9/23/2020

University of Pennsylvania

Protocol title: The Cutaneous Microbiota of Psoriasis: Lesional Variation and a Phase IV, Interventional Study of its Response to Phototherapy.

Short title: Psoriasis Microbiome and Phototherapy

Protocol number: 821876

NCT number: NCT02552316

Statistical Analysis Plan

Paired-end amplicon sequences will be assembled using PandaSEQ36 and custom scripts, and processed in QIIME (Quantitative Insights Into Microbial Ecology) open source software package. To analyze microbiota, we will apply several metrics to capture multiple dimensions of microbial diversity and composition that may be altered in psoriasis as follows. 1. Alpha-diversity: The diversity present in a given sample will be measured by the Shannon Diversity Index which takes into account the number of species and the evenness of species present in a sample. The number of observed species alone will serve as a measure of richness. 2. Beta-diversity: The diversity shared among samples will be measured by the Jaccard index. The Jaccard index measures shared species amongst samples ("community membership"). 3. Bacterial load: We will use a quantitative real-time PCR assay to amplify the 16S rRNA gene. To calculate bacterial load from real-time PCR data, a standard curve is generated with a well-characterized isolate (i.e., E. coli). The standard curve is then used to convert real-time PCR amplification data to 16S gene copy number, and from gene copy number, the number of bacterial cells in the sample is estimated.

Significance in alpha diversity and bacterial load will be assessed using the nonparametric Wilcoxon rank-sum test (for pairwise comparisons), Wilcoxon signed-rank test (for matched pairwise comparisons), or Kruskal-Wallis test (for >2 comparisons). Beta-diversity metric will be visualized by principle coordinates analysis and plots. The significance of the observed clustering will be assessed using the non-parametric Adonis test through permutations. Statistical tests will be adjusted for multiple comparisons as appropriate. Sample size calculations are based on previously published estimates of bacterial load, alpha-diversity, and beta-diversity in lesional versus non-lesional skin in psoriasis patients. Using a two-sided, 0.0125-level test, a sample size of 30 subjects with at least one sample site will provide 80% power to detect a difference of 2.4% (95% confidence interval [CI] width of 2) in combined bacterial load, 0.24 (95% CI width of 0.2) in alpha-diversity, and 0.012 (95% CI width of 0.01) in beta-diversity. To account for an estimated 10% loss to follow-up, 34 subjects will be recruited.