MXP23001 Statistical Analysis Plan

INTRODUCTION

Scope of the Analyses

Table 1

SAP Version Number/ Date:	SAP version 1.0 13Nov2023
Protocol Number/ Title:	MXP23001: A Retrospective Review Evaluating the Matrix Pro Applicator for Treatment of Wrinkles

NCT: NCT06219278

STUDY OBJECTIVES AND ENDPOINTS

Table 2

Study Description	
Study Objective:	**Study Endpoint:**
Evaluate the safety and efficacy of the Profound Matrix System with the Matrix Pro applicator for treatment of wrinkles	Primary Efficacy Endpoint is pre-defined as the percentage correct before and after treatment photograph determinations made by blinded evaluators based on perception of facial wrinkles. There will be 3 independent blinded evaluators partaking in the study. Each will receive the set of subject photographs in de-identified coded randomized order (randomized for before and after treatment presentation order with respect to each subject photograph set, and with respect to order of subject set presentation). The blinded evaluators will be required to determine within each presented photographic set which of the images is the 'After' image (3-months post-treatment administration) based on his or her perception of the presentation of facial wrinkles. Each blinded evaluator will perform this task independently of the other. A minimum of 2 out of the 3 blinded evaluators correctly determining Before-After assignment for an individual subject's photograph set will indicate success for that individual subject with respect to positive treatment response. 70% overall individual responder rate will be positive for study success. [redacted]
	The Secondary Efficacy Endpoint is subject satisfaction ratings at study endpoint.
	The Safety Endpoint is adverse events that will be tabulated by type, incidence, severity, relatedness to treatment, action taken, and outcome.
	The Tolerability Endpoint is Subject assessment of treatment discomfort/pain immediately post-treatment via Numerical Rating Scale (NRS)
	Exploratory endpoints include local skin responses as assessed immediately after treatment by type and severity for all subjects.

STUDY METHODS

General Study Design and Plan

Table 3

Study Design and Plan	
Study Type and Design:	This study is a retrospective analysis of de-identified subject data collected during implementation of the clinical study, "Functional Usability and Feasibility Testing of the Profound Matrix™ System (FUFT2002)" (source study). The FUFT2002 clinical study was a multi-site prospective clinical trial. The current retrospective study will analyze data extracted from the FUFT2002 database according to prespecified study selection criteria.
Control Group:	☐ Placebo, Treatment with No Treatment Effect ☐ State of the Art Treatment or Medication ☐ No Treatment ☐ Subject Serves as Own Control ☐ Non-randomized Concurrent Control (Observational Study) ☐ Other: _____ ☒ N/A, No Control Group. Explain rationale (e.g., pilot study for feasibility): [redacted]
Type of Comparison:	☐ Inferiority (at least as good as) ☐ Superiority (better than) ☐ Equivalence (equal to) ☒ N/A, No Control Group
Randomization:	☐ Yes, randomized control study. Explain below: ☒ No, study is not randomized. Explain rationale: The study is a retrospective, single-arm study.
Level of Blinding/ Masked:	☐ Yes, double blinded study. Explain below: ☐ Yes, single blinded study. Explain below: ☒ No, study is unblinded/ open label. [redacted]

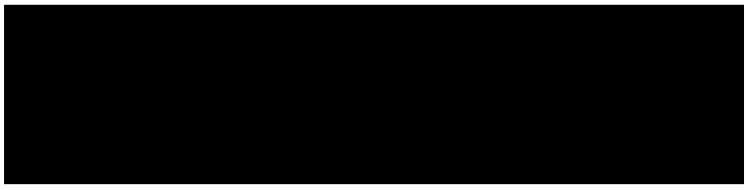
Inclusion-Exclusion Criteria and General Study Population

See study protocol for inclusion-exclusion criteria and general study population.

Study Assessments

See study protocol for table of study assessments by study visit timepoints.

Table 4

Description of Variables	
Efficacy Variables:	• **Subject Global Aesthetic Improvement Scale (GAIS):** A 5-point scale assessing the subject's satisfaction with any perceived overall improvement in their facial appearance following the treatments with the study device.  • **Numerical Rating Scale (NRS):** A horizontal line scale from 0 to 10, with 0 equaling no pain and 10 equaling the worst possible pain. The subject is asked to mark the point on the scale that best represents their current pain level.  • **Post-treatment Severity Scale**: A 0 to 3 categorical scale presented below. 
Safety Variables:	Assessment of safety of Matrix Pro treatment(s) for wrinkle reduction will be through: • Evaluation of the number, severity, and type of any device-related adverse event that occurs throughout the course of the study from baseline through to the 3-month follow-up evaluation visit. • Subject assessment of treatment discomfort/pain recorded immediately post-treatment via the 11-point Numerical Rating Scale (NRS). • Investigator assessment of local skin responses recorded immediately post-treatment, evaluated by type and severity.

HYPOTHESIS

Table 5

Statistical Criteria for Hypothesis	
Efficacy Variables:	Primary Efficacy variable is according to pre-determined responder rate criteria as follows: *Null Hypotheses*: • The proportion of individual subject successes for primary endpoint, i.e., the proportion of individual subjects for whom at least 2 of the 3 blinded evaluators correctly identifies the Before-After photo order, will be less than 70%. *Alternative Hypothesis*: • The proportion of individual subject successes for primary endpoint i.e., the proportion of individual subjects for whom at least 2 of the 3 blinded evaluators correctly identifies the Before-After photo order, will be 70% or greater. Statistical hypotheses for secondary variables are not applicable as secondary variables will be evaluated descriptively only. ☐ NA, Explain rationale: N/A
Safety Variables:	☒ NA, Explain rationale: Statistical hypotheses for safety variables are not applicable as formal safety evaluation is not required for this non-significant risk (NSR) retrospective study. As such, statistical significance evaluation of outcome is limited to the primary efficacy variable only, as explained above.

SAMPLE SIZE

Calculating Sample Size

Table 6

Sample Size Justification	
Clinical Background:	The parameters used for sample size calculation were determined from that employed in prior pivotal clinical trials whose outcome data was used to successfully support an FDA 510(k) clearance for a comparable indication to that being evaluated in this study.
Expected Outcomes:	As this is a retrospective, single arm study, minimally clinically meaningful difference between treatment groups is not applicable. Expected outcome is that within the single arm active treatment group, the pre-determined primary success criteria of at least 2 of the 3 blinded evaluators correctly identifying the Before-After photo order for a minimum of 70% of subjects, will be met.
Estimated Retention	N/A – This is a retrospective clinical study
Effect Size	☒ N/A, Explain rationale: There is no comparator arm.
Significance Level and Power Justification:	Significance Level: $\alpha = 0.05$ Power: 80% Type of t-test: Responder Rate Analysis: percent of successes. ☐ N/A, Explain rationale: N/A

Sample Size Calculation and Justification	Employing these parameters, the minimum pre-determined sample size to provide sufficient power for a statistical comparison of the proportion of treatment responders (P) versus a reasonable cutoff (P0) is based on a power calculation utilizing the (one proportion) binomial exact test and based on the following assumptions. [redacted] Therefore, a sample size of 24 study subjects is determined to be sufficient to provide appropriate sample population for statistical analysis such that the results of this retrospective study can be considered statistically and clinically meaningful and generalizable to the broader intended population. ☐ N/A, Explain rationale: N/A
Target Number of Subjects per Site	A sample size of 24 study subjects has been determined sufficient to provide an appropriate sample population for statistical analysis and generalizability of results.

GENERAL ANALYSIS CONSIDERATIONS

Timing of Analyses

- The final analysis for primary efficacy will be performed after all qualified subjects from the source study have been identified per the retrospective study inclusion and exclusion criteria and enrolled in the retrospective study, after the final data entry has been entered into the current study database, and after each of the three independent blinded evaluators have completed their Before-After blinded photographic assessments.

- The final analysis will be performed on data recorded in the electronic Excel database, having been documented as meeting the cleaning and approval requirements of SOP 10-030-01617 (Global Clinical Data Management Procedure) and after the finalization and approval of this SAP document.

Analysis Populations

Table 7

Study Design and Plan	
Full Analysis Population/ Intention to Treat Population (or Modified Intention to Treat Population):	The Full Analysis Population in this retrospective study will be the per protocol population including all enrolled subjects who had valid recorded baseline and study endpoint outcomes recorded during the source study, including evaluable photographs at both assessments. The Full Analysis Per Protocol population analysis will be the primary and only analysis population in this retrospective study.
Per Protocol Population	The per protocol (PP) population as explained above will be the primary and only analysis population in this retrospective study.

Covariates and Subgroups

Multi-center Studies

- Individual center results will be pooled together to perform the final efficacy analysis.

- The justification for combining of study data across test sites is based on the clinical assessment provided by Meinert (1): the clinical (source) study was conducted under a common protocol for each investigational site, the study sites were monitored for protocol compliance, and the same data gathering instruments and methods were used at every site.
- Justification of the plausibility of combining of study data across test sites will be conducted through Fischer's Exact Test to evaluate for homogeneity of Responder Rates (study Primary Efficacy Outcome assessment) between test sites.

This study has not been explicitly designed with enough power to detect center effects. Therefore, treatment-by-test site interactions will be analyzed descriptively only through tables and charts, using N, mean, standard deviation, and minimum-maximum values. **Missing Data**

As by virtue of the study selection criteria, qualifying subjects will have had valid required measures recorded at baseline and endpoint assessments for the current study, including evaluable digital photographs, handling of missing data is not applicable.

Interim Analyses and Data Monitoring (as applicable)

N/A

Table 8

Interim Analysis Plan	
Purpose of Interim Analysis	☒ N/A, Explain rationale: There is no uncertainty as to the study design, treatment parameters, outcome variables, study endpoints, or endpoint analyses.
Planned Schedule of Interim Analyses	☒ N/A, Explain rationale: No interim analysis is planned for this study.
Scope of Adaptations	☒ N/A, Explain rationale: No interim analysis is planned for this study.
Stopping Rules	☒ N/A, Explain rationale: No interim analysis is planned for this study.
Analysis Methods to Minimize Bias	☒ N/A, Explain rationale: No interim analysis is planned for this study.
Adjustment of Confidence Intervals and p-values	☒ N/A, Explain rationale: No interim analysis is planned for this study.
Interim Analysis for Sample Size Adjustment	☒ N/A, Explain rationale: No interim analysis is planned for this study.
Practical Measures to Minimize Bias	☒ N/A, Explain rationale: No interim analysis is planned for this study.
Documentation of Interim Analyses	☒ N/A, Explain rationale: No interim analysis is planned for this study.

SUMMARY OF STUDY DATA

- All continuous variables will be summarized in tables using the following descriptive statistics: n, mean, standard deviation, maximum and minimum.

- All categorical measures will be summarized in tables and/or charts as frequencies and/or percentages of observed levels.
- Summary tables will be structured with a column for the treatment and rows for the variables.
- Primary efficacy outcome data will additionally be listed by subject, and sorted by site, and when appropriate by visit within subject.

Subject Disposition

The following variables from the CRF will be used to establish subject disposition with respect to those selected from the source study per the current retrospective study selection criteria:

- Number received two or three study treatments and completed 1-month and 3-month follow-ups = Per Protocol population

Determination of population membership and population size per treatment group is not applicable as this study is a single arm design with no sham or control arm.

Derived Variables

The primary endpoint of blinded evaluator before/after photo determination will be derived from the photographs that are source documents recorded at the respective evaluation timepoints during the study from which the current retrospective study data will be drawn.

Protocol Deviations

There are no significant protocol deviations in the current retrospective study that could impact the study primary outcomes analysis. With respect to the source study, given that the subject selection criteria will result in a per protocol analysis population only for the current study data , there will be no major protocol deviations that could impact that study's primary outcomes analysis
Any additional protocol deviations that occurred in the source study that are relevant to the current study will be listed and explored as applicable. There is no criteria for reporting of any protocol deviation that may potentially cause harm to a subject to the IRB as the source study is complete and any such reporting requirements have already been satisfied.

Demographic and Baseline Variables

The demographic or baseline variables that will be recorded in this clinical study are age, gender, race/ethnicity, and Fitzpatrick Skin Type (I through VI).

Depending on the final enrollment sample, age may be transformed into category, within the inclusion range of 18-75 years.

Demographic/baseline variables will also be summarized by test site.

Concurrent Illnesses and Medical Conditions

Subject medical history and concomitant medication use will not be specifically evaluated in this retrospective study. It is a condition of eligibility in this retrospective study that qualified subjects met the eligibility criteria under FUFT2002 and were enrolled into FUFT2002 study at which time any medical history and concomitant medication use that would be pertinent inclusion/exclusion criteria for the current retrospective study would have been satisfied. Given that the current study is retrospective, medical history and concomitant medication use will not be tracked throughout the course of the study, and as such, no coding system needs to be implemented.

Treatment Compliance

As this study is a retrospective study, assessment of treatment compliance is not applicable.

EFFICACY ANALYSES

The primary efficacy variable will be listed by subject. Primary data will be summarized across the pooled subject group. N, Mean, Standard Deviation, Minimum and Maximum will summarize continuous efficacy variables, whereas number and percent will summarize categorical efficacy variables, including responder rate variables.

Responder rate variables will be assessed against a predetermined criterion.

Categorical efficacy measures will be evaluated descriptively within evaluations and for change across evaluations.

Primary Efficacy Analysis

Primary Efficacy Endpoint is pre-defined as the percentage correct before and after treatment photograph determinations made by blinded evaluators based on perception of facial wrinkles.

There will be 3 independent blinded evaluators partaking in the study. Each will receive the set of subject photographs in de-identified coded randomized order (randomized for before and after treatment presentation order with respect to each subject photograph set, and with respect to order of subject set presentation). The blinded evaluators will be required to determine within each presented photographic set which of the images is the 'After' image (3-months post-treatment administration) based on his or her perception of the presentation of facial wrinkles. Each blinded evaluator will perform this task independently of the other. A minimum of 2 out of the 3 blinded evaluators correctly determining Before-After assignment for an individual subject's photograph set will indicate success for that individual subject with respect to positive treatment response. Seventy percent (70%) overall individual responder rate will be positive for study success.

Secondary Efficacy Analyses

Secondary measures efficacy analysis will be conducted descriptively only through summary statistics such as means, standard deviations, and ranges for qualitative data, percentages per category, and in tabular and/or graphical format, as applicable to the data type (qualitative or quantitative, continuous, or categorical, etc.).

Subgroup analysis of the primary efficacy endpoint may be performed for the variables of age, gender, and Fitzpatrick Skin Type.

Secondary Analyses of Primary Efficacy Endpoint

Secondary analysis of the primary efficacy endpoint is not applicable as the per protocol population is the only population being evaluated in this retrospective study.

Exploratory Efficacy Analyses

Evaluation of exploratory endpoints may occur informally.

SAFETY ANALYSES

Each of the following safety outcome variables as recorded during the source study for subjects enrolled in the current study will be reported, evaluated, and discussed numerically and descriptively, as applicable:

- The incidence, severity, and type of any device-related adverse event that occurred throughout the course of the study from baseline through to the 3-month follow-up evaluation visit.
- Subject reports of treatment discomfort/pain recorded immediately post-treatment using the 11-point Numerical Rating Scale (NRS).
- Investigator assessment of local skin responses recorded immediately post-treatment, evaluated by type and severity.

Adverse Events

The incidence rates and percentages of adverse events will be calculated with respect to each of number of subjects and number of treatment administrations. Wherein subject is the denominator, each subject will only be counted once, and any repetitions of adverse events will be ignored. All adverse events will be included in the summary and analysis.

SUBJECT SATISFACTION QUESTIONNAIRE

[redacted]

QUALITY ASSURANCE OF STATISTICAL ANALYSIS

Study data extracted from the source study database will be recorded on paper CRFs and then entered in the study database. Photographs will be stored electronically. Other source documentation (i.e., evaluations performed post-hoc from the photographs by the independent blinded evaluators) will be entered into the database at completion. Verification of the entry of the paper source documentation into the database will occur after the final entry has been made. Subsequently, the fully verified database will be locked. The database will be constructed using Microsoft Office Excel (Microsoft, Redmond, WA). The final dataset will be forwarded to an independent contracted biostatistician for analysis per the protocol Statistical Analysis Plan using SAS Version 9.4 or equivalent, as per the time of writing.

ROLES AND RESPONSIBILITIES OF SAP

Table 9

Roles and Responsibilities	
Name/ Title	**Function**
Elvira Cawthon, B.S., M.S., AEMT – *Independent Clinical Consultant/Biostatistician*	Provide statistics for sample size calculation and input to data analysis plan and the final analysis.
Maya Duffy – *Clinical Affairs Manager*	Oversee study related procedures
Bhumi Vinay Patel, MPH – *Senior Clinical Research Associate and Data Lead*	• *Oversee data monitoring and management.* will review the data or analyses and make decisions. • what changes if any will be made to the study protocol, device, etc.
Katherine Coleman, Liuping Li, and Nardeen Badarny . –*Clinical Research Associates*	Oversee study related procedures, site monitoring, and collection of data
Principal Investigator(s) of Clinical Trial Sites	Ensuring that the source clinical study is conducted according to the investigational plan, and applicable FDA regulations. Protecting the rights, safety, and welfare of study subjects.

REFERENCES

1. Meinert, C. (1986). Clinical Trials: Design, Conduct, and Analysis. Oxford University Press, New York