Predictors of anti-TNF treatment failure: a prospective multi-centre study of biologic naïve patients with active luminal Crohn's disease

Nicholas A Kennedy,1,2,3 MBBS; Graham A Heap,1 PhD; Harry D Green,4 PhD; Benjamin Hamilton,1 MBBS; Claire Bewshea,2 MSc;  Gareth J Walker,1,2 MBBS ; Amanda Thomas,1,2 MBBS; Rachel Nice,5 MSc; Mandy H Perry,5 PhD;   Sonia Bouri,6 MBBS; Neil Chanchlani,2 MBChB;  Neel M Heerasing,1 MBBS; Peter Hendy,1 MBBS; Simeng Lin,2 MBChB;  Daniel R Gaya,7 DM ; JR Fraser Cummings,8,9 DPhil; Charlie W Lees,3, 10 PhD; Ailsa L Hart,6 PhD; Miles Parkes,11 DM;  Shaji Sebastian,12 MD;  John C Mansfield,13 MD; Peter M Irving,14 MD; James Lindsay,15 PhD; Richard K Russell,16 PhD; Timothy McDonald,5 PhD; Dermot McGovern,17 DPhil;\* James R Goodhand,1,2 MBBS; Tariq Ahmad,1,2 DPhil, UK IBD Pharmacogenetics study group

1.Department of Gastroenterology, Royal Devon and Exeter Hospital NHS Foundation Trust, Exeter.

2. Exeter IBD Pharmacogenetics Research Group, University of Exeter.

3. Institute of Genetic and Molecular Medicine, University of Edinburgh.

4.Genetics of Complex Traits, University of Exeter Medical School, Exeter.

5.Department of Blood Science, Royal Devon and Exeter Hospital NHS Foundation Trust, Exeter.

6. Department of Gastroenterology, St Mark's Hospital, London North West Healthcare NHS Trust, Harrow.

7. Department of Gastroenterology, Glasgow Royal Infirmary, NHS Greater Glasgow and Clyde, Glasgow.

8. Department of Gastroenterology, University Hospital Southampton NHS Foundation Trust, Southampton.

9. Faculty of Experimental Medicine, University of Southampton, Southampton.

10. Department of Gastroenterology, Western General Hospital, NHS Lothian, Edinburgh.

11. Department of Gastroenterology, Addenbrooke's Hospital, Cambridge University Hospitals NHS Foundation Trust, Cambridge.

12.Gastroenterology and Hepatology, Hull and East Yorkshire Hospitals NHS Trust, Hull.

13. Department of Gastroenterology, Newcastle Upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne.

14. Department of Gastroenterology, Guy’s and St Thomas’ NHS Foundation Trust, London.

15. Department of Gastroenterology, The Royal London Hospital, Barts Health NHS Trust, London.

16.  Department of Paediatric Gastroenterology, Royal Hospital for Children, NHS Greater Glasgow and Clyde, Glasgow.

17. F. Widjaja Foundation Inflammatory Bowel and Immunobiology Research Institute, Cedars-Sinai Medical Center, Los Angeles. USA

\*Supplementary appendix

**Address for correspondence**

Dr Tariq Ahmad D. Phil FRCP

Exeter IBD and Pharmacogenetics Research Group, RILD building, Barrack Road, Exeter, EX2 5DW. Direct Dial: + 44 (0) 1392 406218

Email: tariq.ahmad1@nhs.net

The protocol can be found at https://www.ibdresearch.co.uk/pants/

ClinicalTrials.gov Identifier: NCT03088449

Abstract

**Background**

Anti-tumour necrosis factor (TNF) drugs have revolutionised Crohn’s disease management but treatment failure is common. We sought clinical and pharmacokinetic factors that predict and mitigate primary non-response at week 14, non-remission at week 54, and adverse events leading to drug withdrawal.

**Methods**

The personalised anti-TNF therapy in Crohn’s Study (PANTS) is a prospective observational UK-wide study in 1610 biologic naïve patients with active luminal Crohn’s disease (955 infliximab [753 Remicade; 202 biosimilar CT-P13] and 655 adalimumab).

**Findings**

Primary non-response (PNR) occurred in 23·8% (95% CI 21·4%-26·2%), non-remission in 63·1% (95% CI 60·3%-65·8%), and adverse events curtailed treatment in 7·8% (95% CI 6·6%-9·2%) patients. The only independent risk factor for PNR was low drug level. The optimal week 14 drug levels associated with remission at weeks 14 and 54 were 7 mg/L and 12 mg/L for infliximab and adalimumab, respectively. Continuing standard dosing regimens after PNR was rarely helpful: only 12·4% patients (14/113; 95% CI 6·9%-19·9%) entered remission by week 54. Obesity, smoking, low albumin levels, higher baseline markers of disease activity, and development of anti-drug antibodies were associated with low drug levels, which mediated week 54 non-remission. Immunogenicity rates at week 54 were 62·8% (95% CI 59·0-66·3%) for infliximab and 28·5% (95% CI 24·0-32·7%) for adalimumab. For both drugs, sub-optimal week 14 drug levels were the main determinant of immunogenicity; whilst subsequent anti-drug antibody formation was the principle factor accounting for low drug levels. Combination thiopurine or methotrexate therapy mitigated this risk with similar effect sizes for both anti-TNF drugs and for infliximab led to lower week 54 non-remission rates.

**Interpretation**

Anti-TNF treatment failure is common and associated with low drug levels, partly mediated by immunogenicity. Clinical trials are required to investigate whether personalised induction regimens and treatment-to-target dose intensification improve outcomes.

**Funding**

Charities: CORE, CCUK, C3. Unrestricted grants: Abbvie, Merck Sharp & Dohme, NAPP, Pfizer, Celltrion.

Evidence before this study

We searched PubMed from Jan 1, 1974, to Sept 18, 2018, with the terms “Crohn's disease” AND “antitumor necrosis factor” or “anti-tumor necrosis factor” or “infliximab” or “adalimumab” or “anti TNF” or “anti-TNF” or “anti-tumour necrosis factor” AND “clinical response” or efficacy or “primary non-response" or “immunogenicity.” We cross-checked the reference lists of review articles and landmark studies.

We identified 11 previous systematic reviews, five with meta-analyses: 127 original articles reported factors associated with treatment failure. These studies were mostly retrospective or cross-sectional in design (n=85), conducted in adults (n=110), subject to tertiary centre bias (n=64), restricted to the use of infliximab (n=85), and/or too small to permit predictive multivariable analyses (n=75). In summary, multiple patient, disease, and pharmacokinetic factors, including anti-TNF drug and anti-drug antibody levels, have been implicated in anti-TNF treatment failure, but their relative effects and interactions have not been fully explored and target drug levels have not been validated.

Added value of this study

In the PANTS study, 1610 patients with active luminal Crohn’s disease treated with infliximab (originator [Remicade] and biosimilar [CT-P13]) or adalimumab were recruited from 120 UK hospitals, reflecting ‘real-life’ practice in specialist and non-specialist IBD centres.

Consistent with the registration studies, about one-quarter of patients’ experienced primary non-response, one-third of initial responders lost response, and only one-third were in remission at week 54. Treatment failure, safety, and rates of immunogenicity of infliximab originator were no different to biosimilar.

Effects of clinical variables influencing treatment failure were largely mediated through drug level and/or immunogenicity. The optimal week 14 drug levels associated with remission after induction and at week 54 were 7 mg/L and 12 mg/L for infliximab and adalimumab, respectively. Continuing standard dosing regimens after PNR was rarely helpful: only 12·4% patients entered remission by week 54.

We observed a negative bi-directional relationship between drug level and immunogenicity, – that is low drug levels at week 14 were the main risk factor for immunogenicity by week 54 and conversely immunogenicity through drug clearance was the main cause of low drug levels by week 54. Immunogenicity was twice as common in infliximab-treated than adalimumab-treated patients and combination therapy with a thiopurine or methotrexate mitigated this risk.

Implications of the available evidence

Anti-TNF treatment failure is associated with sub-optimal drug levels suggesting that there is a window of opportunity to improve outcomes. Dose intensification during induction of at-risk individuals (obese patients, smokers and patients with more active disease) and iterative dose adjustment, aiming for higher target drug levels than is currently recommended, may improve the durability and effectiveness of anti-TNF treatment. Reassuringly, treatment failure, safety, and immunogenicity rates of infliximab biosimilar are no different to infliximab originator and therefore remove some of the cost constraints of dose intensification. Thiopurines or methotrexate should be used in all infliximab-treated patients but could be avoided in some patients treated with adalimumab, such as those who do not carry the HLA-DQA1\*05 allele associated with immunogenicity (paper currently under review).

# Background

The anti-TNF monoclonal antibodies, infliximab and adalimumab, are effective treatments for patients with Crohn’s disease refractory to conventional therapies. Successful treatment leads to mucosal healing, reduced hospitalizations and surgeries, and improvements in quality of life.1–5

Unfortunately, anti-TNF treatment failure is common: 10-40% of patients fail to respond to induction therapy (primary non-response, PNR),6–8 24-46% of patients suffer secondary loss of response in the first year of treatment,9 and approximately 10% suffer an adverse drug reaction that curtails treatment.10

Multiple patient, disease, and drug related factors have been implicated in anti-TNF treatment failure11 but their relative effects, interactions, and impact on drug and anti-drug antibody levels have not been explored in an adequately powered prospective study.

Early identification of patients at-risk of treatment failure may help direct monitoring, early dose optimisation, and use of mitigating strategies to allow these drugs to be used in a safer, more cost-effective manner.

The overarching aims of The Personalised Anti-TNF Therapy in Crohn’s Disease study (PANTS) study were to build a biorepository to investigate the genetic and other -omic factors associated with anti-TNF treatment failure in patients with active luminal Crohn’s disease. Here, we report the clinical and pharmacokinetic factors that predict and mitigate anti-TNF treatment failure in the first year of treatment.

# Methods

## Study design and participants

PANTS is a UK-wide, multicentre, prospective observational cohort reporting the treatment failure rates of the anti-TNF drugs infliximab (originator, Remicade [Merck Sharp & Dohme, UK] and biosimilar, CT-P13 [Celltrion, South Korea]) and adalimumab (Humira [Abbvie, USA]) in anti-TNF-naïve patients with active luminal Crohn’s disease.

Patients were recruited at the time of first anti-TNF exposure from 120 NHS trusts across the United Kingdom (appendix, pp 3-10) between February 2013 and June 2016. Patients were studied for 12 months or until drug withdrawal.

Patients were screened for inclusion at time of decision to treat with an anti-TNF drug, and no more than four weeks prior to starting the drug. The eligibility criteria were:

1. Aged ≥6 years.
2. Crohn's disease involving the colon and/or small intestine.
3. Active luminal disease supported by C-reactive protein (CRP) >3 mg/L in 90 days prior to first dose and/or faecal calprotectin >50 µg/g between 90 days prior and 28 days after first dose.

Exclusion criteria included prior exposure to, or contraindications for the use of, anti-TNF therapy.

## ****Procedures****

The choice of anti-TNF was at the discretion of the treating physician and prescribed according to the licensed dosing schedule.

Study visits were scheduled at first dose (week 0), post-induction (week 14), and at weeks 30 and 54. Additional visits were planned for infliximab-treated patients at each infusion and for both groups at treatment failure or exit. In cases where the visit did not exactly occur on the day delineated by the protocol, the following windows of eligibility were specified: week 0 (week -4 to 0), week 14 (week 10 to 20), week 30 (week 22 to 38), and week 54 (week 42 to 66) (appendix pp 12-13).

### Clinical variables

At baseline, sites recorded demographic data, smoking status, age at diagnosis, disease duration, Montreal Classification,15 prior medical and drug history, and previous Crohn’s disease-related surgeries. At every visit, disease activity score, weight, current therapy, and adverse events were recorded.

### Laboratory variables

Blood and stool samples were processed through the central laboratory at the Royal Devon & Exeter NHS Foundation Trust (https://www.exeterlaboratory.com/). Drug and total anti-drug antibody concentration were measured with IDKmonitor**®** ELISA assays (Immundiagnostik AG, Bensheim, Germany) performed on the Dynex DS2 ELISA robot (Dynex technologies, Worthing, UK) (appendix p 11). For all infliximab-treated patients we measured trough drug levels, excluding levels taken at other time points. For adalimumab-treated patients, we asked research sites to take blood samples as near as possible to trough whilst minimising inconvenience to patients.

We chose a drug tolerant anti-drug antibody assay which allowed us to identify all patients with immunogenicity regardless of circulating drug level. We defined immunogenicity by an anti-drug antibody titre ≥10 AU/ml, based on the 99th centile of drug-naïve controls and stratified this by the presence or absence of detectable drug (<0·8 mg/L). Investigators were blinded to these data until week 54.

## ****Outcomes****

Treatment failure endpoints were primary non-response at week 14, non-remission at week 54 and adverse events leading to drug withdrawal. We used composite end-points defined using symptom scores (Harvey Bradshaw Index [HBI] in adults12 and the HBI or Short Paediatric Crohn’s Disease Activity Index [sPCDAI] in children),13 corticosteroid use, and CRP (appendix p 14).

*Primary non-response (PNR)*: exit prior to week 14 for treatment failure (including resectional IBD surgery) or corticosteroid use at week 14 (new prescriptions or failure to taper). Patients who exhibited both a failure of CRP to fall to ≤3 mg/L or by 50% from baseline (week 0) and failure of HBI to fall to ≤4 or by 3 points were also classified as PNR. For children, a failure of sPCDAI to fall to <15 or by more than 12·5 points was used.

*Grey zone* (intermediate between PNR and response): CRP falls to ≤3mg/L or by 50% from baseline (Week 0) or HBI falls to ≤4 or by 3 points from baseline (but not both).

*Response*: both CRP falls to ≤3mg/L or by 50% from baseline (Week 0) and HBI falls to ≤4 or by 3 points from baseline.

*Remission*: CRP of ≤3 mg/L and HBI of ≤4 points (sPCDAI ≤15), no ongoing steroid therapy, and no exit for treatment failure.

*Loss of response* *(LOR)* was defined in patients who did not have PNR as symptomatic IBD activity that warranted an escalation of steroid, immunomodulatory or anti-TNF therapy, resectional surgery, or exit from study due to treatment failure.9 Timing of LOR was defined as the time of treatment escalation, drug withdrawal, or surgery.

*Non-Remission* was assessed at week 54 and defined as CRP of >3mg/L or HBI of >4 points (sPCDAI >15), ongoing steroid therapy, or exit for treatment failure.

*Exit:* Patients exited the study when they stopped anti-TNF therapy or had an intestinal resection. Patients who exited the study for treatment failure were deemed to be in non-remission for subsequent time points. Patients who exited the study for loss to follow-up, withdrawal of consent, or elective withdrawal of drug, including for pregnancy, were censored at the time of study exit and excluded from the denominator for subsequent analyses.

We defined steroid therapy for the purposes of non-remission and PNR as any systemic therapy, either oral or intravenous (including use of steroids for other conditions), but not including single pre-infusion dosing with hydrocortisone.

## ****Adverse events****

Adverse events (AEs) were coded centrally according to the Medical Dictionary for Regulatory Activities (MedDRA) version 20·1. MedDRA is the international medical terminology developed under the auspices of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH).

Serious AEs included those that required hospitalization, were life-threatening, or resulted in persistent/permanent or significant disability/incapacity. Causality was graded according to the Good Clinical Practice framework guidelines as ‘not related’, ‘unlikely’, ‘possibly’, ‘probably’, or ‘definitely’ by the local research sites.14

## Statistical analysis

## Study size

At cohort inception, sample size was based on identifying a genetic predictor of PNR. Assuming a PNR rate of 0·2 and a perfectly-tagged risk allele of 25% frequency, we calculated the need to recruit 240 non-responders and 960 responders to yield 99% and 33% power to detect genome wide significant association (P<5×10-8) for relative risks of 2 and 1·5 respectively. We anticipated an attrition rate of 20% of patients and so our recruitment target was 1600 patients.

Secondly, in February 2015, the infliximab biosimilar, CT-P13, became available in the UK. We calculated that a sample size of 180 CT-P13-treated patients would permit a comparison of non-inferiority of CT-P13 and Remicade based on power of 80%, our observed PNR rate of 25%, non-inferiority margin of 10%, attrition rate of 20%, and a ratio of CT-P13 to Remicade-treated patients of 1:4.15

## Patients excluded from effectiveness analyses

Following central monitoring, we identified three groups of patients who we subsequently excluded from the effectiveness analyses: 1. patients with stomas in whom the HBI and sPCDAI are not validated; 2. patients that were recruited into the study with normal pre-screening and visit 1 calprotectin and CRP levels; 3. patients where the only indication for anti-TNF was perianal disease. Because these patients had received drug, we included them in our immunogenicity and safety analyses.

**Statistical methods**
Because of differences in drug formulation, route of delivery, dosing interval, and potential for inducing immune response, infliximab and adalimumab treatment outcomes were analysed separately.16

Outcomes were assigned using an algorithm written in R 3·5·1 (R Foundation for Statistical Computing, Vienna, Austria). All analyses were two tailed and p-values <0·05 were considered significant.

Patients who exited the study for treatment failure were deemed to be in non-remission for every subsequent time point. Patients that exited the study for loss to follow-up, patient withdrawal of consent or elective withdrawal or drug by physician, including for pregnancy, were censored at the time of study exit and were excluded from the denominator for subsequent analyzes.

We performed univariable analyses using Fisher’s exact and Mann-Whitney U tests to identify differences in baseline characteristics between infliximab- and adalimumab-treated patients, and to determine categorical and continuous factors associated with predefined outcomes. Multivariable logistic regression analyses were used to confirm which factors were independently associated with treatment failure. We included variables with univariable p<0·05 in the model and used Akaike Information Criterion (AIC) and backward stepwise variable selection. We also built predictive models, using forwards and backwards stepwise model selection starting from the null model, again using AIC. We used leave-one-out cross validation to test the model, firstly to ensure the model was not overfitted and secondly to estimate the diagnostic accuracy of the model. For prediction testing, a probability threshold was determined by maximising the sum of sensitivity and specificity.

We explored associations with drug concentration using linear regression, using the same variable selection methods as those detailed above for logistic regression.Rates of immunogenicity and LOR were estimated using the Kaplan-Meier method, and comparative analyses were performed using univariable and multivariable Cox proportional hazards regression. For immunogenicity, patients were censored after their last drug and antibody measurement or at week 54. For LOR, patients were censored if they exited for reasons other than treatment failure or at week 54.

Optimal thresholds for drug levels were determined graphically by plotting outcome against intervals of drug level and looking for the threshold beyond which further increases were not associated with improvement in outcome.

Non-inferiority for biosimilar infliximab was assessed by determining whether the one-sided 95% confidence interval of the absolute difference in proportions was ≥10%. The confidence interval was calculated using the prop.test function in R.

## Role of the funding source

PANTS is an investigator-led study funded by the research charities CORE, CCUK, and C3, and by unrestricted educational grants from Abbvie (USA), Merck Sharp & Dohme (UK), NAPP Pharmaceuticals (UK), Pfizer (USA), and Celltrion Healthcare (South Korea). No funding bodies had any role in study design, data collection or analysis, writing or decision to submit for publication.

# Results

## Participants

1610 patients, 955 [59·3%] treated with infliximab (Remicade 753 [46·8%], CT-P13 202 [12·5%]) and 655 [40·7%] treated with adalimumab were included between 07/03/2013 and 15/07/2016 (figure 1). Differences between demographic and clinical characteristics of infliximab- and adalimumab-treated patients are shown in table 1 and appendix pp 34-35.

There were significant differences at baseline between the infliximab- and adalimumab-treated patients, including age, smoking, body mass index, disease duration, disease location, and disease behaviour. Patients treated with infliximab had more active disease at baseline as evidenced by higher Harvey-Bradshaw indices, serum CRP, and faecal calprotectin levels (table 1). Most differences persisted when the 219 paediatric patients (<18 years at time of first dose) were excluded; almost all of whom were treated with infliximab (appendix p 34). At the initiation of anti-TNF treatment, immunomodulator use was higher in patients treated with infliximab (61% vs. 51% P·<0·0001) but no differences were seen in the proportions of patients treated with corticosteroids.

## Treatment failure rates

PNR occurred in 21·9% (170/775; 95% CI 19·1-25·0%) of patients treated with infliximab and 26·8% (125/466; 95% CI 22·9 – 31·1%) of patients treated with adalimumab (table 2). After excluding primary non-responders, the estimated rate of LOR by week 54 for infliximab was 36·9% (95% CI 32·7-40·9%) and for adalimumab was 34·1% (28·4-39·4%) (appendix pp 15-16). At week 54, non-remission rates were 60·9% (469/770; 95% CI 57·4-64·4%) for infliximab-treated patients and 66·9% (295/441; 95% CI 62·3-71·3%) for adalimumab-treated patients (table 2).

## Factors associated with treatment failure

### Primary non-response

Univariable analyses demonstrated the strongest associations with PNR to infliximab and adalimumab were with week 14 drug and anti-drug antibody levels (table 3, appendix pp 17, 36). PNR to infliximab was also associated with older age at first dose, current smoking status, non-use of an immunomodulator, and lower baseline albumin levels. PNR to adalimumab was associated with obesity.

Multivariable analyses confirmed that, for both drugs, only week 14 drug level was associated independently with PNR (table 4). A dose-response association was seen for week 14 drug concentration and remission up to 7 mg/L for infliximab and 12 mg/L for adalimumab (appendix p 18). For infliximab-treated patients, for whom we also collected week 6 levels, a dose-response association was seen between these levels and increasing week 14 remission rates up to 30-35 mg/L (appendix p 19). Our predictive models of PNR to infliximab and adalimumab, however, were not clinically useful (appendix pp 20, 37). For infliximab, the AUC was 0·527 (0·461-0·593) with sensitivity 0·350, specificity 0·753, PPV 0·275, and NPV 0·812, while for adalimumab the AUC was 0·539 (0·462-0·616) with sensitivity 0·350, specificity 0·952, PPV 0·560, and NPV 0·777.

### Non-remission at week 54

Univariable analysis demonstrated, for both drugs, the major determinant of non-remission at week 54 was clinical status at week 14 (table 3, appendix pp 21-22, 38). Despite meeting PNR criteria, 44·7% (76/170; 95% CI 37·1%-52·5%) of infliximab- and 48·8% (61/125; 95% CI 39·8%-57·9%) of adalimumab-treated patients continued drug beyond week 20: of these, only 14·9% (10/67; 95% CI 7·4%-25·7%) and 8·7% (4/46; 95% CI 2·4%-20·8%), respectively, were in remission at week 54.

The optimal drug level at week 14 associated with remission at week 54 was also 7 mg/L for infliximab and 12 mg/L for adalimumab (appendix p 18). In addition, amongst infliximab- but not adalimumab-treated patients, non-remission was associated with immunogenicity at week 14 (table 3).

Amongst patients who continued treatment beyond week 14, multivariable analyses (table 4) demonstrated, for both drugs, independent associations with drug level and obesity and non-remission at week 54. Smoking at baseline and no prior history of perianal disease were associated with poorer outcomes for both drugs on univariable analyses, but only for adalimumab on multivariable analyses.

### Predicting non-remission at week 54

We devised two diagnostic models informed by the above univariable factors to predict non-remission at week 54 to infliximab and adalimumab. Our first model attempted to predict non-remission using baseline variables only and had limited diagnostic value. Our second model using baseline variables and week 14 pharmacokinetic data had greater predictive power: for infliximab, the AUC was 0·814(0·760-0·868) and for adalimumab 0·752 (0·678-0·826) (appendix pp 20, 39-41).

## Adverse events

Serious AEs, excluding worsening of Crohn’s disease activity, were observed in 17·9% (171/955; 95% CI 15·5%-20·5%) of infliximab- and 14·7% (96/655; 95% CI 12·0%-17·6%) of adalimumab-treated patients. AEs leading to treatment withdrawal occurred in 8·8% (84/955; 95% CI 7·1%-10·8%) and 6·4% (42/655; 95% CI 4·7%-8·6%) patients, respectively (appendix p 42).

The five patients who died (four treated with infliximab) were in the upper quartile for age, and none responded to treatment by the time of death: two died of pneumonia, two died of intra-abdominal sepsis, and one of Crohn’s disease-related malnutrition. Four of the five patients were taking concomitant corticosteroids at time of death and one was taking azathioprine.

Serious infections were reported in 4·0% (38/955; 95% CI 2·8%-5·4%) of infliximab-treated patients, including active tuberculosis in three patients, and 3·2% (21/655; 95% CI 2·0%-4·9%) of adalimumab-treated patients. Concomitant immunomodulatory therapy was not associated with an increased risk of infections, even when stratified by age (appendix p 43).

Infusion reactions within 24 hours of infliximab, which occurred after a median of 5 weeks (IQR 1-14) of starting treatment, were experienced by 3·2% (31/955; 95% CI 2·2-4·6%) patients and were associated with anti-drug antibody titre (median peak antibody 96 AU/mL (IQR 5-313) vs. 8 AU/mL (IQR 5-45), P=0·004). Injection site reactions to adalimumab, which occurred after a median of 14 weeks, were observed in 4·3% (28/655) patients but were not associated with immunogenicity.

## Pharmacokinetics

### Drug Level

Univariable factors associated with low drug levels at weeks 14 and 54 are shown in appendix pp 23-25, 44-45. At both time-points, the strongest independent association with low drug levels was development of anti-drug antibodies. In addition, low drug levels were associated with markers of active disease (high CRP and faecal calprotectin levels at baseline, weeks 14 and 54). Lower albumin levels and obesity were independently associated with week 14 drug levels for infliximab and adalimumab, respectively. In infliximab-treated but not adalimumab-treated patients, low drug levels at week 54 was also associated with anti-TNF monotherapy (table 3).

### Immunogenicity

The estimated rate of immunogenicity by week 54 (defined as anti-drug antibody concentration ≥ 10 AU/mL) was 62·8% (95% CI 59·0-66·3%) for infliximab and 28·5% (95% CI 24·0-32·7%) for adalimumab (appendix p 25). When defined as concentration ≥10 AU/mL and concurrent undetectable drug level, the rates were 31·2% (95% CI 27·6-34·6%) for infliximab and 12·3% (95% CI 8·9-15·6%) for adalimumab (appendix p 27).

For infliximab treated patients, for whom early anti-drug antibody levels were available, the point-prevalence of anti-drug antibody positivity at weeks 2, 6 and 14 were 3·9%, 5·0% and 14·6% respectively.

The univariable factors associated with the time to immunogenicity are shown in figure 2 and appendix p 46. Multivariable analyses demonstrated that week 14 drug level was the major independent risk factor associated with development of immunogenicity for both drugs. In addition, time to immunogenicity was associated with obesity for both drugs and smoking for infliximab-treated patients (table 4).

Immunomodulator use was the major protective factor against immunogenicity, with similar effect sizes for infliximab and adalimumab. There was no difference in time to immunogenicity between thiopurine medications or methotrexate (appendix pp 29-30). Thiopurines reduced immunogenicity to infliximab in a dose-dependent manner with the lowest rates of immunogenicity seen in patients treated with the highest thiopurine doses (appendix p 31).

Sensitivity analyses exploring the effect of combination immunomodulator use on clinical outcomes demonstrated that immunomodulator use was associated with lower week 54 non-remission rates in infliximab-treated patients (combination: 52·6% (95% CI 47·9%-57·1%) vs. monotherapy: 74·0% (95% CI 68·6%-78·9%)), but not adalimumab-treated patients (combination: 64·2% (95% CI 57·6%-70·4%) vs monotherapy: 69·8% (95% CI 63·1%-75·9%)). Further sensitivity analyses of infliximab-treated patients, limited to the modifiable factors of immunomodulator use and drug and antibody levels, demonstrated the benefit of immunomodulators was independent of drug level or immunogenicity status at week 14 (appendix p 47).

## Biosimilar versus originator infliximab

There were no differences in baseline demographic or clinical characteristics between patients treated with CT-P13 and Remicade (appendix p 48). Of 955 patients treated with Remicade, 79 (8·3%) switched to CT-P13 during the first year and were excluded from analyses comparing originator and biosimilar infliximab after the switch date. At week 14, CT-P13 was non-inferior to Remicade for PNR (difference in proportions -3·9%, one sided 95% CI upper bound 2·4%). At week 54, CT-P13 was non-inferior to Remicade for non-remission (difference in proportions -2·2%, one sided 95% CI upper bound 5·6%) (appendix p32).

# **Discussion**

Primary non-response occurred in 23·8%, non-remission in 63·1%, and adverse events curtailed treatment in 7·8% patients. Obesity, smoking, low albumin levels, higher baseline markers of disease activity, and development of immunogenicity were associated with low drug levels, which mediated non-remission.

## Low drug levels

Numerous studies reported a relationship between drug level and clinical outcome, although the therapeutic thresholds, particularly for adalimumab, are poorly defined.17 In our study, low drug levels during induction were associated with PNR at week 14 and non-remission at week 54. Patients with PNR who continued standard dosing regimens rarely entered remission; few had optimal drug levels suggesting true pharmacodynamic-PNR is uncommon. Despite variation in drug level amongst remitters, our data suggests higher target drug levels during induction than those reported in previous studies,18 likely reflecting our more stringent definition of remission. At week 14 the optimal drug levels associated with remission at both week 14 and 54 were 7 mg/L and 12 mg/L for infliximab and adalimumab, respectively. The importance of drug level is further demonstrated by our predictive models which were only clinically useful when pharmacokinetic data were included.

Previous prospective randomised studies of proactive dose increase based on drug levels have failed to demonstrate improved clinical outcomes. For both the TAXIT19 and TAILORIX20 studies, this might in part be explained by inclusion of patients after the critical induction period and adoption of lower infliximab thresholds of 3 mg/L. Further adequately powered clinical trials are required to investigate whether treatment-to-target drug level during the induction period improves outcomes. 21

## **Immunogenicity**

Using analytical platforms that differed only by target antibody, we have shown that immunogenicity is more common in patients treated with infliximab than adalimumab: an observation frequently attributed to the chimeric formulation of infliximab. For both drugs, however, we observed a bi-directional negative relationship between drug level and immunogenicity. The lowest drug levels were seen in patients with high titre antibody levels in keeping with their impact on drug clearance. Conversely, low drug levels at week 14 were associated with an increased risk of immunogenicity by week 54. This is consistent with the discontinuity theory of the immune response which proposes that intermittent exposure to antigen promotes a persistent immune reaction, whilst exposure at constant levels, observed with subcutaneously bi-weekly delivered adalimumab, induces an immune tolerance.22 Immunogenicity, which we have shown may occur earlier than previously appreciated may be mitigated by early dose optimisation, minimising LOR.23 We accept, however, that this observation might be explained by the formation of anti-drug antibodies at sufficient concentration to lower the drug level but not detectable by our assay.

## **Immunomodulators**

Immunomodulator use was associated with lower rates of immunogenicity to both drugs and higher drugs levels for infliximab-treated patients. Methotrexate exerted a similar effect to thiopurine drugs on immunogenicity. In contrast to previous reports24,25 we demonstrated for infliximab-treated patients that thiopurines reduced immunogenicity in a dose-dependent manner without an obvious threshold effect. Post-hoc analyses of the SONIC study suggested that the primary benefit of azathioprine was on pharmacokinetics of infliximab. 26 Conversely, here we demonstrated concomitant immunomodulator use in infliximab-treated patients was associated with higher week 54 remission rates independent of week 54 drug level or immunogenicity status, suggesting that the addition of immunosuppression to anti-TNF therapy may have additional benefits. Consistent with previous studies,27 immunomodulator use was not associated with increased remission rates to adalimumab; however, this may reflect lower rates of immunogenicity and/or the short duration of follow-up.

Obesity and smoking

We have demonstrated that obesity is independently associated with low drug levels and non-remission at week 54. Our data suggest that the previously reported associations of obesity and PNR are likely to mediated by low drug levels.28 For adalimumab-treated patients, fixed dosing was probably a major contributing factor. Infliximab-treated patients, however, received weight-based dosing, so this association suggests a non-linear relationship between weight and volume of distribution. Obesity was also associated with immunogenicity to both drugs: further clinical trials of dose optimisation are needed to clarify if this was because of sub-optimal dosing during induction or whether obesity contributes to immunogenicity directly.

Our observation that cigarette smoking was independently associated with an increased risk of immunogenicity to infliximab may explain the poorer, less durable, anti-TNF response rates reported in patients with Crohn’s disease who smoke.29

Disease activity and albumin

Previous studies investigating the relationship between baseline markers of inflammation and anti-TNF response are conflicting.2,30 In our study, higher baseline markers of inflammation predicted lower drug levels at week 14, suggesting higher inflammatory load, contributes to faster drug elimination. We, like others, have shown that lower baseline albumin levels predict suboptimal week 14 drug levels.31 This association may reflect increased drug clearance as well as higher faecal protein losses.

Adverse events

Data from a nationwide population-based study suggest that benefits of anti-TNF and immunomodulatory combination treatment needs to be balanced against the additional risks of serious and opportunistic infection.32 In this study, accepting our smaller sample size and shorter duration of follow-up, combination therapy was not associated with an increased risk of infection in the first year of treatment, even amongst older patients. However, sepsis was the cause of death in four of the five patients who died in the first year: all were aged over 50 years, all but one was prescribed concomitant corticosteroid, and none had responded to anti-TNF, but nevertheless continued treatment.

## Limitations

Firstly, we used pragmatic definitions of treatment failure combining corticosteroid use with clinical and biochemical markers of disease activity that are closely aligned to routine treatment targets. Whilst we accept that our data would have been strengthened by endoscopic outcomes, we observed a significant association between clinical outcomes at weeks 14 and week 54 and faecal calprotectin (appendix p 33). We are likely to have underestimated the rate of LOR because our definition required an increase in therapy which was not always initiated. In addition, we employed a pragmatic schedule of visits to minimise inconvenience to patients and fewer assessments were undertaken for adalimumab- than infliximab-treated patients. We acknowledge, because CRP is elevated in obesity, that we may have overestimated the effect of BMI on treatment failure. Finally, we did not employ real-time monitoring, therefore, missingness rates are higher in this study compared to registration trials.6–8

## Conclusion

In this, the largest prospective study of anti-TNF therapy in IBD to our knowledge, we have demonstrated that the major modifiable factors associated with treatment failure were low drug levels and immunogenicity. Concomitant immunomodulator use and dose intensification in at-risk individuals during induction may improve the effectiveness and durability of treatment. Reassuringly, treatment failure, safety, and immunogenicity rates of originator infliximab are no different to biosimilar, therefore, removing some of the cost-constraints of dose intensification. Further clinical trials are required to confirm whether these strategies allow us to improve the effectiveness and durability of anti-TNF therapy.

## Author contributions

T.A, C.B, G.A.H, J.R.F.C, A.H, M.P, J.C.M, P.I, J.L, R.K.R, C.L participated in the conception and design of the work: C.B was the project manager and coordinated recruitment. T.M, M.H.P and R.N coordinated all biochemical analysis and central laboratory aspects of the project. N.K, J.R.G, T.A, G.A.H, J.R.F.C, C.W.L, A.H, M.P, S.S, J.C.M, P.M.I, J.L, R.K.R, P.H, N.M.H, D.McG, A.T, G.J.W, N.C, S.L, S.B, D.G were involved in the acquisition, analysis or interpretation of data. The data analysis was performed by N.A.K, G.A.H, H.D.G, B.H. Drafting of the manuscript was conducted by T. A, J.R.G, N.A.K, D.McG, G.A.H, T.M, C.B, N.C, S.L. All the authors contributed to the critical review and final approval of the manuscript. T.A obtained the funding for the study.

## Conflicts of Interest Disclosures

All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest and declare: N.A.K received personal fees from Falk, Takeda, Pharmacosmos and other from Janssen, and non-financial support from Janssen, AbbVie and Celltrion outside the submitted work; G.A.H reports non-financial support from AbbVie, outside the submitted work; and that he is now an employee of AbbVie and owns stock in the company; G.J.W reports grants from UK Crohn's Colitis, personal fees from AbbVie, Janssen, Tillotts, Falk, outside the submitted work; A.H has served as consultant, advisory board member or speaker for AbbVie, Atlantic, Bristol-Myers Squibb, Celltrion, Falk, Ferring, Janssen, MSD, Napp Pharmaceuticals, Pfizer, Pharmacosmos, Shire and Takeda and also serves on the Global Steering Committee for Genentech outside the submitted work;. S.S reports personal fees from AbbVie, Merck, Takeda, grants from Takeda, Tillotts, AbbVie, Merck, outside the submitted work; P.M.I reports personal fees from Abbvie, Janssen, Pfizer, Sandoz, VH squared, Samsung Bioepis, and Ferring , grants from MSD, grants and personal fees from Takeda, non-financial support from Falk, outside the submitted work; D.McG reports grants from NIH and Helmsley Charitable Trust, during the conduct of the study; grants and personal fees from Janssen, Precision IBD Inc, Second Genome, Qu Biologics, Pfizer, Gilead and Takeda, outside the submitted work; C.W.L reports grants and personal fees from Abbvie, GSK, Janssen, Takeda, Amgen, Pfizer, Samsung Bioepis, Cellgene and grants and personal fees from Gilead, outside the submitted work; J.R.F.C reports personal fees from Abbvie, grants and personal fees from Takeda, grants and personal fees from Biogen, personal fees from Janssen, grants and personal fees from Hospira, personal fees from MSD, from Celltrion, personal fees from NAPP, personal fees from Sandoz, outside the submitted work; J.L has served as advisory board member for Atlantic Health, AbbVie UK/Global, MSD UK, Shire UK, Ferring International, Celltrion, Takeda, Pfizer, Janssen, GSK, and consultant for AbbVie UK, Takeda, Bristol Myers Squibb, has received grants from Tadeka, and Hospira (Pfizer) for AbbVie UK, Global, Ferring (International/UK) MSD, Allergen, Shire (UK/International), Takeda, Cornerstone US, Janssen and Pfizer, fees for educational presentations for AbbVie International and Cornerstone UK and travel/accommodation expenses for AbbVie, Warner Chilcot UK and Takeda and has received travel support from AbbVie, Warner Chilcot, Takeda and Pfizer outside the submitted work; R.K.R reports honoraria from Abbvie, Ferring , Therakos and Celltrion, grants from Nestec, outside the submitted work; J.R.G received honoraria from Falk, Abbvie and Shield therapeutics for unrelated topics. T.A reports grants from AbbVie and MSD, grants and other from NAPP, grants from Celltrion, grants from Pfizer, personal fees and non-financial support from Immunodiagnostik, grants from Celgene, during the conduct of the study; personal fees and non-financial support from NAPP, personal fees and non-financial support from AbbVie , personal fees and non-financial support from MSD , personal fees from Celltrion, personal fees from Pfizer, grants and personal fees from Takeda, grants from Janssen, grants and non-financial support from Tillotts, outside the submitted work. No financial relationships with any organizations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work. H.G, B.H, C.B, M.H.P, T.M, A.T, R.N, S.B, N.C, N.M.H, P.H, S.L, M.P, J.C.M have no conflicts of interest to declare.

# Acknowledgements

Laboratory tests were undertaken by the Exeter Blood Sciences Laboratory at the Royal Devon & Exeter (RD&E) NHS Trust (https://www.exeterlaboratory.com/). The Exeter NIHR Clinical Research Facility coordinated sample storage and management.

The sponsor of the study was the Royal Devon and Exeter NHS Foundation Trust. The South West Research Ethics committee approved the study (REC Reference: 12/SW/0323) in January 2013.

T.M is funded by an NIHR Health Education England (HEE) Senior Lectureship. The views expressed in this publication are those of the author(s) and not necessarily those of the NHS, HEE, NIHR or the Department of Health.

Role of the Funder/Sponsor:  None of the listed funders had a role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, and decision to submit the manuscript for publication.

Additional Contributions: We are grateful to the following for their participation in our interim data interpretation meeting: Professors Shomron Ben-Horin, Yehuda Chowers and Geert D’Haens and Drs John Ding and James Lee. We would like to thank the patients who participated in this study, the IBD Pharmacogenetic Study Group (see supplementary appendix), and research nurses who collected clinical data and biological samples at each study visit. We would also like to acknowledge the study co-ordinators of the Exeter IBD Research Group, United Kingdom: Marian Parkinson and Helen Gardner-Thorpe for their on-going administrative support to the study.

# References

1 Lichtenstein GR, Yan S, Bala M, Blank M, Sands BE. Infliximab maintenance treatment reduces hospitalizations, surgeries, and procedures in fistulizing Crohn’s disease. *Gastroenterology* 2005; **128**: 862–9.

2 Colombel JF, Sandborn WJ, Reinisch W, *et al.* Infliximab, azathioprine, or combination therapy for Crohn’s disease. *N Engl J Med* 2010; **362**: 1383–95.

3 D’haens G, Van Deventer S, Van Hogezand R, *et al.* Endoscopic and histological healing with infliximab anti-tumor necrosis factor antibodies in Crohn’s disease: A European multicenter trial. *Gastroenterology* 1999; **116**: 1029–34.

4 Louis E, Löfberg R, Reinisch W, *et al.* Adalimumab improves patient-reported outcomes and reduces indirect costs in patients with moderate to severe Crohn’s disease: results from the CARE trial. *J Crohns Colitis* 2013; **7**: 34–43.

5 Loftus E V, Feagan BG, Colombel J-F, *et al.* Effects of adalimumab maintenance therapy on health-related quality of life of patients with Crohn’s disease: patient-reported outcomes of the CHARM trial. *Am J Gastroenterol* 2008; **103**: 3132–41.

6 Rutgeerts P, D’Haens G, Targan S, *et al.* Efficacy and safety of retreatment with anti-tumor necrosis factor antibody (infliximab) to maintain remission in Crohn’s disease. *Gastroenterology* 1999; **117**: 761–9.

7 Hanauer SB, Sandborn WJ, Rutgeerts P, *et al.* Human anti-tumor necrosis factor monoclonal antibody (adalimumab) in Crohn’s disease: the CLASSIC-I trial. *Gastroenterology* 2006; **130**: 323–33; quiz 591.

8 Colombel J-F, Sandborn WJ, Rutgeerts P, *et al.* Adalimumab for maintenance of clinical response and remission in patients with Crohn’s disease: the CHARM trial. *Gastroenterology* 2007; **132**: 52–65.

9 Ben-Horin S, Chowers Y. Review article: loss of response to anti-TNF treatments in Crohn’s disease. *Aliment Pharmacol Ther* 2011; **33**: 987–95.

10 Sprakes MB, Ford AC, Warren L, Greer D, Hamlin J. Efficacy, tolerability, and predictors of response to infliximab therapy for Crohn’s disease: a large single centre experience. *J Crohns Colitis* 2012; **6**: 143–53.

11 Naviglio S, Giuffrida P, Stocco G, *et al.* How to predict response to anti-tumour necrosis factor agents in inflammatory bowel disease. *Expert Rev Gastroenterol Hepatol* 2018; **12**: 797–810.

12 Harvey RF, Bradshaw JM. A simple index of Crohn’s-disease activity. *Lancet (London, England)* 1980; **1**: 514.

13 Kappelman MD, Crandall W V, Colletti RB, *et al.* Short pediatric Crohn’s disease activity index for quality improvement and observational research. *Inflamm Bowel Dis* 2011; **17**: 112–7.

14 World Health Organisation. The use of the WHO-UMC system for standardised case causality assessment-1-The use of the WHO-UMC system for standardised case causality assessment. .

15 Chow S-C, Shao J, Wang H. Sample size calculations in clinical research. Marcel Dekker, 2003.

16 Kugathasan S, Denson LA, Walters TD, *et al.* Prediction of complicated disease course for children newly diagnosed with Crohn’s disease: a multicentre inception cohort study. *Lancet* 2017; **6736**: 1–9.

17 Vande Casteele N, Herfarth H, Katz J, Falck-Ytter Y, Singh S. American Gastroenterological Association Institute Technical Review on the Role of Therapeutic Drug Monitoring in the Management of Inflammatory Bowel Diseases. *Gastroenterology* 2017; **153**: 835–857.e6.

18 Cornillie F, Hanauer SB, Diamond RH, *et al.* Postinduction serum infliximab trough level and decrease of C-reactive protein level are associated with durable sustained response to infliximab: a retrospective analysis of the ACCENT I trial. *Gut* 2014; **63**: 1721–7.

19 Vande Casteele N, Ferrante M, Van Assche G, *et al.* Trough concentrations of infliximab guide dosing for patients with inflammatory bowel disease. *Gastroenterology* 2015; **148**: 1320–9.e3.

20 D’Haens G, Vermeire S, Lambrecht G, *et al.* Increasing Infliximab Dose Based on Symptoms, Biomarkers, and Serum Drug Concentrations Does Not Increase Clinical, Endoscopic, and Corticosteroid-Free Remission in Patients With Active Luminal Crohn’s Disease. *Gastroenterology* 2018; **154**: 1343–1351.e1.

21 Van Stappen T, Bollen L, Vande Casteele N, *et al.* Rapid Test for Infliximab Drug Concentration Allows Immediate Dose Adaptation. *Clin Transl Gastroenterol* 2016; **7**: e206.

22 Pradeu T, Jaeger S, Vivier E. The speed of change: towards a discontinuity theory of immunity? *Nat Rev Immunol* 2013; **13**: 764–9.

23 Ungar B, Engel T, Yablecovitch D, *et al.* Prospective Observational Evaluation of Time-Dependency of Adalimumab Immunogenicity and Drug Concentrations: The Poetic Study. *Am J Gastroenterol* 2018; **113**: 890–8.

24 Yarur AJ, Kubiliun MJ, Czul F, *et al.* Concentrations of 6-thioguanine nucleotide correlate with trough levels of infliximab in patients with inflammatory bowel disease on combination therapy. *Clin Gastroenterol Hepatol* 2015; **13**: 1118–24.e3.

25 Roblin X, Boschetti G, Williet N, *et al.* Azathioprine dose reduction in inflammatory bowel disease patients on combination therapy: an open-label, prospective and randomised clinical trial. *Aliment Pharmacol Ther* 2017; **46**: 142–9.

26 Colombel J-F, Reinisch W, Mantzaris GJ, *et al.* Randomised clinical trial: deep remission in biologic and immunomodulator naïve patients with Crohn’s disease - a SONIC post hoc analysis. *Aliment Pharmacol Ther* 2015; **41**: 734–46.

27 Chalhoub JM, Rimmani HH, Gumaste V V, Sharara AI. Systematic Review and Meta-analysis: Adalimumab Monotherapy Versus Combination Therapy with Immunomodulators for Induction and Maintenance of Remission and Response in Patients with Crohn’s Disease. *Inflamm Bowel Dis* 2017; **23**: 1316–27.

28 Singh S, Facciorusso A, Singh AG, *et al.* Obesity and response to anti-tumor necrosis factor-α agents in patients with select immune-mediated inflammatory diseases: A systematic review and meta-analysis. *PLoS One* 2018; **13**: e0195123.

29 Juillerat P, Sokol H, Froehlich F, *et al.* Factors associated with durable response to infliximab in Crohn’s disease 5 years and beyond: a multicenter international cohort. *Inflamm Bowel Dis* 2015; **21**: 60–70.

30 Magro F, Rodrigues-Pinto E, Santos-Antunes J, *et al.* High C-reactive protein in Crohn’s disease patients predicts nonresponse to infliximab treatment. *J Crohns Colitis* 2014; **8**: 129–36.

31 Fasanmade AA, Adedokun OJ, Olson A, Strauss R, Davis HM. Serum albumin concentration: a predictive factor of infliximab pharmacokinetics and clinical response in patients with ulcerative colitis. *Int J Clin Pharmacol Ther* 2010; **48**: 297–308.

32 Kirchgesner J, Lemaitre M, Carrat F, Zureik M, Carbonnel F, Dray-Spira R. Risk of Serious and Opportunistic Infections Associated With Treatment of Inflammatory Bowel Diseases. *Gastroenterology* 2018; **155**: 337–346.e10.

# Figure legends

## Figure 1: Flow of patients through the PANTS study

## Figure 2: Univariable associations of time to immunogenicity using Kaplan Meier and Cox proportional hazards methods

Y axis shows proportion without development of any antibody defined as ≥10 AU/mL. P values and hazard ratios are derived from Cox proportional hazards models for each individual variable. The data for week 14 drug quartile excludes anyone who developed immunogenicity or exited the study or prior to week 14 and is based on the log10 of the drug level, therefore, demonstrate the hazard ratio for each ten-fold increase. Abbreviations: HR hazard ratio; BMI body mass index; w: week.