethics Committees
ICF	Informed Consent Form
ISRCTN	International Standard Randomised Controlled Trial Number
MA	Marketing Authorisation
MS	Member State
Main REC	Main Research Ethics Committee
NHS R&D	National Health Service Research & Development
PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control
Participant	An individual who takes part in a clinical trial
RCT	Randomised Controlled Trial
REC	Research Ethics Committee
SAE	Serious Adverse Event
SDV	Source Document Verification
SOP	Standard Operating Procedure
SSA	Site Specific Assessment
TMG	Trial Management Group
TSC	Trial Steering Committee

4 Summary/Synopsis

Title	Biomarkers of relapse in ulcerative colitis patients after tofacitinib dose reduction
Protocol Short Title/Acronym	BRITE
Protocol Version number and Date	Version 1.0, 07/06/2021
IRAS Number	276727
REC Reference	
Sponsor Reference	
Study Duration	44 weeks
Methodology	Prospective observational study
Sponsor name	GSTT
Co-sponsor name	King's College London
Chief Investigator	Dr Peter Irving
Funder Name	Pfizer
Medical condition or disease under investigation	Ulcerative Colitis
Purpose of clinical trial	To identify markers predictive of disease flare in ulcerative colitis patients who responded to the higher dose commencing on maintenance tofacitinib therapy.
Primary objective	To identify biochemical and genetic markers predictive of ulcerative colitis flare
Secondary objective (s)	To identify clinical and disease characteristics predictive of ulcerative colitis flare
Number of Subjects/Patients	50 patients
Trial Design	Prospective observational study
Endpoints	1. Flare requiring drug discontinuation, surgery (colectomy) or addition of new therapy for ulcerative colitis. 2. Endoscopic activity at week 44 endoscopy
Main Inclusion Criteria	Ulcerative colitis patients commencing tofacitinib maintenance dosing, undergoing endoscopy as part of their routine clinical care.
Statistical Methodology and Analysis	Receiver operator curves will be generated to determine optimal cut-offs for continues variables, predictive of disease flare. Kaplan-Meier curves will be generated to allow comparisons of subdivisions of patients based on genetic and biochemical variables, using determined cut-offs. Univariate and Multivariate analyses will be performed on clinical, biochemical and genetic variables to establish predictors of disease flare.

5 Introduction

Tofacitinib has been shown in large-scale randomised clinical trials to be an effective treatment for patients with moderate to severe UC that is resistant to both conventional treatment and to biologic therapy[1]. In the few months it has been available in the UK, our unit has accrued some of the biggest experience in the country. Our initial experience and prospectively collected observational data suggest that the drug performs at least as well as clinical trial results would indicate.

Results from the OCTAVE clinical trial program led to the approval of an induction regimen of 10mg BD, with subsequent dose reduction in responders to 5mg BD after at least 8 weeks. This maintenance regimen was shown to maintain remission with greater efficacy than placebo[1]. However, the clinical trial data further suggests that in patients who have previously failed anti-TNF therapy, the 10mg BD maintenance dose is superior to the 5mg BD dosing[1]. Anecdotally, many clinicians are accordingly favouring continuing the 10mg bd dose in higher risk patients (biologic failures) for up to 52 weeks, rather than dropping to the maintenance dose at week 8.

Inevitably, our early experience is in a largely anti-TNF and vedolizumab experienced cohort; nonetheless, response rates appear to be favourable. The decision to dose reduce these patients must be carefully considered, particularly in view of their previous biologic failures and hence limited options should they fail tofacitinib. Recent extension studies from the original clinical trials have shown that only 64.9% and 49.1% of patients who flare after dose de-escalation are able to recapture response and remission, respectively, on dose re-escalation[2].

The risk of flare with dose reduction needs to be balanced against the potential risk of continuing at the 10mg bd dose. There is a clear dose response in terms of the risk of herpes zoster[1,3,4], and recent communication from a trial of patients with rheumatoid arthritis has suggested that there may be other safety signals in the form of greater risk of venous thromboembolism with the higher dose[2].

Therefore, given that many patients who are receiving tofacitinib will be biologic-experienced, there is an urgent need to identify biomarkers that might predict those more likely to flare should they dose reduce. Such biomarkers would enable us to risk stratify our patients at the point of consideration of dose reduction, to enable assessment of the risk and benefit of dose reduction versus continuation at the higher dose. By extension, predictors would enable us to maintain higher dosing in those more likely to flare with dose reduction, justifying the additional risk. To the best of our knowledge, there is neither any data already in existence that addresses this field, nor are there any ongoing trials. As IBD enters the era of personalised/precision medicine, gaining a deeper understanding of the best use of our most efficacious agents, such as tofacitinib, appears an essential endeavour.

This study aims to explore 3 novel areas for predictive biomarkers, in addition to assessing the predictive capability of baseline (defined here as the point of dose reduction) clinical, endoscopic and histologic markers. We hypothesize that patients who relapse following dose reduction to maintenance dose tofacitinib will have changes in cytokine expression of their pro-inflammatory effector T cells in peripheral blood mononuclear cells (PBMCs), increased

STAT activation in PBMCs, and possibly signature genes in their colonic tissue that may predict relapse. Accordingly, we aim to address 3 novel questions:

1. Is there increased activation of STAT proteins in peripheral blood in patients who relapse?

The direct molecular targets of cytokine-induced JAK tyrosine kinases are the signal transducers and activators of transcription (STAT) proteins, which when phosphorylated become activated and translocate to the nucleus to bind DNA and induce cytokine specific gene expression and chromatin regulation[5]. STAT proteins are key molecular regulators of CD4-T-helper (Th) cell lineage differentiation programmes, which are directed and propagated following specific signature cytokine cues[6]. Aberrant activation of STATs is, thus, often a critical step in the molecular pathogenesis of several inflammatory and auto-immune diseases, that are associated with dysregulated Th function[7,8]. Of the 7 STAT proteins, increased activation of STAT1, STAT3, and STAT5a/b in particular, and to a lesser extent STAT4 and STAT6, have been associated with the pathophysiology of UC, consistent with their activation by inflammatory cytokines involved in UC pathogenesis, such as IFNγ, IL12 (TH1), IL4, IL6, IL13, IL21 (TH2), IL6, IL23 (TH17), IL9 (TH9)[9-13]. Therefore, we will monitor the phosphorylation of STAT proteins in PBMC from patients enrolled in the study, with blood drawn before dose reduction.

2: Are there changes in cytokine expression in peripheral T cells that predict relapse?

The immunopathogenesis of IBD, including UC, is driven by the dysregulated expression of specific proinflammatory cytokines, secreted primarily by CD4 T-effector cells, which critically drives inflammation and tissue destruction[14]. These cytokines mediate their actions via STAT proteins, which are the subject of evaluation in question 1 above. To understand if there are changes in cytokine expression that predict relapse in specific patients, we will evaluate cytokine expression in peripheral CD4 T cells. The cytokines we will study are the key CD4 T-effector cytokines IFNγ, IL17, and IL9 corresponding to TH1, Th17 and Th9 lineages, that have been implicated in the pathogenesis of UC. These data will inform us of any changes in peripheral levels of pathogenic cytokines that predict patients who will relapse following tofacitinib dose reduction.

3: Can we identify signature gene(s) that serve as a biomarker to predict relapse?

Changes in immune homeostasis that predict relapse following dose reduction are most likely to be identified at the site of inflammation. The distinctive molecular changes that underlie the loss of immune homeostasis prior to relapse can be identified by studying gene expression changes in colonic tissue, as has previously been done to predict response to infliximab treatment[15]. We wish to compare gene expression changes between patients who remain symptomless on the lower dose of tofacitinib with those patients who ultimately show clear clinical signs of relapse. We expect that the differential gene expression (DEG) list may contain negative regulators of cytokine signaling (SOCS proteins) and/or inhibitors of STAT proteins (PIAS proteins), whose activity critically regulates the functional activation of STAT proteins and downstream cytokine induced gene expression. These studies will identify genes that can be used as biomarkers for relapse following tofacitinib dose decrease and also help in identifying genes that can be more rapidly screened in blood for the purpose of predicting relapse in patients in the clinic.

4: Using immune monitoring of the mucosal immune system to identify biomarkers of relapse and remission.

We have the opportunity to systematically collect tissue from patients with UC treated with tofacitinib, as such we will undertake to track the mucosal immune response by multiparametric flow-cytometry study on endoscopic biopsies of the 50 patients within the BRITE study. These biopsies will be excess to these required to undertake the agreed experiments as part of the original study proposal – hence this proposal is of added benefit and will not detract from the funded study. This will facilitate flow cytometric immune monitoring of the architecture of the mucosal immune system (T cells, B cells and the myeloid compartment) at the point of de-escalation. Patients will subsequently be followed to relapse or the end of the study, whichever comes first, and tissue resampled to investigate the longitudinal changes in immunophenotype in the context of response to therapy. Similar immune monitoring has proved crucial at defining immune signatures in SARS-CoV-2 infection (15). This study is complementary to the work being performed in the original BRITE proposal and will provide more information on the effect of the drug on immunophenotypes and identify immunological signatures of response and or relapse – allowing a precision approach to drug selection.

6 Trial objectives and purpose

This is an investigator-initiated study with an overarching objective to identify biochemical and genetic markers of patients more likely to flare after tofacitinib dose reduction. It is hoped that this will eventually allow us to target continued higher dosing of 10mg bd at those patients who are most likely to benefit from it and avoid its use in patients in whom it is potentially unnecessary. Thereby, it would enable using the drug in a safer, more effective and more economical manner.

Primary objective:

- To identify biochemical, histologic and genetic markers predictive of ulcerative colitis flare in patients on tofacitinib commencing on maintenance 5mg BD dosing.

Secondary objectives:

- To identify clinical characteristics predictive of ulcerative colitis flare in patients on tofacitinib commencing on maintenance 5mg BD dosing.
- To identify predictors of endoscopic activity at week 44

7 Study design & Flowchart

7.1 Study Design

This is a prospective observational study. We will prospectively enroll a cohort of 50 UC patients who have achieved an adequate response to tofacitinib at a dose of 10mg bd and whose dose is being reduced to 5mg bd following a routine flexible sigmoidoscopy. Colonic

tissue and blood samples will be collected at study entry, and patients' disease course will be followed-up through routine care.

Week 44 has been chosen for the primary endpoint, as 44 weeks post dose de-escalation was used as the endpoint in OCTAVE Sustain.

Primary endpoint:

- Biomarkers, clinical markers and histological markers associated with ongoing treatment with 5mg bd in the absence of steroids at week 44.

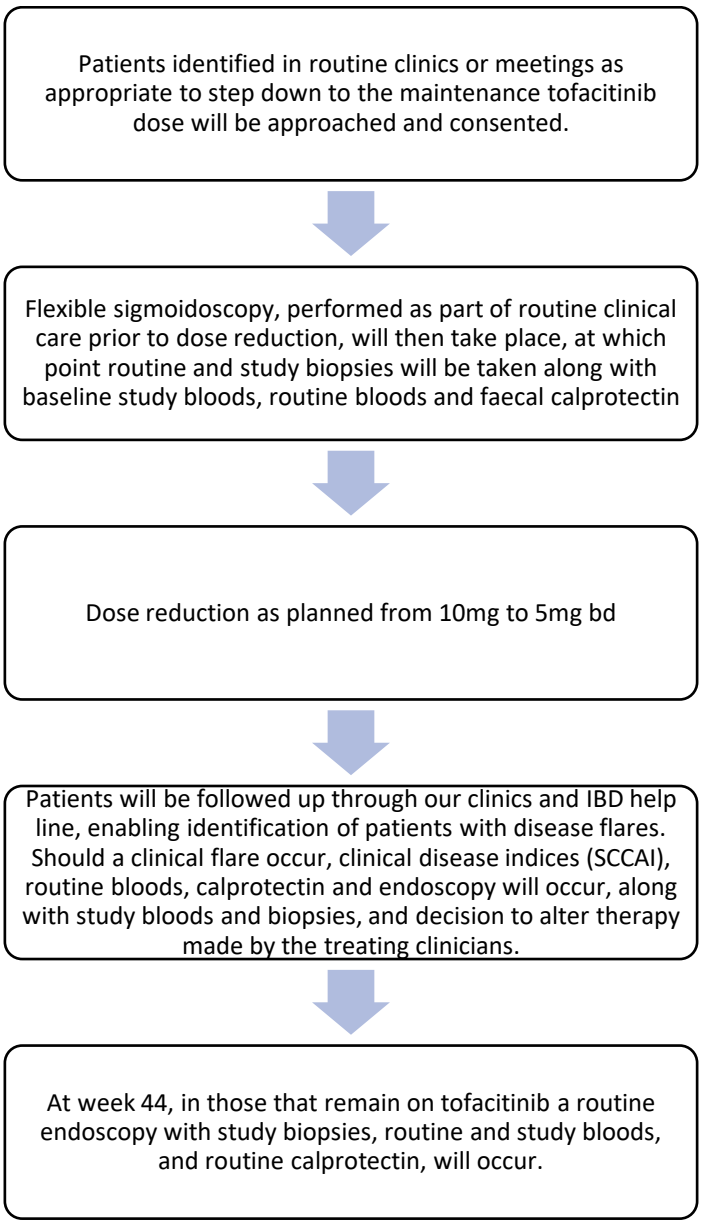
Secondary Endpoints

- Biomarkers, clinical markers and histological markers associated with steroid free endoscopic remission at routine endoscopy at week 44

Biomarkers / outcome measures will include:

- Clinical status (SCCAI)
- CRP and faecal calprotectin (as part of routine care)
- Histological markers of disease activity (Nancy histological index) (as part of routine care)
- STAT activation in peripheral blood mononuclear cells
- Cytokine expression in peripheral T cells
- Gene signatures in colonic biopsies

Flow Diagram



7.2 Flowchart

	Screening	Baseline Endoscopy	Flare	Week 44
Study information provided and information sheet mailed. Inclusion/exclusion criteria reviewed.	X			
Patient information and informed consent completed.		X		
Collection of demographic and baseline clinical data		X		
Collection of clinical activity score (SCCAI)	X	X	X	X
Routine endoscopic assessment and collection of tissue for routine histology		X	X	X
Collection of research blood and tissue samples		X	x	X
Collection of clinical data, routine blood tests and faecal calprotectin		X	X	X

8 Subject selection

Adult patients with ulcerative colitis on tofacitinib 10mg BD who are having their tofacitinib reduced to the maintenance dosing of 5mg BD in view of adequate response will be eligible for recruitment. The patients will be identified from Guy's and St Thomas' cohort of ulcerative colitis patients receiving tofacitinib. We expect there will be approximately 50 patients per year eligible for this trial. Given that the only difference to usual care is the additional blood and tissue samples collected at baseline and flare or week 44, we expect most patients would agree to this study.

8.1 Subject inclusion criteria

1. Aged 18 years or over, either male or female
2. A history of ulcerative colitis, as defined by standard clinical criteria
3. Patients who are having their tofacitinib dose reduced from 10mg BD to 5mg BD in view of adequate response, as decided by their treating clinical team, and are undergoing routine endoscopy.

4. Sufficient English language skills to understand the patient information sheet and consent form.

8.2 Subject exclusion criteria (with justification if necessary – for example consider contra-indications to trial treatments, incompatible concurrent treatments, recent involvement in other research)

1. Patients undergoing dose reduction for reasons other than adequate response to 10mg bd (ie. active infection or VTE risk).
2. Patients being dose reduced without undergoing endoscopy

9 Study procedures

9.1 Subject recruitment

- Patients who have clinically responded to 10mg BD tofacitinib, are approaching their routine endoscopy and who are being considered for dose reduction by their treating team, will be identified.
- Potential participants will most likely be identified from our database of patients currently on tofacitinib but could be identified by any member of the multidisciplinary direct care team, including registrars, clinical research fellows, consultants, as well as clinical nurse specialists, IBD research nurses or pharmacists. Potential participants could be identified at outpatient clinics, or during our weekly multidisciplinary meeting (Virtual biologics and immunosuppressant clinic).
- Prior to their routine endoscopy, potential participants will be approached via telephone or during a clinic consultation. An information sheet and consent form will be given to them or sent by post or via e-mail. Patients will be allowed at least 48 hours to decide if they wish to consent to participate, although patients can be consent earlier than 48 hours if they wish. Consent may also be obtained on the day of endoscopy from one of the study's medical practitioners.
- There will be no payment to participants for their involvement in the study

9.2 Screening Procedures

- Screening phone call:
    - Inclusion/exclusion criteria reviewed
    - Information provided on the study, and information sheet and informed consent form sent to patient in the mail or via e-mail.

9.3 Schedule of Assessment for each visit

Patients in whom the treating team is planning to reduce their Tofacitinib from 10mg BD to 5mg will be referred for consideration of inclusion in the study.

1. Screening phone call
    a. Information regarding the study provided
    b. Inclusion/exclusion criteria reviewed
    c. Patient information sheet, and consent form sent to patient in the mail or via e-mail.
2. Routine baseline endoscopy
    a. Informed consent gained and form signed, prior to the endoscopy.
    b. Baseline clinical data collected
    c. Endoscopy performed and biopsies taken from rectum and sigmoid, to be sent for:

i. Histological assessment of disease activity (Nancy Histological Index)
ii. Genetic analysis
iii. Future exploratory analysis

d. Blood collection, to be sent for:
i. Routine clinical bloods (FBC, CRP, LFTs, U&E)
ii. STAT activation in peripheral blood mononuclear cells
iii. Cytokine expression in peripheral T cells

e. Faecal calprotectin submitted

3. Flare (SCCAI ≥ 5)
a. Should a clinical flare occur, patients will be encouraged to contact our IBD advice line as per current standard of care.
b. Routine and study blood tests, stool, and endoscopic tests will be initiated, as per standard of care, and the decision on how to manage the clinical flare will be taken by the treating team. Additional study biopsies will be taken at the time of the endoscopy.

4. Routine clinical visits
a. These will occur as per the treating team's discretion, with testing performed accordingly.

5. Week 44
a. In patients who remain on tofacitinib at week 44, routine clinical assessment will occur.
b. Tests performed will be
i. Routine endoscopy and biopsies, sent for:
1. Histological assessment of disease activity (Nancy index) (part of routine care)
ii. Study biopsies, sent for:
1. Genetic analysis
2. Future exploratory analysis
iii. Routine blood collection, to be sent for:
1. Routine clinical bloods (FBC, CRP, LFTs, U&E)
2. Study blood tests will also be taken at this time point
iv. Routine faecal calprotectin

9.4 Follow up Procedures

See 9.5

9.5 Radiology Assessments

There are no planned radiology assessments as part of this study. While they may occur at the treating team's decision as part of the care of the patient, they will not be associated with this study.

9.6 End of Study Definition

The end of the trial will be deemed as date of final database lock and completion of analysis of laboratory samples collected from participants.

10 Laboratories

Routine tests including blood tests (FBC, CRP, U&E, LFTs), and faecal calprotectin, will be performed as per standard of care at baseline, week 44, and at the treating team's discretion.
The Nancy Histological Index will be used to score histological disease activity, as per standard of care, at baseline and week 44.
Study specific tests of STAT activation, peripheral T cell cytokine expression, and genetic signature exploration will occur at baseline and flare or week 44.

10.1 Central/Local Laboratories

Routine blood and histological tests will be performed in the ViaPath lab at Guy's and St Thomas' hospitals. Faecal calprotectin will be performed at King's College Hospital.
The STAT activation, cytokine expression and gene expression studies on blood and biopsy samples collected will be conducted in Dr. Susan John's and Dr. Robin Dart's labs in the Dept of Immunobiology, SIMS, 2nd and 3rd Floor Borough Wing, Guy's Hospital, KCL.

10.2 Sample Collection/Labelling/Logging

Biopsy samples will be taken by the endoscopist at the time of flexible sigmoidoscopy. Participants will have been recruited to the study prior to the procedure taking place and as part of that consent process will have had the chance to consider whether donating biopsy samples is acceptable to them. They will also be consented separately by the endoscopy team for the flexible-sigmoidoscopy procedure and biopsy sample acquisition (to be used as part of standard care, rather than research).
Samples will be taken from the rectum and sigmoid. They will be treated, transported and stored in accordance with HTA regulations. The histology specimens will be stored as per standard of care at GSTT. Biopsy samples for genetic analysis will be stored frozen in a laboratory in the Dept. of Immunobiology, SIMS, 3rd Floor Borough Wing, Guy's Hospital, KCL. The custodians will be and Dr. Susan John and Dr. Peter Irving.
Baseline blood tests will be collected by a practitioner qualified in this technique. Samples will be treated, transported and stored in accordance with HTA regulations in the Dept of Immunobiology, SIMS, 3rd Floor Borough Wing, Guy's Hospital, KCL. The custodians will be Dr. Susan John and Dr. Peter Irving.
All routine samples will be labelled at the point of collection as per hospital standards. Study samples will be labelled with patients' study number only. A record of all samples will be made at the point of collection and upon arrival at the laboratory.
None of these samples will be stored beyond the end of the study.

10.3 Sample Analysis Procedures

Standard lab processes will occur for the analysis of routine blood, faecal and histological tests.
STAT activation in peripheral T cells will be assessed by phospho-flow FACS analysis of PBMC using anti-phospho-tyrosine-STAT and anti-CD4, anti-CD3 and CD4 T-effector subset specific antibodies, according to standard protocols routinely used in our laboratory[16]. If

required, T cells maybe purified using magnetic bead isolation, prior to phospho-flow analysis. To confirm the results from the phospho-flow analysis and complement these studies, western blot analysis will be performed on a limited number of T cell samples to detect and quantify phosphorylated and total STAT protein levels. These are well established standard methods for detecting STAT activation, routinely in use in our laboratory.

We will evaluate cytokine expression in peripheral CD4 T cells following PMA/ionomycin stimulation for 4hrs, in the presence of GolgiStop (to prevent cytokine secretion), followed by intracellular cytokine staining and FACS analysis to detect specific cytokine expression. The supernatants from these cultures will be harvested and assessed for secreted cytokine expression using IL17, IFNγ and IL9-specific ELISA assays. Additionally, we will perform a multiplex cytokine array using the Th1/Th2/Th9/Th17/Th22/Treg ProcartaPlex Human Cytokine Array (Thermo Fisher Scientific) of patient plasma (frozen at -80C, upon isolation) to complement the FACS studies above. These are standard methods for detecting cytokine expression, routinely in use in our laboratory.

For genetic studies, RNA will be prepared using Qiaprep microRNA kit, then quantified on a Qubit instrument and 2ug RNA will be sent for library preparation and RNA-sequencing using commercial genomic services at Novogene. Briefly, mRNA is purified from total RNA using poly-T oligo-attached magnetic beads, then isolated mRNA is fragmented, reverse transcribed and stranded libraries will be prepared and sequenced using the Illumina platform to generate at least 20M reads per sample. Bioinformatic analysis of the RNA sequencing data will be carried out at Novogene as part of the service package and will provide comprehensive gene expression analysis. Unsupervised and supervised clustering analysis will be performed to provide a high-level view of the differences between the two groups analysed and to produce a differential gene expression (DEG) list. Enrichment analysis of the DEGs will be performed using the clusterProfiler software for enrichment analysis, including GO Enrichment, DO Enrichment, KEGG and Reactome database, to predict biological functions or pathways are significantly associated with DEGs. The top 5-10 genes from the DEG list will be validated by quantitative real-time PCR using RNA from mucosal tissue and also from PBMC and T cells isolated from peripheral blood.

In addition, obtained biopsies will be defrosted and processed either by:

1. A whole tissue digestion protocol
2. Placed in culture to allow crawl out of lymphocytes as previous described (Di Marco Barros Cell 2016)
3.

The resulting single cell suspension will either be analysed by flow cytometry (in the presence/absence of stimulation) or processed for transcriptomic analysis. Further, a single biopsy will be placed into RNAlater and subsequent RNA/DNA extraction performed. The resulting material will be saved for genotyping, gene expression analysis and T/B cell receptor analysis.

10.4 Sample Storage Procedures

Plasma samples will be stored in a -80 degrees Celsius upright freezer. Biopsy samples will be stored in a 4 degrees Celsius fridge for short term storage prior to processing, and RNA extracted from these samples will be frozen in a -80 degrees Celsius upright freezer. PBMC samples will be stored in a temperature monitored nitrogen vapour tank (≤-175 degrees

Celsius). All storage units are located in the Dept. of Immunobiology, SIMS, 3rd Floor Borough Wing, Guy's Hospital, KCL.

10.5 Data Recording/Reporting

The Chief Investigator will act as custodian for the trial data. The following guidelines will be strictly adhered to:

- Data will be kept in site files/trial master file and will be stored and protected to the standards described in GSTFT and King's College London Information Security Policy and the Data Protection Act.
- Data will be accessed by named researchers only, on password protected, encrypted computers. If data needs to be transferred between the two hospital sites of the same trust (Guy's and St Thomas' Hospitals), it will be on encrypted media and GSTT laptops. Data will be transferred via secure GSTFT email to other GSTFT email.
- If written communication between researchers is required between GSTFT and KCL, secure email with NHS.net accounts to NHS.net accounts will be used.
- Data will be accessed by named researchers only, on password protected, encrypted computers. If data needs to be transferred between the two hospital sites (Guy's and St Thomas' Hospitals), it will be on encrypted media and GSTT laptops. If written communication between researchers is required, secure GSTFT email will be used to other GSTFT email, or NHS.net accounts to NHS.net accounts.
- Data will be pseudoanonymised. A unique study ID will be given to each participant for use in the research database. A password protected Microsoft Excel file will be used to create a database. Access to the secure file linking study ID with personal details will be held on a separate, password-protected NHS computer on an NHS server.
- All study data will be kept in a secure office for clinical research staff at Guy's & St Thomas' Hospital. Only members of the medical and research team with the adequate security clearance will have access to the office. They will also be kept as safe as possible from other damage such as fire or water damage.

10.6 Sample Receipt/Chain of Custody/Accountability

- Study samples will be labelled with patients' study number only at the point of collection, and transported to the laboratory via standard internal measures.
- A record of all samples will be made at the point of collection and upon arrival at the laboratory.
- Upon arrival to the laboratory the physical integrity of the sample will be assessed. If this has been compromised, the study teams and sponsor will be informed.

10.7 Sample Transfer to sites outside the Organisation

In the study, blood and colonic tissue will be collected from patients in accordance with the patient consent form and patient information sheet and shall include all tissue samples or other biological materials and any derivatives, portions, progeny or improvements as well as all patient information and documentation supplied in relation to them. Further, the custodian of the Materials and use the Materials for the Study only. For the avoidance of doubt Recipient shall only use the Material in accordance with the consent provided by the Study

Donors (if applicable) and shall always use the Materials with dignity and respect and shall always use good laboratory practice in handling Materials.

The blood and colonic tissue will be appropriately sent to Dr Susan John, dept. of Immunobiology, SIMS, 3rd Floor Borough Wing, Guy's Hospital, KCL for STAT activation, cytokine analysis, and genetic analysis of the samples to be carried out in accordance with the analytical plan agreed with the Chief Investigator.

Dr Susan John, dept. of Immunobiology, SIMS, 3rd Floor Borough Wing, Guy's Hospital, KCL will process, store and dispose of blood and colonic tissue in accordance with all applicable legal and regulatory requirements, including the Human Tissue Act 2004 and any amendments thereto. While Provider uses reasonable endeavours to ensure the quality of the Materials, the Materials are provided 'as is' and it makes no representation and gives no warranty or undertaking in relation to the Materials, including but not limited to its safety, fitness for purpose or use of any kind.

The blood and colonic tissue will not be transferred to any party not identified in this protocol and are not to be processed and/or transferred other than in accordance with the patients' consent. After ethics approval for the study has expired, the blood and colonic tissue will be disposed of in accordance with the Human Tissue Act 2004, and any amendments thereto, or transferred to a licensed tissue bank.

Stool samples will be collected by the patient, given to a member of the direct care team and will be sent to Pathology services (Viapath) at St Thomas' Hospital. This is required as part of standard of care and no additional specimens are needed for this study.

11 Assessment of Safety

General safety assessments will be made as part of each clinical assessment, as per standard of care by the treating team. These will include clinical history and examination, blood tests, faecal tests, endoscopy and imaging, at the treating team's discretion. Specifically, disease flare, complications of immunosuppression (ie. Infection or malignancy) and specific tofacitinib side effects (ie. Vascular thromboembolism) will be observed for. As per standard of care, patients will be informed about potential adverse events and encouraged to contact our helpline email address should concern arise.

GSTT is the Sponsor and KCL is a co-sponsor, and the SAE is related (that is, it resulted from administration of any of the research procedures), to the study procedures or is an unexpected occurrence (that is, the type of event is not listed in the protocol as an expected occurrence) then it must be reported immediately upon knowledge of the event to R&D and always within 24 hours. For all other AEs these must be reported to GSTT when copied into the Annual Progress Report.

Important Medical Events (IME) & Pregnancy

Events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the patient or may require intervention to prevent one of the other outcomes listed in the definition above should also be considered serious.

Although not a serious adverse event, any unplanned pregnancy will also be reported via the SAE reporting system.

11.1.1 Reporting Responsibilities

Reports of related and unexpected SAEs will be submitted to the Main REC within 15 days of the chief investigator becoming aware of the event, using the NRES template. The form will be completed in typescript and signed by the chief investigator so that the main REC can acknowledge receipt of safety reports within 30 days. A copy of the SAE notification and acknowledgement receipt will be sent to the R&D Directorate. Disease flare requiring treatment is an expected event and will not need to be reported as an SAE.

11.2 Trial Steering Committee (if applicable)

Not applicable. There will not be any data monitoring/steering/safety committees set up for this study.

11.3 Ethics & Regulatory Approvals

This protocol and related documents have been reviewed and approved by the Research Ethics Committee (_____).

12 Compliance and withdrawal

12.1 Subject compliance

No specific compliance testing will be carried out.

12.2 Withdrawal / dropout of subjects

Participants have the right to withdraw from the study at any time for any reason. The investigator also has the right to withdraw patients from the study drug in the event of inter-current illness, AEs, SAE's, SUSAR's, protocol violations, cure, administrative reasons or other reasons. It is understood by all concerned that an excessive rate of withdrawals can render the study un-interpretable; therefore, unnecessary withdrawal of patients should be avoided. Should a patient decide to withdraw from the study, all efforts will be made to report the reason for withdrawal as thoroughly as possible. Because this is a non-interventional trial there won't be an interim analysis or premature termination of the study.

12.3 Protocol Compliance

Protocol violations will be documented on the protocol deviation log (in the trial master file) and reported by the chief investigator to the sponsor by email.

13 Data

13.1 Data to be collected

Data will be collected by the study investigators.

Data at the various time points include:

- Baseline:
    - Demographic data, disease extent history, concomitant medications, previous IBD medications, smoking history from patient history and notes.
    - Simple Clinical Colitis Activity Index (SCCAI), calculated by the assessor
    - Serum CRP and albumin, and faecal calprotectin
    - Endoscopic Mayo Score, recorded by the endoscopist
    - Nancy histological index, reported by the pathologist
    - STAT activation in peripheral blood mononuclear cells, recorded from laboratory data
    - Cytokine expression in peripheral T cells, recorded from laboratory data
    - Gene and T/B cell receptor analysis in colonic biopsies, recorded from laboratory data
- Flare
    - Time point, alteration to treatment, faecal calprotectin, CRP, SCCAI, endoscopic Mayo score (if endoscopy performed), all recorded by the investigator from the patient notes.
    - Gene signatures in colonic biopsies, recorded from laboratory data
- Week 44
    - CRP, albumin, SCCAI, calprotectin, recorded from the patient's notes.
    - Endoscopic Mayo score, recorded by the endoscopist
    - Nancy histological index, reported by the pathologist
    - STAT activation in peripheral blood mononuclear cells, recorded from laboratory data
    - Cytokine expression in peripheral T cells, recorded from laboratory data
    - Gene signatures in colonic biopsies, recorded from laboratory data

In the event of incomplete data, patients will be telephoned to arrange completion.

13.2 Data handling and record keeping

Pseudo-anonymised patient data will be kept on a password-locked computer. Every patient will be appointed a two-digit study number. All patient specific data will be recorded using only this number. The full name and birth date will only be recorded on the informed consent form. The study coordinator will monitor patient inclusion and protocol steps, coordinate data entry, perform data analyses and reporting. Data will be double entered into a Microsoft Excel spreadsheet to endure validity and quality.

All trial data will be stored in line with the Data Protection Act (1998). Records will be retained for a maximum of 7 years.

14 Statistical considerations

14.1 Sample size calculation (some pilot/feasibility studies may not require a formal sample size calculation)

Given the endpoints are exploratory, sample size calculations are not possible.

14.2 Statistical analysis

Dr Sailish Honap, clinical research fellow in gastroenterology, will perform the statistical analyses.

Baseline variables will be presented as either mean and standard deviation or medians and inter quartile range, depending on the normality of the data.
Receiver operator curves will be generated to determine optimal cut-offs for continues variables, predictive of disease flare.

Kaplan-Meier curves will be generated to allow comparisons of subdivisions of patients based on genetic and biochemical variables, using determined cut-offs.

Univariate and Multivariate analyses will be performed on clinical, biochemical and genetic variables to establish predictors of disease flare.

15 Ethical considerations

This is a non-interventional, observational study. Approvals will be sought from REC and HRA. Patients will need to provide informed consent to participate, and the only data collected from patients which occur in addition to standard of care are the additional tissue and blood samples collected at baseline and at week 44 for genetic, immune cell and cytokine analyses. Decisions regarding patient's care will have no relation to the study and will be directed by the patient's care team. Data collection and monitoring will occur in accordance with the rules and regulations set out above. The study design, management, undertaking, analysis and dissemination of results have had patient involvement, via a patient representative who sits on our project approvals board.

16 Financing and Insurance

Guy's and St Thomas' NHS Foundation Trust will take primary responsibility for ensuring that the design of the study meets appropriate standards and that arrangements are in place to ensure appropriate conduct and reporting are adhered to. King's College London as co-sponsor also provides cover under its No Fault Compensation Insurance, which provides for payment of damages or compensation in respect of any claim made by a research subject for bodily injury arising out of participation in a clinical trial or healthy volunteer study (with certain restrictions).

17 Reporting and dissemination

It is intended that the results of the study will be reported and disseminated at international conferences and in peer-reviewed scientific journals. A manuscript of the final study results will be submitted to an IBD specific journal (IBD or JCC) or to the IBD section of a general gastroenterology journal (Gut, Gastroenterology or CGH).

18 Useful reading/websites

Integrated Research Application System (IRAS)
https://www.myresearchproject.org.uk/
Health Research Authority (HRA)
www.hra.nhs.uk

HRA Guidance for Patient Information Sheet and Informed Consent
http://www.hra.nhs.uk/research-community/before-you-apply/participant-information-sheets-and-informed-consent/

CONSORT statement
A set of recommendations for improving the quality of reports of parallel group randomised trials
http://www.consort-statement.org/

ICH Harmonised Tripartite Guidelines for Good Clinical Practice (1996)
http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E6/E6_R1_Guideline.pdf

Martin Bland et al, Statistical guide for research grant applications
http://www-users.york.ac.uk/~mb55/guide/guide.htm
Includes detailed information and definitions of many aspects required for a research protocol as well as information about randomisation software and services

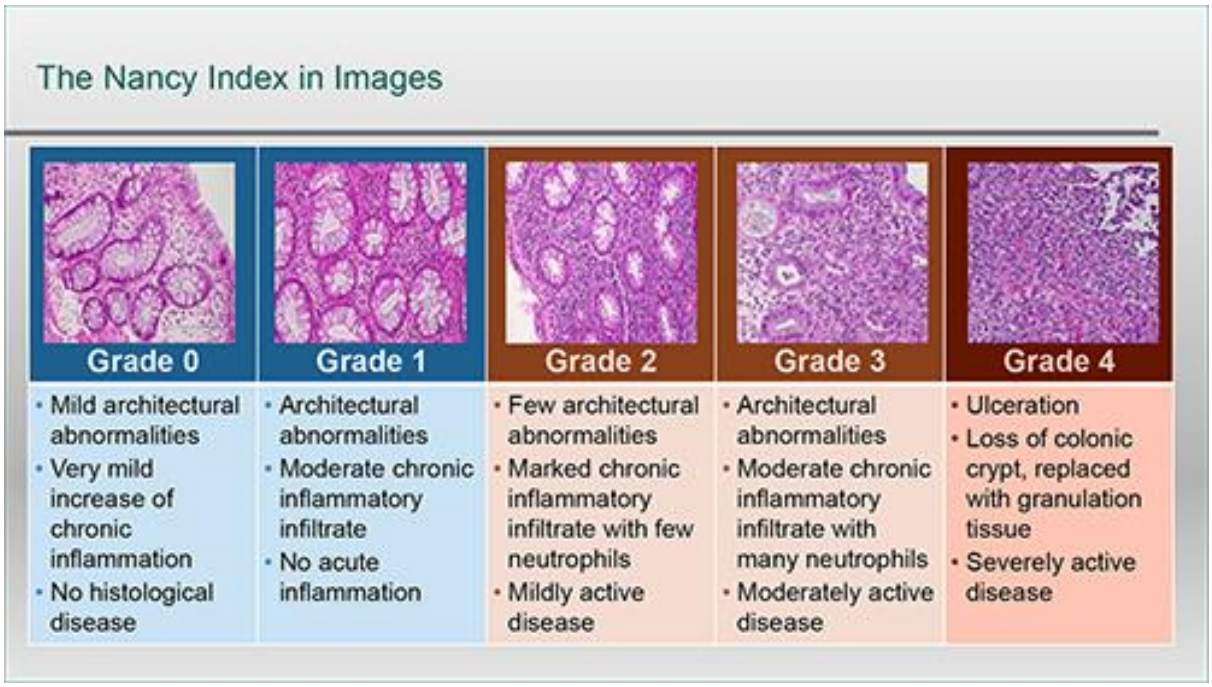
Martin Bland, Directory of randomisation software and services
http://www-users.york.ac.uk/~mb55/guide/randsery.htm

Declaration of Helsinki
(http://www.wma.net/en/30publications/10policies/b3/index.html)

19 Appendix 1 – Information with regards to Safety Reporting in Non-CTIMP Research

	Who	When	How	To Whom
SAE	Chief Investigator	-Report to Sponsor within 24 hours of learning of the event -Report to the MREC within 15 days of learning of the event	SAE Report form for Non-CTIMPs, available from NRES website.	Sponsor and MREC
Urgent Safety Measures	Chief Investigator	Contact the Sponsor and MREC Immediately Within 3 days	By phone Substantial amendment form giving notice in writing setting out the reasons for the urgent safety measures and the plan for future action.	Main REC and Sponsor Main REC with a copy also sent to the sponsor. The MREC will acknowledge this within 30 days of receipt.
Progress Reports	Chief Investigator	Annually (starting 12 months after the date of favourable opinion)	Annual Progress Report Form (non-CTIMPs) available from the NRES website	Main REC
Declaration of the conclusion or early termination of the study	Chief Investigator	Within 90 days (conclusion) Within 15 days (early termination) *The end of study should be defined in the protocol*	End of Study Declaration form available from the NRES website	Main REC with a copy to be sent to the sponsor
Summary of final Report	Chief Investigator	Within one year of conclusion of the Research	No Standard Format However, the following Information should be included:- Where the study has met its objectives, the main findings and arrangements for publication or dissemination including feedback to participants	Main REC with a copy to be sent to the sponsor

20 Appendix 2 – Nancy Histological Index



Source: https://curricula.peervoice.com/ibd

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