STATISTICAL ANALYSIS PLAN

Protocol Number: A-101-DPN-201

A Phase 2 Open Label, Single Arm Pilot Study of A-101 Topical Solution in Subjects with Dermatosis Papulosa Nigra

STATISTICAL ANALYSIS PLAN - TEXT

Title: A Phase 2 Open Label, Single Arm Pilot Study of A-101 Topical Solution in Subjects with Dermatosis Papulosa Nigra

Protocol:	**A-101-DPN-201**
Study Drug:	A-101 Solution, 40%
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List of Abbreviations

Abbreviations/Acroynms

AE	adverse event
ALT	alanine aminotransferase
ANCOVA	analysis of covariance
AST	aspartate aminotransferase
ATC	antomical therapeutic chemical
BUN	blood urea nitrogen
CS	clinically significant
CRF	case report form
DPN	dermatosis papulosa nigra
FST	Fitzpatrick skin type
ITT	intent-to-treat
LDH	lactate dehydrogenase
LSR	local skin reaction
Max	maximum
MedDRA	Medical Dictionary for Regulatory Activities
Min	minimum
mm	millimeters
NCS	non-clinically significant
PLA	physician's lesion assessment
PP	per-protocol
PT	preferred term
SAE	serious adverse event
SSA	subject self-assessment
Std Dev	standard deviation
SOC	system organ class
TEAE	treatment-emergent adverse event
TESAE	treatment-emergent serious adverse event

1 STUDY OBJECTIVES AND SAMPLE SIZE RATIONALE

1.1 STUDY OBJECTIVES

This study is to assess safety and efficacy of A-101 Solution 40% when applied to up to 4 dermatosis papulosa nigra (DPN) Target Lesions in subjects with Fitzpatrick Skin Type (FST) 5 or 6.

There are 2 cohorts in the study. The first cohort of the study will enroll a total of 12 subjects (6 subjects with a FST of 5 and 6 subjects with a FST of 6). The second cohort of the study a total of 24 subjects will be a randomized to a two-arm cohort:

- A-101 40% without medically abrading the identified DPN prior to treatment
- A-101 40% with the identified DPN lesions medically abraded prior to treatment

Primary and Secondary Objectives are summarized below.

1.1.1 Primary Objective

The main objective of this study is to evaluate the safety and efficacy of hydrogen peroxide, A-101 Solution 40% for the treatment of DPN lesions on subjects with a FST of 5 or 6.

1.1.2 Secondary Objectives

The secondary objective of this study is to assess durability of response.

1.1.3 Exploratory Objective

An exploratory objective of this study is to evaluate the subject's assessment of the treatment with A-101 to DPN lesions using a Subject Self-Assessment Scale.

1.1.4 Safety Assessments

Safety will be evaluated based on clinical laboratory studies (hematology and clinical chemistry), vital signs, urine pregnancy tests, assessment of local skin reactions (LSRs), assessment of adverse events (AEs), and concomitant medication review.

1.2 SAMPLE SIZE

A total of 36 subjects are planned to be enrolled/randomized in the study.

2 STUDY DESIGN SUMMARY

All enrolled/randomized subjects will receive at least one application of A-101 40% solution on up to four target DPN lesions on the subject's face or neck. Subjects in cohort 1 may receive a second application of A-101 40% Solution if the lesions meet the criteria for retreatment at Visit 4 (Day 22). Subjects in cohort 2 may receive up to 3 treatment applications if the lesions meet the criteria for retreatment at Visit 3 (Day 15) and Visit 5 (Day 29). All subjects will be followed on study protocol until Visit 8 (Day 106).

Protocol provides a more detailed description of the study.

2.1 VISITS

The protocol defined study visits for cohort 1 are:

- Visit 1 (Day -13 to 0) screening
- Visit 2 (Day 1) enrollment and study medication treatment
- Visit 3 (Day 8) follow up visit
- Visit 4 (Day 22) follow up visit and second application of study medication if DPN target lesions meet criteria for retreatment
- Visit 5 (Day 29) follow up visit
- Visit 6 (Day 50) follow up visit
- Visit 7 (Day 78) follow up visit;
- Visit 8 (Day 106) follow up visit; end of study

The protocol defined study visits for cohort 2 are:

- Visit 1 (Day -13 to 0) screening
- Visit 2 (Day 1) randomization and study medication treatment
- Visit 3 (Day 15) follow up visit and second application of study medication if DPN target lesions meet criteria for retreatment
- Visit 4 (Day 22) follow up visit
- Visit 5 (Day 29) follow up visit and third application of study medication if DPN target lesions meet criteria for retreatment
- Visit 6 (Day 50) follow up visit
- Visit 7 (Day 78) follow up visit;
- Visit 8 (Day 106) follow up visit; end of study

Study procedures for subjects in cohort 1 and 2 are detailed in the following two tables.

Table 1: Study Procedures for Cohort 1 Subjects

Visit	V1 Screening	V2	V3	V4	V5	V6	V7	V8
Treatment Day	-13 to 0	1	8	22	29	50	78	106
Treatment Window	N/A	N/A	+ 7 days	+ 4 days	+7 days	± 7days	± 7 days	± 7 days
Study Procedures								
Informed Consent	X							
Inclusion Criteria/Exclusion Criteria	X	X[1]						
Subject Identifier	X[2]							
Medical history/demographics	X							
Fitzpatrick Skin Type Assessment	X[3]							
Vital Signs	X	X[4]		X				X
Prior Medications/Therapies	X[5]							
Clinical Chemistry and CBC[6]	X							X
Urine Pregnancy Test[7]	X	X						X
Target Lesion Identification[8]	X							
Physician's DPN Lesion Assessment[9]	X	X		X		X	X	X
Subject Self-Assessment Scale[10]	X	X		X		X	X	X
Lesion Dimensions[11]	X	X		X		X	X	X
Standardized Photography[12]	X	X		X		X	X	X
Subject Enrollment		X[13]						
Local Skin Reactions		X[14]	X	X[14]	X	X	X	X
Study Medication Application		X[15]		X[15]				
Wound Care[16]		X		X				
Subject Instructions	X	X	X	X	X	X	X	X
Concomitant therapies[17]		X	X	X	X	X	X	X
Adverse Events[18]		X	X	X	X	X		

[1] Subject inclusion/exclusion criteria will be re-assessed prior to study enrollment during Visit 2.
[2] Investigational sites will assign a unique five-digit subject identifier to each subject at Visit 1. This subject identifier will be used in all study documentation for the duration of the study.
[3] Each subject's skin must be assessed during Visit 1 using the Fitzpatrick Skin Type Assessment. Refer to Protocol Section 9.5.1 for the scale.

[4] Vital signs [including temperature, pulse, respiratory rate, blood pressure, and weight (Visit 1 only)] will be measured by a qualified staff member at Visit 1, Visit 2 prior to study enrollment, and at Visit 8.
[5] Prior medications/therapies will be collected for a time-period of 13 days prior to Visit 2. Refer to Protocol Section 7.7 for a list of permitted and restricted concomitant medications.
[6] A complete blood count (including hematocrit, hemoglobin, platelet count, red blood cell count, white blood cell count and differential (absolute and %) including basophils, eosinophils, lymphocytes, monocytes and neutrophils and a clinical chemistry panel including albumin, alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), bicarbonate, calcium, chloride, creatinine, glucose, lactate dehydrogenase (LDH), phosphorus, potassium, sodium, total bilirubin, total protein, and uric acid.
[7] Woman of child bearing potential will be required to have a urine pregnancy test at Visit 1, at Visit 2 prior to enrollment and at Visit 8.
[8] The treating investigator will identify up to 4 Target DPN Lesions. Each lesion that is identified to be treated must be in an inconspicuous area.
[9] The investigator will assess each DPN target lesion based on a 4- point Physician's DPN Lesion Assessment Scale. In order to be eligible for enrollment at Visit 2, the subject must have at least one DPN lesion that is between 2mm and 5 mm in diameter. At Visit 2 and if applicable at Visit 4, the investigator must assess each DPN lesion that is to be treated prior to application of the study medication.
[10] Subjects will use a Subject Self-Assessment Scale to assess each Target DPN Lesion at Screening Visit 1, at Visit 2 prior to application of the study medication, at Visit 4 (prior to study medication, if applicable), at Visit 6, Visit 7 and Visit 8.
[11] The investigator will measure the diameter and thickness of each identified Target DPN Lesions at Visit 1 and prior to enrollment. at Visit 2. At Visit 4 prior to retreatment (if applicable, at Visit 6, Visit 7 and at Visit 8 the investigator will only measure the diameter of each Target DPN Lesion.
[12] At Visits 1, Visit 2 (prior to study medication application), Visit 4 prior to study medication application if applicable, Visit 6, Visit 7, and at Visit 8, a qualified investigational center staff member will take a photograph of each DPN lesion that has been treated using the Aclaris supplied camera. All photographs will be sent to a central imaging laboratory.
[13] Subjects will be enrolled to the study at Visit 2 prior to application of study medication. Investigational study staff will re-confirm subject eligibility prior to study enrollment.
[14] Both the investigator and the subject will assess each Target DPN Lesion for symptoms associated with irritation. At Visit 2 and Visit 4, the investigator will assess the Target DPN Lesions prior to application of the study medication and 20 (± 4) minutes after treatment with the study medication. At Visit 2 and Visit 4, the subject will assess the Target DPN Lesions prior to application of the study medication and 10 (±4) minutes after the treatment of the study medication.
[15] A-101 study medication will be applied by the treating physician. All Target DPN Lesions will be treated with study medication following enrollment at Visit 2. If a Target DPN Lesion meets the criteria for re-treatment as defined in Section 7.5, the lesion will be re-treated at Visit 4. Following application of study medication, subjects must NOT wash/submerge the Target DPN Lesions for at least 6 hours and they must NOT apply any topical products to the Target DPN Lesions for at least 6 hours.
[16] All subjects will be required to apply Aquaphor to all Target DPN Lesions the morning after study medication application. Sites will be provided supplies of Aquaphor by the Sponsor.
[17] All concomitant therapies including (topical and oral) prescription medications, over the counter medications and natural supplements and non-drug therapies including chiropractic, physical therapy, and energy-based therapy must be documented in the subject CRF. Subjects must not apply any topical products (e.g. moisturizers, sunscreen, etc.) to their Target Lesions within 12 hours prior to any study visit.

[18] The reporting period for Serious Adverse Events (SAEs) begins when the subject signs the informed consent. Refer to Section 10 for instructions on the reporting of SAEs. Non-serious clinical adverse events will be collected following the application of the study medication at Visit 2. Non-serious adverse events that occur between the time of consent and study medication application will be documented as medical history. All safety reporting (AEs and SAEs) will conclude at Visit 5 (approximately 21 days after last study medication application) except for clinical adverse events related to local skin reactions. These events will be collected through V8.

Table 2: Study Procedures for Cohort 2 Subjects

Visit	V1 Screening	V2	V3	V4	V5	V6	V7	V8
Treatment Day	-13 to 0	1	15	22	29	50	78	106
Treatment Window	N/A	N/A	+ 4 days	+ 2 days	+4 days	± 7days	± 7 days	± 7 days
Study Procedures								
Informed Consent	X							
Inclusion Criteria/Exclusion Criteria	X	X[1]						
Subject Identifier	X[2]							
Medical history/demographics	X							
Fitzpatrick Skin Type Assessment	X[3]							
Vital Signs	X	X[4]						X
Prior Medications/Therapies	X[5]							
Clinical Chemistry and CBC[6]	X							X
Urine Pregnancy Test[7]	X	X						X
Target Lesion Identification[8]	X							
Physician's DPN Lesion Assessment[9]	X	X	X	X	X	X	X	X
Subject Self-Assessment Scale[10]	X	X	X	X	X	X	X	X
Lesion Dimensions[11]	X	X	X	X	X	X	X	X
Standardized Photography[12]	X	X	X	X	X	X	X	X
Subject Randomization		X[13]						
Local Skin Reactions		X[14]	X[14]	X	X[14]	X	X	X
Study Medication Application		X[15]	X[15]		X[15]			
Wound Care[16]		X	X		X			
Subject Instructions	X	X	X	X	X	X	X	X
Concomitant therapies[17]		X	X	X	X	X	X	X
Adverse Events[18]		X	X	X	X	X		

[1] Subject inclusion/exclusion criteria will be re-assessed prior to study enrollment during Visit 2.
[2] Investigational sites will assign a unique five-digit subject identifier to each subject at Visit 1. This subject identifier will be used in all study documentation for the duration of the study.
[3] Each subject's skin must be assessed during Visit 1 using the Fitzpatrick Skin Type Assessment. Refer to Protocol Section 9.5.1 for the scale.

[4] Vital signs [including temperature, pulse, respiratory rate, blood pressure, and weight (Visit 1 only)] will be measured by a qualified staff member at Visit 1, Visit 2 prior to study enrollment, and at Visit 8.
[5] Prior medications/therapies will be collected for a time-period of 13 days prior to Visit 2. Refer to Protocol Section 7.7 for a list of permitted and restricted concomitant medications.
[6] A complete blood count (including hematocrit, hemoglobin, platelet count, red blood cell count, white blood cell count and differential (absolute and %) including basophils, eosinophils, lymphocytes, monocytes and neutrophils and a clinical chemistry panel including albumin, alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), bicarbonate, calcium, chloride, creatinine, glucose, lactate dehydrogenase (LDH), phosphorus, potassium, sodium, total bilirubin, total protein, and uric acid.
[7] Woman of child bearing potential will be required to have a urine pregnancy test at Visit 1, at Visit 2 prior to enrollment and at Visit 8.
[8] The treating investigator will identify up to 4 Target DPN Lesions.
[9] The investigator will assess each DPN target lesion based on a 4-point Physician's DPN Lesion Assessment Scale. In order to be eligible for enrollment at Visit 2, the subject must have at least one DPN lesion that is between 2mm and 5 mm in diameter. The investigator must assess each DPN lesion that is to be treated prior to application of the study medication at Visit 2, if applicable at Visit 3 and Visit 5.
[10] Subjects will use a Subject Self-Assessment Scale to assess each Target DPN Lesion at Screening Visit 1, Visit 2 (prior to application of the study medication), Visit 3 (prior to study medication, if applicable), Visit 4, Visit 5 (prior to study medication, if applicable), Visit 6, Visit 7 and Visit 8.
[11] The investigator will measure the diameter and thickness of each identified Target DPN Lesions at Visit 1 and prior to enrollment. at Visit 2. At Visit 3 (prior to treatment, if applicable), Visit 4, Visit 5 (prior to retreatment if applicable), Visit 6, Visit 7 and Visit 8 the investigator will only measure the diameter of each Target DPN Lesion.
[12] At Visits 1, Visit 2 (prior to study medication application) Visit 3 (prior to study medication application, if applicable), Visit 4, Visit 5 (prior to study medication application if applicable), Visit 6, Visit 7, and Visit 8, a qualified investigational center staff member will take a photograph of each DPN lesion that has been treated using the Aclaris supplied camera. All photographs will be sent to a central imaging laboratory.
[13] Subjects will be enrolled to the study at Visit 2 prior to application of study medication. Investigational study staff will re-confirm subject eligibility prior to study enrollment.
[14] Both the investigator and the subject will assess each Target DPN Lesion for symptoms associated with irritation. At Visit 2, Visit 3 and Visit 5, the investigator will assess the Target DPN Lesions prior to application of the study medication and 20 (± 4) minutes after treatment with the study medication, if applicable. At Visit 2, Visit 3, and Visit 5, the subject will assess the Target DPN Lesions prior to application of the study medication and 10 (±4) minutes after the treatment of the study medication, if applicable.
[15] A-101 study medication will be applied by the treating physician. All Target DPN Lesions will be treated with study medication following enrollment at Visit 2. Subjects randomized to ARM B will be required to have their Target DPN Lesions medically abraded prior to treatment with A-101 study medication. If a Target DPN Lesion meets the criteria for re-treatment as defined in Section 7.5, the lesion will be re-treated at Visit 3, and Visit 5. Following application of study medication, subjects must NOT wash/submerge the Target DPN Lesions for at least 6 hours and they must NOT apply any topical products to the Target DPN Lesions for at least 6 hours.
[16] All subjects will be required to apply Aquaphor to all Target DPN Lesions the morning after study medication application. Sites will be provided supplies of Aquaphor by the Sponsor.

[17] All concomitant therapies including (topical and oral) prescription medications, over the counter medications and natural supplements and non-drug therapies including chiropractic, physical therapy, and energy-based therapy must be documented in the subject CRF. Subjects must not apply any topical products (e.g. moisturizers, sunscreen, etc.) to their Target Lesions within 12 hours prior to any study visit.

[18] The reporting period for Serious Adverse Events (SAEs) begins when the subject signs the informed consent. Refer to Section 10 for instructions on the reporting of SAEs. Non-serious clinical adverse events will be collected following the application of the study medication at Visit 2. Non-serious adverse events that occur between the time of consent and study medication application will be documented as medical history. All safety reporting (AEs and SAEs) will conclude at Visit 5 (approximately 21 days after last study medication application) except for clinical adverse events related to local skin reactions. These events will be collected through V8.

2.2 SUBJECTS

2.2.1 Number of Subjects

A total of 36 subjects will be treated on the study at 2 investigational sites in US.

2.2.2 Diagnosis and Main Criteria for Inclusion

Adult subjects with up to 4 DPN Target Lesions with FST 5 or 6.

2.2.3 Study Population Characteristics

Subjects with a clinical diagnosis of DPN who meet all the inclusion criteria and none of the exclusion criteria will be eligible to enroll/randomize in the study. Detailed inclusion and exclusion criteria, see protocol sections 5.1 and 5.2.

2.2.4 Replacement Subjects

If a subject is enrolled to the study but does not receive a dose of study drug, then the subject will be replaced.

2.3 TREATMENT AND RANDOMIZATION

2.3.1 Study Medications, Treatment, and Mode of Administration

A-101 Solution 40% is hydrogen peroxide that will be supplied in a glass ampule with an applicator to be applied to a DPN lesion.

2.3.2 Randomization

Cohort 1 of this study is an open label study. Subject randomization is not applicable for this part of the study.

Subjects that are determined to be eligible for treatment in Cohort 2 will be randomized in a 1:2 ratio to one of the following treatment arms.

- ARM A: A-101 40% Solution; No medical abrasion to the Target DPN Lesions Treatment Days 1, 15 and 29
- ARM B: A-101 40% Solution applied after medically abrading the Target DPN Lesions; Treatment Days 1, 15 and 29

2.3.3 Administration of Treatment

The study medications are for external, topical use on the DPN Target Lesions on the appropriate study subject only.

The treating investigator performing the study medication treatments must comply with the study medication handling warnings. In cohort 1, the treating physician will apply the A-101 Solution 40% to each DPN Target Lesion at Visit 2 and at Visit 4, if applicable.

At Visit 4, any Target Lesion that has a Physician DPN Lesion Assessment **grade of >0** and **ONLY DPN Target Lesions that have a Physician DPN Lesion Assessment grade of >0**, must receive study medication treatment UNLESS either of the following criteria apply to the DPN Target Lesion:

- The DPN Target Lesion has a Visit 4 pre-treatment LSR grade of 3 (severe) for any sign or symptom AND the grade has increased compared to the Visit 3
- The DPN Target Lesion is, in the investigator's opinion, not appropriate for a retreatment (the investigator must note the reason on the subject's Comments CRF page).

In cohort 2, for those subjects randomized to ARM B, the treating physician will medically abrade the Target DPN Lesion prior to apply the A-101 study medication. All subjects in cohort 2 will be allowed to have up to 3 treatment applications of the A-101 study medication. If subjects meet the criteria as outlined above, subjects in cohort 2 may receive 2 additional study medication applications at Visit 3 (Day 15) and Visit 5 (Day 29).

2.3.4 Duration of Treatment

The duration of the study participation is anticipated to be a maximum of 127 days per subject. The final visit (Visit 8), has a maximum allowable visit window of 7 days.

2.4 ASSESSMENTS

2.4.1 Evaluators

The investigator, a designated and appropriately trained staff member (e.g., sub investigator) or the subject will perform the study assessments according to the defined schedules.

2.4.2 Evaluations – Efficacy

2.4.2.1 Physician's DPN Lesion Assessment

The Physician's DPN Lesion Assessment is the investigator's assessment of the severity of the Target DPN Lesion at a particular time point. The investigator should NOT refer to any other assessments to assist with these assessments.

At Visits 1, 2, 3 (cohort 2 ONLY), 4 (cohort 1 ONLY), 5 (cohort 2 ONLY), 6, 7 and 8, the investigator will assess the Target DPN Lesion using the scale below and report the one integer that best describes the severity of the Target DPN Lesion. At Visit 2, and if appropriate at Visit 4 for cohort 1 subjects or Visit 3 and Visit 5 for cohort 2 subjects, the investigator must complete the Physician's DPN Lesion Assessment prior to the study medication treatment.

Table 3: Physician's DPN Lesion Assessment Definitions

Grade	Descriptor
0	Clear: no visible DPN lesion;
1	Near Clear: a slightly visible DPN lesion; lesion may be macular
2	Small: a visible DPN lesion with a diameter of less than 3 mm
3	Large: a visible DPN lesion that is elevated with a diameter of ≥3 mm

At Visit 1 and Visit 2, for the subject to be eligible for enrollment to the study the identified DPN lesions must have a Physician's Lesion Assessment of grade ≥ 2.

2.4.2.2 Lesion Dimensions

At Visit 1 and at Visit 2 prior to enrollment the investigator will be required to measure the diameter and thickness of each Target DPN Lesion. For subjects enrolled to cohort 1 the investigator will measure the diameter of each Target DPN Lesion using the ruler at Visit 4 (prior to retreatment, if applicable), Visit 6, Visit 7 and Visit 8. For subjects randomized to cohort 2, the investigator will measure the diameter of each Target DPN Lesion at Visit 3 (prior to retreatment if applicable), Visit 4, Visit 5 (prior to retreatment if applicable) Visit 6, Visit 7 and Visit 8.

At Visit 1 for the subject to be enrolled and at Visit 2 for the subject to be enrolled each Target DPN Lesion must have:

- A diameter that is between 2 mm and less than 5 mm.
- A height or thickness that is ≤ 2 mm.

2.4.2.3 Subject Self-assessment Scale

For subjects enrolled to cohort 1, each subject will use the Subject Self-Assessment Scale to assess each Target DPN Lesions at the following visits: Visit 1, Visit 2 (prior to treatment), Visit 4 (prior to treatment), Visit 6, Visit 7 and Visit 8. For subjects randomized to cohort 2, each subject will use the Subject Self-Assessment Scale to assess each Target DPN Lesion at the following visits: Visit 1, Visit 2 (prior to treatment), Visit 3 (prior to retreatment, if applicable), Visit 4, Visit 5 (prior to retreatment, if applicable) Visit 6, Visit 7 and Visit 8.

Table 4: Subject Self-Assessment Scale

Grade	Descriptor
0	No Visible DPN Lesion
1	Mild; Slightly raised DPN lesion
2	Moderate; Obviously raised DPN lesion
3	Severe; Prominent DPN lesion

2.4.3 Evaluations – Safety

2.4.3.1 Local Skin Reaction (LSR)

The LSR assessment is the investigator's assessment of the signs and the subject's assessment of the symptoms associated with irritation at each Target DPN Lesion site, which includes the Target DPN Lesion and the area immediately surrounding the Target DPN Lesion.

At Visits 2-8, the investigator and the subject will evaluate the LSR signs and the LSR symptoms at each Target DPN Lesion site respectively.

The investigator will assess the LSR signs as follows:

- Visits 2 and 4 (cohort 1) and Visits 2, 3 and 5 (cohort 2):
    - For each Target DPN Lesion site report the severity for all signs prior to any study medication treatment
    - For every treated Target DPN Lesion site, 20 (±4) minutes after the Treatment Completion Time, report the severity for the following signs:
        - Erythema
        - Edema
        - Scaling/dryness
        - Vesicles/bullae
- Visits 3 and 5-8 (cohort 1) and Visits 4 and 6-8 (cohort 2):
    - For each Target DPN Lesion site, report the severity for all signs.

The subject will assess the LSR symptoms as follows:

- Visits 2 and 4 (cohort 1) and Visits 2, Visit 3 and Visit 5 (cohort 2):

  - For each Target DPN Lesion site report the average of the severity over the previous 24 hours for all symptoms prior to any study medication treatment.
  - For every treated Target DPN Lesion site, 10 (±4) minutes after the Treatment Completion Time, report the average of the severity of the LSR for all symptoms since completion of the study medication treatment.
- Visits 3 and 5-8 (cohort 1) and Visits 4 and 6-8 (cohort 2):
  - For each Target DPN Lesion site, report the average of the severity over the previous 24 hours for all symptoms.

Both the subject and the study staff member will initial and date the source document to indicate the subject performed the LSR for symptoms as instructed. The staff member must not influence the subject's assessment.

The investigator should report the one integer that best describes the severity of each LSR sign for each Target Lesion site using the scale below. Each subject should report the one integer that best describes the severity of each LSR symptom for each Target Lesion site using the scale below:

Table 5: Grading of Local Skin Reactions

Grade	Descriptor
0	None
1	Mild
2	Moderate
3	Severe

2.4.3.2 Adverse Events

Non-serious AEs will be recorded starting with the subject's first study medication treatment at Visit 2 and continuing through Visit 6. All SAEs regardless of relationship to study medication will be collected and reported from the time the Informed Consent is signed through Visit 6.

2.4.3.3 Laboratory Sampling

Non-fasting blood samples for clinical laboratory analysis will be collected by a qualified staff member at Visit 1 and at Visit 8. Approximately 7.5 mL of blood will be collected for each chemistry sample and 3ml of blood will be collected for the complete blood count (CBC). These blood samples will be sent to a central laboratory for analysis. Refer to the study specific laboratory manual for instructions regarding handling of the blood samples and shipping instructions.

Laboratory tests (minimum) are as follows:

Chemistry	Hematology
Albumin	Hematocrit
Alkaline phosphatase	Hemoglobin
Alanine aminotransferase	Platelet count
Aspartate aminotransferase	Red blood cell morphology
Blood urea nitrogen	Red blood cell count
Bicarbonate	White blood cell count
Calcium	White blood cell differential
Chloride	% & absolute
Creatinine	Basophils
Glucose	Eosinophils
Lactate dehydrogenase	Lymphocytes
Phosphorus	Monocytes
Potassium	Neutrophils
Sodium	
Total bilirubin	
Total protein	
Uric acid	

2.4.3.4 Vital Signs

Vital signs will be measured by a qualified staff member at Visit 1, Visit 2 prior to randomization, Visit 4 (cohort 1 ONLY) prior to treatment and at Visit 8. The following items will be measured:

- Body temperature
- Pulse rate
- Respiration rate
- Blood pressure (systolic and diastolic) after the subject sits quietly for at least 5 minutes
- Height (at Visit 1 only)
- Weight (at Visit 1 only)

Any measure that is, in the opinion of the investigator, abnormal AND clinically significant (CS) must be recorded as history if found prior to the first study medication treatment or as an AE if found after the first study medication treatment begins.

A systolic blood pressure >140mm Hg or a diastolic blood pressure >90mm Hg is considered abnormal and therefore must be defined as CS or non-clinically significant (NCS) on the Case Report Forms (CRFs).

2.4.4 Other Evaluations

At Visit 1, the investigator or designee will collect demographic information including date of birth, sex at birth, race, ethnicity, and FST for each subject.

Table 6: Fitzpatrick Skin Type (FST) Scoring System

Skin Type Classification	Description
Type I	Always burns, never tans (pale white; blond or red hair; blue eyes; freckles)
Type II	Usually burns, tans minimally (white; fair; blond or red hair; blue, green, or hazel eyes)
Type III	Sometimes mild burn, tans uniformly (cream white; fair with any hair or eye color)
Type IV	Burns minimally, always tans well (moderate brown)
Type V	Very rarely burns, tans very easily (dark brown)
Type VI	Never burns, never tans (deeply pigmented dark brown to darkest brown)

3 STATISTICAL METHODOLOGY

This plan describes methods planned for the analysis and display of efficacy and safety endpoints. Summaries will be based on mainly descriptive summaries for each assessment.

3.1 GENERAL STATISICAL CONSIDERATIONS

Descriptive statistics (mean, standard deviation [Std Dev], median, minimum [Min], and maximum [Max]) will be used for continuous variables; number and percentage of subjects will be used for discrete variables. In general, the last measurement prior to the first dose of study treatment will be used as the baseline value. Nominal visits will be used for by-visit summaries and analyses.

All treatment group comparisons will be made at the 0.05 level using a two-sided hypothesis test, unless otherwise specified.

Summaries will be presented by each arm, ie, Cohort 1 FST 5, Cohort 1 FST 6, Cohort 2 Arm A, Cohort 2 Arm B, and Total (combining both cohorts subjects).

All tabulations of summary statistics, graphical presentations, and statistical analyses will be performed using SAS® Version 9.2 or higher.

3.2 ANALYSIS POPULATION AND DISPOSITION OF SUBJECTS

3.2.1 Data Sets Analyzed

Per-protocol (PP) analysis set

All treated subjects completing all treatment visits and Visit 8 with no major protocol violations.

Intent-to-Treat (ITT) analysis set

Intent-to-Treat (ITT) analysis set includes all treated subjects with at least one post-baseline visit.

Safety analysis set

The Safety Analysis Set includes all treated subjects.

3.2.2 Disposition of Subjects

The numbers of subjects screened (ie, signed informed consent), enrolled/randomized, treated, and discontinued from treatment (by reason), will be summarized for each cohort separately.

3.3 DEMOGRAPHIC AND OTHER BASELINE CHARACTERISTICS

Demographic and baseline characteristics will be summarized for the PP and Safety analysis sets. Gender, race, ethnicity, and FST will be summarized with number and percentage presented for each category. Age, height, weight will be described with summary statistics (n, mean, Std Dev, median, minimum, and maximum).

3.4 MEDICAL HISTORY

Medical history will be summarized by body system class using the Safety analysis sets.

3.5 PRIOR/CONCOMITANT MEDICATIONS

The number and percentage of prior medications, and concomitant medications will be summarized by Antomical Therapeutic Chemical (ATC) and preferred term (PT) separately using Safety analysis set.

Medications taken any time prior to the first study treatment are counted as prior medications. Those taken any time on or after the first application of study medication through 30 days after the last application of study are counted as concomitant.

Subject will be counted only once within each classification. The same subject may contribute to two or more preferred terms within the same ATC classification.

3.6 EFFICACY ANALYSIS

All efficacy analyses and summaries will be performed on the PP analysis population by randomized treatment group. The primary efficacy analysis will be repeated on the ITT population.

3.6.1 Physician's Leasion Assessment (PLA) Analyses

PLA related parameters will be summarized at lesion level and at subject level.

At each visit or applicable visit, the following analyses will be performed:

- Descriptive statistics will be provided for the per-subject mean change from baseline in PLA for each post-baseline scheduled visit separately for the PP population.

- Descriptive statistics will be provided for the per-subject mean change from baseline in PLA for each post-baseline scheduled visit separately for the ITT population.
- The number and percent of subjects having all lesions with PLA score of 0 will be provided for each post-baseline scheduled visit separately (PP population).
- The number and percent of subjects having all lesions with PLA score <= 1 will be provided for each post-baseline scheduled visit separately (PP population).

The following treatment group comparisons will be performed for PLA endpoints from Cohort 2:

- Tretment group comparisons will be made for the change from baseline in per-subject mean PLA score at each post-baseline scheduled visit for the PP population. These comparisons will be made using a repeated measures mixed model where treatment group and time (visit) are included as class variables and baseline per-stubject mean PLA score and number of treated lesions will be included as continuous covariates. The per-subject PLA measures over time will be treated as repeated measures by including subject as a random effect. An unstructured variance covariance matrix will be assumed. If the model experiences convergence issues with the unstructured variance covariance assumption, then a compound symmetric assumption will be used. The difference in least square means for the treatment groups along with corresponding 95% confidence intervals and p-values will be provided for each of the scheduled visits. A plot of the treatment group least square means (and 95% confidence intervals) across time (visit) will be provided.
- Tretment group comparisons will be made for the change from baseline in per-subject mean PLA score at each post-baseline scheduled visit for the ITT population using LOCF for missing data. The model for analysis and output will match that of the PP population analysis and output described above.
- A logistic regression will be used to make treatment comparions in the proportion of subjects having all lesions with PLA score of 0 (PP population). This analysis will be perfomed at each scheduled visit separately. This model will include a class variable for treatment group as well as baseline per-subject mean PLA score and number of treated lesions as a continuous covariate. Model based odds ratios, corresponding 95% confidence intervals and p-values will be provided at each scheduled visit.
- Similarly, a logistic regression will be used to make treatment comparions in the proportion of subjects having all lesions with PLA score of <= 1. This model and output will be the same as described for the PLA score = 0 analysis.

3.6.2 Subject Self Assessment (SSA) Analyses

Descriptive statistics will be provided for the per-subject mean change from baseline in the subject self-assessment scale for each post-baseline scheduled visit separately.

3.7 SAFETY ANALYSIS

The safety and tolerability of the investigational products will be determined by LSR, reported AEs, laboratory tests and vital signs. The Safety analysis set with actual treatment received (as opposed to randomized treatment) will be used for all safety summaries, unless otherwise specified.

3.7.1 Study Drug Exposure And Compliance

Duration of treatment (Last study treatment application date – First study treatment application date + 1) will be summarized descriptively using the Safety analysis set. Number and percentage of subjects at each visit will be summarized by the number of target/non-target lesions being treated. In addition, number and percentage of subjects with re-treatment will be summarized for the applicable visits (Visit 4 for cohort 1 subjects, and Visits 3 and 5 for cohort 2 subjects).

3.7.2 Local Skin Reaction (LSR)

For each visit, all predefined signs and symptoms at pre-medication and post-medication will be summarized by maximum severity cross all lesions for a subject. Number and percentage of subjects with grades 1, 2, 3, 4 will be tabulated for each sign (investigator)/symptom (subject) and at each visit. In addition, the maximum severity for each sign and symptom will also summarized cross all visits. Similar summaries will be presented for lesions. That is, number and percentage of lesions with grades 1, 2, 3, 4 will be tabulated for each sign and symptom at each visit and over all visits.

For treatment visits (ie, Visits 2 and 4 [cohort 1] and Visits 2, 3 and 5 [cohort 2]), for each sign (investigator assessment: Erythema, Edema, Scaling/dryness, Vesicles/bullae) or symptom (subject assessment: Stinging/burning, Pruritus [itch]) will be summarized using shift table. Severity grade shift from pre-medication to post-medication will be analyzed. Number and percentage of lesions with shifts will be presented. Number of treated lesions will be used as the denominator for percentage calculation.

3.7.3 Adverse Events

All subjects will be assessed regularly for the potential occurrence of adverse events (AEs) from the date of informed consent to Visit 6. The incidence of treatment-emergent AEs (TEAEs) will be defined for new or worsening events from the first study treatment until 30

days after the last study treatment. Summaries will be conducted using MedDRA (version 21.1), by System Organ Class (SOC) and Preferred Term (PT).

An overview of adverse events for the ITT analysis set will be provided, summarizing the incidence of the following:

- Count of TEAEs;
- Subjects with TEAEs;
- Count of Treatment-emergent serious AEs (TESAE);
- Subjects with TESAE;
- TEAE by maximum severity;
- TESAE by maximum severity;
- Subjects with TEAEs that lead to discontinuation of study;
- Subjects with TESAEs that lead to discontinuation of study;
- Subjects with Related TEAEs;
- Subjects with Related TESAEs.

3.7.4 Laboratory Tests

For each laboratory test, a summary will be provided for each visit. The absolute value and its change from baseline will be descriptively presented.

3.7.5 Vital Signs

Vital signs measurements include pulse rate, temperature, systolic blood pressure, and diastolic blood pressure. Measures at baseline and changes from baseline to post baseline visit will be summarized. The number and percentage of subjects with abnormal blood pressure will be summarized.

3.8 INTERIM ANALYSES

There is no interim analyses planned for the study.

4 CHANGES TO PLANNED ANALYSES

After communications with the FDA, the sponsor is no longer persuing a separate indication for in patients with DPN. Therefore, an abbreviated report will be produced for A-101-DPN-201. This abbreviated report will contain all the safety of a full report, but only the primary and key secondary efficacy analyses will be reported. As a result, the following descriptive statistics mentioned in the protocol will not be produced:

- Number and percentage of lesions by PLA score categories (clear, near-clear, small, and large): overall, separately for face or neck target lesions, separately for subjects with FST 5 and 6;
- Summary statistics on PLA score (mean of all lesions): overall and separately for subjects with FST 5 and 6;
- Summary statistics on percentage of lesions that are clear (PLA=0): overall, separately for face or neck target lesions, separately for subjects with FST 5 and 6;
- Summary statistics on percentage of lesions that are clear or near clear (PLA=0 or 1): overall, separately for face or neck target lesions, separately for subjects with FST 5 and 6;
- Number and percentage of lesions by location (face, or neck): overall and separately for subjects with FST 5 and 6.

Furthermore, the following hypothesis tests will not be performed:

- Change in percentage of lesions that are clear (PLA=0) from baseline evaluation to the last evaluation of the lesions ANCOVA model;
- Change in percent of lesions that are clear (PLA=0) or near-clear (PLA=1) from baseline evaluation to the last evaluation of the lesions ANCOVA model;
- Change in longest lesion diameter from baseline to the last lesion measurement ANCOVA model;

The protocol also states that efficacy summaries may be presented for the intent-to-treat (ITT) population using LOCF to impute missing data. The primary endpoint will be summarized and analyzed for the ITT population using LOCF to impute missing data, but none of the other efficacy endpoints will be summarized or analyzed for the ITT population.

Lastly, the ANCOVA model described in the protocol for the primary endpoint has been replaced with a repeated measures mixed model in order to capture changes over time in the primary efficacy endpoint and account for within subject correlation in the repeated measures over time. The number of treated lesions is also being added as a covariate in the primary efficacy model and the logistic regression model for responders which was not considered in the protocol.