**Statistical Design and Power**

**Study Design:** The proposed clinical trial will adopt a double-blind, randomized, placebo-controlled design with a 12-week intervention period and four scheduled visits. A total of 80 subjects will be randomized in a 1:1 ratio into two groups:

* **Group 1**: American Ginseng (AG) 3g daily
* **Group 2**: Placebo

Inclusion and exclusion criteria, as detailed in Section 2.2 of the protocol, will ensure uniform baseline characteristics among participants. Identification and recruitment of participants will be carried out by Drs. Joseph Meserve and Thomas Faust, and their assistants at Prisma Health Gastroenterology and Hepatology. Initial contact with potential participants will be made by Prisma Health staff, followed by a phone call to confirm willingness to participate and schedule the first visit.

Participants will attend six visits over the 12-week study period (Weeks 0, 4, 8, and 12), during which clinical endpoints will be assessed, including the Modified Ulcerative Colitis Disease Activity Index (mUCDAI) and Lichtiger Disease Activity Index (LDAI). Blood, fecal samples, and LDAI data will be collected at these visits. Compliance will be monitored through plasma measurements. The primary endpoint is clinical remission, while secondary endpoints include clinical improvement, endoscopic improvement, and histological remission.

**Statistical Analysis Plan:**

**1. Clinical Endpoints:** The primary clinical endpoint is clinical remission, defined by a significant reduction in disease activity based on mUCDAI and LDAI scores. Secondary endpoints include measures of clinical improvement, endoscopic healing, and histological remission.

Statistical analysis will involve comparing baseline values to endpoints within and between the two groups using the following methods:

* **Within-group comparisons**: Paired t-tests or Wilcoxon signed-rank tests, depending on data distribution.
* **Between-group comparisons**: Independent t-tests, Mann-Whitney U tests, χ2 tests, and Fisher’s exact test will be employed to assess differences between the intervention and placebo groups. A p-value of <0.05 will be considered statistically significant for all tests.

**2. Blood Biomarkers:** Blood samples will be collected at Weeks 0, 4, 8, and 12 and analyzed for biomarkers such as C-reactive protein (CRP), TNF-α, IL-1β, IL-4, IL-6, IL-10, and other markers using the Olink® Target 96 Inflammation panel via RT-PCR.

* **Within-group analysis**: Baseline and endpoint comparisons will be made using paired t-tests or Wilcoxon signed-rank tests.
* **Between-group analysis**: Independent t-tests or Mann-Whitney U tests will compare differences between the AG and placebo groups.
* **Significance level**: A p-value of <0.05 will determine statistical significance.

**3. Tissue Biomarkers:** Tissue samples obtained from colonoscopies at Weeks 0 and 12 will undergo histological assessments and immunohistochemistry (iNOS, Cox2, Nf-kB) as well as fecal biomarker analysis (calprotectin, lactoferrin, alpha-1 antitrypsin, occult blood).

* **Within-group analysis**: Paired t-tests or Wilcoxon signed-rank tests will compare baseline and endpoint values.
* **Between-group analysis**: Independent t-tests or Mann-Whitney U tests will evaluate differences between groups.
* **Histological scores**: These will be categorized for statistical analysis (e.g., 0, 1, 2, 3) using appropriate non-parametric tests.
* **Significance level**: A p-value of <0.05 will be used to establish statistical significance.

**4. Microbiota Sequencing:** Fecal samples collected at Weeks 0, 4, 8, and 12 will be subjected to 16S rRNA gene sequencing to assess changes in the gut microbiota composition.

* **Within-group analysis**: Alpha diversity and beta diversity metrics will be compared between baseline and endpoint samples.
* **Between-group analysis**: Differences in microbial composition between the AG and placebo groups will be assessed using PERMANOVA and other relevant statistical tests.
* **Significance level**: A p-value of <0.05 will be applied to determine significance.

**Statistical Software:** All data analyses will be performed using SPSS statistical software. Detailed reports of the methods, results, and statistical significance will be presented in the final study report.

**Power Calculation:**

The power calculation for the sample size in this study is based on an anticipated primary response rate of 50% in the AG group, derived from preclinical studies and previous investigations into herbal therapies for ulcerative colitis. The placebo group is expected to have a 10% response rate. Accounting for a 10% dropout rate, the study aims for an 80% power (β = 0.8) with an alpha (α) of 0.05.

Based on previous studies involving sample sizes of 60, 10, 38, 54, and 48 patients, the required sample size has been calculated as 80 total participants (40 in each group) to detect an effect of the expected magnitude or greater. Randomizing subjects equally between the two groups maximizes the statistical power for detecting clinically meaningful differences.

The power analysis has been designed in collaboration with Drs. Cai and Hebert to ensure adequate sample size and statistical rigor, providing sufficient power to detect the expected treatment effects in both primary and secondary outcomes.