Statistical Analysis Plan
for
final analysis

Version 4.0

Study:	A randomized, placebo-controlled, double-blind, multicenter study to evaluate the efficacy of intranasal kinetic oscillation stimulation in the preventive treatment of chronic migraine
Study-ID:	PM007
Sponsor / Contact:	Chordate Medical AB C/o Regus, Kistagången 20B 164 40 Kista, Sweden
Evaluation:	FGK Clinical Research GmbH
FGK code	[redacted]
Version:	4.0 of 12Oct2022
Previous versions:	1.0 of 09Mar2021 2.0 of 16May2022 3.0 of 18May2022

Revision history

Version	Author	Date	Reason for Revision
1.0	[redacted]	09Mar2021	n.a. first final version
2.0		16May2022	Second final version, update of first final version due to: • protocol amendment (consolidated version 7.0 based on version 5.0 of 27Nov2019 and version 6.0 of 05Nov2020 for Finland) ***Note:*** *no change of statistical analysis due to protocol amendment* • additional unblinded subgroup analysis (the unblinded analysis of the German subjects) • detected findings during programming
3.0		18May2022	Third final version, update of second final version due to detected findings
4.0		12Oct2022	Fourth final version, update of third final version due to detailed description of a group-sequential design with O´Brien & Fleming alpha spending function using for analysis of primary performance variable

Table of Contents

List of Abbreviations

In the following abbreviations are listed as used within this statistical analysis plan or which might occur within the tables, listings and graphs outputs:

AE	Adverse event
ANCOVA	Analysis of covariance
ATC	Anatomical therapeutic chemical classification
BDRM	Blind data review meeting
BMI	Body mass index
CI	Confidence interval
CRF	Case report form
FAS	Full analysis set
ICH	International council of harmonization
HIT	Headache impact test
H_0	Null hypothesis
H_1	Alternative hypothesis
MedDRA	Medical dictionary for regulatory activities
MSQ	Migraine-specific quality of life questionnaire
N	Number of subjects
PGI-S	Patient global impression of severity
PP	Per-protocol
Q1	1st quartile
Q3	3rd quartile
SAE	Serious adverse event
SAF	Safety analysis set
SAP	Statistical analysis plan
SD	Standard deviation
SOC	System organ class
TLG	Tables, listings, graphs
V	Visit

1 General

This Statistical Analysis Plan (SAP) was defined by the Sponsor and the responsible Statistician without knowledge of the randomization code. It is based upon the Study Protocol (version 7.0 of 14Jun2021) and contains detailed description of the statistical methods described therein.

The SAP describes prospectively the analyses to be performed on study data. It was finalized prior to data base lock and unblinding.

This is a randomized, placebo-controlled, double-blind, multicenter clinical investigation of a medical device. The study consists of a 4-week screening period, a 6-week treatment period (2 weeks run-in and a 4-week observation window to assess the treatment effect), and a 4-week follow-up period.

Subjects who provided written informed consent and are eligible for the study will be asked to complete a daily diary for 4 weeks during the screening period. In the diary the subjects will record headache and migraine days, any changes in their health, and concomitant medications they may be using. The data collected in the diary during this screening period will be used as Baseline for the performance assessments.

Subjects who completed the screening period and continue to meet the eligibility criteria after review of diary entries by the investigator will be randomized (stratified by medication overuse) in a 1:1 ratio to receive either active or placebo treatment. The subjects will receive 6 treatments with the Chordate System S211 at weekly intervals at the site (Day 0 to 35; treatment Visit [V] 1 to 6) and continue to maintain a daily diary. One week after the last treatment, an end of treatment visit will be performed on Day 42 (V7).

After completion of the 6-week study treatment period (V1 to V7), subjects will be followed up for another 4 weeks. The final visit will be performed on Day 70, 5 weeks after the last study treatment. During the 4-week follow-up, the subjects will continue to complete a daily diary.

The study will be performed according to a 2-stage group-sequential design with one interim analysis that allows the premature termination of the study due to efficacy or futility or adaption of the sample size.

Approximately 148 subjects will be enrolled to achieve at least 132 subjects evaluable for the primary performance endpoint (assuming a drop-out rate of 10%), with 66 subjects receiving treatment with the Chordate System S211 and 66 subjects receiving treatment with the Chordate System S211 in placebo mode. If, during the conduct of the study, the drop-out rate proves to be higher than expected (>10%), enrollment will continue until at least 132 subjects were assessed for the primary endpoint. A group-sequential design with one interim analysis after approximately 50 subjects have completed the 6-week study treatment period will be used. It will allow the premature termination of the study due to efficacy or futility or adaption of the sample size.

At the time of finalization of this SAP, the interim analysis had been carried out and did not lead to any adaption of the study.

An additional unblinded subgroup analysis (the unblinded analysis of the German subjects) will be performed and will include selected analyses only. Details on which outputs will be produced for additional unblinded subgroup analysis are given in Appendix A.

2 Performance and Safety Endpoints

2.1 Primary Performance Endpoint

The primary performance endpoint is given by the mean change from Baseline (4-week screening period) in 4-week headache days with moderate to severe intensity in 4-week performance assessment period (V3 to V7).

2.2 Secondary Performance Endpoints

The following secondary performance endpoints will be analyzed:

- Mean change from Baseline in 4-week headache days with moderate to severe intensity in follow-up period;
- Mean change from Baseline in 4-week migraine days;
- Mean change from Baseline in 4-week headache days (mild, moderate and severe intensity) in 4-week performance assessment period (V3 to V7);
- Proportion of subjects with 30% or greater reduction in headache days of moderate to severe intensity;
- Proportion of subjects with 50% or greater reduction of headache days of moderate to severe intensity;
- Change in the use of acute (abortive) medication;
- Change in migraine-specific quality of life questionnaire (MSQ), headache impact test-6 (HIT-6), and patient global impression of severity (PGI-S).

2.3 Safety Endpoint

- Frequency, severity, device-relationship, and outcome of AEs

3 Statistical Analysis Sets

3.1 Safety Analysis Set

The safety analysis set (SAF) will include all subjects who were treated with the investigational medical device at least once, irrespective of the duration of use. Subjects will be analyzed as treated.

3.2 Full Analysis Set

The full analysis set (FAS) will include all subjects included in the SAF and having baseline and any post baseline data of any performance endpoint (primary performance endpoint or any of the secondary endpoints). Subjects will be analyzed as randomized.

3.3 Per-Protocol Set

The per-protocol analysis set (PP) will include all subjects who fulfill the following criteria:

- Included in the FAS.
- Do not have any other major protocol violations which will affect the assessment of efficacy. An example of a major protocol violation is a subject who terminates the study participation before the primary endpoint follow-up or violation of the inclusion/exclusion criteria.
- Have no imputation for the primary performance endpoint (i.e. completed the diary for 28 days after V3).
- Have full duration of study treatment.

Subjects will be analyzed as treated.

3.4 Assignment of Analysis Sets to Analysis

Primary efficacy analysis will be based on the FAS. A sensitivity analysis of the primary endpoint will be performed using the PP set. All secondary efficacy outcomes will exploratorily be analyzed using the FAS and PP set. Safety analyses will be based on the SAF.

4 Statistical Evaluation

The investigator will review the patient's diary and enter the number of days with headache and the number of days with migraine in the eCRF. This information will be available for the baseline period, the performance assessment period and the follow-up period.

In case a patient should have documented less than 28 days per period (but has a compliance of at least 80%), the number of headache days per period is derived as follows:

Number of headache days = (Number of headache days x 28) / Number of days observed

If a patient has a diary compliance of less than 80%, this period will not be used for statistical analysis of this endpoint.

For example, if a patient documented 24 days for one period only, and documented 6 days with headache, the derived number of headache days in this period is calculated to (6 x 28) / 24 = 7.

The assessments done before start of treatment will serve as baseline evaluations. Generally, a baseline measurement refers to the last non-missing assessment made before treatment start.

Continuous data are presented with the number of observations, mean value, standard deviation (SD), minimum, 1st quartile (Q1), median, 3rd quartile (Q3), and maximum value. Categorical data are presented as counts and percentages. In general, minimum and maximum will be presented to the same level of precision as the raw data; means and medians, SD, and quartiles will be presented to one further decimal place. Categorical variables will be presented by frequency tables, using number and percentage. Percentages will be presented to one decimal place.

4.1 Dispositions of Subjects and Analysis Sets

Disposition of subjects and analysis sets

The disposition of subjects and analysis sets, subjects per center, inclusion and exclusion criteria, and the status at study termination will be presented using summary statistics.

The number of subjects available per visit will be tabulated.

No inferential assessments will be performed on disposition data.

4.2 Demographics and Other Covariates

Demographic data

Demographic data (age, sex, race, height, weight, body mass index) will be summarized by treatment group to describe the study population.

Medical and surgical history

Relevant past medical history will be collected and listed. Migraine history will be listed separately.

Concomitant medication

Concomitant medication will be coded according to World health organization drug dictionary version March 2018.

Medications will be tabulated by anatomic group (ATC) level 4, and WHO-DD preferred term. The number of entries, as well as the number and percentage of affected subjects will be reported.

Urine Pregnancy test

The proportion of patients with positive or negative pregnancy test results from females with childbearing potential will be tabulated.

Physical examination

All information about physical examination will be listed.

4.3 Performance Analysis

For all tests, a two-sided significance level of 5% ($\alpha = 0.05$) will be applied.

Analysis for the primary performance endpoint will be based on the FAS. Additionally, a sensitivity analysis of the primary endpoint will be performed using the PP.

All statistical tests for secondary performance endpoints are considered exploratory and no adjustment for multiplicity is made.

All secondary endpoints will be evaluated for the performance assessment period and the follow-up period.

The 4-week screening period is defined as Baseline.

4.3.1 Analysis of Primary Performance Variable

Mean change from Baseline in 4-week headache days

The mean change from Baseline (4-week screening period) in 4-week headache days with moderate to severe intensity in 4-week performance assessment period (V3 to V7) will be analyzed as follows:

The primary null hypothesis to be tested is

H_0: $\mu_{active} = \mu_{placebo}$ against the alternative

H_1: $\mu_{active} \neq \mu_{placebo}$,

where μ is the mean change from Baseline (4-week screening period) in 4-week headache days with moderate to severe intensity in 4-week performance assessment period (V3 to V7).

An analysis of covariance (ANCOVA) with treatment, baseline number of headache days, medication overuse as independent variables will be calculated to test above null hypothesis. Check for the normal distribution of data will be performed.

Normality of data cannot be assumed, if at least one of the following items can be demonstrated on the residuals, as determined when applying the ANCOVA as described above:

- p-value using Shapiro-Wilk test on normality < 0.2.
- Visual check of the QQ-plot shows: plotted points do not approximately lie on the diagonal line y = x.

If normality of data cannot be shown, it can be assumed that the data are approximately normal distributed due to the large sample size, i.e. the results of ANCOVA can be implemented in the group-sequential design.

A group-sequential design with O´Brien & Fleming alpha spending function allows adjustment of alpha using spending function according to the information fraction at the interim analysis

available, so that the overall significance level alpha of 0.05 is considered for evaluation at the final analysis.

ANCOVA model as derived from SAS procedure GENMOD will be applied for final analysis to derive a maximum likelihood estimate of the mean difference between active and control group and the corresponding standard error of the mean difference. These parameter estimates will be used in SAS procedure SEQTEST. The implementation details of group-sequential design with O´Brien & Fleming alpha spending function are described in paper (Yuan, 2009). ANCOVA model as derived from SAS procedure GLM provides an approximate maximum likelihood estimate of the mean difference between active and control group in case of normal distribution or naïve estimates from both the mean difference between active and control group and the corresponding standard error of the mean difference in case of not normal distribution. In interim and subgroup analyses SAS procedure GLM was used and therefore this procedure will additionally be applied for checking purposes.

In addition, the number of 4-week headache days and change from Baseline will be presented by descriptive statistics.

4.3.2 Analysis of Secondary Performance Variables

The analyses of the secondary performance variables will be performed using the FAS. All statistical tests will be considered exploratory and no adjustment for multiplicity will be made.

Mean change from Baseline in 4-week headache in follow-up period

The mean change from Baseline in 4-week headache days in follow-up period will be similarly analyzed as the primary performance endpoint for the performance assessment period. If the data is normally distributed an ANCOVA will be calculated to test above null hypothesis. If the data is not normally distributed the van Elteren test will be used with medication overuse as stratification to test above null hypothesis.

The analysis for the performance assessment period is covered by the primary analysis.

Mean change from Baseline in 4-week migraine days

The mean change from Baseline in 4-week migraine days will be similarly analyzed as the primary performance endpoint; however, the number of migraine days instead of number of headache days will be counted. If the data is normally distributed an ANCOVA will be calculated to test above null hypothesis. If the data is not normally distributed the van Elteren test will be used with medication overuse as stratification to test above null hypothesis.

Mean change from Baseline in 4-week headache days (mild, moderate and severe intensity) in 4-week performance assessment period (V3 to V7)

The mean change from Baseline in monthly headache days with mild to severe intensity will be similarly analyzed as the primary performance endpoint; however, the number of headache days with mild intensity will be included. If the data is normally distributed an ANCOVA will be calculated to test above null hypothesis. If the data is not normally distributed the van Elteren test will be used with medication overuse as stratification to test above null hypothesis.

Proportion of subjects with 30% or greater reduction in headache days

The proportion of subjects with 30% or greater reduction from baseline period to performance assessment period and to follow-up period will be presented descriptively using frequency tables

summarizing the number and percentage of patients with a headache reduction of 30% or greater.

Additionally, active and placebo treatment arms will be compared using a Chi-square test. In case of less than 5 subjects per group, the Fisher's exact test will be used.

Proportion of subjects with 50% or greater reduction of headache days

The proportion of subjects with 50% or greater reduction from baseline period to performance assessment period and to follow-up period will be presented descriptively using frequency tables summarizing the number and percentage of patients with a headache reduction of 50% or greater.

Additionally, active and placebo treatment arms will be compared using a Chi-square test. In case of less than 5 subjects per group, the Fisher's exact test will be used.

Change in the use of acute (abortive) medication

Information about the use of abortive medication is given in the patient diary. The number of days with abortive medication will be summed up per 4-week period.

The absolute 4-week use of abortive medication and change from Baseline in 4-week use of abortive medication will be presented by descriptive statistics.

Headache impact test-6 (HIT-6)

The total HIT-6 score and change from Baseline will be presented by descriptive statistics. The number and percentage of patients in each category will be presented for each of the 6 items. The total HIT-6 score change from Baseline will be tested using a Wilcoxon rank sum test.

All HIT-6 items must be answered to receive a total HIT-6 score. The total HIT-6 score will be calculated as the weighted sum of answers. The answers are weighted with points as follows:

Answer	Point
Never	6
Rarely	8
Sometime	10
Very often	11
Always	13

Further details of HIT-6 handling are described in user's guide (Bayliss & Batenhorst, 2002).

In tables and listings the HIT-6 items will be labeled as follows:

Question No.	Question Text in CRF	Label for listings and tables
1.	When you have headaches, how often is he pain severe?	Level of severe pain
2.	How often do headaches limit your ability to do usual daily activities including household work, work, school, or social activities?	Activity
3.	When you have a headache, how often do you wish you could lie down?	Tiredness
4.	In the past 4 weeks, how often have you felt too tired to do work or daily activities because of your headaches?	Fatigue
5.	In the past 4 weeks, how often have you felt fed up or irritated because of your headaches?	Psychological distress
6.	In the past 4 weeks, how often did headaches limit your ability to concentrate on work or daily activities?	Cognitive function

Migraine-specific quality of life questionnaire (MSQ)

The 3 dimensions of the MSQ will be presented by descriptive statistics for absolute values and changes from Baseline. The changes from Baseline will be tested using a Wilcoxon rank sum test for each of the 3 dimensions.

The scoring of the MSQ items is completed in 3 steps:

1. Recoding of MSQ items

The MSQ items are worded with a negative perspective, therefore they must be recoded before the domain scores are calculated.

Item Number	Response categories Precoded	Precoded item value	Final item value
1-14	None of the time	1	6
	A little bit of the time	2	5
	Some of the time	3	4
	A good bit of the time	4	3
	Most of the time	5	2
	All of the time	6	1

2. Computation of raw domain scores: the algebraic sum of the final item value for all items in that domain:

Domain	Cluster of Items
Role function - Restrictive	1 - 7
Role function - Preventive	8 – 11
Emotional function	12 - 14

3. Transformation of raw domain scores to a 0 to 100 scale.

Domain	Raw score range	Transformation formula
Role function - Restrictive	7 to 42	(raw score -7)*100/35
Role function - Preventive	4 to 24	(raw score -4)*100/20
Emotional function	3 to 18	(raw score -3)*100/15

Further details of MSQ scaling and scoring as well as handling of missing data are described in manual (Mapi Research Teust, 2017).

Patient global impression of severity (PGI-S)

The score will be presented descriptively using frequency tables. The change from Baseline will be assessed with a shift table of baseline versus post-baseline assessments.

4.4 Safety Analysis

Safety variables will be analyzed based on the SAF.

Adverse events (AEs)

Adverse events will be coded by the Medical Dictionary for Regulatory Activities (MedDRA) version 21.0.

The analysis will be based on treatment emergent AEs (TEAEs), i.e. AEs which started after first treatment. Incidences of TEAEs will be summarized by preferred term (PT) and system organ class (SOC). TEAEs related to the medical device, TEAEs related to procedure, and serious TEAs will be tabulated separately. TEAEs will be summarized also by preferred term (PT), system organ class and intensity. Listings will be generated for TEAEs, serious TEAEs, TEAEs related to procedure or medical device and non-treatment emergent AEs.

4.5 Missing Values

In case of incomplete diary data, the endpoints will be imputed as described in Section 4. In case of incomplete questionnaires data, handling of missing data will be performed as described within corresponding manual. No other missing value imputation methods will be applied.

4.6 Interim Analysis

The interim analysis was carried out and did not lead to stop of the study or to sample size adaption. The study will continue as originally planned.

4.7 Data Base Closure and Data Review

Due to additional unblinded subgroup analysis (the unblinded analysis of the German subjects) two data base closures are foreseen: additional data base closure - a data base closure for additional unblinded subgroup analysis and final data base closure - data base closure for final analysis.

An additional data base closure will be performed prior to the additional unblinded subgroup analysis. All parameters for German subjects only will be checked, as specified in the data validation plan, and all queries resolved before additional data base closure and additional unblinded subgroup analysis.

The blind data review meeting (BDRM) for German subjects only will be conducted prior to additional data base lock based on clean data to verify the number of subjects allocated to the selected analysis sets, needed for additional unblinded subgroup analysis.

These evaluations and assessments will be done together and in agreement with the Sponsor, however FGK will provide the Sponsor with the appropriate excel file including affiliation to the analysis sets. Data review for additional unblinded subgroup analysis will contain data of German subjects only and will be done in writing.

The affiliation of subjects to the SAF and the FAS (German subjects only) will be done prior to unblinding for additional unblinded subgroup analysis. The identification and classification of the protocol deviation observed within the clinical investigation for German subjects as well as affiliation of subjects to the PP will be done during final BDRM.

Data unblinding for German subjects only based on the randomization listing and the additional unblinded subgroup analysis will be done after data review has been conducted and data review minutes have been signed by both the Sponsor and FGK. However, no final unblinding for German subjects will take place for additional unblinded subgroup analysis. Only unblinded FGK team will perform affiliation of subjects to the treatment groups and the additional unblinded subgroup analysis. The results of additional unblinded subgroup analysis will be provided by unblinded FGK team to the unblinded responsible person of Sponsor. The final unblinding of all subjects will be done after final data base closure.

A final data base closure will be performed prior to the final analysis. All parameters will be checked, as specified in the data validation plan, and all queries resolved before final data base closure and final analysis.

The final blind data review meeting (BDRM) will be conducted prior to final data base lock based on clean data to verify the number of subjects allocated to the analysis sets, and to identify and classify the protocol deviation observed within the clinical investigation.

These evaluations and assessments will be done together and in agreement with the Sponsor, however FGK will provide the Sponsor with the appropriate subject listings (as defined in appendix A). Data review can be done via a telephone conference or in writing.

The affiliation of subjects to the SAF, the FAS, and the PP (inclusive German subjects) will be done prior to unblinding.

Data unblinding based on the randomization listing and the final analysis will be done after data review has been conducted and data review minutes have been signed by both the Sponsor and FGK.

The affiliation of subjects to the treatment groups will be done after final unblinding.

4.8 Miscellaneous

For qualitative variables the frequencies (absolute and relative) are calculated. If no further remark is given in the description of the tables following format will be used for all tables with qualitative variables:

	Y-variable(s) (e.g., treatment group)					
	Category 1		Category 2		Total	
X-variable(s)	N	%	N	%	N	%
category 1	xx	xx.x	xx	xx.x	xx	xx.x
category 2	xx	xx.x	xx	xx.x	xx	xx.x
missing	xx	xx.x	xx	xx.x	xx	xx.x
Total	xx	100.0	xx	100.0	xx	100.0

For this standard format the description of the tables in Appendix A determines only the X- and Y-variables. If another format of table is described in the details to the tables, the real design will be determined by the technical possibilities within SAS and may not look identical to the provided example. However, all information as displayed will be included.

Quantitative parameters will be described by declaring the mean value, standard deviation, minimum, first quartile, median, third quartile, and maximum. In the description of the tables this will be denoted by „basic statistics“.

The listings are always sorted by treatment group, center, and subject. If a different sorting order should be used for some listings this will be remarked separately. The variables for the special listings are explicitly given in the description of listings. All listings will be presented for the safety analysis set, if not stated differently, and indicator variables for further analysis sets, e.g., full analysis set, will be added.

Screened but not treated subjects (e.g. withdrawal before treatment) will be considered in tables and listings describing disposition of subjects, analysis sets and discontinuation as well as listings for subject demographics and migraine history.

The following title will be used for all generated tables, listings, and graphs:

PM007 – Final Analysis Page # of #

<Table/Listing/Graph NNN: Description of contents>
<Subtitle for description of contents - if applicable>
<Analysis set>

The numbering NNN of the tables/listings/graphs will be stated in the detailed description (Appendix A).

Following footnote will be used for all generated tables, listings, and graphs:

Date: <Actual date(ddmmmyyyy)> Program: <Name of program>

The statistical evaluation will be performed using SAS version 9.4 or higher.

5 Changes from Protocol

- The wording “monthly headache days” was changed to “4-week headache days” in order to avoid confusion. The analysis is based on 4 week (28 day) assessment windows and not on calendar months (30 days).
- For PGI-S no Wilcoxon test for change from baseline will be performed, since this in a categorical parameter.
- Secondary efficacy outcomes will also be analyzed for the PP set.
- An additional unblinded subgroup analysis (the unblinded analysis of the German subjects) will be performed.
- No Van Elteren test will be used for primary performance endpoint if the data is not normally distributed as no adjustment of alpha is possible according to group-sequential design with O´Brien & Fleming alpha spending function. The ANCOVA will be used even if the normal distribution cannot be shown. Due to the large sample size, it can be assumed that the data are approximately normal distributed.

6 Literature

Bayliss, M. S., & Batenhorst, A. S. (2002). *The HIT-6 TM A User's Guide.* Research Triangle Park, NC: QualityMetric Incorporated, Lincoln, RI and GlaxoSmithKline.

Mapi Research Teust. (2017). *MSQ Migraine Specific Quality of Life Questionnaire Version 2.1 Scaling and Scoring Version 1.0.* Lyon, France.

Yuan, Y. (2009). *Group Sequential Analysis Using the New SEQDESIGN and SEQTEST Procedures.* SAS Institute Inc., Rockville, MD.

7 Signatures

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