CLINICAL STUDY PROTOCOL

A PHASE 1B/2, DOUBLE-BLIND, PLACEBO-CONTROLLED, RANDOMIZED, PARALLEL-ARM STUDY TO EXPLORE SAFETY, PHARMACOKINETICS, AND EARLY CLINICAL SIGNAL OF EFFICACY OF DS-2325A IN PATIENTS WITH NETHERTON SYNDROME

DS2325-119

IND NUMBER 157483

EU CT NUMBER 2022-502853-32-00

VERSION 3.0, 22 Jan 2024

DAIICHI SANKYO

211 Mount Airy Road, Basking Ridge 07920, USA

INVESTIGATOR AGREEMENT

A PHASE 1B/2, DOUBLE-BLIND, PLACEBO-CONTROLLED, RANDOMIZED, PARALLEL-ARM STUDY TO EXPLORE SAFETY, PHARMACOKINETICS, AND EARLY CLINICAL SIGNAL OF EFFICACY OF DS-2325A IN PATIENTS WITH NETHERTON SYNDROME

Investigator's Signature:

I have fully discussed the objectives of this study and the contents of this protocol with the Sponsor's representative.

I understand that information contained in or pertaining to this protocol is confidential and should not be disclosed, other than to those directly involved in the execution or the ethical review of the study, without written authorization from the Sponsor. It is, however, permissible to provide information to a patient in order to obtain consent.

I agree to conduct this study according to this protocol and to comply with its requirements, patient to ethical and safety considerations and guidelines, and to conduct the study in accordance with the Declaration of Helsinki, International Council for Harmonisation guidelines on Good Clinical Practice (ICH E6), and applicable regional regulatory requirements.

I agree to make available to Sponsor personnel, their representatives and relevant regulatory authorities, my patients' study records in order to verify the data that I have entered into the case report forms. I am aware of my responsibilities as an investigator as provided by the Sponsor.

I understand that the Sponsor may decide to suspend or prematurely terminate the study at any time for whatever reason; such a decision will be communicated to me in writing. Conversely, should I decide to withdraw from execution of the study, I will communicate my intention immediately in writing to the Sponsor.

Print Name	**Signature**
Title	**Date (DD MMM YYYY)**

DOCUMENT HISTORY

Version Number	Version Date
3.0	22 Jan 2024
2.0	05 Jun 2023
1.0	13 Feb 2023

SUMMARY OF CHANGES

Please refer to the comparison document for protocol Version 2.0 (dated 05 Jun 2023) versus protocol Version 3.0 (dated 22 Jan 2024) for actual changes in text. The summary of changes below is a top-line summary of major changes in the current DS2325-119 clinical study protocol (Version 3.0) by section.

Amendment Rationale:

The main purpose of this amendment is to revise the dose administration frequency and add guidance for the evaluation of injection site reactions and minor clarifications throughout the document.

CONVENTIONS USED IN THIS SUMMARY OF CHANGES

All locations (section numbers and/or paragraph/bullet numbers) refer to the current protocol version, which incorporates the items specified in this Summary of Changes document.

Minor edits, such as update to language that does not alter the original meaning, update to version numbering, formatting, change in font color, corrections to typographical errors, use of abbreviations, moving verbiage within a section or table, change in style, or change in case, are not noted in the table below.

Section # and Title	Description of Change	Brief Rationale
Protocol Synopsis: Study Objectives Section 2.1 Study Objectives	Modified the dose administration frequency to every other week for both primary and secondary objectives including the Main Phase and Extension Phase.	Change made to ensure consistency between dose administration frequency and study objectives.
Protocol Synopsis: Dosage Form, Dose and Route of Administration Section 1.4 Dose Selection and Justification of Dose Section 3.1.1 Overview	Modified the maintenance dose of 600 mg SC to be given biweekly. In addition, during both the Main Phase and Extension Phase, a 600 mg SC dose will also be given one week after the first dose of the phase, coinciding with one week before following biweekly doses.	Most patients find weekly visits too frequent to be compatible with their daily life commitments. Nonetheless, patients are inclined to accept an additional visit one week after the first dose of the Main Phase and Extension Phase, coinciding with one week before following biweekly doses.
Protocol Synopsis: Study Endpoints Section 2.3.3 Exploratory Endpoints Section 7.3 Biomarker Assessments	Corrected the endpoint from skin mRNA to skin concentration of mediators of skin inflammation.	Assessment of mRNA coding for mediators of skin inflammation and homeostasis is replaced by the direct assessment of these mediators as proteins.

Section # and Title	Description of Change	Brief Rationale
Protocol Synopsis: Study Endpoints Section 2.3.2 Secondary Endpoints Schedule of Events: Table 2 Appendix 16.3: Schedule of Events: Table 4	Corrected to epidermis-to-dermis DS-2325a concentration ratio instead of skin to plasma concentration ratio.	Clarification change.
Schedule of Events: Table 1 and Table 2 Appendix 16.3 Schedule of Events: Table 3 and Table 4	Modified the dose administration frequency to every other week. Modified the maintenance dose of 600 mg SC to be given biweekly. In addition, during both the Main Phase and Extension Phase, a 600 mg SC dose will also be given one week after the first dose of the phase, coinciding with one week before following biweekly doses.	Change made to ensure consistency between Schedule of Events tables and text.
Section 1.4 Dose Selection and Justification of Dose	Updated the area under the plasma concentration-time curve (AUC), maximum plasma concentration (C_{max}), and C_{ave} values	Change made in consideration of changed dose administration frequency.
Section 1.4 Dose Selection and Justification of Dose	Updated half-life values in healthy subjects for the 1000 mg IV dose and 600 mg SC dose.	Changes made to be consistent with the DS2325-104 CSR.
Section 4.2 Exclusion Criteria 4.2.1 Screening	Systemic treatment (#15) timeline updated to within 4 weeks before Screening.	4 weeks is deemed sufficient instead of 8 weeks.
Section 4.2 Exclusion Criteria 4.2.2 Baseline (Main Phase-Interventional Part)	Updated the Systemic treatment (#6) timeline to within 4 weeks before Baseline.	4 weeks is deemed sufficient instead of 8 weeks.
Section 5.6 Concomitant Medications	Corrected the header to "Concomitant Medications". Added clarification on the occasional and temporary use of corticosteroids.	Clarification change.
Section 5.7.2 Withdrawal Procedures	Minor clarification to withdrawal procedure.	Clarification change.

Section # and Title	Description of Change	Brief Rationale
Section 5.9 Dose Reduction	Minor edits to the frequency of dose administration.	Change made in consideration of changed dose administration frequency.
Protocol Synopsis: Study Endpoints Section 2.3.1 Primary Endpoint Section 8.1 Assessment of Safety Endpoints Section 8.4 Injection Site Reactions	Added a new section with instructions for patients and investigators on how to record injection site reactions as AEs.	Added section to increase accuracy, with the provision of evaluation guidance, of the recording of injection site reactions as AEs.
Section 8.9 Clinical Laboratory Evaluations	Deleted assessments of direct bilirubin and BUN.	Clarification change.
Schedule of Events: Table 1 and Table 2; footnote #6 Section 9 Efficacy Assessments Section 9.5 Skin Concentrations of Mediators of Skin Inflammation and Homeostasis Appendix 16.3 Schedule of Events: Table 3 and Table 4; footnote #6	Updated to provide clarification on the parameters that will be assessed to understand efficacy.	Changes made given that assessment of mRNA coding for mediators of skin inflammation and homeostasis is replaced by the direct assessment of these mediators as proteins.
Section 10.1 General Statistical Considerations	Included efficacy endpoint in the Baseline value for the last non-missing measurement before the first dose of the study drug.	Clarification change.
Section 10.4.5.1 Adverse Event Analyses	Added adverse event collection and analysis for injection site reactions.	Changes made in consideration of the added Section 8.4.
Section 10.2 Analysis Sets Section 10.3 Study Population Data	Added a definition for the All Enrolled Subject Set.	Required for summary analysis based on the Observational Part set of patients.

PROTOCOL SYNOPSIS

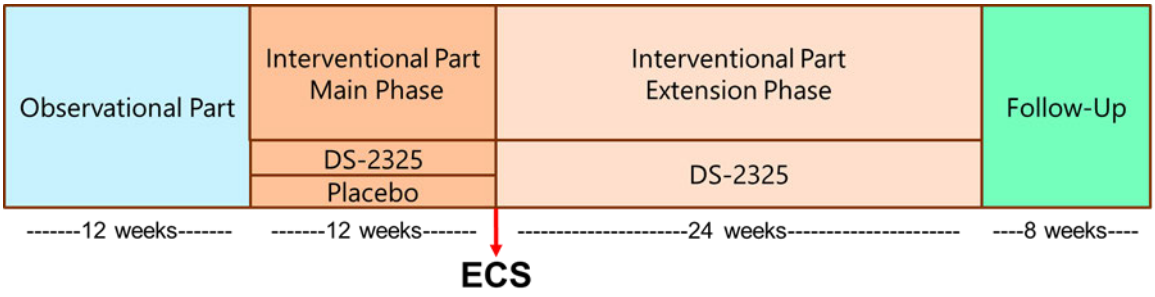
EU CT Number:	2022-502853-32-00
IND Number:	157483
Protocol Number:	DS2325-119
Investigational Product:	DS-2325a
Active Ingredient(s)/INN:	DS-2325a
Study Title:	A Phase 1b/2, Double-Blind, Placebo-Controlled, Randomized, Parallel-Arm Study to Explore Safety, Pharmacokinetics, and Early Clinical Signal of Efficacy of DS-2325a in Patients with Netherton Syndrome
Study Phase:	Phase 1b/2
Indication Under Investigation:	Netherton Syndrome (NS)
Study Objectives:	Observational Part Objectives: • To explore the characteristics of patients with NS for 12 weeks immediately before study treatment administration by assessing the same safety and efficacy (including mechanistic efficacy) endpoints to be assessed during the Interventional Part. Interventional Part Primary Objective: • To explore the safety and tolerability of DS-2325a in patients with NS by administering DS-2325a every other week for 12 consecutive weeks (Main Phase) and to confirm by administering for an additional 24 weeks (Extension Phase). Secondary Objectives: • To explore the pharmacokinetic (PK) properties of DS-2325a in patients with NS by administering DS-2325a every other week for 12 consecutive weeks (Main Phase) and to confirm by administering for an additional 24 weeks (Extension Phase). • To explore the efficacy of DS-2325a in patients with NS by administering DS-2325a every other week for 12 consecutive weeks (Main Phase) and to confirm by administering for an additional 24 weeks (Extension Phase). • To explore the immunogenicity of DS-2325a in patients with NS by administering DS-2325a every other week for 12 consecutive weeks (Main Phase) and to confirm by administering for an additional 24 weeks (Extension Phase).

	Exploratory Objective: • To explore the mechanistic efficacy of DS-2325a in patients with NS by administering DS-2325a every other week for 12 consecutive weeks (Main Phase) and to confirm by administering for an additional 24 weeks (Extension Phase).
Study Design:	The study follows a double-blind, placebo-controlled, randomized, parallel-arm design. The study includes 2 parts: An Observational Part and an Interventional Part.
Study Duration:	The total study duration from first patient-in to last patient-out is expected to be 72 weeks, including Enrollment (expected to take 16 weeks), Observational Part (12 weeks), the entire Interventional Part, ie, both Main Phase (12 weeks) and Extension Phase (24 weeks), and Follow-Up (8 weeks). The duration including Enrollment, Observational Part, and the Interventional Part Main Phase, ie, until early clinical signal (ECS) readout, will be 40 weeks. Each patient will be involved in the study for the total of 56 weeks (12 weeks Observational Part + 12 weeks Interventional Main Phase + 24 weeks Extension Phase + 8 weeks Follow-Up), excluding up to 4 weeks of screening.
Study Sites and Location:	This study will be conducted at a single site in France.
Patient Eligibility Criteria:	Key Inclusion Criteria: • Male or female patients aged 18 to 65 years with clinical diagnosis of NS including at least 3 out of the 4 following clinical criteria: o Neonatal erythroderma o Bamboo hair and/or alopecia o Chronic atopy specified as food allergy and/or asthma and/or rhino-conjunctivitis and/or eczema for at least 2 years o Ichthyosis linearis circumflexa or scaling erythroderma or equivalent. Eligible patients are those who have received ineffective or otherwise inappropriate standard-of-care treatment and therefore remain in need of cure. • Immunohistochemistry documentation of absence of lympho-epithelial Kazal-type-related inhibitor (LEKTI) in the skin or confirmed serine peptidase inhibitor of Kazal type 5 (SPINK5) gene mutations. • NS involvement of ≥20% of Body Surface Area (BSA) at both Screening and Baseline.

	• Patients must give written informed consent to participation in the study prior to Screening. • Patients must be willing to have skin tape harvests collected from lesional and non-lesional skin areas. Key Exclusion Criteria: • Any skin disease that may interfere with the diagnosis or evaluation of NS. • Any infection requiring treatment with systemic antibiotics, antivirals, antiparasitics, or antifungals within 2 weeks before Screening visit.
Dosage Form, Dose and Route of Administration:	DS-2325a will be provided as a sterile solution in CCI [redacted]. Placebo will be prepared from normal saline solution. Treatment will be given according to the maximum feasible dosing regimen of a single initial ("loading") dose of 1000 mg IV followed by biweekly ("maintenance") doses of 600 mg SC. However, during both Main Phase and Extension Phase, a 600 mg SC dose will also be given one week after the first dose of the phase, coinciding with one week before following biweekly doses. Thus, DS-2325a will be given at Weeks 1, 2, 3, 5, 7, 9, etc. Both 1000 mg and 600 mg doses were found to be safe and well-tolerated based on Phase 1a preliminary results.
Study Endpoints:	**Observational Part** The same safety and efficacy (including mechanistic efficacy) endpoints to be assessed during the Interventional Part. **Interventional Part** Primary endpoints: Safety endpoints will be adverse events (AEs), including serious AEs (SAEs), injection site reactions (ISRs), physical examination findings, vital sign recordings (body temperature, blood pressure, heart rate, respiratory rate), results of safety laboratory analyses of blood and urine, and electrocardiogram (ECG) findings. Secondary endpoints: Endpoints descriptive of plasma PK properties will be PK parameters derived from population PK analysis, including, but not limited to, pre-dose trough concentration (C_{trough}), $AUC_{tau,ss}$, total body clearance (CL), and CL/F, and epidermis-to-dermis DS-2325a concentration ratio (K_{ed}). DS-2325a presence will be assessed in the skin if the optional skin biopsies will be available. Endpoints for the assessment of efficacy (ECS) will include Ichthyosis Area Severity Index (IASI) (including IASI-Erythema and IASI Scaling), and Investigator Global Assessment (IGA) and patient-reported outcome (PRO) measures, such as Itch Numerical Rating Scale (NRS) scores and quality-of-life

	assessments obtained using the Skindex-29, and the Dermatology-Life-Quality-Index (DLQI) questionnaires. Anti-drug antibodies (ADAs) against DS-2325a will be the immunogenicity endpoint. Exploratory endpoints: Endpoints for the assessment of mechanistic efficacy will include transepidermal water loss (TEWL); skin concentration of mediators of skin inflammation and homeostasis, such as S100A7, S100A8, S100A9 and IL-36γ; skin KLK5 activity; and circulating cytokines, such as interleukin (IL)-36γ, IL-1β, IL-4, IL-6, IL-8, TSLP, and TNFα and chemokines like CCL17 (TARC), CCL20 (MIP3A), CCL22 (MDC), and CCL27 (CTACK); and circulating immunoglobulin (Ig) E.
Planned Sample Size:	The planned sample size is 9 to 12 patients with NS. In the Interventional Part Main Phase, patients will be randomized to 2 parallel arms to receive DS-2325a or placebo in a 2:1 ratio. In the Extension Phase, all patients will receive DS-2325a.
Statistical Analyses:	All efficacy analyses will be based on the modified Intent-to-Treat Analysis Set (mITT). The efficacy endpoints (IASI, IGA, patient-reported outcome [PRO] measures, and mechanistic endpoints) will be summarized by treatment group using descriptive statistics. Comparison between treatment groups for the change from Baseline (pre-treatment on Day 1 of Week 1 of the Interventional Part Main Phase) or from pre-treatment obtained by grouping Observational Part timepoints, which precede Baseline, to each post-treatment visit in IASI, IGA, PRO measures, and mechanistic endpoints will be summarized. All safety analyses will be based on the Safety Analysis Set. All AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). Incidence summary of patients with treatment-emergent AEs will be presented by maximum severity, SAEs, AEs assessed as related to study, and AEs resulting in discontinuation of study drug. Observations at each visit and changes from Baseline in vital sign recordings, results of laboratory analyses, and ECG findings will be numerically summarized by treatment group over time. Physical examination findings at each evaluation will be listed and clinically significant abnormal findings will be noted in the listings. All PK analyses will be based on the PK Analysis Set. Plasma concentration of DS-2325a will be summarized using descriptive statistics by treatment group and by phase. The PK parameters for both the Main Phase and the Extension Phase of the Interventional Part of study will be analyzed by descriptive statistics, including the mean, geometric mean, standard deviation (SD), coefficient of variation percentage (CV%) or median, minimum, and maximum.

STUDY SCHEMA



ECS = Early Clinical Signal

SCHEDULE OF EVENTS

Table 1: Visit Schedule for Observational Part and Interventional Part-Main Phase

Schedule of Events		Observational Part			Interventional Part-Main Phase						
	Screening*				Baseline^						
Week	**−4 to 0**	**1**	**5**	**9**	**1**	**2**	**3**	**5**	**7**	**9**	**11**
Visit Window allowed (day)		**±2**	**±2**	**±2**	**±1**	**±1**	**±1**	**±2**	**±2**	**±2**	**±2**
Informed Consent	X										
Demographics, height, and BMI	X										
Body weight	X	X	X	X	X			X		X	
Eligibility Criteria	X				X						
Serology (HBV, HCV, and HIV)	X										
COVID-19 test	X				X						
Urine drugs of abuse	X										
Medical History	X										
Adverse Events (1)	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medications (1)	X	X	X	X	X	X	X	X	X	X	X
Vital Signs (1)	X	X	X	X	X	X	X	X	X	X	X
Physical Exam (2)	X	X	X	X	X		X	X	X	X	X
Safety Laboratory Tests, Including Urinalysis (3)	X	X			X			X		X	
ECG (4)	X				X			X		X	
Serum Pregnancy Test	X										
Urine Pregnancy Test (5)					X			X		X	
Clinical Evaluations (IASI, IGA, and PRO) (2)	X	X	X	X	X		X	X	X	X	X
Skin Photography (2)	X	X	X	X	X		X	X	X	X	X
Mechanistic Evaluations (PD biomarkers) - TEWL (6)		X			X						
Mechanistic Evaluations (PD biomarkers) - Skin Tape Harvests (6)		X			X						
Mechanistic Evaluations (PD biomarkers) - Blood (7)		X	X	X	X			X		X	
Randomization					X						
Dose Administration (8)					X	X	X	X	X	X	X
PK (blood) (9)					X (10)		X	X	X	X	X
ADA (blood) (11)					X			X		X	

Abbreviations: ADA = Anti-drug Antibody, BMI = Body Mass Index, COVID-19 = Coronavirus Disease 2019, ECG = Electrocardiogram, EOS = End of Study, EOT = End of Treatment, HBV = Hepatitis B virus, HCV = Hepatitis C virus, HIV = Human Immunodeficiency Virus, IASI = Ichthyosis Area Severity Index, IGA = Investigator Global Assessment, IP = Interventional Part, IV = intravenous, OP = Observational Part, PD = Pharmacodynamics, PK = Pharmacokinetics, PRO = Patient-Reported Outcome, SC = subcutaneous, TEWL = Transepidermal water loss
*Screening will occur during the 28 days preceding Week 1 of OP; ^Baseline is pretreatment on Day 1 of Week 1 of Interventional Part Main Phase
(1) To be recorded at Screening; at Weeks 1, 5, and 9 during OP; every other week during IP before dose administration; and at Weeks 37 EOT and 45 EOS.
(2) To be performed at Screening; at Weeks 1, 5, and 9 during OP; every other week (ie, at odd-numbered weeks, to start with Week 1) during IP before dose administration; and at Weeks 37 EOT and 45 EOS (except clinical evaluations [IASI, IGA, and PRO] and skin photography, which will not be performed at Week 45 EOS).
(3) To be performed at Screening; at Week 1 during OP; at Weeks 1, 5, 9, 13, 21, and 29, during IP before dose administration; and at Weeks 37 EOT and 45 EOS.
(4) To be performed at Screening; at Weeks 1, 5, 9, and 13 during IP before dose administration; and at Weeks 37 EOT and 45 EOS.
(5) To be performed at Weeks 1, 5, 9, 13, 17, 21, 25, 29, and 33 during IP before dose administrations; and at Week 37 EOT.
(6) To be performed at Week 1 during OP and at Weeks 1, 13, and 25 during IP before dose administration; and at Week 37 EOT. Skin tape harvests will allow assessing the skin concentrations of mediators of skin inflammation and homeostasis and skin KLK5 activity.
(7) To be performed at Weeks 1, 5, and 9 during OP; at Weeks 1, 5, 9, 13, 21, 25, and 29 during IP before dose administration; and at Week 37 EOT.
(8) To be given at Weeks 1, 2, and 3 and every other week during IP-Main Phase. Dose administration will be IV at Week 1 and then SC.
(9) To be collected every other week (ie, at odd-numbered weeks, to start with Week 1) during IP before dose administration; and at Weeks 37 EOT and 45 EOS. Blood samples will be collected no more than 1 hour before the start of dose administration.
(10) On Week 1 of IP, PK blood sample will also be collected at the end of infusion (ie, 1 hour after start of dose administration) and 1 hour later (ie, 2 hours after start of dose administration). These two blood samples will be collected ± 10 minutes from the nominal times.
(11) To be collected at Weeks 1, 5, 9, 13, 17, 21, 25, 29, and 33 during IP before dose administration; and at Weeks 37 EOT and 45 EOS.

Table 2: Visit Schedule for Interventional Part-Extension Phase

Schedule of Events	Interventional Part-Extension Phase													Follow-Up	
														EOT	EOS
Weeks	**13**	**14**	**15**	**17**	**19**	**21**	**23**	**25**	**27**	**29**	**31**	**33**	**35**	**37**	**45**
Visits window allowed (Day)	**±1**	**±1**	**±1**	**±2**	**±2**	**±2**	**±2**	**±2**	**±2**	**±2**	**±2**	**±2**	**±2**	**±2**	**±3**
Body weight	X			X		X		X		X		X		X	X
Adverse Events (1)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medications (1)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs (1)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical Exam (2)	X		X	X	X	X	X	X	X	X	X	X	X	X	X
Safety Laboratory Tests, Including Urinalysis (3)	X					X				X				X	X
ECG (4)	X													X	X
Serum Pregnancy Test															
Urine Pregnancy Test (5)	X			X		X		X		X		X		X	
Clinical Evaluations (IASI, IGA, and PRO) (2)	X		X	X	X	X	X	X	X	X	X	X	X	X	
Skin Photography (2)	X		X	X	X	X	X	X	X	X	X	X	X	X	
Mechanistic Evaluations (PD biomarkers) - TEWL (6)	X							X						X	
Mechanistic Evaluations (PD biomarkers) - Skin Tape Harvests (6)	X							X						X	
Mechanistic Evaluations (PD biomarkers) - Blood (7)	X					X		X		X				X	
Randomization															
Dose Administration (8)	X	X	X	X	X	X	X	X	X	X	X	X	X		
PK (blood) (9)	X		X	X	X	X	X	X	X	X	X	X	X	X	X
PK (skin) (10)	X														
ADA (blood) (11)	X			X		X		X		X		X		X	X

Abbreviations: ADA = Anti-drug Antibody, ECG = Electrocardiogram, EOS = End of Study, EOT = End of Treatment, IASI = Ichthyosis Area Severity Index, IGA = Investigator Global Assessment, IP = Interventional Part, IV = intravenous, OP = Observational Part, PD = Pharmacodynamics, PK = Pharmacokinetics, PRO = Patient-Reported Outcome, SC = subcutaneous, TEWL = Transepidermal water loss.
*Screening will occur during the 28 days preceding Week 1 of OP; ^Baseline is pretreatment on Day 1 of Week 1 of Interventional Part Main Phase
(1) To be recorded at Screening; at Weeks 1, 5, and 9 during OP; every other week during IP before dose administration; and at Weeks 37 EOT and 45 EOS.

(2) To be performed at Screening; at Weeks 1, 5, and 9 during OP; every other week (ie, at odd-numbered weeks, to start with Week 1) during IP before dose administration; and at Week 37 EOT and 45 EOS (except clinical evaluations [IASI, IGA, and PRO] and skin photography, which will not be performed at Week 45 EOS).
(3) To be performed at Screening; at Week 1 during OP; at Weeks 1, 5, 9, 13, 21, and 29, during IP before dose administrations; and at Weeks 37 EOT and 45 EOS.
(4) To be performed at Screening; at Weeks 1, 5, 9, and 13 during IP before dose administration; and at Weeks 37 EOT and 45 EOS.
(5) To be performed at Weeks 1, 5, 9, 13, 17, 21, 25, 29, and 33 during IP before dose administrations; and at Week 37 EOT.
(6) To be performed at Week 1 during OP and at Weeks 1, 13, and 25 during IP before dose administration; and at Week 37 EOT. Skin tape harvests will allow assessing the skin concentrations of mediators of skin inflammation and homeostasis and skin KLK5 activity.
(7) To be performed at Weeks 1, 5, and 9 during OP; at Weeks 1, 5, 9, 13, 21, 25, and 29 during IP before dose administration; and at Week 37 EOT.
(8) To be given at Weeks 13, 14, and 15 and every other week during IP-Extension Phase. Dose administration will be SC.
(9) To be collected every other week (ie, at odd-numbered weeks, to start with Week 1) during IP before dose administration; and at Weeks 37 EOT and 45 EOS. Blood samples will be collected no more than 1 hour before the start of dose administration.
(10) Optional skin biopsy will be a 3-mm biopsy to be collected before dose administration at Week 13 to assess epidermis-to-dermis DS-2325a concentration ratio.
(11) To be collected at Weeks 1, 5, 9, 13, 17, 21, 25, 29, and 33 during IP before dose administration; and at Weeks 37 EOT and 45 EOS.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ABBREVIATION	DEFINITION
ADA	Anti-drug antibodies
AE	Adverse Event
ALP	Serum alkaline phosphatase
ALT	Serum alanine aminotransferase
AST	Serum aspartate aminotransferase
AUC	Area Under the Plasma Concentration-time Curve
AUC_t	Area Under the Plasma Concentration-time Curve up to time
$AUC_{t,ss}$	Area Under the Plasma Concentration-time Curve up to time t at steady state
BMI	Body Mass Index
BSA	Body Surface Area
CCL20	Chemokine-C-C motif ligand 20
CFR	Code of Federal Regulations
CK	Creatine Kinase
CL	total body clearance
CL/F	Apparent Total Body Clearance
C_{max}	Maximum Plasma Concentration
COVID-19	Coronavirus disease 2019
CRO	Contract Research Organization
CTCAE	Common Terminology Criteria for Adverse Events
C_{trough}	trough concentration
CV%	Coefficient of variation percentage
DLQI	Dermatology-Life-Quality-Index
DMC	Data Monitoring Committee
EC	Ethics Committee
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
ECS	Early clinical signal
EDC	Electronic Data Capture
EOS	End of Study
EOT	End of Treatment

ABBREVIATION	DEFINITION
EU	European Union
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GGT	γ-Glutamyl Transferase
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HS	healthy subjects
IASI	Ichthyosis Area Severity Index
IB	Investigator's Brochure
IC_{50}	half-maximal inhibition
ICF	Informed Consent Form
ICH	International Council for Harmonisation
Ig	Immunoglobulin
IGA	Investigator Global Assessment
IL	Interleukin
ILC	Ichthyosis linearis circumflexa
INN	International Non-proprietary Name
IRB	Institutional Review Board
ISRs	Injection Site Reactions
IV	intravenous
ITT	Intent-to-Treat
IXRS	Interactive Web/Voice Response System
KLK	Kallikrein-Related Peptidase
K_{ed}	epidermis-to-dermis DS-2325a concentration ratio
LDH	Lactate Dehydrogenase
LEKTI	lympho-epithelial Kazal-type-related inhibitor
MAD	multiple ascending dose
MedDRA	Medical Dictionary for Regulatory Activities
mITT	Modified Intent-to-Treat
NOAEL	no observed adverse effect level
NRS	Numerical Rating Scale

ABBREVIATION	DEFINITION
NS	Netherton Syndrome
NS-ILC	Netherton Syndrome -ichthyosis linearis circumflexa
NS-SE	Netherton Syndrome-scaly erythroderma
PAR2	protease-activated receptor 2
PD	Pharmacodynamic
PK	Pharmacokinetic
PRO	Patient-reported outcome
RNA	Ribonucleic Acid
SAD	single ascending dose
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SC	subcutaneous
SD	Standard deviation
SOP	Standard Operating Procedure
SAVER	Serious Adverse Event Report
SPINK	serine peptidase inhibitor Kazal type
SPINK5	serine peptidase inhibitor of Kazal type 5
SUSAR	Suspected Unexpected Serious Adverse Reaction
TARC	thymus and activation-regulated chemokine
TBL	Total bilirubin level
TEAE	Treatment-emergent Adverse Event
TEWL	Transepidermal water loss
TNF	Tumor necrosis factor
TSLP	thymic stromal lymphopoietin
ULN	upper limit of normal

1. INTRODUCTION

1.1. Background

Netherton Syndrome (NS) is a severe rare disease caused by autosomal recessive mutations in the serine peptidase inhibitor of Kazal type 5 (SPINK5) gene on chromosome 5q32.[1,2,3] It is part of a group of genetically determined skin diseases referred to as ichthyoses, which are characterized by generalized scaling, erythema, and epidermal barrier defects and also include congenital ichthyosiform erythroderma, lamellar ichthyosis, and epidermolytic ichthyosis.[4] The SPINK5 gene encodes lympho-epithelial Kazal-type-related inhibitor (LEKTI), which plays a critical role in maintaining skin barrier function and regulating desquamation of keratinocytes by inhibiting Kallikrein-Related Peptidase 5 (KLK5).[5,6] Since SPINK5 mutations result in loss of functional LEKTI, KLK5 is excessively activated in the skin of NS patients.

Although the prevalence of NS is not well documented, there is a reported worldwide birth incidence of 1 in 200,000.[7] The estimated number of patients in the United States is around 2,000 and in the European Union more than 2,000. Clinically, NS main features include congenital ichthyosiform erythroderma, a hair shaft abnormality known as trichorrhexis invaginata (bamboo hair), and severe atopy. However, NS phenotype is variable. Two main presentations are recognized: NS-ichthyosis linearis circumflexa (NS-ILC) and NS-scaly erythroderma (NS-SE). Aside from the appearance of the skin, pruritus is a constant feature and a substantial quality-of-life issue. The first manifestation of NS is typically in the neonatal period, where erythroderma and sparse hair are present. Netherton Syndrome can be life-threatening in neonates, in whom the compromised skin barrier leads to severe dehydration, hypothermia, weight loss, and sepsis. Failure to thrive is common in infancy as a result of chronic fluid loss, persistent cutaneous infections, and malnutrition.[8,9] Mortality rate has been reported in infancy from 30% to 40%.[10] Most patients survive the neonatal period, however, and show improvement of their symptoms over time, but NS remains a serious and debilitating condition with relentless negative impact also throughout adulthood.[9] Patients continue showing generalized erythroderma and suffering from pruritus and are additionally afflicted by atopic disorders such as food allergies and asthma. Netherton Syndrome often takes a relapsing and remitting course, characterized by flares of intermittent redness and patches of ichthyosis linearis circumflexa (ILC), which may occur every 1 to 2 months, apparently precipitated at times by stress or infection. The etiopathogenesis of NS consists in SPINK5 genetic mutation resulting in loss of functional LEKTI and ensuing excessive activation of KLK5 in the skin. Kallikrein-Related Peptidase 5 (KLK5) plays a central role in shedding epidermis stratum corneum and inciting dermis inflammation. Active KLK5 induces stratum corneum detachment by cleavage of corneodesmosomal proteins, such as desmoglein-1, which join keratinocytes; and up-regulates inflammatory signaling by cleavage of protease-activated receptor 2 (PAR2) on keratinocytes.[11]

Unopposed KLK5 activity results in a compromised skin barrier and an abnormal production of pro-inflammatory and pro-allergic mediators, including tumor necrosis factor (TNF), interleukin (IL)-8 and especially Th2 cytokines, such as thymic stromal lymphopoietin (TSLP) and thymus and activation-regulated chemokine (TARC).[12] There are no approved specific therapeutic agents for the treatment of NS. Emollients are regularly used and topical keratolytic agents, glucocorticoids, and antibiotics represent the mainstay treatment. Part of the standard of care are

also oral antihistamines and retinoids. In certain severe cases, pimecrolimus and psoralen plus phototherapy are used. Despite anecdotal cases of some success, NS poorly responds to biologics that have recently shown important efficacy in the treatment of major inflammatory dermatological disorders such as psoriasis (anti-IL-17A antibodies secukinumab and ixekizumab) and atopic dermatitis (anti-IL-4Rα antibody dupilumab).[13,14,15] A topical Kallikrein-Related Peptidase 7 (KLK7) inhibitor (BPR277) was tested in an early phase clinical trial in NS patients and reported to have had "a treatment effect"; the plan of a late phase clinical trial was presented in 2020.[16]

CCI

[redacted]

1.2. Data Summary

CCI

[redacted]

CCI

To assess the general toxicity profile of DS-2325a, two 4-week intermittent SC dose toxicity studies were conducted, one in mice (CCI) and one in cynomolgus monkeys (CCI), with an 8-week recovery period at the dose levels of 0 (control group), CCI . A 26-week intermittent SC dose toxicity study was also conducted in mice (Q2D, 91 times in total) with an 8-week recovery period at the dose levels of 0 (control group), CCI . In all these studies, no toxicologically significant changes were noted in any animal at dose levels up to CCI included. Therefore, the no observed adverse effect level (NOAEL) of DS-2325a in these studies was considered to be CCI . Additionally, in telemetered male cynomolgus monkeys treated with a single SC dose of DS-2325a, no effects on the cardiovascular system, the respiratory system or the central nervous system were observed at dose levels up to CCI included.

In a cytokine release assay using human peripheral blood mononuclear cells in solid phase, DS-2325a induced higher levels of TNF compared with vehicle control. The extent of induction was less than that of bevacizumab, a reference antibody, for which low incidence of infusion-related reactions has been reported. In cytokine release assays using human peripheral blood mononuclear cells or human whole blood in liquid phase, DS-2325a did not induce the release of any cytokine tested compared with vehicle control. DS-2325a did not show cell proliferation activity in any assay. Therefore, the risk of infusion-related reactions upon DS-2325a administration is considered to be low given the results of these assays.

For further details, please refer to the current Investigator's Brochure (IB).

1.3. Study Rationale

Netherton Syndrome is caused by loss-of-function mutations in the gene coding for LEKTI. LEKTI is the natural inhibitor of KLK5, and its functional loss leads to excessive activation of KLK5. DS-2325a may be an ideal pharmacological remedy for NS pathological defect because of its ability to inhibit KLK5. DS-2325a may replace deficient LEKTI in patients with NS and thus block disease development.

DS-2325a is undergoing 2 Phase 1a studies in healthy subjects (HS): 1) a single ascending dose study (SAD), in which DS-2325a is being investigated through SC (6 dose levels ranging from 30 to 1500 mg) and intravenous (IV) (2 dose levels, ie, 100 and 1000 mg) administration; and 2) a multiple ascending dose study, during which DS-2325a is being investigated through SC administration 4 times once a week (3 dose levels, ie, 300, 600, and 900 mg).

This Phase 1b/2 study will initially explore the safety, pharmacokinetics (PK), and efficacy of DS-2325a in adult patients with NS. If found of adequate safety and efficacy, DS-2325a will later be explored also in pediatric patients with NS. The study will follow DS-2325a Phase 1a studies in HS. It will be articulated into an Observational Part, during which patients will receive no treatment, an Interventional Part, during which patients will receive DS-2325a, and a Follow-Up. The Interventional Part will be in turn articulated into 2 phases: A double-blind, placebo-controlled, randomized, parallel-arm Main Phase, during which patients will receive

DS-2325a or placebo, and an open-label Extension Phase, during which all patients will receive DS-2325a. The Interventional Part Main Phase, the core component of the study, will be immediately and seamlessly preceded by the Observational Part and will be followed, also immediately and seamlessly, by the Interventional Part Extension Phase, as long as early clinical signal (ECS), as initial evidence of efficacy, is found at the end of the Main Phase and warrants study continuation. All patients will transition from the Main Phase to the Extension Phase; however, the latter will be discontinued if ECS is not found. After an initial ("loading") dose of 1000 mg IV, which will be given only in Interventional Part Main Phase, DS-2325a will be given weekly at the maximum feasible dose of 600 mg SC. Both doses were determined to be safe and well tolerated in Phase 1a. DS-2325a is not expected to pose any concerning safety risks, given the results of the toxicology studies and the preliminary results of the Phase 1a studies, but is expected to give initial evidence of efficacy, ie, ECS. This evidence will be based on impact on the Ichthyosis Area Severity Index (IASI) as the main clinical endpoint and on several mechanistic endpoints.

1.4. Dose Selection and Justification of Dose

CCI

This dosing regimen was found to be safe and well tolerated in Phase 1a. Two Phase 1a studies investigated DS-2325a in HS: A SAD study and a multiple ascending dose (MAD) study. In SAD study, DS-2325a was given to eight cohort of 8 subjects each, randomized 6 to 2 to DS-2325a and placebo, at the SC doses of 30, 100, 300, 600, 1000, and 1500 mg and at the IV doses of 100 and 1000 mg. In MAD study, DS-2325a was given four times once a week to two cohorts of 8 subjects each, also randomized 6 to 2 to DS-2325a and placebo, at the SC doses of 300 and 600 mg. Based on the review of preliminary blinded safety data from these Phase 1a studies, DS-2325a is found to be well tolerated by the study subjects at single SC (up to 1500 mg) and IV (up to 1000 mg) doses and at the multiple SC dose of 300 and 600 mg. The adverse events (AEs) reported in these studies were mild in nature with no serious events reported. Additional information on these AEs is provided in the IB.

CCI [redacted] It should be noticed that both in mice and monkeys NOAEL corresponded to the maximum dose tested, since no dose level was found that resulted in toxicities. CCI [redacted]

CCI [redacted]

The rationale for the use of placebo is the provision of treatment control, necessary to interpret DS-2325a activity, including safety and tolerability, not only efficacy, even if the sample size of this study was not calibrated to allow finding a statistically significant difference between DS-2325a- and placebo-treated patients at pre-stipulated levels of probability and power.

1.5. Risks and Benefits for Study Patients

Risk Assessment

Based on the nonclinical findings, it is expected that DS-2325a will be safe and well tolerated and will show ECS as initial evidence of efficacy. During this study, there may be risks related to the blood sampling (eg, bruising and pain at the blood draw site). More detailed information about the known and expected benefits and risks and reasonably expected AEs related to DS-2325a may be found in the IB.

Coronavirus Disease 2019

During special circumstances (eg, coronavirus disease 2019 [COVID-19] pandemic), the specific guidance from local public health and other competent authorities regarding the protection of individuals' welfare must be applied.

Risk Mitigation Strategies

The safety monitoring practices employed by this protocol (ie, AEs, clinical laboratory findings, vital signs, 12-lead electrocardiograms (ECGs), and physical findings) are considered adequate to protect the safety of study patients.

All study activities at the study site will be performed by trained clinical staff authorized by the study investigator.

The Sponsor will immediately notify the investigator if any additional safety or toxicology information becomes available during the study.

This study will be performed in compliance with the protocol, International Council for Harmonisation (ICH) Good Clinical Practice (GCP) and applicable regulatory requirements. Aspects of the study concerned with the investigational medicinal product(s) will meet the requirements of European Union – Good Manufacturing Practice.

Benefit Assessment

By receiving DS-2325a, patient's NS condition might improve.

All patients will undergo a physical medical examination at the first study visit; these evaluations, made by skilled and trained clinical staff, may potentially provide valuable knowledge about the patient's health. In case the study staff discovers any medical condition, the patient may be referred to the local healthcare system in case any abnormalities/diseases are observed.

The patients will contribute to the process of developing therapies in an area of unmet need. They may also help provide valuable data, about the tolerability and immunogenicity of DS-2325a and about its potential ability to provide treatment for NS.

Overall Risk/Benefit Conclusion

Considering the measures taken to minimize possible risks to the patients in this study, the potential risks associated with the study drug and study assessments are balanced by the potential benefits that may be provided to the patients.

2. STUDY OBJECTIVES, HYPOTHESIS, AND ENDPOINTS

2.1. Study Objectives

2.1.1. Observational Part Objectives

To explore the characteristics of patients with NS for 12 weeks immediately before study treatment administration by assessing the same safety and efficacy (including mechanistic efficacy) endpoints to be assessed during the Interventional Part.

2.1.2. Interventional Part Objectives

2.1.2.1. Primary Objectives

The primary objective of this study is to explore the safety and tolerability of DS-2325a in patients with NS by administering DS-2325a every other week for 12 consecutive weeks (Main Phase) and to confirm by administering for an additional 24 weeks (Extension Phase).

2.1.2.2. Secondary Objectives

The secondary objectives of the study are:

- To explore the PK properties of DS-2325a in patients with NS by administering DS-2325a every other week for 12 consecutive weeks (Main Phase) and to confirm by administering for an additional 24 weeks (Extension Phase).
- To explore the efficacy of DS-2325a in patients with NS by administering DS-2325a every other week for 12 consecutive weeks (Main Phase) and to confirm by administering for an additional 24 weeks (Extension Phase).
- To explore the immunogenicity of DS-2325a in patients with NS by administering DS-2325a every other week for 12 consecutive weeks (Main Phase) and to confirm by administering for an additional 24 weeks (Extension Phase).

2.1.2.3. Exploratory Objectives

The exploratory objectives of the study are:

- To explore the mechanistic efficacy of DS-2325a in patients with NS by administering DS-2325a every other week for 12 consecutive weeks (Main Phase) and to confirm by administering for an additional 24 weeks (Extension Phase).

2.2. Study Hypotheses

It is hypothesized that DS-2325a will be safe and well tolerated and will show ECS as initial evidence of efficacy.

2.3. Study Endpoints

2.3.1. Primary Endpoint

The primary endpoints of this study are:

- Safety endpoints, ie, AEs, including serious AEs (SAEs), injection site reactions (ISRs) physical examination findings, vital sign recordings (body temperature, blood pressure, heart rate, respiratory rate), results of safety laboratory analyses of blood and urine, and ECG findings.

2.3.2. Secondary Endpoints

The secondary endpoints of this study are-:

- Endpoints descriptive of plasma PK properties will be PK parameters derived from population PK analysis, including, but not limited to, pre-dose trough concentration (C_{trough}), $AUC_{tau,ss}$, total body clearance (CL), and CL/F, and epidermis-to-dermis DS-2325a concentration ratio (K_{ed}).
- DS-2325a presence will be assessed in the skin if the optional skin biopsies will be available.
- Several endpoints for the assessment of efficacy (ECS), including, but not limited to IASI (including IASI-Erythema and IASI Scaling), Investigator Global Assessment (IGA) and patient-reported outcome (PRO) measures, such as Itch Numerical Rating Scale (NRS) scores and quality-of-life assessments obtained using the Skindex-29 and the Dermatology-Life-Quality-Index (DLQI) questionnaires.
- Anti-drug antibodies (ADAs) against DS-2325a as the immunogenicity endpoint.

2.3.3. Exploratory Endpoints

The exploratory endpoints of this study are:

- Endpoints for the assessment of mechanistic efficacy, including transepidermal water loss; skin concentration of mediators of skin inflammation and homeostasis, such as S100A7, S100A8, S100A9, and IL-36γ; skin KLK5 activity; and circulating cytokines, such as IL-36γ, IL-1β, IL-4, IL-6, IL-8, TSLP, and TNFα and chemokines like CCL17 (TARC), CCL20 (MIP3A), CCL22 (MDC), and CCL27 (CTACK); and circulating IgE.

3. STUDY DESIGN

3.1. Overall Design

3.1.1. Overview

This Phase 1b/2 study will enroll a total of 9 to 12 patients with NS and will be conducted entirely at 1 site in France, an NS center of excellence and referral which is expected to enroll NS patients with well-known disease histories at a relatively high rate.

It will be articulated in an Observational Part (12 weeks), during which patients will not be treated and will be investigated only to understand the course of their disease, an Interventional Part, during which patients will be treated with DS-2325a to understand its properties as a candidate drug product, and a Follow-Up, during which safety will be monitored after treatment.

The Interventional Part will be further articulated into 2 phases: A Main Phase (12 weeks) and an Extension Phase (24 weeks). The Interventional Part Main Phase is the core component of the study. It will be immediately and seamlessly preceded by the Observational Part and will be followed, also immediately and seamlessly, by the Interventional Part Extension Phase, as long as at the end of the Main Phase ECS is found as initial evidence of efficacy and warrants study continuation. All patients will transition from the Main Phase to the Extension Phase; however, the latter will be discontinued if ECS is not found. The Interventional Part Main Phase will be double-blinded and placebo-controlled: Patients will be randomized to 2 parallel arms to receive DS-2325a or placebo in a 2:1 ratio and will receive DS-2325a or placebo SC every other week, except for the initial ("loading") dose, which will be administered by an IV infusion. The Extension Phase will be open-label and all patients will receive DS-2325a SC every other week. However, during both Main Phase and Extension Phase, a 600 mg SC dose will also be given one week after the first dose of the phase, coinciding with one week before following biweekly doses. Thus, eDS-2325a will be given at Weeks 1, 2, 3, 5, 7, 9, etc. Intravenous dose will not be given in Interventional Part Extension Phase. In case of patient(s) showing benefit from treatment with DS-2325a, the protocol of this study will be amended to allow a prolongation of the Extension Phase and, therefore, a continuation of DS-2325a administration within the clinical trial framework beyond the currently foreseen end. This should ensure that benefiting patient(s) will continue receiving cure.

The total study duration from first patient-in to last patient-out is expected to be 72 weeks, including Enrollment (expected to take 16 weeks), Observational Part (12 weeks), the entire Interventional Part, ie, both Main Phase (12 weeks) and Extension Phase (24 weeks), and Follow-Up (8 weeks). The duration including Enrollment, Observational Part, and the Interventional Part Main Phase, ie, until ECS readout, will be 40 weeks. Each patient will be involved in the study for the total of 56 weeks (12 weeks Observational Part + 12 weeks Interventional Main Phase + 24 weeks Extension Phase + 8 weeks Follow-Up), excluding up to 4 weeks of screening.

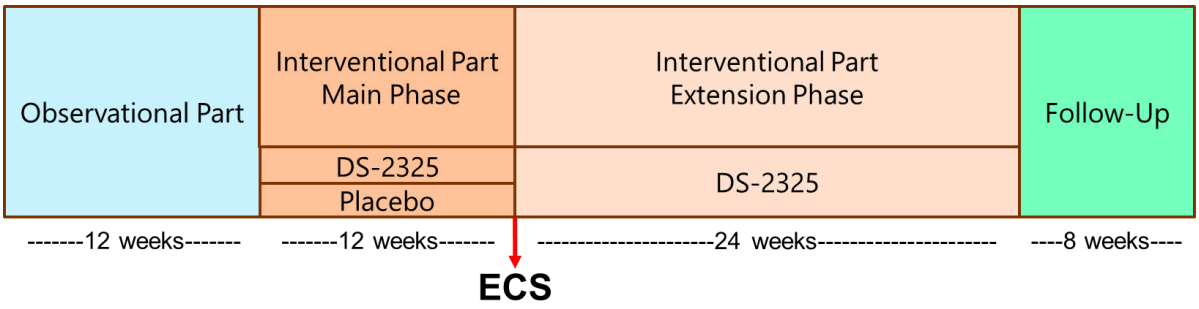
Study eligibility will be assessed at the beginning of the Observational Part and randomization for treatment will be done at the beginning of the Interventional Part, when eligibility will be re-confirmed. Randomization will follow a randomization schedule generated by a biostatistician independent of the study. Patients, investigators, and site staff will be blinded,

except for the unblinded site pharmacist or designee who will be responsible for preparing the study drugs. Sponsor's staff or representative (eg, clinical research organization) will also be blinded.

The DS-2325a dose has been selected based on the safety, tolerability, and PK results of the Phase 1a studies.

Replacing patients will be permitted during the Observational Part and the Interventional Part Main Phase in order to achieve a minimum of 9 patients for ECS evaluation at the end of the Interventional Part Main Phase.

Figure 1: Study Design



ECS = Early Clinical Signal

3.2. Discussion of Study Design

The intent of the Observational Part of the study is to gather ample information on the course of the NS disease of each patient participating in the study during the time immediately preceding study treatment administration. Based on clinical experience with NS patients, Observational Part 12-week duration, which matches the duration of the Interventional Part Main Phase, is thought to be adequate to gather this information. During the Observational Part, patients will be assessed according to the same safety and efficacy (including mechanistic efficacy) endpoints, as the Interventional Part Main Phase. This should give stronger possibility of comparing post-treatment with pre-treatment data than simply assessing patients once at Baseline, enhancing therefore the possibility to conclude if DS-2325a gives ECS or not. Additionally, the information collected during Observational Part will possibly be used to elaborate a mathematical disease progression model that could also facilitate concluding if DS-2325a gives ECS or not. Participation in the Observational Part should also give patients the possibility of acclimatizing with study environment and familiarizing with study procedures, such as use of questionnaires.

The intent of the Interventional Part of the study is to explore DS-2325a safety and efficacy, by comparing post-treatment with pre-treatment NS course and, in the Main Phase, the effect on NS of treatment with DS-2325a with treatment with placebo. Double-blind, placebo-controlled, and randomized design of the Interventional Part Main Phase should minimize bias. In addition to characterizing safety, intent of the Interventional Part Main Phase is to find ECS, as initial evidence of DS-2325a efficacy. Based on clinical experience with NS patients, Interventional Part Main Phase 12-week duration is thought to be adequate treatment time to find ECS, striking a balance between shorter times, which may not be sufficient for treatment to show effect, and

longer times, which may protract treatment to no avail. Early Clinical Signal will be inferred by the impact DS-2325a show on clinical endpoints, mainly IASI, and several mechanistic endpoints. Concomitant and consistent impact on multiple endpoints will represent ECS, especially if this impact is of large extent. This impact will be evinced by comparison of endpoint values between DS-2325a-treated and placebo-treated patients and especially between pre-treatment and post-treatment timepoints, since the Observational Part of the study should allow an in-depth characterization of each patient and thus facilitate pre-and post-treatment comparisons. Given its mechanism of action, there is an expectation that DS-2325a impact on endpoints will be unequivocally self-evident. However, in order to declare ECS finding, at least 50% of treated patients will have to achieve at least 50% decrease in total IASI score at the end of the Interventional Part Main Phase compared to Baseline. This is the minimum impact expected to be of clinical significance, which may justify DS-2325a use as a therapy for NS. The intent of the Interventional Part Extension Phase is to gather additional safety and efficacy information on DS-2325a, albeit under open-label design.

The intent of the Follow-Up component of the study is to monitor DS-2325a safety from the end of treatment, marked by the End of Treatment (EOT) visit, 1 week after the last drug administration, until the time DS-2325a is expected to have been virtually completely eliminated, marked by the End of Study (EOS) visit 8 weeks after EOT.

3.3. Stopping Rules

3.3.1. Subject-level Safety Stopping Rules

For any subject experiencing the following events, treatment will be suspended until further evaluation by the Data Monitoring Committee (DMC).

- Any event severe in nature (eg, severe injection site reaction, severe allergic reaction, etc) deemed by the DMC as necessitating further safety review before treatment can be continued
- Hy's Law event: Alanine aminotransferase [ALT] or aspartate aminotransferase [AST] ≥3 × upper limit of normal [ULN] with simultaneous total bilirubin ≥2 × ULN)
- QTc prolongation: QT interval corrected with Fridericia's formula (QTcF) >500 msec OR increase in QTcF from pre-dose baseline of >60 msec

After an in-depth review of subject-level data is conducted by the DMC, one of the following recommendations will be made:

- To continue with treatment as planned
- To continue treatment, but at a reduced dose
- To terminate participation in the study

3.3.2. Population-level Safety Stopping Rules

The DMC, as part of continuous monitoring of safety, will evaluate the data across the study for the following criteria:

- One active-treated subject experiences an AE of severe intensity (eg, severe injection site reaction, severe allergic reaction, etc) that is related to the study drug OR two active-treated subjects experience the same AE of moderate intensity that is related to the study drug.
- One active-treated subject with ALT or AST >5 × ULN or serum total bilirubin >2 × ULN.

If any of the above criteria are met, an in-depth review of data will be conducted by the DMC, and one of the following recommendations will be made:

- To continue with the study as planned, with no change
- To continue with the study as planned, with modification to the safety monitoring plan
- To continue with the study, but at a reduced dose
- To terminate the study

4. STUDY POPULATION

The patients must sign and date the Informed Consent Form (ICF) provided by the study site before any study-specific qualification procedures are conducted.

4.1. Inclusion Criteria

4.1.1. Screening

Patients must satisfy all of the following criteria to be included in the study:

1. Male or female patients aged 18 to 65 years with clinical diagnosis of NS including at least 3 out of the 4 following clinical criteria:
   - Neonatal erythroderma
   - Bamboo hair and/or alopecia
   - Chronic atopy specified as food allergy and/or asthma and/or rhino-conjunctivitis and/or eczema for at least 2 years
   - Ichthyosis linearis circumflexa or scaling erythroderma or equivalent

   Eligible patients are those who have received ineffective or otherwise inappropriate standard-of-care treatment and therefore remain in need of cure.
2. Immunohistochemistry documentation of absence of LEKTI in the skin or confirmed SPINK5 gene mutations.
3. NS involvement of ≥20% of Body Surface Area (BSA).
4. Patients must give written informed consent to participation in the study prior to Screening.
5. Patients must be willing and able to understand and comply with study requirements.
6. Patients must be willing to have skin tape harvests collected from lesional and non-lesional skin areas.
7. All women must have a negative serum pregnancy test at Screening. Women must not be lactating.
8. Women of childbearing potential (ie, a female after menarche and before becoming postmenopausal [no menstrual period for a minimum of 12 months without a medical cause alternative to natural menopause] or being made permanently sterile by surgery [hysterectomy, bilateral salpingectomy, or bilateral oophorectomy] done at least 1 month before the first dose of study drug) with a male partner must be willing to practice effective contraception during the study, starting at Screening and continuing for 3 months after the last dose of study drug. Methods of highly effective contraception include 1) hormonal administration of estrogen and progestogen combined, which may be given in oral, intravaginal, or transdermal form, or of progestogen only, which may be given in oral, injectable, or implantable form; 2) use of intrauterine devices, including intrauterine hormone-releasing devices; 3) bilateral tubal occlusion; 4) vasectomy of partner; and 5) complete sexual abstinence.

4.1.2. Baseline (Main Phase-Interventional Part)

At Week 1 Baseline Visit, patients must continue to satisfy all of the following criteria before continuing to the Interventional Part of the study:

1. NS involvement of ≥20% of BSA. Eligible patients are those who have received ineffective or otherwise inappropriate standard-of-care treatment and therefore remain in need of cure.
2. All women must have a negative urine pregnancy test at Baseline. Women must not be lactating.
3. Women of childbearing potential (ie, a female after menarche and before becoming postmenopausal [no menstrual period for a minimum of 12 months without a medical cause alternative to natural menopause] or being made permanently sterile by surgery [hysterectomy, bilateral salpingectomy, or bilateral oophorectomy] done at least 1 month before the first dose of study drug) with a male partner must be willing to practice effective contraception during the study, starting at Screening and continuing for 3 months after the last dose of study drug (refer to Section 4.1.1 for methods of highly effective contraception).
4. Patients must agree not to participate in any other investigational study during study drug administration and for 3 months after the last dose of study drug.

4.2. Exclusion Criteria

4.2.1. Screening

Patients who meet any of the following criteria will be disqualified from entering the study:

1. Any skin disease that may interfere with the diagnosis or evaluation of NS.
2. Any infection requiring treatment with systemic antibiotics, antivirals, antiparasitics, or antifungals within 2 weeks before Screening visit.
3. Concomitant systemic disease not controlled by treatment. Stability for 3 months prior to Screening is required.
4. Kidney or liver disease with significant impairment of organ function (creatinine clearance <30 mL/min, calculated using the Cockcroft-Gault Equation, and Child-Pugh Class C; ALT and AST ≥2 × ULN range; total bilirubin ≥1 × ULN).
5. Concomitant disease or condition that may interfere with, or treatment of which may interfere with, the conduct of the study or that would, in the opinion of the investigator, pose an unacceptable risk to the patient in this study.
6. Any significant condition (eg, medical, psychiatric, or social) that according to investigator's judgment would prevent compliance with study protocol and full study participation.
7. Known hypersensitivity to any ingredient of the study drug product.
8. Anticipation of the need for surgery or hospitalization during the study.

9. History of suicide attempt or suicidal ideation within 1 year prior to Screening.
10. History of substance abuse within 6 months prior to Screening or a positive urine drug test at Screening. Medical marijuana may be used per discretion of the investigator.
11. History or positive test result for human immunodeficiency virus (HIV) at Screening.
12. Active hepatitis B virus (HBV) infection, determined by positive test result for hepatitis B surface antigen, at Screening.
13. Active hepatitis C virus (HCV) infection, determined as HCV ribonucleic acid (RNA) above the limit of detection in patients with positive HCV antibody titer, at Screening.
14. Use of topical drugs that may alter the course of NS (eg, topical corticosteroids and topical calcineurin inhibitors) within 2 weeks before Screening or anticipation of need to use these drugs during study drug.
15. Systemic treatment with corticosteroids, immunosuppressants, targeted therapeutics, biologics, and IV Ig within 4 weeks before Screening.
16. Participation in any other clinical study or expanded access program with an investigational drug or device within 4 weeks before Screening.
17. Suspected or confirmed COVID-19 within 4 weeks before or ongoing at Screening and planned vaccination against COVID-19 during study drug.

4.2.2. Baseline (Main Phase-Interventional Part)

At Week 1 Baseline Visit, patients must continue to not meet any of the following criteria before continuing to the Interventional Part of the study:

1. Any infection requiring treatment with systemic antibiotics, antivirals, antiparasitics, or antifungals within 2 weeks before Baseline visit.
2. Concomitant disease or condition that may interfere with, or treatment of which may interfere with, the conduct of the study or that would, in the opinion of the investigator, pose an unacceptable risk to the patient in this study.
3. Any significant condition (eg, medical, psychiatric, or social) that according to investigator's judgment would prevent compliance with study protocol and full study participation.
4. Anticipation of the need for surgery or hospitalization during the study.
5. Use of topical drugs that may alter the course of NS (eg, topical corticosteroids and topical calcineurin inhibitors) within 2 weeks before Baseline or anticipation of need to use these drugs during study drug.
6. Systemic treatment with corticosteroids, immunosuppressants, targeted therapeutics, biologics, and IV Ig within 4 weeks before Baseline.
7. Participation in any other clinical study or expanded access program with an investigational drug or device within 4 weeks before Baseline.
8. Suspected or confirmed COVID-19 within 4 weeks before or ongoing at Baseline and planned vaccination against COVID-19 during study drug.

5. STUDY DRUG

5.1. Assigning Patients to Treatment Group(s)/Sequences and Blinding

5.1.1. Treatment Group

The patients will either receive DS-2325a or placebo.

5.1.2. Method of Treatment Group(s)/Sequences Allocation

Randomization will follow a randomization schedule generated by an independent biostatistician. Patients, investigators, and site staff will be blinded, except for the unblinded site pharmacist or designee who will be responsible for preparing the study drugs. Sponsor's staff or representative (eg, clinical research organization) will also be blinded, except for the unblinded monitor.

5.1.3. Blinding

The Interventional Part Main Phase will be double-blinded.

Blinding will be applied to all personnel related to the study (patients, investigators, and Sponsor), with the exception of the independent biostatistician and other staff involved in the preparation, release, and shipment of study drug.

The independent biostatistician will generate the randomization schedule in accordance with the operating procedure for allocating study drug. Until the study is unblinded, the study drug randomization schedule will be kept securely.

Preventive measures will be taken to ensure that the blinded team is not unblinded.

5.1.4. Emergency Unblinding Procedure

In the case of an emergency where, in the opinion of the investigator, discontinuation of study drug is not sufficient and the study drug must be unblinded in order to evaluate further a course of medical treatment, the investigator can perform the unblinding by opening the paper envelopes stored at the study site. Entry of the adverse event electronic case report form (eCRF) page should be completed before unblinding except in cases where immediate treatment of the patient is required. If immediate treatment is required, the adverse event eCRF page is to be completed within 24 hours of the unblinding.

In the event of an emergency unblinding, the patient will be informed about their treatment assigned. Information about the treatment assignment **must be** restricted to designated study site staff/personnel who are providing immediate care to the patient. Any documentation of the treatment assignment **must be** maintained separately (ie, a secured file). The information **must not be** included in the patient's source files to ensure the treatment assignment will remain blinded to study personnel not involved with the patient's immediate care.

Once the study drug has been unblinded for a specific patient, investigator should consult Sponsor on whether discontinuing treatment for the patient. If discontinuation is confirmed, the patient should leave the study drug phase. The end of treatment and follow-up assessments will be performed as defined in the protocol.

5.2. Study Drug

5.2.1. Description

DS-2325a will be provided as a sterile solution in vials of [redacted]. Placebo will be prepared from normal saline solution. The appearance of DS-2325a solution is virtually indistinguishable from the appearance of saline. Additional information is available in IB and in the Pharmacy Manual. DS-2325a and placebo will be administered by SC injection, with the exception of the initial ("loading") dose, which will be administered by IV infusion.

5.2.2. Labeling and Packaging

DS-2325a will be supplied by the Sponsor. Saline placebo may be sourced locally or provided by Sponsor/Contract Research Organization (CRO). DS-2325a will be labeled in compliance with regulatory requirements and packaged. The packaging will be clearly labeled "For Clinical Trial Use Only" and will display name of the study drug, the lot number, storage condition, protocol number, and other required information as applicable in accordance with local regulations.

5.2.3. Preparation

Preparation of the study drug is detailed in the Pharmacy Manual.

5.2.4. Administration

Treatment will be administered by [redacted] Permitted injection sites are the abdomen, upper arm, and/or thigh (more than one injection site may be used simultaneously).

Preparation and administration instructions will be provided in the Pharmacy Manual.

5.2.5. Storage

DS-2325a and placebo must be stored in a secure, limited access storage area under the labeled storage conditions.

If storage conditions are not maintained per specified requirements, the Sponsor or CRO should be contacted.

5.2.6. Drug Accountability

When study drug shipment is received, the investigator or designee will check the amount and condition of the drug against the shipping documentation.

In addition, the investigator or designee shall contact Sponsor as soon as possible if there is a problem with the shipment.

Drug Accountability will be provided for study drug and the placebo control. The record must be kept current and should contain the dates and quantities of study drug received, patient's (identification number and/or initials or supply number as applicable) for whom the study drug was dispensed, the date and quantity of study drug dispensed and remaining, as well as the initials of the dispenser.

At the end of the study, or as directed, all study drug and placebo, with all discrepancy resolved, including unused, partially used, or empty containers, will be locally destroyed or returned to a designee as instructed by Sponsor. Study drug will be destroyed or returned only after the study monitor has completed a final inventory to verify the quantity to be returned. The return of study drug must be documented, and the documentation included in the shipment. At the end of the study, a final study drug reconciliation statement must be completed by the investigator or designee and provided to the Sponsor. Unused drug supplies may be destroyed by the investigator when approved in writing by the Sponsor and the Sponsor has received copies of the study site's drug handling and disposition standard operating procedures (SOPs) and it is assured that the Sponsor will receive copies of the certificate of destruction which is traceable to the study drug.

5.2.7. Retention Samples

No retention of samples is required for storage at the investigational site.

5.3. Control Treatment

In the Interventional Part Main Phase, patients will be randomized to 2 parallel arms to receive DS-2325a or placebo in a 2:1 ratio. Placebo will be prepared from normal saline solution.

5.4. Dose Interruptions

Protocol prescribed dosing schedule should be followed throughout the entire study. In case a patient misses a scheduled visit and thereby a dose, the dose should be administered at the next visit. Any such dose interruptions should be discussed with the Sponsor and documented. After any dose interruption, the patient should resume the dosing schedule as soon as possible.

5.5. Method of Assessing Treatment Compliance

All the patients will receive study drug administration under the supervision at the study site. The timing of the administration and the amount administered will be recorded.

5.6. Concomitant Medications

5.6.1. Concomitant and Prohibited Medications

Emollients and topical keratolytic agents and oral antihistamines are allowed. Systemic antibiotic therapy to treat possibly emerging skin infections is allowed.

The following medications are prohibited:

- Systemic corticosteroids, immunosuppressives, targeted therapeutics, biologics, and IV Ig.

- Topical corticosteroids and other topical immunosuppressive agents, including topical calcineurin inhibitors. However, some occasional and temporary use of topical corticosteroids may be allowed only with the intent of symptom relief and if it is not deemed by the investigator to be capable to alter NS course and confound the interpretation of study results.

During the study, other concomitant treatment will be given only if deemed strictly necessary by the investigator or co-investigator. In any case, all concomitant treatments will be reported in the eCRF along with their daily dosage, duration, and reasons for administration.

Patients who have received any prohibited concomitant treatment may be withdrawn from the study at the discretion of an investigator.

5.6.2. Dietary and Lifestyle Restrictions

No restrictions other than limiting alcohol consumption to no more than 14 units per week (one unit of alcohol equals to 250 ml of beer, 100 ml of wine, and 10 ml of spirits).

5.7. Patient Withdrawal/Discontinuation

5.7.1. Reasons for Withdrawal

Any patient who is withdrawn from the study drug for any reason will have their reasons for withdrawal recorded.

Patients may be withdrawn from the study after signing the ICF for the following reasons:

- Adverse Event
- Withdrawal by Patient
- Physician Decision
- Death
- Lack of Efficacy
- Pregnancy
- Protocol Deviation
- Study Terminated by Sponsor
- Lost to Follow-up
- Other

5.7.2. Withdrawal Procedures

If a patient is withdrawn from the study, the investigator will complete and report the observations as thoroughly as possible up to the date of withdrawal, including the date of last dose of study drug and the reason for withdrawal. The investigator will try to perform EOT and EOS procedures, unless the patient is withdrawn during the Observational Part of the study.

If the patient is withdrawn due to an AE, the investigator will try to follow the patient until the AE has resolved or stabilized.

5.7.3. Patient Replacement

Replacing patients will be permitted during the Observational Part and the Interventional Part Main Phase in order to achieve a minimum of 9 patients for ECS evaluation at the end of the Interventional Part Main Phase. However, a patient withdrawn because of AEs considered related to study drug will not be replaced.

5.7.4. Patient Re-screening Procedures

Re-screening is permitted for any patient who failed to meet eligibility criteria upon initial screening. The patient identification number must remain different at the time of re-screening. The initial screening information and the reason why the patient is ineligible for the initial evaluation will be recorded on the Screening Log. No data from the initial evaluation will be entered into the clinical database for a patient who is rescreened.

5.8. Criteria for Discontinuing Study Drug

Treatment will be temporarily put on hold until further evaluation by the DMC if the subject experiences

- Any event severe in nature (eg, severe injection site reaction, severe allergic reaction, etc) deemed by the DMC as necessitating further safety review before treatment can be continued
- Hy's Law event: ALT or AST ≥3 × ULN with simultaneous total bilirubin ≥2 × ULN)
- QTc prolongation: QTcF >500 msec OR increase in QTcF from pre-dose baseline of >60 msec

After an in-depth review of the safety data is conducted by the DMC, the decision to either continue with treatment as planned, to continue treatment, but at a reduced dose, or to terminate participation in the study will be made. If the decision is made to continue treatment, but at a reduced dose, the guidelines as specified in the dose reduction section of the protocol will be followed.

In addition, the subject will be discontinued from the study

- If a subject experiences a severe event or abnormal laboratory test result that the investigator deems to pose a safety risk if he/she were to continue in the study.
- If the use of prohibited medications such as topical corticosteroids and other topical immunosuppressive agents, including topical calcineurin inhibitors, or systemic corticosteroids, immunosuppressives, targeted therapeutics, biologics, and IV Ig is deemed necessary in the opinion of the investigator, the subject may be withdrawn to avoid biased interpretation of study results.
- If a subject shows worsening disease or lack of efficacy according to the discretion of the investigator.

5.9. Dose Reduction

When a reduction in administered dose is necessary, first consider a 50% reduction in the administered dose, which may be attained either by halving the dose (from 600 mg to 300 mg) or by reducing the frequency of administration (from Q2W to Q4W), followed by a 75% reduction if needed, which could be obtained by halving both dose and frequency of administration. If it becomes necessary to consider dose reduction, PI should discuss the dose reduction strategy with the Sponsor.

6. STUDY PROCEDURES

6.1. Informed Consent Form and Eligibility Assessment

Before a patient participates in the study, it is the investigator's responsibility to obtain the patient's freely given consent, in writing, after adequate explanation of the aims, methods, limited benefits, and potential hazards of the study and before any protocol-specific procedures or any study drugs are administered. Patients should be given the opportunity to ask questions and receive answers to their inquiries, and they should have adequate time to decide whether or not to participate in the study.

Eligibility for the study will be accomplished by evaluating the patients against the eligibility criteria mentioned in Section 4.

6.2. Study Procedures Performed During the Study

6.2.1. Screening

As indicated in Section 16.3, the screening procedures will include the following:

- Sign ICF
- Review inclusion/exclusion criteria
- Record demographic information and medical history
- Record vital signs and physical examinations
- Record 12-lead ECG
- Take blood and urine samples for clinical laboratory tests
- Record clinical evaluations
- Record concomitant medications
- Skin photography

6.2.2. Observational Part

As indicated in Section 16.3, the study procedure during the Observational Part includes the following:

- Record vital signs and physical examinations
- Take blood and urine samples for clinical laboratory tests
- Record clinical evaluations
- Record mechanistic evaluations
- Record AEs and/or SAEs and treatments (drug and non-drug) given for these AEs and/or SAEs
- Record concomitant medications
- Skin photography

6.2.3. Interventional Part-Main Phase

As indicated in Section 16.3, the study procedures during the Interventional Part-Main Phase include the following:

- Review inclusion/exclusion criteria
- Randomization
- Record vital signs and physical examinations
- Take blood and urine samples for clinical laboratory tests and ADAs
- Record 12-lead ECG
- Record clinical evaluations
- Record mechanistic evaluations
- Record AEs and/or SAEs and treatments (drug and non-drug) given for these AEs and/or SAEs
- Record concomitant medications
- Skin photography
- Study drug administration
- Take blood samples for PK

6.2.4. Interventional Part-Extension Phase

The patients will be moved from the Main Phase to the Extension Phase with no treatment interruption, as the latter is the immediate and seamless continuation of the former. In the Extension Phase, DS-2325a will only be given SC and not IV. The Extension Phase will be discontinued if ECS is not found and may be terminated anytime by the Sponsor in case lack of efficacy is observed.

As indicated in Section 16.3, the study procedures during the Interventional Part-Extension Phase include the following:

- Record vital signs and physical examinations
- Take blood samples for clinical laboratory tests and ADAs
- Record 12-lead ECG
- Record clinical evaluations
- Record mechanistic evaluations
- Record AEs and/or SAEs and treatments (drug and non-drug) given for these AEs and/or SAEs
- Record concomitant medications
- Skin photography

- Study drug administration
- Take blood samples for PK
- Take optional skin biopsy sample for PK (at Week13)

6.3. Demographics and Medical History

Demographic and physical characteristics will be collected at Screening and include sex, age, race, ethnicity, body weight (kg), height (cm), and BMI (kg/m^2).

Patient's medical and medication histories will be collected by the investigator or a qualified designee. Any untoward medical occurrence (including clinically relevant laboratory values/vital signs that are out of range) that is noted prior to Observational Part will be recorded.

This information is necessary to determine study eligibility and/or may be necessary to interpret study results.

6.4. Randomization

The Interventional Part Main Phase will be double-blinded and placebo-controlled: patients will be randomized to 2 parallel arms to receive DS-2325a or placebo in a 2:1 ratio.

7. PHARMACOKINETIC/PHARMACODYNAMIC ASSESSMENTS

7.1. Pharmacokinetic Assessments

The following PK parameters will be derived from population PK analysis:

- $AUC_{tau,ss}$, C_{trough}, CL, and CL/F.

In addition, DS-2325a presence will be assessed in the skin if the optional skin biopsies will be available. Study patients are outpatients.

Please refer to Section 16.3 for plasma PK and skin biopsy time points.

7.2. Pharmacodynamic Assessment

Please see Section 7.3.

7.3. Biomarker Assessments

Several biomarker endpoints indicative of mechanistic efficacy will be assessed. Information is also in Section 9. Biomarker analyses will be used to investigate the correlation of biomarker with safety or efficacy endpoints. Biomarker analyses will also be used to investigate the correlation between impact at molecular and cellular level, on the one hand, and clinical outcome or PK exposure to DS-2325a, on the other.

The following biomarkers will be assessed in the skin:

- Transepidermal water loss
- Concentrations of mediators of skin inflammation and homeostasis, such as S100A7, S100A8, S100A9, and IL-36γ. These concentrations will be assessed using skin tape harvests collected by tape stripping of lesional and non-lesional skin areas
- KLK5 activity, using skin tape harvests

The following biomarkers will be assessed in circulation:

- Cytokines, like IL-36γ, IL-1β, IL-4, IL-6, IL-8, TSLP, and TNFα
- Chemokines, like CCL17 (TARC), CCL20 (MIP3A), CCL22 (MDC), and CCL27 (CTACK)
- IgE

7.4. Immunogenicity

The ADAs against DS-2325a are the immunogenicity endpoint for this study.

7.5. Pharmacogenetic (Inherited Genetic) Analysis

Not applicable.

8. SAFETY EVALUATION AND REPORTING

8.1. Assessment of Safety Endpoints

The safety endpoints/assessments are-

- AEs, including SAEs and injection site reactions
- Physical exam findings
- Vital signs (body temperature, blood pressure, heart rate, respiratory rate)
- Results of safety laboratory analyses of blood and urine
- Electrocardiogram findings

8.2. Adverse Event Collection and Reporting

All clinical AEs (see Section 8.5.1 for definitions) occurring from the time of patient signing the ICF, including those occurring during the Observational, Interventional, and Extension Parts of the study, up until discharge from the study (following completion of the final Return Visit or unscheduled follow-up), whether observed by the investigator or reported by the patient, will be recorded on the adverse event eCRF page.

Medical conditions (including clinically significant laboratory values/vital signs that are out of range) that were diagnosed or known to exist prior to the consent date will be recorded as medical history, and not an AE. Exacerbation of a pre-existing medical condition or symptom, including increase in severity of the symptom will be recorded as an AE.

All SAEs are to be reported according to the procedures in Section 8.6. All SAEs will be followed until resolution, stabilization, the event is otherwise explained, or the patient is lost to follow-up.

All clinical laboratory results, vital signs, and ECG results or findings should be appraised by the investigator to determine their clinical significance. Isolated abnormal laboratory results, vital sign findings, or ECG findings (ie, not part of a reported diagnosis) should be reported as AEs if they are symptomatic, lead to study drug discontinuation, dose reduction, require corrective treatment, or constitute an AE in the investigator's clinical judgment.

At each time visit, the investigator will determine whether any AEs have occurred by evaluating the patient. Adverse events may be directly observed, reported spontaneously by the patient or by questioning the patient at each study visit. Patients should be questioned in a general way, without asking about the occurrence of any specific symptoms. The investigator must assess all AEs to determine seriousness, severity, and causality, in accordance with the definitions in Section 8.5. The investigator's assessment must be clearly documented in the site's source documentation with the investigator's signature.

Always report the diagnosis as the AE or SAE term. When a diagnosis is unavailable, report the primary sign or symptom as the AE or SAE term with additional details included in the narrative until the diagnosis becomes available. If the signs and symptoms are distinct and do not suggest a common diagnosis, report them as individual entries of AE or SAE.

For events that are serious due to hospitalization, the reason for hospitalization must be reported as the SAE (diagnosis or symptom requiring hospitalization). A procedure is not an AE or SAE, but the reason for the procedure may be an AE or SAE. Pre-planned (prior to signing the ICF) procedures or treatments requiring hospitalization for pre-existing conditions that do not worsen in severity should not be reported as SAEs (see Section 8.5.2 for definitions).

For deaths, the underlying or immediate cause of death should always be reported as an SAE.

Any serious, untoward event that may occur subsequent to the reporting period that the investigator assesses as related to study drug and should also be reported and managed as an SAE.

Details related to COVID-19 assessments are mentioned in Section 8.13.

8.3. Adverse Events of Special Interest

No adverse events of special interest have been identified for this study. Based on the mechanism of action of DS-2325a and experience described for small molecule KLK inhibitors like berotralstat and avoralstat, no potential risks have been identified for DS-2325a. Additional information on KLK inhibitors is available in IB.

However, combined elevations of aminotransferases and total bilirubin, either serious or nonserious and whether or not causally related, meeting the laboratory criteria of a potential Hy's Law case (ALT or AST ≥3 × ULN with simultaneous total bilirubin ≥2 × ULN) should always be reported to the Sponsor using a special collection eCRF, with the investigator's assessment of seriousness, causality, and a detailed narrative. These events should be reported within 24 hours of investigator's awareness of the event.

If the patient discontinues study drug due to liver enzyme abnormalities, the patient should have additional clinical and laboratory evaluations in order to determine the nature and severity of the potential liver injury.

8.4. Injection Site Reactions

Patients should be instructed to inspect the site of injection and call the site to report any pain, redness, swelling, or itching and to determine if any treatment is needed to manage the signs and symptoms. The investigator should collect the details of such injection site related AEs using the Injection Site Reaction form, which captures the presence and severity of pain, redness, swelling, and itching at the injection site using a five-point scale. This information should be captured via an electronic data capture (EDC). In addition, the investigator should inspect the injection site at all visits in the Interventional Part (Main Phase and Extension Phase) and Follow-Up part (EOT and EOS) of the study. All ISRs must be reported as AEs, and serious ISRs should follow the reporting process for SAEs.

8.5. Adverse Event

8.5.1. Definition of Adverse Event

An AE is any untoward medical occurrence in a patient administered a pharmaceutical product and that does not necessarily have to have a causal relationship with this treatment. An AE can

therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product (ICH E2A Guideline. Clinical Safety Data Management: Definitions and Standards for Expedited Reporting, Oct 1994).

It is the responsibility of investigators, based on their knowledge and experience, to determine those circumstances or abnormal laboratory findings which should be considered AEs.

8.5.2. Serious Adverse Event

A SAE is any untoward medical occurrence that at any dose:

- Results in death,
- Is life-threatening,
- Requires inpatient hospitalization or prolongation of existing hospitalization,
- Results in persistent or significant disability/incapacity,
- Is a congenital anomaly/birth defect, or
- Is an important medical event.

Note: The term "life-threatening" in the definition of "serious" refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe (ICH E2A Guideline. Clinical Safety Data Management: Definitions and Standards for Expedited Reporting, Oct 1994).

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above. Examples include allergic bronchospasm, convulsions, and blood dyscrasias or development of drug dependency or drug abuse.

Note:

- Procedures are not AEs or SAEs, but the reason for the procedure may be an AE or SAE.
- Pre-planned (prior to signing the ICF) procedures or treatments requiring hospitalizations for pre-existing conditions that do not worsen in severity are not SAEs.

8.5.3. Severity Assessment

The following definitions should be used to assess intensity of AEs:

- Mild: Awareness of sign or symptom, but easily tolerated, ie, does not interfere with patient's usual function.
- Moderate: Discomfort enough to cause interference with usual activity.

- Severe: Incapacitating with inability to work or do usual activity, ie, interferes significantly with patient's usual function.

Severity versus Seriousness: Severity is used to describe the intensity of a specific event while the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as "seriousness", which is based on patient/event outcome at the time of the event.

8.5.4. Causality Assessment

The investigator should assess causal relationship between an AE and the study drug on the basis of his/her clinical judgment and the following definitions. The causality assessment must be made based on the available information and can be updated as new information becomes available.

- Related:
  - The AE follows a reasonable temporal sequence from study drug administration and cannot be reasonably explained by the patient's clinical state or other factors (eg, disease under study, concurrent diseases, and concomitant medications).

    or
  - The AE follows a reasonable temporal sequence from study drug administration and is a known reaction to the drug under study or its chemical group or is predicted by known pharmacology.
- Not Related:
  - The AE does not follow a reasonable sequence from study drug administration or can be reasonably explained by the patient's clinical state or other factors (eg, disease under study, concurrent diseases, and concomitant medications).

8.5.5. Action Taken Regarding Study Drug

- Dose Not Changed: No change in study drug dosage was made.
- Dose Reduced: The study drug dose was decreased.
- Drug Withdrawn: The study drug was permanently stopped.
- Drug Interrupted: The study drug was temporarily stopped.
- Not Applicable: The patient died, the study drug had been completed prior to a reaction/event, or the reaction/event occurred prior to the start of treatment.

8.5.6. Other Action Taken for Event

- None.
- No treatment was required.
- Medication required.
- Prescription and/or over-the-counter medication was required to treat the AE.

- Hospitalization or prolongation of hospitalization required.
  - Hospitalization was required or prolonged due to the AE, whether or not medication was required.
- Other.

8.5.7. Adverse Event Outcome

- Recovered/Resolved
  - The patient fully recovered from the AE with no residual effect observed.
- Recovering/Resolving
  - The AE improved but has not fully resolved.
- Not Recovered/Not Resolved
  - The AE itself is still present and observable.
- Recovered/Resolved with Sequelae
  - The residual effects of the AE are still present and observable.
  - Include sequelae/residual effects.
- Fatal
  - Fatal should be used when death is a direct outcome of the AE.
- Unknown

8.6. Serious Adverse Events Reporting–Procedure For Investigators

All SAEs will be reported in the eCRF.

The following types of events should be reported by the investigator in the eCRF (or on a Serious Adverse Event Report [SAVER] Form when the eCRF is unavailable) within 24 hours of awareness:

- SAEs (see Section 8.5.2 for definition)
- Hepatic events meeting combination abnormalities (ALT or AST ≥3 × ULN with simultaneous total bilirubin level ≥2 × ULN) (potential Hy's Law case), both serious and non-serious (Section 8.3)
- Pregnancy
- Overdose

All events (serious and non-serious) must be reported with investigator's assessment of the event's seriousness, severity, and causality to the study drug. A detailed narrative summarizing the course of the event, including its evaluation, treatment, and outcome should be provided. Specific or estimated dates of event onset, treatment, and resolution should be included when available. Medical history, concomitant medications, and laboratory data that are relevant to the event should also be summarized in the narrative. For fatal events, the narrative should state whether an autopsy was or will be performed and include the results if available. Source documents (including medical reports) will be retained at the study site and should not be submitted to the Sponsor for SAE reporting purposes.

Urgent safety queries must be followed up and addressed promptly. Follow-up information and response to non-urgent safety queries should be combined for reporting to provide the most complete data possible within each follow-up.

In the event that eCRF is unavailable, report SAEs by faxing the paper SAVER Form to the CRO using the provided fax cover sheet and the appropriate fax number provided for your country. Once eCRF becomes available, please enter SAEs reported on the SAVER Form into eCRF as soon as possible. Please refer to eCRF Completion Guide for additional instructions.

Please call your study monitor for any questions on SAE reporting.

8.6.1. Medication Error, Misuse, and Abuse

Medication errors and uses outside what is foreseen in the protocol, including misuse and abuse of the product shall be patient to the same reporting obligations as adverse events.

- Medication error: This is an unintended failure in the drug treatment process that leads to, or has the potential to lead to harm to the patient.
- Misuse: This refers to situations in which the medicinal product is intentionally and inappropriately used in a manner inconsistent with the protocol or the product labeling.
- Abuse: This corresponds to the persistent or sporadic, intentional excessive use of a medicinal product, which is accompanied by harmful physical or psychological effects to the user.

8.6.2. Overdose

- An overdose is defined as the accidental or intentional administration of any dose of a product that is considered both excessive and medically important. All occurrences of overdose must be reported to the Sponsor within 24 hours of awareness and recorded via the SAVER/Overdose Form and eCRF.
- An “excessive and medically important” overdose includes any overdose in which either an SAE, a non-serious AE, or no AE occurs and is considered by the investigator as clinically relevant, ie, poses an actual or potential risk to the patient.
- Overdoses are not expected in this study in which the study drug is monitored at all times by the clinical site staff. The pharmacist and the pharmacy staff are responsible for correctly preparing the study drug for delivery by the staff to the patients.
- Occupational exposures must be reported via the SAVER form.

8.7. Notifying Regulatory Authorities, Investigators, and Institutional Review Board/Ethics Committee

Daiichi Sankyo and/or CRO will inform investigators, Institutional Review Boards/Ethics Committees (IRBs/ECs), and regulatory authorities of any Suspected Unexpected Serious Adverse Reactions (SUSARs) occurring in other studies of the investigational drug, as appropriate per local reporting requirements. Daiichi Sankyo and/or CRO will comply with any additional local safety reporting requirements.

8.8. Exposure In Utero During Clinical Studies

Daiichi Sankyo must be notified if any patient or partner of the patient becomes pregnant while receiving or within 3 months after the last dose of discontinuing the study drug.

Although pregnancy is not technically an AE, all pregnancies must be followed to conclusion to determine their outcome. If a pregnancy is reported, the investigator should inform the Sponsor within 24 hours of learning of the pregnancy as this information is important for drug safety and public health concerns.

For reports of pregnancy in a female patient or in a female partner of a male patient, the Exposure In Utero Reporting Form (or SAE form if associated with an adverse outcome) should be completed and the details regarding the female partner should be entered in the narrative section.

The adverse pregnancy outcome, either serious or non-serious, should be reported in accordance with study procedures. If the outcome of the pregnancy meets the criteria for immediate classification as a SAE (ie, post-partum complications, spontaneous or induced abortion, stillbirth, neonatal death, or congenital anomaly, including that in an aborted fetus), the investigator should follow the procedures for reporting SAEs outlined in Section 8.6.

8.9. Clinical Laboratory Evaluations

The following items will be measured as indicated in the Schedule of Events (Section 16.3). For clinical laboratory parameters, the reference range of the institution that performs the measurements will be used.

Information will be entered in the eCRF on whether measured, date of measurement, and measurement results for the following items.

1. Hematology tests

   Red blood cell count, hemoglobin, hematocrit, white blood cell count, differential white blood cell count (neutrophils, lymphocytes, monocytes, eosinophils, basophils), and platelet count.

2. Blood chemistry tests

   Total protein, albumin, A/G ratio, total bilirubin, AST, ALT, ALP, GGT, LDH, CK, creatinine, uric acid, Na, K, Cl, Ca, P, Mg, total cholesterol, triglycerides, glucose, and C-reactive protein.

3. Urinalysis

   Urinary glucose, urinary protein, urinary occult blood, urinary ketone bodies, urinary bilirubin, urinary urobilinogen, urine pH, urine sediments (red blood cells, white blood cells, and urinary casts), and urine drug screen tests.

8.10. Vital Signs

Blood pressure and pulse rate will be measured after the patient has rested in a recumbent position for 5 minutes or more.

Information will be entered in the eCRF on whether or not measured, date of measurement, and measurement results for the following items:

- Systolic blood pressure, diastolic blood pressure, heart rate, respiratory rate, and body temperature.

8.11. Electrocardiograms

The ECG will be measured after the patient has rested in a recumbent position for 5 minutes or more. Whether or not measurement is performed and, if performed - date performed and heart rate, PR interval, RR interval, QRS amplitude, and QT interval findings will be recorded in the eCRF.

8.12. Physical Examinations

Physical examination will be performed for each patient, as indicated in Section 16.3.

8.13. Reporting of Exposure to COVID-19 Virus (SARS-CoV-2)

All confirmed or suspected COVID-19 events must be recorded in the eCRF.

- Patients who test positive for COVID-19 should be reported as “Confirmed COVID-19”, either as an AE or SAE.
- Patients whose medical history and clinical manifestations, signs, and possible exposure are consistent with COVID-19 but for whom no polymerase chain reaction or antibody test for COVID-19 is available should be reported as “Suspected COVID-19”, either as an AE or SAE.

The usual protocol mandated SAE reporting requirements should be followed for confirmed or suspected COVID-19 (or SARS-CoV-2) as done for any other AE, ie, the investigator should assess whether any seriousness criteria are met per protocol, and appropriate protocol reporting requirements should be followed.

In the event that the investigator assesses that a COVID-19 case does not meet any seriousness criteria as outlined in the protocol, it should be reported as a non-serious AE in the eCRF.

When assessing the severity of the COVID-19 AE, the severity grading criteria as defined in Section 8.5.3 will be used.

All study drug interruption or dose reduction or discontinuation due to the COVID-19 event must be recorded on the AE and drug administration eCRFs.

For both serious and non-serious COVID-related AEs, the following information should be provided as a minimum:

- Date and laboratory results confirming the COVID-19 diagnosis (including viral antigen test and/or antiviral antibody serological test) in the laboratory eCRF, if available.
- Clinical course of the case, including presenting signs, symptoms, exposure, actions taken with the investigational products, medications used for treatment or prophylaxis of COVID-19, and outcome in relevant eCRF (eg, concomitant medication, AE).
- Findings from diagnostic imaging (including computed tomography scan or other chest imaging).

8.14. Other Examinations

Not applicable

9. EFFICACY ASSESSMENTS

These include assessments of clinical efficacy (IASI, IGA, and PRO measures) and of mechanistic efficacy (transepidermal water loss, skin concentrations of mediators of skin inflammation and homeostasis, skin KLK5 activity, and circulating cytokines, chemokines, and IgE). IASI and IGA for each patient will be assessed by the same investigator throughout the study or by a sub-investigator familiar with the study. This assessment will include skin photography for documentation. The areas of the skin to be photographed are left to the discretion of the investigator.

9.1. Ichthyosis Area and Severity Index

The IASI measures the severity of the erythema (IASI-Erythema) and scaling (IASI-Scaling), adding them together to a total IASI. It is an instrument specifically designed for patients with ichthyoses like NS.[4]

9.2. Investigator Global Assessment

The IGA measures, using a 5-point scale (0, clear; 1, almost clear; 2, mild; 3, moderate; 4, severe) erythema, scaling, inflammatory papules or plaques, oozing, and lichenification.

9.3. Patient-Reported Outcome Measures

9.3.1. Itch NRS

The Itch NRS is a self-rated single item scale designed for assessing worst pruritus in the past 7 days. The scale utilizes an 11-point NRS, scored from 0 (no itch) to 10 (worst imaginable itch).[22]

9.3.2. Quality-of-life Assessments

9.3.2.1. Skindex-29 Questionnaire

The Skindex-29 is a self-reported measure of skin-related symptoms, functioning, and emotional well-being, designed for use across dermatologic conditions.[23]

9.3.2.2. Dermatology Life Quality Index Questionnaire

The DLQI is a self-reported measure of patients' perception of the impact of skin diseases on different aspects of their quality-of-life over the last week.[24]

9.4. Transepidermal Water Loss

Transepidermal water loss is the amount of water that passively evaporates through the skin to the external environment and is used to characterize skin barrier function. It will be determined using ad hoc instrument AQUAFLUX AF200 by BIOX (BIOX Systems Ltd., Technopark Building, 90 London Road, London SE1 6LN, United Kingdom).

9.5. Skin Concentrations of Mediators of Skin Inflammation and Homeostasis

The production in the skin of S100A7, S100A8, and S100A9 is upregulated in a number of inflammatory skin disorder, including NS. The production in the skin of IL-36γ is also upregulated in NS, representing a hallmark of the disease.[25] These upregulations indicate that the IL-17 and IL-36 pathways drive immune-mediated inflammation in the skin of NS patients.

9.6. Skin KLK5 Activity

Skin KLK5 activity is elevated in NS patients, because of deficiency in LEKTI, which is KLK5 natural inhibitor.

9.7. Circulating Cytokines, Chemokines, and IgE

Circulating cytokines like IL-36γ, IL-1β, IL-4, IL-6, IL-8, TSLP, and TNFα, chemokines like CCL17 (TARC), CCL20 (MIP3A), CCL22 (MDC), and CCL27 (CTACK), and IgE are elevated in NS patients, who are often afflicted by atopic disorders, indicating that the IL-17 and IL-36 pathways of immune-mediated inflammation are activated as well as a Th2/Th9 type of allergic response.[5,25]

10. STATISTICAL METHODS

10.1. General Statistical Considerations

This is a Phase 1b/2 study with safety as primary objective and PK, efficacy (ECS), and immunogenicity as secondary objectives. No formal statistical hypothesis tests are planned.

The study will be considered to have been completed when all subjects have either completed treatment and follow-up or discontinued study prior to that for any reason. Study will be terminated if ECS is not found at the end of the Interventional Part Main Phase. Early Clinical Signal is not found unless at least 50% of treated patients achieve at least 50% decrease in total IASI score at the end of the Interventional Part Main Phase compared to Baseline.

Unless otherwise specified, the Baseline value of a safety or PK variable or efficacy endpoint is the last non-missing measurement before the first dose of the study drug.

In general, missing data will not be imputed for the purpose of data analysis unless otherwise specified below or in the statistical analysis plan (SAP), which will be finalized before database lock and will describe procedures for accounting for missing, unused, and spurious data.

Continuous variables will be summarized by the number of observations, mean, standard deviation, median, and minimum and maximum values. Categorical variables will be summarized using frequency counts and percentages. Summary statistics will be presented for both the Main Phase and the Extension Phase of the Interventional Part of study.

10.2. Analysis Sets

- All Enrolled Subject Set will consist of all patients who are enrolled in the Observational Part of the study.
- Safety Analysis Set will consist of all patients who receive at least 1 dose of study drug.
- Pharmacokinetic Analysis Set will include all patients who receive at least 1 dose of study drug and have at least 1 measurable PK result.
- Intent-to-Treat (ITT) Analysis Set will consist of all patients who are randomized.
- Modified Intent-to-Treat Analysis Set will consist of all randomized patients who received at least 1 dose of study drug.

10.3. Study Population Data

Patient disposition will be summarized for patients in the Intent-to-Treat Analysis Set. The total number of patients for each defined analysis population will also be tabulated. The demographic and Baseline characteristics will be summarized descriptively for this set. Some Baseline characteristics may also be summarized for the Intent-to-Treat Analysis Set. Study drug exposure and treatment duration will be summarized using descriptive statistics for the Intent-to-Treat Analysis Set. In addition, the patient disposition and other summaries of the Observational Part data will be tabulated on the All Enrolled Subject Set.

10.4. Statistical Analysis

10.4.1. Pharmacokinetic Analyses

All PK analyses will be based on the PK Analysis Set. The PK assessments are described in Section 7.1. Plasma concentration of DS-2325a will be summarized using descriptive statistics by treatment group and by phase. The PK parameters for both the Main Phase and the Extension Phase of the Interventional Part of study will be analyzed by descriptive statistics, including the mean, geometric mean, standard deviation (SD), coefficient of variation percentage (CV%), or median, minimum, and maximum. Further details shall be outlined in the SAP.

10.4.2. Pharmacodynamic Analyses

Please refer to Section 10.4.3.

10.4.3. Biomarker Analyses

All biomarker analyses will be based on the Safety Analysis Set. The biomarkers (Section 7.3) will be summarized by treatment group using descriptive statistics. Comparison between treatment groups for the change from Baseline (pre-treatment on Day 1 of Week 1 of the Interventional Part Main Phase) to each visit in biomarkers will be summarized.

10.4.4. Efficacy Analyses

All efficacy analyses will be based on the modified Intent-to-Treat Analysis Set (mITT). The efficacy endpoints (IASI, IGA, PRO measures, and mechanistic endpoints) will be summarized by treatment group using descriptive statistics. Comparison between treatment groups for the change from Baseline (pre-treatment on Day 1 of Week 1 of the Interventional Part Main Phase) or from pre-treatment obtained by grouping Observational Part timepoints, which precede Baseline, to each post-treatment visit in IASI, IGA, PRO measures, and mechanistic endpoints will be summarized. More information on this comparison and its use of data collected during Observational Part will be provided in SAP.

10.4.5. Safety Analyses

All safety analyses will be based on the Safety Analysis Set. All AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). Incidence summary of patients with treatment-emergent AEs will be presented by maximum severity, SAEs, AEs assessed as related to study, and AEs resulting in discontinuation of study drug. Observations at each visit and changes from Baseline in vital sign recordings, results of laboratory analyses, and ECG findings will be numerically summarized by treatment group over time. Physical examination findings at each evaluation will be listed and clinically significant abnormal findings will be noted in the listings.

10.4.5.1. Adverse Event Analyses

- Treatment-emergent AEs (TEAEs) are defined as AEs that occur, having been absent before the study drug administration, or worsen in severity after the initiation of study drug administration.

- This study collects non-serious AEs from the time of signing informed consent. Determination of treatment emergence will be done based on the counting rules of TEAE in Global Guidance on Statistical Analysis in Clinical Trials.
- Any AE that starts prior to the first dosing with any study drug, is considered a pre-treatment AE.

AEs will be coded using the MedDRA. The number and percentage of patients reporting TEAEs will be calculated overall, by system organ class, by preferred term, and by treatment group. TEAEs will be further summarized by severity and relationship to study drug. Similarly, the number and percentage of patients reporting treatment-emergent SAEs will be tabulated, as well as TEAEs leading to discontinuation of study drugs.

A by-patient AE (including treatment-emergent) data listing including but not limited to verbatim term, preferred term, system organ class, Common Terminology Criteria for Adverse Events (CTCAE) grade, and relationship to study drug will be provided. Deaths, other SAEs, and other significant AEs, including those leading to discontinuation of study drugs, will be listed. AEs related to injection site reactions will be summarized and listed. The AEs due to COVID-19 will be summarized and listed.

10.4.5.2. Clinical Laboratory Evaluation Analyses

Descriptive statistics will be provided for the clinical laboratory results by scheduled time of evaluation and by treatment group for the Safety Analysis Set, as well as for the change from Baseline. The Baseline value is defined as the last non-missing value before the initial administration of study drug. In addition, mean change from Baseline will be presented by treatment group for the maximum and minimum post-treatment values and the values at check-out.

Abnormal clinical laboratory results will be graded according to National Cancer Institute (NCI) CTCAE version 4.0, if applicable, and the grade will be presented in a by-patient data listing. A shift table, presenting by treatment group the two-way frequency tabulation for Baseline and the worst post-treatment value according to the CTCAE grade or severity, will be provided for clinical laboratory tests. Abnormal clinical laboratory results deemed of clinical significance or of Grade 3 or 4 will be presented.

10.4.5.3. Vital Sign Analyses

Descriptive statistics will be provided for the vital signs measurements by scheduled time of evaluation and by treatment group for the Safety Analysis Set, as well as for the change from Baseline. The Baseline value is defined as the last non-missing value before the initial administration of study drug.

10.4.5.4. Physical Examination Analyses

Physical examination findings at each evaluation will be listed.

10.4.5.5. Electrocardiogram Analyses

Descriptive statistics will be provided for the ECG measurements by scheduled time of evaluation and by treatment group for the safety population, as well as for the change from

Baseline. The Baseline value is defined as the last non-missing value before the initial administration of study drug. In addition, the number and percentage of patients with ECG interval values meeting the criteria will be tabulated (eg, corrected QT interval [QTc] <450 msec, >450 to ≤480 msec, >480 msec to ≤500 msec, and >500 msec). Data from ECG will also be presented in the data listings.

10.5. Sample Size Determination

This Phase 1b/2 study will enroll a total of 9 to 12 patients.

The sample size selected is not based on statistical power considerations. The number of patients in each arm is considered sufficient to achieve the objectives of this study. Initial evidence of DS-2325a efficacy (ie, ECS) will be inferred by the impact DS-2325a shows on clinical endpoints, mainly IASI, and several mechanistic endpoints. Concomitant and consistent impact on multiple endpoints will represent ECS, especially if this impact is of large extent. This impact will be evinced by comparison of endpoint values between DS-2325a-treated and placebo-treated patients and especially between Baseline (pre-treatment) and post-treatment timepoints, since the Observational Part of the study should allow an in-depth characterization of each patient and thus facilitate pre-and post-treatment comparisons. The results of these comparisons will allow for the determination of whether DS-2325a has given ECS and whether the continuation of the Interventional Part Extension Phase, as well as the further development of DS-2325a, is warranted.

10.6. Statistical Analysis Process

In this study, Interventional Part Main Phase is double-blind and Extension Phase is open-label.

Statistical Analysis Plan will be finalized prior to unblinding to preserve the integrity of the statistical analysis and clinical study conclusions. Changes in the SAP planned analyses will be reported in the Clinical Study Report.

All statistical analyses will be performed using SAS® Version 9.2 or higher (SAS Institute, Cary, NC 27513).

11. DATA INTEGRITY AND QUALITY ASSURANCE

11.1. Monitoring and Inspections

The Sponsor/CRO monitor, and regulatory authority inspectors are responsible for contacting and visiting the investigator for the purpose of inspecting the facilities and, upon request, inspecting the various records of the study (eg, eCRFs, source data, and other pertinent documents).

The verification of adherence to the protocol; completeness, accuracy, and consistency of the data; and adherence to ICH GCP and local regulations on the conduct of clinical research will be accomplished through a combination of onsite visits by the monitor and review of study data remotely. The frequency of the monitoring visit will vary based on the activity at each study site. The monitor is responsible for inspecting the eCRFs and ensuring completeness of the study essential documents. The monitor should have access to patient medical records and other study-related records needed to verify the entries on the eCRFs. Detailed information is provided in the monitoring plan.

The monitor will communicate deviations from the protocol, SOPs, GCP, and applicable regulations to the investigator and will ensure that appropriate action (s) designed to prevent recurrence of the detected deviations is taken and documented.

The investigator agrees to cooperate with the monitor to ensure that any problems detected in the course of these monitoring visits are addressed to the satisfaction of the Sponsor and documented.

In accordance with ICH GCP and the Sponsor's audit plans, this study site may be selected for audit by representatives from the Sponsor. Audit of study site facilities (eg, pharmacy, drug storage areas, laboratories) and review of study-related records will occur in order to evaluate the study conduct and compliance with the protocol, ICH GCP, and applicable regulatory requirements. The investigator should respond to audit findings. In the event that a regulatory authority informs the investigator that it intends to conduct an inspection, the Sponsor shall be notified immediately.

11.2. Data Collection

Daiichi Sankyo or a designee will supply eCRFs. An eCRF must be completed for each patient who signs an ICF and undergoes any screening procedure. If a patient is not treated, the reason must be recorded on the eCRF. All data collected during the study will be recorded in this individual, patient-specific eCRF. Instructions will be provided for the completion of the eCRF, and any corrections made will be automatically documented via the Electronic Data Capture (EDC) software's "audit trail."

Completion of the eCRF should be kept current to enable the monitor to review the patient's status throughout the course of the study. All information and other material to be used by patients and investigative staff must use vocabulary and language that are clearly understood. The eCRF will be completed, reviewed, and signed off or e-signed by the investigator. The investigator will sign and date the indicated places on the eCRF via the EDC system's electronic

signature. These signatures will indicate that the investigator inspected or reviewed the data on the eCRF, the data queries, and the study site notifications, and agrees with the content.

11.3. Data Management

Each patient will be identified in the database by a unique patient identifier as defined by the Sponsor.

To ensure the quality of clinical data across all patients and study sites, a Clinical Data Management review will be performed on patient data according to specifications given to Sponsor/CRO. Data will be vetted both electronically and manually for eCRFs and the data will be electronically vetted by programmed data rules within the application. Queries generated by rules and raised by reviewers will be generated within the EDC application. During this review, patient data will be checked for consistency, completeness, and any apparent discrepancies. To resolve any questions arising from the Clinical Data Management review process for paper eCRFs, data queries, and/or study site notifications will be sent to the study site for completion and return to the Sponsor/CRO; whereas eCRFs queries will be raised and resolved within the EDC application.

Data received from external sources such as central laboratories will be reconciled to the clinical database.

The SAEs in the clinical database will be reconciled with the safety database.

All adverse events will be coded using MedDRA.

Data that may potentially unblind the treatment assignment (ie, study drug serum concentrations, treatment allocation, and study drug preparation/accountability data) will be handled with special care during the data cleaning and review process. These data will be handled in such a way that, prior to unblinding, any data that may unblind study team personnel will be presented as blinded information or otherwise will not be made available. If applicable, unblinded data may be made available to quality assurance representatives for the purposes of conducting independent audits.

11.4. Study Documentation and Storage

The investigator will maintain a Signature List of appropriately qualified persons to whom he/she has delegated study duties. All persons authorized to make entries and/or corrections on eCRFs will be included on the Signature List.

investigators will maintain a confidential screening log of all potential study candidates that includes limited information of the patients, date and outcome of screening process.

investigators will be expected to maintain an Enrollment Log of all patients enrolled in the study indicating their assigned study number.

investigators will maintain a confidential patient identification code list. This confidential list of names of all patients allocated to study numbers on enrolling in the study allows the investigator to reveal the identity of any patient when necessary.

Source documents are original documents, data, and records from which the patient's eCRF data are obtained. These include but are not limited to hospital records, clinical and office charts, laboratory and pharmacy records, diaries, microfiches, X-rays, and correspondence.

Records of patients, source documents, monitoring visit logs, data correction forms, eCRFs, inventory of study drug, regulatory documents (eg, protocol and amendments, IRB/EC correspondence and approvals, approved and signed ICFs, investigator's Agreement, clinical supplies receipts, distribution and return records), and other Sponsor correspondence pertaining to the study must be kept in appropriate study files at the study site (Trial Master File). Source documents include all recordings and observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical study. These records will be retained in a secure file for the period required by the institution or study site policy. Prior to transfer or destruction of these records, the Sponsor must be notified in writing and be given the opportunity to further store such records.

11.5. Record Keeping

The investigator and study staff are responsible for maintaining a comprehensive and centralized filing system (Trial Master File) of all study-related (essential) documentation, suitable for inspection at any time by representatives from the Sponsor and/or applicable regulatory authorities. Essential documents contained in the Trial Master File include:

- Patient files containing completed eCRFs, ICFs, and supporting copies of source documentation (if kept).
- Study files containing the protocol with all amendments, IB, copies of relevant essential documents required prior to commencing a clinical study, and all correspondence to and from the EC/IRB and the Sponsor.
- Records related to the study drug(s) including acknowledgment of receipt at study site, accountability records, and final reconciliation, and applicable correspondence.

In addition, all original source documents supporting entries in the eCRFs must be maintained and be readily available.

All study-related essential documentation will be retained by the investigator as per the local regulations. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by an agreement with the Sponsor. It is the responsibility of the Sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

Patient's medical files should be retained in accordance with applicable legislation and in accordance with the maximum period of time permitted by the hospital, institution, or private practice.

No study document should be destroyed without prior written agreement between Sponsor and the investigator. Should the investigator wish to assign the study records to another party or move them to another location, he/she must notify Sponsor in writing of the new responsible person and/or the new location.

12. FINANCING AND INSURANCE

12.1. Finances

Prior to starting the study, the investigator and/or institution will sign a clinical trial agreement with the CRO, on behalf of DS. This agreement will include the financial information agreed upon by the parties.

12.2. Reimbursement, Indemnity, and Insurance

The Sponsor provides insurance for study patients to make available compensation in case of study-related injury.

Reimbursement, indemnity, and insurance shall be addressed in a separate agreement on terms agreed upon by the parties.

13. PUBLICATION POLICY

Daiichi Sankyo is committed to meeting the highest standards of publication and public disclosure of information arising from clinical trials sponsored by the company. We will comply with US, EU, and Japanese policies for public disclosure of the clinical trial protocol and clinical trial results, and for sharing of clinical trial data. We follow the principles set forward in "Good Publication Practice for Communicating Company-Sponsored Medical Research (GPP3)", and publications will adhere to the "Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals" established by the International Council of Medical Journal Editors (ICMJE).

In order to ensure that we are in compliance with the public disclosure policies and the ICMJE recommendations, and to protect proprietary information generated during the study, all publications (manuscripts, abstracts, or other public disclosure) based on data generated in this study must be accepted, reviewed, and approved in writing by the Sponsor prior to submission.

14. ETHICS AND STUDY ADMINISTRATIVE INFORMATION

14.1. Compliance Statement, Ethics and Regulatory Compliance

This study will be conducted in compliance with the protocol, the ethical principles that have their origin in the Declaration of Helsinki, the International Council for Harmonisation (ICH) consolidated Guideline E6 for Good Clinical Practice (GCP) (CPMP/ICH/135/95), and applicable regulatory requirement(s) including the following:

- Regulation (EU) No 536/2014 of the European Parliament and of the Council of 16 Apr 2014 on clinical trials on medicinal products for human use, and repealing Directive 2001/20/EC and/or;
- US Food and Drug Administration (FDA) GCP Regulations: Code of Federal Regulations (CFR) Title 21, parts 11, 50, 54, 56, and 312 as appropriate and/or;
- Japanese Ministry of Health, Labor and Welfare Ordinance No. 28 of 27 Mar 1997 and/or;
- The Act on Securing Quality, Efficacy and Safety of Pharmaceuticals, Medical Devices, Regenerative and Cellular Therapy Products, Gene Therapy Products, and Cosmetics No. 1 of 25 Nov 2014;
- Other applicable local regulations.

14.2. Patient Confidentiality

The investigators and the Sponsor will preserve the confidentiality of all patients taking part in the study, in accordance with GCP, local regulations, and the rules laid down in the European Data Protection Directive 95/46/EC on the protection of individuals with regard to the processing of personal data and the free movement of such data.

The investigator must ensure that the patient's anonymity is maintained. On the eCRFs or other documents submitted to the Sponsor or the CRO, patients should be identified by a unique patient identifier as designated by the Sponsor. Documents that are not for submission to the Sponsor or the CRO (eg, signed ICF) should be kept in strict confidence by the investigator.

In compliance with ICH GCP Guidelines, it is required that the investigator and institution permit authorized representatives of the company, of the regulatory agency(s), and the IRB/EC direct access to review the patient's original medical records for verification of study-related procedures and data. The investigator is obligated to inform the patient that his/her study-related records will be reviewed by the above-named representatives without violating the confidentiality of the patient.

14.3. Informed Consent

Before a patient's participation in the study, it is the investigator's responsibility to obtain freely given consent, in writing, from the patient after adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the study and before any protocol-specific procedures or any study drugs are administered. Patients should be given the opportunity to ask questions and receive satisfactory answers to their inquiries and should have adequate time to

decide whether or not to participate in the study. The written ICF should be prepared in the local language(s) of the potential patient population.

In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirements, and should adhere to GCP and to the ethical principles that have their origin in the Declaration of Helsinki. ICF and any revision(s) should be approved by the EC or IRB prior to being provided to potential patients.

The patient's written informed consent should be documented in the patient's medical records. The ICF should be signed and personally dated by the patient and by the person who conducted the informed consent discussion (not necessarily the investigator). The original signed ICF should be retained in accordance with institutional policy, and a copy of the signed ICF should be provided to the patient. The date and time (if applicable) that informed consent was given should be recorded on the eCRF.

If the patient lacks the ability to consent, it is the investigator's responsibility to obtain freely given consent, in writing, where allowed by law, legally acceptable representative (based on country and site-specific practice) who has the best interests of the patient in mind. After adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the study and before any protocol-specific procedures or any study drugs are administered. The legally acceptable representative should be given the opportunity to ask questions and receive satisfactory answers to their inquiries and should have adequate time to decide whether or not to participate in the study. The legally acceptable representative's written informed consent should be documented in the patient's medical records. If a legally authorized representative provides consent on behalf of an incapable patient, then, once the patient recovers the ability to consent, he/she should be approached by the investigator or designee to obtain his/her consent (if considered a requirement for the study site as per the local ethical requirements).

If the patient cannot read, then according to ICH GCP Guideline, Section 4.8.9, an impartial witness should be present during the entire informed consent discussion. This witness should sign the ICF after the patient has consented to the patient's participation and, if possible, signed the ICF. By signing the ICF, the witness attests that the information in the ICF and any other written information was adequately explained to and apparently understood by the patient and that informed consent was freely given by the patient.

14.4. Regulatory Compliance

The study protocol, ICF, IB, any patient written instructions to be given to the patient, available safety information, patient recruitment procedures (eg, advertisements), information about payments and compensation available to the patients, and documentation evidencing the investigator's qualifications should be submitted to the EC or IRB for ethical review and approval according to local regulations, prior to the study start.

Changes in the conduct of the study or planned analysis will be documented in a protocol amendment and/or the SAP.

Among other possible duties, the investigator will be responsible for reviewing and approving the Final Clinical Study Report and testifying to the accuracy of the description of the study conduct.

The investigator and/or Sponsor must submit and, where necessary, obtain approval from the EC or IRB for all subsequent protocol amendments and changes to the ICF. The investigator should notify the EC or IRB of deviations from the protocol or SAEs occurring at the study site and other AE reports received from the Sponsor/CRO, in accordance with local procedures.

As required by local regulations, the Sponsor's local Regulatory Affairs group or representative to whom this responsibility has been delegated will ensure all legal aspects are covered, and approval from the appropriate regulatory bodies obtained, prior to study initiation. If substantial changes to the initial protocol and other relevant study documents are made, this representative will also ensure that any revised documents required for submission are submitted to regulatory authorities and implementation of these changes happen only after approval by the relevant regulatory bodies, as required.

In the event of any prohibition or restriction imposed (eg, clinical hold) by an applicable Regulatory Authority(ies) in any area of the world, or if the investigator is aware of any new information which might influence the evaluation of the benefits and risks of the investigational drug, the Sponsor should be informed immediately.

In addition, the investigator will inform the Sponsor immediately of any urgent safety measures taken by the investigator to protect the study patients against any immediate hazard, and of any suspected/actual serious GCP non-compliance that the investigator becomes aware of.

14.5. Protocol Deviations

The investigator should conduct the study in compliance with the protocol agreed to by Sponsor and, if required, by the regulatory authority(ies), and which was given approval/favorable opinion by the IRBs/ECs.

A deviation to any protocol procedure or waiver to any stated criteria will not be allowed in this study except where necessary to eliminate immediate hazard(s) to the patient. Sponsor must be notified of all intended or unintended deviations to the protocol (eg, inclusion/exclusion criteria, dosing, missed study visits) on an expedited basis.

The investigator, or person designated by the investigator, should document and explain any deviation from the approved protocol.

If a patient was ineligible or received the incorrect dose or study drug, and had at least 1 administration of study drug, data should be collected for safety purposes.

If applicable, the investigator should notify the EC or IRB of deviations from the protocol in accordance with local procedures.

14.6. Supply of New Information Affecting the Conduct of the Study

When new information becomes available that may adversely affect the safety of patients or the conduct of the study, the Sponsor will inform all investigators involved in the clinical study, ECs/IRBs, and regulatory authorities of such information, and when needed, will amend the protocol and/or patient information.

The investigator should immediately inform the patient whenever new information becomes available that may be relevant to the patient's consent or may influence the patient's willingness

to continue participation in the study. The communication should be documented on medical records, for example, and it should be confirmed whether the patient is willing to remain in the study.

If the patient information is substantially revised (mere administrative changes are not considered to be substantial), it must be re-approved by the EC/IRB. The investigator should obtain written informed consent to continue participation with the revised written information even if patients were already informed of the relevant information. The investigator or other responsible personnel who provided explanations and the patient should sign and date the revised ICF.

14.7. Protocol Amendments

Any amendments to the study protocol that seem to be appropriate as the study progresses will be communicated to the investigator by Daiichi Sankyo or the CRO. Also, the Sponsor will ensure the timely submission of substantial amendments to regulatory authorities.

The protocol amendments will undergo the same review and approval process as the original protocol. Changes made by such amendments will be documented in a Summary of Changes document.

A protocol amendment may be implemented after it has been approved by the IRB/EC and by regulatory authorities unless implementation is allowed without this approval by local regulations.

14.8. Study Termination

The Sponsor may initiate study termination/site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of a study site may include but are not limited to:

- Failure of the investigator to comply with the protocol, the requirements of the IRB or local health authorities, Sponsor's procedures, or GCP guidelines.
- Inadequate recruitment of patients by the investigator.
- Total number of patients included earlier than expected.
- If the study is prematurely terminated or suspended, Sponsor/CRO shall promptly inform the investigator, the IRB/EC and the regulatory authorities of the reason for termination or suspension, as specified by the applicable regulatory requirements. The investigator shall promptly inform the patients and should ensure appropriate patient therapy and/or follow-up.

14.9. Data and Safety Monitoring Board

A DMC will be assembled to guarantee the safety of study conductance. The DMC will regularly analyze the safety data that will accumulate during the study and will make recommendations concerning study continuation, modification, or termination. The DMC will include members external to the sponsor. The DMC composition and operating conditions will be described in the DMC charter.

14.10. Address List

A list of key study personnel (including personnel at the Sponsor, CRO, laboratories, and other vendors) and their contact information (address, telephone, fax, email) will be kept on file and regularly updated as necessary.

15. REFERENCES

1. Chavanas S, Bodemer C, Rochat A, et al. Mutations in SPINK5, encoding a serine protease inhibitor, cause Netherton syndrome. Nat Genet. 2000; 25(2):141-2.
2. Sprecher E, Chavanas S, DiGiovanna JJ, et al. The spectrum of pathogenic mutations in SPINK5 in 19 families with Netherton syndrome: implications for mutation detection and first case of prenatal diagnosis. J Invest Dermatol. 2001; 117(2):179-87.
3. Descargues P, Deraison C, Bonnart C, et al. Spink5-deficient mice mimic Netherton syndrome through degradation of desmoglein 1 by epidermal protease hyperactivity. Nat Genet. 2005; 37(1):56-65.
4. Paller AS, Renert-Yuval Y, Suprun M, et al. An IL-17-dominant immune profile is shared across the major orphan forms of ichthyosis. J Allergy Clin Immunol. 2017; 139(1):152-65.
5. Petrova E and Hovnanian A. Advances in understanding of Netherton syndrome and therapeutic implications. Expert Opinion in Orphan Drugs. 2020; 8(11):455-87.
6. Hovnanian A. Netherton syndrome: skin inflammation and allergy by loss of protease inhibition. Cell Tissue Res. 2013; 351(2):289-300.
7. Hovnanian A. Netherton syndrome: new advances in the clinic, disease mechanism and treatment. Expert Rev Dermatol. 2012; 7(1):81-92.
8. Jones SK, Thomason LM, Surbrugg SK, et al. Neonatal hypernatraemia in two siblings with Netherton's syndrome. Br J Dermatol. 1986; 114(6):741-3.
9. Judge MR, Morgan G, Harper JI. A clinical and immunological study of Netherton's syndrome. Br J Dermatol. 1994; 131(5):615-21.
10. Hoeger PH and Harper JI. Neonatal erythroderma: differential diagnosis and management of the "red baby." Arch Dis Child. 1998; 79(2):186-91.
11. Furio L, de Veer S, Jaillet M, et al. Transgenic kallikrein 5 mice reproduce major cutaneous and systemic hallmarks of Netherton syndrome. J Exp Med. 2014; 211(3):499-513.
12. Briot A, Deraison C, Lacroix M, et al. Kallikrein 5 induces atopic dermatitis-like lesions through PAR2-mediated thymic stromal lymphopoietin expression in Netherton syndrome. J Exp Med. 2009; 206(5):1135-47.
13. Luchsinger I, Knoepfel N, Theiler M, et al. Secukinumab therapy for Netherton syndrome. JAMA Dermatol. 2020; 156(8):907-11.
14. Barbieux C, Bonnet des Claustres M, de la Brassinne M, et al. Duality of Netherton syndrome manifestations and response to ixekizumab. J Am Acad Dermatol. 2021; 84(5):1476-80.
15. Steuer AB, Cohen DE. Treatment of Netherton syndrome with dupilumab. JAMA Dermatol. 2020; 156(3):350-1.

16. Johnson KW, Hovnanian A, Teng J, et al. Rationale and design for the kallikrein inhibitor in Netherton syndrome (KINS) pivotal clinical trial. J Invest Dermatol. 2020; 140(7):S79.

17. Tomisato W, Siggs OM, Beutler B. Record for crusty2, updated 22 May 2022. MUTAGENETIX (TM), B. Beutler and colleagues, Center for the Genetics of Host Defense, UT Southwestern Medical Center, Dallas, TX. Accessed 19 December 2022. URL: https://mutagenetix.utsouthwestern.edu.

18. Yalcin AD. A case of netherton syndrome: successful treatment with omalizumab and pulse prednisolone and its effects on cytokines and immunoglobulin levels. Immunopharmacol Immunotoxicol. 2016; 38(2):162-6.

19. Fontao L, Laffitte E, Briot A, et al. Infliximab infusions for Netherton syndrome: sustained clinical improvement correlates with a reduction of thymic stromal lymphopoietin levels in the skin. J Invest Dermatol. 2011; 131(9):1947-50.

20. Small AM, Cordoro KM. Netherton syndrome mimicking pustular psoriasis: clinical implications and response to intravenous immunoglobulin. Pediatr Dermatol. 2016; 33(3):e222-3.

21. Andreasen TH, Karstersen HG, Duno M, et al. Successful treatment with dupilumab of an adult with Netherton syndrome. Clin Exp Dermatol. 2020; 45(7):915-17.

22. Newton L, DeLozier AM, Griffiths PC, et al. Exploring content and psychometric validity of newly developed assessment tools for itch and skin pain in atopic dermatitis. J Patient Rep Outcomes. 2019; 3(1):42.

23 Chren MM, Lasek RJ, Quinn LM, et al. Skindex, a quality-of-life measure for patients with skin disease: reliability, validity, and responsiveness. Journal of Investig. Dermatol. 1996; 107(5):707-13.

24 Finley AY, Khan GK. Dermatology Life Quality Index (DLQI)-a simple practical measure for routine clinical use. Clin Exp Dermatol. 1994; 19(3):210-6.

25 Barbieux C, Bonnet des Claustres M, Fahrner M, et al. Netherton syndrome subtypes share IL-17/IL-36 signature with distinct IFN-a and allergic responses. J Allergy Clin Immunol. 2022; 149(4):1358-72.

16. APPENDICES

16.1. Labeling and Packaging

Details are provided in Section 5.2.2.

16.2. Blood Collection Volume by Category and Total

The details are available in the ICF.

16.3. Schedule of Events

Table 3: Visit Schedule for Observational Part and Interventional Part-Main Phase

Schedule of Events		Observational Part			Interventional Part-Main Phase						
	Screening*				Baseline^						
Week	**−4 to 0**	**1**	**5**	**9**	**1**	**2**	**3**	**5**	**7**	**9**	**11**
Visit Window allowed (day)		**±2**	**±2**	**±2**	**±1**	**±1**	**±1**	**±2**	**±2**	**±2**	**±2**
Informed Consent	X										
Demographics, height, and BMI	X										
Body weight	X	X	X	X	X			X		X	
Eligibility Criteria	X				X						
Serology (HBV, HCV, and HIV)	X										
COVID-19 test	X				X						
Urine drugs of abuse	X										
Medical History	X										
Adverse Events (1)	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medications (1)	X	X	X	X	X	X	X	X	X	X	X
Vital Signs (1)	X	X	X	X	X	X	X	X	X	X	X
Physical Exam (2)	X	X	X	X	X		X	X	X	X	X
Safety Laboratory Tests, Including Urinalysis (3)	X	X			X			X		X	
ECG (4)	X				X			X		X	
Serum Pregnancy Test	X										
Urine Pregnancy Test (5)					X			X		X	
Clinical Evaluations (IASI, IGA, and PRO) (2)	X	X	X	X	X		X	X	X	X	X
Skin Photography (2)	X	X	X	X	X		X	X	X	X	X
Mechanistic Evaluations (PD biomarkers) - TEWL (6)		X			X						
Mechanistic Evaluations (PD biomarkers) - Skin Tape Harvests (6)		X			X						
Mechanistic Evaluations (PD biomarkers) - Blood (7)		X	X	X	X			X		X	
Randomization					X						
Dose Administration (8)					X	X	X	X	X	X	X
PK (blood) (9)					X (10)		X	X	X	X	X
ADA (blood) (11)					X			X		X	

Abbreviations: ADA = Anti-drug Antibody, BMI = Body Mass Index, COVID-19 = Coronavirus Disease 2019, ECG = Electrocardiogram, EOS = End of Study, EOT = End of Treatment, HBV = Hepatitis B virus, HCV = Hepatitis C virus, HIV = Human Immunodeficiency Virus, IASI = Ichthyosis Area Severity Index, IGA = Investigator Global Assessment, IP = Interventional Part, IV = intravenous, OP = Observational Part, PD = Pharmacodynamics, PK = Pharmacokinetics, PRO = Patient-Reported Outcome, SC = subcutaneous, TEWL = Transepidermal water loss

*Screening will occur during the 28 days preceding Week 1 of OP; ^Baseline is pretreatment on Day 1 of Week 1 of Interventional Part Main Phase
(1) To be recorded at Screening; at Weeks 1, 5, and 9 during OP; every other week during IP before dose administration; and at Weeks 37 EOT and 45 EOS.
(2) To be performed at Screening; at Weeks 1, 5, and 9 during OP; every other week (ie, at odd-numbered weeks, to start with Week 1) during IP before dose administration; and at Weeks 37 EOT and 45 EOS (except clinical evaluations [IASI, IGA, and PRO] and skin photography, which will not be performed at Week 45 EOS).
(3) To be performed at Screening; at Week 1 during OP; at Weeks 1, 5, 9, 13, 21, and 29, during IP before dose administration; and at Weeks 37 EOT and 45 EOS.
(4) To be performed at Screening; at Weeks 1, 5, 9, and 13 during IP before dose administration; and at Weeks 37 EOT and 45 EOS.
(5) To be performed at Weeks 1, 5, 9, 13, 17, 21, 25, 29, and 33 during IP before dose administrations; and at Week 37 EOT.
(6) To be performed at Week 1 during OP and at Weeks 1, 13, and 25 during IP before dose administration; and at Week 37 EOT. Skin tape harvests will allow assessing the skin concentrations of mediators of skin inflammation and homeostasis and skin KLK5 activity.
(7) To be performed at Weeks 1, 5, and 9 during OP; at Weeks 1, 5, 9, 13, 21, 25, and 29 during IP before dose administration; and at Week 37 EOT.
(8) To be given at Weeks 1, 2, and 3 and every other week during IP-Main Phase. Dose administration will be IV at Week 1 and then SC.
(9) To be collected every other week (ie, at odd-numbered weeks, to start with Week 1) during IP before dose administration; and at Weeks 37 EOT and 45 EOS. Blood samples will be collected no more than 1 hour before the start of dose administration.
(10) On Week 1 of IP, PK blood sample will also be collected at the end of infusion (ie, 1 hour after start of dose administration) and 1 hour later (ie, 2 hours after start of dose administration). These two blood samples will be collected ± 10 minutes from the nominal times.
(11) To be collected at Weeks 1, 5, 9, 13, 17, 21, 25, 29, and 33 during IP before dose administration; and at Weeks 37 EOT and 45 EOS.

Table 4: Visit Schedule for Interventional Part-Extension Phase

Schedule of Events	Interventional Part-Extension Phase													Follow-Up	
														EOT	EOS
Weeks	**13**	**14**	**15**	**17**	**19**	**21**	**23**	**25**	**27**	**29**	**31**	**33**	**35**	**37**	**45**
Visits window allowed (Day)	**±1**	**±1**	**±1**	**±2**	**±2**	**±2**	**±2**	**±2**	**±2**	**±2**	**±2**	**±2**	**±2**	**±2**	**±3**
Body weight	X			X		X		X		X		X		X	X
Adverse Events (1)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medications (1)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs (1)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical Exam (2)	X		X	X	X	X	X	X	X	X	X	X	X	X	X
Safety Laboratory Tests, Including Urinalysis (3)	X					X				X				X	X
ECG (4)	X													X	X
Serum Pregnancy Test															
Urine Pregnancy Test (5)	X			X		X		X		X		X		X	
Clinical Evaluations (IASI, IGA, and PRO) (2)	X		X	X	X	X	X	X	X	X	X	X	X	X	
Skin Photography (2)	X		X	X	X	X	X	X	X	X	X	X	X	X	
Mechanistic Evaluations (PD biomarkers) - TEWL (6)	X							X						X	
Mechanistic Evaluations (PD biomarkers) - Skin Tape Harvests (6)	X							X						X	
Mechanistic Evaluations (PD biomarkers) - Blood (7)	X					X		X		X				X	
Randomization															
Dose Administration (8)	X	X	X	X	X	X	X	X	X	X	X	X	X		
PK (blood) (9)	X		X	X	X	X	X	X	X	X	X	X	X	X	X
PK (skin) (10)	X														
ADA (blood) (11)	X			X		X		X		X		X		X	X

Abbreviations: ADA = Anti-drug Antibody, ECG = Electrocardiogram, EOS = End of Study, EOT = End of Treatment, IASI = Ichthyosis Area Severity Index, IGA = Investigator Global Assessment, IP = Interventional Part, IV = intravenous, OP = Observational Part, PD = Pharmacodynamics, PK = Pharmacokinetics, PRO = Patient-Reported Outcome, SC = subcutaneous, TEWL = Transepidermal water loss.
*Screening will occur during the 28 days preceding Week 1 of OP; ^Baseline is pretreatment on Day 1 of Week 1 of Interventional Part Main Phase
(1) To be recorded at Screening; at Weeks 1, 5, and 9 during OP; every other week during IP before dose administration; and at Weeks 37 EOT and 45 EOS.

(2) To be performed at Screening; at Weeks 1, 5, and 9 during OP; every other week (ie, at odd-numbered weeks, to start with Week 1) during IP before dose administration; and at Week 37 EOT and 45 EOS (except clinical evaluations [IASI, IGA, and PRO] and skin photography, which will not be performed at Week 45 EOS).
(3) To be performed at Screening; at Week 1 during OP; at Weeks 1, 5, 9, 13, 21, and 29, during IP before dose administrations; and at Weeks 37 EOT and 45 EOS.
(4) To be performed at Screening; at Weeks 1, 5, 9, and 13 during IP before dose administration; and at Weeks 37 EOT and 45 EOS.
(5) To be performed at Weeks 1, 5, 9, 13, 17, 21, 25, 29, and 33 during IP before dose administrations; and at Week 37 EOT.
(6) To be performed at Week 1 during OP and at Weeks 1, 13, and 25 during IP before dose administration; and at Week 37 EOT. Skin tape harvests will allow assessing the skin concentrations of mediators of skin inflammation and homeostasis and skin KLK5 activity.
(7) To be performed at Weeks 1, 5, and 9 during OP; at Weeks 1, 5, 9, 13, 21, 25, and 29 during IP before dose administration; and at Week 37 EOT.
(8) To be given at Weeks 13, 14, and 15 and every other week during IP-Extension Phase. Dose administration will be SC.
(9) To be collected every other week (ie, at odd-numbered weeks, to start with Week 1) during IP before dose administration; and at Weeks 37 EOT and 45 EOS. Blood samples will be collected no more than 1 hour before the start of dose administration.
(10) Optional skin biopsy will be a 3-mm biopsy to be collected before dose administration at Week 13 to assess epidermis-to-dermis DS-2325a concentration ratio.
(11) To be collected at Weeks 1, 5, 9, 13, 17, 21, 25, 29, and 33 during IP before dose administration; and at Weeks 37 EOT and 45 EOS.

Signature Page for VV-CLIN-112487
ds2325-119-prot-amend-v3.0

Approval with eSign	PPD BDM 23-Jan-2024 19:05:16 GMT+0000

Approval with eSign	PPD Clinical Development/Science 23-Jan-2024 21:34:12 GMT+0000

Approval with eSign	PPD Regulatory 23-Jan-2024 22:33:01 GMT+0000

Approval with eSign	PPD CSPV 24-Jan-2024 00:49:35 GMT+0000

Signature Page for VV-CLIN-112487