Statistical Analysis Plan

Study Title:	A Randomized, Double-Blind, Vehicle-Controlled, Phase 2 Trial to Evaluate the Anti-pruritic Efficacy, Safety, Tolerability, and Pharmacokinetics of ASN008 in Adults with Mild to Moderate Atopic Dermatitis
Protocol Number and Version:	ASN008-201 V3.0
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This statistical analysis plan will be reviewed and revised as needed.

Table of Contents

Abbreviations

• AD	• Atopic Dermatitis
• AE	• Adverse Event
• AR	• Autoregressive
• ATC	• Anatomical Therapeutic Chemical Classification System
• BID	• Twice Daily
• BMI	• Body Mass Index
• BP	• Blood Pressure
• BSA	• Body Surface Area
• CFR	• Code of Federal Regulation
• CL/F	• Apparent Systemic Clearance
• CV	• Coefficient of Variance
• DLQI	• Dermatology Life Quality Index
• EASI	• Eczema Area and Severity Index
• ECG	• Electrocardiogram
• eCRF	• electronic Case Report Form
• ED50	• Median Effective Dose
• ET	• Early Termination
• HCV	• Hepatitis C virus
• HIV	• Human immunodeficiency virus
• IP	• Investigational Product
• IPA	• Investigational Product Application
• MCPMod	• Multiple Comparison Modelling
• MMRM	• Mixed Model Repeated Measures
• NRS	• Numeric Rating Scale
• PK	• Pharmacokinetics
• POEM	• Patient Oriented Eczema Measure
• PT	• Preferred Term
• PP	• Per Protocol
• SAE	• Serious Adverse Event

• SD	• Standard Deviation
• SOC	• System Organ Class
• TEAE	• Treatment Emergent Adverse Event
• ULN	• Upper Limit of Normal
• UN	• Unstructured Covariance Structure
• Vd/F	• Apparent Volume of Distribution
• vIGA	• validated Investigator Global Assessment

1 INTRODUCTION

This statistical analysis plan was drafted for the study protocol ASN008-201 "A Randomized, Double-Blind, Vehicle-Controlled, Phase 2 Trial to Evaluate the Anti-pruritic Efficacy, Safety, Tolerability, and Pharmacokinetics of ASN008 in Adults with Mild to Moderate Atopic Dermatitis". In this document, the contents and methods of statistical analysis will be described in detail.

This statistical analysis plan was based on ASN008-201 Protocol Version 3.0, 05 July 2023 and Case Report Form version 8.0, 31 August 2023.

2 STUDY OBJECTIVES AND ENDPOINTS

Table 1 Objectives and Endpoints

Objectives	Endpoints
Primary Efficacy:	
To evaluate the anti-pruritic effect of ASN008 topical gel compared to matching vehicle in participants with AD following BID topical application for 4 weeks (28 days).	• Percent change of 7-day average daily peak pruritus NRS from Baseline to Week 4.
Secondary Efficacy	
To evaluate the therapeutic effect of ASN008 topical gel on AD.	• Percent change of 7-day average daily peak pruritus NRS from Baseline to Week 1, 2, and 3. • Change of 7-day average daily peak pruritus NRS from Baseline to Week 1, 2, 3 and 4. • Change and percent change from baseline in daily peak pruritus NRS to Days 1 to 7(Week 1). • Pruritus response defined as 7-day average of daily peak pruritus NRS reduction ≥ 4 points from Baseline at Week 1, 2, 3, and 4. • Change from baseline in 5-D Pruritus Scale at Week 2 and 4. • Change and percent change from baseline in the EASI score at Week 2 and 4. • Change from baseline in total BSA at Week 2 and 4. • Change from baseline in the Dermatology Life Quality Index (DLQI) at Week 2 and 4. • Change from baseline in The Patient Oriented Eczema Measure (POEM) at Week 2 and 4.

Objectives	Endpoints
Pharmacokinetics	
To evaluate the pharmacokinetic (PK) profile of ASN008 topical gel in participants with AD following BID topical application.	• ASN008 PK will be characterized using population-based methods. Apparent systemic clearance (CL/F), apparent volume of distribution (Vd/F), inter- and intra-participant variability of these parameters will be estimated.
Safety	
To evaluate the local and systemic safety and tolerability of ASN008 topical gel in participants with AD following BID topical application.	• Number of TEAEs. • Number of IP-related TEAEs. • Changes in vital signs, physical examinations, ECG, and safety laboratory tests. • Incidence of treatment-emergent serious adverse events (TESAEs) from first dose through completion of the follow-up period (up to a maximum of 56 days). • Incidence of TEAEs leading to treatment discontinuation from first dose through completion of follow-up period (up to a maximum 56 days).
Exploratory	
To further explore the efficacy of ASN008 gel on the AD.	• Change from baseline in vIGA at Weeks 2 and 4. • Patients achieving at least a 50% reduction from baseline in EASI (EASI-50) at Week 2 and 4. • Patients achieving At least a 75% reduction from baseline in EASI (EASI-75) at Week 2 and 4. • Patients achieving At least a 2-grade reduction from Baseline to clear (0) or almost clear (1) in vIGA at Week 2 and 4. • Hourly Pruritus Numeric Rating Scale (NRS) at Day 1.

AD: atopic dermatitis; BID: twice daily; BSA: body surface area; DLQI: Dermatology Life Quality Index; EASI: Eczema Area and Severity Index; ECG: electrocardiogram; NRS: numerical rating scale; POEM: The Patient Oriented Eczema Measure; TEAE: treatment emergent adverse event; TESAE: treatment emergent serious adverse event; vIGA: validated Investigator Global Assessment.

3 STUDY DESIGN

3.1 Overall Design

Approximately 120 participants, at least 18 years of age, with mild to moderate AD (validated Investigator Global Assessment [vIGA] of 2 or 3), BSA of ≤20% involved, and a pruritus NRS ≥7 at Screening and at Day 1 (pre-dose), will be included in this randomized, double-blind, placebo-controlled, multicenter, Phase 2 trial.

All participants will sign an informed consent form and undergo screening (within 28 days prior to Day 1). With approval from the medical monitor, the screening period may be extended to 7 days in certain situations (i.e., IP (investigational product) shipping delays, laboratory or ECG re-tests, or other logistical or unforeseen issues) that would cause the Day 1 visit to need to occur more than 28 days after the screening visit). During the screening period, all treatments for AD and/or itch will be stopped to allow for wash out, as applicable and according to eligibility requirements. Participants may be re-screened once if they fail the initial screening evaluation for reasons related to incidental transitory conditions and if they met (and continue to meet) the disease severity and pruritus level specified in the inclusion criteria.

Eligible participants will be randomized in a 1:1:1:1 ratio to receive ASN008 gel 1.25%, ASN008 gel 2.5%, ASN008 gel 5.0%, or matching vehicle BID for 4 weeks (28 days), followed by a 4-week (28-day) follow-up period. The first dose of study treatment will be applied on Day 1 and the last dose will be applied during the Day 28 study visit. Participants are not to receive study treatment for more than 28 days. Refer to the Study Schema (Figure 1) and Schedule of Events (Table 1) for more details.

Participants will be required to participate in 8 scheduled visits: Screening; Randomization (remote visit); Day 1; Week 1 (Day 8); Week 2 (Day 15); Week 3 (Day 22); Week 4 (Day 28); and Week 8 (Day 56)/early termination (ET).

The trial duration per participant is up to 12 weeks (84 days): including up to 4 weeks (28 days) for the screening period, 4 weeks (28 days) for the treatment period, and up to 4 weeks (28 days) for the follow-up period.

A participant is considered to have reached the end of the trial when they have completed their Day 56 (Week 12) or ET (early termination) visit. The trial will be considered complete when the last participant has completed their last trial visit.

3.2 Schedule of Events

Table 1 Schedule of Events

Study Visits	Screening (up to D-28)	Randomization[a] (phone)	Treatment Period					Follow-up/ET (Day 56/Week 8)
			D1	D8 (Week 1)	D15 (Week 2)	D22 (Week 3)	D28 (Week 4)	
Window (days)			0 days	±1 day	±2 days	±2 days	±2 days	-2 days
Informed consent	X							
Demographics	X							
Medical and surgical history	X		X					
Inclusion-exclusion criteria	X		X					
Pregnancy test (WOCBP only)	Serum		urine	urine	urine	urine	urine	urine
Clinical laboratory tests (biochemistry, hematology, urinalysis)	X		X		X		X	X
Body weight and height, BMI[b]	X		X		X		X	X
Serology (HIV, HBV, HCV)	X							
Physical examination	X		X		brief		X	brief
Vital signs	X		X	X	X	X	X	X
Electrocardiogram (12-lead, central reading)	X[c]		X[d]		X[e]		X[d]	X[f]
vIGA	X		X		X		X	X
BSA (excluding palms, scalp, axillae, groin, genitals, and folds)	X		X		X		X	X
EASI	X		X		X		X	X
5-D pruritus scale			X		X		X	X
DLQI			X		X		X	X
POEM			X		X		X	X
Pruritus NRS[g]	X		X	X	X	X	X	
Randomization		X						

Study Visits	Screening (up to D-28)	Randomization[a] (phone)	Treatment Period					Follow-up/ET (Day 56/Week 8)
			D1	D8 (Week 1)	D15 (Week 2)	D22 (Week 3)	D28 (Week 4)	
Window (days)			0 days	±1 day	±2 days	±2 days	±2 days	-2 days
Investigational Product Application at study site			X	X	X	X		
Investigational Product BID Application Daily[h]			X---------- X				X[g]	
Participant Daily e-Diary[i]			X---------- X					
Investigational product distribution			X	X	X	X		
Collection of investigational product				X	X	X	X	X[f]
Verification of daily diary			X	X	X	X	X	X
Emollient use	-7 days	X[a]	X---------- X					
Local tolerability assessments			X	X	X	X	X	X
PK samples collection			See Table 2					
Concomitant medication	X	X	X	X	X	X	X	X
Adverse events evaluation	X	X	X---------- X					

BID=twice daily; BMI=body mass index; BSA=body surface area; D=day; DLQI=Dermatology Life Quality Index; EASI=Eczema Area and Severity Index; ECG=electrocardiogram; ET=early termination; HBV=hepatitis B virus; HCV=hepatitis C virus; HIV=human immunodeficiency virus; NRS=numerical rating scale; PK=pharmacokinetics; POEM =Patient-Oriented Eczema Measure; vIGA= Validated Investigator Global Assessment; WOCBP=women of childbearing potential.

Note: In general, non-invasive procedures should be completed prior to invasive procedures. Unless otherwise specified, the study assessments scheduled during the study visits will be performed before the study product application. If assessments are scheduled for the same nominal time, then the assessments should occur in the following order, when possible: vital signs, 12-lead ECG, local tolerability, pruritus NRS, and blood draw for PK evaluation.

a The Randomization visit will be conducted remotely via phone after all screening assessments have been completed and the results of those assessments are determined to be in line with the trial entry criteria (i.e., based on central read ECG, central laboratory results, and all clinical outcome assessments and patient-reported outcome assessments). During this remote check-in, study staff will verify the participant's desire to continue in the trial, record any adverse events, assess concomitant medications, and confirm that nonprescription emollient has been (or will be) used consistently for at least 7 days prior to Day 1. Study staff will then acquire a randomization number for eligible participants, which will prompt shipment of investigational product to the study site. The interval between the Randomization Visit and Day 1 Visit should allow enough time for the investigational product to arrive at the study site (i.e., approximately 1 week).

b Height will be measured only at screening and the same value will be used for BMI calculation at other visits.

c If participant has clinically significant 12-lead ECG abnormalities (per Investigator) or QTcF >450 ms, abnormal ECG may be repeated once. For participants with QTcF >450 ms on initial ECG, the mean of the 2 QTcF assessments will determine eligibility.

d On Day 1 and Day 28, ECGs will be time matched with PK sample collection and will be performed at time 0 (pre-dose, within 1 hour of dosing), 1 (±15 min), 2 (±30 min), and 4 (±1 hour) hours post-dose. The ECG should be performed prior to the PK sample collection.

e ET only.

f Hourly starting immediately post dose until bedtime on Day 1 only.

g Should not be applied to palms, scalp, axillae, groin, genitals, or folds. The last dose of investigational product should be applied during the Day 28 study visit; participants are not to receive study treatment for more than 28 days.
h Participant will complete daily e-diary onsite during visit if not already completed.

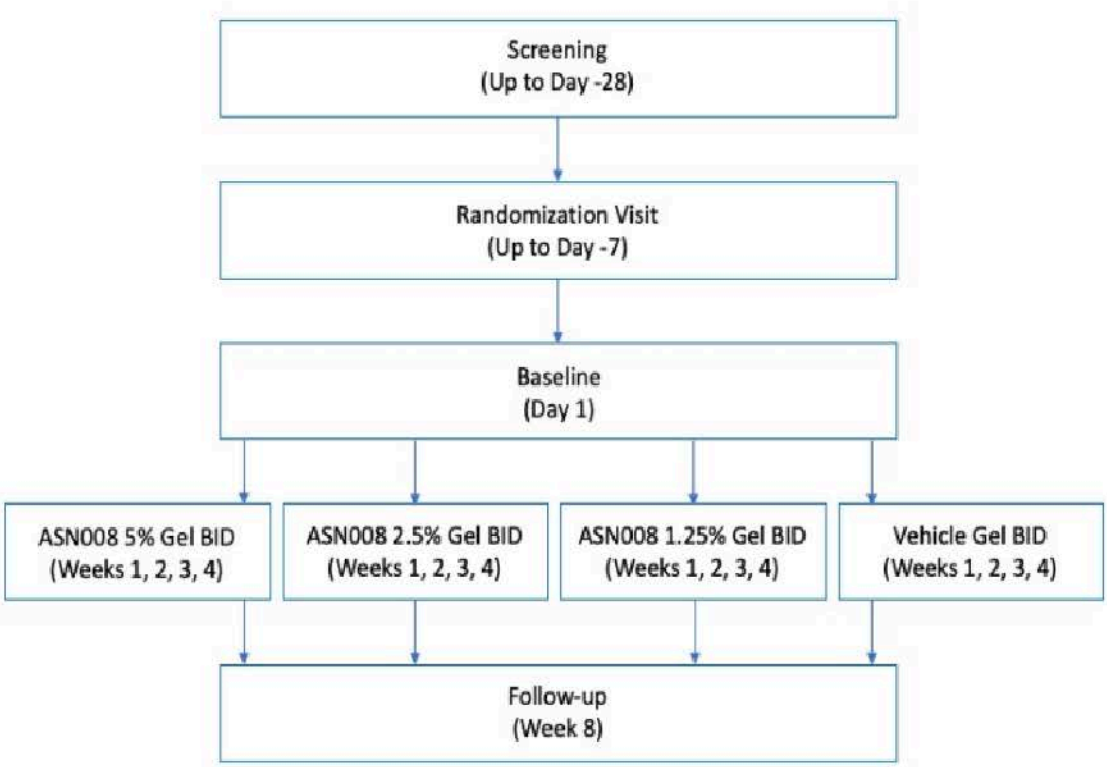
Table 2 Pharmacokinetic Sampling

Window	Predose	Post IP Administration		
		1 hour	2 hours	4 hours
	within 1 hour of dosing	± 15 min	± 30 min	± 1 hour
Day 1	X	X	X	X
Day 28 (-2 days)	X	X	X	X

IP=investigational product

3.3 Study Design Schema

Figure 1 Trial Design



4 ANALYSIS SETS

4.1 Modified Intent-to-Treat Analysis Set

Modified intent-to-treat analysis (mITT) analysis set includes all randomized participants who meet inclusion and exclusion criteria at the Day 1 visit and receive at least 1 dose of treatment. Participants will be attributed to the treatment they are randomized regardless of the actual treatment they receive.

4.2 Per-Protocol Set

The Per-Protocol (PP) set includes all participants who were randomized, received at least 1 dose of study product without any major protocol deviations that may affect the efficacy outcome, received the treatment they were randomized to, who don't have missing baseline pruritus NRS, who don't have 50% or more missing pruritus NRS data in week 4. Those who discontinue the study treatment prior to NRS week 4 will also be excluded. The participants to be excluded from the per protocol set due to the major protocol deviations will be evaluated and determined prior to database lock by the sponsor.

4.3 Safety Set

The safety set includes all participants who receive at least 1 dose of treatment. Participants will be attributed to the initial actual treatment they receive.

4.4 Pharmacokinetic Set

The Pharmacokinetic (PK) set will include all participants who received at least 1 dose of treatment and have ASN008 concentration data.

5 GENERAL CONSIDERATIONS

5.1 Sample Size

For the primary endpoint analysis of the percent change of 7-day average daily peak pruritus NRS from Baseline to Week 4, N = 24 / group will give us 80% power to detect a standardized effect of 0.72 with 2-sided significance level of 0.1 (28.8% improvement over placebo in % change from baseline in pruritus, assuming standard deviation [SD] = 40%).

5.2 Baseline

Unless otherwise specified, baseline value will be defined as the last non-missing assessment prior to or on the first study treatment application date and time (including unscheduled assessments). If the last non-missing assessment is performed on the same date as the first study treatment and time is not available, the assessment will be considered as baseline, except for adverse events (AEs) and medications starting on the first study treatment dose date, which will be considered post-baseline.

5.3 Study Day

Study day will be calculated from the first study treatment date and will be used to show start/end day of assessments or events.

- For dates on or after the first treatment date, Study Day = Date – First Study Treatment Date + 1.
- For dates before the first treatment date, Study Day = Date – First Study Treatment Date.
- Study day will be set to missing when the assessment/event date is partial or missing.

5.4 Descriptive Statistics

All continuous variables will be summarized by the number of participants, mean, SD, median, minimum, and maximum. Categorical variables will be presented as frequencies and percentages. Summary tables will be presented by treatment and visit, when applicable.

- Change from baseline will be calculated as:
  - Assessment value at post-baseline visit X – baseline value.
- Percent change from baseline will be calculated as:
  - (Assessment value at post-baseline visit X – baseline value) / baseline value × 100.

5.5 Treatment Grouping

The participants will be grouped by ASN008 gel at 1.25, 2.5, or 5%, or Vehicle BID.

5.6 Handling of Retests, Unscheduled Visits, and Early Termination Data

When retests measurements are done, the retest measurement will be used for the summary analysis. All data from all visits will be listed.

Unscheduled visit assessment will be listed and will not be included in by-visit outputs.

All safety data will be included in the non-by-visit summary tables/figures. Besides, data from unscheduled visits will be listed.

Early Termination visit assessments will be summarized as a separate visit in by-visit outputs.

5.7 Analysis Visit Windows and Endpoints

5.7.1 Non-Peak Pruritus NRS

For efficacy analyses and safety analyses by visit (NRS excluded), assessments at scheduled and unscheduled visits will be mapped to the appropriate analysis visit window as detailed in Table 1 Schedule of Events.

5.7.2 Peak Pruritus NRS

The Peak Pruritus NRS is a single self-reported item designed to measure peak pruritus, or 'worst' itch, over the previous 24 hours.

On Day 1, pruritus NRS will be assessed at several time points: pre-dose to confirm eligibility and then hourly in the eDiary starting immediately post-dose until bedtime.

Following Day 1, participants will record the peak pruritus NRS daily in the eDiary before the morning investigational product application, and their compliance on the peak pruritus NRS will be followed at each clinic visit. During study visits, the NRS data will be recorded by site staff in the eCRF.

The baseline is Day 1 pre-treatment in-clinic peak pruritus NRS.

Post-treatment daily peak pruritus will be derived as follows:

- The worst hourly pruritus NRS on study day 1.
- First from eDiary data, then diary paper backup, then in-clinic for post study day 1. If there are multiple worst ones, the first one is flagged for analysis. Missing values will not be imputed.

The 7-day daily peak pruritus by week will be the average of all non-missing values from

- NRS week 1: study day 1 post-treatment to study day 7.
- NRS week 2: study day 8 to study day 14.
- NRS week 3: study day 15 to study day 21.
- NRS week 4: study day 22 up to study day 28.

The 7-day average of the Peak Pruritus NRS which is used for primary analysis will be calculated as the average of non-missing values of the daily NRS if there are 50% or more days with non-missing values within each visit window, otherwise it will be set to missing. In addition, any data after the on-study initiation of the prohibited medication will be set to missing values.

The all available 7-day average of the Peak Pruritus NRS which is used for sensitivity analysis will be calculated as the average of all non-missing values of the daily NRS within each visit window. The NRS will be set to missing after the on-study initiation of prohibited medication. If multiple records appear in one source, only the first one will be used for analysis.

5.7.3 7-day Average of the Peak Pruritus NRS - Primary

Calculated as the average of non-missing values of the daily NRS if there are 50% or more days with non-missing values within each visit window, otherwise it will be set to missing. In addition, any data after the on-study initiation of prohibited or rescue medication will be set to missing values.

5.7.4 The All Available 7-day Average of the Peak Pruritus NRS - Sensitivity

Calculated as the average of all non-missing values of the daily NRS within each visit window. The NRS will be set to missing after the on-study initiation of prohibited or rescue medication.

5.7.5 5D-Pruritus Scale

The 5-D Pruritus Scale is a 1-page, 5-question, validated questionnaire used in clinical trials to assess 5 dimensions of background itch: degree, duration, direction, disability, and distribution.

Each question corresponds to 1 of the 5 dimensions of itch; subjects will rate their symptoms over the preceding 2-week period as "present" or on a 1 to 5 scale, with 5 being the most affected.

The scores of each of the five domains are achieved separately and then summed together to obtain a total 5-D score. 5-D scores can potentially range between 5 (no pruritus) and 25 (most severe pruritus). The score will be set to missing in case where at least one of the five domain results is missing.

- Single-item domain scores (duration, degree, and direction) are equal to the value in the response choice (range 1–5).
- The disability domain includes four items that assess the impact of itching on daily activities: sleep, leisure/social activities, housework/errands, and work/school. The score for the disability domain is achieved by taking the highest score on all available items.
- For the distribution domain, the number of affected body parts is tallied among all available items (potential sum 0–16) and the sum is sorted into five scoring bins: sum of 0–2 = score of 1, sum of 3– 5 = score of 2, sum of 6–10 = score of 3, sum of 11–13 = score of 4, and sum of 14–16 = score of 5.

5.7.6 Eczema Area and Severity Index (EASI)

The EASI is a composite score ranging from 0 to 72 that takes into account the degree of erythema, induration/infiltration (papules), excoriation, and lichenification (each scored from 0 to 3 separately) for each of 4 body regions, with adjustment for the percentage of BSA involved for each body region and for the proportion of the body region to the whole body.

5.7.7 Validated Investigator Global Assessment (vIGA)

The vIGA is a global assessment of the current state of the disease. It is a 5-point morphological assessment of overall disease severity. The vIGA (excluding hands and feet) will be assessed before the morning investigational product administration.

5.7.8 Body Surface Area Involvement of Atopic Dermatitis

The overall BSA affected by AD (total BSA) will be evaluated (from 0% to 100%).

5.7.9 The Patient-Oriented Eczema Measure (POEM)

The POEM is a self-assessment of disease severity by the participant; the participant's response to 7 questions is scored from 0 to 4; the POEM is the sum of the 7 scores, which ranges from 0 to 28. The higher the POEM, the severer the disease.

If missing items occur the following rules will be followed:

- If 1 question is left unanswered this is scored 0 and the scores are summed and expressed as usual out of a maximum of 28.
- If > 1 questions are left unanswered the questionnaire is not scored.
- If > 1 response options are selected for a single question, the response option with the highest score should be recorded.

5.7.10 Dermatology Life Quality Index (DLQI)

The DLQI is a 10-item questionnaire used to measure the impact of skin disease on the quality of life of an affected patient.

Generally, questions are scored on a four-point Likert scale:

- Very much = 3
- A lot = 2
- A little = 1
- Not at all = 0
- Not relevant = 0
- Question unanswered = 0

For question 7, the first part asks: "Over the last week, has your skin prevented you from working or studying?"

If working or studying are not relevant to the subject, the response is "Not relevant" (scored 0).

If the skin disease has prevented the subject from working or studying, the answer is "Yes". As "prevention" is the biggest possible impact it is scored the maximum, 3.

If the skin disease has not prevented the subject from working or studying, the answer is "No". It is therefore assumed that as the skin disease has not prevented the subject from working or studying, the subject is able to continue to work or study, but that the skin disease may be a problem while doing so.

The subject is therefore asked the following question about the magnitude of the impact thus: "If 'No' (in other words 'If the skin disease has not prevented you from working or studying'), over the last week how much has your skin been a problem at work or studying?"

There are three possible responses to the question "How much has your skin been a problem at work or studying": "A lot" (scored 2), "A little" (scored 1) or "Not at all" (scored 0).

The DLQI can be classified into 6 subscales: symptoms and fillings (questions 1 and 2), daily activities (questions 3 and 4), leisure (questions 5 and 6), work and school (question 7), personal relationships (question 8 and 9), and treatment (question 10).

The DLQI is calculated by adding the score of each question, ranging from 0 to 30. The higher the score, the more quality of life is impaired. A score higher than 10 indicates that the patient's life is being severely affected by their skin disease.

For this study, if one question is unanswered, this is allocated a score of 0 and the DLQI score summed in the usual way. If two or more questions are unanswered, the questionnaire is not scored.

5.8 Handling of Missing Values

Missing values will not be imputed unless specified otherwise.

For the efficacy analysis of 7-day average of the daily peak pruritus NRS as specified in section 5.7 the missing values will be imputed implicitly with MMRM modeling under assumption of missing at random (MAR).

Refer to Appendix 1 for the handling of partial dates in AE or concomitant medication data.

6 STATISTICAL CONSIDERATIONS

6.1 General Statistical Considerations

The significance level for this trial is set to be 2-sided 0.05 unless otherwise specified. For PK data, geometric mean and percent of coefficient of variance (%CV) will be presented.

All decimal places will be kept to 3 places or less.

6.2 Interim Analysis

No interim analysis is planned.

7 STUDY PARTICIPANTS

7.1 Disposition of Participants

All participants who provide informed consent will be accounted for this study. The number of participants screened, and screen failure will be summarized.

Screen failures:

- Screen failures and the reason for screen failure will be listed.
- Those who were rescreened and did not fail the re-screening(s) are not considered as screen failures.
- A participant can still be a screen failure post randomization but before Day 1 due to the lack of drug availability at the time of randomization when a participant's eligibility is re-evaluated post randomization and prior to the first dose.

The number and percent of participants who are

- Randomized.
- Withdrew consent post randomization.
- Screen failure post-randomization but before Day 1.
- Screen failure at Day 1.
- Eligible to continue and receive the first dose of IP on Day 1.

will be summarized descriptively.

Study completion status and the reason for study treatment discontinuation will also be presented.

Unless otherwise specified, the denominator of the percentages will be based on the number of participants in the mITT analysis set.

The percentage of screen failures will be calculated based on the total number of participants screened as the denominator. The percentage of screen failure by reasons will be based on the number of total screen failures as the denominator.

Number of days in the study will be calculated as follows:

Number of days in study = Date of completion/discontinuation – 1st dose date +1

A listing of participant's disposition will be provided. For those who were rescreened, data on the initial screening, including the rescreening participant identifier, will be presented under the initial screening participant identifier. A listing of participant's randomization data and a listing of participants included in each of the study analysis sets will also be provided.

In addition, participants who are excluded from the per protocol set and the reason of being excluded will be provided in a separate listing.

7.2 Protocol Deviations

The number of events and the number and percentage of participants with at least 1 major protocol deviation, as identified during the conduct of the clinical trial, will be summarized by deviation category and treatment group using the safety set. A listing of all major protocol deviations will also be provided, with a flag indicating if any protocol deviations led to a participant being excluded from the PP Set.

8 DEMOGRAPHIC AND OTHER BASELINE CHARACTERISTICS

Demographics and baseline characteristics will be summarized with descriptive statistics using the safety set.

A listing of all demographics and baseline characteristics will be provided.

9 MEDICAL AND SURGICAL HISTORY

Medical and surgical history will be coded according to the Medical Dictionary for Regulatory Activities (version 26.1 or later).

Medical and surgical history will be summarized by system organ class (SOC), preferred term (PT), and treatment using the safety set. A participant who experienced the same medical and surgical history event multiple times will be counted only once for the corresponding PT. Similarly, if a participant experienced multiple medical and surgical history events within the same SOC, the participant will be counted only once for that SOC. Medical and surgical history events will be sorted alphabetically by SOC and within each SOC the PT will be presented by decreasing order.

A listing of all medical and surgical history events will be provided.

10 PRIOR AND CONCOMITANT MEDICATIONS

Medications will be coded according to the World Health Organization Drug Dictionary (WHODD).

Prior medications are defined as any medication discontinued prior to the first study treatment dose. Concomitant medications are defined as any medication whose stop date is after the first study treatment dose. See Appendix 1 for handling of missing dates for prior and concomitant medications.

Incidence of prior and concomitant medications will be tabulated by Anatomical Therapeutic Chemical Classification System (ATC) level 3, and PT using the safety set. A participant with the same medication taken multiple times will be counted only once for the corresponding PT. Similarly, if a participant has taken more than 1 medication within the same ATC level, the participant will be counted only once for that ATC.

In addition, prohibited medications will be reviewed and determined from the concomitant medications used prior to the database lock. A listing of all prior and concomitant medications will be provided, with a flag indicating prohibited medication used.

11 STUDY TREATMENT EXPOSURE AND TREATMENT COMPLIANCE

The study treatment will be applied twice daily (except on Day 28), at approximately the same times each day; the date and time of each application can be recorded in the eDiary, paper back up and in clinic. It is recommended that the application times be approximately 12 hours apart (±2 hours).

If an application is missed participants should apply the study treatment as soon as they remember on the same day. However, if it is the next day, the missed application should be skipped and the next application should be applied as normal. Participants are not to receive study treatment for more than 28 days.

In the case of application data are found in multiple sources, all available records will be used for analysis. An application recorded within 10 minutes on eDiary and paper back up is considered as one dose.

A summary of exposure will be summarized descriptively using the safety set by each treatment group including the number of days treated, total number of applications received and compliance during treatment, which is calculated as:

$$\frac{\text{The total number of IP Applications}}{(\text{The number of days between the last dose date and the first dose date } + 1)\text{x}2 - 1} \times 100$$

where the total number of IP applications include all valid doses even if they are over 28 days and the last dose date is calculated up to 28 days.

Descriptive statistics for compliance will be presented for each treatment group. Frequency distribution will also be presented for the following categories:

- $\leq 80\%$
- 80-100% (100% included)
- 100-120% (120% included)
- $> 120\%$

Study exposure and drug accountability data will be provided in a separate listing.

12 PHARMACOKINETIC ANALYSIS

Due to the sparse PK data collected from individual participants, non-compartmental analysis will not be performed.

Descriptive statistics of the concentration data will be summarized based on nominal timepoints by treatment and will be presented in a table using the PK set. PK concentration data, including actual sampling time, will be provided in a listing. For computation of mean plasma concentrations, data that are below the limit of quantification will be set to the limit of quantification.

13 EFFICACY ANALYSIS

Efficacy analysis will be performed on the modified intent-to-treat set and per protocol set; the modified intent-to-treat set will be used as the primary analysis set.

13.1 Primary Efficacy Endpoint

The aims of the analyses of the primary endpoint, percent change from baseline at Week 4 in daily peak pruritus NRS, are to test for treatment effect; to characterize the gel concentration-response curve; to estimate the minimum effective and optimal concentration. The MCPMod approach will be applied for this study. If any candidate model given below is significant, the significant treatment effect will be concluded, then the significant models will be used to estimate the minimum and optimal concentration.

In essence, the aim of the primary analysis is to establish the ASN008 gel concentration-response curve, the response is defined as the change from baseline in 7-day average of the daily peak pruritus NRS at Week 4 for mild and moderate atopic dermatitis participants who do not use any prohibited medications during the entire study and have not safety concerns. For the primary analysis, any data after use of the prohibited medication will set to missing values. The missing data will be imputed implicitly using MMRM modeling under assumption of missing at random.

For the MCPMod approach, the candidate gel concentration-response models to be applied are given follows:

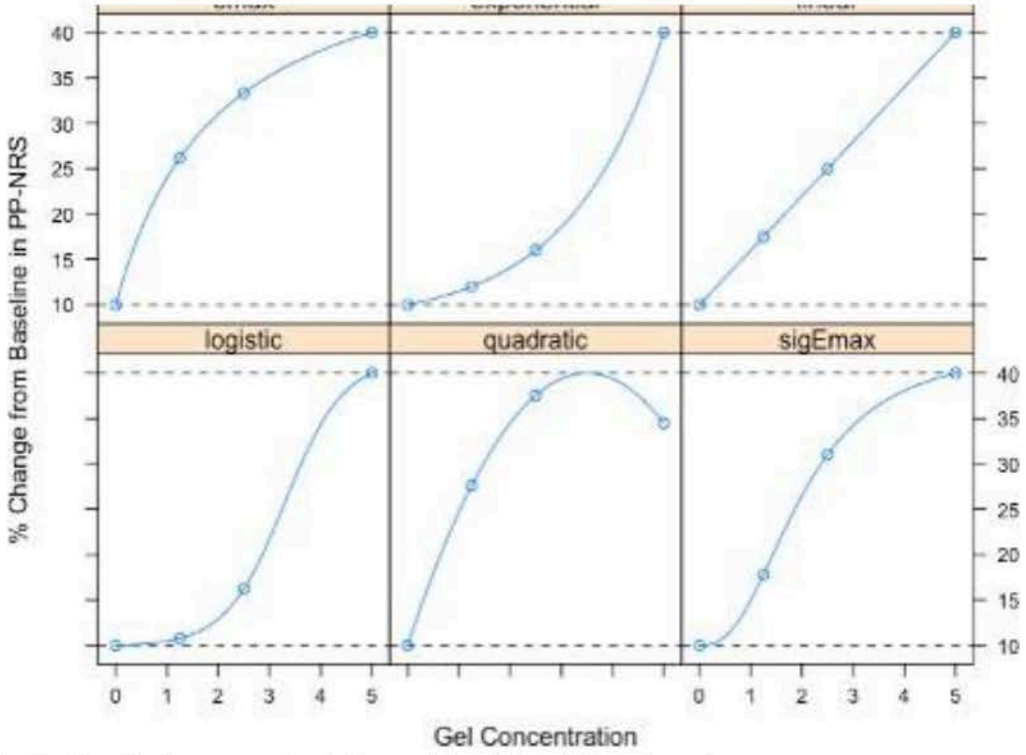
- Linear model.
- Quadratic model with maximum effect reached at 3.5%, the model parameter is - 0.1428571.
- Exponential model which reaches 20% of the effects at 2.5%, the model parameter is 1.803368.
- E_{max} model with ED50 = 2.5%.
- Sigmoid Emax model with ED50=2.5% and Hill's parameter = 2.5.
- Logistic model which reaches 20% and 95% effects at concentrations 2.5% and 5%, respectively, the model parameters are ED50% = 3.3002654, delta = 0.5772694.

The minimum effective concentration is defined as the lowest concentration whose standardized effect size is at least 0.25 and is significantly different from placebo at a 2-sided significance level of 0.2.

The optimal concentration is defined as the highest concentration of ASN008 used in this clinical trial without safety concerns for linear and exponential models, or the lowest concentration which maintains 90% of the maximum effect for the rest of candidate models.

Figure 2 depicts the shapes of curves for each candidate model, assuming the vehicle effect is 10% and treatment effect is 40% (30% improvement over vehicle).

Figure 2 Candidate Concentration - Response Models



Analysis of primary endpoint consists of the following steps:

Mean and variance estimation: A MMRM model with visit, treatment and visit by treatment interaction as fixed effects, baseline NRS as covariate, participant as random effects will be fitted first, the ls means and their variance-covariance matrix at Week 4 will be estimated using the fitted model.

1. MCP-step: the candidate models are tested using the estimated means and their variance- covariance matrix from the previous step. If any of the models is statistically significant at two-sided significance level of 0.1, the treatment effect is established, the analysis moves to the next step.
2. Mod-step: the concentration-response curve will be characterized, and the minimum effective concentration and optimal concentration will be estimated. All significant models indicated by the previous step will be fitted, and minimum effective concentration and optimal concentration will be estimated based on the fitted models.
3. Finally, weighted estimates for minimum effective concentration and optimal concentration will be calculated using weighing in Buckland et al. (Buckland 1997).

For MMRM model fitting, the unstructured (UN) variance-covariance matrix will initially be assumed. If the model does not converge using the UN covariance structure, the autoregressive (order 1) AR(1) structure will be used. If the AR(1) structure also does not converge, other covariance structures, such as the Toeplitz structure, deemed appropriate to fit the data will be used.

To estimate the degree of freedom, the Kenward Roger method will be used.

13.1.1 The Primary Analyses – Percent Change of 7-day Average of the Peak Pruritus NRS at Week 4

The primary analysis of the primary efficacy end point is the percent change of 7-day average of the peak pruritus NRS as defined in section 5.7.3 using the analysis method described in section 13.1 using the mITT analysis set.

13.1.2 The Sensitivity Analyses – Percent Change of the All Available 7-day Average of the Peak Pruritus NRS at Week 4

The percent change of all available 7-day average of the peak pruritus NRS as defined in section 5.7.4 will be repeated using method described in section 13.1 using the mITT analysis set as a sensitivity analysis. In addition, both the percent change of 7-day average of the peak pruritus NRS and the all available 7-day average of the peak pruritus NRS analyses will be repeated using the PP set.

13.2 Key Secondary Endpoints

The following continuous secondary efficacy endpoints, as defined in section 5.7, will be analyzed using MMRM model with visit, treatment and visit by treatment interaction as fixed effects, baseline NRS as covariate, participant as random effects will be fitted first, the ls means and their variance-covariance matrix at Week 4 will be estimated using the fitted model. Percent change of 7-day average daily peak pruritus NRS from Baseline to Week 1, 2, and 3.

- Change of 7-day average daily peak pruritus NRS from Baseline to Week 1, 2, 3 and 4.
- Change and percent change from baseline in daily peak pruritus NRS from Days 1 to 7.
- Change from baseline in 5-D Pruritus Scale at Week 2 and 4.
- Change and percent change from baseline in the EASI score at Week 2 and 4.
- Change from baseline in total BSA at Week 2 and 4.
- Change from baseline in the Dermatology Life Quality Index (DLQI) at Week 2 and 4.
- Change from baseline in The Patient Oriented Eczema Measure (POEM) at Week 2 and 4.

The endpoints will be summarized by visit presenting LS Mean, 95% CI and associated p-value from the above MMRM model, using mITT analysis set and PP set.

The following categorical secondary efficacy endpoints will be analyzed based on Fisher's exact test. In addition, odds ratio (OR) and 95% Wald confidence intervals will also be presented when applicable:

- Pruritus response is defined as the 7-day average of daily peak pruritus NRS reduction ≥ 4 points from Baseline at Week 1, 2, 3, and 4.

13.3 Exploratory Endpoints

The following categorical exploratory endpoints will be summarized using mITT analysis set. P-values and 95% confidence intervals (CI) will be presented based on Fisher's exact test. In addition, odds ratio (OR) and 95% Wald confidence intervals will also be presented when applicable:

- At least a 50% reduction from baseline in EASI-50 at Week 2 and 4.
- At least a 75% reduction from baseline in EASI-75 at Week 2 and 4.
- At least a 2-grade reduction from Baseline to clear (0) or almost clear (1) in vIGA at

Week 2 and 4.

The following continuous exploratory efficacy endpoints will be analyzed using an MMRM model similar to the continuous secondary efficacy endpoints:

- Change from baseline in vIGA at Weeks 2 and 4.

The following exploratory endpoint will be summarized and listed using mITT analysis set and PP set:

- Hourly Pruritus Numeric Rating Scale (NRS) on Day 1.

14 SAFETY ANALYSIS

The safety analysis will include reported local and systemic TEAEs and other safety information (i.e., clinical laboratory evaluations, vital signs, physical examinations, ECGs and local tolerability assessments results).

14.1 Adverse Events

Adverse events will be coded according to the latest version of the Medical Dictionary for Regulatory Activities (Version 26.1 or later).

Analyses of adverse events will include all events reported from the time of informed consent to 28 days after the last dose of study drug.

See Appendix 1 for handling of completely or partially missing dates for AEs. In the case where it is not possible to define an AE as treatment emergent or not, the AE will be classified as treatment emergent.

A TEAE is any condition that was not present prior to treatment with the trial product but appeared following first dose, was present at treatment initiation but worsened during treatment, or was present at treatment initiation but resolved and then reappeared while the individual was on treatment (regardless of the intensity of the AE when the treatment was initiated) up to and including 28 days post the last dose of the study treatment.

An overall summary table of AEs will be provided. The number of events and the number and percentage of participants who experienced AE, TEAE, TEAE by highest relationship, TEAE by relationship, TEAE by maximum severity, TEAE by severity, related TEAE by maximum severity, related TEAE by severity, serious AE, serious TEAE, serious TEAE by highest relationship, TEAE and related TEAE leading to study treatment discontinuation, TEAE and related TEAE leading to treatment interruption, and AE leading to death will be presented.

Unless otherwise specified, a participant experiencing the same TEAE multiple times will be counted only once for the corresponding PT. Similarly, if a participant experience multiple TEAEs within the same SOC, the participant will be counted only once for that SOC. The summary table of TEAEs will be sorted alphabetically by SOC and within each SOC the PT will be presented by decreasing order of the overall occurrence.

The TEAE will be summarized:

- by SOC and PT within SOC;
- by SOC, PT, and relationship. If a participant experiences more than 1 TEAE within different relationship categories within the same SOC/PT, only the worst case (highest relationship) will be summarized. A TEAE with an unknown relationship will be considered treatment related.
- by SOC, PT, and severity (mild/moderate/severe). If a participant experiences more than 1 TEAE within different severity categories within the same SOC/PT, only the worst case (maximum severity) will be reported. A TEAE with an unknown severity will be considered as severe. A listing of AEs with changing severity will be provided.
- by SOC, PT, relationship, and severity (mild/moderate/severe). Each participant will be counted only once within a SOC or a PT by using (1) the highest relationship followed by (2) the maximum severity. A TEAE with an unknown severity will be considered as severe.

The serious TEAE will be summarized:

- by SOC and PT;
- by SOC, PT, and relationship. If a participant experiences more than 1 serious TEAE within different relationship categories within the same SOC/PT, only the worst case (highest relationship) will be reported.

Listings of all AEs, all AEs leading to death, all SAEs, all TEAEs leading to study drug discontinuation, all TEAEs leading to study drug interruption, and most frequent TEAEs will be provided.

14.2 Clinical Laboratory

Descriptive statistics will be presented for data related to chemistry, hematology, and quantitative urinalysis. Change from baseline values will be presented for each post-baseline assessment. Frequencies and percentages for each result will be provided for qualitative urinalysis data.

Shift tables from baseline to each post-baseline visits describing shifts to abnormality will be provided as well. Only participants with a baseline result and a result at the specified visit for the parameter will be considered.

Separate listings of all data for chemistry, hematology, urinalysis, serology, and pregnancy test will be provided.

In addition, separate listings of data for chemistry, hematology, and urinalysis will be provided for each parameter where a participant had at least 1 abnormal result.

14.3 Vital Signs

Descriptive statistics will be presented for data related to vital signs (systolic blood pressure diastolic blood pressure, pulse rate, and body temperature). Change from baseline values will be presented for each post-baseline assessment.

Shift tables from baseline to each post-baseline visit describing shifts to abnormality will also be provided. Only participants with a baseline result and a result at the specified visit for the parameter will be considered.

A listing of all vital sign assessments will be provided. In addition, a listing will be provided for each parameter where a participant had at least 1 abnormal result.

14.4 Electrocardiogram

Descriptive statistics will be presented for data related to ECGs (heart rate, RR interval, PR interval, QRS duration, QT interval, and QTcF interval). Change from baseline values will be presented for each post-baseline assessment.

Shift tables from baseline to each post-baseline visit and to each timepoint describing shifts to abnormality will be provided for overall interpretation. Only participants with a baseline result and a result at the specified visit for the parameter will be considered.

A listing of ECG assessments will be provided. In addition, a listing will be provided for each parameter where a participant had at least 1 abnormal result.

14.5 Brief Physical Examinations and Ophthalmology

Brief physical examinations will be summarized by treatment and visit.

A listing of physical examinations will be provided.

14.6 Local Tolerability Assessment

The local tolerability assessment data will be summarized and listed descriptively.

15 CHANGES FROM PROTOCOL

- Added “analyses of adverse events will include all events reported from the time of informed consent to 28 days after the last dose of study drug” to AE definition.
- Added “receive at least 1 dose of treatment” to the definitions of all the analysis sets.
- Updated the definition of the Per protocol population.
- Updated the analysis of categorical efficacy endpoints to use Fisher exact test instead of the CMH test.

APPENDICES

Appendix 1: Algorithm for Imputation of Start/End Date of Adverse Events and Prior/Concomitant Medications

Event Start Date Imputation

- Imputation of event end date should be done before imputation of event start date.
- Completely missing: Impute to the first study treatment date.
- Missing day and month: Impute to January 1st, unless year is the same as year of first study treatment dose then impute to the first study treatment date.
- Missing day: Impute to the 1st day of the month unless both the month and year are the same as the month and year of the first study treatment date, which then will impute to the first study treatment date.
- If the imputed event start date is after the event end date (imputed or not), the event start date will be set to the imputed event end date.

Event End Date Imputation

- Completely missing (and not flagged as "ongoing"): Impute to the last contact date.
- Missing day and month: Impute to December 31st, unless the year is the same as the last contact date which then will impute to the last contact date.
- Missing day: Impute to the last day of the month unless both the year and month are the same as the year and month of the last contact date which then will impute to the last contact date.

Appendix 2: Sample R Codes for Primary Analysis

R version 4.3.1 and "sas7bdat", "dplyr", "DoseFinding", "MCPMod", "mmrm", "nlme", "lme4" packages will be used to generate the outputs:
Model parameters:

For the MCPMod approach, the candidate gel concentration-response models to be applied are given follows:

- Linear model
- Quadratic model with maximum effect reached at 3.5%, the model parameter is -0.1428571
- Exponential model which reaches 20% of the effects at 2.5%, the model parameter is 1.803368
- E_{max} model with ED50 = 2.5%
- Sigmoid Emax model with ED50=2.5% and Hill's parameter = 2.5
- Logistic model which reaches 20% and 95% effects at concentrations 2.5% and 5%, respectively, the model parameters are ED50% = 3.3002654, delta = 0.5772694

Specify model parameters as following:

```
quad<-guesst(d=3.5,p=1,"quadratic")
exp<-guesst(d=2.5,p=0.2,"exponential",Maxd=5)
emax<-guesst(d=2.5,p=0.5,"emax")
sigemax<-guesst(d=c(1.25,2.5),p=c(0.2,0.5),"sigEmax")
logis<-guesst(d=c(2.5,5),p=c(0.2,0.95),"logistic")
models<-Mods(linear=NULL,quadratic=quad,exponential = exp,emax=emax,sigEmax
=sigemax,logistic=logis,doses=c(0,1.25,2.5,5))
```

The optimal concentration is defined as the highest concentration of ASN008 used in this clinical trial without safety concerns for linear and exponential models, or the lowest concentration which maintains 90% of the maximum effect for the rest of candidate models.
Optimal concentration:

```
ED(fit,0.9)
```