Protocol CO-170726100607-SACT

RANDOMIZED, EXPLORATORY STUDY TO EVALUATE CHANGES TO SKIN MICROBIOME WITH TAPE-STRIPPED WOUNDS

Statistical Analysis Plan (SAP)

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CONTENTS

1 INTRODUCTION

The skin is colonized by diverse microorganisms, and the genetic contributions of these microorganisms is collectively referred to as the skin microbiome. These microorganisms, in combination with mammalian cells of the host produce a diverse array of metabolites, which are unique chemical fingerprints left behind by specific cellular processes, important for normal cell function. The microorganisms and metabolic processes exist in a dynamic equilibrium and are believed to contribute to acute wound healing. However, when the skin is compromised (i.e. a wound occurs), adverse organisms can also colonize and metabolite profiles can shift in the wound leading to impaired or prolonged healing 1,2. Thus, characterizing the skin microbiome and metabolites during the wound healing process is critical to understanding their role in wound repair.

This exploratory study is being conducted to characterize the microbial load and diversity of the skin microbiome and metabolites of induced wounds in relation to specific skin properties, and after treatment with various adhesive bandages to further establish the role of microorganisms in wound healing.

1.1 Study Objectives

The primary objective of this study is to evaluate skin microbiome changes of induced wounds covered with adhesive bandages in comparison to uncovered wounds and intact skin in healthy subjects.

The secondary objective of this study is to evaluate the following for induced wounds covered with adhesive bandages in comparison to uncovered wounds and intact skin: changes in skin barrier function, and skin redness. [redacted]

1.2 Study Design

This single center, 15-day clinical trial is being conducted to assess the changes to the skin microbiome of induced wounds on the backs of approximately 35 healthy adult subjects aged 18-55 years, with Fitzpatrick Skin Types I – III. There will be 6 test sites randomly assigned to treatments for each subject. Four of the test sites will be wounded and covered with various marketed adhesive bandages, one test site will be wounded and un-covered (positive control) and one test site will remain intact and un-covered (negative control). Following a screening visit (Day -3 to -14) and a 3-day washout period, subjects will be assessed at Baseline (Day 0), Days 1 – 7 and Day 14; subjects will also return to the study site daily for bandage changes.:

The following assessments will be performed by trained study staff at Baseline (Day 0), Days 1-7, and Day 14:

- Microbiome [redacted] Swabs will be used to collect bacteria [redacted] from the skin surface. A total of 2 swabs will be taken per site per timepoint.
- Trans Epidermal Water Loss (TEWL; tewameter): Noninvasively measures water evaporation from the skin, which is a measure of skin barrier function. These measurements will be done three times for each test site.
- [redacted]
- Skin pH (Skin pH Meter PH905): Noninvasively measures skin surface pH. [redacted]
- Diffuse Reflectance Spectroscopy (DRS): Noninvasively measures redness by oxy-, deoxy-hemoglobin, and melanin levels in the skin. Five repetitive spectra acquisition will be implemented for each test site.
- [redacted].

A randomization code will designate which test site will receive the appropriate treatment. A subgroup of 17 subjects will also be randomly assigned for additional metabolite analysis. Study bandages will be changed daily (by study staff on each day of the study Days 1 -14). Subjects will report back to the study facility for daily bandage changes (Days 1-14) and for assessments on Days 1-7 and 14.

Adverse events will be monitored throughout the study.

2 INTERIM ANALYSES

No interim analysis is planned for this trial.

3 ANALYSIS SETS

3.1 Primary Analyses Set

The efficacy analysis will be based on the Intent-to-treat (ITT) principle, i.e., all subjects who received induced wounds and started the bandage or no treatment will be included in the analysis.

[redacted]

3.2 Per-Protocol Set

Not applicable.

3.3 Safety Analysis Set

The safety analysis will be based on all subjects who received induced wounds.

4 EFFICACY ASSESSMENTS AND ENDPOINTS

4.1 Efficacy Assessments

- Predominant microflora based on the following criteria:
  - Microbial Community Richness on the back at Baseline (Day 0), Days 1-7, and Day 14, based on the total number of different bacterial taxa detected in the sample.
  - Microbial Community Diversity on the back at Baseline (Day 0), Days 1-7, and Day 14 based on the Shannon Index.
  - Microbial Community Evenness on the back at Baseline (Day 0), Days 1-7, and Day 14 based on Pielou's evenness index.
- Skin barrier function as assessed by mean TEWL measurements of all test sites at Baseline (Day 0), Days 1-7 and Day 14.
- Redness as assessed by mean DRS measurements of oxy-, deoxy-hemoglobin, and melanin levels at all test sites at Baseline (Day 0), Days 1-7 and Day 14.
- [redacted]
  - [redacted]

4.2 Efficacy Endpoints

[redacted]

The following efficacy endpoints will be analyzed by the statistical group of J&J Consumer Group.

- Change from baseline in TEWL measurements at Days 1-7 and Day 14.
- Change from baseline in DRS measurements at Days 1-7 and Day 14.
- [redacted]
- Change from baseline in Skin pH at Days 1-7 and Day 14.

4.3 Safety Assessments and Endpoints

Safety assessments consist of Adverse Events.

- Number and percentage of subjects with treatment-emergent adverse event
- Number and percentage of subjects with treatment-related adverse event
- Number and percentage of subjects with serious adverse event

4.4 Other Endpoints

Not applicable.

4.5 Covariates

For the analyses of Change from Baseline in TEWL, [redacted], skin pH and DRS measurement, corresponding baseline value will be adjusted in the models as covariate.

5 HANDLING OF MISSING VALUES

No imputation of missing data will be performed.

6 STATISTICAL METHODOLOGY AND STATISTICAL ANALYSES

6.1 Statistical Hypotheses

For each between-product comparison, the null hypothesis that the product mean change from baseline are equal will be tested against the alternative hypothesis that the product mean change from baseline are not equal.

In other words, the following hypothesis will be tested:

$$H_0\colon\ \mu_1 = \mu_2$$

against the two-sided alternative

$$H_1\colon \mu_1 \neq \mu_2$$

where μ_1 and μ_2 are the mean change from baseline for the two product groups, respectively.

6.2 Statistical Decision Rules

The comparison between products will be performed at the 0.05 level of significance, two-sided and all analyses are exploratory. Thus, for any given comparison, the null hypothesis of no difference in treatment means will be rejected if, and only if, the corresponding test p-value is equal to or smaller than 0.05.

6.3 Statistical Methods

TEWL, [redacted], skin pH and DRS are measured at Baseline, Days 1-7 and Day 14. The triplicate values at each assessment time point will be averaged as the final response value, with the exception of the DRS which will have 5 replicates at each timepoint.

The final response values of TEWL, DRS, SKICON and skin pH will be summarized at each assessment time point by the assigned test products.

The within product comparison will be performed using the paired t-test to compare the values of each product at each post baseline time point with its own baseline value.

The between products comparison will be performed based on the change from baseline (post baseline value minus baseline value). The change from baseline of each product will be analyzed using mixed effect ANCOVA model at each post baseline time point. The ANCOVA model will include the product as the factor and the baseline value as the covariate. The model will include the subject as a random effect to incorporate the within subject correlation. The adjusted mean of each product from the ANCOVA model will be compared between the test products and the negative control, between the test products and the positive control, and between the negative control and the positive control.

The mixed effect analysis of covariance model can be performed by the following SAS codes

```
PROC MIXED DATA = <dataset>;
    CLASS subjid treatment;
    MODEL chg = treatment baseline/solution ddfm = contain;
    RANDOM subjid ;
    LSMEANS treatment/diff cl;
    ESTIMATE “Positive Control vs. Negative Control” treatment -1 1 0 0 0 0 /cl;
    ESTIMATE “Product #1 vs. Negative Control” treatment -1 0 1 0 0 0/cl;
    …
RUN;
```

Note:

1. Assume Negative Control = “A”, Positive Control = “B”, Product #1 = “C”, Product #2 = “D”, Product #3 = “E”, Product #4 = “F”.

6.4 DEMOGRAPHIC AND BASELINE CHARACRISTICS

Summary statistics (number of subjects, mean, standard deviation, median, minimum and maximum) will be provided for numerical variable age.

Frequency summary (number and percentage of subjects) will be provided for categorical variables including sex, race ethnicity and Fitzpatrick skin type classification.

6.5 SAFETY ANALYSIS

All subjects who received induced wounds will be included in the safety analysis. All adverse events reported during the adverse-event reporting period will be listed by subject number.

The number and percentage of subjects experiencing treatment-emergent adverse events will be presented by body system and preferred term. Treatment-emergent adverse events are those with a start date and time on or after the date and time of first use of investigational product, or the AEs start before the first use of investigational product and worsened after it. The latest version of the Medical Dictionary for Regulatory Activities (MedDRA) will be used as the adverse event classification system.

Number and percentage of subjects with serious adverse events will be summarised by MedDRA system organ class and preferred term.

Number and percentage of subjects with treatment-related adverse events will be summarized by MedDRA system organ class and preferred term. Treatment-related AEs are events evaluated by the investigator as possible, probable or very likely related to study medication. AEs with unknown relationship to treatment will be considered as treatment-related.

Number and percentage of subjects with serious adverse events will be summarized by MedDRA system organ class and preferred term.

APPENDICES

APPENDIX 1: SUMMARY TABLES AND FIGURES

The following tables and figures are planned for subsections in Sections 14 of the Clinical Study Report. The numbering and titles of tables and figures in this document serves as guidance; the exact numbers and titles may be modified as appropriate.

Section / Table No	Title	Population / Analysis Sets
14.1 Subject Disposition and Baseline Information		
Table 14.1.1	Disposition of Subjects	All randomized subjects
Table 14.1.2	Demography and Baseline Characteristics	All randomized subjects
14.2 Efficacy		
Table 14.2.1	Analysis of Change from Baseline in Trans Epidermal Water Loss (TEWL) Measurement	Intent-to-Treat Subjects
Table 14.2.2	Analysis of Change from Baseline in Diffuse Reflectance Spectroscopy (DRS) Measurement	Intent-to-Treat Subjects
Table 14.2.3	Analysis of Chance from Baseline in Skin pH Measurement	Intent-to-Treat Subjects
Table 14.2.4	Analysis of Change from Baseline in [redacted] Measurement	Intent-to-Treat Subjects
14.3 Safety		
Section / Table No	**Title**	**Population / Analysis Sets**
Table 14.3.1	Subjects with Treatment-Emergent Adverse Events by System Organ Class and Preferred Term	Safety Analysis Set
Table 14.3.2	Subjects with Treatment-related Adverse Events by System Organ Class and Preferred Term	Safety Analysis Set
Table 14.3.3	Subjects with Serious Adverse Events by System Organ Class and Preferred Term	Safety Analysis Set

FIGURES:

None .

APPENDIX 2: DATA LISTINGS

The following listings are planned for subsections in Appendix of Clinical Study Report. The numbering and titles of data listings in this document serve as guidance; the exact numbers and titles may be modified as appropriate.

Listing No.	Title	Population
16.1.7	Randomization Scheme and Codes	All randomized Subjects
16.2.1.1	Subject Disposition Listing	All randomized Subjects
16.2.1.2	Discontinued Subjects	All randomized Subjects
16.2.2	Subjects with Protocol Deviations	All randomized Subjects
16.2.3	Subjects Excluded from the Primary Analysis	All randomized Subjects
16.2.4.1	Demographic and Baseline Characteristics	All randomized Subjects
16.2.6.1	Trans Epidermal Water Loss (TEWL) Measurements	All randomized Subjects
16.2.6.2	[redacted] Measurements	All randomized Subjects
16.2.6.3	Skin pH Measurements	All randomized Subjects
16.2.7.1	Subjects with Adverse Events	All randomized Subjects
16.2.7.2	Subjects with Serious Adverse Events	All randomized Subjects
16.2.7.3	Subjects Withdrawn from Investigational Product due to Adverse Events	All randomized Subjects