Statistical Analysis Plan

Clinical Evaluation of Metal Panel Allergens: Aluminum, Copper, Manganese, Molybdenum, Tin, Titanium, Vanadium and Zinc Dose Response Study

Sponsor Study Number:	SP 14 8MP 201
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STATISTICAL ANALYSIS PLAN

Clinical Evaluation of Metal Panel Allergens:
Aluminum, Copper, Manganese, Molybdenum, Tin, Titanium, Vanadium and Zinc
Dose Response Study

Sponsor Study No. SP 14 8MP 201

(Version Amendment V, dated 27 JUN 2018)

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Revision History

VERSION	DATE	DESCRIPTION OF REVISIONS	REVISED BY

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List of Abbreviations and Definition of Terms

AE	Adverse Event
CI	Confidence Interval
CRF	Case Report Form
DBL	Database Lock
GST	Gold Sodium Thiosulfate
HPC	Hydroxypropyl cellulose
ICH	International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use
OTC	Over the Counter
MedDRA	Medical Dictionary for Regulatory Activities
FAS	Full Analysis set
PT	Preferred Term
PVP	Polyvinylpyrrolidone
RA	Reference Allergen
SAE	Serious Adverse Event
SOC	System Organ Class
SSAR	Serious Suspected Adverse Reaction
TEAE	Treatment Emergent Adverse Event
T.R.U.E. Test	Thin Layer Rapid Use Epicutaneous Test

1. Introduction to the Statistical Analysis Plan

This Statistical Analysis Plan (SAP) provides a thorough description of statistical analysis methods and presentation of study data from the clinical trial described in Study Protocol SP 14 8MP 201, (Amendment V) dated 27 JUN 2018.

SAP will be finalized prior to database lock (DBL) and statistical methods described in this document supersede the statistical section mentioned in the study protocol.

Table & Listing (TLs) shells are only template purpose and modifications might be done in final TLs as identified during the review of data.

2. Objective of the Study

2.1 Objective:

- To evaluate the diagnostic performance and safety of metal allergens proposed for inclusion in Metal Panel T.R.U.E. Test. The study will compare the diagnostic performance (primary) and safety (secondary) of ascending patch test doses of aluminum, copper, manganese, molybdenum, tin, titanium, vanadium and zinc allergens.
- To determine if subjects who have not had previous patch testing are allergic to any of the most common metal allergens; nickel, cobalt chromium and gold.

3. Investigational Plan

3.1 Overall Study Design

This is a prospective, multi-center, randomized, double-blind design. That is, the allergen doses on each panel will be randomized into three different configurations, which will be randomly assigned to subjects as they enter the study.

Although the investigators and subjects will know which allergen is being tested, they will be blinded to the placement of the allergen doses within each panel.

A 48-hour application (approximate) of investigational allergen panel(s), an excipient control and corresponding reference petrolatum (or aqueous) allergen(s) will be applied to the skin of human subjects to test for potential positive allergic responses.

Test sites will be evaluated at 3-4, 7-8, 10-14 and 19-23 days after application.

3.2 Investigational Allergen Panels:

Investigational Panel 1

Aluminum Dilution Series

0.040 mg/cm² aluminum chloride hexahydrate
0.12 mg/cm² aluminum chloride hexahydrate
0.36 mg/cm² aluminum chloride hexahydrate
0.72 mg/cm² aluminum chloride hexahydrate

0.047 mg/cm² aluminum lactate
0.14 mg/cm² aluminum lactate
0.42 mg/cm² aluminum lactate
0.84 mg/cm² aluminum lactate

Investigational Panel 2- (Copper, Zinc and Tin Dilution Series):
Copper Dilution Series:
0.013 mg/cm² copper sulfate anhydrous
0.040 mg/cm² copper sulfate anhydrous
0.080 mg/cm² copper sulfate anhydrous
0.12 mg/cm² copper sulfate anhydrous

Tin Dilution Series:
0.018 mg/cm² tin chloride dihydrate
0.037 mg/cm² tin chloride dihydrate
0.11 mg/cm² tin chloride dihydrate
0.33 mg/cm² tin chloride dehydrate

Zinc Dilution Series:
0.013 mg/cm² zinc chloride
0.040 mg/cm² zinc chloride
0.080 mg/cm² zinc chloride
0.24 mg/cm² zinc chloride

Investigational Panel 3 - (Manganese and Molybdenum Dilution Series):

0.013 mg/cm² manganese chloride tetrahydrate
0.040 mg/cm² manganese chloride tetrahydrate
0.080 mg/cm² manganese chloride tetrahydrate

0.24 mg/cm² manganese chloride tetrahydrate

Molybdenum Dilution Series:
0.0067 mg/cm² ammonium molybdate
0.020 mg/cm² ammonium molybdate
0.040 mg/cm² ammonium molybdate
0.12 mg/cm² ammonium molybdate

Investigational Panel 4 - (Titanium Dilution Series):
Titanium Citrate
Ammonium titanium peroxo citrate (0.055 mg Ti/cm²)
Ammonium titanium peroxo citrate (0.11 mg Ti/cm²)
Ammonium titanium peroxo citrate (0.22 mg Ti/cm²)

Titanium Lactate
Ammonium titanium lactate (0.070 mg Ti/cm²)
Ammonium titanium lactate (0.14 mg Ti/cm²)
Ammonium titanium lactate (0.28 mg Ti/cm²)

Titanium Oxide Oxalate
Potassium titanium oxide oxalate (0.060 mg Ti/cm²)
Potassium titanium oxide oxalate (0.12 mg Ti/cm²)
Potassium titanium oxide oxalate (0.24 mg Ti/cm²)

Ammonium titanium oxide oxalate (0.055 mg Ti/cm²)
Ammonium titanium oxide oxalate (0.11 mg Ti/cm²)
Ammonium titanium oxide oxalate (0.22 mg Ti/cm²)

Investigational Panel 5 - (Vanadium Dilution Series):.

Vanadium chloride (0.0042 mg V/cm²)
Vanadium chloride (0.0083 mg V/cm²)
Vanadium chloride (0.025 mg V/cm²)
Vanadium chloride (0.050 mg V/cm²)

Vanadium oxide sulfate (0.0042 mg V/cm²)
Vanadium oxide sulfate (0.0083 mg V/cm²)
Vanadium oxide sulfate (0.025 mg V/cm²)

Vanadium oxide sulfate (0.050 mg V/cm²)

Investigational Panel 6 - T.R.U.E. Test Panel 6 (Common Allergens/ Excipient Controls):

0.2 mg/cm^2 nickel sulfate
0.054 mg/cm^2 potassium dichromate
0.02 mg/cm^2 cobalt dichloride
0.075 mg/cm^2 gold sodium thiosulfate (GST)
Blank patch
Polyvinylpyrrolidone (PVP)
Hydroxypropyl cellulose (HPC)

Reference Allergens:

Following reference allergen will be tested concurrently with each allergen panel to determine skin reaction concordance and discordance.

- The Aluminum Dilution Series will be tested concurrently with
  - Aluminum chloride hexahydrate,10 % w/w in petrolatum
  - Aluminum lactate, 12% w/w in petrolatum
- The Copper Dilution Series will be tested concurrently with copper sulfate anhydrous, 2 % w/w in petrolatum.
- The Manganese Dilution Series will be tested concurrently with Manganese chloride tetrahydrate, 2 % w/w in petrolatum.
- The Molybdenum Dilution Series will be tested concurrently with Ammonium molybdate, 1% aqueous solution.
- The Tin Dilution Series will be tested concurrently with tin chloride dihydrate, 1% w/w in petrolatum.
- The Titanium Dilution Series will be tested concurrently with
  - Ammonium titanium peroxo citrate, 17% w/w in petrolatum
  - Ammonium titanium lactate, 34% aqueous solution
  - Potassium titanium oxide oxalate, 22% w/w in petrolatum
  - Ammonium Titanium oxide oxalate 19% w/w in petrolatum
- The Vanadium Dilution Series will be tested concurrently with
  - Vanadium chloride, 1% w/w in petrolatum
  - Vanadium oxide sulfate, 1.5% w/w in petrolatum
- The Zinc Dilution Series will be tested concurrently with zinc chloride, 2% w/w in petrolatum

NOTES:

1) The common allergens (Panel 6) will not be tested against reference allergens
2) Subjects enrolled in Germany will not be tested with the reference allergens

3.3 Sample Size Justification

A minimum of 15 subjects per dilution series allergen, who exhibit a positive skin response (score of 1+, +2 or 3+ during at least one reaction assessment visit) to the dilution series allergen and/or at least one of its corresponding reference allergens, is needed to complete the study.

Subjects with a past positive patch test response to at least one of the dilution series allergens will be tested with the allergen panel and corresponding reference allergen(s) to which they have had the previous response. Subjects with suspicion of metal contact allergy will be tested with all dilution series and reference allergens. All subjects will be tested with the excipient controls. The common allergens will be tested at the discretion of the investigator.

Past dose response study populations have included 20 adult subjects (per allergen) with a historical positive patch test to the corresponding reference allergen. In these studies, determination of the optimal test allergen dose was the lowest concentration eliciting a 1+, 2+ or 3+ positive reaction in 70-90% of subjects with the fewest number of 3+ reactions; therefore a minimum of 14 subjects with positive reactions was needed to determine optimal test allergen dose.

Due to the fact that not all of the metals being tested on this study have a large database of patients with past patch-test positive reactions, the inclusion criteria was modified to include subjects with a suspicion of metal allergy, in addition to those with a historical positive patch test. Because it is anticipated that not all subjects will test positive, the study will conclude when a total of 400 subjects have been tested.

The option of further testing may be considered for any allergen that elicits a minimum of 8 positive responses. In such a case, the protocol will be amended.

3.4 Schedule of Events

The schedule of events can be found in Figure 2, in Section 10.0, page 33 of 82, of the protocol- Amendment V.

3.5 Change to Analysis from Protocol

There is no change in analysis from protocol for this study.

3.6 ANALYSIS POPULATIONS

3.6.1 FAS Population

The FAS population will include all randomized subjects.

3.6.2 Modified FAS (mFAS) Population

The mFAS population will include all randomized subjects who have under-gone at least one efficacy evaluation.

All the efficacy analysis will be performed on mFAS and safety analysis will be performed on FAS populations.

3.7 Population Derivation

Conditions for the population derivation:

1) Informed consent form sign date is not missing
2) Randomization number should not be missing.

   Subjects who satisfied conditions # 1 and 2, will be considered in FAS population.

3) Subjects who satisfied all inclusion/exclusion criteria
4) Did subject complete at least one efficacy evaluation = "Yes"

   Subjects who are in the FAS population and satisfied conditions # 3 and 4, will be considered in mFAS population.

4. Efficacy and Safety Variables

4.1 Primary Endpoint (Efficacy)

Determination of optimal test allergen dose as:

- The lowest concentration of each dilution series allergen eliciting positive responses in a minimum of 15 subjects. Positive responses are defined as score of 1+, 2+ or 3+ during at least one reaction assessment visit. If a significant number of 3+ responses are elicited, the dose will be selected based on 1+ and 2+ responses.

- For all sites with the exception of Germany: Concordance will be measured using Cohen's kappa where less than 0% indicates no agreement, 0-20% indicates poor agreement, 20-40% indicates fair agreement, 40-60% indicates moderate agreement, 60-80% indicates good agreement and 80% or higher indicates very good agreement.

  Concordance will be measured using all subjects who are tested with each allergen and corresponding reference allergen.

4.2 Secondary Endpoint (Safety)

Determination of allergen safety:

- Frequency of tape and polyester chip induced irritation or allergic reactions at Visits 2 through 6.
- Frequency of subject reported sensations of itching and/or burning for each allergen panel at patch removal.
- Frequency of positive (1+, 2+, 3+) skin reactions for each investigational and reference allergen dose at each post removal visit and overall.

- Frequency of negative, doubtful, irritant, late and persistent skin responses for each investigational and reference allergen dose at each post removal visit (late and persistent reactions at visits 4, 5 and 6 only).
- Frequency of all adverse events. Documentation for all local and systemic adverse reactions classified by the investigator as possibly or definitely related to the study product (e.g., erythema, hyperpigmentation, hypo-pigmentation, skin thinning or dermatitis flare) will include grade (mild, moderate or severe) and time point (clinic visit).

5. Statistical Considerations

5.1 General Considerations

General considerations are as follows:

- Unless otherwise specified, all statistical tests will be performed using a 2-sided, 0.05 significance level.
- Descriptive statistics such as n, mean, median, standard deviation (SD) and range (minimum, maximum) will be used for summarizing the continuous variables. Frequencies and percentage frequency will be computed for categorical data.
- The summaries will be sorted by visits and allergen. Listings will be sorted by subject number and visit.
- Unscheduled visits data, will be listed only. Summary tables will not utilize the unscheduled data.
- All statistical analysis will be performed using SAS®, Version 9.4 or higher (SAS Institute Inc., NC Cary, USA).

5.2 Derived Variable

- AE duration = AE Stop Date – AE Start Date +1
- The Skin score at visit 4, 5 and 6 are positive (1+, 2+ or 3+) and visit 3 are doubtful or irritant then it will be considered as Positive (1+) at visit 3.

5.3 Site Pooling

- This section is not applicable for the study.

5.4 Reporting Precision

All outputs will be generated based on the specifications mentioned in the document of Mock TLFs.

Summary statistics will be presented to the following degree of precision:

Statistic	Degree of Precision

Mean and Median	One more decimal place than the raw data
Minimum and Maximum	Same as the raw data
Standard deviation	Two more decimal places than the raw data
Percentage, ratios and CI	Two decimal places
p-value	Four decimal places or as <0.0001

Examples for the Decimal Places:

If percentage value is 0 or 100, it will be reported as '0' and '100' respectively.

Ratios and/or Confidence Interval (CI) will be reported as percentages with at least 2 decimal places (e.g. xx.xx, (xx.xx, xx.xx)).

Rounding of data will be followed as mentioned in below examples (if required):

Requirement	Original values	Rounded values
4 decimal points	0.07542	0.0754
2 decimal points	0.75462	0.75
1 decimal point	0.75462	0.8

Statistical significance will be based on two-sided hypothesis testing. P-values less than 0.05 will be considered to be significant.

5.5 Multiple Comparison/ Multiplicity

This section is not applicable for the study.

5.6 Handling of Dropout or Missing Data

Missing data will not be imputed.

Imputation of partial dates:

If medication end date is missing or partially known, the following algorithm will be used to determine if the medication is prior or concomitant:

- The latest possible date matching the partially known date will be imputed. That is, if the year and month are known, the last day of this month will be imputed; if only year is known, consider last month of that year and then the last day of the same month will be imputed.
- The medication will be considered concomitant if this imputed end date is on or after the date of the first use of the study medication (any study medication).
- If end date is unknown, the medication will be considered concomitant.

5.7 Interim Analysis

This section is not applicable for the study.

6. Statistical Analysis

6.1 Primary Endpoint Analysis

Primary Endpoint 01:

The lowest concentration of each dilution series allergen eliciting positive responses in a minimum of 15 subjects. Positive responses are defined as score of 1+, 2+ or 3+ during at least one reaction assessment visit. If a significant number of 3+ responses are elicited, the dose will be selected based on 1+and 2+ responses.

The frequency and percentages of skin responses; "Negative, Irritant, Doubtful and Positive (1+, 2+, 3+)" will be calculated for each dilution series investigational allergen dose and reference allergen.

The optimal allergen dose will be based on following criteria:

1. The optimal allergen dose based on reaction scores will be based on lowest dose eliciting positive reactions.

   Example:

   Step#1: Subject recorded with either of score i.e. 1+, 2+, 3+ then subject's corresponding position allergen dose will be considered as "Positive Allergen Response".

   Step#2: The allergen dose from the respective dilution series eliciting positive reactions (i.e. 1+, 2+, 3+) will be considered as lowest concentration eliciting positive reactions.

   This procedure will be repeated for all visits.

   If a significant number of 3+ responses are elicited, the optimal allergen dose will be selected based on 1+ and 2+ positive responses.

Frequency and percentage analysis of skin reaction score will be carried out for investigational and reference allergen to obtain the optimal dosage.

Primary Endpoint 02:

For all sites with the exception of Germany (i.e. Site 6): Concordance will be measured using Cohen's kappa where less than 0% indicates no agreement, 0-20% indicates poor agreement, 20-40% indicates fair agreement, 40-60% indicates moderate agreement, 60-80% indicates good agreement and 80% or higher indicates very good agreement.

Concordance will be measured using all subjects who are tested with each allergen and corresponding reference allergen.

Data from all the sites by investigational allergen and corresponding Reference Allergen (RA) will be considered to assess the concordance.

Subjects with Missing Response at Investigator Determination of either panel (i.e. Investigational or respective Reference Panel) will not be considered for assessment of concordance.

Overall concordance of skin reaction responses from each allergen dose will be compared with reference allergen by using Kappa statistic as described below,

Kappa statistics (K) = $(P_0-P_e) / (1-P_e)$

Where P_0 is the relative observed agreement among raters (identical to accuracy), and P_e is the hypothetical probability of chance agreement and the Kappa agreement statistics score is interpreted as,

≤ 0 as indicating no agreement

0.01 – 0.20 as poor agreement

0.21 – 0.40 as fair agreement

0.41 – 0.60 as moderate agreement

0.61 – 0.80 as good agreement

0.81 – 1.00: very good agreement

Concordance will be measured using all subjects who are tested with each allergen and corresponding reference allergen.

Note: Data from the subjects with the completely detached patches for the intended wear period (approximately 48 hours or two days) will not be included in the analysis of positive responses necessary to determine optimal dose.

6.2 Secondary Endpoint Analysis

Frequency and percentage analysis will be carried out for below mentioned endpoints for investigational and reference allergen to obtain the safety of allergen.

Determination of allergen safety:

- Frequency of tape and polyester chip induced irritation or allergic reactions at Visits 2 through 6.
- Frequency of subject reported sensations of itching and/or burning for each allergen panel at patch removal.
- Frequency of positive (1+, 2+, 3+) skin reaction will be tabulated for the each investigational and reference allergen dose tested.
- Frequency of negative, doubtful, irritant, late and persistent skin responses for each investigational and reference allergen dose at each post removal visit (late and persistent reactions at visits 4, 5 and 6 only).
- Frequency of all adverse events. This will be brief in the section 6.2.1.

6.2.1 Overall Summary of AEs or TEAEs

Adverse Events (AEs) will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) dictionary Version 20.0.

This table will include the number of events (Adverse Events or Treatment Emergent Adverse Events), number and percent of subjects who experienced,

- AEs or TEAEs
- Serious AEs or TEAEs
- Related AEs or TEAEs (i.e. Relationship to study panel)
- AEs or TEAEs leading to study discontinuation

6.2.2 Summary of AEs or TEAEs by SOC and PT

A summary of number and percent of subjects who experienced at least one AE or TEAE as well as the number of events, number and percent of subjects who experienced each specific SOC and PT will be presented.

If a subject has more than one occurrence of the same preferred term (PT) for an AE then the PT will be counted only once for that subject under the SOC at which it was experienced.

In case AE start date is missing then “Date of randomization” will be considered for start date and if the stop date is missing then “Date of last contact” is considered for AE duration calculation.

6.3 Physical Examination

Physical examination will be performed on visit 1 (Day 0).

Results of physical examinations performed will be presented in listings Current symptoms associated with metal exposure and Additional Sites of Dermatitis. The listing will include subject number, current symptoms, additional site dermatitis, age at diagnosis and severity.

6.4 Subject Disposition and Withdrawals

Frequency of following categories will also be displayed:

- Number of all screened subject
- Number of screened failure subjects

The following frequencies (number and percent) will be displayed for all the randomized subjects:

- randomized subjects who has under-gone at least one efficacy evaluation
- Subjects who discontinued early, as well as reasons for subject discontinuation

The denominators for the percent calculations will be the number of all randomized subjects.

Subjects' discontinuation status will be listed and will include subject number, Sample number, Date of completion or discontinuation and specific reason for discontinuation (if reason for discontinuation will be "Lost to follow-up" then "Date of last contact:" will be listed).

6.5 Demographic and Baseline Characteristics

Demographic will be performed on Visit 1 (Day 0).

Demographics (age, sex, and ethnicity) will be summarized using descriptive statistic (n (number of subjects), mean, standard deviation, minimum, median and maximum for quantitative variables, and number of subjects and percent of subjects for qualitative variables).

Demographic data will also be listed.

6.6 Protocol Deviation

All the protocol deviations will be listed. A summary of protocol deviation will also be produced as frequency and percentages.

6.7 Medical History (Type of Metal Exposure Questionnaire)

A complete medical history of contact allergy associated with metal exposure, including a complete review of all current and past diseases and their respective treatments, shall be done on screening visit.

Listing of medical history will include the parameters as subject number, Condition/ Procedure, date of diagnosed/procedure, Medication, duration and related information.

6.8 Urine Pregnancy Test

Urine Pregnancy Test performed at Visit 1 (Day 0) will be listed by subject number, date of urine pregnancy test performed, birth control and results.

6.9 Prior and Concomitant Medication

Any medication other than study drug, either prescription drug or OTC will be treated as concomitant medication.

Prior Medication: If the end date is known and medication consumed by subject prior to first use of patch application.

Concomitant Medication: Medication consumed by subject that are ongoing or ended after the first use of the patch application.

Listing of prior/concomitant medication will include Condition/ Procedure, Date of diagnosed/ procedure, Medication, Frequency of use, Route of administration, Dose, and Start date and Stop date. No summary table will be generated.

7. Summary of Analyses

Endpoint	**Analyses**	**Population(s)**
Primary endpoint	Kappa statistics	FAS
Secondary endpoint	Frequency Counts (%)	FAS
Safety Variables: Adverse events	Frequency Counts (%)	FAS
Disposition	Summary Statistics	FAS
Demographic and Baseline characteristics	Summary Statistics	FAS

8. Data Management Considerations

All data collected in the Case Report Forms (CRFs) using validated Trianz Acceliant, Version 7.0.1.14. Prior to performing the analyses, a series of edits will be performed to identify missing data, inconsistent data and errors in database. Protocol deviations will be identified. All reported adverse events (AEs) will be coded to standard 'preferred terms' and 'System Organ Class' using the Medical Dictionary for Regulatory Activities (MedDRA) version 20.0.

After all the modifications to the database are made, protocol deviations identified, and adverse events coded, database will be locked. All request to modify the database after treatment assignment is added will require the written approval of the statistician and medical monitor responsible for the study.

All summaries, statistical analyses, and individual subject data listings will be completed using Statistical Analytical System (SAS®) version 9.4 or higher, SAS Institute, Cary, North Carolina, United States of America (USA).

9. Tables, Listings and Figures

The TLs shells for this study is provided in a separate document "SAP Mock TLs". These shells may not be reflective of every aspect of this study but are intended to show the general layout of the Tables, Listings and Figures that will be included in the final report.

TLs are numbered following the ICH E3 "Structure and Content of Clinical Study Report".

10. References

- Protocol Version: Amendment V.
- ICH E3: Structure and Content of Clinical Study Reports, July 1996, CPMP.
- ICH E9: Statistical Principles for Clinical Trials, September 1998, CPMP.