The fecal microbiome as a biomarker for monitoring and predicting remission in Ustekinumab-treated, anti-TNF-alpha refractory Crohn's Disease patients.

Running title: Microbiome of Ustekinumab-treated Crohn's Disease patients.

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Abstract

Abstract: Crohn's disease (CD) is a global health concern characterized by patches of ulceration and inflammation along the gastrointestinal tract, as well as reduced gut microbial diversity. We investigated the association between the fecal microbiome and clinical phenotypes in subjects with moderate to severe CD, refractory to anti-TNFα, that was treated with Ustekinumab (UST) in a double-blinded, placebo-controlled, Phase 2b clinical trial. We hypothesized that the fecal microbiome would be different between treatment response groups. Stool samples from 306 subjects, at screening, were obtained over the course of 22 weeks. The V4 region of the 16S rRNA gene was amplified and sequenced to determine the structure of the fecal bacterial communities.

The α-diversity of UST treated clinical responders increased over time, in contrast to nonresponsive subjects. Using Random Forest models, differences in the fecal microbiome following treatment could classify patients in remission from those with active disease. The baseline microbiome and clinical metadata could effectively predict therapeutic response at week 6, especially for remission. Baseline α-diversity was higher in treated subjects in remission at week 6. Baseline β-diversity was different based on response to UST treatment. Two OTUs, *Faecalibacterium* and *Bacteroides*, were significantly more abundant in treated subjects who were in remission at week 6. The fecal microbiome at baseline was also associated with markers for disease severity, such as Crohn's Disease Activity Index (CDAI), stool frequency, CRP, fecal lactoferrin, fecal calprotectin, corticosteroid use, disease duration, and tissue involvement. The observed baseline differences in fecal microbiota and changes due to therapeutic response support using the microbiome as a biomarker for establishing and maintaining CD remission.

Importance: Finding biomarkers that give clinicians the ability to predict response to CD treatment at diagnosis will increase the likelihood of faster induction and maintenance of remission. The fecal microbiome could be a useful non-invasive biomarker for directing or monitoring the treatment of CD patients. OTUs associated with remission following treatment induction, especially *Faecalibacterium*, could be biomarkers for successful UST treatment of TNF-α refractory CD patients.

Keywords: Crohn's Disease, IBD, fecal microbiome, microbiota, biologics, prediction, biomarkers, remission, Faecalibacterium, Ustekinumab, Stelara, machine learning, random forest

Introduction

Crohn's disease (CD), an incurable inflammatory bowel disease (IBD), is a global health concern causing large economic and healthcare utilization impacts on society (1–3). CD is characterized by patches of ulceration and inflammation along the entire gastrointestinal tract, though mostly the ileum and colon. Currently, individuals with CD are treated based on disease location and risk of complications using escalating immunosuppressive treatment, and/or surgery, with the goal of achieving and sustaining remission (4, 5). Faster induction of remission following diagnosis reduces the risk of irreversible intestinal damage and disability (5–7). Ideally, clinicians would be able to determine personalized treatment options for CD patients at diagnosis that would result in faster achievement of remission (8). Therefore, recent research has been focused on identifying noninvasive, prognostic biomarkers to monitor CD severity and predict therapeutic response (9–11).

The precise etiology of CD remains unknown, but host genetics, environmental exposure, and the gut microbiome appear to be involved (1, 12). Individuals with CD have reduced microbial diversity in their guts, compared to healthy individuals, with a lower relative abundance of *Firmicutes* and an increased relative abundance of *Enterobacteraciae* and *Bacteroides*, at the phylum level (13–17). Additionally, genome-wide association studies of individuals with CD identified several susceptibility loci, including loci involved in the IL-23 signaling pathway, which could impact the gut microbiome structure and function (4, 13, 18–24). If the fecal microbiome can be used to monitor disease severity and predict response to specific treatment modalities, then clinicians could use it as a noninvasive tool for prescribing therapies that result in faster remission (25).

The microbiome has been correlated with a variety of diseases and has shown promise as a predictive tool for disease outcome for gingivitis (26), cardiovascular disease (27), *Clostridium difficile* infection (28–30), and colorectal cancer (31, 32). In relation to IBD, previous studies have shown that the gut microbiome correlates with disease severity in new-onset, pediatric CD patients (17, 33). Additionally, recent studies have shown promise for the microbiome as it relates to IBD and therapeutic response (34, 35). It remains to be determined, however, whether the fecal microbiome can predict and monitor response to therapy in CD (13).

The FDA recently approved Ustekinumab (UST), a monoclonal antibody directed against the shared p40

subunit of IL-12 and IL-23, for the treatment of CD (5, 36–38). Given the potential impact of IL-23 on the microbiome (18–24), we hypothesized that UST treatment may alter the fecal microbiome and that response to UST could be predicted or influenced by differences in patients' gut microbiota. We analyzed the fecal microbiomes of individuals who participated in a double-blinded, placebo-controlled Phase II clinical trial of UST in treating CD (36). Using 16S rRNA gene sequence data from these patients' stool samples, we tested whether the microbiome changed in subjects treated with UST and if clinical responders had a microbiome that was distinct from non-responders. We also determined associations between clinical metadata, disease severity, and the fecal microbiome. Our study demonstrates that the fecal microbiome is associated with baseline clinical metadata and that these associations are useful in predicting and monitoring UST treatment outcome.

Results

Characteristics of the study population and sequencing results

We characterized the fecal microbiota in a subset of TNF-α refractory CD patients, with moderate to severe CD, who took part in the double-blinded, CERTIFI clinical trial (36). Demographic and baseline disease characteristics of this subset are summarized in Table 1. Patients were randomly assigned to a treatment group in the induction phase of the study and at week 8 patients were re-randomized into maintenance therapy groups based on their induction response (Figure 1A). Subjects provided stool samples at screening (week 0), week 4, week 6, and week 22 post induction for analysis using 16S rRNA gene sequencing (Figure 1B).

Following sequence curation using the mothur software package (39), we obtained a median of 13,732 sequences per sample (IQR = 7,863-21,978). Parallel sequencing of a mock community had an error rate of 0.017%. To limit effects of uneven sampling, we rarefied the dataset to 3,000 sequences per sample. Samples from subjects that completed the clinical trial and had complete clinical metadata were included in our analysis. Of these samples, 306 were provided prior to treatment as well as 258 provided at week 4, 289 at week 6, and 205 at week 22 post-treatment, for a total of 1,058 samples.

The diversity of the microbiome changes in UST responders

Given the potential impact of IL-23 pathways on the microbiome (18–22), we hypothesized that UST

treatment would alter the fecal microbiome. The effects of biologic treatment of IBD on the microbiome are not yet well described, but are hypothesized to be indirect, as these drugs act on host factors (4). We tested whether treatment with UST alters the microbiome by performing a Friedman test comparing α-diversity at each time point within each treatment group based on subjects' week 22 response status. We included 48 subjects induced and maintained with UST (20 responders, 28 non-responders) and 14 subjects induced and maintained with placebo (10 responders, 4 non-responders), who provided samples at every time point (Figure 1). As shown in Figure 2, we saw no significant difference in α-diversity over time in subjects who did not respond at week 22, regardless of treatment. However, the α-diversity of week 22 UST responders changed over time ($p = 0.005$). Multiple comparisons following the Friedman test showed the inverse Simpson index at week 22 was significantly higher than week 0 ($p < 0.05$). No change was observed in subjects induced and maintained with placebo who responded at week 22.

The microbiome following treatment can distinguish between treatment outcomes

Having observed that α-diversity increases in subjects who responded to treatment, we hypothesized that we could use the fecal microbiome to distinguish between subjects who responded to treatment from those who did not. A recent study demonstrated a link between the microbiome and disease activity, where specific microbes were associated with remission compared to active CD (25). We hypothesized that the microbiome could be used to monitor response to UST therapy in a similar manner. We used the AUCRF package in R to develop a random forest classification model to distinguish between subjects by treatment response based on their fecal microbiome (32, 40). The study design resulted in only 75 week 22 stool samples from subjects induced and maintained with UST, so we focused our analysis on the 220 week 6 stool samples from subjects induced with UST. As shown in Figure 3, the model could distinguish week 6 responders from non-responders using week 6 stool samples with an AUC of 0.708 (sensitivity $= 0.769$, specificity $= 0.606$). For week 6 remission, using week 6 samples the model had an AUC of 0.866 (sensitivity $= 0.833$, specificity $= 0.832$). We were better able to distinguish remitters from non-remitters compared to responders from non-responders. We hypothesize that this is due to the relative nature of the response criteria compared to the threshold used to determine remission status, as response was defined as a decrease in a subject's initial CDAI of 30% or more, while remission was defined as a CDAI below the threshold of 150.

Prediction of remission based on the microbiome at screening

Having demonstrated that the microbiome at week 6 following induction could classify week 6 treatment outcomes, we hypothesized that the week 0 fecal microbiome could predict week 6 response to therapy. To test this hypothesis, we again used the AUCRF package in R to classify week 6 responders from non-responders, as well as week 6 remitters from non-remitters, based on the relative abundance of fecal microbiome community members at week 0, clinical metadata at week 0, and the combination of microbiome and clinical data (32, 40). We ran these models on 232 week 0 stool samples from subjects induced with UST. The optimal models for response and remission at the primary endpoint (Week 6) are shown in Figure 4A and C. Using only microbiome data, the model predicted response with an AUC of 0.737 (specificity = 0.807, sensitivity = 0.585). When combining clinical metadata with the microbiome, the model predicted response with an AUC of 0.745 (specificity = 0.727, sensitivity = 0.744). With respect to week 6 remission, using only fecal microbiome data the model had an AUC of 0.838 (specificity = 0.766, sensitivity = 0.806). Finally, when combining clinical metadata with the microbiome we achieved an AUC of 0.844 (specificity = 0.831, sensitivity = 0.774) for remission at week 6. Prediction with clinical metadata alone did not perform as well as models using the week 0 fecal microbiome. Also, the models were again better able to classify remission compared to response.

The majority of OTUs identified as optimal classifiers in our model for remission were low in abundance across our cohort (Figure 4B and D). However, two OTUs appeared to be differentially abundant for subjects in remission at week 6. The relative abundance of *Escherichia/Shigella* (OTU00001) appeared lower in remitters (median = 1.07 IQR = 0.033-3.7) compared to non-remitters (median = 4.13, IQR = 0.667-15.4). Also, the relative abundance of *Faecalibacterium* (OTU00007) was not only higher in remitters (median = 7.43, IQR = 1.43-11.9) than non-remitters (median = 0.167, IQR = 0-5.1), it was present prior to the start of treatment in every subject who was in remission at week 6 post induction.

Comparison of clinical responders and non-responders

As our random forest models identified OTUs abundant across our cohort that were important in classifying response and remission, we further explored differences in the week 0 microbiome that could serve as potential biomarkers for successful UST treatment. We compared the week 0 microbiomes of all 306 subjects who provided a sample at screening based on treatment group and response at week 6. With respect to α-diversity, subjects induced with UST and in remission at week 6 had significantly higher diversity based on inverse Simpson than non-remitters treated with UST (respective median

values = 11.6 (IQR = 4.66-13.9), 6.95 (IQR = 4.4-11.8), p = 0.020). No other treatment or response groups were significantly different. β-diversity was significantly different for response and remission in UST treated subjects at week 6 (response p = 0.012, week 6 remission p = 0.017). Wilcoxon rank sum tests were performed on the relative abundance of each phylum and OTU based on treatment group and week 6 response and then corrected for multiple comparisons. No phyla were significantly different by treatment and response (Supplemental Figure 1). No OTUs were significantly different among subjects receiving placebo for induction, regardless of response and remission status at week 6. Two OTUs were significantly more abundant in UST-induced, week 6 remitters compared to non-remitters; *Bacteroides* (OTU0019) (p = 0.022) and *Faecalibacterium* (OTU0007) (p = 0.0026), as shown in Figure 5.

The baseline microbiome associates with clinical variables

Since we found associations between week 0 microbial diversity and week 6 response, we hypothesized that there were associations between the microbiome and clinical variables at baseline that could support the use of the microbiome as a non-invasive biomarker for disease activity (25). To test this hypothesis, we compared the week 0 microbiome with clinical data at week 0 for all 306 samples provided at screening (Supplemental Table 1). We compared α-diversity at baseline to clinical variables using the inverse Simpson index with Spearman correlation, Wilcoxon rank sum, or Kruskal-Wallis rank sum, tests where appropriate. We compared β-diversity with a PERMANOVA using the adonis function in the vegan R package. Following multiple comparison correction, we observed small, but significant correlations for lower α-diversity correlating with higher CDAI (rho = -0.161, p = 0.014), higher frequency of loose stools per week (rho = -0.193, p = 0.003), and longer disease duration (rho = -0.225, p = 0.001). Corticosteroid use was associated with higher α-diversity (p = 0.001). No significant association was observed between α-diversity and CRP, fecal calprotectin, or fecal lactoferrin. However, the β-diversity was significantly different based on CRP (p = 0.033), fecal calprotectin (p = 0.006), and fecal lactoferrin (p = 0.004). The β-diversity was also significantly different based on weekly loose stool frequency (p= 0.024), age (p = 0.033), the tissue affected (p = 0.004), corticosteroid use (p =0.01), and disease duration (p = 0.004). No significant differences in the microbiome were observed for BMI, weight, or sex.

Discussion

With this study we sought to gain a more detailed understanding of if and how UST treatment affects the microbiome and to determine whether the microbiome can be used to identify patients who will respond to UST therapy. We also aimed to gain a better understanding of the interaction between the human gut microbiome and CD pathogenesis in adult patients refractory to anti-TNF-α therapies with moderate to severe CD. We found the fecal microbiome to be useful in uncovering associations between the microbiome and aspects of CD severity metrics and treatment outcomes. We also showed that the microbiome of treated responders changed over time. Finally, we demonstrated that the microbiome could be useful in predicting remission due to UST therapy, compared to clinical metadata alone, in our unique patient cohort.

We determined whether or not the microbiome is affected by treatment with biologics by looking at the microbiome of our CD subjects across multiple time points during treatment. We observed that the α-diversity of clinical responders increased over time, in contrast to nonresponsive subjects. This observation could be due to lower inflammation and changes in disease activity corresponding to improved health in subjects who responded to UST. We were able to classify subjects who responded to treatment based on their microbiome following treatment and were better able to predict remission status compared to response status. Response may be more difficult to classify due to the relative nature of a decrease ($>$30%) in CDAI compared to a threshold (CDAI$<$150) for remission. We also addressed whether response to therapy can be predicted with the microbiome by developing a random-forest model that used relative microbial abundance data and/or clinical metadata for input. Again, we were better able to predict remission/non-remission than response/non-response, using samples provided prior to treatment induction. These findings are consistent with other studies suggesting the microbiome could be useful as a biomarker in detecting remission versus active disease (25).

The presented prognostic model is useful for biomarker discovery and hypothesis generation about the biology of CD as it relates to the microbiome. Similar models could be further developed into a clinically useful prognostic tool. *Faecalibacterium* was the most frequently occurring OTU in our models. It is associated with health, comprising up to 5% of the relative abundance in healthy individuals, and has been shown to be low in CD patients (13, 15, 41, 42). Each subject induced with UST and in remission

at week 6 had measurable *Faecalibacterium* present at baseline. This supports the hypothesis that *Faecalibacterium* impacts CD pathogenesis. *Escherichia/Shigella* also occurred frequently in our models. This OTU is associated with inflammation and has been shown to negatively impact CD pathogenesis (42). Many other taxa observed in our analysis had low abundance or were absent in the majority of subjects. However, in many cases these taxa are related and may serve similar ecologic and metabolic roles in the gut environment. We hypothesize that these microbes may have genes that perform similar metabolic functions. These functions could be revealed by performing metagenomics on stool samples in future studies, especially in subjects who achieve remission.

We observed several associations between the microbiome and clinical variables that could impact how CD is monitored and treated in the future. Serum CRP, fecal calprotectin, and fecal lactoferrin are used as biomarkers to measure intestinal inflammation and CD severity. We found that the microbial community structure is different among patients based on these markers and that fecal lactoferrin and fecal calprotectin were important classifiers in our random forest models combining clinical metadata and the microbiome. This supports the hypothesis that the microbiome could function as a biomarker for measuring disease activity in patients, especially in concert with established inflammatory biomarkers (25, 43, 44). We also found that higher CDAI was associated with lower microbial diversity. This is consistent with other studies on the microbiome in individuals with CD compared to healthy individuals and studies looking at active disease compared to remission (17, 25, 45). However, these differences may have been driven by weekly stool frequency (Supplementary Table 1), which is consistent with previous studies (46). We also observed differences in the microbial community structure based on disease localization, which is consistent with a study by Naftali et al (41). Our study also found that corticosteroid use impacts the composition of the human fecal microbiome, which is consistent with observations in mouse models (47). As corticosteroid use appears to impact diversity, corticosteroid therapy may be useful when trying to positively impact microbial diversity during biologic therapy and thereby increase the possibility of response to CD therapies. We also observed that longer disease duration is associated with a reduction in fecal microbial diversity. This decreased diversity may be due to the long duration of inflammatory conditions in the gut. This observation and the increased likelihood of remission and mucosal healing in individuals treated with biologics earlier in the course of their disease is an argument for earlier biologic intervention (48–50). Hypothetically, earlier biologic

intervention would occur before chronic inflammation resutled in reduced microbial diveristy. A more diverse microbiome may then promote remission and reduce the likelihood of relapse. However, the cost of biologics for patients is hindrance to early biologic intervention. Using aptamers in place of monoclonal antibodies may alleviate this expense (51).

The positive and negative associations between the microbiome and CD allow us to hypothesize on ways to alter the microbiome in order to increase the likelihood therapeutic response. Prior to the initiation of therapy, patients could get a fecal microbiome analysis. The community data could then be used to direct the patient to undergo a round of antibiotics to target and reduce the levels of *Escherichia* in the patient's gut. Alternatively, the microbes found to be positively associated with response could be formulated into a daily probiotic patients could take while receiving therapy with the goal of increasing the likelihood of remission and mucosal healing. Additionally, further research into the microbiome as a prognostic biomarker could eventually allow for the screening of patients with stool samples at diagnosis to better inform treatment decisions. If the fecal microbiome can be used as a prognostic tool to non-invasively predict response to specific treatment modalities or inform treatment, then more personalized treatment could result in faster achievement of remission, thereby increasing patients' quality of life and reducing economic and healthcare impacts.

Methods

Study Design and Sample Collection

Janssen Research and Development conducted a placebo-controlled, phase II clinical study of approximately 500 patients to assess the safety and efficacy of UST for treating anti-TNF-α refractory, moderate to severe CD patients (36). Both patients and clinicians were blinded to their induction and maintenance treatment groups. Participants provided a stool sample prior to the initiation of the study and were then divided into 4 groups of 125 individuals receiving placebo or 1, 3, or 6 mg/kg doses of UST by IV. Additional stool samples were provided at week 4. At week 6 an additional stool sample was collected, patients were scored for their response to UST based on CD Activity Index (CDAI), and then divided into groups receiving either subcutaneous injection of UST or placebo at weeks 8 and 16 as maintenance therapy. Finally, at 22 weeks patients provided an additional stool sample and were then scored using CDAI for their response to therapy. Response was defined as a decrease in a subject's initial CDAI of 30% or more. This value was determined by using the approximate percent change in CDAI from mild-moderate CD (220) to remission (150). Remission was defined as a CDAI below the threshold of 150. Frozen fecal samples were shipped to the University of Michigan and stored at -80°C prior to DNA extraction.

DNA extraction and 16S rRNA gene sequencing

Microbial genomic DNA was extracted using the PowerSoil-htp 96 Well Soil DNA Isolation Kit (MoBio Laboratories) and an EPMotion 5075 pipetting system, as previously described (31, 32). The V4 region of the 16S rRNA gene from each sample was amplified and sequenced using the Illumina MiSeq™ platform as described elsewhere (44). Sequences were curated as described previously using the mothur software package (v.1.34.4) (39, 52). Briefly, we reduced sequencing and PCR errors (53), aligned the resulting sequences to the SILVA 16S rRNA sequence database (54), and removed any chimeric sequences using UCHIME (55). Sequences were clustered into operational taxonomic units (OTU) at a 97% similarity cutoff using the average neighbor algorithm (56). All sequences were classified using a naive Bayesian classifier trained against the RDP training set (version 14) and OTUs were assigned a classification based on which taxonomy had the majority consensus of sequences within a given OTU

(57). All fastq files and the MIMARKS spreadsheet with de-identified clinical metadata are available at **TBD**.

Gut microbiome biomarker discovery and statistical analysis

R v.3.3.2 (2016-10-31) and mothur were used for our data analysis (58). To assess α-diversity, the inverse Simpson index was calculated for each sample in the dataset. Spearman correlation tests were performed to compare the inverse Simpson index and continuous clinical data. Wilcoxon rank sum tests were performed for pairwise comparisons and Kruskal-Wallis rank sum tests for comparisons with more than two groups (59, 60). To measure β-diversity, the distance between samples was calculated using the thetaYC metric, which takes into account the types of bacteria and their abundance to calculate the differences between the communities (61). These distance matrices were assessed for overlap between sets of communities using the non-parametric analysis of molecular variance (AMOVA) and homogeneity of variance (HOMOVA) tests in mothur (62), as well as the adonis function in the R package vegan (v.2.4.2) (63). Change in α-diversity over time based on week 22 response was assessed using a Friedman test on subjects who provided a sample at each time point (64). The Friedman test is a function in the R package stats (v.3.3.2). Multiple comparisons following a Friedman test were performed using the friedmanmc function in the package pgirmess (v.1.6.5) (65). Change in beta-diversity over time by treatment group and response was assessed using the adonis function in vegan stratified by subject. We used the relative abundance of each OTU across the samples and clinical metadata as input into the AUCRF R package (v.1.1) (66), in order to identify phylotypes or clinical variables that distinguish between various treatment and response groups, as well as to predict or determine response outcome (67). Differentially abundant OTUs and phyla were selected through comparison of clinical groups using Kruskal-Wallis and Wilcox tests, where appropriate, to identify OTUs/phyla where there was a p-value less than 0.05 following a Benjamini-Hochberg correction for multiple comparisons (68). Other R packages used in our analysis included ggplot2 v.2.2.1 (69), dplyr v.0.5.0 (70), pROC v.1.9.1 (71), knitr v.1.15.1 (72–74), gridExtra v.2.2.1 (75), devtools v.1.12.0 (76), knitcitations v.1.0.7 (77), scales v.0.4.1 (78), tidyr v.0.6.1 (79), Hmisc v.4.0.2 (80), and cowplot v.0.7.0 (81).

Tables

Table 1: Summary of clinical metadata of chort at baseline

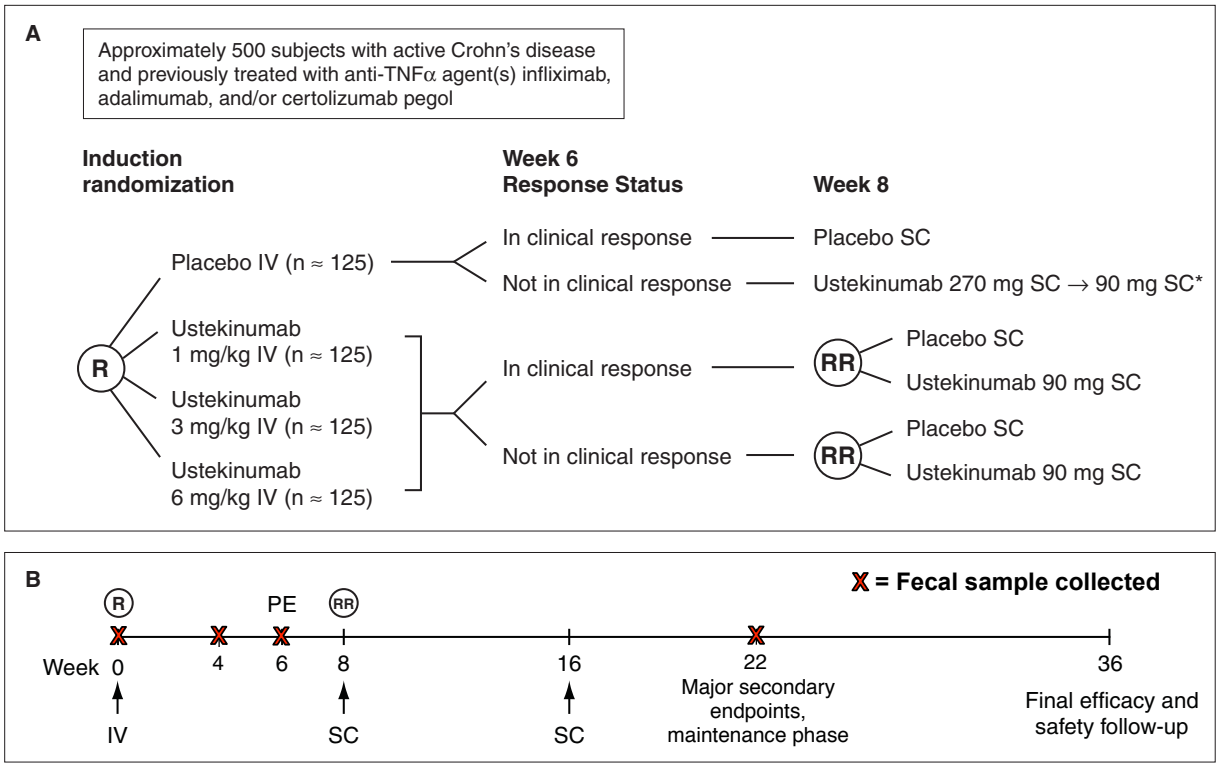
Clinical Variable	Treated	Placebo	Total
	n = 232	n = 74	n = 306
Age (years)	38 ± 13	40 ± 14	39 ± 13
Sex (% Male)	36.6	43.2	38.2
Race (% Caucasian)	91.8	93.2	92.2
Corticosteroid Use (%)	40.1	52.7	43.1
BMI (kg/m^2)	26 ± 6.7	25 ± 4.9	25 ± 6.3
Disease Duration (years)	12 ± 8.4	13 ± 10	12 ± 8.8
CDAI	330 ± 62	310 ± 69	320 ± 64
Bowel Stricture (%)	12.5	10.8	12.1
Tissue Involvement (%) Colon/Ileocolic/Ileal	28.9/51.3/19.8	24.3/39.2/36.5	27.8/48.4/23.9

Supplemental Table 1: Diversity differences based on clinical metadata of chort at baseline

Clinical Variable	Correlation	Alpha−Diversity (p−value)	Beta−Diversity (p−value)
CDAI	rho = −0.2	0.014	0.324
Loose Stool Frequency (per week)	rho = −0.2	0.003	0.024
C−Reactive Protein (mg/L serum)	rho = 0.06	0.394	0.033
Fecal Calprotectin (µg/g)	rho = 0.08	0.254	0.006
Fecal Lactoferrin (µg/g)	rho = 0.1	0.07	0.004
BMI	rho = 0.07	0.299	0.277
Weight (kg)	rho = 0.07	0.299	0.112
Age (years)	rho = −0.05	0.472	0.033
Sex (F/M)	−	0.539	0.277
Corticosteroid Use (Y/N)	−	0.001	0.01
Disease Duration (years)	rho = −0.2	0.001	0.004
Tissue Involvement	−	0.19	0.004

Figures

Figure 1: Experimental design as adapted from Sanborne et al 2012. (A) Diagram of experimental design. Participants were divided into 4 groups of 125 individuals receiving placebo or 1, 3, or 6 mg/kg doses of UST by IV. At week 8, patients were divided into groups receiving either subcutaneous injection of UST or placebo at weeks 8 and 16 as maintenance therapy, based on response at week 6. Finally, at 22 weeks patients were scored using CDAI for their response to therapy. (B) Stool sampling, treatment, and response evalution timeline.



IV = Intravenous; SC = Subcutaneous; ↑ = Study agent administration

PE = Primary Endpoint; R = Randomization; RR = Rerandomization only for subjects receiving ustekinumab induction therapy

* Subjects receiving placebo at Week 0 who are not in clinical response at Week 6 will receive ustekinumab 270 mg SC and 90 mg SC at Weeks 8 and 16, respectively.

Figure 2: Change in alpha diversity over time by induction treatment and week 22 response status. The α-diversity of 48 subjects induced and maintained with UST and 14 subjects induced and maintained with placebo was assess at each time point. Friedman test were perfomed within each teatment and responder group. * indicates week 22 is significantly different from week 0 ($p < 0.05$).

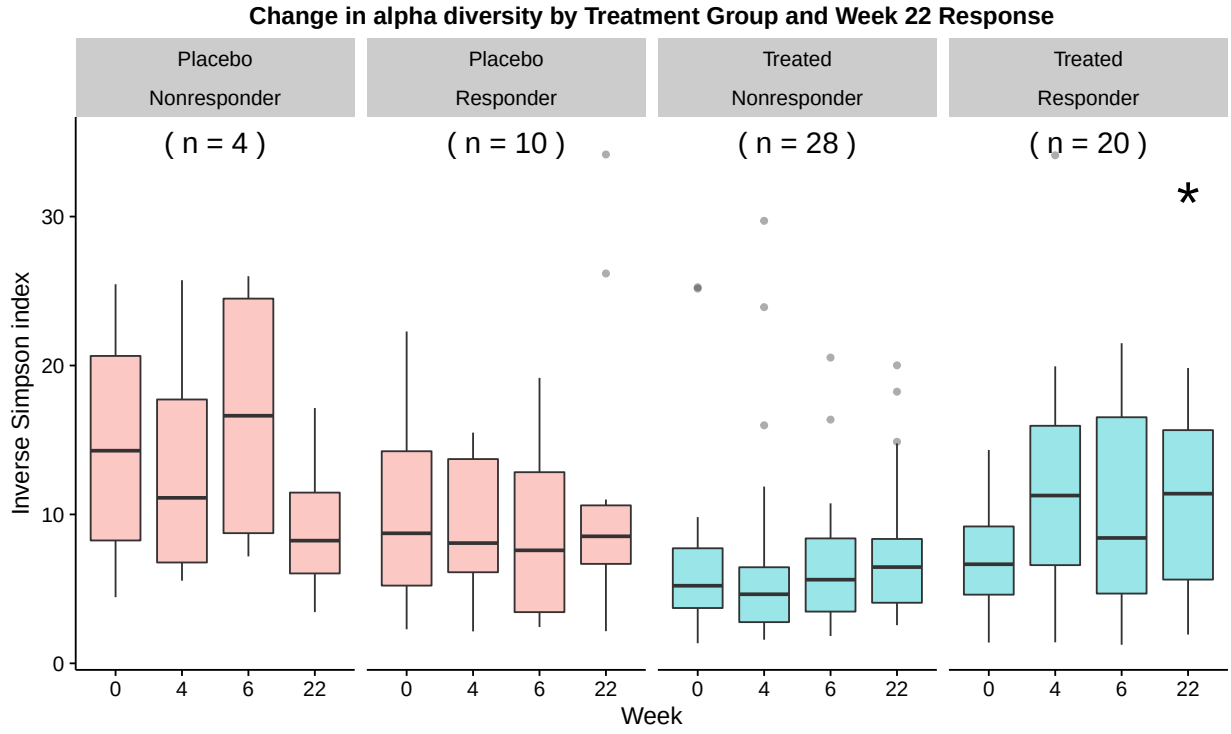


Figure 3: Classification of week 6 response or remission status using week 6 stool samples from subjects treated with UST (A) ROCs for week 6 outcome based on the week 6 microbiome. (B) Top predictive taxa from week 6 stool for remission status at week 6, based on MDA.

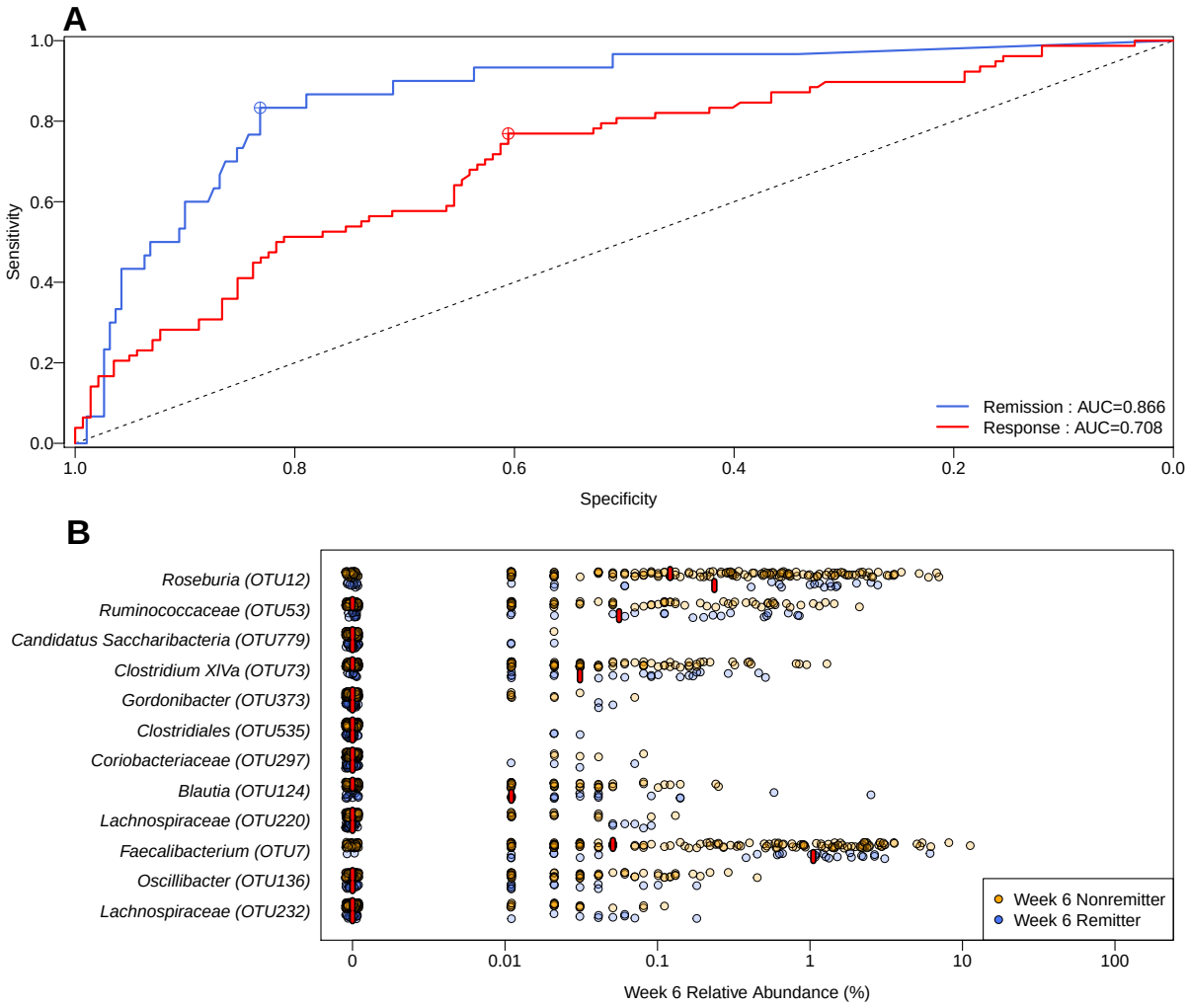
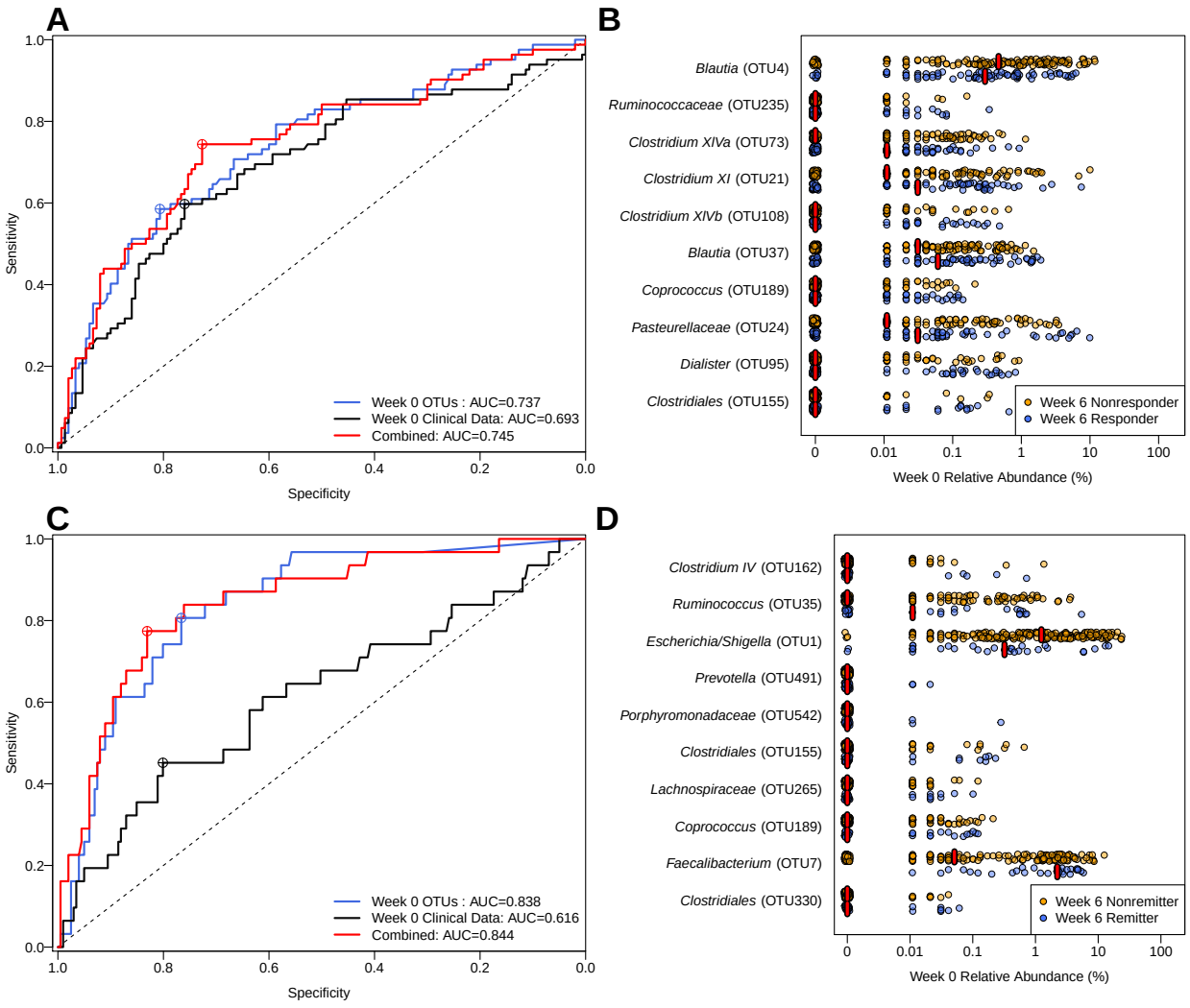


Figure 4: Prediction of week 6 disease status in subjects treated with UST, using week 0 samples ROCs for (A) response and (C) remission using microbiome data, clinical metadata, and a combined model. Top predictive taxa for the microbiome model based on MDA for (B) response and (D) remission.



Supplemental Figure 1: Phyla from week 0 stool samples in subjects treated with UST by week 6 outcome Compared the relative abundance of each phylum in UST teated subjects based on (A) response and (B) remission status using a Wilcoxon rank sum test and to identify phyla where there was a p-value less than 0.05 following a Benjamini-Hochberg correction for multiple comparisons.

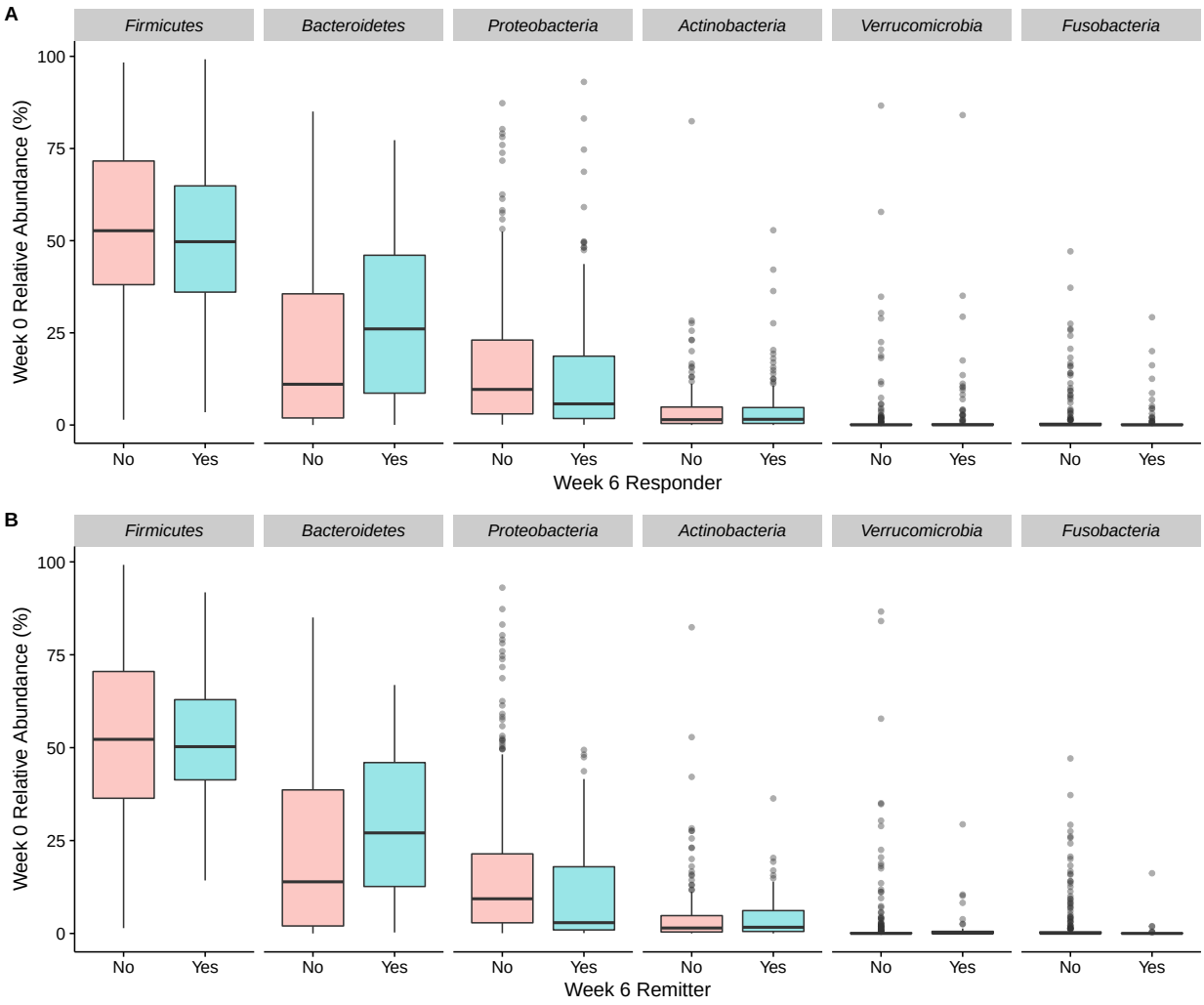
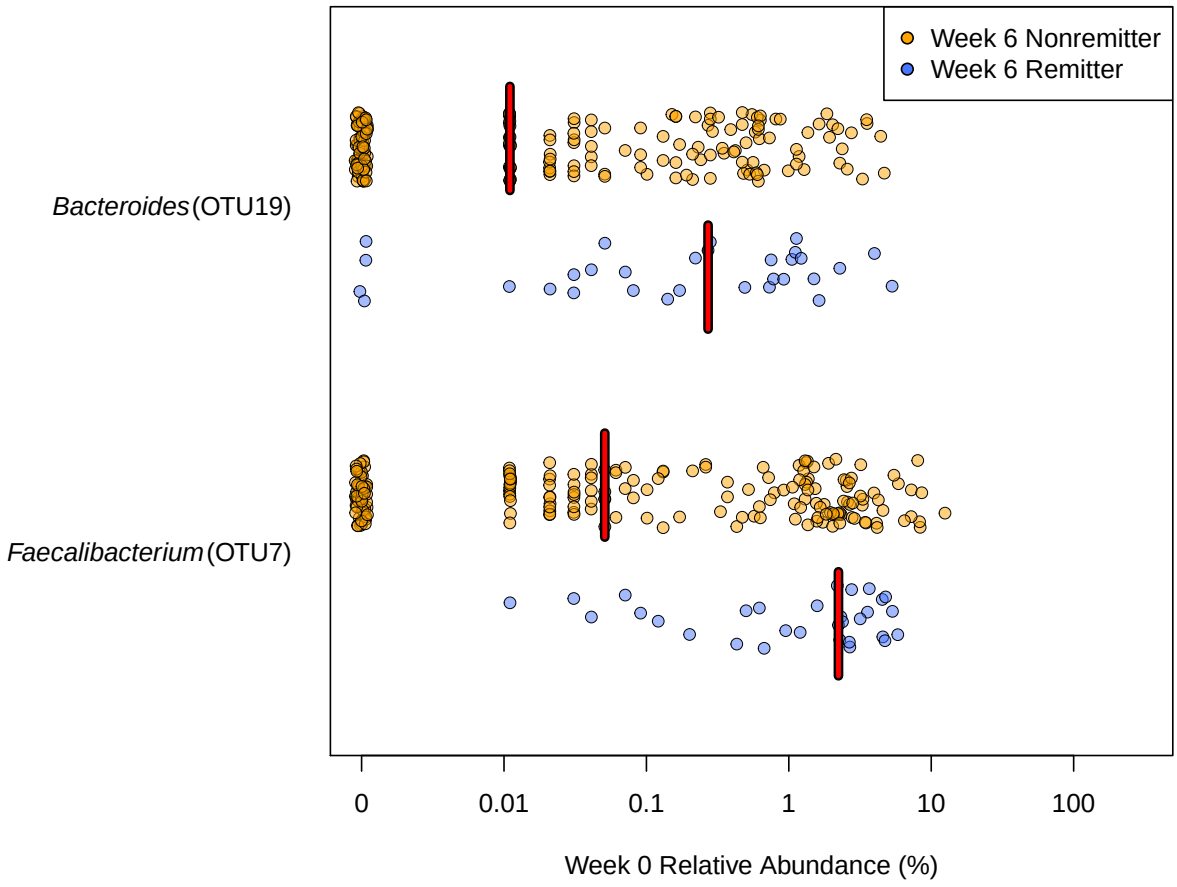


Figure 5: Differential taxa in week 0 stool samples from subjects treated with UST, based on week 6 remission status Compared the relative abundance of each OTU in UST teated subjects based on (A) response and (B) remission status using a Wilcoxon rank sum test to identify OTUs where there was a p-value less than 0.05 following a Benjamini-Hochberg correction for multiple comparisons.



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