

Non-Interventional Study (NIS) Protocol

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Title:	Non-interventional study of patients with Netherton Syndrome to characterise natural history of the disease
Brief lay title:	Natural History of Netherton Syndrome
Protocol version identifier:	Version 2.0
Date of last version of protocol:	20 March 2023 (v1.0)
EU PAS register number:	EUPAS103733
Research question and objectives:	The goal of this non-interventional study (NIS) is to collect real-world data to describe natural history of Netherton Syndrome (NS). The NIS will (1) leverage abstraction of information from existing medical records and (2) conduct new primary data collection on clinician- and patient-reported outcome assessments to achieve the following objectives: Primary objective: - To assess severity of NS by the Ichthyosis Area Severity Index (IASI). Secondary objective: - To assess severity of NS by the Investigator Global Assessment (IGA). Further objectives: - To describe the socio-demographics and clinical characteristics of patients diagnosed with NS. - To describe the treatment patterns in patients with NS. - To describe the healthcare resource utilisation (HCRU) in patients with NS. - To characterise episodes of flares of NS and frequency of occurrence.

	To describe the severity of manifestations and Health-Related Quality of Life (HRQoL) including pain, itch, mental health, sleep, hair assessment, disease burden on family and loss of work and productivity.
Countries of study:	North America, Europe, and Asia.
Author:	██████████ Global Epidemiology & RWE (Real World Evidence)
Date:	28 November 2023
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2. LIST OF ABBREVIATIONS

AE	Adverse Event
ATC	Anatomical Therapeutic Chemical
BI	Boehringer Ingelheim
BSA	Body Surface Area
CA	Competent Authority
CDLQI	Children's Dermatology Life Quality Index
CFR	Code of Federal Regulations
CI	Confidence Interval
CIE	Congenital Ichthyosiform Erythroderma
ClinRO	Clinician-Reported Outcomes
COA	Clinical Outcome Assessment
CRO	Clinical Research Organization
DBP	Diastolic Blood Pressure
DLQI	Dermatology Life Quality Index
eCRF	Electronic Case Report Form
ED	Emergency Department
EDC	Electronic Data Capture
EU	European Union
FDA	Food And Drug Administration
FDLQI	Family Dermatology Life Quality Index
FLACC	Face, Legs, Activity, Cry, Consolability scale
FPS-R	Face Pain Scale- Revised
GCP	Good Clinical Practice
GPP	Good Pharmacoepidemiology Practice
HADS	Hospital Anxiety and Depression Scale
HCRU	Healthcare Resource Utilisation
HRQoL	Health-Related Quality of Life
IASI	Ichthyosis Area Severity Index
IASI-E	Ichthyosis Area Severity Index – Erythema

IASI-S	Ichthyosis Area Severity Index – Scaling
ICF	Informed Consent Form
ICMJE	International Committee of Medical Journal Editors
ICU	Intensive Care Unit
ID	Identity
IEC	Independent Ethics Committee
IGA	Investigator Global Assessment
IgE	Immunoglobulin E
ILC	Ichthyosis Linearis Circumflexa
IRB	Institutional Review Board
IVIg	Intravenous Immunoglobulins
LEKTI	Lympho-Epithelial Kazal-Type-Related Inhibitor
MOS	Medical Outcomes Study
N	Non missing values
N/A	Not Applicable
NASA	Netherton Area Severity Assessment Score
NIS	Non-Interventional Study
NRS	Numeric Rating Scale
NS	Netherton Syndrome
NIS	Non-Interventional Study
PGI-S	Patient Global Impression of Severity
PIQ-C	Paediatric Itch Questionnaire - Child
PRO	Patient-Reported Outcomes
PROMIS	Patient-Reported Outcomes Measurement Information System
PT	Preferred Term
QA	Quality Assurance
QoL	Quality of Life
SD	Standard Deviation
SDV	Source Data Verification
SE	Scaly Erythroderma

SEAP	Statistical and Epidemiological Analysis Plan
SBP	Systolic Blood Pressure
SHA	Scalp Hair Assessment
SOPs	Standard Operating Procedures
SPINK5	Serine Protease Inhibitor of Kazal Type 5
TI	Trichorrhexis Invaginata
UK	United Kingdom
UV	Ultraviolet
VAS	Visual Analogue Scale
WPAI-SHP	Work Productivity and Activity Impairment – Specific Health Problem

3. RESPONSIBLE PARTIES

Study Contact Name	Address	Telephone/Email
Study Sponsor – Boehringer Ingelheim International GmbH		
Study Lead: [REDACTED]	[REDACTED]	[REDACTED] ☎Tel.: [REDACTED]
Global Epidemiology product Lead: [REDACTED]	[REDACTED]	Email: [REDACTED] ☎Tel.: [REDACTED]
Contract Research Organization (CRO) – [REDACTED]		
Scientific Lead: [REDACTED]	[REDACTED]	Email: [REDACTED] ☎Tel.: [REDACTED]
Sr. Principal Statistician: [REDACTED]		Email: [REDACTED] ☎Tel.: [REDACTED]
Operations Lead: [REDACTED]		Email: [REDACTED] ☎Tel.: [REDACTED]

Contact details and a list of investigators will be made available upon request.

4. ABSTRACT

Name of company:		Boehringer Ingelheim	
Name of finished medicinal product:		N/A	
Name of active ingredient:		N/A	
Protocol date:	Study number:	Version/Revision:	Version/Revision date:
20 Mar 2023(v1.0)	1368-0121	2.0	28 Nov 2023
Title of study:	Non-interventional study in patients with Netherton Syndrome (NS) to characterise the natural history of disease		
Rationale and background:	Netherton syndrome (NS) is a rare autosomal recessive disorder that manifests as congenital ichthyosis form erythroderma (CIE) at birth or shortly thereafter. Epidemiological data on NS is scarce with no reliable data on the disease incidence. At present, there is no approved therapy for NS from a regulatory perspective. Effective management of the symptoms of NS requires a multi-disciplinary approach that targets the specific clinical characteristics of individual patients. In the context of clinical development of spesolimab for NS, a better understanding of the natural history and real-world management and assessment of NS is key to put results from clinical studies in perspective. It is also important to understand the burden of NS in terms of the management of its complications and comorbidities. A multi-country study with medical chart extraction and new data collection is necessary to reliably identify patients diagnosed with NS in real-world clinical practice settings and to collect data to describe patient characteristics, disease course, treatment patterns, healthcare resource utilisation and clinical outcomes. As well, to better understand the factors that lead to the high burden of NS, primary data on clinician- and patient-reported outcomes should be collected using validated instruments that measure status and progression of skin and hair conditions and patient's health-related quality of life (HRQoL).		
Research question and objectives:	Primary objective: - To assess severity of NS by the Ichthyosis Area Severity Index (IASI). Secondary Objective: - To assess severity of NS by the Investigator Global Assessment (IGA). Further objectives:		

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	- To describe the socio-demographics and clinical characteristics of patients diagnosed with NS. - To describe the treatment patterns in patients with NS. - To describe the healthcare resource utilisation (HCRU) in patients with NS. - To characterise episodes of flares of NS and frequency of occurrence. - To describe the severity of manifestations and HRQoL including pain, itch, mental health, sleep, hair assessment, disease burden on family and loss of work and productivity.		
Study design:	This is a non-interventional, multi-centre cohort study to be conducted in multiple countries to collect real-world data on patients managed for NS in routine clinical practice. The study will enrol approximately 100 patients. To achieve the study objectives of collecting data to describe the natural history of NS and to assess longitudinal assessment of clinical outcomes, a two-part study is proposed: [Part 1] inclusion of patients diagnosed with NS (including deceased patients and patients whose survival status are not known) to collect existing data in medical records. [Part 2] inclusion of patients who are not participating in any clinical trial to collect new data on clinical outcome assessments at enrolment, within 2 weeks of enrolment, and at approximately 16 and 52 weeks. Clinical data will continue to be abstracted from patient's charts during the 52-week follow-up. Informed consent/assent will be obtained, if required by local and country regulations, for study participation in Part 1 and Part 2. Patients will have the choice to enrol in either Part 1 only or both Parts 1 and 2.		

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Protocol date:	Study number:	Version/Revision:	Version/Revision date:
20 Mar 2023(v1.0)	1368-0121	2.0	28 Nov 2023
Population:	Patients diagnosed and managed for NS in routine clinical practice will be enrolled by the participating sites. The following eligibility criteria will be used for patient selection. Inclusion criteria <i>[for Part 1 and Part 2]</i> 1. Confirmed diagnosis of NS by <u>at least</u> one of the following: a. Genetic testing of mutations in serine protease inhibitor Kazal-type 5 (SPINK5); b. Absence or major deficiency of the protein Lympho-epithelial Kazal-type-related inhibitor (LEKTI) in skin biopsy; c. Clinical diagnosis based on signs and symptoms 2. Provision of consent or assent as required by local regulations: a. [Part 1] to authorise access to existing medical records for study data collection; b. [Part 2] to participate in the longitudinal 52-week evaluation of disease severity and clinical outcome assessments. <i>[for Part 2 only]</i> 3. Not participating in any clinical trial at the time of study enrolment for Part 2. Exclusion criteria <i>[for Part 1 and Part 2]</i> 1. Patient who has died prior to 2002. 2. Patient whose last known survival status is dated prior to 2002 (i.e., patient has been lost to clinical follow-up since 2002).		

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Variables:		For Part 1, clinical data will be abstracted from patients' medical records. The variables of interest include socio-demographics, first clinical signs, or symptoms of NS (where data is available), diagnosis of NS, skin colour classification, vital signs and anthropometrics, gestational information, dietary history, lifestyle habits, hair assessment, NS-related complications, co-morbidities, flare characteristics, treatments for NS and flares. Information on hospital admissions, emergency care visits, outpatient and specialist visits will also be abstracted from medical records for the evaluation of health resource utilisation. For Part 2, during the 52-week follow-up, clinical information will continue to be abstracted from patients' medical records, and the clinical outcome assessments (COAs) to be collected using questionnaires will include: <i>Clinician-reported outcomes (ClinRO)</i> • Ichthyosis Area Severity Index (IASI) (All age groups) • Investigator Global Assessment (IGA) (All age groups) • Netherton Area Severity Assessment (NASA) (All age groups) • Face, Legs, Activity, Cry, Consolability scale (FLACC) (≤ 2 years) <i>Patient-reported outcomes (PRO)</i> • Itch assessment using age-appropriate tools: ○ Itch Numeric Rating Scale (NRS) (≥ 12 years) ○ 5-D Itch Scale (≥ 8 years) ○ Patient Reported Outcomes Measurement Information System (PROMIS) Pediatric Short Form – Itch 6a (PIQ-C) (8–11 years) ○ PROMIS Parent Report/Proxy Short Form – Itch 6a (PIQ-C) (1–7 years) • Pain assessment using age-appropriate tool: ○ Pain NRS (≥ 12 years) ○ Face Pain Scale – Revised (FPS-R) (3–11 years)	

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	- Quality of life assessment using age-appropriate tools: - Dermatology Life Quality Index (DLQI) (≥ 16 years) - Children's Dermatology Life Quality Index (CDLQI) (4–15 years) - Family Dermatology Life Quality Index (FDLQI) (to be completed by a family member who is ≥ 16 years) - Patient Global Impression of Severity (PGI-S) for erythema (≥ 12 years) - PGI-S for scaling (≥ 12 years) - Scalp Hair Assessment PRO™ (SHA) (≥ 12 years) - Hospital Anxiety and Depression Scale (HADS) (≥ 12 years) - Sleep disturbance assessment using age-appropriate tools: - Medical Outcomes Study (MOS) Sleep Scale (≥ 18 years) - PROMIS Pediatric Short Form – Sleep Disturbance 4a (8–17 years) - PROMIS Parent Report/Proxy Short Form – Sleep Disturbance 4a (1–7 years) - Work Productivity and Activity Impairment – Specific Health Problem (WPAI-SHP) (≥ 16 years) - Adult caregiver WPAI-SHP (≥ 16 years)		
Data sources:	The two main data sources of this non-interventional study (NIS) are: - Existing medical records - Primary data collected from clinicians and patients		
Study size:	The NIS is aiming to include approximately 100 patients across multiple sites in North America, Europe, and Asia for Parts 1 and 2 combined.		
Data analysis:	There is no pre-specified hypothesis to be tested. Data analysis will be descriptive. Quantitative variables will be summarised by number of non-missing values (N), mean, standard deviation (SD), standard error of the mean (SE), minimum		

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	(min), median, and maximum (max). Categorical variables will be summarised by counts and percentages for each category. The primary outcome is the IASI and its two subscales (IASI-E [erythema] and IASI-S [scaling]) collected in Part 2. The secondary outcome is the IGA collected in Part 2. The primary and secondary outcomes will be analysed descriptively on the overall population as well as for any subgroups deemed appropriate and informative. Percent changes from baseline in the IASI will also be analysed descriptively to depict temporal change in disease severity. Full details of the statistical analysis methods for the study will be included in the Statistical and Epidemiological Analysis Plan (SEAP).		
Milestones:	Protocol finalisation - Oct 2023 IRB/ IEC approval - Feb 2024 Start of data collection - Feb 2024 End of data collection - Oct 2025 Final report of study results - Jun 2026		

5. AMENDMENTS AND UPDATES

Number	Date	Section of study protocol	Amendment or update	Reason
Amendment 1	20Oct23	Author	██████████ removed. ██████████ added.	BI staff change
Amendment 1	20Oct23	Responsible Parties	██████████ removed. Study Lead, ██████████ added. Global Epidemiology Lead title added to ██████████	BI staff change
Amendment 1	20Oct23	Abstract	Aligning with protocol changes.	Protocol amendment 1
	20Oct23	Milestones	Milestones dates updated.	Delays with study set up
Amendment 1	20Oct23	9.2.2 Study Population Inclusion criteria	- Replaced “clinical assessment” with “clinical diagnosis based on signs and symptoms.” - Added in inclusion criteria 3 Part 2 only: Not participating in any interventional clinical trial at the time of study enrolment for Part 2.	Verbiage updated for more clarity
Amendment 1	20Oct23	9.2.2 Study Population Exclusion criteria	- Patient who has died prior to 2002. - Patient whose last known survival status is dated prior to 2002 (i.e., patient has been lost to clinical follow-up since 2002).	Verbiage updated for more clarity

Number	Date	Section of study protocol	Amendment or update	Reason
Amendment 1	20Oct23	9.1 Study Design, 9.2.3 Study Visits, 9.3 Variables, 9.3.2 Outcomes	- Updated 6 months (± 3 weeks) to 16 weeks (± 2 weeks) - Updated 52 weeks (± 3 weeks) to 52 weeks (± 4 weeks)	To align with BI clinical trial design and ensure time windows allow for minimal recall bias
Amendment 1	20Oct23	9.3 Variables	- Replaced mentioning of “alopecia” with “hair loss,” as we are asking physician to indicate whether there is evidence of hair loss, not to evaluate for specific type of baldness. - Replaced mentioning of “bamboo hair” with “Trichorrhexis invaginata.” As “bamboo hair” is a microscopic description we are trying to ask if typical trichorrhexis invaginata is seen by microscopy.	Verbiage updated for more clarity
Amendment 1	20Oct23	9.3 Variables, Table 1	Table 1 variables updated to align with DCT v1.0. (including longitudinal assessment of clinical signs and symptoms of NS)	Provided additional information for more clarity and editorial changes

Number	Date	Section of study protocol	Amendment or update	Reason
			- Details added for each ClinRO and PRO (name and age group). - Footnote added under Table1.	
Amendment 1	20Oct23	9.3 Variables, Data Collection Schedule by Data Source for Part 1, and Part 2	- Updated table 2 and to align with DCT v1.0 - Updated timepoints of data collection - Revision of footnote	For alignment with changes made in previous sections of the protocol and for clarity
Amendment 1	20Oct23	9.1 Study Design 9.3.1 Exposures and Covariates. 9.3.2 Outcomes 9.6.1 Data Collection 9.6.2 Data Motoring 9.7.1 Main Analysis 9.7.2 Further Analysis 9.8 Quality Control 9.9 Limitations of the Research Methods	Reworded and additional information added	Editorial changes for more clarity and completeness

Number	Date	Section of study protocol	Amendment or update	Reason
		9.10.2 Study Records 9.10.3 Completion of Study		
Amendment 1	20Oct23	9.5 Study size	Target sample size is 100 patients for parts 1 and 2 combined.	Verbiage update for more clarity
Amendment 1	20Oct23	Annex 3	Added investigator protocol signature page	Administrative alignment

6. MILESTONES

Milestone	Planned Date
Protocol finalisation	V1.0 Mar 2023; V2.0 Oct 2023
IRB/IEC approval	Feb 2024
Start of data collection	Feb 2024
End of data collection	Oct 2025
Final report of study results:	Jun 2026

7. RATIONALE AND BACKGROUND

7.1 DISEASE BACKGROUND

Netherton syndrome (NS) is a rare autosomal recessive disorder that manifests as congenital ichthyosiform erythroderma (CIE) at birth or shortly thereafter. The typical clinical presentations of NS are ichthyosis linearis circumflexa (ILC), trichorrhexis invaginata (TI, or “bamboo hair”) and atopic predisposition with high serum levels of immunoglobulin E (IgE) (Yoneda et al., 2016; Saleem et al., 2018). Chavanas et al., 2000a discovered the positional cloning of the NS gene on chromosome 5q32 and described different mutations in the serine protease inhibitor of Kazal type 5 gene (SPINK5) which encode the lympho-epithelial Kazal-type-related inhibitor (LEKTI) protein (Chavanas et al., 2000b; Volc et al., 2020). Most SPINK5 gene mutations result in a deficiency or lack of LEKTI protein. In the epidermis, LEKTI forms a tight binding complex with kallikrein serine proteases limiting desquamation to ensure epidermal barrier integrity (Bellon et al., 2021; Hernandez-Martin et al., 2015; Kubo et al., 2012). As a consequence, patients with NS have unregulated desquamation (Volc et al., 2020), which can lead to cutaneous, gastrointestinal, endocrinal, and pulmonary symptoms with high incidence of severe metabolic abnormalities (Bellon et al., 2021). Additionally, loss of LEKTI inhibition of allergen proteases activity combined with increased allergen penetration caused by severe barrier defect may contribute to the allergic sensitisation observed in patients with NS (Hannula et al., 2014).

Epidemiological data on NS is scarce with no reliable data on the incidence. A French population-based study reported a prevalence estimate for the condition to be 0.80 per million (95% confidence interval [CI] 0.66–1.06) (Dreyfus et al., 2014). A Dutch national cohort study reached out to all 16 known cases of NS in the Netherlands, representing a prevalence of approximately 0.9 per million (Stuvel et al., 2020), which is comparable with the French study. Although the standard clinical signs should address a clear diagnosis of NS, diagnosis remains challenging because the triad (ichthyosis, bamboo hair and atopy) is not always complete (Bilgili et al., 2016). Some differential diagnoses that share clinical signs with NS include non-bullous congenital ichthyosiform erythroderma, erythrodermic psoriasis, lamellar ichthyosis, primary immunodeficiencies (e.g., Hyper IgE syndrome or Wiskott-Aldrich syndrome), atopic or seborrheic dermatitis and acrodermatitis enteropathy (Orphanet, 2008). As well, bamboo hair does not generally become apparent until childhood. For this reason, it is recommended that eyebrow hair be examined in addition to scalp hair, as there was strong evidence on increased density of the diagnostic lesions in eyebrows compared with that of scalp hairs in patients with NS (Powell et al., 1999; Bhattarai et al., 2021).

Immunohistochemistry of skin biopsies revealing LEKTI deficiency has been proposed as a useful diagnostic test for NS, and identification of the disease-causing mutation (SPINK5 gene) allows

molecular confirmation of the diagnosis ([Orphanet, 2008](#)). To-date, there are 108 registered mutations of SPINK5 (e.g., weak expression of LEKTI, no expression of LEKTI, production of truncated LEKTI, higher risk of mortality) found in patients with NS, and their consequences lead to the wide variety of clinical phenotypes ([Pan et al., 2022](#)). The nine lethal mutations identified are, c.153delT, c.238insG, c.375_376delAT, c.C649T, c.997C>T, c.1111C>T, c1431–12G>A, c.715insT and 375delAT ([Sarri et al., 2017](#); [Pan et al., 2022](#)). Analysis of foetal DNA is possible, and there had been a case of prenatal diagnosis of homozygous c.153delT which led to termination of the pregnancy ([Bitoun et al., 2002](#)).

7.2 BURDEN OF ILLNESS

NS places a considerable burden on the patient, the family, and the healthcare system from early on in life of the inflicted individual. Infants are born erythrodermic, and the neonatal period is a critical time with high morbidity and life-threatening complications including hypernatraemic dehydration and hypothermia due to severe skin barrier defect, recurrent skin, respiratory tract and systemic infections and gastrointestinal symptoms, such as abdominal pain, vomiting and diarrhoea ([Hernandez-Martin et al., 2015](#); [Ozyurt et al., 2019](#); [Petrova & Hovnanian, 2020](#); [Chavanas et al., 2000a](#)). The cutaneous manifestations often remain severe during infancy through childhood and tend to improve in teenage years and adulthood.

Compared with the general population, patients with skin diseases have reduced well-being, health-related quality of life (HRQoL) and are prone to mental health concerns, such as anxiety and depression ([Dures et al., 2011](#); [Gånemo et al., 2010](#); [Verhoeven et al., 2007](#)). Real-world cross-sectional studies on patients with NS (child and adult) and their parents showed high burdens with neuropsychological and psychosocial issues ([Sun et al., 2020](#); [Versteegh et al., 2020](#)). Cognitive evaluation showed below-average scores on processing speed in NS patients. Physical appearance related problems and negative social consequences were reported by patients/parents ([Versteegh et al., 2020](#)), and bullying was commonly reported as well. It was found in Japanese patients with congenital ichthyosis that patients with NS had a significantly higher risk of various food and/or environmental allergies than patients with other syndromic forms of congenital ichthyosis ([Murase et al., 2018](#)). Patients with NS also reported one of the lowest scores of Dermatology Life Quality Index (DLQI) for quality of life compared with patients with other forms of severe ichthyosis ([Murase et al., 2018](#)).

7.3 CURRENT MANAGEMENT OF DISEASE

At present, there is no approved therapy for NS. Effective management of the symptoms of NS requires a multi-disciplinary approach that targets the specific clinical characteristics of individual patients. Current treatment options include, but are not limited to, emollients, topical keratolytic,

antipruritic, antibiotics, antiseptics, antihistamines, topical corticosteroids, oral retinoids, intravenous immunoglobulins (IVIg), topical calcineurin and protease inhibitors and phototherapy (Barbati et al., 2021). While symptoms may stay well-controlled with some of these treatments or a combination of therapies, frequent and severe flares have been reported with ultra-violet (UV) exposure (Berthold et al., 2016) and use of etretinate (a second-generation retinoid) in the presence of atopic eczema (Happle et al., 1987). A case of treatment-related death associated with the misuse of topical corticosteroids was reported in an adult patient (Valette et al., 2020). The event was likely due to the systemic effect of the topical agent being amplified by the major defective skin barrier in NS. Although symptoms of NS tend to improve with age, because no effective treatment is available, burden of illness remains significant and poses a major unmet medical need.

Currently, there are several agents under development such as, topical protease inhibitors alpha1-antitrypsin and SXR1096, a reactive aldehyde species inhibitor ADX-102, a KLK7 and epidermal elastase 2 small molecule inhibitor (BPR 277), monoclonal antibodies (adalimumab, infliximab, dupilumab, omalizumab and ustekinumab), phage therapy and gene therapy (Barbati et al., 2021; Joosten et al., 2022). In the published literature, there had been reports of promising treatment outcomes with the monoclonal antibodies infliximab (Fontao et al., 2011; Cicek et al., 2021; Roda et al., 2017) and dupilumab (Murase et al., 2021; Steuer et al., 2020; Galdo et al., 2022). Spesolimab (BI 655130; Food and Drug Administration (FDA) Approved Drug Products: SPEVIGO, 2022) a recently commercialized monoclonal antibody for the treatment of generalized pustular psoriasis, may be another potential candidate for the treatment of NS.

7.4 RATIONALE

In the context of clinical development of spesolimab for NS, a better understanding of the natural history and real-world management and assessment is key to put results from clinical studies in perspective and assist in the design of future studies. It is also important to understand the burden of NS in terms of the disease characteristics at diagnosis, management of its complications and comorbidities.

A multi-country study with medical chart extraction and new data collection is necessary to reliably identify patients diagnosed with NS in real-world clinical practice settings and to collect data to describe patient characteristics, disease course, treatment patterns, healthcare resource utilisation and clinical outcomes. As well, to better understand the factors that lead to the high burden of NS, primary data on clinician- and patient-reported outcomes should be collected using validated instruments that measure status and progression of skin and hair conditions and patient's HRQoL.

8. RESEARCH QUESTION AND OBJECTIVES

A non-interventional study (NIS) will be conducted to generate evidence on the natural history of NS. The study will enrol patients with NS who were diagnosed and treated in routine clinical practice. This is a descriptive study with no *a priori* hypothesis to be tested. Data collection will leverage review of existing medical records and longitudinal documentation of NS disease characteristics, including severity, using clinician-reported outcomes (ClinRO) and patient-reported outcomes (PRO) to achieve the following study objectives:

Primary objectives:

- To assess severity of NS by the Ichthyosis Area Severity Index (IASI).

Secondary objectives:

- To assess severity of NS by the Investigator Global Assessment (IGA).

Further objectives:

- To describe the socio-demographics and clinical characteristics of patients diagnosed with NS.
- To describe the treatment patterns in patients with NS.
- To describe the healthcare resource utilisation (HCRU) in patients with NS.
- To characterise episodes of flares of NS and frequency of occurrence.
- To describe the severity of manifestations and HRQoL including pain, itch, mental health, sleep, hair assessment, disease burden on family and loss of work and productivity.

9. RESEARCH METHODS

9.1 STUDY DESIGN

This is a non-interventional, multi-centre cohort study to be conducted in multiple countries to collect real-world data on patients with NS being treated in routine clinical practice. To achieve the study objectives, a two-part study is proposed to leverage existing data in medical records and to collect new data from patients and clinicians in a 52-week follow-up period from the time of study enrolment.

- In Part 1 of the study, following documentation of provision of study consent/assent, clinical data will be abstracted from patients' existing medical records from the date of study enrolment back to the date of initial diagnosis of NS. Date of initial diagnosis will be used as index date. As many eligible patients as possible will be included in Part 1, including deceased patients and patients whose survival status is not known.
- In Part 2, patients who are not enrolled in any clinical trial at the time of study inclusion will be asked to provide additional consent/assent to participate in a 52-week follow-up for continuous clinical data collection from medical records and completion of ClinRO and PRO assessments.
 - Clinician and patient will complete the ClinRO and PRO assessments separately, once at the time of enrolment and then again within 2 weeks of enrolment, for repeatability evaluation (test-retest) of the assessment tools. Patient's travel expenses will be reimbursed.
 - Clinician and patient will complete the ClinRO and PRO assessments at approximately 16 weeks (± 2 weeks) and end of the 52-week follow-up (± 4 weeks). Patient's travel expenses will be reimbursed.
 - Upon completion of assessments at each data collection time point, compensation will be provided to patient according to each country's regulation (if permitted).

Patients will have the choice to enrol in either Part 1 only or both Parts 1 and 2. Not all patients included in Part 1 will or are possible to be enrolled in Part 2 of the study. Moreover, a patient who becomes a subject of any interventional trial at any time during participation of Part 2 of the study, their participation in this natural history study will be early terminated by the date of their inclusion in the trial.

Considering the rarity of NS and the paucity of epidemiologic data on its prevalence, it was not possible to conduct a formal sample size estimation for this study. A target sample size of

approximately 100 patients, in Parts 1 and 2 combined, was selected to collect sufficient data to describe natural history of NS. As well, the study will aim to include as many patients as possible within a reasonable enrolment timeframe to document ClinRO and PRO assessments in Part 2 to help contextualize the clinical trial results.

The primary and secondary outcomes of interest are clinician's assessment of severity of erythema and scaling as measured by the IASI (including the IASI-E erythema and IASI-S scaling subscales) and IGA.

In both Parts 1 and 2, further outcomes will be collected as listed in [Table 2](#). Further outcomes include socio-demographic and clinical characteristics of patients diagnosed with NS, frequency, and reason of visits to emergency department (ED) and outpatient clinics and frequency, duration and reason of hospital and intensive care unit (ICU) admissions. Furthermore, treatments used to manage NS will be summarised by type/drug class, sequence, and duration of use. Reason of interruption or discontinuation of treatment will be described by type of therapy (e.g., topical, systemic, biologic). Flares of NS will be characterised in terms of symptoms and complications, frequency of occurrence, duration of episode, type of treatment used at the time of flare, how the flare was managed with related ED and/or hospital and ICU admissions and the outcome of flare. Scores and ratings of any ClinRO and PRO used and documented in the medical records for the evaluation of NS disease status and symptoms will be collected and summarised as secondary outcomes as well.

In Part 2 of the study, the following instruments for skin and scalp hair evaluations, sleep as well as HRQoL assessments will be used: Netherton Area and Severity Assessment (NASA), patient-reported severity of pain and itch using a numeric rating scale (NRS), 5-D Itch Scale, Patient Global Impression of Severity (PGI-S) for erythema and for scaling, Scalp Hair Assessment (SHA), Medical Outcomes Study (MOS) Sleep Scale, Dermatology Life Quality Index (DLQI)/Children's Dermatology Life Quality Index (CDLQI), and Hospital Anxiety and Depression Scale (HADS). Age-appropriate versions of the assessment tools will be used if available and as applicable since some assessment tools may require assistance from parents for completion (i.e., parent-report or parent-proxy). In addition, adult patients will also complete the Work Productivity and Activity Impairment – Specific Health Problem (WPAI-SHP) questionnaire. An adult family member of the patient (any age) will be asked to complete the Family Dermatology Life Quality Index (FDLQI).

9.2 SETTING

9.2.1 Study sites

This study will be conducted in hospitals/clinical centres where patients with NS are typically managed in real-world clinical practice. The study will include study sites across several countries in North America, Europe, and Asia.

For practical reasons, site selection will prioritize centres where a higher number of patients can be enrolled to achieve the target study sample size. Approximately 22 centres will participate in this study; more sites may be included to achieve the target study sample size.

9.2.2 Study population

Sites will identify all patients diagnosed and treated for NS in routine clinical practice to form the base cohort for study eligibility assessment. Patients in the base cohort will be further reviewed for study eligibility based on the criteria below.

9.2.2.1 Inclusion criteria

[for Part 1 and Part 2]

1. Confirmed diagnosis of NS by at least one of the following:
 - Genetic testing of mutations in SPINK5;
 - Absence or major deficiency of the protein LEKTI in skin biopsy;
 - Clinical diagnosis based on signs and symptoms.
2. Provision of consent or assent (i.e., by parent or legal guardian) as required by local regulations:
 - [Part 1] to authorise access to existing medical records for study data collection;
 - [Part 2] to participate in the longitudinal 52-week evaluation of disease severity and clinical outcome assessments.

[for Part 2 only]

3. Not participating in any interventional clinical trial at the time of study enrolment for Part 2.

9.2.2.2 Exclusion criteria***[for Part 1 and Part 2]***

1. Patient who has died prior to 2002.
2. Patient whose last known survival status is dated prior to 2002 (i.e., patient has been lost to clinical follow-up since 2002).

9.2.3 Study visits

For Part 1 of the study, data will be collected solely from existing medical records. Patients who participate in Part 2 of the study will be evaluated using the ClinRO and PRO assessments:

- at the time of study enrolment,
- within 2 weeks of enrolment assessment (for test-retest evaluation),
- at approximately 16 weeks (± 2 weeks), and
- at the end of the 52-week study follow up (± 4 weeks).

At all time-points of ClinRO and PRO assessments, patients will be reimbursed for their travel expenses and be compensated for their time spent on completing the assessments if permitted by local regulations.

9.2.4 Study discontinuation

Boehringer Ingelheim reserves the right to discontinue the study overall or at a particular study site at any time for the following reasons:

- Failure to meet expected enrolment goals overall or at a particular study site.
- Violation of Good Pharmacoepidemiology Practice (GPP), the study protocol, or the contract by a study site, investigator, or research collaborator, disturbing the appropriate conduct of the study.

The investigator/study site/research collaborator will be reimbursed for reasonable expenses incurred in case of study/site termination (except in case of the second reason).

9.3 VARIABLES

Data collection will include variables as assessed, measured, and documented in patients' medical records as permitted by local routine clinical care. Table 1 summarises the study variables by category of study outcomes.

Table 1 Study Variables*

Cohort characterisation/ Study outcomes	Variables
Socio-demographics	• Age • Sex • Race (where allowed/recorded) • Ethnicity (where allowed/recorded) • Marital status • Education level • Employment status • Consanguinity (i.e., parents first cousins) • Family history of NS • Residential location of patient at time of diagnosis (urban/rural)
Diagnosis of Netherton Syndrome (First clinical signs, initial diagnosis, and changes over time)	• Skin colour classification (Fitzpatrick scale; Ward et al., 2017) • First clinical signs or symptoms ○ Diagnostic method(s) ○ Date of initial onset of signs and symptoms ○ Possible diagnosis ○ Treatments for possible diagnosis • Date of referral to the site • Initial diagnosis of NS ○ Date or year or age at initial diagnosis ○ Diagnostic method(s) ○ Genetic test (date, specify SPINK5 mutation) ○ Skin biopsy (date), LETKI protein absent or reduced level ○ Clinical diagnosis (ILC, TI) • Phenotype at initial diagnosis and subsequent clinical follow-up assessments • Severity of erythroderma • Severity of erythema • Severity of scaling • Severity of skin thickening • Severity of elevated red bumps on the skin • Number of defined lesions • Worst Itch (e.g., NRS if documented in charts) • Worst Pain (e.g., NRS, VAS if documented in charts) • Hair assessment (e.g., date of hair microscopy, severity of hair loss, TI assessment)

Cohort characterisation/ Study outcomes	Variables
Longitudinal assessment of NS signs/symptoms (worst severity)	• Scaly erythroderma • Ichthyosis Linearis Circumflexa (ILC) • Itch • Pain • Skin infections
Vital signs and anthropometrics (Initial diagnosis and change over time)	• Height • Weight • Blood pressure (SBP and DBP) • Temperature • Respiratory rate • Pulse oximetry • Pulse rate • Vital status (i.e., information to be collected at study enrolment and during 52-week follow-up; date of study discontinuation if prior to week 52, date of death if known or date last known to be alive)
Gestational information	• Preterm birth (yes/no) • Gestational age • Birth weight • Birth length • Presence of colloidal membrane at birth • Ability to be breastfed • Neonatal allergy to cow's milk (yes/no) • Age when Failure to thrive (weight for length <5th percentile or length <5th percentile) detected • Complication during delivery
Dietary history	• Feeding (date of initiation and duration) ○ Oral ○ Tube feeding ○ Gastrotomy ○ Parenteral ○ Parenteral and gastrotomy
Lifestyle habits	• Substance use ○ Type (tobacco, nicotine, vaping, alcohol, caffeine, cannabis) ○ Status (never, current, former) ○ Duration of use

Cohort characterisation/ Study outcomes	Variables
Co-morbidities and NS-related complications with associated treatments and healthcare resource utilization (if applicable)	• Allergies and intolerance • Asthma • Atopic eczema • Cardiovascular • Covid (date of infection, vaccination status) • Developmental delay type (e.g., cognitive, language, other) • Encephalitis • Gastrointestinal disorder requiring medication or intervention and/or healthcare resource utilization (e.g., chronic diarrhoea, recurrent vomiting, gastro-oesophageal reflux, constipation, irritable bowel syndrome) • Growth hormone deficiency • Hyperactivity • Hyper-IgE syndrome (i.e., IgE > 22.8 kU L-1) • Hypereosinophilia • Hyponatraemia (i.e., Na⁺ >145 mmol L-1) • Hypothyroidism • Infections, type (e.g., viral, bacterial, fungal), location of infection and specify organism (if applicable and available) • Skin cancer • Other malignancies • Malnutrition • Pruritus
Drug and Non-Drug Treatments for NS/ comorbidities/ complications	• Treatment (type,* dose provided, frequency, start and stop date, route of administration, reason for discontinuation) • Indication • Any clinical trial participation (as a treatment sequence, collect information on date of informed consent and name of investigative agent, as available in the medical chart)
Flare	• Date of occurrence • Outcome of flare • Symptoms (exacerbation of prior symptom or new symptom) • Treatment given (including medication, visits to ED, ICU/hospital admission) • Potential source(s) of flare • Severity of erythroderma, scaling, skin infection, pain, itch
Health resource utilisation (All cause and NS-related as primary reason)	• Outpatients visit (date, reason) • ED visit (date, reason) • Hospital admission (date of admission, discharge, reason) • ICU admission (date of admission, discharge, death, and reason)
Clinical outcome assessments Clinician-reported**	• Ichthyosis Area Severity Index (IASI) (All age groups) • Investigator Global Assessment (IGA) (All age groups) • Netherton Area Severity Assessment (NASA) (All age groups) • Face, Legs, Activity, Cry, Consolability (FLACC) scale (≤ 2 years)

Cohort characterisation/ Study outcomes	Variables
Patient-reported outcomes Patient-reported**	• Itch assessment using age-appropriate tools: ○ Itch Numeric Rating Scale (NRS) (≥ 12 years) ○ 5-D Itch Scale (≥ 8 years) ○ PROMIS Pediatric Short Form v1.0 – Itch 6a (PIQ-C) (8–11 years) ○ PROMIS Parent Report/Proxy Short Form v1.0 - Itch 6a (PIQ-C) (1–7 years) • Pain assessment using age-appropriate tools: ○ Pain Numeric Rating Scale (NRS) (≥ 12 years) ○ Face Pain Scale – Revised (FPS-R) (3–11 years) • Quality of life assessment using age-appropriate tools: ○ Dermatology Quality of Life Index (DLQI) (≥ 16 years) ○ Children’s Dermatology Life Quality Index (CDLQI) (4–15 years) ○ Family Dermatology Life Quality Index (FDLQI) (to be completed by a family member who is ≥ 16 years) • Patient Global Impression of Severity (PGI-S) for erythema (≥ 12 years) • PGI-S for scaling (≥ 12 years) • Scalp Hair Assessment Patient Reported Outcome™ (SHA PRO™) (≥ 15 years, but due to the simplicity of the question it will be administered in ≥ 12 year of age group) • Hospital Anxiety and Depression Scale (HADS) (≥ 12 years) • Sleep disturbance assessment using age-appropriate tools: ○ Medical Outcomes Study (MOS) Sleep Scale (≥ 18 years) ○ PROMIS Pediatric Short Form – Sleep Disturbance 4a (8 -17 years) ○ PROMIS Parent Report/Proxy Short Form – Sleep Disturbance 4a (1–7 years) • Work Productivity and Activity Impairment-Specific Health Problem (WPAI-SHP) (≥ 16 years) • Adult caregiver WPAI-SHP (≥ 16 years)
* Treatment may be any therapy use over-the-counter or prescribed as per real-world clinical standard including treatment received through a compassionate use program. Information on investigational treatment will be abstracted for Part 1. Patients who become participants of any investigational trial during Part 2 participation will be early terminated for the NIS; information on date consent and name of investigational treatment (if known) will be documented as part of description of treatment patterns in this NIS. **Data availability will vary across sites. Age ranges specified in parentheses for the COA tools reflect the specific age groups where the COAs have been administered for other related contexts of use and will be administered to the age groups in this study.	

The schedule of data collection is displayed in [Table 2](#). For patients included in Part 1, data collection occurs only once at enrolment based on information documented in their existing medical records.

For patients included in Part 2, data collection from medical records will continue up to 52 weeks from enrolment, and ClinRO and PRO will be collected once at enrolment, again at approximately 2 weeks and 16 weeks (± 2 weeks) after enrolment and at the end of the 52-week follow-up (± 4 weeks).

Table 2 Data collection schedule by data source for Part 1 and Part 2

	At enrolment (one-time data collection)		During longitudinal follow-up	
			Within 2 weeks of enrolment	At approximately 16, 52 weeks*
	Patients in Part 1	Patients in Part 2	Patients in Part 2	Patients in Part 2
Cohort entry				
Eligibility assessment	X	X		
Consent/Assent – Part 1	X			
Consent/Assent – Part 2		X		
From medical records				
Socio-demographic variables	X	X		
NS first clinical signs/ symptoms information	X	X		
Diagnosis of NS	X	X		
Gestational information	X	X		
Dietary history	X	X		X
Lifestyle habits	X	X		
Hair assessment	X	X		X
NS Phenotype, signs, and symptoms	X	X		X
Co-morbidities and NS complications	X	X		X
Vital signs and anthropometrics	X	X		X
Treatments for comorbidities and NS	X	X		X
Flares	X	X		X***
Healthcare resource utilisation (HCRU)	X	X		X
COA (Clinician-/ Patient-reported)	X**	X**		
ClinRO – Clinician-reported outcomes				
- IASI - IGA *** - NASA - FLACC		X	X	X
PRO – Patient-reported outcomes				

	At enrolment (one-time data collection)		During longitudinal follow-up	
			Within 2 weeks of enrolment	At approximately 16, 52 weeks*
	Patients in Part 1	Patients in Part 2	Patients in Part 2	Patients in Part 2
• Itch† ○ Numeric Rating Scale (NRS) ○ PROMIS PIQ-C (Pediatric) ○ PROMIS PIQ-C (Parent report/proxy) • Pain† ○ NRS ○ Faces Pain Scale - Revised (FPS-R) • Quality of life† ○ DLQI ○ CDLQI ○ FDLQI • PGI-S for erythema • PGI-S for scaling • WPAI-SHP		X	X	X
• 5D-Itch Scale • Scalp Hair Assessment (SHA) • Hospital Anxiety and Depression Scale (HADS) • Sleep Disturbance† ○ Medical Outcomes Study (MOS) Sleep Scale ○ PROMIS Sleep Disturbance (Parent proxy) ○ PROMIS Sleep Disturbance (Paediatric)		X		X
*At approximately 16 weeks (±2 weeks) and 52-week follow-up (±4 weeks). The 52-week assessment is end of study observation. **For Part 1, data collection from existing medical records, information on the date, type and result of clinical outcome assessment already documented in charts will be collected. Variable availability will likely vary by site. ***Flares occurring during the 52-week follow-up and managed at the study site as assessed by the local investigator per their clinical expertise. Flares are defined as exacerbated inflammation with periods of temporary remission presented as a stable/improved phenotype (source: Petrova E et al. 2020 Expert Opinion on Orphan Drugs) based on physician's clinical evaluation. †Age-appropriate versions will be used for each assessment.				

9.3.1 Exposures and covariates

This is a natural history of disease study. As part of the study objectives, treatment patterns in patients will be described, but there is no evaluation of a specific exposure. The study cohort will include patients diagnosed with NS, per study inclusion criteria to assess patient socio-demographics and clinical characteristics, treatment patterns, flares, HCRU, COAs (ClinROs and PROs).

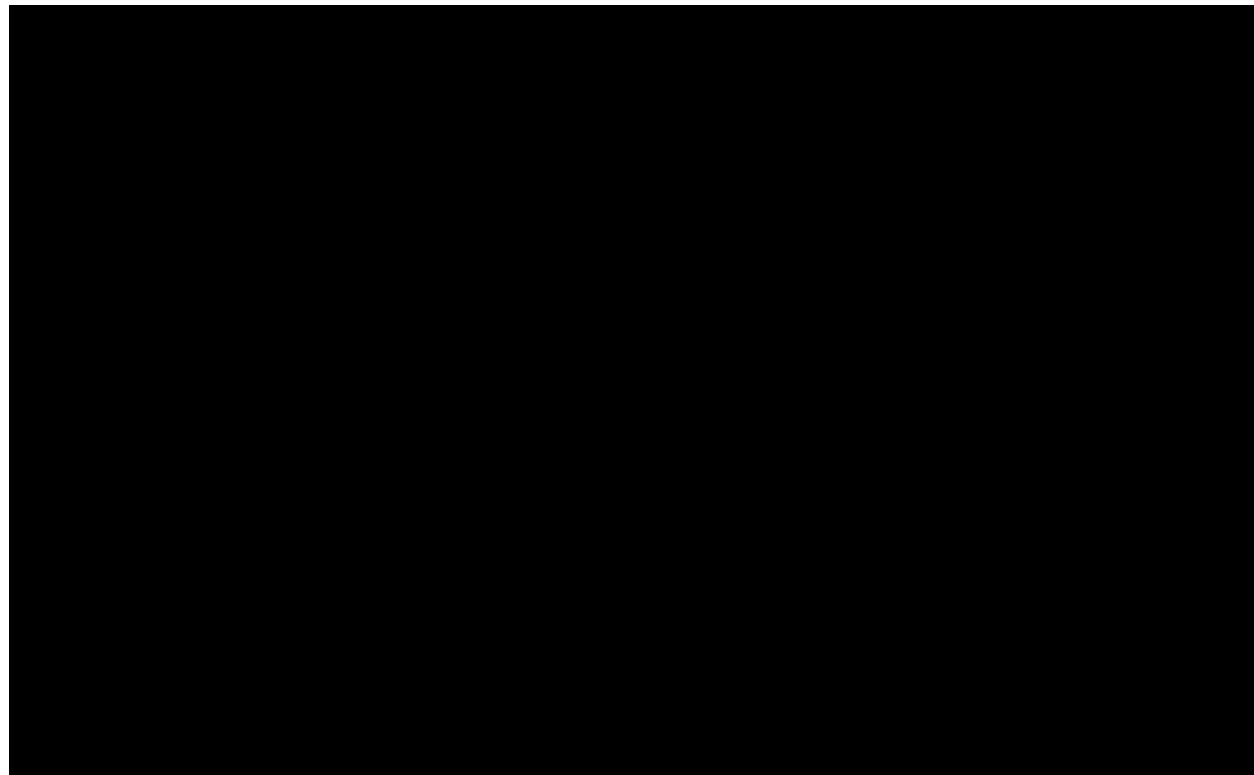
9.3.2 Outcomes

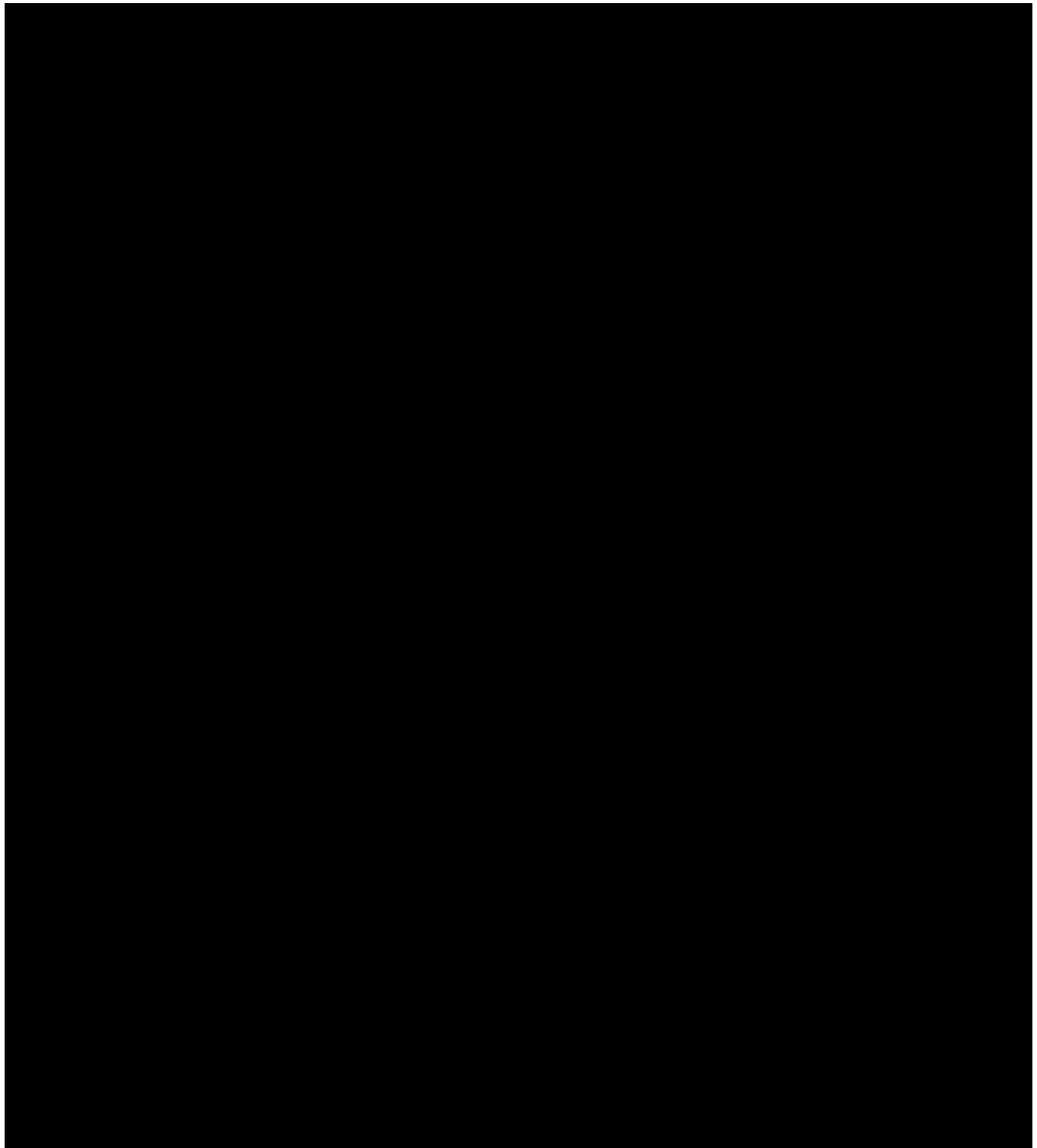
9.3.2.1 Primary and secondary outcomes

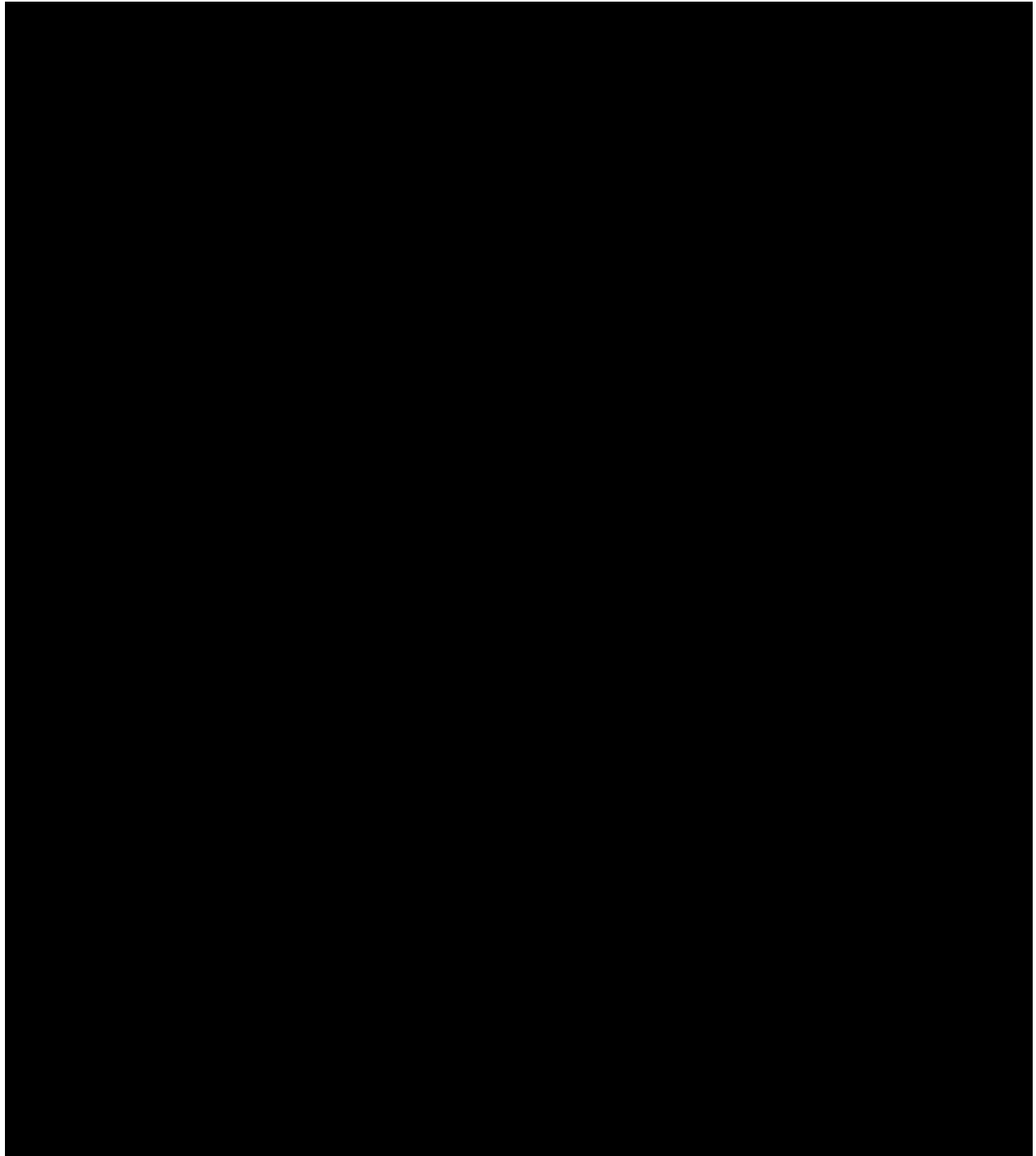
For patients participating in Part 2 of the study, clinical assessment of severity of NS by IASI (primary outcome) and IGA (secondary outcome) will be conducted in the clinic, once at enrolment, within 2 weeks of enrolment and at approximately 16 weeks (± 2 weeks) and 52-week follow-up (± 4 weeks).

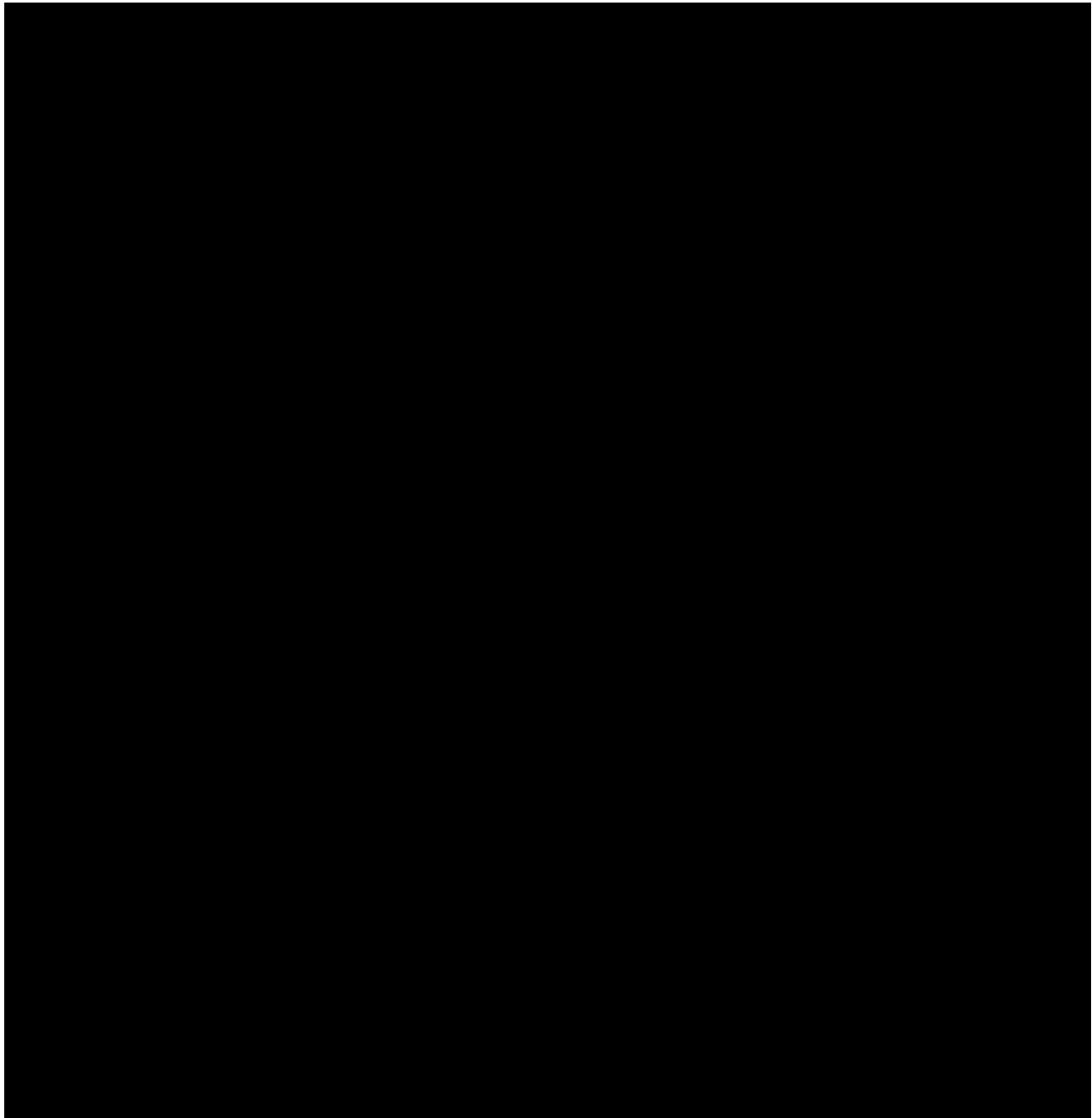
IASI is a ClinRO that results in a composite score comprising severity of erythema and scaling in different body regions as a function of their respective body surface areas. Severity of erythema and scaling is rated on a 5-point Likert scale of 0–4 in each of 4 body regions: head and neck (including scalp), arms (including palms), legs (including soles) and trunk, prorated based on body surface area in these body regions and the percentage of involvement in each of these body regions. The total IASI score ranges between 0–48 (i.e., sum of a maximum score of 24 for erythema and maximum score of 24 for scaling). Higher score denotes worse clinical severity.

IGA is a ClinRO that will assess the global severity of erythema and scaling in NS patients using a 5-point Likert scale ranging from 0=clear, to 4=severe.









9.4 DATA SOURCES

The two main data sources of this NIS are (1) existing medical records and (2) new, primary data collected from clinicians and patients.

9.5 STUDY SIZE

Considering the rarity of NS and the paucity of epidemiologic data on its prevalence, it was not possible to conduct a formal sample size estimation for this study. As the study is descriptive in nature, no minimum sample size is required. The number of patients with NS that can be enrolled at each participating centre will be evaluated and projected during site feasibility. A target sample size of approximately 100 patients, for Parts 1 and 2 combined, was selected to collect sufficient data to describe natural history of NS.

9.6 DATA MANAGEMENT

9.6.1 Data collection

A web-based electronic data capture (EDC) system will be provided to serve as an integrated, transparent tool to collect and manage data and track study progress at the centre- and patient-level. The structure and programming of the EDC system permits the contract research organization (CRO) to monitor the data closely as the study data accrue. Data in the EDC system are kept in a central location and all data will be transmitted to a central database.

The EDC system meets approved established standards for the security of health information and is validated. The system is developed in accordance with a rigorous system development life cycle and quality program, which ensures compliance with regulatory agency guidelines including 21 CFR Part 11. Prior to deployment of the study-specific EDC database, user acceptance testing will be performed. Data will be backed up at intervals that comply with the CRO's standard operating procedures and details on frequency of back-ups will be provided in a study specific Data Management Plan.

Study eligibility results for inclusion and exclusion criteria will be captured in the EDC system for all patients. One electronic case report form (eCRF) will be completed in the EDC system for each enrolled patient. The completed original eCRFs are the sole property of the Sponsor and will not be made available in any form to third parties without written permission from the Sponsor, except for authorized representatives of the Sponsor or appropriate regulatory authorities.

Each study investigator has ultimate responsibility for the collection and reporting of all data entered in the eCRFs and any other data collection forms (source documents) and ensuring that they are accurate, authentic/original, attributable, complete, consistent, legible, timely (contemporaneous), enduring, and available when required. The eCRFs must be electronically signed prior to database lock by the study investigator or by an authorized staff member to attest that the data contained on the eCRFs are correctly recorded. The CRO will inform sites when it is time for eCRF sign-off to occur.

In the present case, the source documents are the patient medical charts (including imaging reports, laboratory results, pharmacy records and other relevant records); therefore, data collected on the eCRFs should match the data in the charts. ClinRO and PRO data collected on paper forms (during Part 2) will be included as source document and maintained by site staff. Virtual training will be provided for the following ClinRO: IASI, NASA, FLACC and IGA.

Site staff will be provided with secure usernames and passwords in order to enter study data into the EDC system. All participating sites will only have access to view and enter the data for their own patients. Site staff will be trained by the CRO to perform data entry and how to retrieve and respond to data queries in the EDC system. The training will include eCRF completion guidelines. It is assumed that all sites will be able to complete data entry into the eCRFs via the EDC system.

The EDC system includes logic checks to minimise data entry errors. Data inconsistencies outside the logic checks will be managed by manual queries issued by data management within the EDC system for site completion. All queries will be monitored until resolution within the EDC through the electronic query report. Details regarding query management will be outlined within the Data Management Plan and/ or Data Review Plan.

9.6.2 Data monitoring

The CRO will remotely supervise and monitor de-identified data abstraction by site staff into the EDC system. The structure and programming of the EDC system permits the CRO to monitor the data closely as the study data accrue. Source data verification (SDV) may be conducted on select critical variables.

Quality control mechanisms (e.g., verification of data completeness, validations, and edit checks), which will be automated at time of data entry, will be built into the EDC. Manual queries will be generated by the CRO for resolution by site staff within the EDC system.

9.6.3 Data cleaning

The EDC system will have built-in methods for data validation (e.g., drop down lists, value range controls, and standardised response formats). However, a data cleaning method will furthermore be employed in order to correct inconsistencies or errors that were not captured during data entry (e.g., outliers or conflicting data). Data queries will be identified on an ongoing basis during data collection. Queries will be generated for site completion within the EDC system. Sites will receive an email weekly notifying them of any new or outstanding queries. Sites will have approximately 5 business days to complete queries following email notification. As database lock approaches, the duration to resolve queries may be less than 5 business days. Source data verification (SDV) may be conducted on select critical variables.

9.6.4 Data retention

To enable evaluations and/or audits from regulatory authorities or the Sponsor, the investigator agrees to keep records, including the identity of all participating patients (sufficient information to link records, e.g., eCRFs and medical charts), source documents, detailed records of patient disposition, and adequate documentation of relevant correspondence (e.g., letters, meeting minutes, telephone calls reports). The records should be retained by the investigator for the length of time specified in local regulations, or in the Site Contract Agreement (whichever time period is longer). The investigator must obtain the Sponsor's written permission before disposing of any records, even if retention requirements have been met.

If the investigator becomes unable for any reason (e.g., retirement or relocation) to continue to retain study records for the required period, the Sponsor should be prospectively notified. The study records must be transferred to a designee acceptable to the Sponsor, such as another investigator, another institution, or to an independent third party arranged by the Sponsor. The investigator must obtain the Sponsor's written permission before disposing of any records, even if retention requirements have been met.

9.7 DATA ANALYSIS

The statistical analysis methods for the study are summarised below. Full details of the statistical analysis will be documented in the SEAP, which will be finalized before the end of data collection.

9.7.1 Main analysis

There is no pre-specified hypothesis to be tested. Data analysis will be descriptive.

Quantitative variables will be summarised by number of non-missing values (N), mean, standard deviation (SD), standard error of the mean (SE), minimum (min), median, and maximum (max). Categorical variables will be summarised by counts and percentages for each category.

Socio-demographic and clinical characteristics of patients diagnosed with NS will be summarised by descriptive statistics for the overall population and for the following subgroups, as far as sufficient data are available:

- Age at enrolment (< 4 years, 4 to <6 years, 6 to <12, 12 to <18, 18 years or older)

Additional subgroup analysis can be performed as deemed informative (see [9.7.2 Further analysis](#)).

The IASI total score and its two subscales (IASI-E and IASI-S) will be analysed descriptively on the overall population as well as for the subgroups described above. Changes in the IASI from

baseline to the last assessment will also be analysed descriptively overall and for the above subgroups.

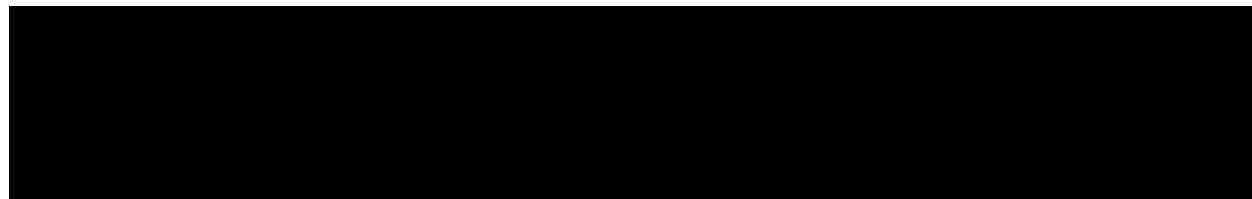
The IGA score will be analysed descriptively on the overall population as well as for the subgroups described above. Changes in the IGA from baseline to the last assessment will also be analysed descriptively overall and for the above subgroups.

HCRU will be analysed as the annual number of outpatient visits, ED visits, hospital admissions and ICU admissions, total hospital admission time per year, and total time spent in ICU per year. HCRU will also be described in the above subgroups if sufficient data are available.

Medication will be coded according to WHO Drug Dictionary and analysed on Anatomical Therapeutic Chemical (ATC) level III (Therapeutic/pharmacological subgroup) and on Preferred Term (PT) level. Treatment patterns will be described by the frequency of medication used to treat NS stratified by age group at treatment initiation. Treatment sequence (i.e., sequence of type of therapy or class of drug) will also be analysed overall and for the above subgroups.

Annualised incidence of flares will be calculated overall and for the above subgroups. Duration of an episode of flare will be summarised using descriptive statistics for continuous variables. Severity of flare based on IGA (captured in Part 2) will be summarised using descriptive statistics for categorical variables.

Each ClinRO and PRO instrument will be scored according to the instructions provided by the developers and summarised either as a categorical or continuous variable as instructed. For the analysis of potential predictors of ClinRO and PRO variables (dependent variables), summary statistics will be calculated for the different categories of socio-demographic, clinical and treatment variables (i.e., independent covariates) as a first-step exploratory analysis. A multivariate variance analysis model may then be developed to analyse the possible correlation between these independent variables with values of the ClinRO and PRO variables. Depending on the available data, potential predictors of flare and change in the ClinRO and PRO variables between enrolment and follow-up assessments will be analysed in the same way. To understand probability of NS flares, multivariate regression analyses of the data with selected variables may be used as independent covariates in regression models to identify significant risk factors of flares. All planned analyses will be detailed in the Statistical and Epidemiological Analysis Plan (SEAP).



9.8 QUALITY CONTROL

Systems with procedures will be implemented to ensure the quality of every aspect of the study.

The development of the protocol and SEAP will follow internal standard operating procedures (SOPs) of the CRO, which include detailed review rounds. Quality control of the statistical programming will follow the CRO's Standard Operating Procedures (SOPs).

The EDC system meets approved established standards for the security of health information, is validated and is 21 CFR Part 11/EudraLex Annex 11 compliant. In order to ensure that patient data (as well as other confidential data) remain secure and intact, the CRO follows SOPs and quality control processes that address patient data security. The EDC system has built in edit checks and validations and supports electronically generated and manual queries.

Patient confidentiality will be strictly maintained. Access to the EDC system will be regulated via a hierarchical username and password control. Subject data will be de-identified through design of data entry fields that do not permit the entry of identifying information such as initials, date of birth, or centre-assigned patient identifiers. Only trained site staff will enter data into the eCRFs. Patients' ages in whole years, but not date of birth, will be entered. No patient identifiers used by sites will be entered; rather the EDC program will automatically assign a study patient ID number to each patient. The de-identified data as entered into the EDC system will be visible to the CRO and the Sponsor, but only centre staff will be able to trace a study ID number back to a patient identity, a necessary measure to allow centre staff to respond to data queries raised by the CRO later.

9.9 LIMITATIONS OF THE RESEARCH METHODS

There are several challenges and limitations to this study. First, NS is an ultra-rare disease; it is anticipated that recruiting patients will be challenging. Enrolment for Part 2 may be further impacted by several clinical trials (including one to be launched by BI) that are currently competing to recruit subjects. To achieve the target sample size of 100 patients, at least for Part 1, within a reasonable study timeline, as many sites will be recruited as possible.

Since BI will conduct a pivotal clinical trial in parallel in some of the same sites, there is a possibility of a selection bias for the inclusion of patients into Part 2 of this study, as patients already included in the trial will not be eligible. Lack of external validity could occur as a convenient sample of centres of reference will be included to the study. Centres agreeing to take

part may not be representative of other facilities treating patients with NS. Potential selection bias could be mitigated by adding additional sites for enrolment into the NIS.

Note: In the EDC, Part 1 patients will be flagged with an indicator if they are included in the BI clinical trial.

Furthermore, depending on the prevalence of the disease in each study country and the number of patients each site identifies and able to invite to participate, there is a risk of resulting in a study cohort that is not representative of the patient population of NS. Part 1 may include patients who have deceased and for whom the entire disease and treatment history will be available for analysis. An exploratory analysis may be conducted to assess differences between the deceased and alive cohort.

To help assess selection bias, each site will provide a log that documents eligibility assessment indicating the following:

- total number of patients diagnosed with NS with medical records at the site,
- the number of patients deemed eligible for the study, screen failures with reasons, and
- the final number of patients enrolled in Part 1 and Part 2.

An exploratory analysis may be conducted to assess differences between the enrolled and non-enrolled patients based on the limited variables that are accessible without consent, such as age, sex, and year of diagnosis/referral to the site. This log will also document the consent/assent process and reasons for not being able to enrol an eligible patient.

Moreover, the objectives of this study are descriptive. Considering the lack of data on the epidemiology of NS, it is not possible to forecast enrolment. Given the low prevalence of the disease, the final sample size will likely be limited. Therefore, robust quantitative analyses may not be possible, and only descriptive analyses may be conducted.

Missing data is an inherent limitation of studies using secondary data sources (e.g., existing medical records). The quality of the data collected for the study is dependent on completeness and accuracy of the data available documented in real-world clinical practice. Availability of information in patient records will vary by physician practice and by country and will reflect differences in practice patterns, recording practices, and medical norms. Information bias could occur if patients received care throughout their disease and treatment history at different sites and the data is not centralised in the requested medical records for study data collection. Site feasibility

will assess these aspects as well as the site's ability to obtain records from external centres to optimize completeness of data collection.

9.10 OTHER ASPECTS

9.10.1 Data quality assurance

A quality assurance (QA) audit/inspection of this study may be conducted by the Sponsor or Sponsor's designees or by Institutional Review Board (IRBs)/Independent Ethics Committee (IECs) or by regulatory authorities. The QA auditor will have access to all medical records, the investigator's study-related files and correspondence, and the informed consent documentation of this study.

9.10.2 Study records

Electronic Case Report Forms (eCRFs) for individual patients will be provided by the Sponsor (via the CRO).

9.10.2.1 Source documents

Source documents provide evidence for the existence of the patient and substantiate the integrity of the data collected. Source documents are filed at the investigator's site.

Data entered in the eCRFs that are transcribed from source documents must be consistent with the source documents, or the discrepancies must be explained. The investigator may need to request previous medical records or transfer records, depending on the variables being collected; current medical records must also be available.

For eCRFs, all data must be derived from source documents.

9.10.2.2 Direct access to source data and documents

The investigator/institution will permit study-related monitoring, audits, IRB/IEC review and regulatory inspection, providing direct access to all related source data/documents. eCRFs and all source documents, including progress notes and copies of laboratory and medical test results must be available at all times for review by the Sponsor's clinical study monitor, auditor, and inspection by health authorities (e.g., United States FDA). BI study staff and auditor may review all eCRFs and written informed consents. The accuracy of the data will be verified by reviewing the documents described in Section [9.10.2.1](#).

9.10.3 Completion of study

For sites in Japan when the study is completed, the investigator should inform the head of the study site of the completion in writing, and the head of the study site should promptly inform the IRB and Sponsor of the completion in writing.

For sites in the European Union (EU), United Kingdom (UK) and United States (US), the IRB/IEC/competent authority in each participating EU member state needs to be notified about the end of the study (last patient/patient out, unless specified differently in Section 9.2) or early termination of the study.

9.10.4 Protocol deviations

The investigator or sub-investigator should record all study protocol deviations.

For sites in Japan, the investigator should provide and submit the Sponsor and the head of the study site the records of deviations infringing the Japanese Good Clinical Practice (GCP) or deviations to eliminate an immediate hazard to study subjects and for other medically inevitable reasons.

9.10.5 Compensation in the event of study related injury (Japan only)

In the event of health injury associated with this study, the Sponsor is responsible for compensation based on the contract signed by the study site.

10. PROTECTION OF HUMAN SUBJECTS

The study will be carried out in compliance with the protocol, the principles laid down in the Declaration of Helsinki, Guidelines for GPP, and the relevant BI SOPs. Standard medical care (prophylactic, diagnostic and therapeutic procedures) remains the responsibility of the treating physician of the patient.

The investigator should inform the Sponsor immediately of any urgent safety measures taken to protect the study subjects against any immediate hazard, and also of any serious breaches of the protocol.

In addition, for sites in Japan, the rights of the investigator and of the Sponsor with regard to publication of the results of this study are described in the investigator contract. As a general rule, no study results should be published prior to finalization of the Study Report.

10.1 STUDY APPROVAL, PATIENT INFORMATION, AND INFORMED CONSENT

The NIS will be initiated only after all required legal documentation has been reviewed and approved by the respective IRB/IEC and Competent Authority (CA) according to national and international regulations. The same applies for the implementation of changes introduced by amendments.

Prior to patient participation in the study, written informed consent must be obtained from each patient (or the patient's legally accepted representative) per GPP and according to the regulatory and legal requirements of the participating country, if applicable.

By signing to attest agreement with procedures and methods stated in this protocol, Investigators commit to complying with all related applicable local laws and regulations when conducting this study. Investigators are responsible to ensure that the requirements for informed consent are met according to local laws and regulations and obtain approval for the Informed Consent Form (ICF) from the respective IEC/IRB.

If consent/assent is deemed required by local and national regulations, the Investigator or designee at each centre will ensure that the patient and patient's parent(s) or legal guardian(s) (if applicable), are given full and adequate oral and written information about the nature, purpose, possible risks, and benefits of the study. Patients must also be notified that they are free to discontinue from the study at any time. The patient and patient's parent(s) or legal guardian(s) (if applicable) will be given the opportunity to ask questions and allowed time to consider the information provided.

10.2 STATEMENT OF CONFIDENTIALITY

Individual patient medical information obtained as a result of this study is considered confidential and disclosure to third parties is prohibited with the exceptions noted below. Patient confidentiality will be ensured by using patient identification code numbers.

Treatment data may be given to the patient's personal physician or to other appropriate medical personnel responsible for the patient's welfare. Data generated as a result of the study need to be available for inspection on request by the participating physicians, the Sponsor's representatives, by the IRB/IEC and the regulatory authorities.

10.3 IRB/EC REVIEW AND HEALTH AUTHORITIES

Before study initiation, the protocol will be submitted for review and approval to the appropriate Central and/or Local IRB/IEC. With assistance from the Clinical Research Organization (CRO), each Principal Investigator will be responsible for obtaining the necessary approval from the central/local IRBs/ECs and for ensuring that the study complies with local legislation relating to data protection and privacy. When local approval is obtained, the documentation indicating the IRB/ECs approval or favourable opinion and the names and qualifications of the Committee members must be sent by the Principal Investigator to the CRO who will send to the study Sponsor before the recruitment process begins.

Health authorities in each country will also be notified of the study protocol by the CRO.

11. MANAGEMENT AND REPORTING OF ADVERSE EVENTS/ADVERSE REACTIONS

This is a natural history study of a disease with no objective to evaluate exposure to any specific treatment. Therefore, no safety outcomes including adverse events will be collected in the eCRF. BI has no marketed product for the disease in scope hence adverse event (AE) safety management and reporting is not applicable.

12. PLANS FOR DISSEMINATING AND COMMUNICATING STUDY RESULTS

The rights of the investigator and of the Sponsor with regard to publication of the results of this study are described in the investigator contract. As a general rule, no study results should be published prior to finalization of the Study Report.

The study documents and database developed during the conduct of the NIS are strictly confidential. Any study result or information may be disclosed only as deemed necessary by the Sponsor. At the conclusion of this study, a report will be prepared. In addition, a manuscript may be prepared for publication in a peer-reviewed journal under the direction of the Sponsor. The Sponsor will publish and communicate the results. Publications will adhere to the International Committee of Medical Journal Editors (ICMJE) guidelines. If a site wishes to publish information from the study, a request must be made to the Sponsor.

13. REFERENCES**13.1 PUBLISHED REFERENCES**

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R23-0418	Bhattarai D, Banday AZ, Sadanand R, Arora K, Kaur G, Sharma S, Rawat A. Hair microscopy: an easy adjunct to diagnosis of systemic diseases in children. <i>Appl Microsc</i> 2021; Nov 29;51(1):18. doi: 10.1186/s42649-021-00067-6.
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R22-1070	Chavanas S, Bodemer C, Rochat A, Hamel-Teillac D, Ali M, Irvine AD, Bonafe JL, Wilkinson J, Taieb A, Barrandon Y, Harper JI, de Prost Y, Hovnanian A. Mutations in SPINK5, encoding a serine protease inhibitor, cause Netherton syndrome. <i>Nat Genet</i> 2000b;25:141-2.
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R number	Reference
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R22-0752	Fontao L., Laffitte E., Briot A., Kaya G., Roux-Lombard P., Fraitag S., Hovnanian A., Saurat JH. Infliximab infusions for Netherton syndrome: sustained clinical improvement correlates with a reduction of thymic stromal lymphopoietin levels in the skin. Journal of Investigative Dermatology (2011) 131, 1947–1950.
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R number	Reference
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13.2 UNPUBLISHED REFERENCES

N/A

14. ANNEXES**ANNEX 1. ENCEPP CHECKLIST FOR STUDY PROTOCOLS**

Doc.Ref. EMA/540136/2009

European Network of Centres for
Pharmacoepidemiology and
Pharmacovigilance**ENCEPP Checklist for Study Protocols (Revision 4)**

Adopted by the ENCePP Steering Group on 15/10/2018

The European Network of Centres for Pharmacoepidemiology and Pharmacovigilance (ENCEPP) welcomes innovative designs and new methods of research. This Checklist has been developed by ENCePP to stimulate consideration of important principles when designing and writing a pharmacoepidemiological or pharmacovigilance study protocol. The Checklist is intended to promote the quality of such studies, not their uniformity. The user is also referred to the ENCePP Guide on Methodological Standards in Pharmacoepidemiology, which reviews and gives direct electronic access to guidance for research in pharmacoepidemiology and pharmacovigilance.

For each question of the Checklist, the investigator should indicate whether or not it has been addressed in the study protocol. If the answer is "Yes", the section number of the protocol where this issue has been discussed should be specified. It is possible that some questions do not apply to a particular study (for example, in the case of an innovative study design). In this case, the answer 'N/A' (Not Applicable) can be checked and the "Comments" field included for each section should be used to explain why. The "Comments" field can also be used to elaborate on a "No" answer.

This Checklist should be included as an Annex by marketing authorisation holders when submitting the protocol of a non-interventional post-authorisation safety study (PASS) to a regulatory authority (see the Guidance on the format and content of the protocol of non-interventional post-authorisation safety studies). The Checklist is a supporting document and does not replace the format of the protocol for PASS presented in the Guidance and Module VIII of the Good pharmacovigilance practices (GVP).

Study title:

Non-interventional study of patients with Netherton Syndrome to characterise natural history of the disease

EU PAS Register® number: EUPAS103733**Study reference number (if applicable):**

<u>Section 1: Milestones</u>	Yes	No	N/A	Section Number
1.1 Does the protocol specify timelines for				
1.1.1 Start of data collection ¹	☒	☐	☐	6
1.1.2 End of data collection ²	☒	☐	☐	6
1.1.3 Progress report(s)	☐	☐	☒	
1.1.4 Interim report(s)	☐	☒	☐	
1.1.5 Registration in the EU PAS Register®	☒	☐	☐	
1.1.6 Final report of study results.	☒	☐	☐	6

Comments:

Progress report of study enrolment will be generated by the study coordinating center on an ongoing basis until enrolment is closed.

<u>Section 2: Research question</u>	Yes	No	N/A	Section Number
2.1 Does the formulation of the research question and objectives clearly explain:	☒	☐	☐	8
2.1.1 Why the study is conducted? (e.g. to address an important public health concern, a risk identified in the risk management plan, an emerging safety issue)	☒	☐	☐	7.4
2.1.2 The objective(s) of the study?	☒	☐	☐	8
2.1.3 The target population? (i.e. population or subgroup to whom the study results are intended to be generalised)	☒	☐	☐	9.2.2
2.1.4 Which hypothesis(-es) is (are) to be tested?	☐	☐	☒	
2.1.5 If applicable, that there is no <i>a priori</i> hypothesis?	☒	☐	☐	8

Comments:

Descriptive data to be collected.

¹ Date from which information on the first study is first recorded in the study dataset or, in the case of secondary use of data, the date from which data extraction starts.

² Date from which the analytical dataset is completely available.

Section 3: Study design	Yes	No	N/A	Section Number
3.1 Is the study design described? (e.g. cohort, case-control, cross-sectional, other design)	☒	☐	☐	9.1
3.2 Does the protocol specify whether the study is based on primary, secondary or combined data collection?	☒	☐	☐	9.1
3.3 Does the protocol specify measures of occurrence? (e.g., rate, risk, prevalence)	☐	☐	☒	
3.4 Does the protocol specify measure(s) of association? (e.g. risk, odds ratio, excess risk, rate ratio, hazard ratio, risk/rate difference, number needed to harm (NNH))	☐	☐	☒	
3.5 Does the protocol describe the approach for the collection and reporting of adverse events/adverse reactions? (e.g. adverse events that will not be collected in case of primary data collection)	☒	☐	☐	11

Comments:

This is a natural history study of Netherton syndrome. Although information on flare-ups will be collected, the study does not intend to estimate rate of occurrence. Frequency of flares will be summarised descriptively in counts.

Section 4: Source and study populations	Yes	No	N/A	Section Number
4.1 Is the source population described?	☒	☐	☐	9.2.2
4.2 Is the planned study population defined in terms of:				
4.2.1 Study time period	☒	☐	☐	9.2.2
4.2.2 Age and sex	☐	☐	☒	
4.2.3 Country of origin	☐	☐	☒	
4.2.4 Disease/indication	☒	☐	☐	9.2.2
4.2.5 Duration of follow-up	☒	☐	☐	9.2.3
4.3 Does the protocol define how the study population will be sampled from the source population? (e.g. event or inclusion/exclusion criteria)	☒	☐	☐	9.2.2.1; 9.2.2.3

Comments:

Section 5: Exposure definition and measurement	Yes	No	N/A	Section Number
5.1 Does the protocol describe how the study exposure is defined and measured? (e.g. operational details for defining and categorising exposure, measurement of dose and duration of drug exposure)	☐	☐	☒	
5.2 Does the protocol address the validity of the exposure measurement? (e.g. precision, accuracy, use of validation sub-study)	☐	☐	☒	
5.3 Is exposure categorised according to time windows?	☐	☐	☒	
5.4 Is intensity of exposure addressed? (e.g. dose, duration)	☐	☐	☒	
5.5 Is exposure categorised based on biological mechanism of action and taking into account the pharmacokinetics and pharmacodynamics of the drug?	☐	☐	☒	
5.6 Is (are) (an) appropriate comparator(s) identified?	☐	☐	☒	

Comments:

Exposure is not relevant for this study since it is a natural history study.

Section 6: Outcome definition and measurement	Yes	No	N/A	Section Number
6.1 Does the protocol specify the primary and secondary (if applicable) outcome(s) to be investigated?	☒	☐	☐	9.3.2
6.2 Does the protocol describe how the outcomes are defined and measured?	☒	☐	☐	9.3.2.1
6.3 Does the protocol address the validity of outcome measurement? (e.g. precision, accuracy, sensitivity, specificity, positive predictive value, use of validation sub-study)	☒	☐	☐	9.3.2.2
6.4 Does the protocol describe specific outcomes relevant for Health Technology Assessment? (e.g. HRQoL, QALYs, DALYS, health care services utilisation, burden of disease or treatment, compliance, disease management)	☒	☐	☐	9.3.2.2

Comments:

There is no outcome assessment tool validated for Netherton syndrome. For primary outcome, the study will use a severity assessment scale that is validated in patients with ichthyosis.

For secondary outcome the study will use a new scale (IGA) and repeatability (test-retest) evaluation will be conducted.

For further objectives all skin, hair and quality-of-life assessments are validated in patient populations with dermatological conditions.

Healthcare resource utilisation will be assessed using information documented in patients' medical records; no additional questionnaire will be administered.

<u>Section 7: Bias</u>	Yes	No	N/A	Section Number
7.1 Does the protocol address ways to measure confounding? (e.g. confounding by indication)	☐	☐	☒	
7.2 Does the protocol address selection bias? (e.g. healthy user/adherer bias)	☒	☐	☐	9.9
7.3 Does the protocol address information bias? (e.g. misclassification of exposure and outcomes, time-related bias)	☒	☐	☐	9.9

Comments:

<u>Section 8: Effect measure modification</u>	Yes	No	N/A	Section Number
8.1 Does the protocol address effect modifiers? (e.g. collection of data on known effect modifiers, sub-group analyses, anticipated direction of effect)	☒	☐	☐	9.7.1

Comments:

Study outcomes will be summarised by age subgroups and any other subgroups that may be informative; however, results will be descriptive for the characterisation of the natural history of Netherton syndrome. There is no causal relationship to be evaluated, and age is being treated as a natural, biological and temporal characteristic, not an effect modifier.

<u>Section 9: Data sources</u>	Yes	No	N/A	Section Number
9.1 Does the protocol describe the data source(s) used in the study for the ascertainment of:				
9.1.1 Exposure? (e.g. pharmacy dispensing, general practice prescribing, claims data, self-report, face-to-face interview)	☒	☐	☐	9.4
9.1.2 Outcomes? (e.g. clinical records, laboratory markers or values, claims data, self-report, patient interview including scales and questionnaires, vital statistics)	☒	☐	☐	9.4
9.1.3 Covariates and other characteristics?	☒	☐	☐	9.3.1

<u>Section 9: Data sources</u>	Yes	No	N/A	Section Number
9.2 Does the protocol describe the information available from the data source(s) on:				
9.2.1 Exposure? (e.g. date of dispensing, drug quantity, dose, number of days of supply prescription, daily dosage, prescriber)	☐	☐	☒	
9.2.2 Outcomes? (e.g. date of occurrence, multiple event, severity measures related to event)	☒	☐	☐	9.3.2
9.2.3 Covariates and other characteristics? (e.g. age, sex, clinical and drug use history, co-morbidity, co-medications, lifestyle)	☒	☐	☐	9.3.1
9.3 Is a coding system described for:				
9.3.1 Exposure? (e.g. WHO Drug Dictionary, Anatomical Therapeutic Chemical (ATC) Classification System)	☐	☐	☒	
9.3.2 Outcomes? (e.g. International Classification of Diseases (ICD), Medical Dictionary for Regulatory Activities (MedDRA))	☒	☐	☐	9.7.1
9.3.3 Covariates and other characteristics?	☒	☐	☐	9.7.1
9.4 Is a linkage method between data sources described? (e.g. based on a unique identifier or other)	☐	☐	☒	

Comments:

The study does not evaluate the outcomes of a specific drug exposure. However, information on therapies used to manage symptoms of Netherton syndrome and comorbidities will be collected from patients' medical records. Medications will be coded using the WHO drug dictionary.

<u>Section 10: Analysis plan</u>	Yes	No	N/A	Section Number
10.1 Are the statistical methods and the reason for their choice described?	☒	☐	☐	9.7
10.2 Is study size and/or statistical precision estimated?	☒	☐	☐	9.5
10.3 Are descriptive analyses included?	☒	☐	☐	9.7.1
10.4 Are stratified analyses included?	☒	☐	☐	9.7.1
10.5 Does the plan describe methods for analytic control of confounding?	☐	☐	☒	
10.6 Does the plan describe methods for analytic control of outcome misclassification?	☐	☐	☒	
10.7 Does the plan describe methods for handling missing data?	☒	☐	☐	9.9

<u>Section 10: Analysis plan</u>	Yes	No	N/A	Section Number
10.8 Are relevant sensitivity analyses described?	☒	☐	☐	9.7.2

Comments:

A formal sample size estimation was not possible due to rarity of Netherton Syndrome and the lack of epidemiologic data on prevalence.

Confounding is not applicable, as the study is not designed to assess any causal relationships. However, an exploratory multivariate analysis may be conducted to identify potential risk factors of flares.

The primary and secondary outcome measures are clinician-reported outcomes (ClinRO) and patient-reported outcomes (PRO), which are descriptive measures. There will be no analytic control of outcome misclassification because PRO are not objective measures. ClinROs are scored based on physician's medical judgement and evaluation. PROs are based on self-report from the patient.

<u>Section 11: Data management and quality control</u>	Yes	No	N/A	Section Number
11.1 Does the protocol provide information on data storage? (e.g. software and IT environment, database maintenance and anti-fraud protection, archiving)	☒	☐	☐	9.6
11.2 Are methods of quality assurance described?	☒	☐	☐	9.8
11.3 Is there a system in place for independent review of study results?	☐	☐	☒	

Comments:

Study data will be abstracted from medical records as well as from assessment questionnaires completed by the clinician and patients. Data quality review will be an ongoing task. The final study data will be verified and signed off by local investigators.

<u>Section 12: Limitations</u>	Yes	No	N/A	Section Number
12.1 Does the protocol discuss the impact on the study results of:				
12.1.1 Selection bias?	☒	☐	☐	9.9
12.1.2 Information bias?	☒	☐	☐	9.9
12.1.3 Residual/unmeasured confounding? (e.g. anticipated direction and magnitude of such biases, validation sub-study, use of validation and external data, analytical methods).	☐	☐	☒	

<u>Section 12: Limitations</u>	Yes	No	N/A	Section Number
12.2 Does the protocol discuss study feasibility? (e.g. study size, anticipated exposure uptake, duration of follow-up in a cohort study, patient recruitment, precision of the estimates)	☒	☐	☐	9.1; 9.5

Comments:

Confounding is not applicable, as the study is not designed to assess any causal relationships. However, an exploratory multivariate analysis may be conducted to identify potential risk factors of flares.

<u>Section 13: Ethical/data protection issues</u>	Yes	No	N/A	Section Number
13.1 Have requirements of Ethics Committee/ Institutional Review Board been described?	☒	☐	☐	10.1; 10.3
13.2 Has any outcome of an ethical review procedure been addressed?	☐	☐	☒	
13.3 Have data protection requirements been described?	☒	☐	☐	10.3

Comments:

The study has not been submitted to any IRB or IEC to-date.

<u>Section 14: Amendments and deviations</u>	Yes	No	N/A	Section Number
14.1 Does the protocol include a section to document amendments and deviations?	☒	☐	☐	5; 9.10.4

Comments:

Current protocol revision is a protocol amendment.

<u>Section 15: Plans for communication of study results</u>	Yes	No	N/A	Section Number
15.1 Are plans described for communicating study results (e.g. to regulatory authorities)?	☐	☒	☐	
15.2 Are plans described for disseminating study results externally, including publication?	☒	☐	☐	12

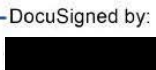
Comments:

The protocol indicates in section 10.3 that health authorities and IRB/IEC will be informed of study completion. However they will not be receiving the study results.

Name of the main author of the
protocol:

[REDACTED]

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ANNEX 2. BI PROTOCOL SIGNATURE PAGE

Study Title: Non-interventional study of patients with Netherton Syndrome to characterise the natural history of disease

Study Number: 1368-0121

Protocol Version: 2.0

I herewith certify that I agree to the content of the study protocol and to all documents referenced in the study protocol.

Position: Project Statistician	Name/Date: [REDACTED]	Signature: _____
Position: Product Lead, Global Epidemiology and RWE	Name/Date: [REDACTED]	Signature: _____
Position: NIS Lead	Name/Date: [REDACTED]	Signature: _____
Position: Senior Global Medical Advisor, TA Inflammation Medicine	Name/Date: [REDACTED]	Signature: _____

ANNEX 3. INVESTIGATOR PROTOCOL SIGNATURE PAGE

SIGNATURE PAGE

Study Title: Non-interventional study of patients with Netherton Syndrome to characterise natural history of the disease

Study Number: 1368-0121

Protocol Version: 2.0

Protocol Date: 28 Nov 2023

I herewith certify that I agree to the content of the study protocol dated 20Oct2023 and to all documents referenced in the study protocol. I agree to conduct the study in compliance with all applicable regulations and guidelines, as stated in the protocol, and other information supplied to me.

Position: PI (Investigator)

PI (Investigator) Name /

Date: _____

Signature: _____

APPROVAL / SIGNATURE PAGE**Document Number:** c41811684**Technical Version Number:**2.0**Document Name:** 1368-0121-nis-protocol

Title: Non-interventional study of patients with Netherton Syndrome to characterise the natural history of disease

Signatures (obtained electronically)

Meaning of Signature	Signed by	Date Signed
Approval-Project Statistician		29 Nov 2023 06:38 CET
Approval-On behalf of Head or VP or Director		29 Nov 2023 10:15 CET
Approval		29 Nov 2023 14:57 CET
Approval		29 Nov 2023 16:24 CET
Approval		04 Dec 2023 17:00 CET

(Continued) Signatures (obtained electronically)

Meaning of Signature	Signed by	Date Signed
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