The fecal microbiome as a tool for monitoring and predicting response outcomes 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 issue characterized by patches of ulceration and inflammation as well as reduced microbial diversity along the gastrointestinal tract. We investigated the association between the fecal microbiome and clinical phenotypes of subjects with moderate to severe CD that were refractory to anti-TNFa and treated with Ustekinumab (UST). We hypothesized that the fecal microbiome at baseline was predictive of disease severity and therapeutic response and that the fecal microbiota would change as a result of therapy. Stool samples from 500 patients taking part in a double-blinded, placebo-controlled, Phase 2b clinical trial 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.

Fecal microbial diversity at baseline was significantly correlated with markers for disease severity, such as Crohn's Disease Activity Index (CDAI), stool frequency, and disease duration. Additionally, stool frequency, CRP, fecal lactoferrin, fecal calprotectin, corticosteroid use, disease duration, and tissue involvement had a significant effect on the overall community structure of the microbiome. Baseline fecal microbiome community structures and species diversity were significantly different based on the outcome of UST treatment. *Faecalibacterium*, among other taxa, was significantly more abundant in responders/remitters. Additionally, the microbiome of clinical responders changed over time, in contrast to nonresponsive subjects. Using Random Forest models, the differences in the baseline microbiome and clinical metadata could effectively predict therapeutic outcome, especially for remission.

Importance: The ability to predict and monitor response to treatment using the microbiome will provide another clinical tool in treating CD patients. Additionally, the observed baseline differences in fecal microbiota and changes due to therapeutic response will allow further investigation into the microbes and/or the metabolic functions important in CD pathogenesis as well as establishing and maintaining CD remission. Finally, beneficial microbes associated with response to treatment could be developed therapeutics to increase the likelihood of response while undergoing treatment.

Keywords: Crohn's Disease, fecal microbiome, biologics, prediction

Introduction

Crohn's disease (CD), an incurable inflammatory bowel disease (IBD), is a global health issue with increasing incidence. CD affects approximately 3 million people worldwide, causing large economic and healthcare utilization impacts on society (1–3). CD is characterized by patches of ulceration and inflammation affecting the entire bowel wall along the gastrointestinal tract, most commonly in the ileum and colon. Individuals with CD experience frequent diarrhea, abdominal pain, fatigue, and weight loss resulting in significant health care costs, lower quality of life, and economic impacts due to loss of productivity (2, 4, 5). Current treatments for CD include antibiotics, anti-inflammatory drugs, immunomodulators, surgery, and biologic agents targeting tumor necrosis factor alpha (TNF-α), such as Infliximab (Remicade). Within 10 years of diagnosis, approximately half of individuals with CD will require surgery and the majority will experience escalating immunosuppressive treatment (6). 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 (5, 7). Faster induction of remission following diagnosis reduces the risk of irreversible intestinal damage and disability (7–9) (7-9). Anti-TNF-α therapy in combination with thiopurines has emerged as the preferred treatment for CD, but up to half of individuals with CD fail to respond or lose response to anti-TNF-α therapy (6, 7). Ustekinumab (UST), a monoclonal antibody directed against the shared p40 subunit of IL-12 and IL-23, has been proposed as an alternative therapy for these patients (10). While clinical trials have demonstrated that UST is a viable option for the treatment of CD (7, 10–12) (7, 10-12), some patients within these trials were non-responsive to UST, which we hypothesized was due to differences in the patients' gut microbiota at the initiation of treatment.

The precise etiology of CD remains unknown, but host genetics, environmental exposure, and the gut microbiome appear involved (1, 13). Genome-wide association studies of individuals with CD identified several susceptibility genes including NOD2, a receptor involved in bacterial killing and innate immunity. Defects in NOD2 function affects microbial sensing, the regulation of IL-23 driven Th17 responses, and indirect modulation of the gut microbiome (5, 14). The gut microbiome has also been shown to play a key role in inflammation, immunity, and IBD (15). 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 (14, 16–19) .

Additionally, previous studies have shown that the gut microbiome correlates with disease severity in new-onset, pediatric CD patients (19, 20).

The microbiome has been correlated with a variety of diseases and has shown promise as a predictive tool for disease outcome and precision therapies (21). Specifically, the severity of gingivitis (22), cardiovascular disease risk (23), and the response to dietary interventions has been shown to be associated or predicted by the microbiome (24, 25). Additionally, the risk of infection with *Clostridium difficile* and recurrence can be determined or predicted using the microbiome (26–28). The Schloss lab has also demonstrated that tumor burden in colorectal cancer can be predicted with the microbiome and that the microbiome can be a diagnostic tool for colonic lesions in combination with fecal immunochemical tests (29, 30). It remains to be determined, however, whether the microbiome can predict response to therapy in CD and if biologic treatment affects the gut microbiome (14), though recent studies have shown promise for the microbiome as it relates to IBD and therapeutic response (31). If the fecal microbiome can be used as a theraprognostic tool to non-invasively determine and monitor disease severity as well as predict response to specific treatment modalities, then more targeted treatment could result in reduced adverse effects of less effective therapies and faster achievement of remission.

Our lab was approached to analyze the gut microbiomes of individuals who participated in a placebo-controlled Phase II clinical trial to determine the safety and efficacy of UST in treating CD (10). Using stool samples taken prior to, and following, the start of the study, 16S rRNA gene sequence data from these patients' stool allowed us to determine associations between clinical metadata, disease severity, and the fecal microbiome, as well as whether clinical responders have a microbiome that is distinct from non-responders at baseline. We also show that the microbiome changed in subjects who responded to treatment. Our study demonstrates that the fecal microbiome is associated with baseline clinical metadata and that these associations and differences are useful in predicting disease severity and treatment outcome.

Results

Characteristics of Study Population

Using 16S rRNA gene sequencing, we studied the fecal microbiota in a subset of TNF-α refractory

CD patients who took park in the CERTIFI clinical trial (10). Demographic and baseline disease characteristics of this subset are summarized in Table 1. Patients with a history of moderate to severe CD were randomly assigned to a treatment group in the induction phase of the study (Figure 1A). At Week 8 patients were re-randomized into maintenance therapy groups. Both patients and clinicians were blinded to their induction and maintenance treatment groups. Subjects provided stool sample at screening (week 0), week 4, week 6, and week 22 post induction (Figure 1B). Therapeutic response was evaluated based on the change in CDAI at week 6 (primary endpoint) and week 22 (secondary endpoint) post induction. For this study, "response" was defined as a decrease in CDAI of 30% from baseline and "remission" was defined as a CDAI below 150.

Comparison of microbiome at screening based on clinical variables

Following sequence curation using the mothur software package, we obtained between 1 and 130,074 sequences per sample (median 13786) (32). Parallel sequencing of a mock community revealed 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 1058 samples.

To determine whether there are any significant associations between microbial diversity and clinical variables of interest, we compared the microbiome with clinical data at week 0 (Supplemental Table 1). We observed a weak, but significant correlation between CDAI and species diversity, with higher CDAI correlating to lower diversity (rho = -0.161, p = 0.00483). The overall community structure was not different based on CDAI. When looking at CDAI sub-scores, we observed a weak, but significant association between species diversity and the frequency of loose stools per week (rho = -0.193, p = 0.000693). The overall community structure was also significantly different based on weekly loose stool frequency (p= 0.012). No significant association was observed between CRP, fecal calprotectin, or fecal lactoferrin and species diversity, following multiple comparison correction. However, the overall community structure was significantly different based on CRP (p = 0.022), fecal calprotectin (p = 0.002), and fecal lactoferrin (p = 0.001). No significant differences in the microbiome were observed for BMI, weight, or sex. Overall community structure was different based on age (p = 0.019). The overall community structure was also different based on the tissue affected (p = 0.001). Species diversity

($p = 0.000196$)and the overall community structure ($p = 0.004$) were significantly different based on corticosteroid use. The community structure was significantly different ($p = 0.001$) based on disease duration and a weak, but significant correlation was seen between species diversity and disease duration (rho = -0.225, $p = 0.0000713$), with lower diversity corresponding to longer disease.

Comparison of clinical responders and non-responders

We hypothesized that there are associations between the microbiome at baseline and response to treatment. To test this, we compared the week 0 microbiomes of subjects based on treatment group and response status at week 6 and week 22. Only week 6 remitters induced with UST were significantly different from non-remitters in terms of alpha diversity, having higher diversity based on inverse Simpson (respective median values = 11.6, 6.95, $p = 0.020$). Baseline community structures were significantly different for each outcome status (response/remission) and treatment group at each clinical endpoint (week 6 response $p = 0.012$, week 6 remission $p = 0.017$, week 22 response $p = 0.012$, week 22 remission $p = 0.012$), as seen in Table 2. No phyla were significantly different by treatment and response, however *Fusobacteria* was less frequently observed in week 6 remitters than non-remitters treated with UST (median relative abundance = 0 and 0.0333, respectively). As seen in Figure 3, two taxa were significantly more abundant in UST-induced, week 6 remitters compared to non-remitters; *Bacteroides* (OTU0019) ($p = 0.022$) and *Faecalibacterium* (OTU0007) ($p = 0.0026$). No individual taxa were significantly different among UST induced subjects at week 22, or those receiving placebo for induction, regardless of response/remission status at week 6 and 22.

The microbiome by treatment and response over time

Having observed differences in the microbiomes of subjects who responded to treatment compared to those who did not, we hypothesized that treatment may affect the microbiome. The effects of biologic treatment of IBD on the microbiome are not yet well described, but are hypothesized to be indirect. We tested whether treatment with UST affects the microbiome using subjects who provided samples at weeks 0, 4, and 6. This allowed for us to analyze 156 treated subjects and 48 placebo subjects at each time point. Using the adonis function in the vegan R package (33), we performed a PERMANOVA stratified on each subject, as a proxy for repeated measures ANOVA, to determine if the community structure of microbiome changed over time. We included induction treatment group, response at each

clinical endpoint, and sample week as parameters.

We found that treatment only affects the microbiome over time in subjects who responded to UST at week 22. No significant difference was seen in community structure or species diversity based on sample date when looking at all treatment groups and week 6 response status, but there was a significant interaction between week 22 response and sample date ($p = 0.001$). There was also a significant interaction and between week 22 responses, induction group, and sample date ($p = 0.044$). This led us to further examining the microbial community structures in week 22 responders and non-responders over time by induction treatment. No significant difference was observed in Week 22 non-responders over time, regardless of treatment. In week 22 responders, we saw a significant change in community structure over time in both placebo ($p = 0.034$) and UST induction groups ($p = 0.018$).

Since we observed significant changes in the community structure of week 22 responders, we also hypothesized that treatment may also affect species diversity. We tested this by performing a Freidman test comparing species diversity at each sample date within each induction treatment group based on their week 22 response status. As seen in Figure 4, we saw no significant difference in species diversity over time in subjects who did not respond at week 22, regardless of induction treatment. However, in UST treated-week 22 responders species diversity increased significantly from week 0 to week 4 ($p = 0.0022$) and remained higher than baseline at week 6. This change was not observed in subjects induced with placebo who responded at week 22, unlike the community structure analysis. We hypothesize that this reflects decreased inflammation in the subjects who responded to treatment.

The microbiome following treatment reflects disease status

A paper recently published by Tedjo et al. demonstrated a link between the microbiome and disease severity, where specific microbes were associated with remission compared to active CD (34). We hypothesized that the microbiome could be used to monitor response to therapy in a similar manner. We used AUC-RF in order to determine if the fecal microbiome at Week 6 could be used to determine if a study participant responded to therapy or was in remission at Week 6. As seen in Figure 5, using the microbiome alone we achieved an AUC of 0.708 for response with a sensitivity of 0.769 and a specificity of 0.606. For remission we had an AUC of 0.866 with a sensitivity of 0.833 and specificity of 0.832. We were better able to distinguish remitters from non-remitters than responders from non-responders.

Prediction of response based on the microbiome at screening

Given the observed differences in the fecal microbiome at baseline and week 6 in responders/remitters compared to non-responders/non-remitters, we hypothesized that the fecal microbiome could predict response to therapy. To test this hypothesis, we used the AUCRF package in R to develop a random forest classification model to classify responders from non-responders, as well as remitters from non-remitters, based on the relative abundance of fecal microbiome community members, clinical metadata, and the combination of microbiome and clinical data (30, 35). We ran these models for response and remission at Week 6 and 22 of the study. The optimal models for response and remission at the primary endpoint (Week 6) are shown in *Figure 6A and C*. Using only clinical metadata, we achieved an AUC of 0.693, a specificity of 0.76, and a sensitivity of 0.598. Using only microbiome data, the model predicted response with an AUC of 0.737 with a specificity of 0.807 and a sensitivity of 0.585. When combining clinical metadata with the microbiome, the model predicted response with an AUC of 0.745, a specificity of 0.727, and a sensitivity of 0.744. With respect to Week 6 remission, using solely clinical metadata we achieved AUC of 0.616 with a specificity of 0.801 and a sensitivity of 0.452. Using only fecal microbiome data we achieved an AUC of 0.838 with a specificity of 0.766 and a sensitivity of 0.806. When combining clinical metadata with the microbiome, we achieved an AUC of 0.844 with a specificity of 0.831 and a sensitivity of 0.774. Across all weeks and responses, prediction with clinical metadata alone did not perform as well as models using the fecal microbiome at screening. Also, combining microbiome data with clinical metadata did not consistently improve prediction compared to microbiome data alone. Additionally we found several OTUs occurred frequently across models including *Faecalibacterium*, among other taxa that were more abundant in responders/remitters (Figure 6B and D).

Discussion

With this study we sought to gain a more detailed understanding of if and how biologic treatment affects the microbiome, to determine whether the microbiome can be used to identify patients who will respond to therapy, and to gain a better understanding of the interaction between the human gut microbiome and CD pathogenesis in adult patients. 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 demonstrated that the microbiome of treated responders changed over time, though it is not yet possible to determine any direct effects of treatment on the microbiome. Finally, we were able to show that the microbiome could be useful in predicting response to therapy, especially clinical remission, compared to clinical metadata alone in our unique patient cohort.

We observed several associations between the microbiome and clinical variables that could play a role in how CD is monitored and treated in the future. Given that serum CRP, fecal calprotectin, and fecal lactoferrin are used as biomarkers to measure intestinal inflammation and CD severity, the observation that the microbial community structure is different among patients based on these markers supports the hypothesis that the microbiome could function as a biomarker for measuring disease activity in patients, especially in concert with these established inflammatory biomarkers (34, 36, 37). Higher CDAI was associated with lower microbial diversity. This appears to be consistent with other studies on the microbiome in individuals with CD compared to healthy individuals and studies looking at active disease compared to remission (19, 34, 38). However, these differences may have been driven by weekly stool frequency, one component of the CDAI, where higher stool frequency is also negatively associated with microbial diversity. This finding is consistent with the association between loose stools and lower diversity (39). We also observed differences in the microbial community structure based on disease localization. These results are consistent with a study by Naftali et al finding distinct microbiotas for ileal versus colonic CD using mucosal tissue (40). Our study also found that corticosteroid use impacts the composition of the human fecal microbiome. This supports data seen in the mouse model where corticosteroid injections altered the fecal mouse microbiome (41). 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 (42–44). Hypothetically, earlier biologic intervention could 'preserve' a more diverse microbiome that promotes remission and reduces 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 reduce this cost and

make earlier intervention possible. Aptamers are short strands of DNA or RNA capable of specifically binding small molecules, proteins, and whole cells. Anti-TNF aptamers have been published that could potentially be used to test this in the mouse model (45).

An important question for the microbiome and IBD is whether or not the microbiome is affected by treatment with biologics. This study attempted to answer that question by looking at the microbiome of our CD subjects across multiple time points during treatment. While we were unable to see direct effects of the drug on the fecal microbiome, we observed that the microbiome of clinical responders changed over time, in contrast to nonresponsive subjects. This was observed for responsive patients regardless of induction treatment, leading us to think we are observing the effects of change in disease activity and health, leading to lower inflammation, rather than any effects from treatment. This interpretation is consistent with studies using the microbiome to distinguish between remission and active CD (34). We did however observe a significant difference in community structure based on treatment and cannot eliminate the possibility of a direct effect on the microbiome in treated responders, however the change in community structure observe in responders treated with placebo supports the hypothesis that the change in community structure reflects a change in inflammation.

Another important question in for the importance of the microbiome in IBD is whether response to therapy can be predicted with the microbiome. We attempted to address this by developing a random-forest model that used relative microbial abundance data and/or clinical metadata for input. We found we were better able to predict remission status compared to response status. Response may be less predictable due to the "floating target" nature of a relative decrease ($>$30% decrease) in CDAI compared to the hard threshold for remission (CDAI$<$150). We were also better able to distinguish remission/non-remission than response/non-response, using samples provided 6 weeks after treatment induction. This is consistent with other studies, again suggesting the microbiome could be useful as a biomarker in detecting remission versus active disease (34).

The presented model is useful for hypothesis generation about the biology of CD as it relates to the microbiome and could be further developed into a clinically useful theraprognostic tool. Some of the frequently occurring factors in our predictive models have already been linked to CD pathogenesis. As far as clinical biomarkers, fecal lactoferrin and fecal calprotectin occurred in the majority of models where clinical metadata was combined with the microbiome, supporting their importance as biomarkers

for CD activity, especially in relation to the fecal microbiome (36, 37). *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 (14, 17, 40, 46). Remission was much more likely in individuals who 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 (46). *Fusobacterium* also appeared in our predictive models and is associated with CD and CRC, something CD patients are more likely to develop than individuals without IBD (46). Many other taxa observed in our analysis had low abundance, but 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 that could be revealed by performing metagenomics on the week 0 stool samples in future studies, especially in subjects who achieved remission. These observations and the positive/negative associations of these microbes and CD also allow us to hypothesize on ways to alter the microbiome 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, altering the weighting or binning of important factors in the model could make prediction of response or remission more reliable. This could eventually allow for pre-screening of patients with stool samples to predict successful treatment or better direct treatment. If the fecal microbiome can be used as a theraprognostic 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 (10). 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 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. 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) using an EPMotion 5075 pipetting system, as previously described (29, 30). The V4 region of the 16S rRNA gene from each sample was amplified and sequenced using the Illumina MiSeq Personal Sequencing platform as described elsewhere (37). Sequences were curated as described previously using the mothur software package (47). Briefly, we reduced sequencing and PCR errors, aligned the resulting sequences to the SILVA 16S rRNA sequence database, and removed any chimeric sequences flagged by UCHIME (48). Sequences were clustered into operational taxonomic units (OTU), as previously described (49). Briefly, OTUs were clustered at a 97% similarity cutoff and the relative abundance was calculated for OTUs in each sample. All sequences were classified using a naive Bayesian classifier trained against the RDP training set (version 11) and OTUs were assigned a classification based on which taxonomy had the majority consensus of sequences within a given OTU (50). All fastq files and the MIMARKS spreadsheet with de-identified clinical metadata are available at TBD.

Gut microbiome biomarker discovery and statistical analysis

Mothur as well as the R software package were used for our data analysis. Alpha diversity metrics (e.g. Inverse Simpson) were calculated for each sample in the dataset, and compared using non-parametric statistical tests (i.e. Kruskal-Wallace and Wilcox Test) (51, 52). Beta diversity was calculated the distance between samples using the theta YC metric, which takes into account the types of bacteria and their abundance to calculate the differences between the communities (53). 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 as well as the adonis function in the R package vegan (33, 54). Change in alpha diversity over time was assessed using a Friedman test, whereas change in beta-diversity over time was assessed using the adonis function in vegan stratified by subject (55). Differentially abundant OTUs and phyla were selected through comparison of clinical groups using non-parametric statistical tests (i.e. Kruskal-Wallace and Wilcox Test) to identify OTUs/phyla where there is a P-value less than 0.05 following a Benjamini-Hochberg correction for multiple comparisons (56). We also used the relative abundance of each OTU across the samples and clinical metadata as input into the AUC-RF R package, in order to identify phylotypes/clinical variables that distinguish between various treatment and response groups, as well as to predict or determine response outcome (57).

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

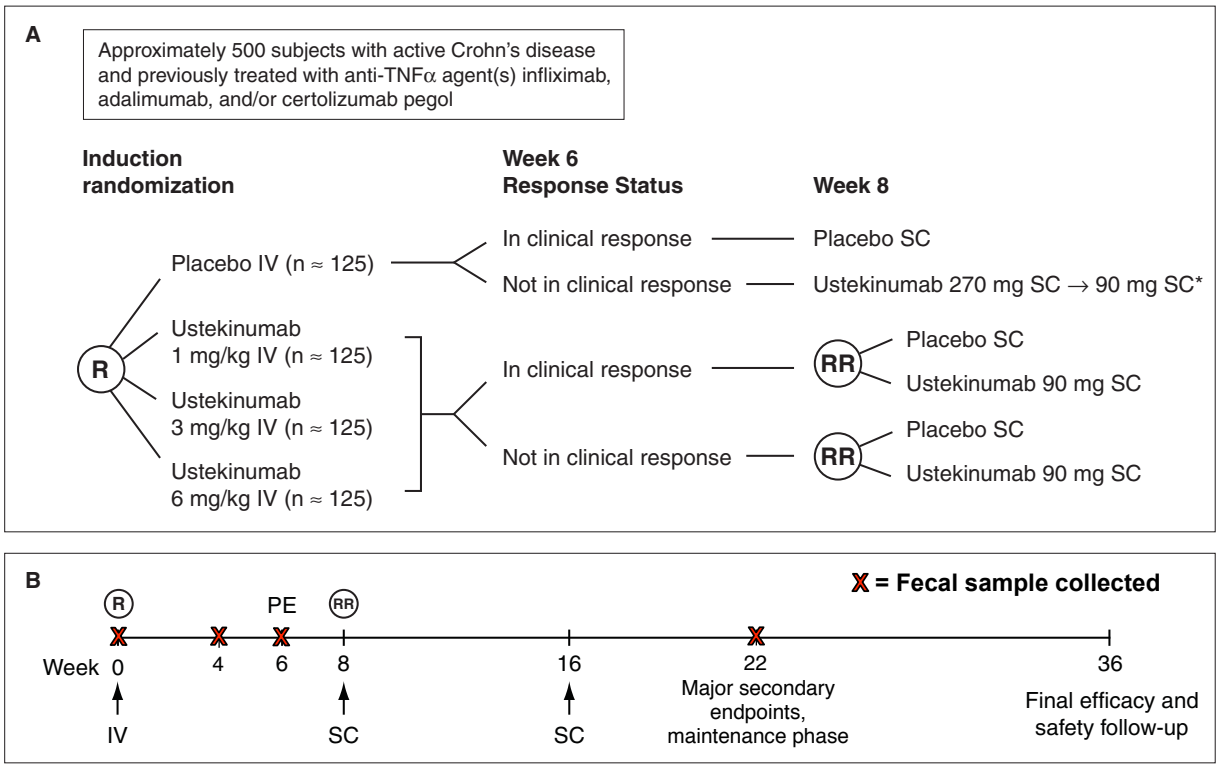
Clinical Variable	Correlation	Species Diveristy (p−value)	Community Structure (p−value)
CDAI	rho = −0.2	0.014	0.324
Loose Stool Frequency (per week)	rho = −0.2	0.003	0.024
C−Reactive Protein (mg/L serum)	rho = 0.06	0.394	0.033
Fecal Calprotectin (µg/g)	rho = 0.08	0.254	0.006
Fecal Lactoferrin (µg/g)	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

Table 2: Diversity differenced bases on Response/Remission in UST treated subjects.

Clinical Variable	Species Diversity (p−value)	Community Structure (p−value)
Week 6 Response (No, Yes)	0.440	0.012
Week 6 Remission (No, Yes)	0.020	0.017
Week 22 Response (No, Yes)	0.900	0.012
Week 22 Remission (No, Yes)	0.440	0.012

Figures

Figure 1: Experimental design as adapted from Sanborne et al 2012. (A) Diagram of experimetnal design and (B) stool sampling, treatment, and response evalution timeline.

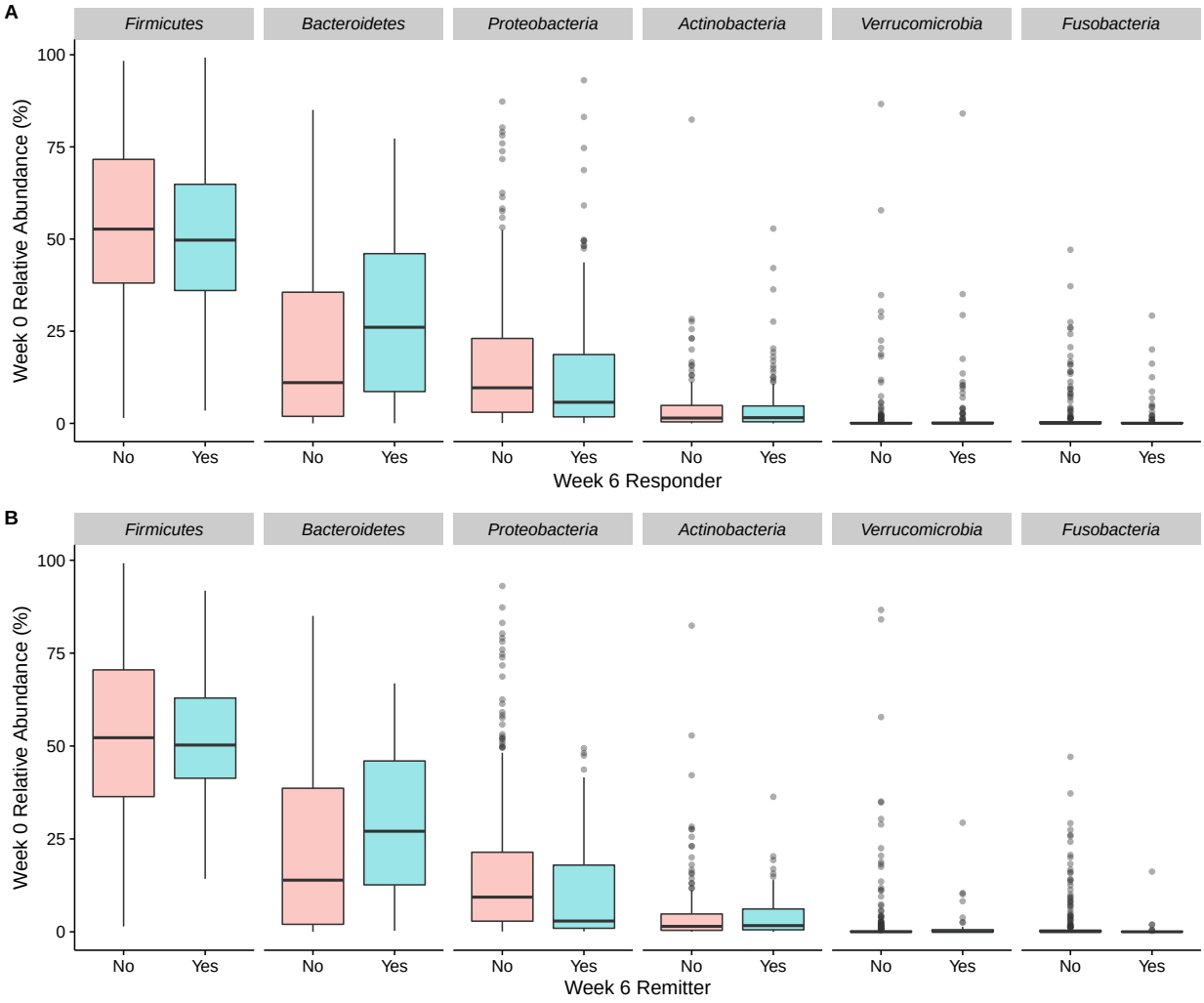


IV = Intravenous; SC = Subcutaneous; ↑ = Study agent administration

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: Phyla from week 0 stool samples in subjects treated with UST by week 6 outcome (A) Response and (B) remission status.



Supplemental Figure 1: Phyla from week 0 stool samples in subjects treated and maintained with UST by week 22 outcome (A) Response and (B) remission status.

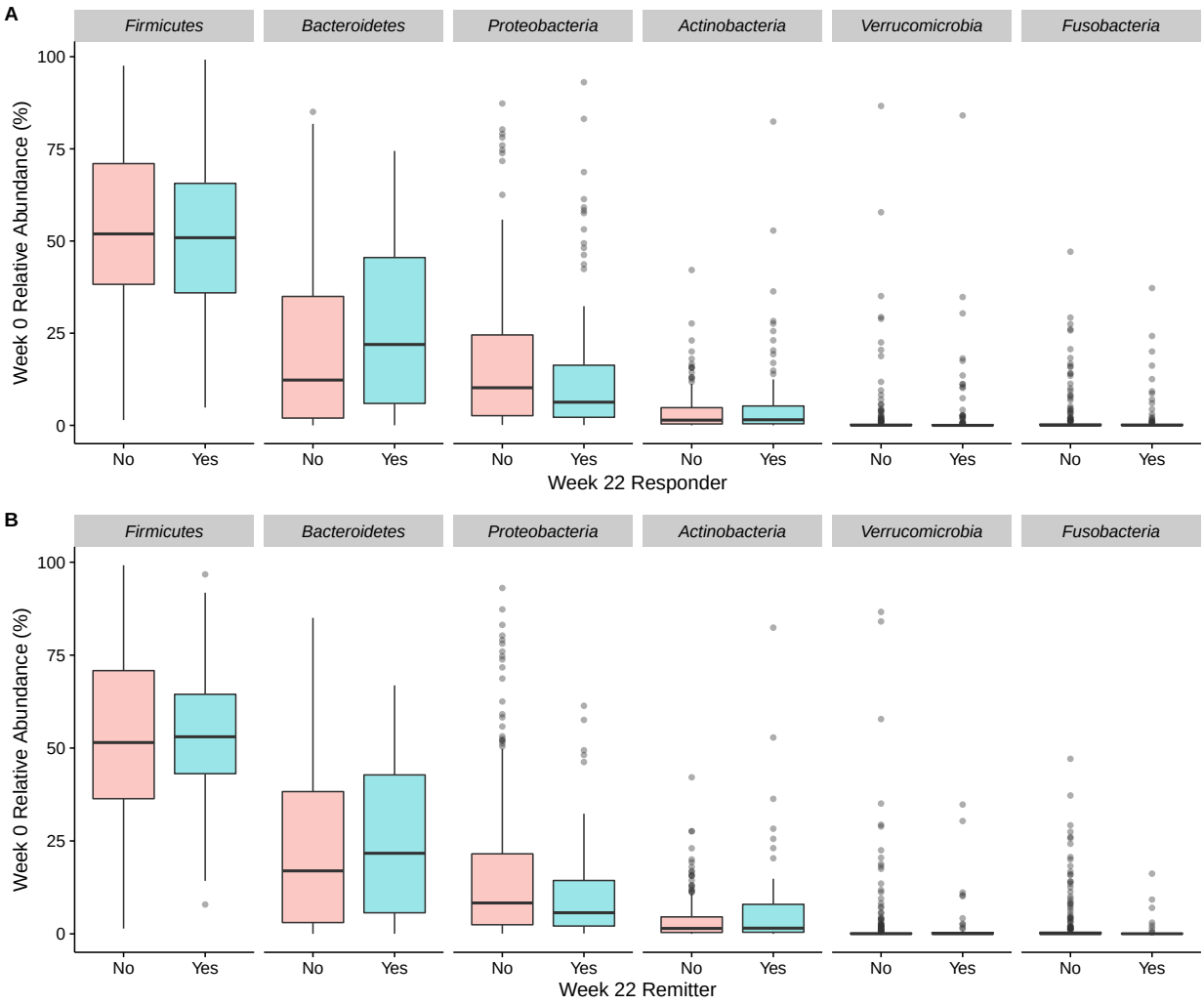


Figure 3: Differential taxa in week 0 stool samples from subjects treated with UST, based on week 6 remission status

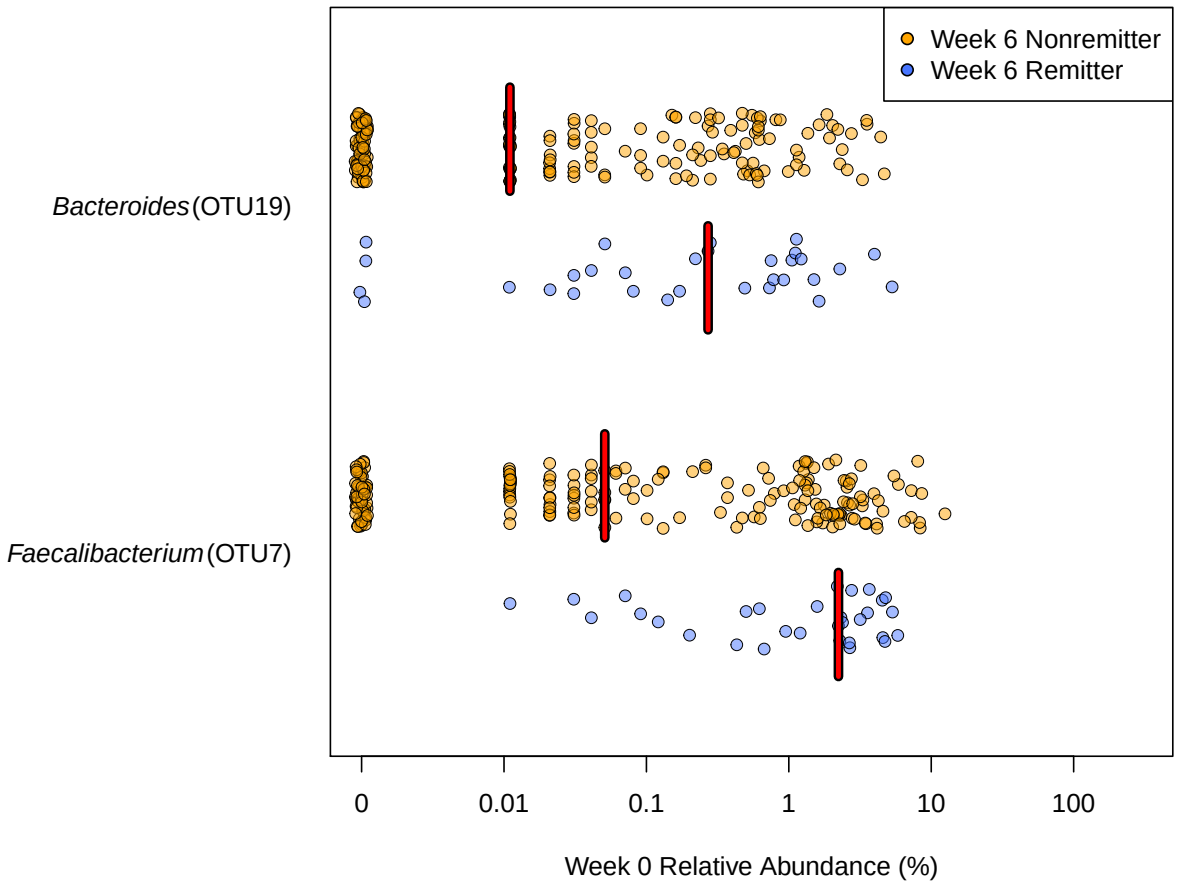


Figure 4: Change in alpha diversity over time by induction treatment and week 22 response status.

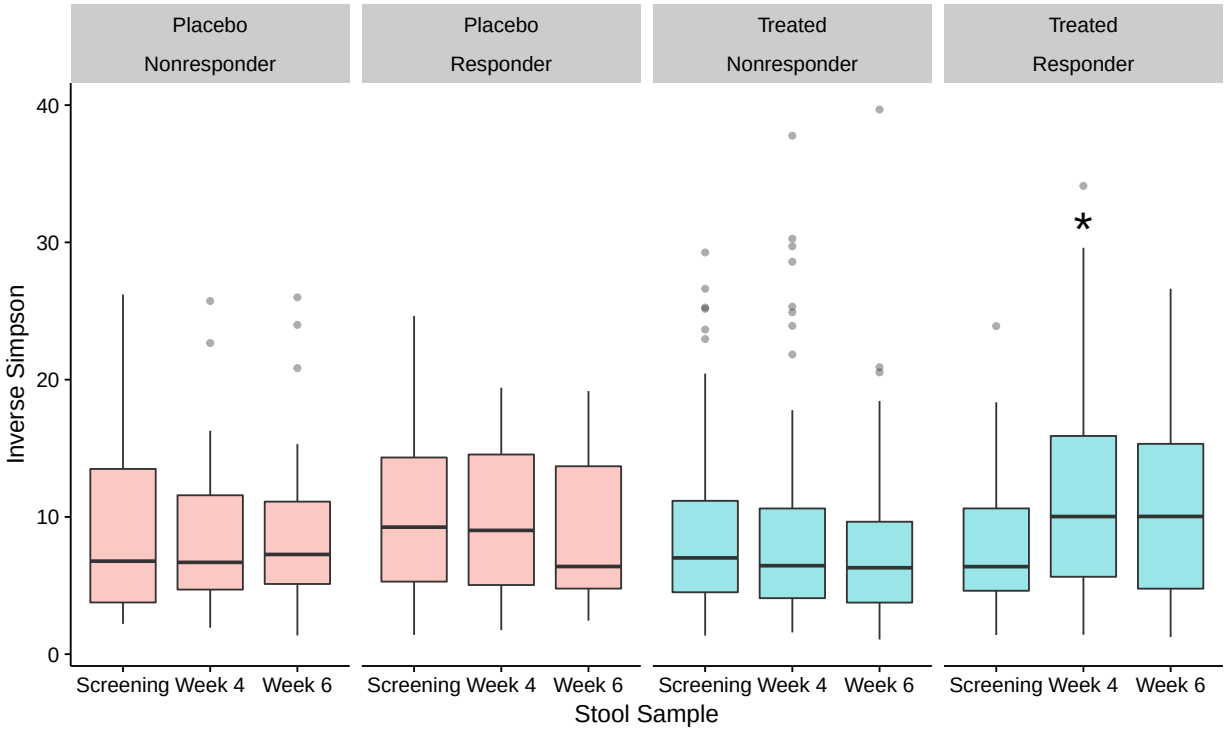


Figure 5: Classification of week 6 response or remission status using week 6 stool samples from subjects treated with UST (A) ROCs for week 6 outcome based on the microbiome. (B) Top predictive taxa from week 6 stool for remission status at week 6.

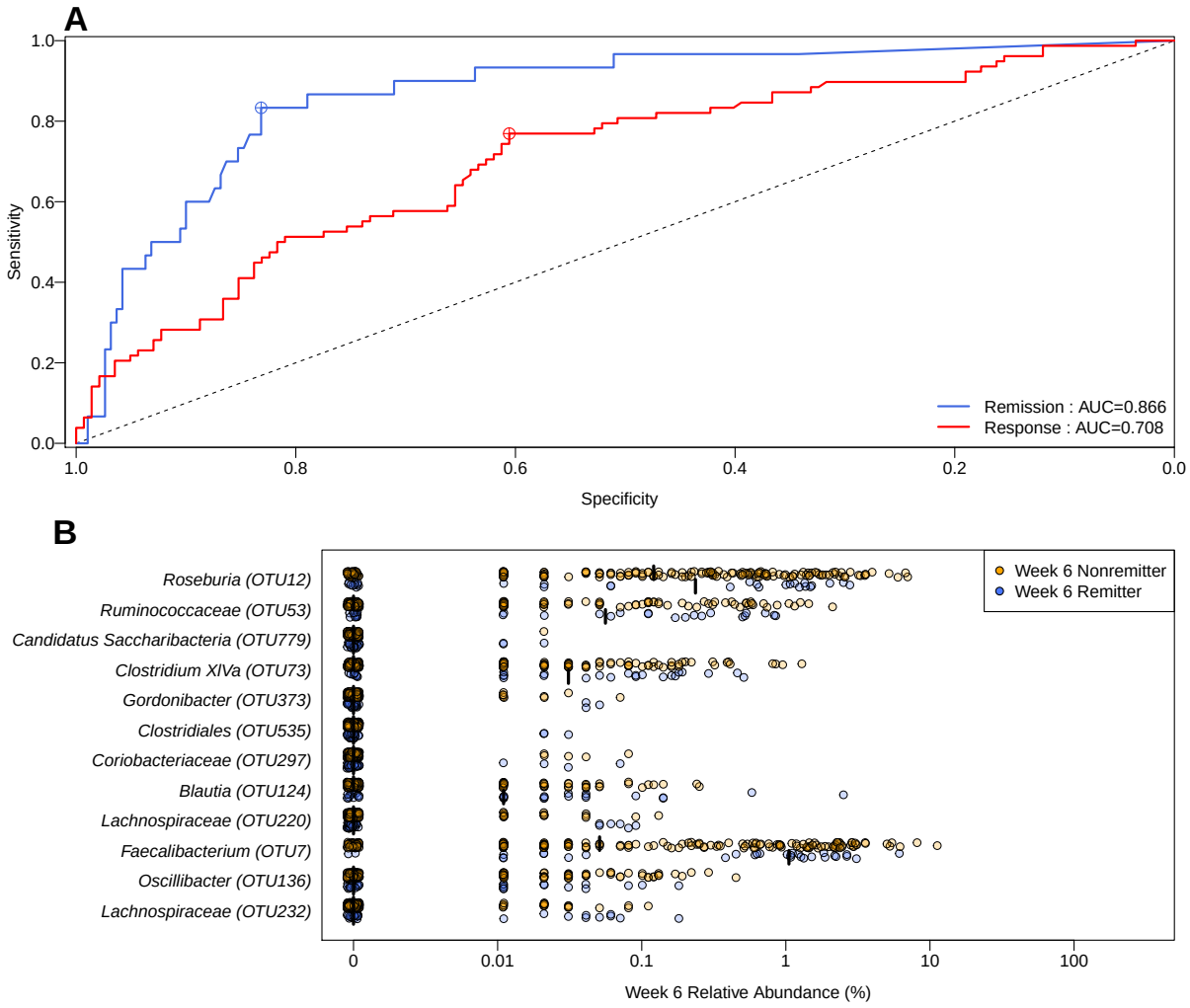
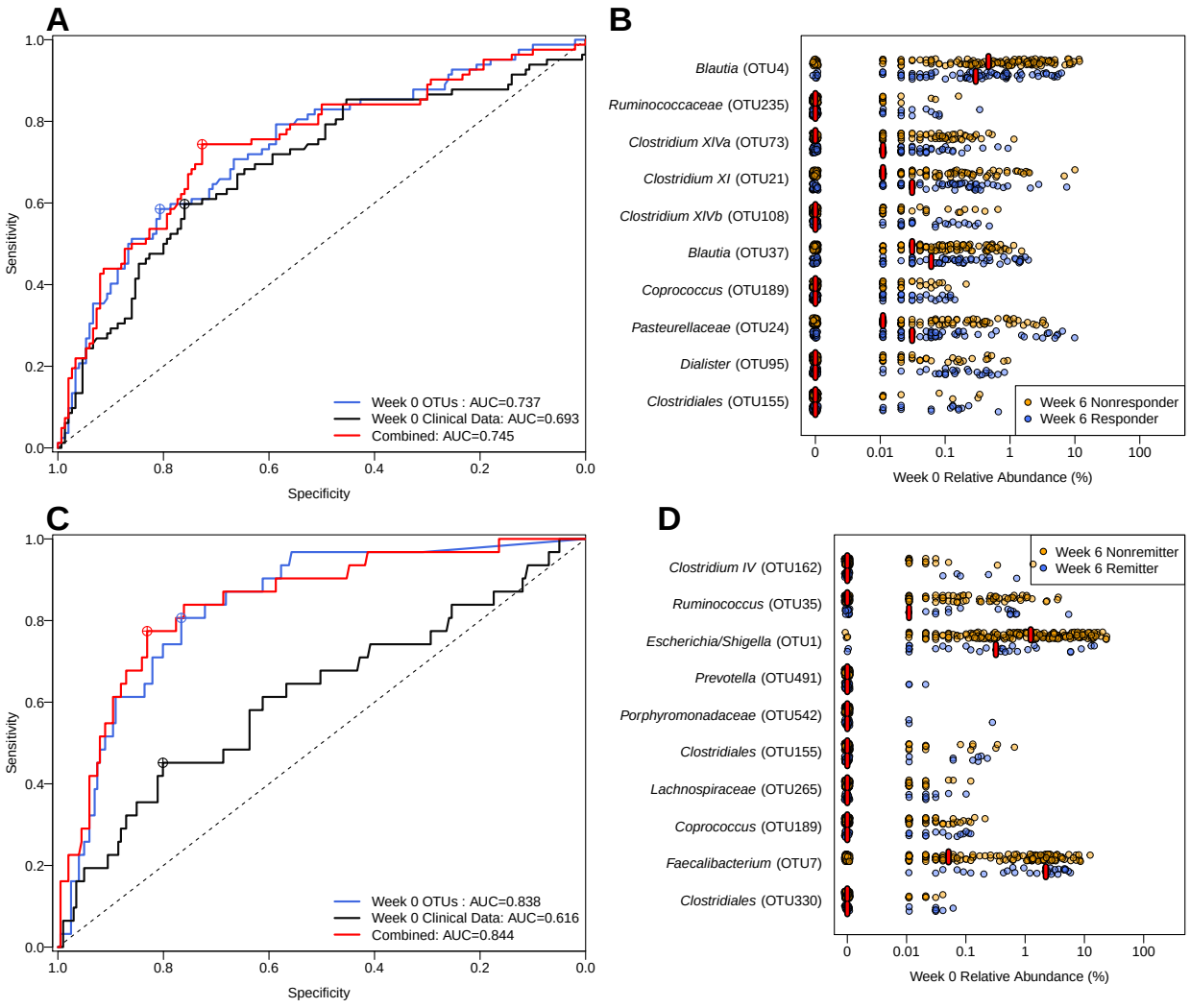
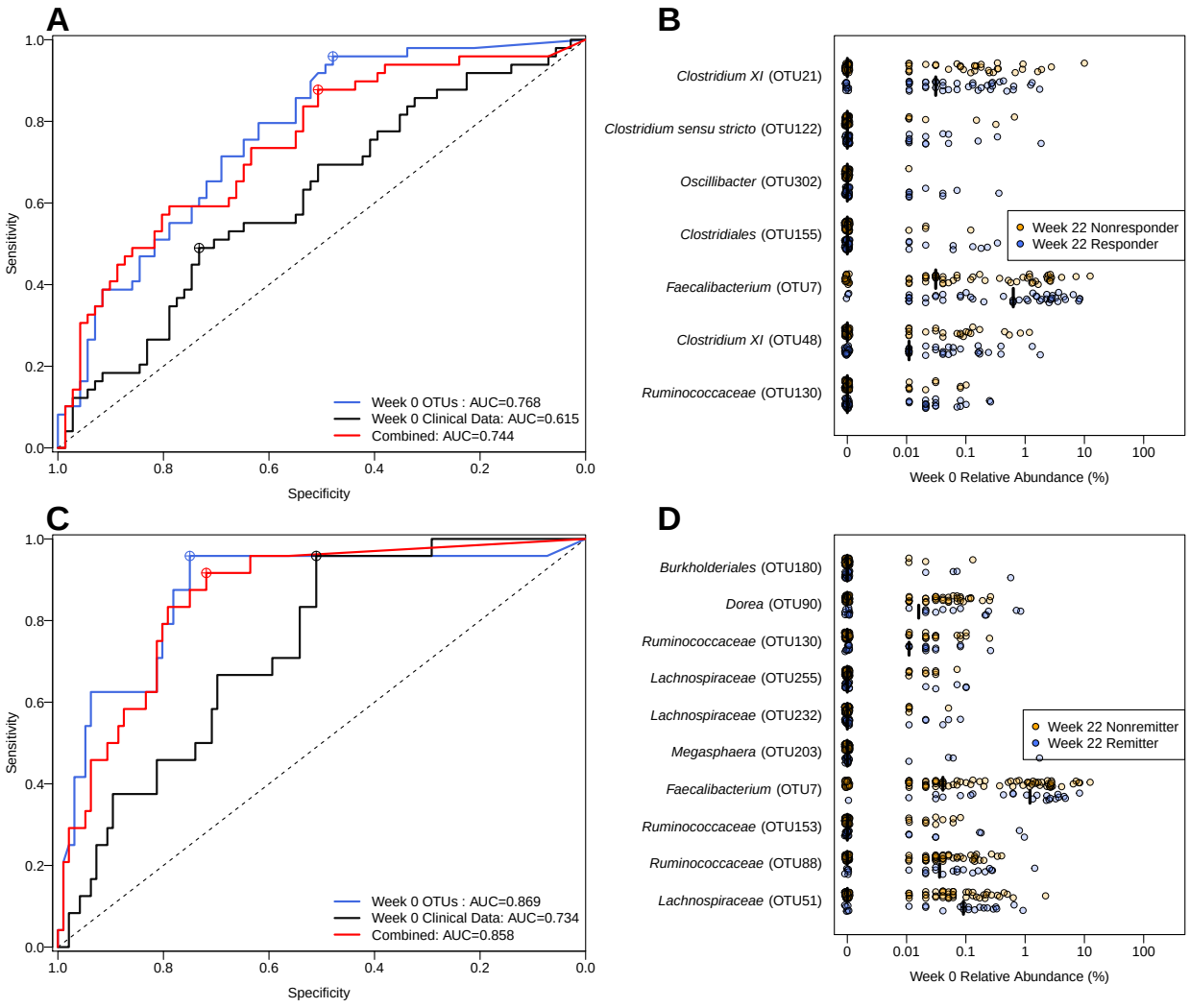


Figure 6: Prediction of week 6 disease status in subjects treated with UST, using week 0 samples ROCs for (A) response and (C) remission using microbiome data, clinical metadata, and the combined model. Top predictive taxa for the microbiome model based on MDA for (B) response and (D) remission.



Supplemental Figure 2: Predicting week 22 disease status in subjects treated and maintained with UST, using week 0 samples ROCs for (A) response and (C) remission using microbiome data, clinical metadata, and the combined model. Top predictive taxa for the microbiome model based on MDA for (B) response and (D) remission.



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