PERSONALISED ANTI-TNF THERAPY IN CROHN’S DISEASE

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PROTOCOL FULL TITLE:

INVESTIGATION OF THE CLINICAL, SEROLOGICAL AND GENETIC FACTORS THAT DETERMINE PRIMARY NON-RESPONSE, LOSS OF RESPONSE AND ADVERSE DRUG REACTIONS TO ANT-TNF DRUGS IN PATIENTS WITH ACTIVE LUMINAL CROHN'S DISEASE

Protocol Short Title/Acronym

PERSONALISED ANTI-TNF THERAPY IN CROHN'S DISEASE / PANTS

Trial Identifiers

REC Number 12/SW/0323

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1. Study Synopsis

Version 6 20th May 2016
<table>
<thead>
<tr>
<th>Title of Research Study</th>
<th>INVESTIGATION OF THE CLINICAL, SEROLOGICAL AND GENETIC FACTORS THAT DETERMINE PRIMARY NON-RESPONSE, LOSS OF RESPONSE AND ADVERSE DRUG REACTIONS TO ANTI-TNF DRUGS IN PATIENTS WITH ACTIVE LUMINAL CROHN'S DISEASE.</th>
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<tr>
<td>Protocol Short Title/Acrylon</td>
<td>Personalised Anti-TNF therapy in Crohn's disease/PANTS</td>
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<td>Study Phase if not mentioned in title</td>
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<td>Sponsor name</td>
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<tr>
<td>Chief Investigator</td>
<td>Dr Tariq Ahmad</td>
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<tr>
<td>REC number</td>
<td>12/SW/0323</td>
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<tr>
<td>Medical condition or disease under investigation</td>
<td>Patients with active luminal Crohn's disease who require treatment with anti-TNF drugs, Infliximab or Adalimumab.</td>
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<td>Purpose of clinical trial</td>
<td>To develop a cost-effective, individualised anti-TNF treatment strategy for patients with Crohn's disease which maximizes benefit and minimises harm.</td>
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<td>Primary objective</td>
<td>The primary objective of this study is to investigate the mechanisms that underlie primary non-response (PNR), loss of response (LOR) and adverse drug reactions (ADRs) to anti-TNF drugs in patients with active luminal Crohn's disease.</td>
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<td>Secondary objective(s)</td>
<td>The secondary objectives are to identify: a) clinically meaningful serological and genetic markers that predict PNR, LOR and ADR b) clinical, biochemical and genetic predictors of durable clinical remission after anti-TNF withdrawal c) To report the initial UK experience of Biosimilar Infliximab (Remsima™ and Inflectra™) including efficacy, safety and pharmacokinetics using a prospective open labelled study design.</td>
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<td>Study Design</td>
<td>Prospective uncontrolled cohort study.</td>
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<td>Sample Size</td>
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<td>Summary of eligibility criteria</td>
<td>All major criteria listed must be met</td>
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<td>Version and date of final protocol</td>
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2. Background & Rationale

The introduction of drugs directed against tumour necrosis factor alpha (anti-TNFα monoclonal antibodies) has greatly advanced treatment of IBD. Infliximab (IFX), a chimeric human-murine monoclonal antibody, and Adalimumab (ADA), a recombinant human monoclonal antibody, are effective in severe Crohn’s disease (CD) refractory to conventional therapies, including immunosuppressive drugs. Clinical efficacy is associated with mucosal healing and improved quality of life. Anti-TNF drugs have had a major impact on important disease parameters such as a reduction in hospital admissions and operations. Recent data suggests that early aggressive therapy with anti-TNF drugs may achieve mucosal healing, reduce the need for surgery, and therefore may alter disease natural history. However anti-TNF drugs are expensive, costing £10,000 - £15,000 per patient per year. Furthermore 10-40% of patients fail to respond to 10-12 weeks of therapy (primary non-response - PNR), and a further 23-46% lose response (loss of response – LOR) after 12 months of treatment. Other patients experience unpredictable drug side effects such as infusion reactions, opportunistic infections, cancers (in particular lymphoma and the rare hepatosplenic T-cell lymphoma) and disorders of the nervous system including demyelination. A recent meta-analysis of all published anti TNF drug trials in Crohn’s disease estimated the “number needed to treat” to induce remission to be 9. Furthermore treatment early in disease course risks exposing about 30% of patients, destined to run a quiescent course, to toxicity without benefit. An ability to predict which patients are unlikely to benefit (PNR), which patients are at risk of developing adverse drug reactions (ADRs) and which patients are likely to lose response (LOR) will allow these drugs to be used in a safer, more cost-effective manner, tailored to the individual patient.

Primary non-response (PNR) refers to the situation where a patient fails to respond to an initial anti-TNF induction regime, typically assessed after 10-12 weeks of treatment. In placebo controlled trials almost one third of patients with Crohn’s disease do not show a response. However, when selecting patients with active Crohn’s disease (assessed by inflammatory markers and/or lesion assessment) the PNR rate is consistently lower. Little is currently known about the factors that determine PNR, although data from clinical trials and single centre series have demonstrated that lower PNR rates are associated with the use of anti-TNF early in disease natural history (within 12 months of diagnosis), immunosuppressive co-treatment, younger age, colonic disease, non-smoking and elevated CRP. The relevance of other factors on PNR such as inadequate early dosing (low / absent serum drug trough levels), early immunogenicity, enhanced drug clearance, and the contribution of non-TNF driven inflammation, is not known. An understanding of possible genetic factors that determine PNR might provide insights into the underlying mechanisms of PNR and might facilitate the development of pre-treatment predictive tests to identify patients unlikely to respond. At the present time therapeutic options to treat moderate to severe Crohn’s disease are limited. Therefore identifying the 10% of patients who are unlikely to respond to anti-TNF therapy might not be a clinical priority and clinicians will continue to favour a trial of treatment. However, with the emergence of new effective therapies, it becomes increasingly important to be able to predict patients unlikely to respond to anti-TNF therapy.

Genetic factors determining PNR have been investigated in small retrospective candidate gene studies but associations have been weak and have failed to replicate in independent cohorts. Comprehensive unbiased techniques to survey the genome such as Genome wide association studies (GWAS) have not previously been used to investigate PNR.

Loss of response (LOR) to anti-TNF drugs poses a very significant challenge in the management of patients with Crohn’s disease. A number of definitions have been used, hampering the ability to estimate the magnitude of this problem. A recent review by Gisbert, defining LOR as the need to dose escalate, calculated an average annual risk of 13%. The majority of these patients lose response in the first year, giving a LOR rate of 23-46% at 12 months. Low drug trough levels predict LOR, although at the present time the clinical utility of measuring trough levels is not known. There are many causes of LOR and not all are due to active Crohn’s disease (e.g. fibrostenotic strictures, bile salt malabsorption, etc). Immunogenicity and anti-drug antibody formation is the most studied mechanism to explain LOR associated with persistent active Crohn’s disease. Up to 60% of patients receiving episodic IFX develop anti-IFX antibodies and such patients are more likely to develop LOR. Why some patients develop anti-drug antibodies and others do not is not known but genetic factors might be important. Immunogenicity does not explain all LOR episodes – in 10-60% of patients with LOR, low drug trough levels are documented without detectable drug antibodies. Whilst this might reflect the timing or technical aspects of the
antibody assays, it seems more likely that other mechanisms are contributing to LOR. Similar to PNR these include enhanced drug clearance, relentless consumption due to excessive TNF production, and a shift of the inflammatory process to non-TNF driven pathways.

Identifying which patients can safely withdraw anti-TNF therapy, and when to do so, remains a major challenge in IBD management. While data in general supports the relative safety of anti-TNF therapy, it remains an expensive treatment accounting for approximately two-thirds of all healthcare costs for people with CD. An ability to predict which patients will remain in clinical remission after anti-TNF withdrawal might allow the drug to be used in a safer, more cost effective manner. The STORI trial (infliximab diSconTinuation in CrOhn’s disease patients in stable Remission on combined therapy with Immunosuppressors) reported a 12 month relapse rate of 44% and identified gender, endoscopic activity, CRP level, haemoglobin and infliximab trough levels as independent predictors of relapse after drug withdrawal. However this study was relatively small with 115 patients, uncontrolled and is yet to be independently validated. Real-life UK experience suggests that many patients who fulfil the STORI favourable parameters for withdrawal are deemed unsuitable for withdrawal because of persistent disease activity and concerns over past disease behaviour. Prospective data from a large contemporary UK secondary care cohort might allow us to identify clinical, biochemical and genetic predictors of durable clinical remission after anti-TNF withdrawal.

In June 2013, the European Medicines Agency’s Committee for Medicinal Products for Human Use (CHMP) recommended the authorisation of Remsima™ and Inflectra™ (both manufactured by Celltrion) as biosimilar medicinal products containing infliximab. Biosimilar Infliximab became available in the UK in February 2015 offering significant savings to the National Health Service. Extensive non-clinical and clinical data have shown comparability with the reference product, Remicade™. However the clinical data to date has emerged from the use of Biosimilar Infliximab in studies of patients with ankylosing spondylitis and rheumatoid arthritis. The PANTS study will provide important post-authorisation data on efficacy (at week 14, 30 and 54), safety (to 3 years) and pharmacokinetics in patients with Crohn’s disease.

This is a prospective uncontrolled cohort study investigating primary non-response (PNR), loss of response (LOR) and adverse drug reactions (ADR) to IFX and ADA in patients with severe active luminal Crohn’s disease. The primary objective of this study is to investigate the mechanisms that underlie PNR, LOR, ADRs and remission after anti-TNF withdrawal. The secondary aims are to develop personalised anti-TNF treatment strategies, through the identification of clinically meaningful serological and genetic predictive markers.

This study builds on the achievements of the UK and international IBDGC in identifying IBD susceptibility genes. These discoveries have provided important insights into disease pathogenesis but are not expected to have an impact in the clinic for a number of years. This study aims to take genetics and biomarker discovery into the IBD clinic to address questions of immediate clinical importance.

The study will commence in February 2013 utilising the network of 120 UK hospitals currently participating in the UK IBDGC pharmacogenetic programme (www.ibdresearch.co.uk). The collection of clinical data is aligned with the data being collected by the Royal College of Physicians UK IBD Biologics Audit. The clinical data for PANTS will be collected separately using a dedicated application held within the N3 network (www.pantsdb.co.uk). In order to avoid duplicate data entry we will share relevant anonymised data with the UK IBD Biologics Audit (and in due course with the UK IBD registry). The PANTS study aims to build a bio-resource for use by the UK IBD scientific community. Anonymised data will be made available to interested parties following appropriate ethical approval and consideration by the scientific management committee.

Patients will not be randomly allocated to one therapy or another and no attempt will be made to match populations including control for disease activity. Therefore the study has not been designed to directly compare PNR or LOR rates between IFX and ADA.

This observational study is funded by CORE, the British Society of Gastroenterology research charity and by unrestricted educational grants from Merck Sharp & Dohme (MSD) and AbbVie. The sponsor of the study is the Royal Devon and Exeter NHS Foundation Trust.
3. Study Objectives and Design

3.1 Study Objectives/Aim

The primary objective of this study is to investigate the mechanisms that underlie primary non-response (PNR), loss of response (LOR) and adverse drug reactions (ADRs) to anti-TNF drugs in patients with active luminal Crohn's disease.

The secondary objectives are:

- To identify clinically meaningful serological and genetic markers that predict PNR, LOR and ADR.
- To identify clinical, biochemical and genetic predictors of durable clinical remission after anti-TNF withdrawal.
- To report the initial UK experience of Biosimilar Infliximab (Remsima™ and Inflectra™) including the efficacy (at week 14, 30 and 54), safety (to 3 years) and pharmacokinetics using a prospective open labelled study design.

The eventual goal is to develop a cost-effective, individualised anti-TNF treatment strategy for patients with Crohn's disease which maximizes benefit and minimises harm.

3.2 Study Design

This is a 4 year prospective uncontrolled observational pharmacogenetic cohort study investigating clinical, serological and genetic factors that determine PNR, LOR and ADR to the anti-TNF drugs Infliximab (IFX – Remicade™, Remsima™, Inflectra™) and Adalimumab (ADA - Humira™) in patients with active luminal Crohn's disease.

The study involves 2 study periods:

1. **PANTS**
   - Patients will be invited to participate in PANTS after the decision to start anti-TNF has been made by their gastroenterologist. Informed consent and recruitment must occur prior to the patient receiving the first dose of anti-TNF medication.
   - A pre-screening assessment may occur at any time after the decision to start anti-TNF has been made. Routine research visits are then scheduled for weeks 0 (first anti-TNF dose), 12, 14, 30 and 54. The timing of study visits are defined from the date of the first anti-TNF infusion / injection.
   - For patients receiving IFX additional serum samples will be taken immediately pre-dose at weeks 2, 6, 22, 38 and 46. To minimise inconvenience to patients these visits will be scheduled to coincide with routine outpatient or infusion clinic appointments.
   - In the event of loss of response (LOR) an additional LOR visit will be scheduled to occur immediately prior to the next anti-TNF infusion / injection.
   - Patients will remain in the study if they continue with the same anti-TNF drug, even if LOR or ADRs occur.
   - If the drug is withdrawn, or the patient switched to another anti-TNF drug, two study exit visits will be scheduled. Exit visit 1 is scheduled for 8 weeks after the last IFX infusion or 2 weeks after the last ADA injection. Exit visit 2 is scheduled for 16 weeks after the last IFX infusion or ADA injection.

2. **PANTS extension**
Patients continuing on the same anti-TNF therapy at the end of one year will be invited to enter the PANTS extension phase. A new consent needs to be obtained at visit 5 (week 54) of PANTS.

Research visits are carried out at weeks 78, 102, 126 and 150. In between these study visits patients will be asked to send a stool sample from home directly to the Exeter laboratory for faecal calprotectin measurement.

If anti-TNF therapy is withdrawn electively any time after 54 weeks, as a result of physician or patient choice, they will continue to be monitored as per the PANTS extension protocol for up to 12 months or until clinical relapse occurs, at which point an additional visit will be scheduled.

This study has been adopted by the UK NIHR CRN and patient recruitment and data entry will be supported by CLRN nurses. Anonymised data will be shared with the UK IBD Biologics Audit (and in due course with the UK IBD registry)

Laboratory investigations including CRP, faecal Calprotectin, drug and drug antibody levels are carried out at the central laboratory at the Royal Devon & Exeter Hospital. Individual CRP and faecal calprotectin data from all research visits (including the pre-screening visit) will be made available to the research sites within 7 working days. The anti-TNF drug and drug antibody level data will be made available to the research sites at week 54, or sooner in the event of primary non-response (PNR), loss of response (LOR), or study exit. In the PANTS extension, patients will be asked to send a faecal calprotectin sample every 8 weeks from home.

3. Anti-TNF switch follow-up visit (single visit, selected patients only)

Patients who stop anti-TNF therapy, exit PANTS and then start a second anti-TNF agent within 12 months of PANTS exit will be invited to a single follow-up visit. This will occur:

- 12 months after starting the second anti-TNF drug if the patient continues on treatment. The visit will be scheduled to occur within 72 hours prior to the next dose of drug.
  
  or

- If treatment with the second anti-TNF drug is stopped before 12 months the visit will occur 8 weeks after the last dose of Infliximab or 2 weeks after the last dose of Adalimumab.

At this visit consent will be taken, a short case report form completed and a blood sample taken to measure drug and antibody levels. These data will be made available to the treating clinician.

3.3 Study Definitions

There is currently no widely accepted definition of PNR or LOR. This study will use clinical and laboratory variables to define these endpoints. Our proposed definitions, which may be modified later based upon data collected, are detailed below.

**Primary non-response (PNR)** is defined at week 12 by all of the following:

- Physician global assessment (PGA) at week 12 suggests non-response.
- HBI fails to fall by 3 or more points from week 0 baseline.
- Both CRP and Calprotectin fail to fall to within the normal range or by 50% from week 0 baseline.

Patients are reviewed at week 12 by their gastroenterologist who will assess response to induction anti-TNF therapy (Physician global assessment). A blood sample for CRP, and stool sample for calprotectin will be sent to the central laboratory and the results made available on the PANTS portal prior to the week 14 infusion / injection. The decision whether to continue, or stop, anti-TNF therapy will be made by the patient's gastroenterologist between week 12 and 14. The patient’s gastroenterologist may wish to utilise the CRP and calprotectin data to inform this decision. This is an observational study and the clinician may decide to continue with anti-TNF therapy even if the patient meets the study definition of primary non-response. Conversely a
gastroenterologist may decide to stop anti-TNF therapy in a patient whose PGA suggests non-response, even if the patient fails to meet the criteria of PNR proposed in this study.

**Loss of response (LOR)** will be defined after week 13 by reviewing the following:

- Initial response to anti-TNF defined at week 12.
- Symptomatic inflammatory IBD activity, which is of sufficient severity and duration to warrant an escalation in steroid, immunomodulatory, or anti-TNF therapy or surgical resection as directed by the patient’s gastroenterologist.

Patients who lose response before week 13 will be categorised as primary non-responders

**Anti-TNF treatment withdrawal after week 54** defined as:

- Withdrawal of anti-TNF treatment electively due to physician or patient choice (e.g. due to prolonged remission or pregnancy)
- Withdrawal of anti-TNF treatment because funding is not available

**Clinical Relapse after Anti-TNF withdrawal**

- Symptomatic inflammatory IBD activity, which is of sufficient severity to warrant an escalation in steroid, immunomodulatory, or reintroduction of anti-TNF therapy as directed by the patient’s gastroenterologist.

**Adverse drug reactions (ADRs)**

This prospective cohort study will focus on infusion reactions only, although all possible adverse events during this study will be reported and recorded for future studies investigating adverse drug reactions. Patients who develop demyelination following exposure to anti-TNF drugs will be invited to participate in PRED4 (NIHR CRN ID 11988).

**Acute Infusion reactions** occur during or within 24 hours of the anti-TNF dose. Symptoms include flushing, chest tightness, dizziness, shortness of breath, headache, hypo/hypertension, nausea, sweating and other symptoms of anaphylaxis, including urticaria and bronchospasm.

*Mild* reactions are self-limiting and resolve spontaneously after temporary cessation of infusion or reduction of infusion speed.

*Moderate* reactions require closer attention and often discontinuation of infusion.

*Severe infusion* reactions involve respiratory symptoms or hypotension and require immediate drug cessation.

**Delayed Infusion reactions** occur between 24 hours and 14 days after the anti-TNF dose and include myalgia, arthralgia, influenza like symptoms, headache, tiredness and rash.

**Other adverse reactions.** Data relating to injection site reactions, opportunistic infections and any hospital admissions will be collected.
4.0 Selection and Withdrawal of Subjects

4.1 Inclusion Criteria

- Age 6 and over.
- Patients with active luminal Crohn’s disease involving the colon and/or small intestine (Montreal classification L1, L2 or L3) where the primary indication for anti-TNFα treatment is NOT fistulising disease.
- Evidence of active inflammatory disease supported by raised CRP (local laboratory) or Calprotectin (local or Exeter laboratory).
- No prior exposure to anti-TNFα medication.
- Written informed consent obtained from patient or parent / guardian.

4.2 Exclusion Criteria

- Patient unwilling to take part.
- Unable to obtain written informed consent.
- Normal CRP and calprotectin at pre-screening.
- Patient is, in the opinion of the investigator, not suitable to participate in the study.
- Patients with contraindications to the use of anti-TNF drugs.

4.3 Participation of Children.

Children will be identified and recruited in the same way as adults, although the study utilises age specific information sheets for children aged 6-10 years, 11-15 years and 16-17 years old. A parent/carer information form will also be provided for the child’s parent/carer. With the exception of blue top RNA tempus tube (more details later in the protocol) the volume of blood taken can be reduced in accordance to the child’s age.

4.4 Consent

All patients recruited to the study will be required to give written informed consent. This will be carried out in person at Visit 1 or before. Patients who lack capacity to consent will not be recruited. All subjects will be given the opportunity to contact a member of the research team to discuss the project in more detail if desired. All subjects will be informed of the nature and purpose of the study, its requirements and possible hazards, and their rights to withdraw at any time from the study without prejudice and without jeopardy to any future medical care at the study site. Parental consent will be obtained for all children under the age of 16. Additionally assent will be sought from children over the age of 11.

Patients opting to participate in the PANTS extension will need to sign a second consent form at week 54.

4.5 Control Subjects

Control subjects with Crohn’s disease are required for a case control study investigating serious adverse reactions to anti-TNF drugs. We will utilise historical cases identified from our large database of anti-TNF treated patients, recruited over the last 5 years by members of the UK IBD Genetics Consortium. These patients have already given informed consent to utilise their stored DNA for genetic studies investigating the genetics of Inflammatory bowel disease and its treatment, and therefore they will not need to attend a research visit or contacted before using their DNA for this study. Criteria for inclusion are:

- History of Crohn's disease.
- History of anti-TNF usage for a minimum of 2 years without occurrence of serious adverse event.
• Written informed consent obtained from patient or parent.

4.6 Withdrawal of Subjects

A subject will be withdrawn from the study for any of the following reasons:
• Withdrawal of consent
• Death
• Principal investigator decision (in the best interest of the patient)

If a subject is lost to follow-up, every reasonable effort must be made by the study site personnel to contact the subject and determine the reason for withdrawal. The measures taken to follow up must be documented.

Subjects may choose to withdraw from the study at any time but their anonymised biological samples will be retained. The reason for withdrawal must be documented in the web-based CRF and a withdrawal log should be completed.

4.7 Expected Duration of Study

Patients participating in PANTS will remain in the study for 54 weeks. The majority of patients who choose to continue with PANTS extension will remain in the study up to week 150.

For patients who stop anti-TNF therapy, 2 Exit visits are required, with the second visit scheduled for 16 weeks after the last drug dose.

Patients in PANTS extension who electively withdraw anti-TNF therapy will be followed up for up to a further 12 months after drug withdrawal.

Recruitment will occur over a 2 year period. The anonymised study data will be kept for 7 years.

5. Study Procedures

5.1 Pre-screening of potential study participants

Pre-screening is to assess patient eligibility into the PANTS study. Pre-screening occurs prior to the enrolment stage and does not require consent, or adding information into the PANTS database. The study may be discussed with the patient once the decision to start anti-TNF therapy has been made by the gastroenterology team.

The inclusion criteria includes objective evidence of active inflammatory disease, supported by a raised CRP or Calprotectin, determined in the local laboratory. A small proportion of patients with active Crohn’s disease will not have a raised CRP and therefore local investigators may wish to test a stool sample for calprotectin if they feel the patient has active Crohn’s disease, but the CRP is normal.

Faecal calprotectin is currently not available in all UK hospitals and the Royal Devon and Exeter NHS Foundation Trust offers this as a clinical service to any UK hospital if it is not locally available. Therefore if the Exeter clinical service is utilised, the calprotectin results will be emailed back to you.

The local research nurse will confirm eligibility before giving, or sending, the patient an invitation letter, patient information sheet and stool pot. If the patient expresses an interest in the study they will then be offered an appointment with a local research nurse for visit 1 of the study. Visit 1 must occur in the 7 days prior to commencing anti-TNF medication, and preferably on the day of the first infusion / injection. Informed consent
and collection of clinical data and biological specimens occurs at Visit 1 or in the 7 days prior to the first infusion/injection.

This is an observational study and the choice of anti-TNF drug is a decision for the clinical team and the patient.

5.2 Data collection and sharing with the UK IBD Biologics Audit / Registry

Local research staff will enter data into a web based database. This database is implemented in Flask, a web development framework, working against a backend relational database (MySQL). The research staff interact with the password-protected system via the web (Secure Sockets Layer). The back-end database is only manipulated by the application, and will not have user level access for either reading or writing. The application and all dependencies will be running on a Linux system updated with the latest security patches at time of launch. Data backups will be made at regular and frequent time-points.

All technologies employed in this solution - Flask, MySQL, Web-SSL - are state of the art in terms of security and robustness and are employed in hundreds of global solutions. The database has been developed to meet the recommended standards set out in the Connecting for Health “Securing Web Infrastructure and supporting Good Practice Guideline”

Data is entered into the application via a secure web interface, where a named list of users will have access, via high-grade-security acceptable passwords. All modifications of the database including adding new records are logged, so user errors can be easily tracked if needed.

The collection of clinical data is aligned with the data being collected by the Royal College of Physicians UK IBD Biologics Audit. The clinical data for PANTS will be collected separately but shared with the UK IBD Biologics Audit (and in due course with the UK IBD registry) to avoid duplicate collection and entry of data. Patients consent will be sought to share their anonymised data with the UK IBD Biologics Audit, although this is not a current requirement for collection of data into the national audit

5.3 Patient Reported Outcome Measures (PROMs)

This study will use PROMs to measure patient’s health-related quality of life in order to calculate the health gain from using anti-TNF drugs. The questionnaires will be given to the patients to complete at each research visit and at LOR 1 or exit 1,2):

The questionnaires will then be transferred into the PANTS database
Age 17 and under IMPACT-III
Age 18 and older EQ5-D and CCQ-12 (IBD-PROM)

5.4 Anti TNF patient Choice Questionnaire

The Anti TNF Patient Choice questionnaire will be given to the patient at visit 1 and sent back to the research site in the pre-paid envelope in an anonymised form.
All patients: Pre-screening to assess eligibility
(Consent not required at this point as patient may not be eligible)

Visit 1 all patients - Baseline assessment and Informed Consent
1 of each sample > Yellow top clotted blood serum + Purple top EDTA whole blood + Blue top RNA Tempus + Stool sample

IFX - Visit A (week 2) 1 x Yellow top clotted blood serum

IFX - Visit B (week 6) 1 x Yellow top clotted blood serum

Visit 2 - Primary Non Response Assessment (week 12) all patients - (PI or IBD nurse specialist required)
1 of each sample > Yellow top clotted blood serum + Stool sample

Visit 3 (week 14) all patients
1 of each sample > Yellow top clotted blood serum + blue top RNA Tempus

IFX Visit C
1 x Yellow top clotted blood serum

IFX Visit D – if required.
1 x Yellow top clotted blood serum

Visit 4 (week 30) all patients
1 of each sample > Yellow top clotted blood serum + blue top RNA Tempus + Stool sample collected

IFX visit E
1 x Yellow top clotted blood serum

IFX visit F
1 x Yellow top clotted blood serum

IFX visit G – if required
1 x Yellow top clotted blood serum

Visit 5 (week 54) - all patient
1 of each sample > Yellow top clotted blood serum + Purple top EDTA whole blood + Blue top RNA Tempus + Stool sample and Consent for PANTS EXTENSION

Pants extension:
Calprotectin monitoring Every 8 weeks stool sample will be sent in by the patient to evaluate calprotectin.

Research Visits: all patients one of each sample > Yellow top clotted blood serum + Purple top EDTA whole blood + Blue top RNA Tempus + Stool sample

Exit visit (1 + 2) to be completed if the Anti-TNF drug is stopped

Adverse events relating to Anti-TNF therapy and hospitalisations relating to their Crohn's Disease can occur at any stage of the study and will need to be recorded in the database.

Please go to the adverse events and hospital admissions page and complete as applicable.

For patients who dose escalate (lose response): LOR 1, LOR 2 and LOR ES can be utilised as well as research visits 3-9. The patient will be required to be followed every 3 months for a year.
5.6 Research visits

Patients will be asked to attend up to 10 research visits. Additional visits might be required in the event of loss of response or drug withdrawal. To minimise inconvenience to patients these visits will be scheduled to coincide with infusion clinic or outpatient appointments.

The major research visits for both Infliximab and Adalimumab patients are the numbered visits at weeks 0 (Visit 1), 12 (Visit 2), 14 (visit 3), 30 (visit 4), 54 (visit 5), week 78 (visit 6), week 102 (visit 7), week 126 (visit 8) and week 150 (visit 9). Please try and schedule these visits (and infusions / injections) as closely to these time points as possible.

The additional lettered visits are for patients receiving Infliximab only and are there to capture infliximab infusions that the patient may or may not receive. Not all of the additional IFX visits may be required. The lettered visit should correspond to the nearest visit time point that the infusion occurs at. If you have any doubt about which visit to select please contact the Exeter office.

Patients who lose response will be asked to attend visits every 3 months for 12 months after dose escalation. For patients treated with Infliximab these visits will coincide with infusions. For patients treated with Adalimumab additional research costs to cover the nursing time for Adalimumab patient visits will be provided and these patients will be offered a £10 Amazon token.

Labelling of samples

- It is crucial to only use the blood collection tubes and labels supplied by the lead site for the PANTS study. If any sample tubes/labels are missing from your site pack, then please contact the lead Research Office as soon as possible and more supplies will be sent.

- The handling and processing of samples sent to the main Exeter laboratory is automated. Therefore it is essential that the correct labels are used for the particular visit and sample type. The labels are pre-printed with Visit number and week.

- It is important that the local site completes the date of birth, Study ID number and the date the sample was taken on each tube.

Pre-screening – see section 5.1

Visit 1 (Week 0 - Immediately prior to commencement of Anti-TNF medication)

This visit must occur in the 7 days prior to commencing anti-TNF medication, and preferably on the day of the first infusion / injection.

Research biological specimens must be collected at visit 1 or anytime in the 7 days prior to commencing anti-TNF drug. We would prefer if these samples are taken immediately prior to the dose of anti-TNF but the samples may be taken at any time point in the 7 days prior to infusion / injection.

- The study will be explained to the patient. Any questions regarding study participation will be discussed.

- The consent form is signed. It is photocopied and a copy given to the patient, a copy placed in the hospital notes, and one held in the site file.
The patient must be registered on the database before you start Visit 1 as the registration process generates the study ID. The rest of the visit information must be completed within 48 hours and preferably on the day of visit.

The research nurse will take the following blood samples using the bottles supplied in the study pack. The tubes should be fully filled and the labels applied.

- One purple top EDTA whole blood tube
- One yellow top clotted blood serum tube
- One blue top RNA Tempus tube filled with 3mls of blood which must be shaken vigorously immediately after blood collection in order to mix blood with RNA preservative

The purple top EDTA whole blood tube is placed in a single transport tube and the yellow top clotted blood serum tube and blue top RNA Tempus tube are placed in a second transport tube. Both transport tubes are then placed in the plastic bag labelled FAO Chemistry MTOs, PANTS study and sent to the RD&E on the day of collection.

The patient will have been provided with:

- a plain stool pot
- x1 stool collection tube containing a preservative RNaLater.

They will be asked to bring both these samples with them to this research visit. Send these labelled samples to the RD&E along with the blood samples. The patient or research nurse must complete the stool pot label detailing the date the stool was produced.

The patient will complete the appropriate questionnaire (IBD-PROM or IMPACT III) which may take up to 25 minutes.

Input the required clinical data using the medical records, and the most recent local hospital blood test results. The patient completed relevant questionnaire (adults: IBD-PROM or children: IMPACT III) should also be inputted when prompted.

The recruitment log, which contains no patient identifiable data, should be completed and sent /faxed to the central Exeter team to alert them of the pending samples.

The Anti-TNF Patient Choice questionnaire will be given to the patient at visit 1; this is at the discretion of the Principal Investigator and is optional. If the patient wishes to complete it they are provided with a pre-paid envelope for its return to the lead research centre.

Visit 1X (Visit 0 + 3 days – 3 days after first dose of anti-TNF therapy - OPTIONAL)

The research nurse will take the following blood samples using the bottles supplied in the study pack. The tubes should be fully filled and the labels applied.

- One yellow top clotted blood serum tube
- One blue top RNA Tempus tube filled with 3mls of blood which must be shaken vigorously immediately after blood collection in order to mix blood with RNA preservative

The yellow top clotted blood serum tube) and blue top RNA Tempus tube are placed in the transport tube. The transport tube is then placed in the plastic bag labelled FAO Chemistry MTOs, PANTS study and sent to the RD&E on the day of collection.

A £10 Amazon voucher is offered to the patient for attending this visit. This represents a contribution to the costs of travelling to hospital for this visit.
Visit 2 (Week 12 after first infusion / injection) - Identifying primary non-response (PNR)

- Visit 2 occurs 12 weeks after the first infusion / injection. Note this may or may not occur at 12 weeks after visit 1 if the first infusion / injection did not occur on the day of visit 1.

- Patients are reviewed at week 12 by their own gastroenterologist who will assess response to induction anti-TNF therapy (Physician global assessment). The patient's gastroenterologist will decide whether to continue with the next infusion / injection at week 14, or stop, anti-TNF therapy. The patient's gastroenterologist may wish to wait 5-7 days for the CRP and calprotectin data to inform this decision. This is an observational study and the clinician may decide to continue with anti-TNF therapy even if the patient meets the study definition of primary non-response. Conversely a gastroenterologist may decide to stop anti-TNF therapy in a patient whose PGA suggests non-response, even if the patient fails to meet the strict criteria of PNR used in this study.

- The research nurse will take the following blood sample using the bottle supplied in the study pack. The tube should be fully filled and the label applied.
  - One yellow top clotted blood serum tube

- The yellow top clotted blood serum tube is placed in a transport tube. The transport tube is then placed in the plastic bag labelled FAO Chemistry MTOs, PANTS study and sent to the RD&E on the day of collection.

- The patient will have been provided with:
  - a plain stool pot at visit 1
  - x1 stool collection tube containing a preservative RNAlater.

  The patient will be asked to bring these samples with them to this research visit. This pot and tubes must be labelled with the date the stool was produced and sent to the RD&E along with the blood sample. The stool samples should be produced as near to the study visit as possible, and preferably no earlier than 3 days prior to the infusion / injection, and no later than 24 hours after the infusion / injection. If the patient does not bring the stool samples to the visit they may return the pot directly to the RD&E lab in a pre-paid jiffy bag fully labelled.

- The patient will complete the appropriate questionnaire (IBD-PROM or IMPACT III)

- Input the required clinical data using the medical records, and the most recent local hospital blood test results. The patient completed relevant questionnaire (adults: IBD-PROM or children: IMPACT III) should also be inputted when prompted.

- The recruitment log should be completed and sent to the central Exeter team to alert them of the pending samples.

NB if after this visit, the anti-TNF drug is stopped, or switched to an alternative anti-TNF drug then the patient’s next visits will be Exit visit 1 (scheduled for week 14) and Exit visit 2 (scheduled for week 22, patients on IFX, week 28 patients on ADA)

Visit 3 (Week 14 after first infusion / injection - Immediately prior to IFX infusion, ADA injection)

This visit is scheduled for patients who continue on Anti-TNF therapy.

- The research nurse will take the following blood samples using the bottles supplied in the study pack. The tubes should be fully filled and the labels applied.
  - One yellow top clotted blood serum tube
• One blue top RNA Tempus tube filled with 3mls of blood which must be shaken vigorously immediately after blood collection.

• The yellow top clotted blood serum tube and the blue top RNA Tempus tube are placed in a transport tube. The transport tube is then placed in the plastic bag labelled FAO Chemistry MTOs, PANTS study and sent to the RD&E on the day of collection.

• There is no stool sample collected at this visit but a further stool pot will be provided for the next visit.

• The recruitment log should be completed and sent to the central Exeter team to alert them of the pending samples.

Visit 4 (Week 30 after first infusion / injection - Immediately prior to IFX infusion, ADA injection)

• The research nurse will take the following blood samples using the bottles supplied in the study pack. The tubes should be fully filled and the labels applied.
  o One yellow top clotted blood serum tube
  o One blue top RNA Tempus tube filled with 3mls blood which must be shaken vigorously immediately after blood collection.

• The yellow top clotted blood serum tube and the blue top RNA Tempus tube are placed in a transport tube. The transport tube is then placed in the plastic bag labelled FAO Chemistry MTOs, PANTS study and sent to the RD&E on the day of collection.

• The patient will have been provided with a stool pot at visit 3 and will be asked to bring this sample with them to this research visit. This pot will be sent to the RD&E along with the blood samples. A further stool pot will be provided for the next visit. The stool sample should be produced as near to the study visit as possible, and preferably no earlier than 3 days prior to the infusion / injection, and no later than 24 hours after the infusion / injection. If the patient does not bring the stool sample to the visit they may return the pot directly to the RD&E lab in a pre-paid jiffy bag fully labelled.

• The patient will complete the appropriate questionnaire (IBD-PROM or IMPACT III).

• If a patient fulfils the LOR criteria then they must also complete an IBD CONTROL PROM.

• Input the required clinical data using the medical records, and the most recent local hospital blood test results. The patient completed relevant questionnaire (adults: IBD-PROM or children: IMPACT III) should also be input when prompted.

• The recruitment log should be completed and sent to the central Exeter team to alert them of the pending samples.

Visit 5 (Week 54 after first infusion / injection - Immediately prior to IFX infusion, ADA injection)

• Repeat the steps as for visit 4.
• In addition to visit 4 blood samples a purple top EDTA whole blood tube is collected and sent to the RD&E.
• Invite the patient to participate in PANTS extension study. Take informed consent if the patient is willing to participate.

Additional blood tests
• For patients receiving IFX, additional yellow top clotted blood serum samples will be taken immediately pre-dose at weeks 2, 6, 22, 38 and 46 when patients attend for infusions. The labelled blood sample is sent to the RD&E laboratory. For patients treated with ADA, blood tests are taken at the scheduled research visits only.

Extension visit {6, 7, 8, 9} every 24 weeks

• The research nurse will take the following blood samples using the bottles supplied in the study pack. The tubes should be fully filled and the labels applied.
  - One purple top EDTA whole blood tube
  - One yellow top clotted blood serum tube
  - One blue top RNA Tempus tube filled with 3mls of blood which must be shaken vigorously immediately after blood collection in order to mix blood with RNA preservative
• The purple top EDTA whole blood tube is placed in a single transport tube and the yellow top clotted blood serum tube) and blue top RNA Tempus tube are placed in a second transport tube. Both transport tubes are then placed in the plastic bag labelled FAO Chemistry MTOs, PANTS study and sent to the RD&E on the day of collection.
• The patient will have been provided with a stool pot at the previous visit and will be asked to bring this sample with them to this research visit. This pot will be sent to the RD&E along with the blood samples. A further stool pot will be provided for the next visit. The stool sample should be produced as near to the study visit as possible, and preferably no earlier than 3 days prior to the infusion / injection, and no later than 24 hours after the infusion / injection. If the patient does not bring the stool sample to the visit they may return the pot directly to the RD&E lab in a pre-paid jiffy bag
• The patient will complete the appropriate questionnaire (IBD-PROM or IMPACT III).
• **If a patient fulfils the LOR criteria then they must also complete an IBD CONTROL PROM.**
• Input the required clinical data using the medical records, and the most recent local hospital blood test results. The patient completed relevant questionnaire (adults: IBD-PROM or children: IMPACT III) should also be input when prompted.
• The recruitment log should be completed and sent to the central Exeter team to alert them of the pending samples.

Give the patient the calprotectin kits to send in from home every 8 weeks.

**ADDITIONAL VISITS**

**Loss of response (LOR)**

A LOR visit should be scheduled any time after week 14 if the LOR criteria are met (page 11) and the patient's gastroenterologist has escalated steroid, immunomodulatory or anti-TNF therapy. Escalation of anti-TNF therapy includes an increase in the dose, or a shortening of the interval between doses.

The LOR visit should be scheduled immediately prior (preferably on the same day) to the next (modified) anti-TNF infusion / injection and will replace any standard visits. In the event of anti-TNF dose intensification this visit may occur earlier than planned or may involve a higher dose of anti-TNF drug.

If in any doubt about visit scheduling please call the Exeter office.

**Loss of response in IFX treated patients**

• Conduct LOR 1 visit which will replace the standard visit.
• Standard visits will continue to be scheduled for each subsequent IFX infusion up to week 54.
If LOR occurs during the PANTS extension study visits will continue to be scheduled every 3 months for 12 months and then revert to standard visits every 24 weeks.

Loss of response in ADA treated patients

- Conduct LOR 1 visit which may replace a standard visit if this coincides with the scheduled ADA research visits at week 30 and week 54.
- After the LOR1 visit subsequent visits are scheduled every 3 months for 12 months. These visits may coincide with the scheduled research visits 4, 5, 6, 7, 8 and 9. If this is the case simply use these visits and labels. **If not then use LOR 2 or LOR ES visits and labels.**
- A £10 Amazon voucher is offered to the patient for each additional visit made due to loss of response to Adalimumab. This represents a contribution to the costs of travelling to hospital for research visits.
- After this 12 month period visits revert to standard visits every 24 weeks.

LOR visits

- At the LOR visits the research nurse will take the following blood samples using the bottles supplied in the study pack. The tubes should be fully filled and the label applied.
  - One purple top EDTA whole blood tube
  - One yellow top clotted blood serum tube
  - One blue top RNA Tempus tube filled with 3mls of blood which must be shaken vigorously immediately after blood collection in order to mix blood with RNA preservative.
- The purple top EDTA whole blood tube is placed in a single transport tube and the yellow top clotted blood serum tube) and blue top RNA Tempus tube are placed in a second transport tube. Both transport tubes are then placed in the plastic bag labelled FAO Chemistry MTOs, PANTS study and sent to the RD&E on the day of collection.
- The patient will have been provided with a stool pot at an earlier visit and will be asked to bring this sample with them to this visit. This pot will be sent to the RD&E along with the blood samples. The stool sample should be produced as near to the study visit as possible, and preferably no earlier than 3 days prior to the infusion / injection, and no later than 12 hours after the infusion / injection. If the patient does not bring the stool sample to the visit they may return the pot directly to the RD&E lab in a pre-paid jiffy bag.
- The patient will complete the appropriate questionnaire (IBD-PROM or IMPACT III) and also complete the IBD control PROM.
- Input the required clinical data using the medical records, and the most recent local hospital blood test results. The patient completed relevant questionnaire (adults: IBD-PROM and IBD control PROM or children: IMPACT III) should also be inputted for LOR visits when prompted.
- The visit log should be completed and sent to the RD&E team to alert them of the pending samples.

Exit Visit 1 (Withdrawal of anti-TNF therapy before week 54 or non-elective withdrawal after week 54).

- If the drug is withdrawn for any reason two exit visits will be scheduled. Exit visit 1 is scheduled for 8 weeks after the last IFX infusion or 2 weeks after the last ADA injection.
- The research nurse will take the following blood samples using the bottles supplied in the study pack. The tubes should be fully filled and the labels applied.
  - One purple top EDTA whole blood tube.
- One yellow top clotted blood serum tube
- One blue top RNA Tempus tube filled with 3mls of blood which must be shaken vigorously immediately after blood collection in order to mix blood with RNA preservative

- The purple top EDTA whole blood tube is placed in a single transport tube and the yellow top clotted blood serum tube (and blue top RNA Tempus tube) are placed in a second transport tube. Both transport tubes are then placed in the plastic bag labelled FAO Chemistry MTOs, PANTS study and sent to the RD&E on the day of collection.

- The patient will have been provided with a stool pot and will be asked to bring this sample with them to this research visit. Send this labelled pot to the RD&E along with the blood samples. The patient or research nurse must complete the stool pot label detailing the date the stool was produced.

- The patient will complete the appropriate questionnaire (IBD-PROM or IMPACT III). If the patient fulfils the LOR criteria then they must also complete an IBD CONTROL PROM.

- Input the required clinical data using the medical records, and the most recent local hospital blood test results. The patient completed relevant questionnaire (adults: IBD-PROM or children: IMPACT III) should also be inputted when prompted.

- The recruitment log, which contains no patient identifiable data, should be completed and sent /faxed to the central Exeter team to alert them of the pending samples.

Exit Visit 2 (Withdrawal of anti-TNF therapy before week 54 or non-elective withdrawal after week 54).

- Exit visit 2 is scheduled for 16 weeks after the last IFX infusion or ADA injection

- The research nurse will take the following blood samples using the bottles supplied in the study pack. The tubes should be fully filled and the labels applied.
  - One yellow top clotted blood serum tube

- The yellow top clotted blood serum tube is placed in a transport tube. The transport tube is placed in the plastic bag labelled FAO Chemistry MTOs, PANTS study and sent to the RD&E on the day of collection.

- The patient will complete the appropriate questionnaire (IBD-PROM or IMPACT III). If the patient fulfils the LOR criteria then they must also complete an IBD CONTROL PROM.

- Input the required clinical data using the medical records, and the most recent local hospital blood test results. The patient completed relevant questionnaire (adults: IBD-PROM or children: IMPACT III) should also be inputted when prompted.

- The recruitment log, which contains no patient identifiable data, should be completed and sent /faxed to the central Exeter team to alert them of the pending samples.

Faecal Calprotectin Monitoring (after week 54) – every 8 weeks

Calprotectin test kits will be given to patient by the local research site. The patient will be asked to return the sample by post every 8 weeks directly to Exeter.
Clinical relapse visit (after elective withdrawal of anti-TNF)

- The clinical relapse visit is scheduled as soon as the patient reports symptomatic inflammatory IBD activity, which is of sufficient severity to warrant an escalation in steroid, immunomodulatory, or reintroduction of anti-TNF therapy as directed by the patient’s gastroenterologist after week 54.

- The research nurse will take the following blood samples using the bottles supplied in the study pack. The tubes should be fully filled and the labels applied.
  - One purple top EDTA whole blood tube
  - One yellow top clotted blood serum tube
  - One blue top RNA Tempus tube filled with 3mls of blood which must be shaken vigorously immediately after blood collection in order to mix blood with RNA preservative

- The purple top EDTA whole blood tube is placed in a single transport tube and the yellow top clotted blood serum tube and blue top RNA Tempus tube are placed in a second transport tube. Both transport tubes are then placed in the plastic bag labelled FAO Chemistry MTOs, PANTS study and sent to the RD&E on the day of collection.

- The patient will have been provided with a stool pot and will be asked to bring this sample with them to this research visit. Send this labelled pot to the RD&E along with the blood samples. The patient or research nurse must complete the stool pot label detailing the date the stool was produced.

- The patient will complete the appropriate questionnaire (IBD-PROM or IMPACT III)

- Input the required clinical data using the medical records, and the most recent local hospital blood test results. The patient completed relevant questionnaire (adults: IBD-PROM or children: IMPACT III) should also be inputted when prompted.

- The recruitment log, which contains no patient identifiable data, should be completed and sent /faxed to the central Exeter team to alert them of the pending samples.

12 Month Follow Up Visit - selected patients only - after elective withdrawal of anti-TNF – Year 4)

We aim to follow patients for 12 months after elective withdrawal of anti-TNF therapy. The 12 Month Follow Up visit allows the patient to be assessed 24 and 48 weeks after withdrawal of anti-TNF. This visit is only required if anti-TNF is withdrawn after week 102 (visit 7). Standard study visits will capture the required information for patients who withdraw anti-TNF before week 107 (visit 7) and the 12 Month Follow Up visit will not be required.

- The research nurse will take the following blood samples using the bottles supplied in the study pack. The tubes should be fully filled and the labels applied.
  - One purple top EDTA whole blood tube
  - One yellow top clotted blood serum tube
  - One blue top RNA Tempus tube filled with 3mls of blood which must be shaken vigorously immediately after blood collection in order to mix blood with RNA preservative

- The purple top EDTA whole blood tube is placed in a single transport tube and the yellow top clotted blood serum tube and blue top RNA Tempus tube are placed in a second transport tube. Both transport tubes are then placed in the plastic bag labelled FAO Chemistry MTOs, PANTS study and sent to the RD&E on the day of collection.
• The patient will have been provided with a stool pot and will be asked to bring this sample with them to this research visit if appropriate. Send this labelled pot to the RD&E along with the blood samples. The patient or research nurse must complete the stool pot label detailing the date the stool was produced.

• The patient will complete the appropriate questionnaire (IBD-PROM or IMPACT III)

• Input the required clinical data using the medical records, and the most recent local hospital blood test results. The patient completed relevant questionnaire (adults: IBD-PROM or children: IMPACT III) should also be inputted when prompted.

• The recruitment log, which contains no patient identifiable data, should be completed and sent /faxed to the central Exeter team to alert them of the pending samples.

Anti-TNF switch visit

Patients who stop anti-TNF therapy, exit PANTS and then start a second anti-TNF agent within 12 months of PANTS exit will be invited to a single follow-up visit. This will occur:

• 12 months after starting the second anti-TNF drug if the patient continues on treatment. The visit will be scheduled to occur within 72 hours prior to the next dose of drug.

  or

• If treatment with the second anti-TNF drug is stopped before 12 months the visit will occur 8 weeks after the last dose of Infliximab or 2 weeks after the last dose of Adalimumab.

At this visit consent will be taken, a short case report form completed and a blood sample taken to measure drug and antibody levels. These data will be made available to the treating clinician.

• The consent form is signed. It is photocopied and a copy given to the patient, a copy placed in the hospital notes, and one held in the site file.

• The research nurse will take the following blood sample using the bottle supplied in the study pack. The tubes should be fully filled and the labels applied.

  • One yellow top clotted blood serum tube

  • The case report form is completed and entered into the PANTS database
5.7 Summary of visit activity – guide as to which patient samples are to be collected at which visit

It is crucial that the sample labels used are the correct labels for the correct visit time point and sample. Labels must be clearly completed with: Study ID (patient number), DOB and importantly, the date the sample was taken. These samples can then be posted to as the Exeter laboratory soon as possible after collection, using the prepaid envelopes supplied.

**INFLIXIMAB (IFX) PATIENTS**

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<tr>
<th>Samples to collect:</th>
<th>Visit 1 (wk 0) All patients</th>
<th>IFX A (wk 2)</th>
<th>IFX B (wk 6)</th>
<th>Visit 2 (wk 12) All patients</th>
<th>IFX C</th>
<th>IFX D (wk 14) All patients</th>
<th>IFX E</th>
<th>IFX F</th>
<th>IFX G (wk 30) All patients</th>
<th>Visit 5 (wk 54) All patients</th>
<th>Extension Visit 6, 7, 8, 9 All patients every 24 weeks</th>
<th>LOR 1</th>
<th>LOR 2 (after wk 54)</th>
<th>LOR ES (after wk 54)</th>
<th>Exit 1</th>
<th>Exit 2</th>
<th>Clinical relapse visit</th>
<th>12 Month Follow Up visit</th>
<th>Anti-TNF switch visit</th>
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* Please note that if Visit 2&3 are merged the research nurse should collect two yellow top serum tubes – if they are only able to fill one serum tube please label it Visit 3.
# ADALIMUMAB (ADA) PATIENTS

<table>
<thead>
<tr>
<th>Samples to collect:</th>
<th>Visit 1 (week 0)</th>
<th>Visit 2 (week 12)</th>
<th>Visit 3 (week 14)</th>
<th>Visit 4 (week 30)</th>
<th>Visit 5, (week 54)</th>
<th>Extension Visit 6, 7, 8, 9 All patients every 24 weeks</th>
<th>LOR 1</th>
<th>LOR 2</th>
<th>LOR ES (after wk 54)</th>
<th>Exit 1</th>
<th>Exit 2</th>
<th>Clinical relapse visit</th>
<th>12 Month Follow Up visit</th>
<th>Anti-TNF switch visit</th>
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* Please note that if Visit 2&3 are merged the research nurse should collect two yellow top serum tubes – if they are only able to fill one serum tube please label it Visit 3.
## Scenario

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Visit 1</th>
<th>IFX A (wk 2)</th>
<th>IFX B (wk 6)</th>
<th>Visit 2 (wk 12)</th>
<th>Visit 3 (wk 14)</th>
<th>IFX C (wk 22)</th>
<th>IFX D</th>
<th>Visit 4 (wk 30)</th>
<th>IFX E (wk 38)</th>
<th>IFX F (wk 46)</th>
<th>IFX G</th>
<th>Visit 5 (wk 54)</th>
<th>Exit 1</th>
<th>Exit 2</th>
<th>LOR 1</th>
<th>LOR 2</th>
<th>LOR ES</th>
<th>Extension</th>
<th>Clinical relapse after withdrawal</th>
<th>12 month follow up</th>
<th>Ant-TNF switch visit</th>
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<tr>
<td>IFX patient remains in remission for 150 weeks with standard IFX regime</td>
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<tr>
<td>ADA patient remains in remission for 150 weeks with standard ADA regime</td>
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<td>IFX patient demonstrates PNR at week 12 (visit 2) and stops the drug</td>
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<td>IFX patient loses response after week 14 but continues IFX treatment for duration of study period</td>
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<td>IFX patient loses response after week 14 and then also stops IFX later on</td>
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<td>ADA patient loses response after week 14 and also stops ADA later on</td>
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<td>IFX is electively withdrawn after week 54 and clinical relapse occurs</td>
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<td>ADA is electively withdrawn after week 54 and clinical relapse occurs</td>
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5.8 Possible scenarios - examples

ADA and IFX exit the PANTS study due to switching to either IFX or ADA
5.9 Handling of Clinical Data

Data entered onto the database will be reviewed for discrepancies, missing data and queries. Research nurses and data managers will liaise with the relevant clinicians regarding data corrections. The Chief Investigator will be updated on a regular basis and will review the reports to ensure consistency and accuracy of the data.

Patient confidentiality will be maintained at all times and will be protected in accordance with local data storage policy and legislation. Data will be pseudo-anonymised. A unique study ID will be given to each participant at the local research site and no personal details will be sent to the central research site. The local research site will be able to link the unique study ID code to the individual participating patient.

5.10 Storage and testing of DNA

DNA will be extracted at the Exeter Molecular Genetics Laboratory, by members of Professor Sian Ellard's team. All DNA samples will be stored at the NIHR Exeter Clinical Research Facility in locked, alarmed -80C freezers. Designated members of the research team will have access to the samples. Dr Tariq Ahmad will act as custodian.

All DNA samples will be pseudo-anonymised as we may wish to recruit patients to future pharmacogenetic based upon their genotype. This is explained to the patients on the patient information sheet. Only the local research sites will be able to link the unique study ID code to the individual patient and therefore any future requests to patients to participate in studies will need to be made by the local centre.

Coded DNA samples will be sent to other centres in the UK, including the Wellcome Trust Sanger Institute, Hinxton, for genotyping but all samples will be fully anonymised.

We will use the latest GWAS genotyping platform (e.g. Illumina 1M chip) and additionally carry out high-resolution class I and II HLA typing. We will draw on publicly available Wellcome Trust Case Control Consortium (WTCCC) data for country and sex-matched population controls.

Genotypic data will be kept on a secure password protected computer server by the Department of Gastroenterology at the Royal Devon and Exeter Hospital in accordance with the data storage policy. Participants and/or their GP’s will not be told of the genotypes identified from the studies at any stage during the study.

5.11 Stool samples for Calprotectin levels including Calprotectin monitoring (visit 5 onwards)

The patient will be asked to bring a stool sample with them to each research visit or send one directly to the Exeter laboratory. The stool must be produced within 72 hours prior to the research visit or 24 hours after. The patient will need to record the date sample produced on the label supplied. The stool pot will be placed in transport tube, then in the plastic bag labeled ‘FOA Chemistry MTO’s PANTS study’ and sent to the RD&E in the pre-paid envelope supplied. We will use the Immunodiagnostik monoclonal ELISA kit to measure calprotectin. Results will be made available to local PIs within 7 working days.
5.12 Antibody and Anti-TNF Drug Levels

Venous blood will be taken from the patient and sent to the Royal Devon and Exeter Hospital by first class post. Serum does not need to be separated or frozen before transportation. To measure anti-TNF drug levels and anti-TNF drug antibody levels we will use the Immunodiagnostik ELISA kits. Results will be made available to local PIs within 10 working days of the sample being received in the laboratory.

5.13 NIBSC Collaboration

The National Institute for Biological Standards and Control (NIBSC) is currently a department within the Health Protection Agency (will become part of the Medicines and HealthCare regulatory agency from April 1, 2013), and its mission is to assure the quality of biological medicines. It is responsible for developing and producing World Health Organisation (WHO) International Standards for world-wide use and is the UK's Official Medicines Control Laboratory, responsible for testing of biological medicines within the framework of the European Union. It supports health policy development and implementation through provision of expert evidence-based advice and technical support and is an important component of the Department of Health's risk management strategy for public health.

Senior scientists from the Biotherapeutics Group, NIBSC have considerable experience and expertise in the area of unwanted immunogenicity of biotherapeutic proteins. This has led to active involvement in drafting of European Medicines Agency guidelines on ‘Immunogenicity assessment of biotechnology derived therapeutic proteins’ and more recently ‘Immunogenicity assessment of monoclonal antibodies intended for in vivo clinical use’. Current work is directed towards developing and refining assays to measure serum levels of a selection of biotherapeutics and induced antibodies, including Infliximab and Adalimumab. Such assays are complex and measurements obtained by different assay systems can differ depending the biological components and precise protocol of the assay systems. Use of different systems can provide complementary information which may facilitate disease and patient management.

The NIBSC will contribute to this project by measuring levels of the therapeutic antibody and antibodies against it in serum samples from patients receiving Infliximab or Adalimumab at NIBSC research sites. NIBSC hope that their results would complement results from our laboratory.

It is proposed that the RD&E laboratory will send a small volume of serum to NIBSC from patients who are naïve to Anti-TNF i.e. at visit 1 and then at different time point throughout the study including an additional sample 16 weeks post LOR or exit.

6. Assessment of Safety

6.1 Risks and benefits for the participant

There is a risk of bruising at the venepuncture site. To minimize this risk venepuncture will only be carried out by healthcare professionals competent at carrying out this procedure.

There are no direct patient benefits from participation. However calprotectin stool test results which provide an objective assessment of disease activity will be reported back to their physician. Similarly if the patient loses response to the anti-TNF therapy data regarding drug and antibody levels will be reported back to the patient's physician. This might help guide decision making in the event of LOR, although scientific evidence to support this is extremely limited.
In the unlikely event that the patient should experience an untoward event as a direct consequence of participation in the research project, the event will be logged on the serious adverse event form and faxed to the central research office and reported to the sponsor.

7. Sample Size

7.1 Primary non-response

Assuming a primary non-response (PNR) rate of 0.2 (PNR occurs in 10-30% of anti-TNF treated patients) and a perfectly tagged multiplicative risk allele of 25% frequency, our study design of 240 non-responders and 960 responders yields 100% and 30% power to detect genome wide significant association (P<5x10^{-8}) given the odds ratios of 2 and 1.5 respectively.

7.2 Serious adverse drug reactions

A sample size of 200 cases and 1200 controls provides greater than 95% power to identify an association at “beyond doubt” p values of 1x10^{-12} at odds ratios of at least 5.0 with an allele frequency of greater than 2%.

8. Genotyping/ Biomarker Assays and Analyses

8.1 Genotyping

At the end of year 3 anonymised DNA samples will be shipped to the Sanger Centre, Cambridge for genotyping and whole exome sequencing. The latest genome wide association study platform and exome sequence technology will be used.

8.2 Calprotectin, Drug and Antibody Levels

Immunodagnostik ELISA kits will be used to measure TNF levels, anti-TNF drug levels, anti-TNF drug antibody levels and Calprotectin levels. Results will be made available to local PIs.

8.3 Analyses and development of predictive algorithm

At year 3 after routine quality control steps to exclude poorly performing samples and SNP’s, we will conduct genome wide association studies for drug response and side effects. Significant associated loci will give insight into biological mechanisms underlying these important clinical outcomes, with potential implications for future drug design. Furthermore, we will use collated clinical serological and genetic data to build predictive algorithms for anti-TNF response and toxicity. Regression and machine learning methodology will be applied to find the most parsimonious predictive models in order to maximise cost-efficiency of the predictive algorithm. This will be achieved by applying penalised-likelihood methods such as ridge-regression, elastic net and lasso regression to prevent over-fitting. The predictive power of the model (R2) will then be tested using cross-validation and/or bootstrapping approaches. Finally we will demonstrate the potential clinical utility of our algorithm by creating a receiver operator characteristic curve.

9. Direct Access to Source Data and Documents

The Investigator(s) will permit monitoring, audits, REC review, and regulatory inspections by providing the Sponsor(s), Regulators and REC direct access to source data and other documents (e.g. consent forms etc.). Study monitoring will be carried out by the central research team and sponsor. CV’s, delegations logs, electronic CRF’s, hospital notes and questionnaires will be deemed source data.
10. Ethics & Regulatory Approvals

10.1 Ethics

The study will be conducted in compliance with the principles of the Declaration of Helsinki (1996), the principles of GCP and in accordance with all applicable regulatory requirements including, but not limited to, the Research Governance Framework.

This protocol and related documents have been reviewed by the Sponsor and the South West - Exeter Research Ethics Committee (REC). Annual progress and safety reports and a final report at conclusion of the study will be submitted to the Sponsor and the REC. All protocol amendments will be submitted to the REC, R&D Offices and Regulatory Authorities for approval.

10.2 Confidentiality

This study adheres to the Caldicott principles for the use of identifiable data. Data will be pseudoanonymised. A unique study ID will be given to each participant by the local research site and no personal details will be sent to the main research site. Access to the secure file linking study ID with personal details will be held by the local research site only on a protected computer. Access will be limited to the local principal investigator and research nurse. The patients’ GP will be informed of their participation.

Individual genetic data will not be made available to participants or their doctors.

10.3 Use of tissue samples in future research

Consent will be sought in line with the Human Tissue Act (2004) from the patients to use their DNA samples in future work, which may involve recruitment based on their genotype. Pseudoanonymisation of the data will allow us to go back to individual patients via the local principal investigators. We will submit a new ethics application for any future studies using these samples.

Patients will be told that their DNA sample will be:

- Considered a gift to the Royal Devon and Exeter NHS Foundation Trust, which will act as custodian of the sample.
- Tested for multiple genes in the future using new genetic techniques.
- Made available in an anonymised form only to other researchers working in the field after careful consideration of their study protocol and approval by the relevant REC.
- Any commercial use of the findings is unlikely to occur in the short-term and that this is a long-term project.
- Furthermore, any commercial exploitation of the findings is unlikely to be due to single samples, but is more likely to be due to the findings in a large number of patient samples.

11. Quality Assurance

Monitoring of this study will ensure compliance with Good Clinical Practice. Scientific integrity will be managed and oversight retained, by the Royal Devon & Exeter Hospital NHS Foundation Trust Research and Development Directorate.
12. Data Handling

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

- Patient data will be anonymised once it leaves the local research site.
- All anonymised data will be stored on a password protected computer.
- All investigators and research site staff involved in this trial must comply with the requirements of the Data Protection Act 1998, and Trust Policy with regards to the collection, storage, processing and disclosure of personal information, and will uphold the Act's core principles. All biological specimens will be stored in line with the Human Tissue Act.

13. Data Management

All clinical data will be captured in the web based application. This will be provided to the researchers in an anonymised form. All data collected will be coded and not hold any identifiable information.

14. Publication Policy

It is intended that the results of the study will be reported and disseminated at international conferences and in peer-reviewed scientific journals.

15. Insurance / Indemnity

NHS Indemnity will apply.

16. Financial Aspects

Funding to conduct the study is provided by:

1. CORE Grant Award – Award letter dated 22nd July 2011
2. Merck – Unrestricted educational award
3. AbbVie – educational award.

17. Signature

[Signature]

Dr Tariq Ahmad D.Phil FRCP  
Consultant Gastroenterologist  
Date  
Chief Investigator