The Role of Centralized Reading of Endoscopy in a Randomized Controlled Trial of Mesalamine for Ulcerative Colitis

Short title: Centralized Reading of Endoscopy in UC

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Abbreviations: CI, confidence interval; ITT, intent to treat; UC, ulcerative colitis; UCCS, Ulcerative Colitis Clinical Score; UCDAI; Ulcerative Colitis Disease Activity Index;

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Abstract

BACKGROUND & AIMS: Inter-observer differences in endoscopic assessment by Site investigators contributes to variation in placebo response rates in ulcerative colitis (UC) trials. Centralized review of images might minimize this variability.

METHODS: We performed a 10-week, randomized, double-blind, placebo-controlled, study in 281 patients with mildly to moderately active UC (sigmoidoscopy score of the Ulcerative Colitis Disease Activity Index (UC-DAI) score $\geq$2) that evaluated the efficacy of delayed release mesalamine (Asacol™ 800 mg tablet) 4.8 g/day. Endoscopic images were reviewed by a single expert reader.

RESULTS: The primary outcome, clinical remission ([UCDAI] stool frequency and bleeding scores of 0 and no fecal urgency) at week 6, was observed in 30.0% of Asacol™ treated patients and 20.6% of those assigned to placebo for an absolute difference of 9.4% (*P* = .069; 95% confidence interval [CI] for the between group difference: [0.7%–19.4%]). Multiple secondary analyses were significant in favor of Asacol™. Thirty one percent of participants deemed eligible by Site investigators were considered ineligible by the Central reader. Although removal of these patients from analysis markedly reduced statistical power, the estimate of treatment effect for the primary outcome was substantially greater and the placebo effect was lower (29% vs 13.8%; *P* = .011; 95% CI for the between group difference: [3.5%–26.0%]) than that observed in the intent to treat (ITT) analysis.

CONCLUSIONS: Although Asacol™ 4.8 g/day was not statistically different from placebo based on an ITT analysis, the totality of the data support a benefit of treatment. Central review of endoscopic images is critical to the conduct of induction studies in UC. ClincialTrials.gov number, NCT01059344.

Key words: Ulcerative colitis; mesalamine; 5-ASA; randomized controlled trial; therapy; endoscopy; inflammatory bowel disease, central reading, inter-observer agreement

Mesalamine (5-ASA) is recommended as a first-line induction therapy for ulcerative colitis (UC) by multiple international guidelines.[1–3] Although effective doses for mildly-to-moderately active disease range from 2.0 to 4.8 g per day,[1,4,5] patients previously treated with corticosteroids, rectal therapies, or multiple UC medications may derive greater benefit from high dose therapy.[6-8] Additionally, patients who fail 8 weeks of low dose treatment may respond to dose escalation to 4.8 g/day for an additional 8 weeks.[9] Thus, a substantial proportion of patients may ultimately require high dose mesalamine therapy.

Multi-dose mesalamine regimens (3 or 4 times daily) evolved from prior experience with sulfasalazine whereby tolerability was improved by splitting the total dose over the course of the day. Recognition that sulfapyridine was responsible for the majority of sulfasalazine side effects, and the advent of sulfa-free formulations, has led to a reappraisal of these regimens. A recent Cochrane meta-analysis concluded that once daily induction therapy with mesalamine is as effective as conventional multi-dose regimens in patients with active disease.update to Cochrane reference[10] New high-dose formulations of mesalamine have been developed to reduce both pill burden and dosing frequency.[11-13] It is noteworthy that many UC patients, particularly those with quiescent disease, are non-adherent with conventional multi-dose regimens.[13–16] Both the frequency of dosing and a high pill burden contribute to this problem.[15, 17] Accordingly, this randomized, placebo controlled study evaluated the efficacy and safety of a high concentration, 800 mg, formulation of Asacol™.

The study also had an important secondary goal. Inter-observer variation in the endoscopic assessment of disease activity is a well-documented challenge that has

contributed to the wide variation in placebo responses observed in UC trials.[18] A potential solution to this problem is centralized review of endoscopic images by an expert Central reader, an approach that might be advantageous for two reasons. First, ensuring that patients reliably meet a minimum degree of endoscopic severity might minimize the rate of response to placebo. Second, minimizing or eliminating inter-observer variability should increase statistical efficiency. Central reading to monitor the consistency of endoscopic assessment by Site investigators was first reported in 2009 in a multi-center trial of delayed-release oral mesalamine, however no assessment of the potential effects of this practice on trial outcomes was performed.[7] In the current study, use of a Central reader for quality control and site-training allowed evaluation of the potential benefits of Central reading on both patient selection and trial outcomes. Furthermore, an ancillary study, based on videos obtained in the trial, provided us with a unique opportunity to assess inter- and intra-observer agreement by Central readers for existing endoscopic scoring systems in UC.

Materials and Methods

Patients

This randomized, double-blind, placebo-controlled, multicenter, phase 3 study was conducted in patients with mildly-to-moderately active UC at 26 centres in Belarus, India, Turkey, and Ukraine (Appendix 1). The study was approved by the independent ethics committee at each center, and all patients provided written informed consent.

Adult patients (≥18 years) with a documented diagnosis of UC were eligible to participate if they met the following criteria: (1) disease extending at least 15 cm from the anal verge and (2) mildly-to-moderately active UC defined by a modified Ulcerative Colitis Disease Activity Index (UCDAI)[11] score of 4-10 with a sigmoidoscopy component score ≥2 and a rectal bleeding component score ≥1. Patients with severe UC, defined as the presence of ≥6 bloody stools daily with one or more of the following: (1) an oral temperature >37.8°C or >100°F, (2) pulse >90/min, or (3) a hemoglobin concentration of <10 g/dL, were excluded. Other exclusion criteria were; previous failure or current treatment with a mesalamine dose of >2.0 g/day; a current disease relapse lasting >6 weeks; systemic antibiotic therapy for UC, probiotics, anti-diarrheals, or a nicotine patch within 1 week; systemic or rectal steroid therapy within 4 weeks; azathioprine, 6-mercaptopurine or immunosuppressives within 6 weeks; infliximab or other biologic treatment within 3 months; or administration of any investigational drug within 30 days prior to randomization. Patients were also excluded who had: a history of colectomy or partial colectomy; colonic dysplasia; Crohn's disease; bleeding disorders; toxic megacolon; hypersensitivity to salicylates, aspirin, sulfasalazine or 5-ASA; a serum

creatinine > 1.5 times the upper limit of normal, or a serum aspartate transaminase, alanine transaminase, total bilirubin or alkaline phosphatase concentration >2 times the upper limit of normal; a serious underlying condition other than UC; a history of drug or alcohol abuse; or a stool culture positive for *Clostridium difficile*. Pregnant or lactating women were not eligible.

Study design

Eligible patients were randomized, in a 1:1 ratio, to receive Asacol™ 4.8 g/day or placebo for 10 weeks. Patients received either three 800 mg Asacol™ or placebo tablets twice daily. The randomization schedule was generated in permutated blocks by computer. An interactive voice/web response system was used to manage the randomization procedure and to dispense study drug.

Efficacy and Safety Evaluations

Patients were assessed at the screening visit and weeks 0, 3, 6, 10, and 14. Disease activity was assessed using a modified UC-DAI and the Ulcerative Colitis Clinical Score (UCCS).[19,20] The UCDAI is the sum of 4 component scores: (1) stool frequency score; (2) rectal bleeding score; (3) flexible sigmoidoscopy score; and (4) physician global assessment score. The modified UCDAI used for this study incorporated a more stringent sigmoidoscopic scoring criterion whereby patients with any friability of the colonic mucosa were given a minimum score of 2, rather than 1.The intent of this modification, which has been used in other trials[7,9] was to provide clarity around the definition of friability (i.e. a "mild" friability score of 1, is a non-sequitur as the presence of friability is binary) and to minimize the placebo response. For the purposes of this

study friability was defined as the presence of bleeding following gentle contact with the endoscope during the insertion phase of the procedure. The UCCS is a modification of the UC-DAI in which the endoscopic score is omitted and a 4 point patient-reported assessment of functional status substituted.[20] This modification allows continued assessment of disease activity in the absence of a sigmoidoscopic exam. Each of the components of the modified UCDAI and the UCCS are scored on a scale of 0 to 3, with total scores ranging from 0 to 12; higher scores mean more severe disease.

Flexible sigmoidoscopy for scoring of the modified UCDAI by the investigators was performed at screening, week 6 and week 10. Site readers were trained on the UCDAI sigmoidoscopy score at investigator meetings and site initiation visits. Video recordings of the distal sigmoid colon were obtained at approximately 15–25 cm from the anal verge. Sites were selected who had access to high quality endoscopic equipment. A standardized image resolution of 640 x 480 pixels was utilized for evaluation of the videos. These videos were reviewed by a single expert Central reader (JWDM); without knowledge of the treatment assignment.

Patients were provided with a diary to record stool frequency and the amount of blood seen with each stool. These records were used to calculate the corresponding component scores of the modified UCDAI and UCCS. Patients were also asked to record the presence or absence of fecal urgency by answering the question “Do you have to urgently visit the toilet to pass stool?” The modified UCDAI was obtained at the screening visit, week 6, and week 10, whereas the UCCS was obtained at screening and all post-randomization visits.

A physical exam was performed at the screening visit, week 6, and week 10. Blood and urine samples were collected at screening, and weeks 6, 10, and 14 for hematology, serum chemistry, and urinalysis. Stool samples were obtained at screening for *C. difficile* culture. Compliance with the study drug was assessed at each visit using patient interviews and pill counts. Study drug compliance was calculated based on the total required drug usage for 100% compliance among patients with complete pill counts. All adverse events (AEs) that occurred were classified according to the Medical Dictionary for Regulatory Affairs Version 11.0.

Statistical Methods

The primary efficacy outcome was the proportion of patients in clinical remission, defined as a score of 0 for stool frequency and rectal bleeding, and absence of fecal urgency at week 6.

Secondary efficacy outcomes were clinical remission at week 10, clinical remission at both weeks 6 and 10, endoscopic remission (defined as a sigmoidoscopic score of ≤1) at week 6, endoscopic remission at week 10, improvement (defined as a decrease of at least 3 points from baseline in the modified UCDAI score) at week 6, improvement at week 10, and the mean changes in the modified UCDAI and UCCS from baseline to week 10.

Endoscopic video recordings were submitted by the sites for assessment by a Central reader for the purpose of quality review and site training. Although eligibility disagreements between the Site investigator and Central reader's evaluation of the baseline endoscopic score were discussed with the sites for training purposes, the Site

investigator's score was used as the criterion for eligibility and to generate the data used in the primary intent to treat (ITT) analysis. No modifications to scoring based on the Central reader assessment, were allowed. This decision was based on technological considerations that were operative in 2009. Namely, adequate file compression technology and sufficient internet band width did not exist to allow transmission of videos to Central readers for evaluation of eligibility to sites in a timely fashion given the imperative to screen patients quickly. Thus, Site investigator assessment was the sole criterion for inclusion.

To assess the methodological effects of Central reading on trial outcomes, we compared results in the ITT population, in which both screening and outcome assessments were performed by the Site investigator to those observed in (1) the population of patients ruled eligible by the Central reader when the Site investigator scores were used for outcome assessment and/or (2) the population of patients ruled eligible by the Central reader when the Central reader's scores were used for outcome assessment. Since endoscopic assessment was not included in the definition of clinical remission in this trial, this latter comparison was performed using other commonly accepted definitions of response (a decrease from baseline in the total UCDAI score of 3 points with an accompanying decrease in the sub-score for rectal bleeding of ≥1 point or an absolute sub-score for rectal bleeding ≤1) and remission (a UC-DAI score of ≤2 points with no individual sub-score >1 point).[21]

All of the pre-specified efficacy outcomes were analyzed according to the ITT principle. Patients who withdrew prior to week 6 or for whom remission status was not evaluable due to incomplete and/or invalid data were considered to not be in clinical remission.

The proportions of patients who met the criteria for the pre-specified outcomes of clinical remission, endoscopic remission, and improvement were compared between the Asacol™ and placebo treatment groups using the chi-square test. The 95% confidence interval for the difference obtained was estimated using Newcombe's method-10.[22] Analyses that excluded patients who were deemed ineligible by the blinded central reader were performed accordingly. A similar approach was used in all of the secondary analyses of binary outcomes. The changes from baseline in modified UCDAI, sigmoidoscopic (mucosal) appearance, rectal bleeding and stool frequency, and UCCS were compared by Student's t-test.

The population for analysis of safety included all patients who received at least one dose of study drug.

The sample size for the clinical trial was determined assuming a remission rate of 20% in the placebo group. To detect a treatment effect of 15% (i.e., 20% remission rate in placebo compared to 35% in the Asacol™ treatment group), 136 evaluable patients per group were required to provide 80% power at the two-sided 0.05 level of significance. A total sample size of 280 patients was estimated to account for a non-evaluable population of approximately 3%.

In an independent study, we also assessed inter- and intra-observer agreement of central reading for all of the existing UC endoscopic indices. Seven expert readers, including JWDM, independently reviewed 50 sigmoidoscopy recordings that were randomly selected from the videos obtained at either the week 6 or 10 visit. Endoscopic disease activity was assessed for each video on three separate occasions using the

sigmoidoscopy score of the UCDAI, the Modified Baron Score (MBS), and the Ulcerative Colitis Endoscopic Index (UCEIS). A 100 mm Visual Analog Scale (VAS) global rating of severity was also completed.

Inter- and intra-observer agreement was estimated for the three endoscopic indices by calculating interclass correlation co-efficients (ICCs). This statistic is mathematically equivalent to a weighted Kappa co-efficient. A two-way random effects ANOVA model was used to analyze the data.

The sample size calculation for the agreement study was based on the ANOVA model. Assuming a true ICC of 0.75, we verified, by simulation, that rating of 50 videos by a minimum of 4 Central readers would yield an 83% chance of obtaining the one-sided 95% lower bound that is greater than 0.6, the “substantial” agreement criterion established by Landis and Koch.[23] For administrative purposes 7 readers participated in the study.

Results

Patient disposition and characteristics

A total of 343 patients were screened for eligibility; 281 met the eligibility criteria and were randomized and received at least one dose of study medication (ITT and safety populations) (**Figure 1).** Of the 281 patients randomized, 140 received Asacol™ and 141 received placebo. There was a single relevant protocol deviation: a patient was randomized with an investigator-defined sigmoidoscopic component score of 1. In total, 213 patients completed the study (84.3% in the Asacol™ group and 67.4% in the placebo group). AEs were the most frequent cause of early withdrawal and worsening of UC was the most common AE leading to withdrawal (10 of 12 patients in the Asacol™ group and 30 of 30 patients in the placebo group). Estimated compliance with the study drug was 93.5% in the Asacol™ group and 84.4% for patients assigned to placebo.

The baseline characteristics of the treatment groups were similar (**Table 1**). Of the 281 patients randomized, 194 (69.0%; Asacol™, n=107 and placebo, n=87) had a sigmoidoscopy score ≥2 confirmed by the Central reader. Approximately one-third (98 of 281; 34.5%) of the scores were graded lower than the site investigator by the Central reader and the majority of these (83 of 98; 85%) had a sigmoidoscopy score <2 and therefore would have been ineligible for participation had the Central reader's assessment been considered the gold standard for inclusion. This difference was not incongruent between the treatment groups. None of the assessments that were graded higher (57 of 281) by the Central reader would have resulted in a change in study eligibility; all involved a change from a score of 2 to 3. **Table 2** provides a summary of

the Site and Central-reader endoscopic assessments of the randomized population. The number of patients evaluated in the subgroup of patients considered eligible by the Central reader's assessment was considerably less than the ITT population. However, the demographics of the ITT population and the Central-reader confirmed population did not differ (data not shown). Disagreement was greatest at the baseline visit and improved, to a substantial degree at the week 6 and 10 visits. This trend was present for all of the participating jurisdictions but was most pronounced for India.

Efficacy

Primary Outcome

Clinical remission occurred in 30% (42 of 140) of patients in the Asacol™ group and 20.6% (29 of 141) of patients assigned to placebo (*P* = .069; 95% CI for the between group difference:[-0.7%–19.4%]). (**Figure 2A, ITT)** During performance of this analysis, a highly significant treatment-by-country interaction effect (*P* = .008) was found using the Gail-Simon test for qualitative interactions.[24] When the 57 patients enrolled from the outlier country were removed in a post-hoc analysis, as suggested in Section 3.2 of the International Conference on Harmonization guidance document "Statistical Principles for Clinical Trials" for the interpretation and analysis of heterogeneous data,[25] clinical remission was present in 35.1% (40 of 114) patients in the Asacol™ group and 20.9% (23 of 110) of patients in the placebo group resulting in an absolute difference in remission rates of 14.2% (*P* = .018; 95% CI of the between group difference: [2.4%–25.4%]).

Secondary Outcome Measures

The proportion of patients in the ITT population in clinical remission at week 10 was significantly higher in the Asacol™ group compared with the placebo group and numerically higher in the Asacol™ group at both weeks 6 and 10 (**Figure 2A, ITT).** The proportion of patients who improved at week 6 or at week 10 was significantly higher at both time points in the Asacol™ group compared with placebo-treated patients (59.3% vs 33.3% at week 6 [*P*< .001] and 62.9% vs 40.4% at week 10 [*P*< .001]), as was the proportion of patients with endoscopic remission (**Figure 2B, ITT)**.

The mean change in the modified UC-DAI and flexible sigmoidoscopic scores from baseline to week 10 or the end of treatment (EOT) visit was significantly greater in the Asacol™ group compared to the placebo group, as were the changes in the UCCS and component scores, with the exception of the subject's global assessment score (**Table 3**).

Post-hoc analyses based on other commonly used definitions of response and remission were also consistent with a benefit of Asacol™ therapy (**Figure 3, ITT)**.

Effects of Central Reading on Outcome Measures

In the population of 194 patients with active disease, defined by a Central reader sigmoidoscopic score of ≥2 at the screening visit, all of the predefined primary and secondary outcome measures were statistically significant (**Figures 2A and 2B)**. When commonly used definitions of response and remission (that included endoscopic components) were analyzed, the results at week 6 were significant irrespective of whether the site investigator's or the Central reader's rating were used for assessment

(Figure 3). Importantly, in all of these analyses the absolute magnitude of the estimates of the treatment effects did not change substantially in the Asacol™ group, but were consistently lower in the placebo group, resulting in a greater treatment difference between the experimental groups. The ICCS[26] between Central and Site investigator reading for the ITT population, were 0.11 (0.04 to 0.17), 0.31 (0.24 to 0.39), and 0.44 (0.36 to 0.52) at screening, week 6, and week 10, respectively. These results, (Table 2) indicate that substantial disagreement between Site readers and the Central reader existed at the screening visit. Moreover, Site readers irrespective of jurisdiction, were more likely to generate higher scores than those of the Central reader. Agreement improved, but still did not reach acceptable levels by the end of the trial.

Safety

At least one AE was reported by 44.3% of the patients in the Asacol™ group and 48.2% of the patients assigned to placebo (**Table 4)**. The most frequently occurring AEs were gastrointestinal disorders. All the events were of mild to moderate severity. Approximately 17% of patients in both groups experienced AEs that were considered related to the study medication. AEs leading to drug discontinuation occurred in 8.6% of patients in the Asacol™ group and 21.3% of patients in the placebo group. Worsening of UC was reported in 9.3% of patients in the Asacol™ group and 21.3% of patients in the placebo group (*P* =.005). Worsening of UC was the primary reason for early termination due to an AE in both the Asacol™ and placebo groups.

Results of the Agreement Study

Table 5 shows the results of the Agreement study. Near perfect intra-observer and inter-observer agreement was demonstrated by the Central readers for the scoring of all four instruments. As expected, intra-observer agreement was greater than inter-observer agreement. JWDM had a similarly high degree of agreement to the other Central readers. This is an important observation that means that the poor correlation observed between the Central and Site readers at the baseline visit in the trial was not due to inconsistent readings by JWDM.

Discussion

These results provide consistent evidence that Asacol™ 800 mg tablets, administered at a dose of 4.8 g/day is more effective than placebo for the treatment of mildly to moderately active UC. Although the observed difference between the treatment groups for the primary endpoint, clinical remission at week 6 in the ITT population, was marginally significant (30.0 vs. 20.6%, *P* =.069), analyses of the pre-specified secondary outcome measures consistently favored Asacol™. Thus, the totality of the data supports a treatment benefit.

Several plausible explanations exist for the failure to show a statistically significant difference between Asacol™ and placebo for the analysis of the primary outcome measure. The most relevant explanation was enrollment of a substantial proportion (31%) of patients who did not have sufficient endoscopic disease activity, based on the blinded Central reader's review. Inclusion of these patients increased the placebo rate of response. Restricting analysis of the primary outcome measure to patients who were deemed eligible by the Central reader markedly reduced the placebo remission rate, which in turn increased the difference observed between the experimental groups. Accordingly, in this analysis a highly significant ($P < .001$) absolute difference of 15.2% in week 6 remission rates was observed favoring Asacol™ despite the reduced statistical power that resulted from exclusion of 31% of the total trial population. This 15.2% difference is very similar to the magnitude of difference reported in other trials of mesalamine for the treatment of mildly to moderately active ulcerative colitis.[4] Thus, although the 9.4% difference observed in the ITT analysis was lower than what might be expected, in the population deemed eligible by the Central reader, the magnitude of the

treatment effect was in line with previous studies. Furthermore, although statistical significance was already evident in the ITT analyses for the majority of the secondary efficacy outcomes, the magnitude of the treatment effect observed was consistently greater in the analyses performed on the population of patients deemed eligible by the Central reader. A second possible explanation concerns the primary outcome measure used in this study. This measure, which was mandated by European regulatory authorities, has not been used in other trials of induction therapy for UC. In other commonly used definitions of clinical remission it is possible to have a stool frequency score of 1. This difference, and inclusion of the "absence of the urgency" criterion (for which no information regarding responsiveness to change is available), likely resulted in an outcome measure that is extremely difficult to achieve, leading to that classification of patients who did experience a clinically relevant change from baseline, with near-complete but not complete resolutions of symptoms, as not being in clinical remission. It is noteworthy that the post-hoc analyses that evaluated more conventional definitions of remission consistently identified significant differences between the treatment groups. Finally, the large degree of statistical heterogeneity observed in the analysis of the primary outcome measure may have been responsible for the marginal statistical significance observed. One of the four participating countries was an outlier in which the estimate of the treatment effect was greater for placebo than for Asacol™. We investigated this observation using the Gail-Simon test for qualitative interaction[24] which was highly significant in spite of the low power of this procedure. Although we could not definitively determine the reason for this finding, despite our performance of intensive country site audits, it is relevant to note that an analysis performed by excluding the

country identified as an outlier, using prescribed analytical techniques, showed a statistically significant result in favor of Asacol™. It is also relevant that India had the highest proportion of patients in whom the Site reader's score at baseline was "downgraded" by the Central reader (Table 2). As noted previously, inclusion of these patients may have obscured efficacy differences between the treatment groups.

Asacol™ was well tolerated. All AEs were mild or moderate in intensity, and an equal proportion of patients in each treatment group experienced AEs that were considered related to study medication. Nearly 3 times as many patients in the placebo group experienced AEs that led to drug discontinuation, with worsening of UC as the primary cause of early termination due to an AE in both groups.

These results have important implications for the conduct of clinical trials. In a systematic review of randomized controlled UC induction studies published between 1966 and 2005, Su *et al* documented that considerable heterogeneity exists in placebo remission rates.[27] While some of this variability is due to differences in study duration and design, number of follow-up visits, and baseline disease severity, it is now well established that inconsistency exists among gastroenterologists in assessing endoscopic disease severity.[18] For example, although Travis *et al* found 76% agreement between raters for videos of severe UC, very poor agreement was observed for those categorized as normal (27%) or moderately severe (37%).[18] Therefore variability in the assessment of endoscopic activity has the capacity to greatly influence the results of clinical trials.

Our study confirms the potential magnitude of this problem in patients with mildly to moderately active UC. Thirty one percent of the patients enrolled in this study did not meet the eligibility criterion for active disease as determined by the Central reader. Inclusion of these patients was associated with a substantially increased rate of remission in the placebo group. Scoring of mucosal friability was the most common source of disagreement between the Central reader and the Site investigator (data not shown), with the Site reading generating, on average, higher scores. Previous studies have identified scoring of friability as a cause for concern. In the ASCEND 3 trial an attempt to formally standardize the assessment of friability by tenting the mucosa with closed biopsy forceps proved futile and an international collaboration that examined the reliability of all existing endoscopic index items also found friability to be highly unreliable.[7] Consequently, it was not included as an item for new index development.[18] In the current trial, our interpretation of the data is that “upgrading” of eligibility scores by site investigators resulted in the inclusion of a number of patients who lacked active inflammation and thus had an increased chance of responding to placebo. This occurrence reduces the probability of detecting a treatment effect, should one exist, by diminishing between group differences. It is interesting to note that the inter-class correlation co-efficients derived by the Central reading readers were not appreciably different between the baseline read , which was integral to eligibility assessment, and the week 6 and 10 reads, which were integral to the assessment of efficacy. In contrast, we found that the baseline by site scores were considerably higher than those of the Central reader (Table 2).Given that the trial Central reader was shown to have a high degree intra and inter-observer agreement, measurement variance among Site readers

was responsible for this finding. Furthermore, the observation that the frequency of "upgrading" was highest at the baseline visit and subsequently decreased post randomization is consistent with the notion that bias was present in the initial assessment of eligibility such that investigators entered patients who did not meet the specified endoscopic inclusion criteria of the trial. An alternative explanation for this trend, specifically the presence of a training effect, is not plausible, since all of the site investigators were experienced in endoscopic scoring who had received multiple training sessions prior to initiation of the study. Furthermore, when we compared agreement over time between the first 50 percent of patients entered at sites and the last fifty percent, no difference was identified (data not shown). This finding is inconsistent with the presence of a training effect.

Based on these findings some very specific recommendations can be made regarding the conduct of future UC induction trials and methodological research in this area. First, patients should enter trials with a minimum degree of disease activity that is verified by an expert Central reader. Currently, an acceptable minimum standard is the presence of friability; however existing data raise questions as to whether this item is an optimal choice. The newly developed Ulcerative Colitis Endoscopic Index of Severity, which has eliminated the friability item, may ultimately prove to be a superior instrument as compared with existing measures; however future studies are needed to further evaluate this possibility. Second, although we could not confirm, in the current study, that central review of endoscopic images reduces inter-observer variability and therefore improves trial efficiency, we strongly suspect this is the case. Central

adjudication of images in other clinical circumstances has become the gold standard for this reason.[28–37]

In summary, we have shown that Asacol™ (800 mg) administered twice daily for 10 weeks at a dose of 4.8 g/day is effective for induction of clinical and endoscopic remission in patients with mildly to moderately active UC. Importantly, we also show that evaluation of endoscopic eligibility criteria by a Central reader has the potential to improve the efficiency of randomized controlled trials of induction therapy in UC by minimizing inclusion of patients with low endoscopic disease activity who increase placebo rates.

References:

1. Kornbluth A, Sachar DB; Practice Parameters Committee of the American College of Gastroenterology. Ulcerative colitis practice guidelines in adults: American College Of Gastroenterology, Practice Parameters Committee. Am J Gastroenterol. 2010;105:501-23.
2. Mowat C, Cole A, Windsor A, et al; IBD Section of the British Society of Gastroenterology Guidelines for the management of inflammatory bowel disease in adults. Gut. 2011;60:571-607.
3. Dignass A, Eliakim R, Magro F, et al. Second European evidence-based consensus on the diagnosis and management of ulcerative colitis Part 2: Current management. J Crohns Colitis. 2012;6:991-1030
4. Feagan BG, Macdonald JK. Oral 5-aminosalicylic acid for induction of remission in ulcerative colitis. Cochrane Database Syst Rev. 2012 Oct 17;10:CD000543. doi: 10.1002/14651858.CD000543.pub3.
5. Sutherland L, MacDonald JK. Oral 5-aminosalicylic acid for induction of remission in ulcerative colitis. Cochrane Database Syst Rev. 2006;Apr 19(2):CD000543.
6. Hanauer SB, Ramsey D, Sandborn WJ. Efficacy of delayed-release oral mesalamine in patients who received previous ulcerative colitis treatment. Gastroenterology 2008;134(Suppl 1):A490.
7. Sandborn WJ, Regula J, Feagan BG, et al. Delayed-release oral mesalamine 4.8 g/day (800-mg tablet) is effective for patients with moderately active ulcerative colitis. Gastroenterology. 2009;137:1934-43.

8. Sandborn WJ, Ramsey D, Sninsky C. Rapid symptom resolution with delayed release mesalamine 4.8g/d compared to 2.4 g/d in moderately active ulcerative colitis patients with a history of more difficult to treat disease. Am J Gastroenterol. 2009;104(Suppl 3):A1209.
9. Kamm MA, Lichtenstein GR, Sandborn WJ, et al. Effect of extended MMX mesalamine therapy for acute, mild-to-moderate ulcerative colitis. Inflamm Bowel Dis 2009;15:1–8.
10. Feagan BG, Macdonald JK. Once daily oral mesalamine compared to conventional dosing for induction and maintenance of remission in ulcerative colitis: A systematic review and meta-analysis. Inflamm Bowel Dis. 2012 May 29.update
11. Kamm MA, Sandborn WJ, Gassull M, et al. Once-daily, high-concentration MMX mesalamine in active ulcerative colitis. Gastroenterology. 2007;132:66-75.
12. Lichtenstein GR, Kamm MA, Boddu P, et al. Effect of once- or twice-daily MMX mesalamine (SPD476) for the induction of remission of mild to moderately active ulcerative colitis. Clin Gastroenterol Hepatol. 2007;5:95-102.
13. Kruis W, Kiudelis G, Racz I, et al. Once daily versus three times daily mesalazine granules in active ulcerative colitis: a double-blind, double-dummy, randomised, non-inferiority trial. Gut. 2009;58:233–40.
14. Kane S, Huo D, Aikens J, et al. Medication nonadherence and the outcomes of patients with quiescent ulcerative colitis. Am J Med. 2003;114:39–43.

15. Kane SV, Brixner D, Rubin DT, et al. The challenge of compliance and persistence: focus on ulcerative colitis. J Manag Care Pharm. 2008;14(1 Suppl A):s2–12.
16. Kane SV, Cohen RD, Aikens JE, et al. Prevalence of nonadherence with maintenance mesalamine in quiescent ulcerative colitis. Am J Gastroenterol. 2001;96:2929–33.
17. Ediger JP, Walker JR, Graff L, et al. Predictors of medication adherence in inflammatory bowel disease. Am J Gastroenterol. 2007;102:1417–26.
18. Travis SP, Schnell D, Krzeski P, et al. Developing an instrument to assess the endoscopic severity of ulcerative colitis: the Ulcerative Colitis Endoscopic Index of Severity (UCEIS). Gut. 2012;61:535-42.
19. Sutherland LR, Martin F, Greer S, et al. 5-Aminosalicylic acid enema in the treatment of distal ulcerative colitis, proctosigmoiditis, and proctitis. Gastroenterology 1987;92:1894–98.
20. Feagan BG, Greenberg GR, Wild G, et al. Treatment of ulcerative colitis with a humanized antibody to the alpha4beta7 integrin. N Engl J Med. 2005;352:2499-507.
21. Rutgeerts P, Sandborn WJ, Feagan BG, et al. Infliximab for induction and maintenance therapy for ulcerative colitis. N Engl J Med. 2005;353:2462–76.
22. Newcombe RG. Interval estimation for the difference between two independent proportions: Comparison of eleven methods. Statistics in Medicine. 1998;17:873-90.

23. Landis J.R., Koch, G.G. The measurement of observer agreement for categorical data. Biometrics. 1977;33 (1):159–74.

24. Gail MH and Simon R. Testing of qualitative interactions between treatment effect and patients subsets. Biometrics 1985;41:361-72.

25. Lewis JA. Statistical principles for clinical trials (ICH E9): an introductory note on an international guideline. Stat Med.1999;18:1903-42.

26. Fleiss, J. L. and Cohen, J. The equivalence of weighted kappa and the intraclass correlation coefficient as measures of reliability. Educational and Psychological Measurement.1973; 33:613-19.

27. Su C, Lewis JD, Goldberg B, et al. A meta-analysis of the placebo rates of remission and response in clinical trials of active ulcerative colitis. Gastroenterology. 2007;132:516-26.

28. Achiron A, Gicquel S, Miron S, et al. Brain MRI lesion load quantification in multiple sclerosis: a comparison between automated multispectral and semi-automated thresholding computer-assisted techniques. Magn Reson Imaging. 2002;20:713-20.

29. Molyneux PD, Brex PA, Fogg C, et al. The precision of T1 hypointense lesion volume quantification in multiple sclerosis treatment trials: a multicenter study. Mult Scler. 2000;6:237-40.

30. Molyneux PD, Miller DH, Filippi M, et al. Visual analysis of serial T2-weighted MRI in multiple sclerosis: intra- and interobserver reproducibility. Neuroradiology.1999;41:882-8.

31. Parodi RC, Sardanelli F, Renzetti P et al. Growing Region Segmentation Software (GRES) for quantitative magnetic resonance imaging of multiple sclerosis: intra- and inter-observer agreement variability: a comparison with manual contouring method. Eur Radiol. 2002;12:866-71.
32. Gasperini C, Rovaris M, Sormani MP, et al. Intra-observer, inter-observer and inter-scanner variations in brain MRI volume measurements in multiple sclerosis. Mult Scler. 2001;7:27-31.
33. Guillemin F, Billot L, Boini S, et al. Reproducibility and sensitivity to change of 5 methods for scoring hand radiographic damage in patients with rheumatoid arthritis. J Rheumatol. 2005;32:778-86.
34. Filippi M, Barkhof F, Bressi S, et al. Inter-rater variability in reporting enhancing lesions present on standard and triple dose gadolinium scans of patients with multiple sclerosis. Mult Scler.1997;3:226-30.
35. Filippi M, Horsfield MA, Bressi S, et al. Intra- and inter-observer agreement of brain MRI lesion volume measurements in multiple sclerosis. A comparison of techniques. Brain.1995;118:1593-600.
36. Rovaris M, Rocca MA, Sormani MP, et al. Reproducibility of brain MRI lesion volume measurements in multiple sclerosis using a local thresholding technique: effects of formal operator training. Eur Neurol. 1999;41(4):226-30.
37. Sharp JT, Wolfe F, Lassere M, et al. Variability of precision in scoring radiographic abnormalities in rheumatoid arthritis by experienced readers. J Rheumatol. 2004;31:1062-72.

Table 1. Patient Demographics and Baseline Characteristics (ITT Population)

	Asacol™ (n=140)	**Placebo (n=141)**
Age, y		
Mean (SD)	42.4 (14.3)	40.4 (13.8)
Range	18.5-79.4	18.6-75.2
Male, n (%)	87 (62.1)	75 (53.2)
White, n (%)	114 (81.4)	110 (78%)
Height, cm		
Mean (SD)	169.6 (8.7)	168.7 (10.4)
Range	149-192	146-194
Weight, kg		
Mean (SD)	68.7 (13.6)	65.7 (14.1)
Range	39-109.7	38-117.3
Smoking status, n (%)		
Current	5 (3.6)	12 (8.5)
Ex-smoker	28 (20)	16 (11.3)
Non-smoker	107 (76.4)	113 (80.1)
Time since symptoms, months		
Mean (SD)	65.4 (82.1)	61.3 (79.7)
Time since diagnosis, months		
Mean (SD)	54.3 (72.7)	51.8 (77.5)
Number of acute exacerbations in past 12 months		
Mean (SD)	1.7 (1.1)	1.4 (1.0)
Hospitalized in past 12 months	47 (33.6)	47 (33.3)
Extent of disease, n (%)		
Proctitis	9 (6.4)	3 (2.1)
Proctosigmoiditis	59 (42.1)	68 (48.2)
Left sided colitis	42 (30)	51 (36.2)
Portion of transverse colon	7 (5)	4 (2.8)
Pancolitis	22 (15.7)	15 (10.6)
Other	1 (0.7)	0 (0.0)
Prior oral mesalamine or 5-ASA	98 (70)	97 (68.8)

Table 2. Site and Central reader Endoscopic Assessments

		Jurisdiction				
		Belarus (91)	**India (56)**	**Turkey (24)**	**Ukraine (108)**	**Total (N=279)**
Visit						
Screening	Downgrade	32 (35.2)	24 (42.9)	7 (29.2)	35 (32.4)	98 (35.2)
	Upgrade	22 (24.2)	7 (12.5)	6 (25.0)	22 (20.4)	57 (20.4)
	Same	37 (40.6)	25 (44.6)	11 (45.8)	51 (47.2)	124 (44.4)
	Downgrade/Upgrade Ratio	1.45	3.43	1.12	1.59	
		Belarus (80)	**India (42)**	**Turkey (16)**	**Ukraine (79)**	**Total (N=217)**
Week 6	Downgrade	32 (40.0)	7 (16.7)	4 (25.0)	12 (15.2)	55 (25.3)
	Upgrade	9 (11.2)	5 (11.9)	2 (12.5)	13 (16.5)	29 (13.4)
	Same	39 (48.8)	30 (71.4)	10 (62.5)	54 (68.3)	13 (61.3)
	Downgrade/Upgrade Ratio	3.56	1.40	2.00	0.92	
		Belarus (78)	**India (40)**	**Turkey (16)**	**Ukraine (75)**	**Total (N=209)**
Week 10	Downgrade	20 (25.6)	2 (5.0)	0(0)	5 (6.7)	27 (12.9)
	Upgrade	8 (10.3)	4 (10.0)	2 (12.5)	9 (12.0)	23 (11.0)
	Same	50 (64.1)	34 (85.0)	14 (87.5)	61 (81.3)	159 (76.1)
	Downgrade/Upgrade Ratio	2.50	2.00	0.05	0.56	

Table 3. Change in UC-DAI and UCCS Scores from Baseline

	ITT				Central Reader-confirmed			
	Asacol™ (N=140)	Placebo (N=141)	Δ	p-value	Asacol™ (N=107)	Placebo (N=87)	Δ	p-value
Change in[a]								
Modified UC-DAI	-3.8±2.3	-2.1±2.7	1.7	<0.001	-3.9±2.3	-1.7±2.8	2.2	<0.001
Flexible proctosigmoidoscopic score	-0.8±0.8	-0.5±0.7	0.3	0.002	-0.8±0.9	-0.4±0.8	0.4	0.002
UCCS	-3.2±2.5	-1.5±3.0	1.7	<0.001	-3.4±2.4	-1.3±3.1	2.1	<0.001
Stool frequency score	-0.9±0.9	-0.3±1.1	0.6	<0.001	-1.0±0.9	-0.2±1.1	0.8	<0.001
Rectal bleeding score	-1.0±0.8	-0.5±0.9	0.5	<0.001	-1.1±0.8	-0.5±0.8	0.6	<0.001
Physician global assessment score	-0.8±0.9	-0.4±0.8	0.4	<0.001	-0.8±0.9	-0.3±0.8	0.5	<0.001
Subject's global assessment score	-0.5±0.8	-0.3±0.8	0.2	0.165	-0.5±0.7	-0.3±0.9	0.2	0.160

A Mean ± SD of change from baseline to week 10 or EOT assessment

Table 4. Treatment Emergent Adverse Events

	Asacol™ (N=140)	**Placebo (N=141)**
Any AE, n (%)	62 (44.3)	68 (48.2)
Severe AE, n (%)	0 (0.0)	0 (0.0)
Drug-related AE, n (%)	24 (17.1)	25 (17.7)
Serious AE, n (%)	0 (0.0)	3 (2.1)
Drug-related serious AE, n (%)	0 (0.0)	0 (0.0)
AE leading to drug interruption, n (%)	1 (0.7)	1 (0.7)
AE leading to drug discontinuation, n (%)	12 (8.6)	30 (21.3)

Table 5. Estimates of Intra- and inter- rater agreement based on data from 50 random videos evaluated three times by 7 blinded Central readers, including the trial Central reader.

	Instrument			
Reader Number	**UCDAI Sigmoidoscopy Score**	**Modified Baron Score**	**UCEIS**	**VAS**
Intra-Observer Agreement				
All 7 Central Readers	0.89 (0.85 to 0.92)	0.88 (0.84 to 0.92)	0.89 (0.85 to 0.93)	0.91 (0.88 to 0.94)
Trial Central Reader	0.88 (0.83 to 0.92)	0.79 (0.71 to 0.86)	0.84 (0.78 to 0.89)	0.89 (0.85 to 0.93)
2	0.85 (0.77 to 0.90)	0.87 (0.81 to 0.92)	0.90 (0.84 to 0.94)	0.91 (0.86 to 0.94)
3	0.89 (0.83 to 0.93)	0.85 (0.78 to 0.91)	0.85 (0.77 to 0.90)	0.92 (0.88 to 0.95)
4	0.89 (0.83 to 0.93)	0.86 (0.79 to 0.91)	0.90 (0.85 to 0.94)	0.88 (0.81 to 0.93)
5	0.81 (0.72 to 0.88)	0.87 (0.80 to 0.92)	0.89 (0.84 to 0.93)	0.89 (0.83 to 0.93)
6	0.92 (0.88 to 0.95)	0.94 (0.91 to 0.97)	0.91 (0.86 to 0.94)	0.93 (0.90 to 0.96)
7	0.91 (0.86 to 0.94)	0.92 (0.88 to 0.95)	0.92 (0.87 to 0.95)	0.90 (0.85 to 0.94)
Inter-Observer Agreement				
All 7 Central Readers	0.79 (0.72 to 0.85)	0.78 (0.71 to 0.85)	0.83 (0.77 to 0.88)	0.78 (0.70 to 0.85)

*All values are interclass correlation co-efficients (statistically identical to the weighted Kappa statistic) and 95% confidence intervals.

UCDAI = Ulcerative Colitis Disease Activity Index, UCEIS = Ulcerative Colitis Endoscopic Index of Severity; VAS = Visual Analog Scale

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