Statistical Analysis Plan

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Version Number	Date (DDMMMYYYY)	Summary of Changes, including rationale for changes
Original (v1.0)	01OCT2020	Original version
Amendment 1 (v1.1)	24MAR2023	• Update section 3.2 sample size according to protocol amendment Sample size updated to 1400. • Update section 4.1: update model adjusted variables • Update 5.1 Definition of terms: add definition of Migraine classification • Added section 5.4.3 study period • Updated definition of exposed to investigational product in section 5.5 • Added Dose switch definition in section 5.5 • Added Duration of IP exposure definition in section 5.6 • Added description of imputation rules for missing IP administration dates of the last IP dose in section 8.3 • Updated description of 9.5.1 • Added ANCOVA methods description in section 9.5.2 • Added adverse events of interest in section 9.6.1 • Added section 10 changes from protocol-specified analysis. • Added section 13 changes for data not covered in this plan.
Amendment 1(v1.2)	20Apr2023	• Update Migraine Pain Intensity to Peak Migraine Pain Intensity • Update Monthly Average Migraine Pain Intensity to Monthly Average Peak Migraine Pain Intensity

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List of Abbreviations and Definition of Terms

Abbreviation or Term	Definition/Explanation
AE	Adverse event
AHMD	acute headache medication days
AMSMD	acute migraine-specific medication days
ANCOVA	**Analysis of covariance**
CGRP	calcitonin gene-related peptide
CM	chronic migraine
CTCAE	Common Terminology Criteria for Adverse Events
DARS	**Data Acquisition Requirements Specification**
DMP	**Data Management Plan**
DXDT	diagnosis date
EAS	efficacy analysis set
eCRF	electronic case report form
eDiary	electronic diary
EM	episodic migraine
EOS	End of study
FAS	full analysis set
GCP	Good Clinical Practice
GBS	Global Biostatistical Science
GSO-DM	Global Study Operations – Data Management
IP	Investigational Product
IPD	**important protocol deviations**
LSM	**Least squares means**
MAF	minor allele frequency
MedDRA	Medical Dictionary for Regulatory Activities
MFIQ	Migraine Functional Impact Questionnaire
MMD	monthly migraine day
mPRS	migraine polygenic risk score
NCT	National Clinical Trials
OLTP	Open-label Treatment Period
PRS	polygenic risk score
Q4W	Every 4 weeks
SAP	statistical analysis plan
SAE	serious adverse events
SAS	safety analysis set
SC	Subcutaneous
SNP	single-nucleotide polymorphism
TEAE	treatment-emergent adverse event

1. Introduction

The purpose of this Statistical Analysis Plan (SAP) is to provide details of the statistical analyses that have been outlined within the **Protocol Amendment 2** for Study 20190006, Erenumab dated **23 February 2022**. The scope of this plan, includes the portion of the final analysis which will be executed by the Amgen Global Biostatistical Science department. The remaining final analysis will be executed by deCODE as specified in the deCODE Analysis Plan (Appendix B).

2. Objectives, Endpoints and Hypotheses

2.1 Objectives and Endpoints

Objectives	Endpoints
Primary	
• To explore the relationship between clinical response to erenumab and genetic biomarkers	• Achievement of at least a 50% reduction from baseline in mean monthly migraine days (MMD) over months 4, 5, and 6 in relation to each individual genome-wide significant single-nucleotide polymorphism (SNP) contributing to the migraine polygenic risk score (mPRS)

Objectives	Endpoints
Exploratory	
• To describe the relationship between monthly AMSM use and mPRS in erenumab-treated subjects	• Change from baseline in monthly AMSMD over months 4, 5, and 6, in relation to mPRS
• To describe the relationship between baseline calcitonin gene-related peptide (CGRP) levels and clinical response with erenumab treatment as assessed by MMD in erenumab-treated subjects	• Change from baseline in MMD over months 4, 5, and 6 in relation to baseline CGRP level • Achievement of at least a 50% reduction from baseline in mean MMD over months 4, 5, and 6 in relation to baseline CGRP level • Achievement of at least a 75% reduction from baseline in mean MMD over months 4, 5, and 6, in relation to baseline CGRP level
• To describe the relationship between baseline CGRP levels and clinical response with erenumab treatment as assessed by monthly acute migraine-specific medication days (AMSMD) in erenumab-treated subjects	• Change from baseline in monthly AMSMD over months 4, 5, and 6, in relation to baseline CGRP level

Objectives	Endpoints
Exploratory (Continued)	
• To describe the relationship between mPRS and baseline CGRP	• mPRS in relation to baseline CGRP level
• To describe the relationship between baseline CGRP levels and migraine disease characteristics	• Baseline CGRP level in relation to baseline MMD • Baseline CGRP level in relation to baseline AMSMD • Baseline CGRP level in relation to baseline AHMD • Baseline CGRP level in relation to each MFIQ individual domain score (physical function, usual activities, social function, and emotional function) and overall impact usual activities global item score at baseline • Baseline CGRP in relation to presence of aura during baseline • Baseline CGRP in relation to baseline mean migraine severity
• To describe the relationship between mPRS and migraine disease characteristics	• mPRS in relation to baseline MMD • mPRS in relation to baseline AMSMD • mPRS in relation to baseline acute headache medication days (AHMD) • mPRS in relation to Migraine Functional Impact Questionnaire (MFIQ) individual domain scores (physical function, usual activities, social function, and emotional function) and overall impact usual activities global item score at baseline • mPRS in relation to presence of aura during baseline • mPRS in relation to baseline mean migraine severity

Objectives	Endpoints
Exploratory (Continued)	
• To evaluate the effect of erenumab as assessed by change in MMD in erenumab-treated subjects	• Change from baseline in mean MMD over months 4, 5, and 6 • Change from baseline in MMD at assessment timepoints • Achievement of at least a 50% reduction from baseline in mean MMD over months 4, 5, and 6 • Achievement of at least a 50% reduction from baseline in MMD at assessment timepoints • Achievement of at least a 75% reduction from baseline in mean MMD over months 4, 5, and 6 • Achievement of at least a 75% reduction from baseline in mean MMD at assessment timepoints
• To evaluate the effect of erenumab as assessed by change in monthly AMSMD in erenumab-treated subjects	• Change from baseline in mean monthly AMSMD over months 4, 5, and 6 • Change from baseline in monthly AMSMD at assessment timepoints
• To evaluate the effect of erenumab as assessed by change in monthly AHMD in erenumab-treated subjects	• Change from baseline in mean monthly AHMD over months 4, 5, and 6 • Change from baseline in monthly AHMD at assessment timepoints
• To evaluate the effect of erenumab on functional impact as assessed by the change from baseline MFIQ score in erenumab-treated subjects	• Change from baseline in MFIQ individual domain scores (physical function, usual activities, social function, and emotional function) and overall impact usual activities global item score at month 6
• To describe the relationship between change from baseline in MFIQ score and mPRS in erenumab-treated subjects	• Change from baseline in MFIQ individual domain scores (physical function, usual activities, social function and emotional function) and overall impact usual activities global item score at month 6, in relation to mPRS

2.2 Hypotheses and/or Estimations

No formal hypothesis testing will be performed. All analyses will be descriptive in nature.

3. Study Overview

3.1 Study Design

This is a phase 4 open-label study that will enroll subjects with episodic migraine (EM) or chronic migraine (CM) without a predetermined allocation ratio. The study will comprise the following periods:

- A screening period of up to 3 weeks (21 days) for the assessment eligibility criteria. The screening visit may be performed on the same day as the baseline visit
- A baseline period of 4 weeks (28 up to 35 days) to collect data on migraine headaches and acute headache medication use using electronic diary (eDiary) and to assess compliance in eDiary usage **at day 1 visit**
- A 24-week open-label treatment period

Subjects will be screened at the screening visit, and **if eligible, subjects may enter the baseline period at the same visit as screening or may begin baseline procedures up to 3 weeks after the screening visit**. At completion of the baseline period, those who **meet the eligibility criteria and** demonstrate ≥ 75% compliance with eDiary use will be enrolled and will enter the open-label treatment period to receive erenumab either 70 mg or 140 mg subcutaneously (SC) every 4 weeks (Q4W) at the discretion of the investigator.

3.2 Sample Size

The primary objective is the identification of genome-wide significant SNPs contributing to the mPRS that are significantly associated with clinical response to erenumab treatment (based on achievement of at least a 50% reduction from baseline in mean MMD over months 4, 5, and 6). Based on erenumab pivotal EM and CM studies, the ≥ 50% response rate with respect to MMD reduction is expected to be approximately 50% after 24 weeks of erenumab treatment. It is hypothesized that the mPRS SNPs with genome-wide significance ($p < 5 \times 10^{-8}$) have an additive effect on clinical response, ie, for each of these mPRS SNPs, each additional copy of the allele (with 0 as the reference) is associated with increased clinical response and will result in an odds ratio of 1.8 or higher (allelic odds ratio**). Given the currently known 123 migraine** SNPs with genome-wide significance and a minor allele frequency (MAF) of at least 0.1, **a** study of **1400** subjects with 10% dropouts (resulting an effective sample size of **1260** at week 24) is at least 90% powered to identify **five of the** genome-wide significant **migraine** SNPs that are statistically significantly associated with clinical response. **This is based on a two-sided power analysis, with Bonferroni adjustment, bootstrapping the data from the pivotal EM Study 20120296 (Mikol et al, 2020), using a logistic regression model to predict clinical response with each SNP, and adjusting for sex, age, BMI and principal components.**

4. Covariates and Subgroups

4.1 Planned Covariates

All models will be adjusted for age and sex. In addition, all model-adjusted analyses of efficacy endpoints **assessing change from baseline** will include the corresponding baseline value for the endpoint being analyzed. **All models that compare baseline measurements only will be adjusted for country. However, country will not be included as a covariate in any deCODE performed analyses since all deCODE analyses will be performed separately by country (Denmark/Iceland) and subsequently combined in a meta-analysis. Due to the high correlation between baseline dose and country, models assessing change from baseline will instead be adjusted for baseline dose (70mg/140mg) and dose switch only.**

Principal components of genotype data will be adjusted for population stratification **in mPRS related analysis**. Calculation of principal components data will be described in the analysis plan developed by deCODE.

4.2 Subgroups

The primary endpoint will be summarized in the subgroups defined by the following:

- sex (female/male)
- **presence of aura during baseline period** (yes/no)
- menstrual-related migraine **at screening** (yes/no)
- **country** (Denmark/Iceland)
- migraine classification **at screening** (EM/CM)
- prior migraine **preventive** treatment failure (yes/no)

The subgroups will be re-examined for appropriateness and may be re-categorized (due to small sample size, for example, if there are < 10% of subjects within a subgroup).

5. Definitions

5.1 Definition of Terms Included in Study Endpoints

5.1.1 Efficacy Endpoints Based on Daily Data Collection

Subjects' daily headache information will be collected through the following questions. The most severe case will be used in the analysis when there are duplicates in the following daily questions. Examples of possible outcomes on migraine-related endpoints are summarized in Table 1:

Table 1. Examples of possible outcomes on migraine-related endpoints.

	Scenario 1	Scenario 2	Scenario 3	Scenario 4	Scenario 5
Daily eDiary questions					
Migraine (Y/N)	Y	Y	Y	N	N
If Y, **Peak** pain intensity (1 to 10)	1	10	7	NA	NA
Acute medication for migraine (Y/N)	Y	Y	N	Y	Y
If Y, Triptan (Y/N)	Y	N	NA	Y	N
Aura (Y/N)	**Y**	**N**	**Y**	**Y**	**N**
Outcomes on migraine-related endpoints					
Migraine day	Yes	Yes	Yes	**No**	No
Acute headache medication day	Yes	Yes	No	Yes	Yes
Acute migraine-specific medication day	Yes	No	No	Yes	No

Migraine Day

A calendar day (00:00 to 23:59) in which the subject reports any migraine headache or takes any triptan-based acute migraine-specific medication.

Peak Migraine Pain Intensity

Worst or peak pain intensity collected on a migraine headache **which** ranges from 1 to 10 with a higher score indicating more severe pain **(10-point numerical rating scale).**

Acute Headache Medication Day

A calendar day (00:00 to 23:59) in which the subject takes any acute headache medication (AHM)

Acute Migraine-specific Medication Day

A calendar day (00:00 to 23:59) in which the subject takes triptan

Diary Day

A calendar day (00:00 to 23:59) with complete migraine headache, aura, and acute migraine headache medication data recorded in the eDiary device.

Migraine Classification

In subgroup analysis, we will define EM as subjects with < 15 headache days per month at baseline and CM as subjects with ≥ 15 headache days per month at baseline to ensure all subjects belong to either subgroup.

Monthly Frequency Variable in Days

Number of days of interest during **each** monthly interval as defined in Table 2. Monthly frequency variables include

- Monthly migraine days (MMD)
- Monthly acute migraine-specific medication days
- Monthly acute headache medication days

The following proration rule will be applied to all monthly frequency variables for each monthly interval.

Table 2. Calculation for monthly frequency variables within each monthly interval

Within Each Monthly Interval	Monthly Frequency Variable
≥ 14 diary days	Prorate to 28-day equivalent without rounding $\frac{Number\ of\ frequency\ days\ within\ each\ monthly\ interval}{Number\ of\ \boldsymbol{diary}\ days\ within\ each\ monthly\ interval}\ x\ 28$
< 14 diary days	Set to missing

Monthly Average Peak Migraine Pain Intensity

Peak pain intensity will be collected for **days with migraine headache reported**. Monthly average **peak** migraine pain intensity is defined as the sum of the **reported peak** pain intensity **scores** during a monthly interval as defined in Table 3 divided by the total number of **reported peak pain intensity scores**. If there is no migraine headache observed during the monthly interval, then the monthly average **peak** migraine pain intensity will be set to 0. If less than 14 days of eDiary data in each monthly interval are recorded, then the monthly average **peak** migraine pain intensity will be set to missing.

5.1.2 Efficacy Endpoints Based on Monthly Collection

Migraine Functional Impact Questionnaire (MFIQ)

The MFIQ version 2.0 is a self-administered 26-item instrument measuring the impact of migraine on broader functioning including 4 domains: Impact on Physical Functioning (5 items), Impact on Usual Activities (10 items), Impact on Social Functioning (5 items), and Impact on Emotional Functioning (5 items). In addition, there is 1 stand-alone global item assessing the overall impact on usual activities. Subjects respond to items using a 5-point scale assigned scores from 1 to 5, with 5 representing the greatest burden. Each domain score will be calculated as the sum of the item responses, and the sum will be rescaled to a 0 to 100 scale, with higher scores representing greater burden. The recall period is the past 7 days.

5.1.3 Safety Endpoints

Treatment-emergent Adverse Event (TEAE)

An event categorized as “Adverse Event” on the Events eCRF starting on or after first dose of investigational product, as determined by “Did event start before first dose of investigational product” equal to “No” or missing, and up to the End of Study date.

Serious Treatment-emergent Adverse Event

A treatment-emergent adverse event with the indicator flag “Serious” equal to “Yes” on the Events eCRF.

Treatment-emergent Adverse Device Effect

A treatment-emergent adverse event with the indicator flag “Is there a reasonable possibility that the event may have been caused by the investigational device” equal to “Yes” on the Events eCRF.

5.2 Study Dates

Informed Consent Date

The date on which subject signs the informed consent form.

eDiary Device Assignment Date

The date on which an eDiary device is assigned to a subject for the first time after completion of initial screening.

First Dose Date

First Dose Date is the date on which a subject is administered the first dose of IP following completion of the Baseline period as recorded on the IP Administration eCRF.

Last Dose Date

Last Dose Date is the date on which a subject is administered the last dose of IP during the study, where receiving IP is defined as above.

End of Study (EOS) Date

End of study (EOS) date is defined as the last date on which the subject participates in the study as recorded on the End of Study eCRF.

5.3 Study Points of Reference

Baseline Assessment

Baseline assessment for the endpoint of the interest is defined as the last non-missing measurement taken or the monthly interval assessed (for endpoints derived from daily eDiary collection) before the first dose of investigational product. In cases where baseline measurements are taken on the same day as IP, it will be assumed that these measurements are taken prior to IP being administered.

Study Day 1

Study Day 1 is defined as the first IP dose date.

Study Day

Study Day is defined as the number of days from Study Day 1.

Before Study Day 1:

Study Day = (Date of Interest – Date of Study Day 1)

On or after Study Day 1:

Study Day = (Date of Interest – Date of Study Day 1) + 1

Therefore, the study day prior to Study Day 1 is -1.

5.4 Study Time Intervals

5.4.1 Monthly Intervals for Efficacy Endpoints Derived From Daily Diary Collection

The (4-week) monthly intervals for efficacy endpoints derived from daily eDiary collection will be determined based on each subject's monthly IP dosing dates. When an IP is

missed, discontinued, or no longer required, a 28-day monthly interval will be used. Any eDiary data occurring after EOS date will not be included in the analysis.

Applicable efficacy endpoints utilizing the monthly intervals in **Table 3** include:

- Monthly migraine days (MMD)
- Monthly acute migraine-specific medication days
- Monthly acute headache medication days
- Monthly average migraine pain severity

Table 3. Monthly Intervals for Efficacy Endpoints Derived From Daily Data Collection

Study Period	Assessment Timepoint	Monthly Interval	
		Start Date (Day) [a]	**End Date (Day)** [b]
Baseline Period	Baseline	Device **assignment** date	Day prior to study day 1
Open-label Treatment Period	Week 4 (Month 1)	Study Day 1	Week 4 dose date – 1
	Week 8 (Month 2)	Week 4 dose date	Week 8 dose date – 1
	Week 12 (Month 3)	Week 8 dose date	Week 12 dose date – 1
	Week 16 (Month 4)	Week 12 dose date	Week 16 dose date – 1
	Week 20 (Month 5)	Week 16 dose date	Week 20 dose date – 1
	Week 24 (Month 6)	Week 20 dose date	Week 20 dose date + 27

[a] Start Date (Day) = End date (day) of previous monthly interval + 1 if IP dose is not **received**
[b] End Date (Day) = Start date (day) of current monthly interval + 27 if IP dose is not **received**

5.4.2 Analysis Visits for Endpoints Based on Monthly Collection

Since the actual visit for a subject may not exactly coincide with their targeted visit date, the actual visit date is mapped to the analysis visit as in **Table 4**. Any data occurred after EOS date will not be included in the analysis.

For by-visit summaries, if more than one visit with non-missing measurement (including the unscheduled visits, ie, CPEVENT = 'UNSCHED') fall within the same visit window, the following rules will be applied according to the order described below for selecting one visit per visit window for summary:

1. Scheduled visit will be used regardless of the distance from the target day. Unscheduled visit will only be used when there is no measurement from scheduled visit in the visit window.
2. The visit closest to the target day among the same type of visits (scheduled vs. unscheduled) will be considered for analysis.
3. If two assessment dates are equidistant from the target date, the latter visit will be considered for analysis.

Table 4. MFIQ Analysis Visit Windows

Study Period	Analysis Visit	Target Day	Visit Window (Study Day)
Baseline Period	Day 1 (Baseline)	1	Last measurement ≤ Day 1
Open-label Treatment Period	Week 24 (Month 6)	169	156 to 183

5.4.3 Study Period

Baseline period is defined in Table 3 and Table 4 respectively. The efficacy and safety analyses will be summarized for the OLTP. The analysis window will be defined with the starting time point given by study day 1 after the 1st IP dose and end time point given by EOS date.

5.5 Subject Disposition

Exposed to Investigational Product

Subjects are defined as exposed if they receive at least one dose of investigational product. **Subjects will be defined as having received IP if the observed data indicates they have received IP (IP date is non-missing) on a specific date, even if the IP quantity field is missing (IPQTY = NULL).**

Completing the Investigational Product

Subjects are defined as completing investigational product if **they complete IP at week 20. It will be derived from the End of IP eCRF with "Completed" as** the primary reason for ending IP.

Completing Study

Subjects are defined as completing study if they complete the entire 24 weeks of study evaluation**.** It will be derived from the End of Study eCRF with "Completed" as the primary reason for ending study.

On-study

Subjects are considered on-study if they **are enrolled and** have not yet completed their EOS visit.

Dose Switch

Per protocol, dose switch is permitted at the discretion of the investigator at week 12. However, subjects could switch dose before or after week 12 if needed (e.g., adverse event). We define dose switch as an indicator variable indicating is a subject's dosage changed from their baseline dose at any time during the study.

5.6 Arithmetic Calculations

Migraine Disease Duration

The number of years from the diagnosis date (DXDT) of migraine (migraine with aura or migraine without aura, whichever is earlier) to the date informed consent is signed.

Observed Portion	Missing Portion	Duration of Migraine (Years)
Year, Month, Day	NA	(Informed Consent Date – DXDT) / 365.25
Year, Month	Day	[Year(Informed Consent Date) – Year(DXDT)] + [Month(Informed Consent Date) – Month(DXDT)] / 12 [a]
Year	Month, Day	[Year(Informed Consent Date) – Year(DXDT)] [a]

[a] If it equals 0, add 1/12 years (ie, 1 month) to avoid a disease duration of 0.

Age at Onset of Migraine

Age at onset of migraine is defined as Age – duration of migraine.

Duration of IP Exposure

If subject switches dose,

- **Duration before the First Switch = Minimum (Last Initial Dose Date + 27, First Switch Dose Date – 1) – First Dose Date + 1**
- **Duration on or after the First Switch = Minimum (Last Dose Date + 27, EOS Date) – First Switch Dose Date + 1**

Otherwise,

- **Duration = Minimum (Last Dose Date + 27, EOS Date) – First Dose Date + 1**

Change From Baseline

Post-baseline monthly value – Baseline, as defined in Section 5.4.

If the baseline or post-baseline value is missing, the change from baseline value will be set to missing.

Percent Change From Baseline

The change from baseline divided by baseline and multiplied by 100:

(Post-baseline – Baseline) * 100 / Baseline

If the baseline value is 0 and the post-baseline value is also 0, the percent change from baseline is set to 0. If the baseline value is 0 and the post-baseline value is non-zero, **then** the percent change from baseline is set to missing.

Mean Monthly Change From Baseline Over Multiple Months

Arithmetic Mean monthly change from baseline is the arithmetic mean of the observed monthly change from baseline values for the months with non-missing change from baseline values.

Subject Incidence

The subject incidence for a given event in a given period is defined as the number of subjects with at least one reported occurrence of the event divided by the number of subjects who enter that period (ie, number of at-risk subjects). For subjects with multiple occurrences of the same event, the event will only be counted once per subject.

5.7 Disease Characteristics

Treatment Failure of Prior Migraine Preventive Medications

Treatment failure of prior migraine preventive medications is determined by "Reason for Stopping" as "Lack of efficacy" or "Adverse reaction" on the Prior Migraine Prophylactic Medication eCRF.

Menstrual-related migraine

If a female patient responds yes to the question "Does the subject report migraine attacks that occurred on day 1 +/- 2 of menstruation (ie Days -2 to +3, day 1 = start of menstruation) in at least two out of the last three menstrual cycles prior to screening?" in the Headache and Migraine Frequency History eCRF then the patient will be considered in the subgroup menstrual-related migraine.

6. Analysis Sets

6.1 Full Analysis Set

The full analysis set (FAS) consists of all subjects who enroll in the study. Analysis of disposition, demographic and baseline characteristics, and important protocol deviations (IPD) will utilize the FAS.

6.2 Safety Analysis Set

The safety analysis set (SAS) will consist of a subset of subjects in FAS who receive at least 1 dose of investigational product. Analysis for safety endpoints and summary of investigational product administration will utilize the SAS.

6.3 Study-specific Analysis Sets

6.3.1 Observed Data Analysis Set

The observed data analysis set consists of a subset of subjects in FAS who receive at least 1 dose of investigational product and have an observed value for the endpoint of interest and pass the quality control procedures of genetic analysis. Primary analysis of

the efficacy endpoints related to the genetic biomarkers will utilize the observed data analysis set. Details of this analysis set is mentioned in the analysis plan developed by deCODE.

6.3.2 Efficacy Analysis Set

The efficacy analysis set (EAS) consists of a subset of subjects in FAS who receive at least 1 dose of investigational product. Primary analysis of the exploratory efficacy endpoints will utilize **observed data for subjects in** the EAS.

7. Planned Analyses

7.1 Interim Analysis and Early Stopping Guidelines

Not applicable.

7.2 Primary Analysis

The primary analysis (or final analysis, as this study has only 1 milestone analysis) will be performed after all subjects have complete**d** their week-24 or end-of-study visit. The data will be cleaned, and the database will be locked for the primary analysis.

8. Data Screening and Acceptance

8.1 General Principles

The objective of the data screening is to assess the quantity, quality, and statistical characteristics of the data relative to the requirements of the planned analyses.

8.2 Data Handling and Electronic Transfer of Data

The Amgen Global Study Operations-Data Management (GSO-DM) department will provide RAVE database, **data outside of RAVE database** (eDiary data and biomarkers). The database will be subjected to edit check outlined in the Data Management Plan (DMP). Additional details **for data outside of RAVE** will be provided in the DMP and Data Acquisition Requirements Specification (DARS).

8.3 Handling of Missing and Incomplete Data

Subjects may miss specific data points for a variety of reasons. In general, data could be missing because of a subject's early withdrawal from the study, a missed visit, or inability to evaluate an endpoint at a time point. For this study, efficacy endpoints will be collected via eDiary and subjects could miss entering several days of data in each monthly interval. The general procedures outlined below describe how missing data will be handled.

For the eDiary data, if at least 14 days of eDiary are collected in the monthly interval, then the monthly frequency measurements (eg, migraine days or acute migraine-specific

medications days [AMSMD]) will be prorated based on the number of days with available

The analysis of **all efficacy and safety** endpoints will be performed **by using observed data only**. Missing **efficacy** assessments (eg, **MMD**, MFIQ etc.) will not be imputed.

Missing safety endpoints will not be imputed. Missing or incomplete dates will be listed as reported, except for incomplete start date of an adverse event or concomitant medication (Table 5).

Table 5 Imputation for Incomplete Start Date of an Adverse Event or Concomitant Medication

Missing	Imputation	Exception on Adverse Event Start Date
Day	01	Default to study day 1 if an adverse event started the same year and month as study day 1 and the flag indicates that the adverse event started on or after the first dose of investigational product on the Events eCRF
Day/Month	01 Jan	Default to study day 1 if an adverse event started the same year as study day 1 and the flag indicates that the adverse event started on or after the first dose of investigational product on the Events eCRF
Day/Month/Year	None	—

eCRF = electronic case report form

Imputation Rules for Missing IP administration Dates of the last IP dose

If the date of last exposure treatment weeks is partial or entirely missing but receiving IP is confirmed, impute the date by the study Day 1 + last exposure treatment weeks * 7 days. Ensure the imputed last exposure to treatment date is on or before the end of study date.

Imputation of missing genotype data

The details for the imputation of genotype data will be described in the deCODE Analysis Plan (Appendix B).

8.4 Detection of Bias

The factors that may bias the results of the study include:

- important protocol deviations likely to impact the analysis and interpretation of the efficacy endpoints
- investigational product dosing non-compliance
- the timing of and reasons for early withdrawal from treatment and from study

The incidence of these factors may be assessed. Important protocol deviations will be tabulated **and/or** listed.

The reasons for early withdrawal from treatment and from study will be tabulated and/or listed.

8.5 Outliers

Histograms will be examined to identify outliers in any of the continuous variables used in the analyses. Unexpected and/or unexplained values in categorical data will be identified by utilizing frequency tables.

Outliers due to data entry errors will be corrected by the study team before final database lock. The validity of any questionable values or outliers will be confirmed. Outliers or any questionable values with confirmed validity will be included in the analyses presented in this statistical analysis plan unless there is sufficient justification to exclude them. However, ad-hoc sensitivity analyses may be conducted to evaluate the influence of extreme values in the data.

8.6 Distributional Characteristics

Continuous endpoints of change from baseline value will be analyzed under normality assumption. **Transformations may be used if necessary. For example, baseline CGRP levels will be log-transformed before analysis if high skewness is present.**

8.7 Validation of Statistical Analyses

Programs will be developed and maintained, and output will be verified in accordance with current risk-based quality control procedures.

Tables, figures, and listings will be produced with validated standard macro programs where standard macros can produce the specified outputs.

The production environment for statistical analyses consists of Amgen-supported versions of statistical analysis software; for example, the SAS System version 9.4 or later.

9. Statistical Methods of Analysis

9.1 General Considerations

Summary statistics will be tabulated at each assessment time point, as applicable. For continuous endpoints, the descriptive statistics include the following: number of observations, means, medians, standard deviations, standard errors, first and third quartiles, minimums and maximums, and 2-sided 95% CIs of the means (CIs will be provided for efficacy endpoints only). For categorical endpoints, the summaries will contain the number and percentage of subjects in each category.

The primary (or final) analysis for the study will be performed on a locked database after all subjects have completed the study or discontinued from the study.

Weighted Bonferroni adjustment will be used to maintain a family-wise error rate of 0.05 (2-sided) for primary endpoint. Analyses involving endpoints related to genetic features will be performed by deCODE.

Summary of efficacy endpoints by treatment group will be based on treatment received on study day 1. Dose **switching** between 70 mg and 140 mg erenumab are permitted at the week 12 visit per investigator's discretion.

9.2 Subject Accountability

The disposition of all enrolled subjects will be tabulated by treatment dose group **received on study day 1**. The summary will include the number of subjects who are enrolled, the number and percent of subjects who received/never received the IP, who completed IP, discontinued IP and reasons for discontinuing, who completed the **study**, and who withdrew prematurely from the study before completion of the 24-week treatment period and their reasons for withdrawal.

Key study dates for the first subject enrolled, last subject enrolled, last subject's end of investigational product, last subject's end of study will be presented.

The number and percent of subjects enrolled will be tabulated by study site.

9.3 Important Protocol Deviations

Important Protocol Deviations (IPDs) categories are defined by the study team before the first subject's initial visit and updated during the IPD reviews throughout the study prior to database lock. These definitions of IPD categories, subcategory codes, and descriptions will be used during the course of the study. Eligibility deviations are defined in the protocol.

9.4 Demographic and Baseline Characteristics

Subject demographic and baseline characteristics will be summarized using descriptive statistics by country and overall study population using FAS. If multiple races have been reported for a subject, the subject will be categorized as multiple races.

The following demographic and baseline characteristics will be summarized:

- Age
- Sex
- Ethnicity
- Race
- Targeted neurological disease diagnosis at baseline
- Disease duration of migraine
- Age at onset of migraine
- Monthly migraine days during baseline period
- Acute headache medication days (AHMD) during baseline
- **Acute headache medication used during baseline**
- MFIQ domain scores of function (physical, usual activities, social, and emotional) and overall impact on usual activities global item score at baseline
- **Female subjects of child bearing potential and those with menstrual related migraine (yes/no)**
- Plasma CGRP levels during baseline
- Migraine **classification at screening** (EM/CM)
- Aura (yes/no) **during baseline**
- Prior migraine **preventive** treatment use
- **Triptan medication used during baseline**
- **Triptan medication days during baseline**

9.5 Efficacy Analyses

Detailed analysis methods and covariates included in the models are summarized in the table below.

Table 6. Analysis of Primary Efficacy Endpoint to be Performed by deCODE

Endpoint	Primary Summary and Analysis Method
Achievement of at least a 50% reduction from baseline in mean monthly migraine days (MMD) over months 4, 5, and 6 in relation to each individual genome-wide significant single-nucleotide polymorphism (SNP) contributing to	This analysis is planned to be performed by deCODE and details of the analysis are described in deCODE Analysis Plan (Appendix B).

Endpoint	Primary Summary and Analysis Method
the migraine polygenic risk score (mPRS)	

Table 7. Analysis of Exploratory Efficacy Endpoint to be Performed by deCODE

Endpoint	Primary Summary and Analysis Method
Change from baseline in monthly AMSMD over months 4, 5, and 6, in relation to mPRS	This analysis is planned to be performed by deCODE and details of the analysis are described in deCODE Analysis Plan (Appendix B).
mPRS in relation to baseline CGRP level	
mPRS in relation to baseline MMD	
mPRS in relation to baseline AMSMD	
mPRS in relation to baseline acute headache medication days (AHMD)	
mPRS in relation to Migraine Functional Impact Questionnaire (MFIQ) individual domain scores (physical function, usual activities, social function, and emotional function) and overall impact usual activities global item score at baseline	
mPRS in relation to presence of aura during baseline	
mPRS in relation to baseline mean migraine severity	
Change from baseline in MFIQ individual domain scores (physical function, usual activities, social function and emotional function) and overall impact usual activities global item score at month 6, in relation to mPRS	
Published migraine SNPs in relation to baseline CGRP	

Table 8. Analysis of Exploratory Efficacy Endpoints to be Performed by Amgen

Endpoint	Primary Summary and Analysis Method
Change from baseline in MMD over months 4, 5, and 6 in relation to baseline CGRP level	1. Summary statistics by visit using observed data at each visit, and the calculated mean monthly migraine days over month 4, 5 and 6. 2. Estimated slope for baseline CGRP, associated 95% CI and nominal p-value from a linear

Endpoint	Primary Summary and Analysis Method
	regression model including baseline CGRP, age, sex, **dose switch (yes/no), baseline dose (full sample analysis only),** and baseline MMD value as covariate using observed data. **Dose switch was excluded for 140 mg treatment group.** **3. Spearman correlation between baseline non-transformed CGRP and Change from baseline in MMD over months 4, 5, and 6.** **4. A scatterplot with loess will developed for CGRP level with change from baseline in monthly MMD over months 4, 5, and 6.**
Achievement of at least a 50% reduction from baseline in mean MMD over months 4, 5, and 6 in relation to baseline CGRP level	1. Summary statistics based on **observed data**. 2. Odds ratio estimate for baseline CGRP, associated 95% CI, and nominal p-value from a logistic regression model with baseline CGRP level as covariate along with baseline MMD, **dose switch (yes/no), baseline dose (full sample analysis only),** age and sex. **Dose switch was excluded for 140 mg treatment group.** 3. Predicted probabilities of achieving at least a 50% reduction in MMD
Achievement of at least a 75% reduction from baseline in mean MMD over months 4, 5, and 6, in relation to baseline CGRP level	Same as above.
Change from baseline in monthly AMSMD over months 4, 5, and 6, in relation to baseline CGRP level	1. Estimated slope for baseline CGRP, associated 95% CI and nominal p-value from a linear **regression** model including baseline CGRP, age, sex, **dose switch (yes/no), baseline dose (full sample analysis only),** and baseline AMSMD value as covariate using observed data. **Dose switch was excluded for 140 mg treatment group.** 2. Spearman correlation analysis will be applied between baseline CGRP and change from baseline in monthly AMSMD over months 4, 5, and 6. 3. **A scatterplot with loess will developed for CGRP level with change from baseline in monthly AMSMD over months 4, 5, and 6.**
Baseline MMD in relation to baseline CGRP level	1. Summary statistics using observed data. 2. Estimated slope for CGRP, associated 95% CI and nominal p-value from a linear regression model including age, sex, **country (full sample analysis only),** and baseline CGRP as covariate using observed data. 3. **Spearman correlation between baseline CGRP and baseline MMD.**

Endpoint	Primary Summary and Analysis Method
Baseline AMSMD in relation to baseline CGRP level	1. Summary statistics using observed data. 2. Estimated slope for CGRP, associated 95% CI and nominal p-value from a linear regression model including age, sex, **country (full sample analysis only),** and baseline CGRP value as covariate using observed data. 3. **Spearman correlation between baseline CGRP and baseline AMSMD.**
Baseline AHMD in relation to baseline CGRP level	1. Summary statistics using observed data. 2. Estimated slope for CGRP, associated 95% CI and nominal p-value from a linear regression model including age, sex, **country (full sample analysis only),** and baseline CGRP value as covariate using observed data. 3. **Spearman correlation between baseline CGRP and baseline AHMD.**
Baseline mean migraine severity in relation to baseline CGRP level	1. Summary statistics using observed data. 2. Estimated slope for baseline CGRP, associated 95% CI and nominal p-value from a linear regression model including age, sex**, country (full sample analysis only),** and baseline CGRP as covariate using observed data. 3. **Spearman correlation between baseline CGRP and baseline mean migraine severity.**
Presence of aura during baseline in relation to baseline CGRP level	1. Summary statistics using observed data. Estimated **odds ratio** for baseline CGRP, associated 95% CI and nominal p-value from a logistic regression model including age, sex**, country (full sample analysis only),** and baseline CGRP using observed data.
Each MFIQ individual domain score (physical function, usual activities, social function, and emotional function) and overall impact usual activities global item score at baseline in relation to baseline CGRP level	1. Summary statistics using observed data. 2. Estimated slope for baseline CGRP, associated 95% CI and nominal p-value from a linear regression model including age, sex**, country (full sample analysis only),** and baseline CGRP as covariate using observed data. 3. **Spearman correlation between baseline CGRP and baseline MFIQ score.**
Change from baseline in MFIQ individual domain scores (physical function, usual activities, social function, and emotional function) and overall impact usual activities global item score at month 6	1. Summary statistics using observed data. 2. Estimated least squares means and associated 95% CI from **an ANCOVA** model including age, sex, dose switch (yes/no), **baseline dose,** and baseline MFIQ score as covariate using observed data.

Endpoint	Primary Summary and Analysis Method
Change from baseline in mean MMD over months 4, 5, and 6 Change from baseline in MMD at assessment timepoints Change from baseline in mean monthly AMSMD over months 4, 5, and 6 Change from baseline in monthly AMSMD at assessment timepoints Change from baseline in mean monthly AMHD over months 4, 5, and 6 Change from baseline in monthly AHMD at assessment timepoints	1. Summary statistics using observed data. 2. Least squares means and associated 95% CI from a generalized linear mixed effect model including **visit**, age, sex**, dose switch (yes/no), baseline dose (full sample analysis only),** and baseline value as covariate using observed data for all assessment timepoints. **Dose switch was excluded for 140 mg treatment group.** A LSM will be presented for change from baseline at each assessment timepoints, and a LSM comparing change from baseline over months 4, 5, 6 will be presented using contrasts.
Achievement of at least a 50% reduction from baseline in mean MMD over months 4, 5, and 6 Achievement of at least a 50% reduction from baseline in MMD at assessment timepoints Achievement of at least a 75% reduction from baseline in mean MMD over months 4, 5, and 6 Achievement of at least a 75% reduction from baseline in mean MMD at assessment timepoints	1. Summary statistics based on observed data.

9.5.1 Analyses of Primary Efficacy Endpoint(s)

Analyses for the primary endpoint will identify the migraine SNPs significantly associated with clinical response of achievement of at least a 50% reduction from baseline in mean MMD over months 4, 5, and 6 and will be performed by deCODE. Details of this analysis are outlined in deCODE Analysis Plan (Appendix B).

9.5.2 Analyses of Exploratory Efficacy Endpoint(s)

The exploratory endpoints except the endpoints related to genetic biomarkers will utilize the EAS. **All models described below will include covariates as specified in Section 4.1.**

The continuous exploratory efficacy endpoints at each assessment time point or as the mean over months 4, 5, and 6 of the 6-month OLTP will be analyzed using the generalized linear mixed effects model that includes **visit and** baseline values of the endpoint, without any imputation for missing data. Least squares means and associated 95% CI will be reported.

The continuous exploratory efficacy endpoints involving two cross-sectional measures (eg. Change from baseline in MMD at week 12 [endpoint], in relation to change from baseline in CGRP level at week 12 [factor]) will be analyzed using a linear regression model that includes corresponding baseline values and the **variable** of interest as covariates without any imputation for missing data. Estimated slope for the **variable of interest**, associated 95% CI, and nominal p-value from the same model will be reported.

The continuous exploratory efficacy endpoints with relation to a factor (eg. baseline CGRP) will be analyzed using the generalized linear **regression** model that includes corresponding baseline values and the factor as covariate without any imputation for missing data. Estimated slope for the factor, associated 95% CI and nominal p-value from the same will be reported. Least square means and 95% CI corresponding to selected baseline values of the factor (eg. quintiles) will be reported.

The continuous exploratory efficacy endpoints involving change from baseline MFIQ at week 24 will be analyzed using an ANCOVA model including baseline score as covariates without any imputation for missing data. Least squares means and associated 95% CI will be reported.

The dichotomous efficacy endpoints with relation to a factor (eg. baseline CGRP) will be analyzed using logistic regression model **including the factor of interest as a covariate and** without any imputation of missing data. Odds ratios with associated 95% confidence intervals and p-values will be reported. Otherwise, descriptive statistics using **observed data** will be summarized.

The analysis of exploratory endpoints with mPRS will be performed by deCODE and details of the analysis are **outlined** in deCODE Analysis Plan (Appendix B).

9.6 Safety Analyses

9.6.1 Adverse Events

The Medical Dictionary for Regulatory Activities (MedDRA) version 22.0 or later will be used to code all events categorized as adverse events to a system organ class and a

preferred term. All AEs will be graded using the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.03.

The subject incidence of adverse events will be **presented for the following groups: (1) subjects always receiving 70mg, (2) subjects who switched from 70mg to 140mg at any time during the study, (3) subjects who switched from 140mg to 70mg at any time during the study, (4) subjects always receiving 140mg, (5) overall – all subjects combined. The subject incidence rates will summarized by SOC in alphabetical order and PT in descending order of frequency** for all treatment-emergent adverse events, serious treatment-emergent adverse events, treatment-emergent adverse events leading to withdrawal of investigational product, treatment-emergent adverse device effect, **treatment-related adverse events, treatment-related serious adverse events,** and fatal adverse events**, and adverse events of interest (EOI) including:**

- **Ischaemic Central Nervous System Vascular Conditions SMQ (Narrow)**
- **Ischaemic Heart Disease SMQ (Narrow and Broad)**
- **Peripheral Arterial Disease AMQ (Narrow)**
- **Hypertension SMQ (Narrow and Broad)**
- **Constipation AMQ (Narrow and Broad)**
- **Alopecias AMQ (Broad)**
- **COVID-19 SMQ (Narrow)**

Summaries of treatment-emergent adverse events and serious treatment-emergent adverse events will be tabulated by system organ class, preferred term, and CTCAE grade.

9.6.2 Exposure to Investigational Product

The total length of investigational product exposure **and exposure by dose level**, the total dose of investigational product, and the proportion of subjects receiving each dose will be summarized using descriptive statistics.

9.6.3 Exposure to Concomitant Medication

Number and proportion of subjects receiving **acute headache medication or** triptan will be summarized **for baseline and post-baseline**.

9.7 Other Analyses

9.7.1 Analyses of Biomarker Endpoints

Summary statistics will be provided for **baseline** CGRP Biomarker **measurements**.

Endpoints related to **baseline** CGRP **measurements** have been described in Section 9.5.2 and Tables 7 & 8.

10. Changes From Protocol-specified Analyses

The proteomics analyses performed by deCODE are not specified in the protocol but will be performed and included in the final CSR.

The primary endpoint analyses have evolved based on deCODE's recommendation. We will no longer present summaries for any genetic variant that is found to be statistically associated with a 50% reduction in MMD over months 4, 5, and 6. Instead, we will only consider associations for a pre-determined set of genetic variants, where these genetic variants will be selected because they have shown genome-wide significant association with migraine in previous studies (~135 SNPs total expected). The reasoning for this change is that the Bonferroni threshold to determine statistical significance will be stricter if we considered all genetic variants (0.05/1,000,000 = 5E-8) than if we only considered a subset of genetic variants (using 135 SNPs gives a threshold of 0.05/135 =3.7E-4). This will substantially improve power to detect statistical significance for individual genetic variants.

11. Literature Citations / References

Mikol DD, Picard H, Klatt J, Wang A, Peng C, Stefansson K. Migraine Polygenic Risk Score Is Associated with Severity of Migraine–Analysis of Genotypic Data from Four Placebo-controlled Trials of Erenumab. Neurology. 2020;94 (15 supplements): 1214.

12. Prioritization of Analyses

Prioritization of analyses has not been identified at this time.

13. Data Not Covered by This Plan

The Global Biostatistical Science department will not perform any analyses using genomics or proteomics data in this study and will not analyze post-baseline CGRP data at this time.

14. Appendices

Appendix A. Reference Values/Toxicity Grades

Adverse event severity and laboratory toxicity are graded based on NCI Common Toxicity Criteria version 4.03 or higher, which is available at the following:

https://www.eortc.be/services/doc/ctc/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf

Appendix B. deCODE Analysis Plan

English: Genetic research methods and data analyses for the Biomarker and Genetic Predictors of Erenumab Treatment Response, a Phase 4 Investigational Open-label Study (INTERROGATE)

Methods and analysis plan:

Whole genome sequencing, genotyping and imputation.

DNA samples will be genotyped using Illumina chips and automated allele-calling software. The front end of the Illumina LIMS system has been integrated with deCODE's blood and DNA sample tracking LIMS system. The data output will feed into deCODE's genotype QC system. We have confirmed the high quality of the data through inheritance checking of large parent-offspring sets. SNPs will be excluded if they have (i) yield lower than 95%, (ii) minor allele frequency less than 1% in the population or (iii) significant deviation from Hardy-Weinberg equilibrium in the controls ($P< 0.001$). All samples with a call rate below 98% will be excluded from the analysis and SNP imputations.

Whole-genome sequencing (WGS) may be performed on selected samples using Illumina technology (1) if they show evidence of rare variants of interest. The sequencing will be performed using NEBNext Ultra™ II DNA Library Preparation and Illumina NovaSeq sequencers.

Sequence variants discovered by deCODE's whole genome sequencing (WGS) project (1) will be imputed. The basis for this imputation is the long range phasing of all chip-genotyped individuals as performed with methods described previously (2,3). Currently, whole genome sequences have been obtained for over 50,000 Icelandic individuals at high coverage of at least 10x (average coverage close to 30x). Sequence variants from these individuals have been propagated using our genealogy and high density genotype databases throughout Iceland's entire population resource. Approx. 36 million variants (SNPs and indels) WGS of ~ 50,000 Icelanders are imputed into >155,000 chip-typed individuals and their first and second degree relatives (1) according to their long range phasing results by the same model as used by IMPUTE (4). For all participants we will impute the SNPs identified and genotyped through sequencing, using imputation assisted by long-range haplotype phasing, the genotypes of all the variants identified by sequencing will be determined for >155,000 Icelanders who have been genotyped with Illumina SNP chips (300K to 2.5 million SNP chips). The nationwide Icelandic genealogical database then allows the propagation of this genotype information into close relatives (for whom we have neither SNP chip nor sequence data) of the chip-genotyped individuals (familial imputation). Such imputed genotypes will be estimated and used temporarily for association analysis, but never stored or made accisible to the researchers.

In addition sequence variants will be imputed from the publicly available SNP data of the HapMap (5) European CEU sample1 (60 triads) and the 1000 Genomes Project data (6) (179 individuals). Approximately 2.5 million SNPs will be imputed based on the HapMap (6) data, and 6.9 million SNPs based on the 1000 Genomes Project data.

Analysis plan:

Analysis of primary and exploratory objectives will be performed by deCODE. Amgen will provide the subject level clinical endpoints and covariates of interest to be analysed in relation to mPRS, sequence variants, and proteins. The analysis set for all deCODE performed analyses consists of individuals who receive at least one dose of investigational product, have an observed value for the endpoint of interest, pass all quality control procedures, and who are determined to be of Icelandic or Danish ancestry based on admixture analysis. Age, sex, dose switch (yes/no), and the baseline value of the clinical endpoint of interest (when applicable) will be used as covariates in all analyses. Additionally, principal components will be included as covariates in all Denmark related genomic analyses to account for population structure. Analyses will be run both with and without the dose switch (yes/no) and baseline clinical value

covariates to assess model robustness. All described analyses will be run separately in the Iceland and Denmark samples, and subsequently combined in a meta-analysis.

Genomic analyses will focus on a subset of 136 migraine variants found to be associated with migraine in previous literature instead of genome-wide analyses (9, 23). The rationale for focusing on this subset of migraine variants is to substantially reduce the multiple testing burden, since a Bonferroni genome-wide significance threshold = 5E-8 and a Bonferroni corrected threshold for the migraine variant subset = 0.05/136 = 3.7E-4.

Primary objective

To explore the relationship between clinical response to erenumab and genetic/proteomic biomarkers

Endpoint:

Achievement of at least a 50% reduction from baseline in mean monthly migraine days (MMD) over months 4, 5, and 6 in relation to each individual genome-wide significant single-nucleotide polymorphism (SNP) contributing to the migraine polygenic risk score (mPRS).

Method:

Test for association using logistic regression between migraine variants and the mPRS and whether individuals treated with erenumab achieve 50% reduction from baseline in MMD over months 4, 5, and 6. Individuals who do not achieve at least a 50% reduction are considered controls and those who achieve a least a 50% reduction are considered cases.

Additionally, this analysis will be performed separately in each of the following subgroups: sex (female/male), presence of aura during baseline period (yes/no), menstrual-related migraine at screening (yes/no), migraine classification at screening (EM/CM), and prior migraine prophylactic treatment failure (yes/no). The subgroups will be re-examined for appropriateness and may be re-categorized (due to small sample size, for example, if there are < 10% of subjects within a subgroup).

Exploratory objectives

1. **To explore the relationship between effect of erenumab on AMSMD and the mPRS**

Endpoint: Change from baseline in mean monthly AMSMD over months 4, 5, and 6, in relation to the mPRS

Method:

Test for association between the mPRS and the mean change from baseline in monthly AMSMD over months 4, 5, and 6 using linear regression.

2. **To describe the relationship between the mPRS and baseline CGRP**

Endpoint: The mPRS in relation to baseline CGRP level

Method:

Test for association between the mPRS and baseline CGRP using linear regression. Association between the migraine SNPs and baseline CGRP will also be analyzed. If high skewness is present, baseline CGRP will be transformed using a log-transformation and rank-based inverse normal transformation to assess model robustness. See Exploratory Objective 1.

3. **To describe the relationship between the mPRS and migraine disease characteristics**

Endpoints:

- mPRS in relation to baseline MMD
- mPRS in relation to baseline AMSMD
- mPRS in relation to baseline acute headache medication days (AHMD)
- mPRS in relation to Migraine Functional Impact Questionnaire (MFIQ) individual domain scores (physical function, usual activities, social function, and emotional function) and overall impact usual activities global item score at baseline
- mPRS in relation to presence of aura during baseline
- mPRS in relation to baseline mean migraine severity

Method:

Test for association between the mPRS and baseline migraine disease characteristic endpoints using linear regression. See Exploratory Objective 1.

4. **To describe the relationship between change from baseline in MFIQ score and mPRS in erenumab treated subjects**

Endpoint: Change from baseline in MFIQ individual domain scores (physical function, usual activities, social function and emotional function) and overall impact usual activities global item score at month 6, in relation to mPRS

Method:

Test for association between the mPRS and the specified MFIQ endpoints using linear regression. See Exploratory Objective 1.

Methods:

Association analysis and meta-analysis.

Genomic associations will only be assessed for SNPs previously validated as being genome-wide associated with migraine. This comprises 136 SNPs to date (9, 23), and this list will be updated with any additional migraine SNPs that may be published at the time of analysis. Associations will be assessed separately in each sample (Iceland, Denmark) and then combined in a meta-analysis. A Bonferroni correction will be used to account for multiple testing.

Calculation of migraine polygenic risk scores (mPRS)

Complex traits such as migraine, are affected by many genes and sequence variants, most of which have a small effect on disease risk (8,9). Polygenic scores can be constructed for any complex genetic phenotype for which a GWAS is available, such as from the large migraine GWAS meta-analyses by Gormley et al. including 375,000 individuals (8) and the most recent, even larger (approximately 1 million individuals) by Hautakangas et al (9). The migraine polygenic risk score (mPRS) will be calculated using migraine GWAS summary statistics from the largest cohorts available to deCODE genetics at the time of analysis (excluding data from Denmark and Iceland). The polygenic risk score (mPRS) values can then be calculated for the individuals participating in the clinical trial by summing their identified risk alleles, which are weighted by effect sizes derived from the GWAS results (8). Linkage disequilibrium will be accounted for using the LDPred software package (10). Principal components will be used to account for population structure within Denmark. No overlap between training and testing datasets is essential to maintain independence of predictions, and the removal of related individuals is also needed, as demonstrated and described in detail by Wray et al. (11).

Effect of sequence variants on levels of plasma proteins and CGRP.

Proteins (n=~7,000) in plasma samples from the study participants will be measured using SomaLogic® (SomaLogic, Inc.) as described elsewhere (16,17). In short, aptamers are modified to recognize specific

proteins and an assay quantifies them on a DNA microarray after the protein concentrations are converted into DNA aptamer concentrations. We will perform a proteome-wide association study (PWAS) and evaluate whether migraine associating sequence variants associate with protein levels, after taking into account other pQTLs at the locus that could be driving the signal. Statistical analyses (PWAS) will be performed using linear regression of log-transformed protein levels against erenumab-response, SNP allele count and mPRS. A Bonferroni correction will be used to account for multiple testing.

CGRP will be measured by Meso Scale Diagnostics (MSD). Association of baseline CGRP levels with migraine SNPs and mPRS will be tested.

The ethics of extensive genetic testing

Extensive genetic testing (GWA chip analysis and genome sequencing) is used to assess common complex diseases, i.e., migraine in this study, where the individual risk variant is neither sufficient nor necessary to cause disease. The research results of the extensive genetic testing are therefore important at the group level for scientific purposes, but not valid for making clinical judgments on the individual level on the health of the participants. The research participants will, in general, not be informed of the results of their genetic makeup apart for incidental findings (see below).

Incidental findings

The Infinium® Global Screening Array (GSA) from Illumina® includes a large number of clinically relevant variants (derived fromClinVar, CPIC and PharmGKB) and whole genome sequencing using the Illumina HiSeqX sequencer together with imputation may detect most clinically relevant variants. Therefore, clinically important variants included on the list of genes defined by the American college of Medical Genetics and Genomics (ACMG) that are well studied and would require feed-back to the research participants could incidentally appear in the analysis of genetic associations to specific traits within this study. Because it is not the purpose of these studies to find these variants, detection of these variants will be considered an incidental finding (21).

In the unlikely event that we find a known disease-specific mutations with high penetrance even though it is not part of the specific purpose of the study, we have established an independent committee that will advise us on how to confirm that the mutation is indeed present (confirmation analysis by use of a CE marked assay, see the recommendations given by the National Scientific Ethics Committee doc. no.: 228077, November 2016). The committee will include clinical immunologists, bioinformaticians and molecular biologists. Elsebet Østergaard, chief physician, professor from the Department of Clinical Genetics at Rigshospitalet will also be part of the committee. If technically validated, the committee will carefully assess the level of evidence for pathogenicity. This will be done using all available literature and exome sequence databases of variants seen in the control population. Such a careful assessment is necessary as recent studies have indicated that many variants have erroneously been reported as pathogenic in monogenic diseases, caused by biased study designs (22). If a mutation is found within the ACMG actionable disorder panel, the participant will be contacted by the research group, provided the participant had previously provided consent.

Contact to the participants with information on incidental findings

For research participants that have a confirmed clinical relevant genetic variant as an incidental finding, the expert committee will appoint a doctor within the specific speciality in the region of the research participants to take care of the clinical management. It is arranged that the research participant has an appointment at the specialist department before the research participant is contacted by phone by the project manager. Only research participants that have agreed to be contacted in case of incidental findings will be contacted. The contact by phone will confirm the research participant's current stance on feedback on genetic findings and will inform the research subject about the finding and the immediate handling of

this. If the research participant agrees to further clinical treatment for the finding he/she will immediately be scheduled for an appointment at the specialist department. At this stage, it will be obvious for the research participant that we have a significant genetic finding. However, the research participant will be offered time for consideration whether he/she wants to be told what the specific genetic finding is – and what it means. If the research participant wants the information – at the same telephone consultation – or at a later consultation – the precise information is given, and the further clinical managements will be in accordance with the management offered for the specific genetic finding. The research participant will be given a phone number on which the project manager can be contacted in case of questions. The research participant will also be offered to invite a representative (e.g. a family member or a friend) to participate in the consultation at the hospital or at telephone conference. At the consultation at the hospital specialist department, the research participant will be given thorough genetic counselling including information about the general knowledge about genetic aetiologies in many diseases and the clinical application of such findings and ethical and legal aspects will be addressed. The research participant will be told about the potential use of genetic findings including the options in relation to work-up of families with inherited diseases. The research participant's rights to know and rights not to know will be discussed.

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