CLINICAL STUDY DOCUMENT

STATISTICAL ANALYSIS PLAN (SAP)

Official Title: Estrogen Herbals

Brief Title: Hormone Estradiol Replacement Therapy Additional Herbals (WH)

Unique Protocol ID: Estrogen Herbals

NCT Number: NCT02618148

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1. INTRODUCTION

This Statistical Analysis Plan (SAP) outlines the detailed statistical methodologies and analyses for the clinical trial NCT02618148. The objective is to evaluate the pharmacokinetic profile of estradiol and the thrombosis-related safety parameters when combining conventional HRT with garlic oil, rutin, and nattokinase.

2. STUDY POPULATIONS

- Intent-to-Treat (ITT) Population: Includes all randomized participants who received at least one dose of the study medication and provided at least one post-baseline measurement.

- Per-Protocol (PP) Population: Includes all randomized participants who completed the specified follow-up periods (1-year and 2-year endpoints) without major protocol deviations.

- Safety Population: Includes all participants who received at least one dose of the assigned intervention.

3. ENDPOINT DEFINITIONS AND VARIABLES

- Primary Endpoint: The time (in months) from treatment initiation until the patient's serum estradiol level exceeds 35 pg/ml. Serum estradiol will be assessed at predefined intervals.

- Secondary Endpoints: Longitudinal evaluation of serum estradiol variations up to 2 years, combined with clinical safety parameters, including incidence of thrombotic micro-events, platelet aggregation rates, and bleeding/clotting times.

4. STATISTICAL METHODOLOGY

4.1 Baseline Characteristics:

Descriptive statistics will be used to summarize baseline demographic and clinical data. Continuous variables (e.g., age, baseline estradiol level) will be presented as means with standard deviations (SD) or medians with interquartile ranges (IQR). Categorical variables (e.g., menopausal status, symptomatic classification) will be presented as frequencies and percentages.

4.2 Primary Analysis:

The primary outcome variable—time to reach serum estradiol > 35 pg/ml—is a time-to-event variable.

- Kaplan-Meier survival curves will be generated to estimate the cumulative probability of achieving the target estradiol level over time for both treatment arms (ESTROGEN HERBALS 21 vs. ESTROGEN HERBALS 28).

- The Log-Rank test will be implemented to assess statistically significant differences between the cumulative curve profiles of the two parallel interventional arms.

- Cox Proportional Hazards Regression models will be constructed to compute Hazard Ratios (HR) along with 95% Confidence Intervals (CI), adjusting for baseline covariates such as age and premenopausal versus postmenopausal categorization.

4.3 Secondary Analysis:

- Longitudinal analysis of continuous serum estradiol levels across multiple visits will be conducted using Mixed-Effects Models for Repeated Measures (MMRM) or Generalized Estimating Equations (GEE) to account for intra-subject correlation.

- Comparative analyses between groups for continuous safety biomarkers will be performed utilizing the Student's t-test (for normally distributed data) or the Mann-Whitney U test (for non-normally distributed data).

- Categorical adverse event rates and thrombosis-related safety indicators will be compared using the Chi-square test or Fisher's Exact test as appropriate.

4.4 Significance Level and Software:

- All statistical tests will be two-sided.

- A p-value of less than 0.05 ($p < 0.05$) will be considered statistically significant.

- Statistical analyses will be performed using standard validated software packages (such as SPSS, SAS, or R).

5. HANDLING OF MISSING DATA

Missing clinical data points will be addressed using Last Observation Carried Forward (LOCF) or Multiple Imputation (MI) methodologies to ensure statistical integrity and minimize attrition bias in the ITT population analysis.