Profiling the Skin Microbiome in Response to Altreno in Acne Patients
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Study Design

This is a single-center, prospective study.

Study Population

Up to 60 subjects who meet study inclusion/exclusion criteria will be recruited into the study.

Materials and Methods

We will prospectively recruit healthy controls and acne patients from the BIDMC dermatology clinic as follows:

- **Control Group:** We will recruit up to 20 healthy controls without any skin diseases.
- **Experimental Group:** We will recruit up to 40 patients with acne with severity that warrants initiation of a topical medication and randomize 20 patients into Altreno 0.05% lotion monotherapy (applied once daily) and 20 patients into BPO 2.5% leave-on gel monotherapy (applied once or twice daily as tolerated).

Control Group will undergo skin swab at baseline and approximately 120 days later without any intervention. During the entire study period, the Group will not be allowed to use any antibacterial wash, other than approved OTC cleansers. *If due to the pandemic, subjects cannot return for 2nd visit in 120 days, the subjects will still remain in the study until able to do so.

Experimental Group will undergo skin swab at baseline, as well as clinical scoring of acne. Then, they will be prospectively divided 1 to 1 into Altreno group or BPO group. During the following 90 days, the Group will only be allowed to use either Altreno or BPO. On the second visit (90 days +/- 5 days), their acne will be scored again, and repeat skin swab will be taken. If significant improvement, they will be asked to discontinue the therapy for 30 days. If no significant improvement, they will be instructed to continue the therapy for additional 30 days. On the third visit (120 days +/- 5 days from the first visit), their acne will be scored, and repeat skin swab will be taken. *If due to the pandemic, subjects cannot return for subsequent visits, the subjects will either continue the therapy (or continue without therapy) and remain in the study until able to return for the subsequent visits.

All skin swab samples will be stored at -80 °C until further processing. CoreBiome will receive skin swabs (with subject ID only, no other PHI), they will extract DNA, and perform 16S rRNA gene and/or shotgun metagenomic sequencing. Corebiome will send the BIDMC research team the raw data. The research team will be doing the bioinformatics/analysis, not CoreBiome. If, after sending the skin swabs, subject withdraws, then the PI will ask Corebiome to destroy the sample and they will not perform DNA extraction or 16S rRNA/shotgun metagenomic sequencing.

Control Group:

	Visit 1	**Visit 2**
Procedures and Measurements	Screening Day 0	Day 120 +/- 5 days
Informed Consent, HIPPA Auth, Photo Release	X	
Review Inclusion/Exclusion Criteria	X	
Review of Demographics	X	
Review of Medical/Surgical Histories	X	X
Review of Current Medications	X	X
Physical Examination of Unaffected Skin	X	X
Regular Photography	X	X
Skin Swabs	X	X
Assess for Adverse Events	X	X

Experimental Group:

	Visit 1	**Visit 2**	**Visit 3**
Procedures and Measurements	Screening Day 0	Day 90 +/- 5 days	Day 120 +/- 5 days
Informed Consent, HIPPA Auth, Photo Release	X		
Review of Inclusion/Exclusion Criteria	X		
Review of Demographics	X		
Review of Medical/Surgical Histories	X	X	X
Review of Current Medications	X	X	X
Review of Acne Histories	X		
Review of Past/Current Acne Medications	X		
Physical Examination of Acne Skin	X	X	X
Acne Leeds Score	X	X	X
Acne PGA Score	X	X	X
Regular Photography	X	X	X
Skin Swabs	X	X	X
Assess for Adverse Events	X	X	X

Data Analysis

Primary Study Parameter(s): The primary outcome of this study is assessing for changes in skin microbial composition in acne patients after treatment with Altreno or BPO by specifically comparing their skin microbiomes (1) at baseline, (2) after 3 months of treatment, and (3) 1 month 10 after discontinuation of treatment. Comparisons of species diversity and relative abundance of the identified species will be made across healthy controls, Altreno group, and BPO group.

The raw sequencing data will be first processed into taxonomic features using the standardized DADA2 protocol as implemented in the bioBakery. Then, we will analyze the

three available microbial features (taxa, alpha diversity summary statistics, and community structure via beta diversity) across the three groups defined above.

Secondary Study Parameter(s): The clinical severity will also be correlated to the presence and/or dominance of *P. acnes* and other populations using the following measures:

- **Physician Global Assessment of Acne:** Categorical variable. A chi-square test will be performed for analysis.
- **Leeds Acne Score:** Ordinal variable. A correlation analysis and depending on distribution, one-way ANOVA, a Kruskal Wallis or a Wilcoxon rank sum test will be performed for analysis.

Other Study Parameter(s): Patient characteristics and demographics data will be presented using descriptive statistics. At every visit, adverse events and changes in medications will be registered.

Statistical Analysis Plan (per Diversigen, who performed computational analysis)

"To examine differences in abundance for taxa between treatment groups and across time, we examined taxa abundances at the Phylum, Family, Genus and ASV levels using the ASV count table as inputs. At each level of interest, count abundances were aggregated then transformed using the centered log ratio (CLR) transformation as implemented in the ALDEx2 R package. Briefly, transformation was performed using the aldex.clr() function, which estimates sequencing technical variation using Monte-Carlo sampling (n = 128) and generates CLR-transformed tables for each instance. A median CLR value for each taxon across all MC instances calculated and used for downstream plotting purposes.

Next, we performed differential abundance testing using LME models of the following model:

- taxon_abundance ~ Treatment_Cohort*Timepoint + (1|SubjectID)

Pairwise contrast p-values for all relevant Treatment_Cohort and Timepoint comparisons were extracted from the model using emmeans(). Next, p-values were corrected for multiple hypothesis correction across all taxa tested using the FDR method. Finally, all adjusted p-values generated by testing were averaged across all 128 MC instances to obtain a padj value for each taxon for each pairwise comparison of interest.

Using our standard significance thresholds of padj < 0.05, we found no taxa as significantly different in abundance at any level in any of the pairwise comparisons we examined. Therefore, we relaxed our threshold to padj < 0.2, which is less conservative but has been reported in the literature. Under this threshold, we found a few taxa that were significantly different."