Randomised trial of ciprofloxacin doxycycline and hydroxychloroquine versus budesonide in active Crohn's disease

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Short title:

Antibiotics and hydroxychloroquine in Crohn's

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Conflicts of interest

JMR has been a member of advisory boards for Atlantic, Pharmacosmos, Procter and Gamble, Vifor and Falk, has received speaking honoraria from Abbott, Allergan, Falk, Ferring, Glaxo Smith Kline, Merck, Procter and Gamble, Schering Plough, Shire, and Wyeth, and with the University of Liverpool and Provexis UK, holds a patent for use of a soluble fibre preparation as maintenance therapy for Crohn's disease plus a patent for its use in antibiotic-associated diarrhoea. Patent also held with the University of Liverpool and others in relation to use of modified heparins in cancer therapy. SS has received speaker fee from MSD, Actavis, Abbvie, Dr Falk pharmaceuticals, Shire and received educational grant from MSD, Abbvie, Actavis and is an advisory board member for Abbvie, Dr Falk Pharma and Vifor. PKF received a Shire Innovation Fund award and has received funded conference travel from Shire and Tillotts. GWH has provided consultancy for Provexis, Nutricia and 4DPharma. AH has served as consultant, advisory board member or speaker for AbbVie, Arena, Atlantic, Bristol-Myers Squibb, Celgene, Celltrion, Falk, Ferring, Janssen, MSD, Napp Pharmaceuticals, Pfizer, Pharmacosmos, Shire and Takeda. She also serves on the Global Steering Committee for Genentech. CP has received speaker fees from Vifor, payment for advisory board attendance from Dr Falk Pharma, and support for attendance to other meetings from Dr Falk Pharma and Vifor. The other authors have no competing interests.

Abstract

Background

Increased mucosa-associated *E.coli* are present in Crohn's disease but their role in pathogenesis is uncertain.

Aims

To assess efficacy and safety of an antibiotic/hydroxychloroquine combination effective against *E.coli* inside macrophages.

Methods

Adults with moderately active disease (CDAI>220-450 plus C reactive protein≥5mg/l and/or faecal calprotectin >250ug/g) were randomised to receive (open label) oral budesonide (Entocort CR 9mg/day 8 weeks, 6mg/day 2 weeks, 3mg/day 2 weeks) or oral ciprofloxacin 500mg bd, doxycycline 100mg bd, hydroxychloroquine 200mg tds for 4 weeks, followed by doxycycline 100mg bd and hydroxychloroquine 200mg tds for 20 weeks. Primary endpoints were remission (CDAI</=150) at 10 weeks, remission maintained to 24 weeks, and remission maintained to 52 weeks. Patients not responding (CDAI fall by >70) by 10 weeks were invited to cross-over onto the alternative therapy.

Results

Fifty-nine patients were recruited across 8 sites. Including cross-over, 39 patients received antibiotics/hydroxychloroquine and 39 received budesonide. At 10 weeks, 24 weeks, 52 weeks on initial therapy only 2/27, 2/27, 1/27 were in remission on antibiotics/hydroxychloroquine compared with 8/32, 1/32, 1/32 on budesonide (P=0.092 at 10 weeks). Withdrawals by 10 weeks due to adverse events were seen in 15 receiving antibiotics/hydroxychloroquine and 6 budesonide. Results including crossover were more promising with 9/24 patients receiving antibiotics/hydroxychloroquine per protocol in

remission by 24 weeks. No correlation was seen between response to antibiotics/hydroxychloroquine and ASCA/OmpC antibody status or disease location.

Conclusion

Overall results with this antibiotic/hydroxychloroquine combination were unimpressive but long-term remission is seen in some patients and justifies further study.

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Keywords: Crohn's disease; antibiotics; ciprofloxacin; doxycycline; hydroxychloroquine; *E. coli*

Short title: Antibiotics and hydroxychloroquine in Crohn's

Introduction

Mucosa-associated *E. coli* are found in Crohn's disease adherent to the intestinal epithelial surface [1], within granulomas [2], mesenteric lymph-nodes [3] and in peripheral blood [4].In the laboratory they translocate across the microfold (M) cells that overlie Peyer's patches and colonic lymphoid follicles [5,6]. They replicate within macrophage vesicles [7,8] and induce granulomas [9]. They lack any consistent genotype [10], and it is unclear whether they are part of the pathogenic process or merely secondary bystanders.

Proof of a pathogenic role depends on showing that therapy targeted to eradicate *E. coli* is beneficial. This entails killing the bacteria replicating within macrophage vesicles [11]. This is not straightforward for some antibiotics such as penicillins and gentamicin are unable to penetrate macrophages. In Crohn's disease meta-analysis has not shown convincing benefit with antibiotics [12]. However, there are positive signals with rifaximin [13], ciprofloxacin (as single agent in one study [14] but usually in combination with metronidazole) [15], and azithromycin with metronidazole [16]. None of these effects have been sufficiently clear for antibiotic therapy to become "mainstream".

Whipple's disease, caused by *Tropheryma whipplei,* and Q-fever, caused by *Coxiella burnetii*, are both unequivocally due to replication of bacteria within intestinal macrophages and are difficult to cure even with long-term antibiotics. One effective strategy is to combine long-term doxycycline, with the antimalarial hydroxychloroquine [17,18]. The rationale for hydroxychloroquine is that many bacteria, including Crohn's disease mucosal *E. coli*, require an acid environment to replicate [8]. Hydroxychloroquine is a mild base that enters

macrophage vesicles and reverses their normal acidification [19]. In the laboratory this substantially enhances killing of *E. coli* by macrophages [20].

We have therefore assessed the efficacy and safety of a combination of short-term ciprofloxacin and longer-term doxycycline with hydroxychloroquine in the treatment of active Crohn's disease. Budesonide was prescribed as comparator as this was standard care for patients with moderately active Crohn's disease at the inception of this trial (2013) [21].

Materials and Methods

Study design

This was an open-label investigator-led randomised controlled trial in adults with moderate to severe Crohn's disease. The aim was to provide an unbiased estimate of the remission rate for the antibiotic/hydroxychloroquine combination to inform subsequent phase 3 trial design. Patients were recruited from 8 Hospitals in England between 2013 and 2018.

The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the North West-Liverpool East Research Ethics Committee of the NHS Health Research Authority on February 6th, 2012 (08/H1002/71). Ethics approval required use of standard-of-care at the time of application as comparator rather than placebo. The study was co-sponsored by University of Liverpool and the Royal Liverpool and Broadgreen Hospitals University Trust and adopted onto the National Institute for Health Research portfolio of supported clinical research studies. An independent Data Monitoring Committee monitored the trial at approximately 6month intervals. All patients gave informed written consent.

Participants

Eligible patients were aged ≥18 with moderately active Crohn's disease (CDAI>220-450) involving small intestine, colon, or both, with serum C reactive protein (CRP) ≥5mg/l and/or faecal calprotectin >250 microgram/gram. Patients receiving thiopurines had to have stable dose for ≥ 3 months. Patients receiving anti-TNF, other biologics, or methotrexate within previous 3 months were excluded. Concurrent use of prednisolone >5mgs/day or budesonide >3mg/day was also excluded as were patients receiving antibiotics within the previous 4 weeks. Patients receiving mesalazine must have a stable dose for at least one month.

Randomisation and stratification

Patients were randomised centrally 1:1 to either antibiotic/hydroxychloroquine combination or budesonide (Entocort CR©). Patients were stratified prior to randomisation into four groups: isolated colonic Crohn's disease versus small intestine ± colon, immunosuppression (thiopurines) versus no immunosuppression. Randomisation was by random number generation in Microsoft Excel, in blocks of 10 patients (by sequence of joining trial) within these four groups. Opaque allocation envelopes labelled by patient number were filled by the trial statistician at a remote site and posted to the trial co-ordinator.

Interventions and crossover

Patients randomised to antibiotics/hydroxychloroquine received oral ciprofloxacin 500mg bd, doxycycline 100mg bd, hydroxychloroquine 200mg tds before meals for 4 weeks followed by 20 weeks continued therapy with doxycycline 100mg bd and hydroxychloroquine 200mg tds. Patients randomised to budesonide received oral budesonide (Entocort CR©) 9mg/day taken in the morning for 8 weeks followed by 6mg/day for 2 weeks as per Rutgeerts et al [22] followed by 3 mg/day for a further 2 weeks as per current standard steroid tailing practice. Patients failing to respond (CDAI fall by >70) by 10 weeks were offered crossover onto the alternative treatment (Figure 1).

Outcomes

The primary endpoint was remission (CDAI ≤150) without addition of any other treatment for Crohn's disease and assessed at three time points: 10 weeks; remission at 10 weeks maintained through to 24 weeks; remission at 10 weeks maintained through to 52 weeks. The 10 weeks' time point was chosen to conform to the comparator budesonide trial data [22], 24 weeks to correspond with the end of the antibiotic/hydroxychloroquine therapy

period, and 52 weeks to determine whether any therapeutic effect might persist after cessation. Secondary endpoints included remission (CDAI $\leq$150) at 4 weeks, remission or response (CDAI fall by >70 points) at 10 weeks, patient global assessment of symptom severity by IBD10 (10 cm visual analogue score) [23] at 4,10,24,52 weeks, faecal calprotectin (assayed using whichever assay was in routine use at the recruiting site) 10,24,52 weeks. Adverse events were assessed at each visit.

Power calculation and early closure

Budesonide was predicted to have a 53% remission rate (95% CI 43%, 64%) at 10 weeks [22]. A sample size of 50 in each group, was estimated to have 80% power at 5% significance level to detect a difference between the predicted 53% remission rate for budesonide and a remission rate of 80% in the antibiotic/hydroxychloroquine group. Recruitment commenced late 2013 and initially progressed reasonably but gradually fell below target probably due to increasing availability of biologics. With 59 patients recruited, 78 treatment courses with crossover, over the five-year recruitment period, the Data Monitoring Committee considered that we had data sufficient to address the primary aims of the trial and that trial closure was appropriate.

Serology

Serum samples were obtained on recruitment, blind labelled, and tested for relevant antibodies: OmpC (outer membrane porin C of *E. coli* and other Gram negative bacteria), FlaX, Fla2 , CBir1 (all flagellar antigens present in Clostridiales) and anti-*Saccharomyces cerevisiae* antibodies (ASCA) by quantitative ELISA performed by Prometheus® Laboratories Inc., San Diego as part of the Prometheus IBD sgi Diagnostic® Cat.#1800 and with reference to their pre-established normal ranges.

Statistical analysis

Primary analysis of categorical outcomes of remission at 10 weeks and maintenance of remission at 24 weeks and 52 weeks was by Fisher's exact test. This was also performed with crossover data included. A pre-planned subgroup analysis assessed the possibility that serology, particularly anti-*Saccharomyces cerevisiae* antibody (ASCA) status might predict response to antibiotics/hydroxychloroquine. To account for multiple testing in these secondary analyses, significance was set at P-values of 0.02 (Fisher's test including crossover) and 0.01 for the other tests, implying an overall type I error risk of approximately 0.05 (Bonferroni calculations). Secondary outcomes were analysed using Fisher’s exact test for categorical outcomes, and Wilcoxon Rank Sign test for numerical values (patient global index [IBD10] and faecal calprotectin).

Results

Eighty-three patients were screened and 61 randomised of whom 59 took the study medication and were eligible for participation (Supplementary Figure 1). There were no significant differences at baseline between the treatment groups in demographics or disease activity (Table 1).

Response to therapy

Initial therapy

Twenty-seven patients received antibiotics and hydroxychloroquine as initial therapy and 32 received budesonide. Response was not significantly different between antibiotics/hydroxychloroquine and budesonide at any of the three primary outcome timepoints: 10 weeks, 24 weeks and 52 weeks (Table 2). Only patients who were in remission at 10 weeks were eligible for the primary analyses of maintenance in remission at 24 and 52 weeks. However, a post-hoc analysis that includes 3 patients who responded (CDAI fall by >70 but still >150) by 10 weeks and had gone into remission by 24 weeks on continued therapy showed a trend towards benefit with antibiotics/hydroxychloroquine, particularly when assessed per protocol (5/17 in remission at 24 weeks versus 1/26 for budesonide; P=0.028 [not reaching predetermined significance threshold of 0.01]. There was a non-significant tendency towards a higher remission rate with budesonide at 10 weeks (8/32 versus 2/27 on intention to treat (ITT) analysis (P=0.092), but by 24 weeks only one budesonide-treated patient remained in remission. Only one patient in each treatment group remained in remission through to 52 weeks – plus one additional patient receiving antibiotics/hydroxychloroquine who had been a responder at 10 weeks.

Initial therapy and crossover combined

Including crossover, there were 39 treatment courses in each group. Intention to treat analysis showed no significant differences in primary endpoint data at any of the three time points, 10, 24, and 52 weeks (Table 3, Figure 2).

When analysed per protocol, and including (post hoc) the three 10 week responders who were in remission by 24 weeks, 9 of 24 patients receiving antibiotics/hydroxychloroquine were in remission at 24 weeks compared with 1 of 32 receiving budesonide (P=0.001). By 52 weeks only one of 31 who had received budesonide per protocol remained in remission compared with three of 23 patients who received antibiotics/hydroxychloroquine plus one additional patient who had responded (CDAI fall >70 but still >150) by 10 weeks.

Impact of phenotype and serology

There was no signal that patients with isolated colonic Crohn's disease (L2) responded differently to the antibiotic/hydroxychloroquine combination (Supplementary Table 1; P=1.0).

Out of 59 patients recruited, 50 had sera available for testing, 21 were positive for ASCA, 6 were positive for OmpC, and 26 were positive for either ASCA or OmpC. ASCA, OmpC, separately or in combination, and FlaX and CBir serology were not associated with response to the antibiotic/hydroxychloroquine combination (Supplementary Table 2).

The phenotypic profile of the 9 patients who were in remission at 24 weeks on antibiotics/hydroxychloroquine showed no obvious features that might be used to predict response (Table 4).

Secondary endpoints

Rates of remission at 4 weeks and the combined endpoint of remission or response at 10 weeks were similar in both treatment groups, on initial therapy (Table 2) and including cross-over (Table 3).

Patient global index of severity (IBD10) correlated well with CDAI (r=-0.745; P<0.0001 based on 219 data sets from the 59 patients) with IBD10 >8 equivalent to CDAI ≤150 (remission) as previously reported[23] (Supplementary Figure 2). Patients showed similar improvement in both treatment groups. IBD10 improved from week 0 median (IQR) 3.3 (2.1-4.9) to week 10 5.9 (4.2-8.1) with antibiotics/hydroxychloroquine (P=0.001) and from week 0 median (IQR) 4.1 (2.2-5.3) to week 10 6.35 (2.7-9.1) with budesonide (P=0.006) (Supplementary Figure 3). Again there was evidence of better response at 24 weeks with antibiotics/hydroxychloroquine median (IQR) 8.75 (6.75-9.3) compared with budesonide median 6 (3.3-6.5) but numbers at this endpoint were small (n=12 antibiotics/hydroxychloroquine; n=6 budesonide).

Faecal calprotectin correlated less closely with CDAI (r=0.331; P<0.0001 based on 136 datasets from all 59 patients) (Supplementary Figure 4). Improvements in both treatment groups were similar but modest – falling from median (IQR) 497 (265-726) ug/g at week 0 to 331.5 (89-600)ug/g at week 10 with antibiotics/hydroxychloroquine (P=0.03) and from 573.5 (307-924)ug/g at week 0 to 454 (167-634)ug/g at week 10 with budesonide (P=0.15) (Supplementary Figure 5). Based on per protocol analysis, 10/17 patients receiving antibiotics/hydroxychloroquine achieved faecal calprotectin <250 ug/g at week 10 compared with 8/27 receiving budesonide (p=0.07).

Adverse events

There were 100 adverse events affecting 34 of 39 (87%) patients receiving antibiotics/hydroxychloroquine and 55 adverse events in 31 of 39 (79%) patients receiving budesonide (Table 5). Withdrawals by 10 weeks for adverse events were seen in 15 receiving antibiotics/hydroxychloroquine and 6 budesonide. Nausea affected 24 of 39 (62%) receiving antibiotics/hydroxychloroquine but was usually transient and only led to discontinuation of therapy in two; photosensitivity affected 11 of 39 (28%) even though all patients were warned to use high sun protective factor creams and minimise exposure and led to discontinuation of therapy in three; tendinopathy or arthralgia affected 7 of 39 (18%) and tendinopathy led to discontinuation in two but resolved without tendon rupture; 6 of 39 (15%) developed candidiasis of whom two received topical antifungals. Five patients receiving antibiotics/hydroxychloroquine suffered severe adverse events including two drug-related (vomiting and photosensitivity) and three unrelated – a fall in an elderly patient complicated by rhabdomyolysis, perianal abscess, and non-fatal pulmonary embolism. There was one severe adverse event (incisional hernia) in a patient receiving budesonide.

Discussion

In this open-label study we found no significant difference in remission or response rates at 10, 24, or 52 weeks between the antibiotic/hydroxychloroquine combination and a standard 12 week course of budesonide. The study was not powered for non-inferiority. On initial therapy there was a non-significant trend towards better results at 10 weeks with budesonide however, with crossover data included, similar remission and response rates were seen with both treatments. The 24 week data also showed no significant difference between treatment groups when assessed on intention to treat using pre-defined thresholds for statistical significance. However, when assessed per protocol, the results were encouraging for the antibiotic/hydroxychloroquine combination with 9 of 24 in remission at 24 weeks. Sustained remission to 24 weeks was only seen in one of 32 (per protocol) budesonide-treated patients – who had of course tailed off their therapy by 12 weeks. This might sound "unfair" given that the doxycycline and hydroxychloroquine were continued through to 24 weeks but is justified given that the efficacy of budesonide is only sustained for around three months, with longer maintenance ineffective [24]. The 52 week data showed a modest signal to support prolonged efficacy with the antibiotic/hydroxychloroquine combination, with 4/23 in remission at that time point on per protocol analysis. Patients who crossed over to the antibiotic/hydroxychloroquine combination fared better (remission at 10 weeks in 5/12, 42%; remission maintained to 24 weeks in 4/12, 33%; and remission maintained to 52 weeks in 2/12, 17%) raising the possibility that prior steroids followed by antibiotics/hydroxychloroquine might have been better than either treatment alone.

Remission rates with budesonide were lower than predicted, 8/32 (25%) at 10 weeks compared with 53% in the original trial by Rutgeerts *et al* [22]. The reason for this is unclear, but might in part be due to the inclusion of patients with isolated colonic Crohn's disease in the current study (Supplementary Table 1). When these are excluded, the remission rate, including crossover, with budesonide was 9/24 (38%) when analysed per protocol at 10 weeks. Average CDAI at baseline was 277 which is almost identical to that in the Rutgeerts *et al* trial (275). CRP was lower (median 8.5 mg/l compared with mean 25 mg/l in Rutgeerts *et al*). Moreover most (7/8; 87.5%) of the patients who were in remission at 10 weeks with budesonide had relapsed by 24 weeks off therapy.

The patient global assessment (IBD10) correlated very well with CDAI scores, confirming that an IBD10 score of 8 is equivalent to a CDAI of 150 [23], and showed treatment responses comparable to the primary outcome data, with improvements by 10 weeks similar for both treatment groups. There was a much weaker correlation between faecal calprotectin and CDAI and improvements seen in both treatment arms were modest. Faecal calprotectin is known to correlate better with mucosal healing and 4 of 9 patients in remission at 24 weeks had faecal calprotectin <70ug/g, one of the lower proposed cut-off values for indicating likely endoscopic remission.

Prevalence of mucosal *E. coli* in Crohn's disease ranges from 21 to 63% of cases [1]. The antibiotic/hydroxychloroquine combination would also be effective against other organisms, particularly Gram negative bacteria, that could invade the gut and replicate within macrophages. We had hoped that serological testing might identify patients more likely to be affected by *E. coli* and thus predict response to antibiotics/hydroxychloroquine. OmpC is

an outer membrane porin protein found in Gram negative organisms including *E. coli* but only 6 of 50 patients tested positive for OmpC antibody. Anti-*Saccharomyces cerevisiae* (baker's yeast) antibody (ASCA) is the best established antibody associated with Crohn's disease. Its origin is unclear as baker's yeast is not the only source of its antigen, an oligomannan with a specific mannose alpha 1,3 mannose alpha 1,2 mannose terminal sequence [25,26]. Other possible microbial sources include *Candida albicans* and *Mycobacterium avium subspecies paratuberculosis* [27,28]. There is an intriguing possibility that a key ASCA antigen could be the mannan receptor for Gram negative bacterial fimH on glycoprotein 2 (GP2) on microfold (M) cells [29]. M cells are the initial sites for bacterial invasion and are also thought to be the sites of the initial lesions in Crohn's disease. There is considerable overlap between ASCA and anti-GP2 serology in Crohn's disease sera [30]. Moreover, although the mannan on GP2 has yet to be characterised, the bladder equivalent of GP2 is uroplakin and its mannan receptor for fimH expressed by uropathogenic *E. coli* is oligomannose-3 which contains the same terminal mannose alpha 1,3 mannose sequence as the ASCA epitope [31]. It therefore seemed plausible that ASCA positivity might identify Crohn's disease patients with *E. coli* invasion. However, in this study there was no correlation between ASCA, either separately, or combined with ompC positivity, and response to the antibiotic/hydroxychloroquine combination.

There is a good case for considering isolated colonic Crohn's disease as a separate condition from Crohn's disease affecting the small intestine [32,33]. There has also been a suggestion that it might respond better to antibiotics [32]. In this study patients with isolated colonic disease showed no significant difference in response to antibiotics/hydroxychloroquine. Careful analysis of the nine patients who were in remission at 24 weeks on the

antibiotic/hydroxychloroquine combination again failed to show association with phenotype or serotype. It is possible that a more specific bacterial DNA-based technique is needed, possibly based on detection of circulating Gram negative bacterial DNA which has been shown to predict relapse [34].

Side effects were an issue with the ciprofloxacin, doxycycline, hydroxychloroquine combination, particularly in the first month when patients were taking all three drugs. Nausea was common but was usually very transient but photosensitivity (all three drugs are known to cause this) and Achilles tendon pain were also issues. Hydroxychloroquine is associated with risk of retinopathy but this is related to cumulative dosage, usually over years of therapy. All patients were screened prior to trial entry for any history of visual impairment not corrected by wearing glasses and by visual acuity testing repeated at 10 weeks or early withdrawal. No patient failed screening on these grounds. One patient was withdrawn because of transient blurred vision but this was thought unlikely to be related to the hydroxychloroquine. Six patients developed candidiasis but none stopped trial medication because of this.

We believe that the signals of efficacy seen with the antibiotic/hydroxychloroquine combination, particularly the longer-term remissions, justify further study. It is unfortunate that neither phenotypic information or serology seem to identify patients likely to respond. It is possible that a DNA-based approach probing for *E. coli* or other Gram negative organisms on faecal or peripheral blood samples might better identify patients likely to respond but this was beyond the funding available for this study. Alternatives deserving

further study include serological testing for serum anti-*E. coli* /salivary CEACAM6 index [35] and quantification of faecal IgA-coated *E. coli* [36].

The choice of antibiotics is also an issue. Ciprofloxacin was selected mainly because it showed the greatest *in vitro* potency against *E. coli* replicating within macrophages [11]. It does however seem to have added considerably to side-effects. A future trial might be confined to long term therapy with the doxycycline hydroxychloroquine combination. This has proved successful in Whipple's disease [19]and Q fever [18]. Alternative strategies targeting the altered Crohn's disease microbiota could include bacteriophages or bacterial function-editing substrates [37].

Conclusion

Further study of long term doxycycline plus hydroxychloroquine as an adjunct to biologic therapy in Crohn's disease should be considered.

Authors contributions

JMR, PF, GH designed the trial. JMR and then CP were Chief Investigators.

SS,PF,JM,MP,AH,HD,TI,JB,CP all recruited and treated patients. KM and KC collected and monitored the data. GH and JMR analysed the data. JMR, CP, and GH drafted the manuscript. All authors critically revised the manuscript for important intellectual content.

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Data accessibility statement

Anonymised patient level data can be made available on reasonable request after approval by the Trial Management Committee and after signing a data access agreement. Proposals should be directed to the corresponding author. Full trial protocol is available on journal website.

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Table 1 Demographic data

Baseline:	Antibiotic / hydroxychloroquine			Budesonide			P value (Initial AB/HCQ vs Initial budesonide)	P value (Total AB/HCQ vs Total budesonide)
	Initial	Cross-over	Total	Initial	Cross-over	Total		
	n=27	n=12	n=39	n=32	n=7	n=39		
Male	18 (66.6%)	9 (75.0%)	27 (69.2%)	18 (56.3%)	4 (57.1%)	22 (56.4%)	0.44	0.35
Age (years)	44.9+/-17.7	44.2 +/-24.2	44.7 +/-19.6	43.3 +/-18.4	46.0 +/-16.5	43.8 +/-17.9	0.74	0.84
Duration (years)(median[IQR])	6 (1-11)	4 (1.5-7.5)	5 (1-10)	3.5 (1.5-12)	10 (2-19)	5 (2-13)	0.89	0.64
Age of onset: (Montreal classification)								
A1 (</=16)	2 (7.4%)	1 (8.3%)	3 (3.3%)	3 (9.4%)	0	3 (7.8%)	1.00	1.00
A2 (17-40)	15 (55.6%)	5 (41.7%)	20 (51.3%)	15 (46.9%)	4 (57.1%)	19 (48.7%)	0.60	1.00
A3 (>40)	10 (37.0%)	6 (50.0%)	16 (15.4%)	14 (43.8%)	3 (42.9%)	17 (43.6%)	0.79	1.00
Location: (Montreal classification)								
L1	8 (29.6%)	2 (16.7%)	10 (25.6%)	8 (25.0%)	3 (42.9%)	11 (28.2%)	0.77	1.00
L2 **(stratified before randomisation)**	8 (29.6%)	3 (25.0%)	11 (28.2%)	11 (34.4%)	1 (14.3%)	12 (30.8%)	0.78	1.00
L3	11 (40.7%)	7 (58.3%)	18 (46.2%)	13 (40.6%)	3 (42.9%)	16 (41.0%)	1.00	0.82
L2/L3 (colonic involvement)	19 (70.4%)	10 (83.3%)	29 (74.4%)	24 (75.0%)	4 (57.1%)	28 (71.8%)	0.77	1.00
L4 (proximal small bowel disease)	1 (3.7%)	0	1 (2.6%)	0	1 (14.3%)	1 (2.6%)	0.46	1.00
Perianal disease	4 (14.8%)	1 (8.3%)	5 (12.8%)	3 (9.4%)	2 (28.6%)	5 (12.8%)	0.69	1.00
Type:								
B1	23 (85.2%)	9 (75.0%)	32 (82.1%)	26 (81.3%)	5 (71.4%)	31 (79.5%)	0.74	1.00
B2 (stricturing)	4 (14.8%)	2 (16.7%)	6 (15.4%)	4 (12.5%)	2 (28.6%)	6 (15.4%)	0.69	1.00
B3 (penetrating)	0	1 (8.3%)	1 (2.6%)	2 (6.3%)	0	2 (5.1%)	0.50	1.00
Baseline CDAI (mean/SD)	287.1+/-56.3	281.9+/-53.5	285.5+/-54.8	282.3+/-50.7	250.6+/-48.2	276.6+/-51.2	0.75	0.46
Baseline CRP (mg/l, normal <3)(median [IQR])	9 (5-26)	5.5 (5-11.5)	9 (5-20)	8 (5-22) (n=31)	9 (5-13)	8.5 (5-19) (n=38)	0.82	0.83
Baseline calprotectin (ug/g, normal <100)(median [IQR])	487.5 (265-726) (n=26)	497 (255-952) (n=11)	497 (265-726) (n=37)	627.5 (404-963) (n=30)	209 (70-286) (n=6)	573.5 (307-924)	0.56	0.66
Current smoker	6 (22.2%)	0	6 (15.4%)	2 (6.3%)	0	2 (5.1%)	0.13	0.26
Current thiopurine **(stratified before randomisation)**	6 (22.2%)	4 (33.3%)	10 (25.6%)	7 (21.9%)	3 (42.9%)	10 (25.6%)	1.00	1.00

Table 2 Response to initial therapy course (Primary endpoint data in bold)

Intention to treat analysis					
	Remission 10w	**Remission maintained to 24 weeks**	**Remission maintained to 52 weeks**	Rem. &/or response 10w	Remission 4wk
AB/HCQ	**2/27**	**2/27 (plus 3 10wk responders in remission @24 weeks)**	**1/27 (plus 1 10wk responder in remission at 52 weeks)**	9/27	4/27
Budesonide	**8/32**	**1/32**	**1/32**	11/32	8/32
Per protocol analysis					
AB/HCQ	**2/20**	**2/17 (plus 3 10wk responders in remission @24 weeks)**	**1/17 (plus 1 10wk responder in remission at 52 weeks)**	9/20	4/21
Budesonide	**8/28**	**1/26**	**1/25**	11/28	8/30

Table 3 Response to therapy including crossover (Primary endpoint data in bold)

Intention to treat analysis					
	Remission 10w	**Remission maintained to 24 weeks**	**Remission maintained to 52 weeks**	Rem. &/or response 10w	Remission 4wk
AB/HCQ	**7/39**	**6/39** **(plus 3 10wk responders in rem @ 24 weeks)**	**3/39** **(plus 1 10wk responder in rem @ 52 weeks; plus 1 clin rem but withdrawn UTI)**	15/39	8/39
Budesonide	**10/39**	**1/39**	**1/39**	13/39	9/39
Per protocol analysis					
AB/HCQ	**7/27**	**6/24** **(plus 3 10wk responders in rem @ 24 weeks) P=0.035 (0.01 needed for significance)**	**3/23** **(plus 1 10wk responder in rem @ 52 weeks; plus 1 clin rem but withdrawn UTI)**	15/27	8/30
Budesonide	**10/34**	**1/32**	**1/31**	13/34	9/37

Table 4. Phenotypic and serological features of the 9 patients who achieved remission at 24 weeks on antibiotics/hydroxychloroquine.

Sex	Age	Duration(y)	Montreal	Colon (L2/L3)	Antibiotic/ HCQ initial/crossover	Thiopurine	Smoker Y(current) N (former/never)	CRP At week 0	ASCA	OmpC	FlaX	CBir	Fla2
M	21	10	A1L1 B1	N	Initial	N	N	8	N	N	N	N	N
F	30	1	A2L3 B1	Y	Initial	Y	Y	74	N	N	Y	N	Y
M	54	6	A2L3 B1	Y	Initial	N	Y	9	N	N	N	N	N
M	59	0.5	A3L1 B1	N	Initial	N	N	9	N	N	Y	N	N
F	37	1	A2L3 B1	Y	Initial	N	Y	7	N	N	N	N	N
M	89	5	A3L2 B1	Y	Crossover	Y	N	30	N	Y	N	N	Y
M	47	5	A3L1 B2P	N	Crossover	Y	N	6	Y	N	N	N	N
M	29	16	A1L3 B1	Y	Crossover	N	N	5	Y	N	N	Y	N
M	27	1	A2L2 B1	Y	Crossover	N	N	5	N	N	N	N	N

Table 5. Adverse events (AEs)

	Budesonide	Antibiotics/HCQ
	n=39	n=39
Subjects with any AEs	31	34
Intensity of worst AE		
Mild	12	8
Moderate	18	19
Severe	1	5
Subjects with AEs leading to discontinuation	**2**	**11**
Adverse events by system		
Total	55	100
Gastrointestinal		
Nausea +/-vomiting	6	24
Diarrhoea (worsened)	7	9
Reflux/heartburn	1	4
Anorexia	0	2
Abdominal pain (worsening)	3	2
Proctalgia	1	2
Tenesmus (worsened)	2	1
Rectal bleeding	2	0
General disorders		
Mouth ulcers	3	1
Fatigue	2	1
Hair loss	1	1
Infections and infestations		
Respiratory	7	4
UTI	0	2
Candidiasis	0	6
Nervous system disorders		
Sleep disturbance	6	8
Headache	4	5
Mood disturbance	5	3
Dizziness	1	1
Panic	1	1
Skin and subcutaneous disorders		
Photosensitivity	0	8
Rash	0	3
Acne	1	1
Eczema (worsening)	1	1
Respiratory, thoracic, and mediastinal disorders		
Pulmonary embolism	0	1
Dyspnoea	0	1
Musculoskeletal disorders		
Tendopathy	0	2
Cramp	1	1
Arthralgia	0	5

Legends to Figures

Figure 1. Study design.

Figure 2. Remission rates at 10, 24, 52 weeks, including crossover data: (a) Intention to treat data; (b) Per protocol data.

Supplementary Figures:

Figure 1. Consort flow diagram.

Figure 2. Correlation between CDAI and patient global index (IBD10) - 219 datasets from the 59 patients.

Figure 3. Patient global index (IBD10) scores including crossover (per protocol). P values for 0 vs 10 weeks by Wilcoxon Signed Rank test on paired samples.

Figure 4. Correlation between CDAI and faecal calprotectin (136 datasets from 59 patients)

Figure 5. Faecal calprotectin levels including crossover (per protocol). P values for 0 vs 10 weeks by Wilcoxon Signed Rank test on paired samples.

Figure 6. Consort check list.

Supplementary Table 1. Response to therapy including crossover in the subgroup of patients with isolated colonic Crohn's disease (L2) (Primary endpoint data in bold).

Intention to treat analysis					
	Remission 10w	**Remission maintained to 24 weeks**	**Remission maintained to 52 weeks**	Rem. &/or response 10w	Remission 4wk
AB/HCQ	**2/12**	**2/12**	**1/12**	6/12	3/12
Budesonide	**1/12**	**0/12**	**0/12**	4/12	2/12
Per protocol analysis					
AB/HCQ	**2/9**	**2/8**	**1/7**	6/9	3/11
Budesonide	**1/10**	**0/10**	**0/10**	4/10	2/12

Supplementary Table 2.

(a) Response to therapy including crossover in patients positive versus negative for ASCA serology

Positive for ASCA					
Intention to treat analysis					
	Remission 10w	**Remission maintained to 24 weeks**	**Remission maintained to 52 weeks**	Rem. &/or response 10w	Remission 4wk
AB/HCQ	**3/11**	**2/11**	**1/11**	3/11	4/11
Budesonide	**5/17**	**0/17**	**0/17**	6/17	2/17
Per protocol analysis					
AB/HCQ	**4/21**	**4/21 (+3 10 week responders in rem @ 24weeks)**	**2/21**	11/21	4/21
Budesonide	**3/15**	**1/15**	**1/15**	4/15	4/15
Negative for ASCA					
Intention to treat analysis					
	Remission 10w	**Remission maintained to 24 weeks**	**Remission maintained to 52 weeks**	Rem. &/or response 10w	Remission 4wk
AB/HCQ	**4/21**	**4/21 (+3 10 week responders in rem @ 24 weeks)**	**2/21**	11/21	4/21
Budesonide	**3/15**	**1/15**	**1/15**	4/15	4/15
Per protocol analysis					
AB/HCQ	**4/16**	**4/15 (+3 10 week responders in rem @ 24 weeks)**	**2/14 (+1 10 week responder in rem @ 52 weeks)**	11/16	4/17
Budesonide	**3/13**	**1/11**	**1/11**	4/13	4/13

(b) Response to therapy including crossover in patients positive versus negative for ASCA and/or OmpC serology

Positive for ASCA and/or OmpC					
Intention to treat analysis					
	Remission 10w	**Remission maintained to 24 weeks**	**Remission maintained to 52 weeks**	Rem. &/or response 10w	Remission 4wk
AB/HCQ	**4/15**	**3/15**	**1/15**	5/15	5/15
Budesonide	**6/19**	**0/19 P=0.076**	**0/19**	7/19	4/19
Per protocol analysis					
AB/HCQ	**4/9**	**3/8**	**1/7**	5/9	5/11
Budesonide	**6/17**	**0/16 P=0.028**	**0/16**	7/17	4/19
Negative for both ASCA and OmpC					
Intention to treat analysis					
	Remission 10 weeks	**Remission maintained to 24 weeks**	**Remission maintained to 52 weeks**	Rem. &/or response 10w	Remission 4wk
AB/HCQ	**3/17**	**3/17**	**2/17**	9/17	3/17
Budesonide	**2/13**	**1/13**	**1/13**	3/13	2/13
Per protocol analysis					
AB/HCQ	**3/13**	**3/13**	**2/13**	9/14	3/15
Budesonide	**2/12**	**1/12**	**1/11**	3/12	2/11

(c) Response to therapy including crossover in patients positive versus negative for FlaX serology:

Positive for FlaX					
Intention to treat analysis					
	Remission 10w	**Remission maintained to 24 weeks**	**Remission maintained to 52 weeks**	Rem. &/or response 10w	Remission 4w
AB/HCQ	**1/6**	**0/6**	**0/6**	3/6	1/6
Budesonide	**4/12**	**1/12**	**1/12**	5/12	2/12
Per protocol analysis					
AB/HCQ	**1/5**	**0/5**	**0/4**	3/5	1/5
Budesonide	**4/11**	**1/11**	**1/11**	5/11	2/11
Negative for FlaX					
Intention to treat analysis					
	Remission 10w	**Remission maintained to 24 weeks**	**Remission maintained to 52 weeks**	Rem. &/or response 10 w	Remission 4 w
AB/HCQ	6/26	6/26	3/26	11/26	7/26
Budesonide	4/20	0/20	0/20	5/20	4/20
Per protocol analysis					
AB/HCQ	6/18	6/17	3/16	11/18	7/21
Budesonide	4/18	0/17	0/16	5/18	4/19

Figure 1.

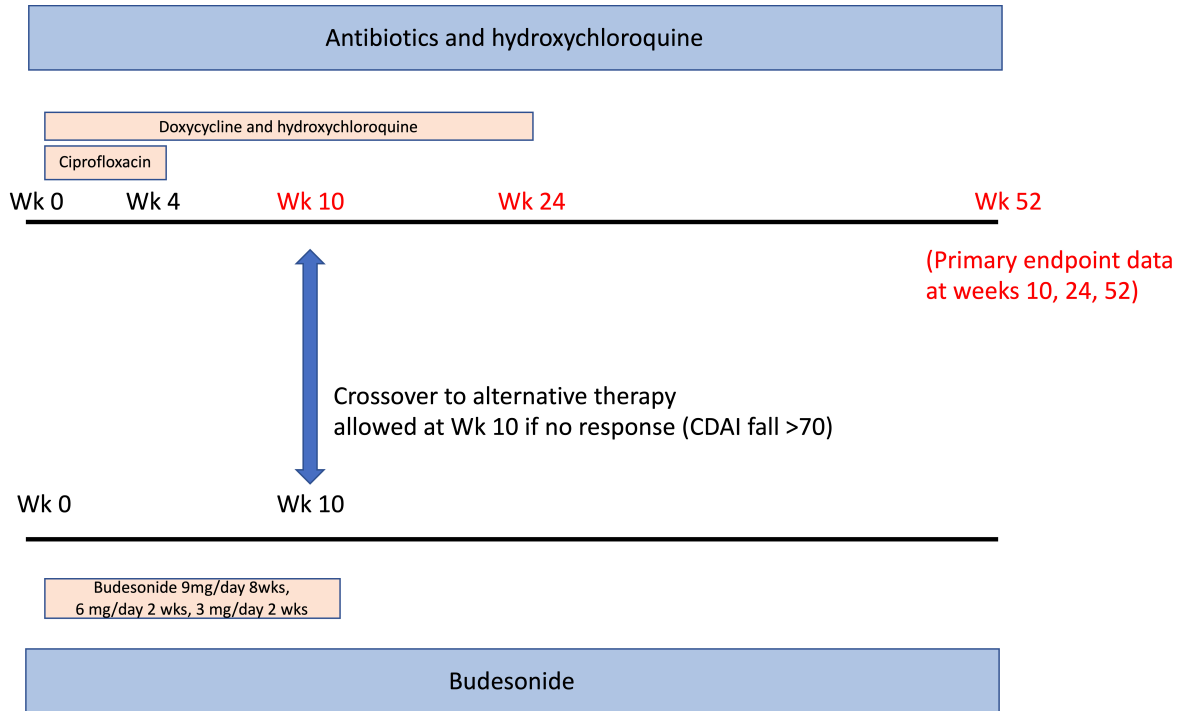
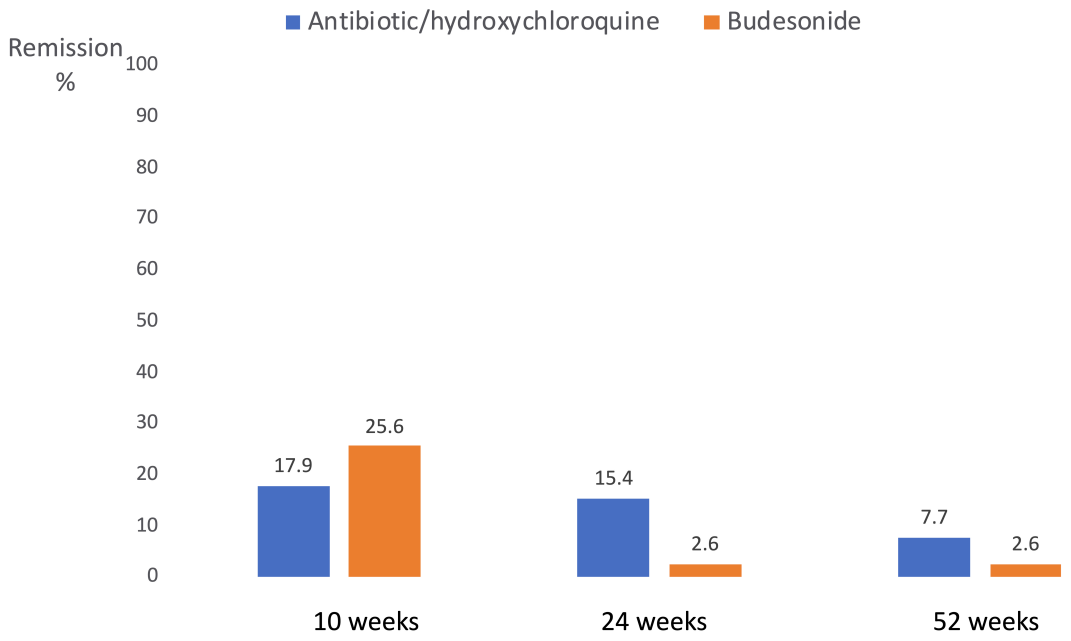
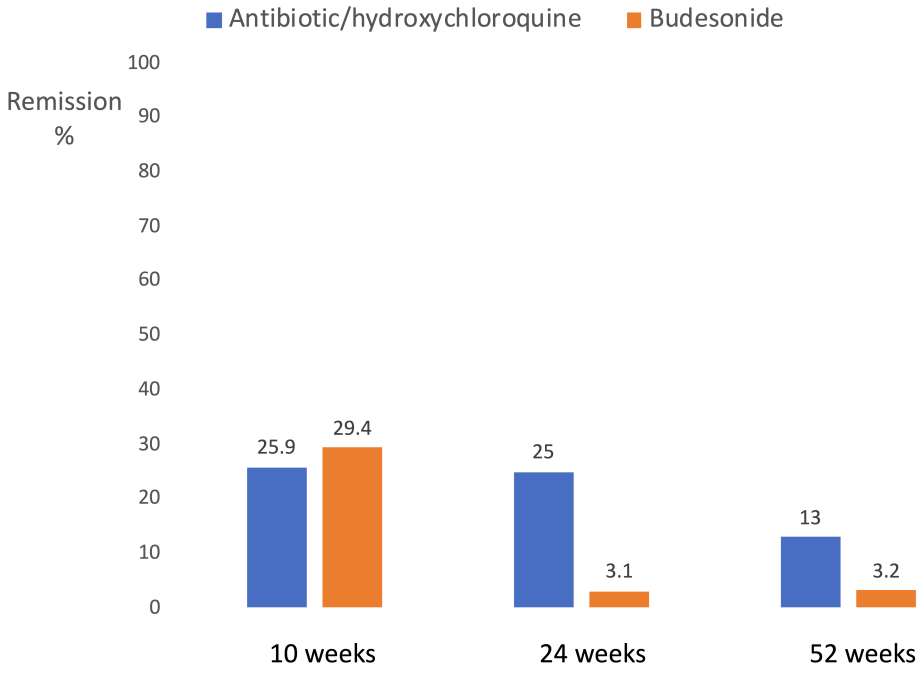

Antibiotics and hydroxychloroquine
Doxycycline and hydroxychloroquine
Ciprofloxacin
Wk 0
Wk 4
Wk 10
Wk 24
Wk 52
(Primary endpoint data at weeks 10, 24, 52)
Crossover to alternative therapy allowed at Wk 10 if no response (CDAI fall >70)
Wk 0
Wk 10
Budesonide 9mg/day 8wks, 6 mg/day 2 wks, 3 mg/day 2 wks
Budesonide

Figure 2A



ITT

Figure 2B

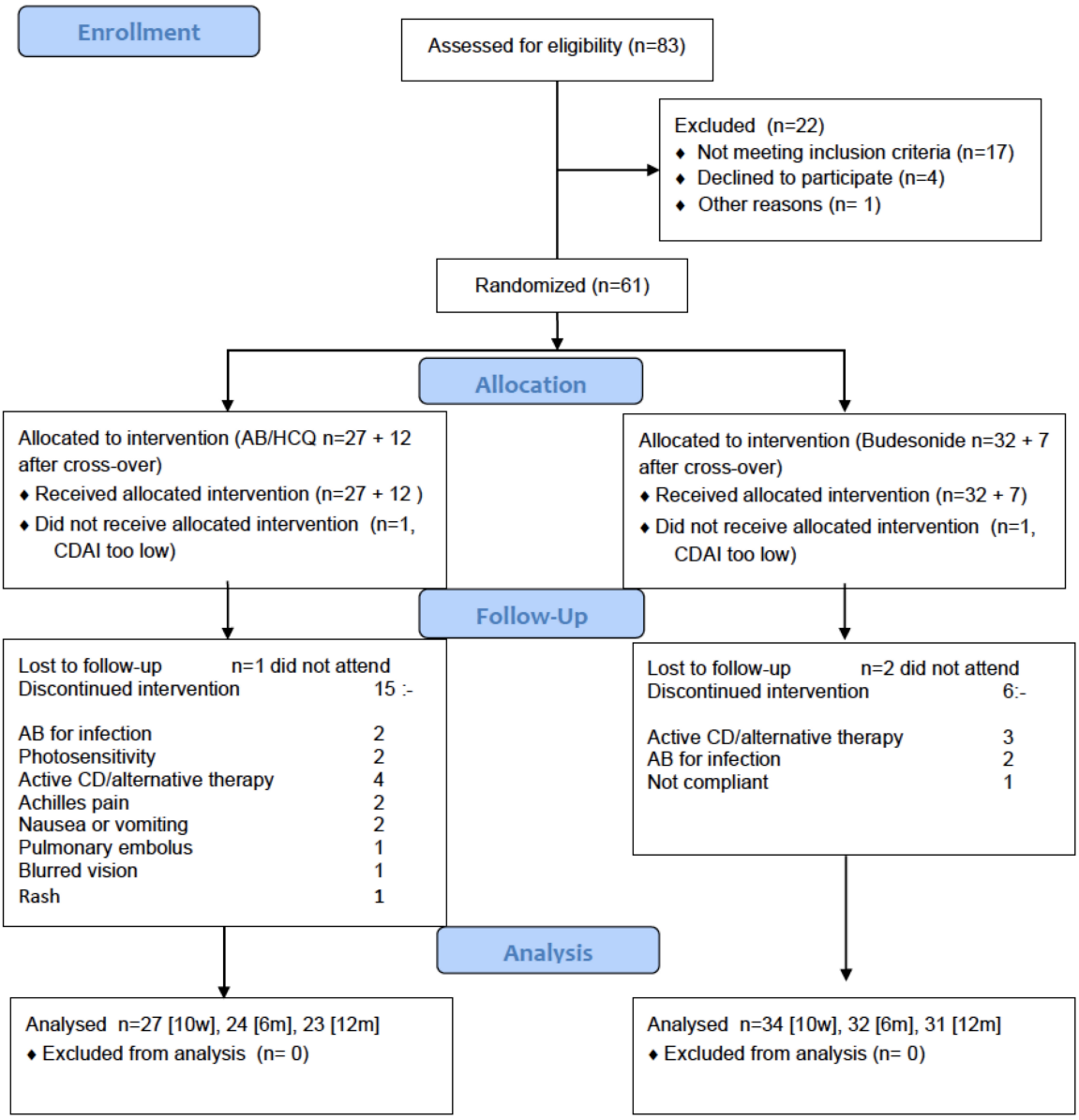


PP

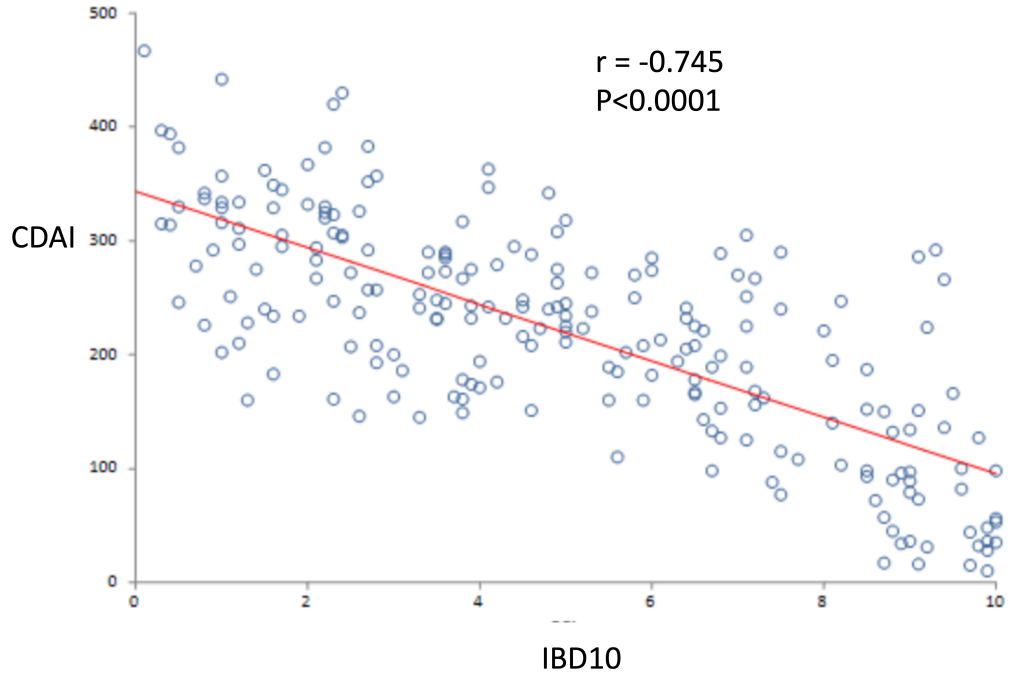
Suppl Figure 1

CONSORT
TRANSPARENT REPORTING of TRIALS

CONSORT 2010 Flow Diagram

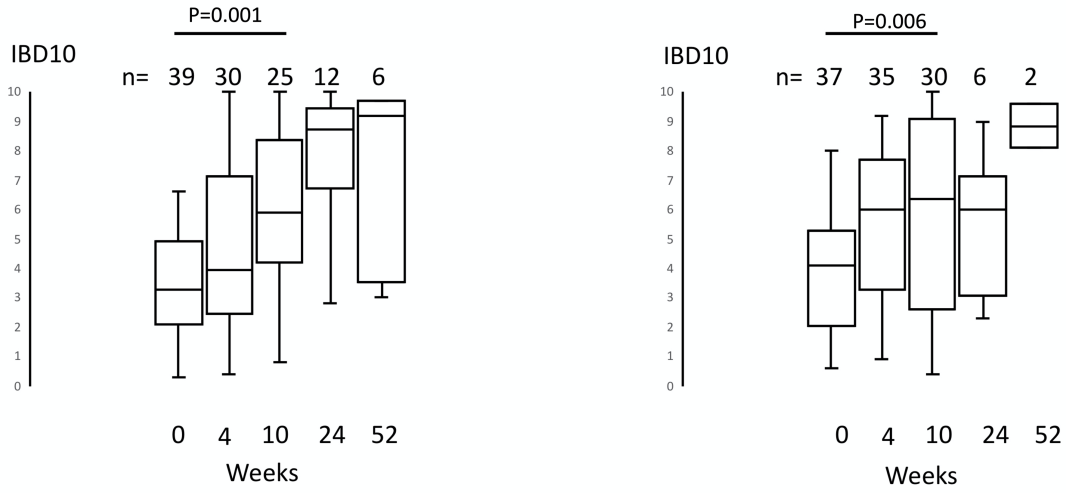


Suppl Fig 2

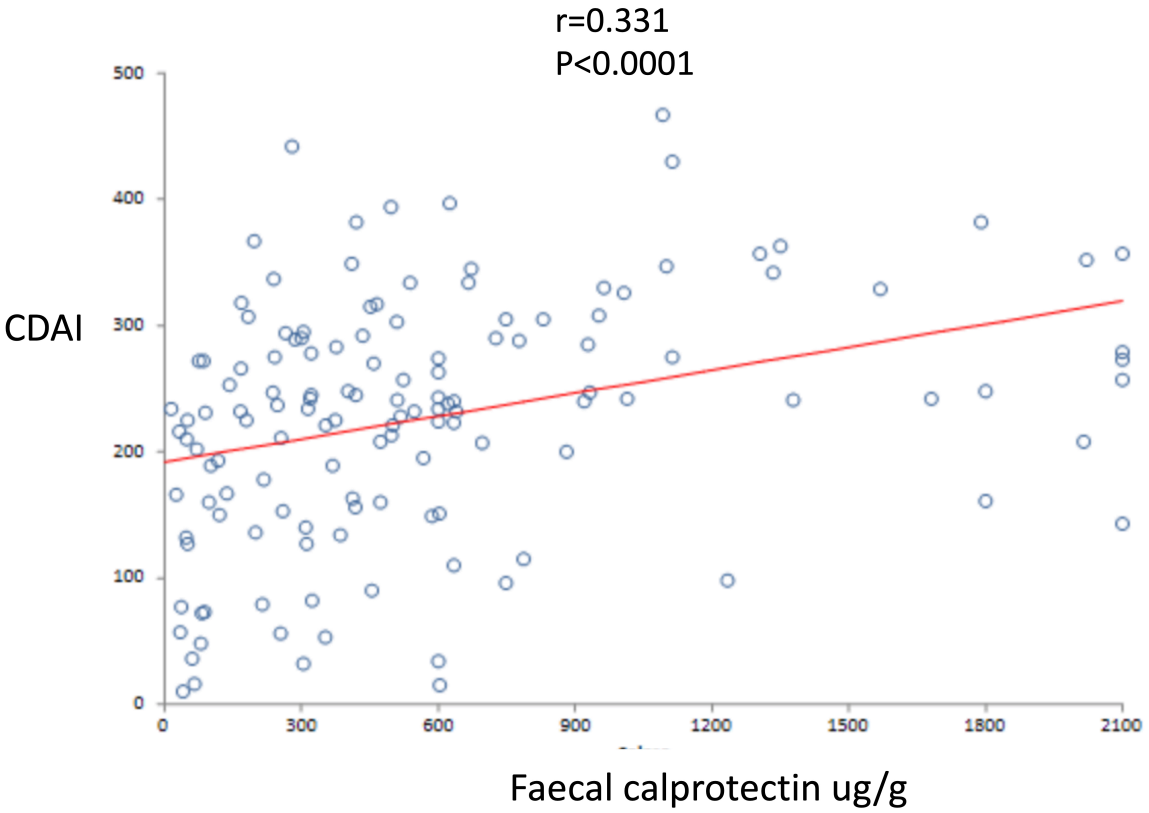


CDAI compared with Patient Global Index (IBD10) [219 data sets from 59 patients]

Suppl Fig 3



Suppl Fig 4



Suppl Fig 5