CLINICAL TRIAL PROTOCOL

Protocol title:	**Open-label exploratory study to evaluate the effect of dupilumab on skin barrier function in pediatric patients with moderate-to-severe atopic dermatitis**
Protocol number:	**LPS16764**
Amendment number:	**Not applicable**
Compound number (INN/Trademark):	**SAR231893/REGN668 dupilumab/Dupixent®**
Study phase:	**Phase 4**
Short title:	**Dupilumab-PEdiatric skin barrier function and LIpidomics Study in patients with Atopic Dermatitis - PELISTAD**
Sponsor name:	**Sanofi-Aventis Recherche & Développement**
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1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Protocol title: Open-label exploratory study to evaluate the effect of dupilumab on skin barrier function in pediatric patients with moderate-to-severe atopic dermatitis.

Short title: Dupilumab-PEdiatric skin barrier function and LIpidomics STudy in patients with Atopic Dermatitis - PELISTAD

Rationale:

There is a growing body of literature to suggest that patients with early-onset atopic dermatitis (AD) have a higher risk of developing other Type 2 comorbidities (the atopic march). The early optimal and successful treatment of AD may prevent or attenuate the development other atopic conditions (1, 2). Literature indicates that samples acquired using a non-invasive skin tape stripping (STS) can be reliably used for analyzing transepidermal water loss (TEWL), TEWL area under the curve (AUC) responses, lipidomics, filaggrin (FLG) breakdown products, proteomics, and transcriptomics. Results from these analyses demonstrated that children with AD pediatric and food allergy exhibit elevated epidermal barrier defects even in non-lesional skin (3). The proposed study will evaluate the effect of dupilumab treatment on the skin barrier in a pediatric population with early-onset moderate-to-severe AD using non-invasive methodologies such as TEWL, STS for skin barrier function assessment, Optical Coherence Tomography (OCT), and Fourier-Transform Infrared (FTIR) spectroscopy for skin barrier structure evaluation.

Objectives and endpoints

Objectives	Endpoints
Primary	
• Evaluate changes in skin barrier function with transepidermal water loss (TEWL) assessed after skin tape stripping (STS) in predefined lesional skin in pediatric patients with moderate-to-severe atopic dermatitis (AD) treated with dupilumab.	• Percent change from baseline in TEWL after 5 STS assessed on lesional skin at Week 16 in AD patients.
Secondary	
• Evaluate changes in skin barrier function with TEWL assessed after STS in predefined lesional and non-lesional skin in pediatric patients with moderate-to-severe AD treated with dupilumab in reference to normal skin of healthy volunteers.	• Change (percent and absolute) from baseline in TEWL after 20 STS assessed on lesional skin at Week 16 in AD patients. • Change (percent and absolute) from baseline in TEWL after 20 STS assessed on non-lesional skin at Week 16 in AD patients. • Change (percent and absolute) from baseline in TEWL after 20 STS assessed on normal skin at Week 16 in healthy volunteers.

Objectives	Endpoints
	• Change (percent and absolute) from baseline in TEWL after 15 STS assessed on lesional skin at Week 16 in AD patients. • Change (percent and absolute) from baseline in TEWL after 15 STS assessed on non-lesional skin at Week 16 in AD patients. • Change (percent and absolute) from baseline in TEWL after 15 STS assessed on normal skin at Week 16 in healthy volunteers. • Change (percent and absolute) from baseline in TEWL after 10 STS assessed on lesional skin at Week 16 in AD patients. • Change (percent and absolute) from baseline in TEWL after 10 STS assessed on non-lesional skin at Week 16 in AD patients. • Change (percent and absolute) from baseline in TEWL after 10 STS assessed on normal skin at Week 16 in healthy volunteers. • Change (percent and absolute) from baseline in TEWL after 5 STS assessed on non-lesional skin at Week 16 in AD patients. • Change (percent and absolute) from baseline in TEWL after 5 STS assessed on normal skin at Week 16 in healthy volunteers.
• Evaluate time course of change in skin barrier function with TEWL assessed before and after STS in predefined lesional and non-lesional skin in pediatric patients with moderate-to-severe AD during dupilumab treatment phase and follow-up period in reference to normal skin of healthy volunteers.	• Change (percent and absolute) in TEWL before STS on lesional skin in AD patients over time. • Change (percent and absolute) in TEWL before STS on non-lesional skin in AD patients over time. • Change (percent and absolute) in TEWL before STS on normal skin in healthy volunteers over time. • Change (percent and absolute) in TEWL area under the curve (TEWL AUC: a composite measure before and after 5, 10, 15, and 20 STS) for skin barrier function in lesional skin in AD patients over time. • Change (percent and absolute) in TEWL AUC (a composite measure before and after 5, 10, 15, and 20 STS) for skin barrier function in non-lesional skin in AD patients over time. • Change (percent and absolute) in TEWL AUC (a composite measure before and after 5, 10, 15, and 20 STS) for skin barrier function in normal skin in healthy volunteers over time. • Change (percent and absolute) in TEWL after STS assessed on lesional skin in AD patients over time. • Change (percent and absolute) in TEWL after STS assessed on non-lesional skin in AD patients over time. • Change (percent and absolute) in TEWL after STS assessed on normal skin in healthy volunteers over time.

Objectives	Endpoints
Tertiary/exploratory	
• Evaluate dupilumab treatment effect on skin lipidomics using STS samples in both predefined lesional and non-lesional skin in pediatric patients with moderate-to-severe AD during dupilumab treatment phase and follow-up period in reference to normal skin of healthy volunteers.	• Changes (percent and absolute) in lipidomics parameters in lesional and non-lesional skin including the ratio of highly hydrophobic omega-esterified fatty acid sphingosine ceramides (EOS CER) and non-hydroxy fatty acid sphingosine ceramides (NS CER), and filaggrin (FLG) breakdown products of urocanic acid (UCA) and pyroglutamic acid (PCA) concentrations over time. • Global characterization of protein-bound CER over time.
• Evaluate dupilumab treatment effect on skin proteomics using STS samples in both predefined lesional and non-lesional skin in pediatric patients with moderate-to-severe AD during dupilumab treatment phase and follow-up period in reference to normal skin of healthy volunteers.	• Changes (percent and absolute) in the expression of proteins associated with skin barrier function including keratin intermediate filaments, proteins associated with inflammatory response, and glycolysis and oxidative stress response proteins in STS protein extracts over time.
• Evaluate dupilumab treatment effect on epidermal hypertrophy and structure of skin barrier changes measured by Optical Coherence Tomography (OCT) and Fourier-Transform Infrared (FTIR) spectroscopy in both predefined lesional and non-lesional skin in pediatric patients with moderate-to-severe AD during dupilumab treatment phase and follow-up period in reference to normal skin of healthy volunteers.	• Change (percent and absolute) in epidermal hypertrophy parameters including epidermal thickness (µm), superficial plexus depth (µm), blood vessel diameter (µm) and density (segments/mm^2) measured by OCT over time. • Change (percent and absolute) in stratum corneum lipid structure measured by FTIR spectroscopy in conjunction with tape-stripping over time.
• Evaluate dupilumab treatment effect on skin transcriptome using STS samples in both predefined lesional and non-lesional skin in pediatric patients with moderate-to-severe AD during dupilumab treatment phase and follow-up period in reference to normal skin of healthy volunteers.	• Changes in expression of genes associated with epidermal differentiation, barrier and lipid metabolism, and Type 2 inflammation over time.
• Explore the association of skin barrier function measured by TEWL with disease impact assessed by standard AD severity assessments (Eczema Area and Severity Index [EASI], and Individual Signs Score [ISS]), patient reported outcomes (PRO) (Patient Oriented Eczema Measure [POEM], Children Dermatology Life Questionnaire Index [CDLQI], worst itch Numerical Rating Scale [NRS], sleep disturbance NRS, skin pain NRS), standardized photos, the biomarker profiles of lipidomics, proteomics, and transcriptomics assessed in the STS sample, and the skin barrier hypertrophy and structure profiles assessed by OCT and FTIR.	• Change (percent and absolute) in EASI over time. • Change (percent and absolute) in ISS for target lesion over time. • Change (percent and absolute) in POEM over time. • Change (percent and absolute) in CDLQI over time. • Change (percent and absolute) in assessment of worst itch NRS over time. • Change (percent and absolute) in sleep disturbance NRS over time. • Change (percent and absolute) in skin pain NRS over time. • Change (percent and absolute) in photograph outputs (eg, severity score) obtained from skin imaging over time. • Correlation between baseline values of TEWL before and after STS, and TEWL AUC in lesional and non-lesional skin of AD patients with the following baseline measures:

Objectives	Endpoints
	- EASI - Targeted lesion ISS - PRO measures (POEM, CDLQI, worst itch NRS, sleep disturbance NRS, and skin pain NRS) - Lipidomics in STS (ratio of EOS CER to NS CER) - Filaggrin breakdown products of UCA and PCA concentrations in STS - Image-derived severity score in targeted lesional skin - Key components of skin proteomics in STS (expression of proteins associated with skin barrier function) - Quantified epidermal hypertrophy and structure changes in OCT and FTIR - Key components of gene expression form transcriptomics • Correlation between percent change from baseline in TEWL before and after STS, and TEWL AUC in lesional and non-lesional skin of AD patients at Week 8, Week 16 and Week 28 with corresponding change from baseline in the following measures: - EASI - Targeted lesion ISS - PRO measures (POEM, CDLQI, worst itch NRS, sleep disturbance NRS, and skin pain NRS) - Lipidomics in STS (ratio of EOS CER to NS CER) - Filaggrin breakdown products of UCA and PCA concentrations in STS - Image-derived severity score in targeted lesional skin - Key components of skin proteomics in STS (expression of proteins associated with skin barrier function) - Quantified epidermal hypertrophy and structure changes in OCT and FTIR - Key components of gene expression from transcriptomics

Overall design:

- Phase 4.
- Two study sites (Dr Leung, Denver, USA; Dr Cork, Michael J, EU [UK]). Patients with moderate-to-severe AD will be included in the US site and patients with severe AD will be included in the EU site (UK). More study sites could be initiated if recruitment at 1 or both of above-mentioned study sites is insufficient.
- Open-label, exploratory study.
- Approximately 24 pediatric patients with moderate-to-severe AD will be enrolled to achieve 20 evaluable patients. These enrolled AD patients will receive Investigational medicinal product (IMP) dupilumab treatment during the study.
- Approximately 24 healthy volunteers will be enrolled to achieve 20 evaluable subjects. These enrolled healthy volunteers will not receive any IMP treatment during the study.

This is a 32-week, open-label, exploratory study with a 4-week screening period, 16-week treatment phase designed to investigate dupilumab's effect on skin barrier function as measured by TEWL before and after STS in approximately 20 pediatric patients with moderate-to-severe AD (not more than 24 patients aged ≥6 and <12 years old), and a 12-week follow-up period. Patients will have 1 on-site visit/week, up to Week 4, 1 on-site visit every 2 weeks from Week 4 to Week 8, and 1 on-site visit every 4 weeks from Week 8 to Week 16 End of Treatment (EoT) phase visit, and every 6-weeks thereafter during the follow-up period and by this will end the study for each participant (End of Study [EoS]). The maximum duration of the study per participant will be 32 weeks (including screening period).

Lesional and non-lesional skin areas for TEWL assessment and STS will be identified on the upper limbs or lower limbs (including trunk, if needed) at baseline ("predefined skin area"). These predefined, lesional and non-lesional skin areas for skin barrier assessment should be separated by a distance of about 4 cm.

Within these predefined skin areas 3 closely adjacent spots without an overlap for the skin tapes will be identified for subsequent skin barrier function assessment (3 spots on lesional skin, 3 spots on non-lesional skin).

The skin barrier function without and before STS will be assessed using TEWL measurement on each of the 3 spots within these predefined skin areas at each visit except Weeks 10 and 14, for which there will be no study assessments, and only study drug administration.

Repeated TEWL assessment in predefined lesional and non-lesional skin areas of AD patients after STS and lipidomics, proteomics, and transcriptomics analysis in STS samples will be conducted at baseline (Week 0, Day 1), Week 2, Week 4, Week 8, Week 12, EoT at Week 16, and Follow-ups at Week 22 and Week 28.

In order to allow the skin to recover from the STS, the skin barrier function assessment with STS will be performed within the predefined lesional and non-lesional skin area as follows: STS assessment on baseline (Week 0), Week 8, Week 16, and Week 28 will be conducted on the first spot; STS assessment on Week 2 will be conducted on the second spot; and STS assessment on Week 4, Week 12, and Week 22 will be conducted on the third spot.

TEWL will be measured before STS (on each of the 3 spots) and after 5, 10, 15, and 20 STS (on the spot defined for the visit) (5 TEWL assessments per visit) on targeted, predefined lesional skin in AD patients at baseline, Week 2, Week 4, Week 8, Week 12, Week 16, Week 22 and Week 28 on the predefined first, second or third spot, respectively.

TEWL will be measured before (on each of the 3 spots) and after 5, 10, 15, and 20 STS (on spot defined for the visit) (5 TEWL assessments per visit) on targeted, predefined non-lesional skin in AD patients at baseline, Week 2, Week 4, Week 8, Week 12, Week 16, Week 22, and Week 28 on the predefined first, second or third spot, respectively (see Figure 3 and Figure 5 in Section 8.1.1.2).

Skin barrier function in approximately 20 healthy volunteers (not more than 24 healthy participants aged ≥6 and <12 years old) matched for age (match on age ±2 years), gender, location of targeted lesion area, and study site to the AD cases will be assessed in a similar manner at baseline, and will have 1 on-site visit/week, up to Week 4, 1 on-site visit every 2 weeks from Week 4 to Week 8, and 1 on-site visit every 4 weeks from Week 8 to Week 16 EoT phase visit, and every 6-weeks thereafter during the follow-up period, serving as a reference comparator for skin barrier function.

Within the targeted skin area of healthy volunteers-the location of it to be identical to the lesional area of the patient to which a healthy volunteer is matched-3 closely adjacent spots without an overlap for the skin tapes will be identified for subsequent skin barrier function assessment.

The skin barrier function without and before STS will be assessed using TEWL measurement on each of the 3 spots at each visit, except Weeks 10 and 14.

On normal skin in healthy volunteers at each visit at baseline (Week 0), Week 2, Week 4, Week 8, Week 12, Week 16, Week 22, and Week 28 TEWL will be measured before and after 5, 10, 15, and 20 STS on the predefined first, second or third spot respectively for skin barrier function assessment (5 TEWL assessments per visit in normal skin) at the same location as for the lesional skin in the matching AD patient and as described above (See Figure 4 and Figure 5 in Section 8.1.1.2).

In particular, STS assessment on baseline (Week 0, Day 1), Week 8, Week 16, and Week 28 will be conducted on the first spot within the location-matched, normal skin. STS assessment on Week 2 will be conducted on the second spot. STS assessment on Week 4, Week 12, and Week 22 will be conducted on the third spot.

Study drug will be administered on-site by study site staff at baseline (Week 0, Day 1), Weeks 4, 8, and 12 for dose regimen 1; and at baseline (Week 0, Day 1), Weeks 2, 4, 6, 8, 10, 12, and 14 for dose regimen 2, after the TEWL assessment has been completed.

Intervention groups and duration:

The study is an open-label study of dupilumab with a 16-week treatment phase in moderate-to-severe AD pediatric patients. Age-, gender-, targeted lesion area- and study-site-matched healthy volunteers will be included for a non-treatment, 16-week evaluation period. Total study duration including screening and follow-up will be 32 weeks.

Study intervention(s)

Investigational medicinal product(s)

Dupilumab 200 mg

- Formulation: a 175 mg/mL dupilumab solution in a pre-filled syringe to deliver 200 mg in a 1.14 mL injection.
- Route(s) of administration: subcutaneous (SC) injection.

Dupilumab 300 mg

- Formulation: a 150 mg/mL dupilumab solution in a pre-filled syringe to deliver 300 mg in a 2 mL injection.
- Route(s) of administration: SC injection.

Dose regimen

Dose regimen 1:

- Children with baseline 15 kg ≤ body weight <30 kg will receive an SC loading dose of dupilumab 600 mg (2 injections of dupilumab 300 mg) on Day 1 (Week 0), followed by every 4-week SC dosing of dupilumab 300 mg from Week 4 to Week 12. The IMP will be administered by study site staff at baseline (Week 0, Day 1), Weeks 4, 8, and 12.

Dose regimen 2:

- Children with baseline 30 kg ≤ body weight <60 kg will receive a SC loading dose of dupilumab 400 mg (2 injections of dupilumab 200 mg) on Day 1 (Week 0), followed by bi-weekly SC dosing of dupilumab 200 mg from Week 2 to Week 14. The IMP will be administered by study site staff at baseline (Week 0, Day 1), Weeks 2, 4, 6, 8, 10, 12, and 14.

Post-trial access to study medication

- After the study treatment phase is completed, patients will stop the study medication. Rescue treatment with dupilumab for AD may be provided to patients during follow-up period. Investigators will be required to perform an Investigator Global Assessment (IGA) evaluation prior to starting rescue treatment and initiate rescue treatment only in patients who either have an IGA score of 4, or have intolerable symptoms. After the study is completed at Week 28, patients will not be provided with any further study medication as part of this protocol.

Duration of study period (per participant)

Study duration for each AD patient and healthy volunteer will be approximately 32 weeks including:

- Screening period: Up to 4 weeks (Day -28 to Day -1) from signing the informed consent.
- Open-label dupilumab treatment (AD patients) and observation period (healthy volunteers) for 16 weeks from baseline on Day 1 (Week 0).
- Follow-up period for 12 weeks after the EoT visit (Week 16). Dupilumab may be used as a rescue therapy for AD patients, if needed during follow-up period.

Statistical considerations: Analyses will be descriptive and explorative.

A first analysis (treatment phase), will be performed after all patients and healthy volunteers have completed the Week 16 assessments, the data up to this time will be cleaned, and locked; all endpoints relating to Week 16 will be analyzed and reported at this time. A second analysis (follow-up period) will be run once all patients and healthy volunteers have completed the Week 28 assessments. The analysis of the follow-up period will be based on all participants and all data.

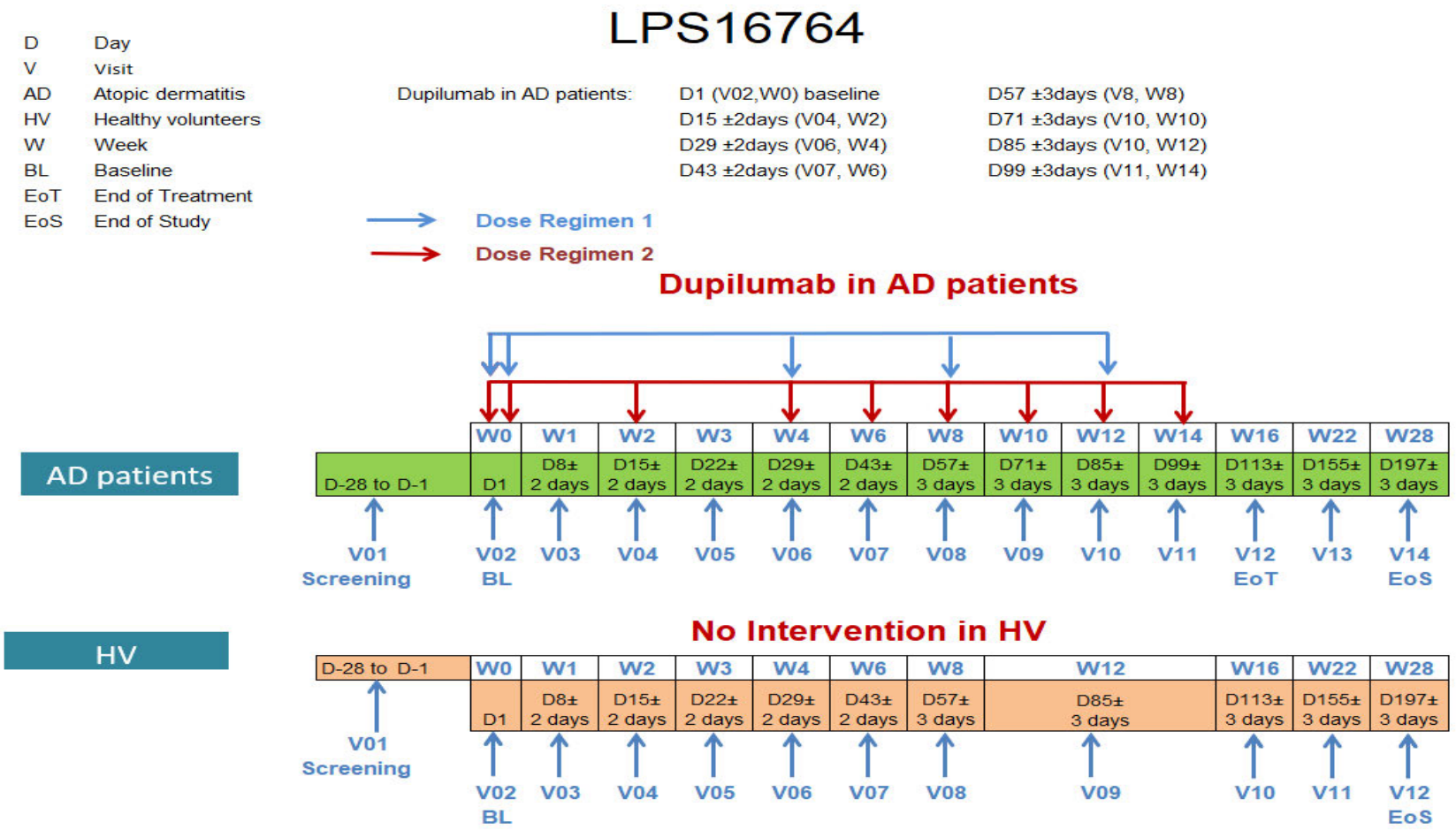
- **Sample size calculations**
  - Sample size for this exploratory study was based on medical/clinical judgment and is consistent with the sample size from similar studies in the literature (4). No formal sample size calculation was performed. TEWL data collected in similar settings as planned for this study were not available: ie, TEWL values after 5 STS, and pre- and post-dupilumab treatment are unknown.
  - Allowing for drop-out rate of 15%, a total of approximately 24 pediatric patients with moderate-to-severe AD will be enrolled to achieve 20 evaluable patients: ie, patients with no major or critical deviations related to IMP and/or TEWL measurements, for whom the TEWL data for primary analysis: ie, TEWL at baseline and Week 16, are considered sufficient and interpretable. An approximately equal number of age, gender, location of targeted lesion area and site matched healthy volunteers serving as a reference comparator cohort will be enrolled. Drop-out rate in healthy volunteers is expected to be minimal. Any drop-out healthy volunteer for whom the matched patient is considered as evaluable will not be replaced.
- **Analysis of primary, secondary, and exploratory endpoints:**
  - This is an exploratory study. No formal statistical testing will be conducted.
  - Descriptive statistics will be generated by group, time point and type of skin area (lesional or non-lesional, if applicable) for selected parameters of interest. Raw data and changes from baseline: ie, absolute and percent changes, for selected parameters will be summarized in descriptive statistics and summary plots.
  - Evolution over time of TEWL before STS and TEWL AUC by type of skin area will be analyzed. Matched healthy participants will provide normal reference values.

- **Safety/other analysis:**
  - The safety evaluation will be conducted on all AD patients who receive at least 1 dose of dupilumab or had at least 1 TEWL/STS assessment performed, and on all healthy volunteers, who had at least 1 TEWL/STS assessment. The analysis will be based on descriptive statistics providing summary statistics for vital signs parameters (including summary of Potentially Clinically Significant Abnormalities [PCSA]) and reported adverse event (AE).

Data Monitoring Committee: None

1.2 SCHEMA

Figure 1 - Graphical study design



1.3 SCHEDULE OF ACTIVITIES/AD PEDIATRIC PATIENTS

Phase	Screening	Baseline	Treatment phase									EoT	Follow-up period		Unscheduled visit	Premature EoT
Day	**D-28 to D-1**	**D 1**	**D8 ±2 days**	**D15 ±2 days**	**D22 ±2 days**	**D29 ±2 days**	**D43 ±2 days**	**D57 ±3 days**	**D71 ±3 days**	**D85 ±3 days**	**D99 ±3 days**	**D113 ±3 days**	**D155 ±3 days**	**EoS D197 ±3 days**	**UNSCH**	
Week		**W0**	**W1**	**W2**	**W3**	**W4**	**W6**	**W8**	**W10**	**W12**	**W14**	**W16**	**W22**	**W28**		
Visits	**V01**	**V02**	**V03**	**V04**	**V05**	**V06**	**V07**	**V08**	**V09**	**V10**	**V11**	**V12**	**V13**	**V14**		
Informed consent/assent form	X															
Inclusion/exclusion criteria	X	X														
Medical/surgical history/IGA/demographics	X															
Pregnancy test (urine), WOCBP only[a]	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Prior/concomitant medications/procedures	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Confirm emollient and washing compliance[b]	X	X[b]	X[b]	X[b]	X[b]	X[b]	X[b]	X[b]	X[b]	X[b]	X[b]	X[b]	X[b]	X[b]	X	X[b]
Study treatment administration																
SC administration of dupilumab, dose regimen 1[c]		X				X		X		X					(X)	
SC administration of dupilumab, dose regimen 2[c]		X		X		X	X	X	X	X	X				(X)	

Phase	Screening	Baseline	Treatment phase									EoT	Follow-up period		Unscheduled visit	Premature EoT
Day	D-28 to D-1	D 1	D8 ±2 days	D15 ±2 days	D22 ±2 days	D29 ±2 days	D43 ±2 days	D57 ±3 days	D71 ±3 days	D85 ±3 days	D99 ±3 days	D113 ±3 days	D155 ±3 days	EoS D197 ±3 days	UNSCH	
Week		W0	W1	W2	W3	W4	W6	W8	W10	W12	W14	W16	W22	W28		
Visits	V01	V02	V03	V04	V05	V06	V07	V08	V09	V10	V11	V12	V13	V14		
Skin barrier function test in lesional and non-lesional skin[d]																
TEWL before STS[e, f]		X	X	X	X	X	X	X		X		X	X	X	(X)	X
TEWL and STS assessment[f]		X[g]		X[g]		X[g]		X[g]		X[g]		X[g]	X[g]	X[g]	(X)[h]	X[h]
Lipidomics, proteomics, FLG breakdown products, and transcriptomics assessment from skin tape strips		X[g]		X[g]		X[g]		X[g]		X[g]		X[g]	X[g]	X[g]	(X)[h]	X[h]
Optical Coherence Tomography (OCT)[i]		X	X	X	X	X	X	X		X		X		X	(X)	X
Fourier-Transform Infrared (FTIR) Spectroscopy[i]		X						X				X		X	(X)	X
Standardized photographs of predefined lesional and non-lesional skin areas used for TEWL		X	X	X	X	X	X	X		X		X	X	X	(X)	X
Standardized full body photographs		X	X	X	X	X	X	X		X		X	X	X	(X)	X
EASI	X	X	X	X	X	X	X	X		X		X	X	X	(X)	X
ISS	X	X	X	X	X	X	X	X		X		X	X	X	(X)	X

Phase	Screening	Baseline	Treatment phase									EoT	Follow-up period		Unscheduled visit	Premature EoT
Day	D-28 to D-1	D 1	D8 ±2 days	D15 ±2 days	D22 ±2 days	D29 ±2 days	D43 ±2 days	D57 ±3 days	D71 ±3 days	D85 ±3 days	D99 ±3 days	D113 ±3 days	D155 ±3 days	EoS D197 ±3 days	UNSCH	
Week		W0	W1	W2	W3	W4	W6	W8	W10	W12	W14	W16	W22	W28		
Visits	V01	V02	V03	V04	V05	V06	V07	V08	V09	V10	V11	V12	V13	V14		
Patient reported outcomes (PRO)																
POEM		X	X	X	X	X	X	X		X		X	X	X	(X)	X
CDLQI		X	X	X	X	X	X	X		X		X	X	X	(X)	X
Worst itch NRS[i]	X	X	X	X	X	X	X	X		X		X	X	X	(X)	X
Sleep disturbance NRS[i]	X	X	X	X	X	X	X	X		X		X	X	X	(X)	X
Skin pain NRS[i]	X	X	X	X	X	X	X	X		X		X	X	X	(X)	X
eDiary[k]	X	X	X	X	X	X	X	X		X		X	X	X	(X)	X
Safety																
AE/SAE collection	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height		X										X		X		X
Body weight	X	X										X		X		X
Vital signs[l]	X	X		X		X	X	X		X		X		X	(X)	X
Physical examination[m]	X	X[m]										X[m]		X[m]		X[m]
Saliva swab sample[n]		X														
Optional assessments																
Optional Biomarker research samples (blood)[o]		X										X				

a For the purpose of this study, any female who has had her first menstrual period (menarche) and is sexually active will be considered to be of childbearing potential. Female patients who are not of childbearing potential at the start of the study but have the onset of menarche during the course of the study and are sexually active will also have to follow adequate birth control methods to continue participation in the study. These females must have a negative urine β-HCG pregnancy test at screening and at each visit (except patients who assigned to dose regimen 1 will not attend visits V9 (W10) and V11 (W14), therefore there's no pregnancy test for these patients on both visits). Pregnancy will lead to definitive treatment discontinuation in all cases.

b Emollients should NOT be applied from Day -7 to the EoS (Week 28) to the targeted, predefined skin areas that will be used for TEWL assessment. Participants should not take showers or soaking in a bathtub within 6 hours before TEWL assessment. Emollients for use during the study will be provided by the study site to study participants. Use of emollients should be documented in a diary provided by the study site.

c Dose regimen 1: Children with baseline 15 kg ≤ body weight <30 kg will receive a SC loading dose of dupilumab 600 mg (2 injections of dupilumab 300 mg) on Day 1 (V02), followed by every 4-week SC dosing of dupilumab 300 mg until Week 12 (at Week 0 [(Day 1], Weeks 4, 8, and 12).

Dose regimen 2: Children with baseline 30 kg ≤ body weight <60 kg will receive a SC loading dose of dupilumab 400 mg (2 injections of dupilumab 200 mg) on Day 1 (V02), followed by bi-weekly SC dosing of dupilumab 200 mg until Week 14 (at Week 0 (Day 1), Weeks 2, 4, 6, 8, 10, 12, and 14).

d Assessments on treatment days have to be conducted before administration of dupilumab.

e Predefined lesional and non-lesional skin areas. TEWL will be measured at each of the 3 closely adjacent spots within the predefined skin areas at all visits without STS and before STS at visits with STS.

f TEWL will be conducted before STS and then after 5, 10, 15, and 20 STS in lesional skin and non-lesional skin.

g At Weeks 0, 8, 16, and 28, STS will be conducted on the first spot within the predefined skin area. At Week 2 STS will be conducted on the second spot within the predefined skin area. At Weeks 4, 12, and 22 STS will be conducted on the third spot within the predefined skin area. TEWL will be assessed in lesional skin and non-lesional skin before STS and after 5, 10, 15, and 20 STS. All skin tape strips should be collected and stored.

h In case STS assessment is to be conducted at an unscheduled visit or at a premature EoT visit the assessment should be conducted at that spot of the skin area, for which the period passed since the last STS assessment is the longest.

i OCT will be done before STS/TWL, and FTIR will be done before and during STS.

j Worst itch NRS, Sleep disturbance NRS and Skin pain NRS will be assessed daily from Day -7 to Day -1; daily from Day 1 to Week 4 and then 7 days prior to Week 6, Week 8, Week 12, Week 16, Week 22, and to Week 28 by e-diary.

k eDiary will be dispensed to the patients at screening (Visit 01) and will be collected on Week 28 (Visit 14). The PROs will be administered to patients via eDiary. Patients will bring the eDiary to the site at each visit, and it will be reviewed for any questionnaires omission and dispensed back to patients at each visit.

l Vital signs, including heart rate, systolic and diastolic blood pressure (mmHg), pulse rate (beats per minute), body temperature (°C), and respiratory rate will be measured.

m Limited to skin-related physical examination at baseline, EoT visit (Week 16) and EoS (Week 28).

n Saliva swab samples for DNA isolation should be collected on Day 1/Baseline (predose) for FLG gene sequencing analysis, but may be collected at any study visit.

o Please refer to Section 8.8.6 for details.

AE = Adverse Events; AD = Atopic Dermatitis; CDLQI = Children's Dermatology Life Quality Index; D= Day; EASI = Eczema Area and Severity Index; EoT = End of Treatment; EoS = End of Study; FLG = Filaggrin; IGA = Investigator Global Assessment; ISS = Individual Signs Score; NRS = Numerical Rating Scale; POEM = Patient Oriented Eczema Measure; SAE = Serious Adverse Events; SC = Subcutaneous; STS = Skin Tape Stripping; TEWL = Transepidermal Water Loss; UNSCH = unscheduled; V = Visit; W = Week; W = Women of Childbearing Potential.

1.4 SCHEDULE OF ACTIVITIES/HEALTHY VOLUNTEERS

Phase	Screening	Baseline								EoT	Follow-up period		Unscheduled visit	Premature EoT
Day	**D-28 to D-1**	**D 1**	**D8 ±2 days**	**D15 ±2 days**	**D22 ±2 days**	**D29 ±2 days**	**D43 ±2 days**	**D57 ±3 days**	**D85 ±3 days**	**D113 ±3 days**	**D155 ±3 days**	**EoS D197 ±3 days**		
Week		**W0**	**W1**	**W2**	**W3**	**W4**	**W6**	**W8**	**W12**	**W16**	**W22**	**W28**		
Visits	**V01**	**V02**	**V03**	**V04**	**V05**	**V06**	**V07**	**V08**	**V09**	**V10**	**V11**	**V12**		
Informed consent/assent form	X													
Inclusion/exclusion criteria	X	X												
Matching by age (±2 years) and gender	X													
Medical/surgical history/demographics	X													
Pregnancy test (urine), WOCBP only[a]	X	X	X	X	X	X	X	X	X	X	X	X		
Prior/concomitant medications/procedure	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Confirm emollient and washing compliance[b]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Skin barrier function test in predefined normal skin														
TEWL before STS[c, d]		X	X	X	X	X	X	X	X	X	X	X	X	X
TEWL and STS assessment[d]		X[e]		X[e]		X[e]		X[e]	X[e]	X[e]	X[e]	X[e]	X[f]	X[f]
Lipidomics, proteomics, FLG breakdown products and transcriptomics assessment from skin		X[e]		X[e]		X[e]		X[e]	X[e]	X[e]	X[e]	X[e]	X[f]	X[f]

Phase	Screening	Baseline								EoT	Follow-up period		Unscheduled visit	Premature EoT
Day	**D-28 to D-1**	**D 1**	**D8 ±2 days**	**D15 ±2 days**	**D22 ±2 days**	**D29 ±2 days**	**D43 ±2 days**	**D57 ±3 days**	**D85 ±3 days**	**D113 ±3 days**	**D155 ±3 days**	**EoS D197 ±3 days**		
Week		**W0**	**W1**	**W2**	**W3**	**W4**	**W6**	**W8**	**W12**	**W16**	**W22**	**W28**		
Visits	**V01**	**V02**	**V03**	**V04**	**V05**	**V06**	**V07**	**V08**	**V09**	**V10**	**V11**	**V12**		
tape strips[e]														
Standardized photographs of healthy skin area used for TEWL		X	X	X	X	X	X	X	X	X	X	X	(X)	X
Standardized full body photographs		X	X	X	X	X	X	X	X	X	X	X	(X)	X
Optical Coherence Tomography (OCT)[g]		X	X	X	X	X	X	X	X	X		X	(X)	X
Fourier-Transform Infrared (FTIR) Spectroscopy[g]		X						X		X		X	(X)	X
Safety														
AE/SAE collection	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height		X								X		X		X
Body weight	X	X								X		X		X
Vital signs[h]	X	X						X		X				X
Physical examination[i]	X	X[i]								X[i]		X[i]		X[i]
Saliva swab sample[j]		X												
Optional assessments														
Biomarker research samples (blood)[k]		X								X				

a For the purpose of this study, any female who has had her first menstrual period (menarche) and is sexually active will be considered to be of childbearing potential. Female patients who are not of childbearing potential at the start of the study but have the onset of menarche during the course of the study and are sexually active will also have to follow adequate birth control methods to continue participation in the study. These females must have a negative urine ß-HCG pregnancy test at screening and at each visit. Pregnancy will lead to definitive treatment discontinuation in all cases.

b Emollients should NOT be applied from Day -7 to the EoS (Week 28) to the targeted, predefined skin areas that will be used for TEWL assessment. Participants should not take showers or soak in a bathtub within 6 hours before TEWL assessment. Emollients for use during the study will be provided by the study site to study participants. Use of emollients should be documented in a diary provided by the study site.

c TEWL will be measured at each of the 3 closely adjacent spots within the predefined skin areas at all visits without STS and before STS at visits with STS.

d TEWL will be conducted before STS and then after 5, 10, 15, and 20 STS for skin barrier function assessment in normal skin and at the same location as for the lesional skin in the matching AD patient. All skin tape strips will be collected and stored.

e At Weeks 0, 8, 16, and 28, STS will be conducted on the first spot within the predefined skin area. At Week 2 STS will be conducted on the second spot within the predefined skin area. At Weeks 4, 12, and 22 STS will be conducted on the third spot within the predefined skin area. TEWL will be assessed in NORMAL skin before STS and 5, 10, 15, and 20 STS. All skin tape strips should be collected and stored.

f In case STS assessment is to be conducted at an unscheduled visit or at a premature end of treatment visit the assessment should be conducted at that spot of the skin area, for which the period passed since the last STS assessment is the longest.

g OCT will be done before STS/TWL, and FTIR will be done before and during STS.

h Vital signs, including heart rate, systolic and diastolic blood pressure (mmHg), pulse rate (beats per minute), body temperature (°C), and respiratory rate will be measured.

i Limited to skin-related physical examination at baseline, EoT visit (Week 16), and EoS (Week28).

j Saliva swab samples for DNA isolation should be collected on Day 1/Baseline (predose) for FLG gene sequencing analysis, but may be collected at any study visit.

k Please refer to Section 8.8.6 for details.

AE = Adverse Events; AD = Atopic Dermatitis; CDLQI = Children's Dermatology Life Quality Index; D = Day; EASI = Eczema Area and Severity Index; EoT = End of Treatment; EoS = End of Study; FLG = Filaggrin; IGA = Investigator Global Assessment; NRS = Numerical Rating Scale; POEM = Patient Oriented Eczema Measure; SAE = Serious Adverse Events; SC = Subcutaneous; STS = Skin Tape Stripping; TEWL = Transepidermal Water Loss; V = Visit; W = Week; W = Women of Childbearing Potential.

2 INTRODUCTION

Dupilumab is a human monoclonal antibody (5, 6) that blocks the shared receptor subunit for interleukin (IL) -4 and IL-13, thus inhibiting signaling of both IL-4 and IL-13, cytokines that are key drivers of Type 2 inflammatory diseases (7).

Dupilumab is recently approved in the US for SC administration Q2W/Q4W (depends on the body weight) for the treatment of pediatric patients ≥6 and <12 years of age with moderate-to-severe AD whose disease is not adequately controlled with topical prescription therapies or when those therapies are not advisable.

For adults and adolescents, dupilumab was already approved in the US for SC administration Q2W for the treatment of patients aged ≥12 years with moderate-to-severe AD inadequately controlled with topical prescription therapies or when those therapies are not advisable, for the treatment of adult AD patients not adequately controlled with existing therapies in Japan, and for use in adults and adolescents with moderate-to-severe AD who are candidates for systemic therapy in the European Union.

Recently Guttman et al (8) demonstrated that dupilumab-mediated inhibition of IL-4 and IL-13 signaling through IL-4 receptor α blockade significantly suppressed cellular and molecular cutaneous markers of Type 2 inflammation and systemic Type 2 inflammation mediators like thymus and activation-regulated chemokine (TARC) and immunoglobulin (Ig) E, and reversed AD-associated epidermal phenotype abnormalities; namely significantly reduced epidermal hyperplasia proliferation measured by Keratin-16 and Ki-67, significantly increased the expression of epidermal differentiation, lipid metabolism, and barrier junction genes measured by FLG, loricrin, claudins, and elongation of very long-chain fatty acids protein (ELOVL3), and significantly reduced lesional epidermal thickness (8, 9). This evidence supports the hypothesis that blockade of Type 2 inflammation, achieved with dupilumab treatment, can repair skin barrier function. It is well known that permeability of skin barrier is critical, and its impairment leads to downstream signals that aim to restore barrier homeostasis (10).

2.1 STUDY RATIONALE

Literature shows that patients with early-onset AD have a higher risk of developing other Type 2 comorbidities (the atopic march). It has been suggested that early optimal and successful treatment of AD may prevent or attenuate the development of other atopic conditions (1, 2). Leung et al used a non-invasive STS sampling technique can be reliably analyzing TEWL, TEWL AUC responses, lipidomics, FLG breakdown products, proteomics, and transcriptomics. Results from these analyses demonstrated that children with AD and food allergy exhibit elevated epidermal barrier defects even in non-lesional skin (3). The proposed study will evaluate the effect of dupilumab on the skin barrier in a pediatric population with early-onset moderate-to-severe AD using non-invasive methodologies such as TEWL, STS, OCT, and FTIR spectroscopy for skin barrier structure evaluation. The effect of dupilumab treatment on skin barrier function will be evaluated using TEWL in conjugation with STS, lipidomics, proteomics, and

transcriptomics using tape stripping samples. Skin barrier structure changes will be evaluated using OCT and FTIR spectroscopy in both predefined lesional and non-lesional areas in pediatric patients.

TEWL is one of the most broadly used, non-invasive methods for measuring the function of the skin barrier. The first measurement approaches were described in 1911, and today this parameter is regarded as the standard in a variety of dermatological and skin research contexts. TEWL is influenced by many environmental and individual factors, including age, sex, race, anatomical region, skin temperature, environmental conditions, season, smoking status, measurement technique, and many others (11). Therefore, including a "normal" TEWL from an age-, gender-, targeted lesion area- and study site-matched healthy volunteers cohort assessed at the same time in the same measurement conditions on the same anatomical region with reference thresholds of skin barrier function evaluation indicating pathological relevance is important (11, 12). For this reason, an age-, gender-, targeted lesion area- and study-site-matched healthy volunteer cohort is included as a reference that could help with interpreting study results.

2.2 BACKGROUND

Atopic dermatitis is a chronic systemic inflammatory skin disease with a prevalence of up to 25% in children and up to 7% in adults. A large proportion of patients experience sleep disturbance and impaired quality of life (QOL) (13). Additionally, AD places a heavy economic burden on patients and their family (14, 15). Atopic dermatitis is caused by the complex interplay between epithelial dysfunction and dysregulated/over-activated Type 2 immune response in the skin, with a special role for IL-4/IL-13-driven signaling in AD pathogenesis (16). This Type 2 hyperactivation blocks terminal differentiation of skin keratinocytes and formation of a mature stratum corneum that is mainly responsible for the skin barrier function (17).

Clinical manifestations and skin pathology in AD are driven by impaired skin barrier, and Type 2-skewed immune responses. Impaired skin barrier function is caused by changes in the expression of key structural cornified barrier proteins and skin barrier lipids (18). Filaggrin mutations are the most profound single-gene defects involved in AD (19). Filaggrin deficiency promotes inflammation and inflammatory cell infiltration in the skin. Changes in FLG expression alter skin acidification, which, in turn, supports activation of skin proteases that alter skin barrier homeostasis by interfering with lipid lamellae assembly and support the onset of Type 2 inflammatory responses. Type 2-skewed immune responses in AD favor epidermal barrier disruption by inhibiting the expression of *FLG* and other structural proteins in skin. Type 2 cytokines also inhibit production of skin barrier lipids in the skin. These changes are already present in non-lesional, normal-appearing AD skin, and are further aggravated in AD lesional skin (18).

Healthy epidermis has lipids that are mostly composed of ceramides (CER), free fatty acids, and cholesterol (20, 21), with very little presence of other lipids. A very specific, highly hydrophobic group of CER, called esterified omega-hydroxy sphingosine (EOS) CER, is present only in the skin. Fatty acids are also unique in skin CER, as they are unusually very long (up to C38) and hydrophobic; this also contributes to the overall requirement for a highly rigid and hydrophobic structure to provide an efficient barrier. Several groups of Investigators have already reported that lesional and non-lesional skin of AD patients has decreased proportion of EOS CER and other

CER with very long–chain fatty acids (C22-C30), and short-chain non-hydroxy fatty acids sphingosine CER (C16-C20). Ultralong-chain lipids, such as EOS CER, control water retention in the skin and prevent allergen penetration. Such changes in skin lipid composition result in aberrant lipid organization in the lipid layers and positively correlate with the degree of TEWL in AD skin (17). The greatest decrease in the ratio between EOS CER and non-hydroxy fatty acid sphingosine (NS) CER indicates the maximum loss of skin hydrophobicity due to a decline of highly hydrophobic EOS CER and the increase in short-chain NS CER. This suggests the entire skin surface of AD is at risk for allergen penetration (3).

Leung et al have pioneered novel methods to profile skin through STS analysis combined with lipidomics, proteomics and transcriptomics (3). Using an STS protein mass spectrometry analysis, AD skin exhibits significantly lower expression of skin barrier proteins (FLG2, corneodesmosin, DSG1, DSC1, and TGM3) and enzymes (arginase-1, caspase-14, and γ-glutamyl cyclotransferase) involved in generating NMF. The transcriptome sequencing together with lipidomics and proteomics has proven a powerful technique for agnostic examination of genomic, lipidomic, and proteomic expression profiles in non-lesional and lesional AD skin (14).

Optical coherence tomography (OCT) is a non-invasive, depth resolved imaging modality conceptually similar to ultrasound, but using near-infrared light (22); as it can image down to 1 to 2 mm depth, it is ideal for studying epithelial tissues including skin. It has become the established method of choice for imaging the retina and is emerging strongly into coronary vascular imaging and dermatology. Angiographic OCT is used to measure epidermal hypertrophy and dermal vascular changes as non-invasive biomarkers of sub-clinical AD.

Attenuated Total Reflectance (ATR) FTIR spectroscopy is a form of molecular spectroscopy useful for the analysis of surfaces, including skin. It has proved valuable for quantifying the lipid, water, and carboxylate content of the skin barrier and for analyzing lipid arrangement/structure (23, 24, 25, 26, 27).

Therefore, the proposed study will use these non-invasive innovative technologies to assess skin barrier function change over in pediatric patients with moderate-to-severe AD.

2.3 BENEFIT/RISK ASSESSMENT

Dupilumab has demonstrated a positive benefit-risk profile and is approved for treatment of moderate-to-severe AD in pediatric patients, ≥6 and <12 years of age in the US.

The safety profile in the pediatric population (≥6 and <12 years of age) was comparable to that previously observed in adult and adolescent patients with AD for whom the drug is already approved. Dupilumab dose in this study is consistent with the approved label.

The safety of dupilumab with concomitant topical corticosteroid (TCS) was assessed in a study of 367 subjects ≥6 and <12 years of age with severe AD. The safety profile of dupilumab + TCS in these subjects through Week 16 was similar to the safety profile from studies in adults and adolescents with AD.

The long-term safety of dupilumab + TCS was assessed in an open-label extension study of 368 subjects ≥6 and <12 years of age with AD. The safety profile of dupilumab + TCS in subjects followed through Week 52 was similar to the safety profile observed at Week 16. The long-term safety profile of dupilumab + TCS observed in pediatric patients was consistent with that seen in adults and adolescents with AD.

In the US, Dupilumab is approved for the treatment of patients ≥6 and <12 years of age with moderate-to-severe AD whose disease is not adequately controlled with topical prescription therapies or when those therapies are not advisable. Dupilumab can be used with or without TCS, whereby in the EU dupilumab is under review for the treatment of severe AD in children ≥6 and <12 years of age who are candidates for systemic therapy. Therefore, in this study a pediatric population with moderate-to-severe AD will be included in the US and severe AD patients in the EU.

More detailed information about the known and expected benefits and risks and reasonably expected AEs of dupilumab may be found in the Investigator's Brochure (28).

2.3.1 Risk assessment

Risks Associated with Skin Barrier TEWL Measurement

There are no known risks associated with this non-invasive skin measurement.

Risks Associated with Skin Tape Strip Collection

Risks associated with STS, theoretically, include a rare possibility of an allergic reaction to the tape or a skin infection. Since the tape is removed immediately after application, the risk of an allergic reaction is low.

In previous and ongoing studies involving tape stripping, it has been noted that a mild erythema may develop immediately after a series of tape strips on 1 localized area of skin, presumably due to the mild mechanical disturbance. The erythema is expected to resolve within 12 hours without sequelae. Skin tape stripping should be ceased, if the skin is broken (ie, bleeding), which is very rare based on investigator's experience.

The risk of skin infection is very low since only superficial skin layers are removed. A bandage will be applied to the area of tape stripping to reduce the small likelihood of an infection.

Possible bleeding and/or bruising may also occur at the area. Participants with a history of moderate-to-severe and serious life-threatening reaction to tape or adhesives known to be used will be excluded from participating, per study exclusion criteria.

Risks Associated with OCT and FTIR

There are no known risks associated with this non-invasive OCT and FTIR spectroscopy assessments.

Risks Associated with Health Questionnaires

There is a possibility that participants or their parents may find questions too personal. Participants including their parents may refuse to answer any questions that make them feel uncomfortable.

Risks Associated with Stopping the Use of Protocol-Prohibited Medications/Procedures

Risks associated with stopping the use of protocol-prohibited medications/procedures may include worsening of the condition being treated and will be reported as such. In an effort to minimize these risks, participants with very severe AD who may have difficulty tolerating periods without medication/procedure use will be excluded from participating, per study exclusion criteria.

Risks Associated with Blood Collection

Risks associated with drawing blood (if any) include possible pain when the needle is inserted, as well as bleeding, bruising and/or infection at the puncture site. Some people may experience lightheadedness, nausea, or fainting. A topical anesthetic (eg, topical lidocaine/prilocaine cream) may be placed on the skin before the blood draw to reduce the pain of the needle insertion. Side effects from this cream (mainly skin rash) may occur. Institution-specific guidelines for blood collection (amount and frequency based on age) will be followed.

2.3.2 Benefit assessment

There may or may not be any direct benefits for the participants who elect to enroll in this study. Participants with AD disease in the AD cohort will receive dupilumab treatment for a total of 16 weeks, and participants in the health volunteer's cohort will receive no treatment during the study.

One potential benefit for participants in the AD cohort is that their AD may be improved and itch may be reduced while on dupilumab treatment; however, there is no guarantee that the product will help the participant's condition. The participant's skin condition may even get worse by withholding his/her previous/regular AD treatment.

Healthy volunteers will receive the same test and procedure for skin barrier function assessment that the patient group receives. Investigators learn about the disease process by comparing the patient group to the clinical research volunteers. Participants in the healthy volunteers' cohort will not benefit directly from participation in this study, other than the nominal reimbursements provided for completing study procedures and visits. However, the results obtained from healthy volunteers in comparison with AD patients will allow the Sponsor to better understand the immune, epidermal, and barrier defects observed in AD patients. The potential benefit to society is significant if it improves our understanding of what affects AD disease severity and skin barrier function. Therefore, the expectation is that the results will benefit others in the future.

Although the results of this study may be of commercial value, it will be explained to participants that they will not have ownership of the study results, and will not be benefited financially from participation in this study, other than the nominal reimbursements provided for completing study procedures and visits.

Benefit/risk related to Coronavirus Disease 2019 (COVID-19)

Dupilumab has shown clinical benefit in several Type-2 driven immunological disorders, such as AD, asthma, chronic rhinosinusitis with nasal polyposis. In asthma and AD clinical benefit has also been established in certain pediatric patients (for asthma in adolescents and for AD in 6 to 18-year-old) and a similar benefit-risk profile to adults has been observed.

To date, more than 8000 subjects have been treated with dupilumab during the clinical development program in several indications, of which atopic dermatitis, asthma and chronic rhinosinusitis with nasal polyposis are licensed in some countries.

Currently, we do not have sufficient data in patients with COVID-19 who are being treated with dupilumab. Thus, the safety and efficacy of dupilumab in COVID-19 patients is unknown. During the course of the clinical trial program, respiratory infections including viral infections were monitored and these events are not listed as adverse drug reactions with dupilumab

The target population of LPS16764 is pediatric patients with moderate-to-severe AD and healthy volunteers as reference. AD, the most common form of eczema, is a chronic inflammatory disease that often appears as a rash on the skin. Moderate-to-severe AD is characterized by rashes that can potentially cover much of the body and can include intense, persistent itching, skin lesions and skin dryness, cracking, redness or darkness, crusting and oozing. Itch is one of the most burdensome symptoms for patients and can be debilitating. Skin barrier dysfunction is a major pathogenic factor in AD. Literature shows that children with AD and food allergy exhibit elevated epidermal barrier defects even in non-lesional skin.

Based on the aforementioned potential benefits to patients participating in LPS16764, the Sponsor assessment is that the benefit-risk remains favorable for patient to participate in this trial. The proposed study will evaluate the effect of dupilumab on the skin barrier in a pediatric population with early-onset moderate-to-severe AD. The efficacy and safety of dupilumab were confirmed based on data that includes a pivotal Phase 3 trial in which dupilumab was used in combination with TCS and compared to TCS alone in children with severe AD. In the trial, children treated with dupilumab and TCS experienced significant improvements in overall disease severity, skin clearance and quality of life. Therefore, the operation of LPS16764 will allow enrolled AD patients the opportunity to receive a therapy which may provide benefit in improving their skin barrier function, overall disease severity, and quality of life. Participants in the healthy volunteers' cohort will not benefit directly from participation in this study, other than the nominal reimbursements provided for completing study procedures and visits. However, the results obtained from healthy volunteers in comparison with AD patients will allow the Sponsor to better understand the immune, epidermal, and barrier defects observed in AD patients. The Sponsor also recognizes that the "Coronavirus Disease 2019" (COVID-19) pandemic may have an impact on the conduct of clinical trials. The Sponsor will monitor the situation closely and ensure the integrity of the trial conduct and data (see Section 8).

2.3.3 Overall benefit: risk conclusion

Taking into account the measures taken to minimize the risk to participants in this study, the potential risks identified in association with dupilumab, TEWL and STS are justified by the anticipated benefits that may be afforded to participants with AD. For healthy volunteers no benefit is expected, but there is also no relevant risk identified for those participants.

3 OBJECTIVES AND ENDPOINTS

Table 1 - Objectives and endpoints

Objectives	Estimands/Endpoints
Primary	
• Evaluate changes in skin barrier function with transepidermal water loss (TEWL) assessed after skin tape stripping (STS) in predefined lesional skin in pediatric patients with moderate-to-severe atopic dermatitis (AD) treated with dupilumab.	• Percent change from baseline in TEWL after 5 STS assessed on lesional skin at Week 16 in AD patients.
Secondary	
• Evaluate changes in skin barrier function with TEWL assessed after STS in predefined lesional and non-lesional skin in pediatric patients with moderate-to-severe AD treated with dupilumab in reference to normal skin of healthy volunteers.	• Change (percent and absolute) from baseline in TEWL after 20 STS assessed on lesional skin at Week 16 in AD patients. • Change (percent and absolute) from baseline in TEWL after 20 STS assessed on non-lesional skin at Week 16 in AD patients. • Change (percent and absolute) from baseline in TEWL after 20 STS assessed on normal skin at Week 16 in healthy volunteers. • Change (percent and absolute) from baseline in TEWL after 15 STS assessed on lesional skin at Week 16 in AD patients • Change (percent and absolute) from baseline in TEWL after 15 STS assessed on non-lesional skin at Week 16 in AD patients. • Change (percent and absolute) from baseline in TEWL after 15 STS assessed on normal skin at Week 16 in healthy volunteers. • Change (percent and absolute) from baseline in TEWL after 10 STS assessed on lesional skin at Week 16 in AD patients. • Change (percent and absolute) from baseline in TEWL after 10 STS assessed on non-lesional skin at Week 16 in AD patients. • Change (percent and absolute) from baseline in TEWL after 10 STS assessed on normal skin at Week 16 in healthy volunteers. • Change (percent and absolute) from baseline in TEWL after 5 STS assessed on non-lesional skin at Week 16 in AD patients. • Change (percent and absolute) from baseline in TEWL after 5 STS assessed on normal skin at Week 16 in healthy volunteers.

Objectives	Estimands/Endpoints
• Evaluate time course of change in skin barrier function with TEWL assessed before and after STS in predefined lesional and non-lesional skin in pediatric patients with moderate-to-severe AD during dupilumab treatment phase and follow-up period in reference to normal skin of healthy volunteers.	• Change (percent and absolute) in TEWL before STS on lesional skin in AD patients over time. • Change (percent and absolute) in TEWL before STS on non-lesional skin in AD patients over time. • Change (percent and absolute) in TEWL before STS on normal skin in healthy volunteers over time. • Change (percent and absolute) in TEWL area under the curve (TEWL AUC: a composite measure before and after 5, 10, 15, and 20 STS) for skin barrier function in lesional skin in AD patients over time. • Change (percent and absolute) in TEWL AUC (a composite measure before and after 5, 10, 15, and 20 STS) for skin barrier function in non-lesional skin in AD patients over time. • Change (percent and absolute) in TEWL AUC (a composite measure before and after 5, 10, 15, and 20 STS) for skin barrier function in normal skin in healthy volunteers over time. • Change (percent and absolute) in TEWL after STS assessed on lesional skin in AD patients over time. • Change (percent and absolute) in TEWL after STS assessed on non-lesional skin in AD patients over time. • Change (percent and absolute) in TEWL after STS assessed on normal skin in healthy volunteers over time.
Tertiary/exploratory	
• Evaluate dupilumab treatment effect on skin lipidomics using STS samples in both predefined lesional and non-lesional skin in pediatric patients with moderate-to-severe AD during dupilumab treatment phase and follow-up period in reference to normal skin of healthy volunteers.	• Changes (percent and absolute) in lipidomics parameters in lesional and non-lesional skin including the ratio of highly hydrophobic omega-esterified fatty acid sphingosine ceramides (EOS CER) and non-hydroxy fatty acid sphingosine ceramides (NS CER), and filaggrin (FLG) breakdown products of urocanic acid (UCA) and pyroglutamic acid (PCA) concentrations over time. • Global characterization of protein-bound CER over time.
• Evaluate dupilumab treatment effect on skin proteomics using STS samples in both predefined lesional and non-lesional skin in pediatric patients with moderate-to-severe AD during dupilumab treatment phase and follow-up period in reference to normal skin of healthy volunteers.	• Changes (percent and absolute) in the expression of proteins associated with skin barrier function including keratin intermediate filaments, proteins associated with inflammatory response, and glycolysis and oxidative stress response proteins in STS protein extracts over time.
• Evaluate dupilumab treatment effect on epidermal hypertrophy and structure of skin barrier changes measured by Optical Coherence Tomography (OCT) and Fourier-Transform Infrared (FTIR) spectroscopy in both predefined lesional and non-lesional skin in pediatric patients with moderate-to-severe AD during dupilumab treatment phase and follow-up period in reference to normal skin of healthy volunteers.	• Change (percent and absolute) in epidermal hypertrophy parameters including epidermal thickness (µm), superficial plexus depth (µm), blood vessel diameter (µm) and density (segments/mm^2) measured by OCT over time. • Change (percent and absolute) in stratum corneum lipid structure measured by FTIR spectroscopy in conjunction with tape-stripping over time.

Objectives	Estimands/Endpoints
• Evaluate dupilumab treatment effect on skin transcriptome using STS samples in both predefined lesional and non-lesional skin in pediatric patients with moderate-to-severe AD during dupilumab treatment phase and follow-up period in reference to normal skin of healthy volunteers.	• Changes in expression of genes associated with epidermal differentiation, barrier and lipid metabolism, and Type 2 inflammation over time.
• Explore the association of skin barrier function measured by TEWL with disease impact assessed by standard AD severity assessments (Eczema Area and Severity Index [EASI], and Individual Signs Score [ISS]), patient reported outcomes (PRO) (Patient Oriented Eczema Measure [POEM], Children Dermatology Life Questionnaire Index [CDLQI], worst itch Numerical Rating Scale [NRS], sleep disturbance NRS, skin pain NRS), standardized photos, the biomarker profiles of lipidomics, proteomics, and transcriptomics assessed in the STS sample, and the skin barrier hypertrophy and structure profiles assessed by OCT and FTIR.	• Change (percent and absolute) in EASI over time. • Change (percent and absolute) in ISS for target lesion over time. • Change (percent and absolute) in POEM over time. • Change (percent and absolute) in CDLQI over time. • Change (percent and absolute) in assessment of worst itch NRS over time. • Change (percent and absolute) in sleep disturbance NRS over time. • Change (percent and absolute) in skin pain NRS over time. • Change (percent and absolute) in photograph outputs (eg, severity score) obtained from skin imaging over time. • Correlation between baseline values of TEWL before and after STS, and TEWL AUC in lesional and non-lesional skin of AD patients with the following baseline measures: - EASI - Targeted lesion ISS - PRO measures (POEM, CDLQI, worst itch NRS, sleep disturbance NRS, and skin pain NRS) - Lipidomics in STS (ratio of EOS CER to NS CER) - Filaggrin breakdown products of UCA and PCA concentrations in STS - Image-derived severity score in targeted lesional skin - Key components of skin proteomics in STS (expression of proteins associated with skin barrier function) - Quantified epidermal hypertrophy and structure changes in OCT and FTIR - Key components of gene expression from transcriptomics • Correlation between percent change from baseline in TEWL before and after STS, and TEWL AUC in lesional and non-lesional skin of AD patients at Week 8, Week 16, and Week 28 with corresponding change from baseline in the following measures: - EASI - Targeted lesion ISS - PRO measures (POEM, CDLQI, worst itch NRS, sleep disturbance NRS, and skin pain NRS) - Lipidomics in STS (ratio of EOS CER to NS CER) - Filaggrin breakdown products of UCA and PCA concentrations in STS

Objectives	Estimands/Endpoints
	- Image-derived severity score in targeted lesional skin - Key components of skin proteomics in STS (expression of proteins associated with skin barrier function) - Quantified epidermal hypertrophy and structure changes in OCT and FTIR - Key components of gene expression from transcriptomics

3.1 APPROPRIATENESS OF MEASUREMENTS

The primary endpoint is percent change from baseline in TEWL after 5 tape strips assessed on lesional skin at Week 16 in AD patients, and key secondary endpoints include TEWL changes over time measured by TEWL and TEWL AUC after various numbers of STS. The stratum corneum provides skin barrier protection and controls transcutaneous water loss. TEWL measurement, which quantifies water diffusion across the stratum corneum, is commonly used for the physiologic assessment of skin barrier function (29). In addition to basal TEWL to assess the undisturbed permeability of the skin barrier, TEWL measurements have also been conducted together with controlled skin barrier perturbation using STS to measure skin barrier integrity (10). Healthy skin is not highly sensitive to STS, and can withstand mild perturbation while disrupted skin and skin with low structural integrity exhibit greater changes in TEWL after 5, 10, 15, and 20 skin tape strips. TWEL after 5 tape strips in the lesional skin causes a mild perturbation of the skin barrier, is tolerable for patients, and therefore chosen as the primary endpoint. The AUC for TEWL measurements (TEWL AUC) done over a defined number of STS is used to reflect the overall integrity of the stratum corneum. Skin barrier dysfunction and increased TEWL are major pathologic features of AD (18, 30, 31). TEWL has been shown to correlate with AD severity (32, 33, 34). Therefore, this study has selected TEWL in conjunction with STS, and TEWL AUC as the key primary and secondary endpoints.

4 STUDY DESIGN

4.1 OVERALL DESIGN

This is a Phase 4 open-label, exploratory study in 2 sites evaluating the effect of dupilumab on skin barrier function in pediatric patients aged between ≥6 and <12 years old with moderate-to-severe AD with a healthy volunteer cohort as a reference comparator. Patients with moderate-to-severe AD will be included in the US site and patients with severe AD will be included in the EU site (UK). More study sites could be initiated if recruitment at 1 or both of above-mentioned study sites is insufficient.

The maximum study duration per participant will be 32 weeks. The study will comprise of:

- Screening period 1 (Day -28 to Day -1): Participants will be evaluated according to inclusion and exclusion criteria.
- Baseline visit (Week 0, Day 1): Participants who remain eligible will be enrolled.
- A 16-week treatment phase for AD patients and a 16-week observation period for healthy volunteers.
- A 12-week follow-up period.

The overall study schema is provided in Section 1.2. TEWL and STS procedures are detailed in Section 1.1 and Section 8.1.

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

The study design includes 2 parallel study cohorts: the AD patients cohort with open-label dupilumab treatment for 16-weeks, and follow-up period for 12 weeks, and the healthy volunteers cohort without any treatment for the whole study duration, which serves as a reference comparator group for the skin barrier assessment parameters. The study applies comprehensive skin barrier function assessments including TEWL in conjunction with STS, lipidomics, proteomics, and transcriptomics from STS samples, OCT and FTIR, standardized full body photographs, photographs from the targeted lesional and non-lesional areas, clinical disease severity, and patient reported outcomes (PRO).

TEWL is commonly used for physiologic assessment of skin barrier function. In addition to basal TEWL to assess the undisturbed permeability of skin barrier, recently TEWL measurements have also been combined with controlled skin barrier perturbation using STS to measure skin barrier function and integrity (10). So far, most studies investigating epidermal biomarkers have used skin biopsies for studies on immunological parameters as well as skin barrier function. The STS technique uses a standardized tape for removing the epidermis layer by layer. Advantages of this technique are it is non-invasive, causes no pain in the patients and leave no scars. With this technique it is possible to follow reactions within the same skin area over time, and to study precisely in which depth of the epidermis the different substances are located (35, 36). Through

these assessments, the study will explore the interplay between skin barrier function kinetics, and clinical disease severity, and therapeutic response.

TEWL can be influenced by many environmental and individual factors, including age, sex, race, anatomical region, skin temperature, environmental conditions, season, smoking status, measurement technique and many others. Therefore, including a "normal" TEWL from an age-, gender-, targeted lesion area-, and study-site-matched healthy volunteers' cohort assessed at the same time in the same measurement conditions on the same anatomical region with reference thresholds of skin barrier function evaluation indicating pathological relevance is important in interpreting study assessment results (11, 12).

4.2.1 Participant input into design

Not applicable.

4.3 JUSTIFICATION FOR DOSE

The dose (2 dose regimens based on body weight) selected in this study is the dose approved for the treatment of moderate-to-severe pediatric (≥6 and <12 years of age) AD patients for dupilumab in the US and is under review for the treatment of severe pediatric (≥6 and <12 years of age) AD patients for dupilumab in the EU.

4.4 END OF STUDY DEFINITION

A participant is considered to have completed the study if he/she has completed all phases of the study including EoS visit as shown in the Schedule of Activities (SoA) in Section 1.3.

The EoS is defined as the date of the last visit of the last participant in the study as shown in the SoA for the last participant in the study.

5 STUDY POPULATION

Prospective approval of protocol deviations to recruitment and enrollment criteria, also known as protocol waivers or exemptions, is not permitted.

The purpose of this study is to understand the effect of dupilumab treatment on well-described pathophysiological features of AD (eg, barrier, epidermal activation, epidermal lipids, etc). This study will be limited to male and female participants aged ≥6 to <12 years, with moderate-to-severe AD.

5.1 INCLUSION CRITERIA

Participants are eligible to be included in the study only if all of the following criteria apply:

Age

I 01. Participant must be between ≥6 to <12 years of age (inclusive), at the time of signing the informed consent.

Type of participant and disease characteristics

Atopic dermatitis patients:

I 02. Male or female pediatric patients.

I 03. Patients with AD diagnosis according to Hanifin and Rajka criteria at least 1 year before screening.

I 04. Investigator Global Assessment score of ≥3 (for US patients) or IGA ≥4 (for EU patients) at screening (on the 0 to 4 scale) depending on approved label indication in the country.

I 05. Patients with moderate-to-severe AD are eligible to be treated with dupilumab according to product label.

I 06. Patients with AD must have active lesions on the upper limbs or lower limbs (including trunk, if needed), with severity for lesion erythema or edema/papulation ≥2 at screening on the 0 to 3 scale of the ISS.

I 07. Participants should have a non-lesional (normal looking) skin area 4 cm from the edge of the lesional area. If unable to identify non-lesional skin 4 cm from the lesional area, it is acceptable to identify normal looking skin as close to the lesion as possible.

I 08. Willing to refrain from applying any topical medications on the target assessment areas (including lesional and non-lesional) throughout the study until EoS unless necessary to alleviate intolerable symptoms.

I 09. Willing to refrain from showers or soak in a bathtub with soaps and body washes within 6 hours before TEWL assessments.

I 10. Willing to NOT apply any moisturizers to the areas of the skin that are targeted assessment areas (lesional and non-lesional) during the entire study from Day -7 to Week 28 (EoS).

I 11. Willing and able to comply with all clinic visits and study-related procedures.

Healthy volunteers:

I 12. Age and gender matched (match on age ±2 years) to a selected AD patient by study site.

I 13. No current dermatologic or systemic condition that could interfere with the assessments.

Weight

I 14. 15 kg ≤ body weight <60 kg.

Sex

I 15. Male or female

Contraceptive use by female of childbearing potential and sexually active should be consistent with local regulations regarding the methods of contraception for those participating in clinical studies.

Informed consent

I 16. Capable of understanding and giving signed informed consent/assent as will be described in the protocol, which includes compliance with the requirements and restrictions listed in the informed consent form (ICF) and in this protocol. Participant's legally authorized representative must provide signed informed consent. Participant must also provide separate informed assent to enroll in the study, and sign and date either a separate informed assent form (IAF) or the ICF signed by the parent/legally authorized representative (as appropriate based on local regulations and requirements).

5.2 EXCLUSION CRITERIA

Participants are excluded from the study if any of the following criteria apply:

Medical conditions

E 01. Previous treatment with dupilumab within 6 months prior to screening.

E 02. Skin conditions other than AD that can confound assessments in the area of TEWL assessments in the opinion of the Investigator (ie, skin atrophy, ichthyosis, tinea infection, contact dermatitis).

E 03. Cracked, crusted, oozing, or bleeding AD lesions in the designated lesional assessment area leaving insufficient skin that is adequate for TEWL assessments.

E 04. Hypersensitivity to the active substance or to any of the excipients of dupilumab.

E 05. Ocular disorder that in the opinion of the Investigator could adversely affect the individual's risk for study participation. Examples include, -but are not limited to-individuals with a history of active cases of herpes keratitis; Sjogren's syndrome, keratoconjunctivitis sicca, or individuals with ocular conditions that require the use of ocular corticosteroids or cyclosporine.

E 06. Systemic AD treatment, cyclosporine A (CsA), systemic corticosteroids, azathioprine (AZA), methotrexate (MTX), mycophenolate mofetil (MMF), or Janus kinase (JAK) inhibitors or phototherapy within 4 weeks of baseline.

E 07. Topical AD treatment within 1 week of baseline. Face and neck may be treated with topical steroids during the washout period if approved by the Investigator.

E 08. Severe concomitant illness(es) that, in the Investigator's judgment, would adversely affect the patient's participation in the study. Examples include, but are not limited to patients with endoparasitic infections, short life expectancy, uncontrolled diabetes (hemoglobin A1c ≥9%), cardiovascular conditions (eg, Class III or IV cardiac failure according to the New York Heart Association classification), severe renal conditions (eg, patients on dialysis), hepato-biliary conditions (eg, Child-Pugh class B or C), neurological conditions (eg, demyelinating diseases), active major autoimmune diseases (eg, lupus, inflammatory bowel disease, etc), other severe endocrinological, gastrointestinal, metabolic, pulmonary, psychiatric (known suicidal intentions), or lymphatic diseases. The specific justification for patients excluded under this criterion will be noted in study documents (chart notes, electronic case report form [eCRF], screening logs, etc).

E 09. History of hypersensitivity reaction to tape or adhesives used in desquamme discs.

Prior/concomitant therapy

E 10. Treatment with an investigational medication within 16 weeks or within 5 half-lives (if known) prior to Day 1, whichever is longer.

E 11. Patients who received a live vaccine within 4 weeks of baseline.

Prior/concurrent clinical study experience

E 12. Current participation in another investigational or interventional clinical study.

Diagnostic assessments

Not applicable.

Other exclusions

E 13. Individuals (including parents or legal guardians) accommodated in an institution because of regulatory or legal order; prisoners or participants who are legally institutionalized.

E 14. Participants (including parents or legal guardians) are dependent or relative of the Sponsor or Investigator (in conjunction with Section 1.61 of the International Council for Harmonisation (ICH)-Good Clinical Practice [GCP] Ordinance E6).

E 15. Individuals (including parents or legal guardians) directly involved in the conduct of the study, or immediate family members of such individuals.

E 16. Any specific situation during study implementation/course that may raise ethical considerations.

E 17. Planned or anticipated major surgical procedure during the patient's participation in this study.

E 18. Healthy volunteers with a personal history of an atopic condition.

E 19. Healthy volunteers with use of any topical treatment anywhere except Cetaphil®, Vanicream™, E45 cream or the preferred moisturizer not containing additives on non-targeted skin areas.

E 20. Female of childbearing potential* and sexually active, who is unwilling to use highly effective methods of contraception prior to the initial dose, during the study and for at least 12 weeks after the last dose of study drug. Highly effective contraceptive measures include stable use of combined (estrogen and progestogen containing) hormonal contraception (oral, vaginal, transdermal) or progestogen-only hormonal contraception (oral, injectable, implantable) associated with inhibition of ovulation initiated 2 or more menstrual cycles prior to screening; intrauterine device (IUD); intrauterine hormone-releasing system (IUS); bilateral tubal ligation; vasectomized partner; and or sexual abstinence**.

* For the purpose of this study, any female who has had her first menstrual period (menarche) and is sexually active will be considered to be of childbearing potential. Female patients who are not of childbearing potential at the start of the study but have the onset of menarche during the course of the study and are sexually active will also have to follow adequate birth control methods to continue participation in the study.

** Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatments. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the subject.

NOTE: Periodic abstinence (eg, calendar, ovulation, symptothermal, post-ovulation methods), declaration of abstinence for the duration of a study, and withdrawal are not acceptable methods of contraception.

5.3 LIFESTYLE CONSIDERATIONS

- Study participants should not take a shower or a bath within 6 hours before assessment of TEWL.
- Participants should apply Cetaphil, Vanicream, E45 cream or preferred same emollients not containing additives except at the predefined skin assessment areas up to 2 times daily from Day -7 to Week 16 (EoT).
- Participants should not apply any emollients on or within 5 cm of the predefined skin assessment areas during the entire study period from Day -7.
- Emollients for use during the study will be provided by the study site to study participants.
- Use of emollients should be documented in a diary provided by the study site.

5.3.1 Meals and dietary restrictions

Patients with a known food allergy should not consume any foods from an outside home provider on the day of the dupilumab injection.

5.3.2 Caffeine, alcohol, and tobacco

Not applicable.

5.3.3 Activity

Not applicable.

5.4 SCREEN FAILURES

Screen failures are defined as participants who consent to participate in the clinical study but are subsequently found to be not eligible to enroll in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure reasons, eligibility criteria, and any serious AE (SAE).

Note: "Enrolled" means a participant's, or their legally acceptable representative's, agreement to participate in a clinical study following completion of the informed consent process. Potential participants who are screened for the purpose of determining eligibility for the study, but do not participate in the study, are not considered enrolled, unless otherwise specified by the protocol.

6 STUDY INTERVENTION

Study intervention is defined as any investigational intervention(s), marketed product(s), placebo, or medical device(s) intended to be administered to a study participant according to the study protocol.

6.1 STUDY INTERVENTION(S) ADMINISTERED

Study participants will be assigned to study intervention as described below:

- Up to 24 pediatric patients with moderate-to-severe AD will receive dupilumab.
- Dose regimen 1: children with baseline 15 kg ≤ body weight <30 kg will receive an SC loading dose of dupilumab 600 mg (2 injections of dupilumab 300 mg) on Day 1 (Week 0), followed by every 4-week SC dosing of dupilumab 300 mg from Week 4 to Week 12.
- Dose regimen 2: children with baseline 30 kg ≤ body weight <60 kg will receive an SC loading dose of dupilumab 400 mg (2 injections of dupilumab 200 mg) on Day 1 (Week 0), followed by bi-weekly SC dosing of dupilumab 200 mg from Week 2 to Week 14.

  Note: Treatment regimen will be applied based on the approved label for the study population.
- One kit number list by dosage strength is generated centrally by Sanofi and IMPs are packaged in accordance with these lists. The treatment will be given to the pediatric patient (≥6 and <12 years of age) depending on their body weight.

Study intervention will be dispensed at the study visits summarized in SoA (see Section 1.3). Returned study intervention should not be re-dispensed to the participants.

Table 2 - Overview of study interventions administered

ARM name	Dupilumab 200	Dupilumab 300
Intervention name	Dupilumab 200 mg	Dupilumab 300 mg
Type	Biological/Vaccine	Biological/Vaccine
Dose formulation	A 175 mg/mL dupilumab solution in a pre-filled syringe to deliver 200 mg in 1.14 mL injection	A 150 mg/mL dupilumab solution in a pre-filled syringe to deliver 300 mg in 2 mL injection
Unit dose strength(s)	200 mg	300 mg
Dosage level(s)	200 mg every 14 ±2 or 3 days after an initial loading dose of 400 mg	300 mg every 28 ±2 or 3 days after an initial loading dose of 600 mg
Route of administration	Subcutaneous[a]	Subcutaneous[a]
Use	Experimental	Experimental

ARM name	Dupilumab 200	Dupilumab 300
IMP	IMP	IMP
Storage	2°C to 8°C	2°C to 8°C
Packaging and labeling	One glass pre-filled syringe packed in a patient kit box. Both pre-filled syringe and the box will be labeled as required per country requirement.	One glass pre-filled syringe packed in a patient kit box. Both pre-filled syringe and the box will be labeled as required per country requirement.
Current/Former name(s) or alias(es)	Dupixent	Dupixent

a Subcutaneous injection sites should alternate between the upper thighs, 4 quadrants of the abdomen, or the upper arms, so that the same site is not injected twice during consecutive administrations. Injection in the upper arms can only be done by the study site staff and/or by a trained person or health care professional but not the participant themselves.

IMP: investigational medicinal product.

Investigational medicinal product(s)

When the participant has a study visit, the IMP will be administered as per the clinical procedures and blood collection (if any) should be performed.

The IMP will be administered on-site by study site staff at baseline (Day 1, Week 0), Weeks 4, 8, and 12 for AD patients who receives dose regimen 1 and at baseline (Day 1, Week 0), Weeks 2, 4, 6, 8, 10, 12, and 14 for AD patients who receives dose regimen 2, after the TEWL assessment has been completed. No IMPs will be administered to healthy volunteers.

Between the protocol-scheduled on-site visits, interim visits may be required for IMP dispensing. As an alternative to these visits, dupilumab may be supplied from the site to the participant (patient, parent, caregiver or legal guardian) via a Sponsor-approved courier company where allowed by local regulations and agreed by the parent or legal guardian. In this case the injections will be administered by a qualified site personnel, a home nurse, a trained caregiver or by a trained parent/legal guardian.

6.1.1 Devices

No devices for administration of study drug will be used in this study.

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 Storage and handling

1. The Investigator or designee must confirm that 2°C to 8°C temperature conditions have been maintained during transit for all study intervention received and any discrepancies are reported and resolved before use of the study intervention.
2. Only authorized site staff may supply or administer study intervention. At study site, all study intervention must be stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labeled storage conditions with access limited to the Investigator and authorized site staff.

3. The Investigator, institution, or the head of the medical institution (where applicable) is responsible for study intervention accountability, reconciliation, and record maintenance (ie, receipt, reconciliation, and final disposition records).

6.2.2 Responsibilities

Any quality issue noticed with the receipt or use of an IMP (deficiency in condition, appearance, pertaining documentation, labeling, expiration date, etc) must be promptly notified to the Sponsor. Some deficiencies may be recorded through a complaint procedure (see Section 8.3.9).

A potential defect in the quality of IMP may be subject to initiation of a recall procedure by the Sponsor. In this case, the Investigator will be responsible for promptly addressing any request made by the Sponsor, in order to recall the IMP and eliminate potential hazards.

Under no circumstances will the Investigator supply IMP to a third party, except for Direct to patient (DTP) shipment, for which a courier company has been approved by the Sponsor, allow the IMP to be used other than as directed by this clinical study protocol, or dispose of IMP in any other manner.

6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

The study is open-label study without a treatment control group in patients. To control bias, the following measures are taken:

- Age-, gender-, targeted lesion area-, and study site-matched healthy volunteers will serve as a reference comparator cohort for interpreting study results.
- Efficacy data (TEWL and OCT) will be measured by the same device model.
- Biomarker data (lipidomics, proteomics, and transcriptomics) will be measured by an experienced, central laboratory.
- Study is limited in 2 study centers which has experience in conducting studies.
- One central laboratory for lipidomics, proteomics, and transcriptomics analysis to reduce skin barrier assessment variabilities.
- One central center for image and spectra processing and analysis.
- Standardized study assessment procedures, including TEWL, STS, OCT, FTIR in skin barrier function assessments.
- Standardized photography for targeted lesional and non-lesional areas, and clinical disease severity and PRO evaluations.
- Reason for screen failures (if any) will be documented.

6.4 STUDY INTERVENTION COMPLIANCE

Investigator or his/her delegate must ensure that IMP will be administered to each participant according to the labeling instructions.

IMP accountability:

- The Investigator counts the number of remaining unused kits/pre-filled syringes, and fills in the IMP accountability and inventory forms.
- The Investigator or his/her delegate records the dosing information on the appropriate page(s) of the eCRF.
- The monitor in charge of the study then checks the eCRF data by comparing them with the IMP and source documents.

Participants will receive the study intervention (see Section 1.3) directly from the Investigator or designee, under medical supervision. The date and time of each dose administered in the clinic will be recorded in the source documents and eCRF. The dose of study intervention and study participant identification will be confirmed at the time of dosing by a member of the study site staff other than the person administering the study intervention.

6.5 CONCOMITANT THERAPY

Any medication or vaccine (including over-the-counter or prescription medicines, vitamins, and/or herbal supplements, [live vaccine is prohibited]) that the participant is receiving at the time of enrollment or receives during the study must be recorded along with:

- Reason for use.
- Dates of administration including start and end dates.
- Dosage information including dose and frequency.

Participants must abstain from taking prohibited prescription drugs within 7 days (or 14 days if the drug is a potential enzyme inducer) or 5 half-lives (whichever is longer) before the start of study intervention until completion of the follow-up visit, unless, in the opinion of the Investigator and Sponsor, the medication will not interfere with the study.

Paracetamol/acetaminophen, at doses of ≤2 g/day, is permitted for use any time during the study. Other concomitant medication may be considered on a case-by-case basis by the Investigator in consultation with the Medical Monitor, if required.

Cetaphil, Vanicream, E45 cream, or the preferred moisturizer not containing additives will be dispensed by the study site and its use is allowed on all body areas except the targeted assessment areas up to 2 times daily from Day -7 to Week 16 (EoT). No moisturizers or emollients are allowed at any time during the study for the targeted lesional and non-lesional skin areas including a buffer zone of 5 cm in AD patients and the targeted skin areas in healthy volunteers for TEWL assessments from Day -7 to Week 28 (EoS).

6.5.1 Rescue medicine

During treatment phase, if absolutely medically necessary (ie, to control intolerable AD symptoms on face and genital areas), rescue treatment for AD may be provided to study patients at the discretion of the Investigator (ie, topical treatment with high potency TCS). Investigators should make every attempt to conduct efficacy and safety assessments (eg, disease severity scores, safety laboratory assessments) immediately before administering any rescue treatment. An unscheduled visit may be used for this, if necessary. Rescue treatment with dupilumab for AD may be provided to patients during follow-up period (Weeks 17 to 28). Investigators will be required to perform an IGA evaluation prior to starting rescue treatment and initiate rescue treatment only in patients who either have an IGA score of 4, or have intolerable. The date and time of rescue medication administration as well as the name and dosage regimen of the rescue medication must be recorded in the eCRF.

6.5.1.1 Prohibited medications and procedures

Treatment with the following concomitant medications and procedures is prohibited until the Week 16 (EoT) Visit:

1. Medications used for the treatment of AD or for super-infection:
   - Topical calcineurin inhibitors (tacrolimus or pimecrolimus)
   - Topical phosphodiesterase inhibitors (crisaborole)
   - Topical corticosteroids
2. Topical antibiotics.
3. Vitamins and dietary or herbal supplements (unless approved by the Investigator).
4. Use of any moisturizers other than those approved and dispensed by the study staff.
5. Systemic treatment for AD with an immunosuppressive/immunomodulating agent (including, but not limited to, systemic corticosteroids, cyclosporine A (CsA), azathioprine (AZA), methotrexate (MTX), mycophenolate mofetil (MMF), Interferon gamma (IFN-γ), or other biologics).
6. Treatment with immune modulating biologics including, but not limited to, the following:
   - Any cell-depleting agents (eg, rituximab)
   - Infliximab, adalimumab, golimumab, certolizumab pegol, abatacept, etanercept, or anakinra
7. Procedures used for the treatment of AD (these are considered rescue procedures):
   - Phototherapy (such as ultraviolet B [UVB], narrowband UVB [NBUVB], ultraviolet A1 [UVA1], or psoralen-UVA [PUVA])
   - Bleach baths
   - Use of a tanning booth/parlor

8. In addition, participants will be asked to abstain from live (attenuated) vaccinations through Week 28. If a participant requires a vaccination prior to 28-weeks after discontinuing treatment with dupilumab, titers should be checked post-vaccination. Live (attenuated) vaccinations include but are not limited to the following:
   - Bacillus Calmette-Guérin (BCG)
   - Chickenpox (Varicella)
   - FluMist-Influenza
   - Intranasal influenza
   - Measles (Rubeola)
   - Measles-mumps-rubella (MMR) combination
   - Measles-mumps-rubella-varicella (MMRV) combination
   - Mumps
   - Oral polio (Sabin)
   - Oral typhoid
   - Rotavirus
   - Rubella
   - Smallpox (Vaccinia)
   - Varicella Zoster (shingles)
   - Yellow fever
9. Taking showers or soaking in a bathtub with soaps and body washes within 6 hours before TEWL assessments should be avoided until Week 28. Moisturizers other than Cetaphil, Vanicream, E45 cream, or the preferred moisturizer not containing additives are prohibited.

6.5.1.2 Permitted medications

Other than the prohibited medications and procedures listed in Section 6.5.1.1, treatment with concomitant medications and procedures is permitted during the study. This includes nasal and inhaled corticosteroids, and oral antihistamines. The use of these medications should be recorded.

6.6 DOSE MODIFICATION

No change in IMP dose is allowed.

6.7 INTERVENTION AFTER THE END OF THE STUDY

Any intervention after the EOS Visit will be at the discretion of Investigator or treating physician.

7 DISCONTINUATION OF STUDY INTERVENTION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION

7.1.1 Definitive discontinuation

In rare instances, it may be necessary for a participant to permanently discontinue (definitive discontinuation) study intervention. If study intervention is definitively discontinued, the participant will remain in the study to be evaluated for TEWL and lipidomics, proteomics, transcriptomics, OCT, and FTIR spectroscopy. See the SoA (Section 1.3 and Section 1.4) for data to be collected at the time of discontinuation of study intervention and follow-up, and for any further evaluations that need to be completed.

Pregnancy in a female participant will lead to definitive intervention discontinuation in all cases.

Handling of participants after definitive intervention discontinuation

Participants will be followed-up according to the study procedures specified in this protocol up to the scheduled date of study completion, or up to recovery or stabilization of any AE to be followed-up as specified in this protocol, whichever comes last.

If possible, and after the definitive discontinuation of intervention, the participants will be assessed using the procedure normally planned for the last dosing day with the IMP.

All cases of definitive intervention discontinuation must be recorded by the Investigator in the appropriate pages of the eCRF when considered as confirmed.

7.1.2 Temporary discontinuation

Temporary intervention discontinuation may be considered by the Investigator because of suspected AEs. For all temporary intervention discontinuations, duration should be recorded by the Investigator in the appropriate pages of the eCRF.

The following definition can be considered:

For example, temporary intervention discontinuation decided by the Investigator corresponds to first dose not administered to the participant.

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

- A participant may withdraw from the study at any time at his/her own request, or may be withdrawn at any time at the discretion of the Investigator for safety, behavioral, compliance, or administrative reasons. This is expected to be uncommon.

- At the time of discontinuation from the study, if possible, an early discontinuation visit should be conducted, as shown in the SoA (Section 1.3 and Section 1.4). For data to be collected at the time of study discontinuation and follow-up, and for any further evaluations that need to be completed, as shown in SoA (Section 1.3 and Section 1.4).
- The participant will be permanently discontinued both from the study intervention and from the study at that time.
- If the participant withdraws consent for disclosure of future information, the Sponsor may retain and continue to use any data collected before such a withdrawal of consent.
- If a participant withdraws from the study, he/she may request destruction of any samples taken and not tested, and the Investigator must document this in the site study records.

If participants no longer wish to take the IMP, they will be encouraged to remain in the study. The patients may withdraw from treatment with the IMP, if they decide to do so, at any time and irrespective of the reason, or this may be the Investigator's decision. All efforts should be made to document the reason(s) for treatment discontinuation and this should be documented in the eCRF.

The Investigators should discuss with them about the key visits to be attended. The value of all their study data collected during their continued involvement will be emphasized as important to the public health value of the study.

Participants who withdraw from the study intervention should be explicitly asked about the contribution of possible AEs to their decision, and any AE information elicited must be documented. Preferably the patient should withdraw consent in writing and, if the patient or the patient's representative refuses or is physically unavailable, the site should document and sign the reason for the patient's failure to withdraw consent in writing.

All study withdrawals should be recorded by the Investigator in the appropriate screens of the eCRF and in the participant's medical records. In the medical record, at least the date of the withdrawal and the reason should be documented.

In addition, a participant may withdraw his/her consent to stop participating in the study. Withdrawal of consent for intervention should be distinguished from withdrawal of consent for follow-up visits and from withdrawal of consent for non-participant contact follow-up, eg, medical record checks. Patients requesting withdrawal should be informed that withdrawal of consent for follow-up may jeopardize the public health value of the study. The site should document any case of withdrawal of consent.

Participants who have withdrawn from the study cannot be reallocated (treated) in the study. Their inclusion and intervention numbers must not be reused.

7.3 LOST TO FOLLOW-UP

A participant will be considered lost to follow-up if he or she repeatedly fails to return for scheduled visits and is unable to be contacted by the study site.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site must attempt to contact the participant and reschedule the missed visit as soon as possible and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain whether or not the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the Investigator or designee must make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record.
- Should the participant continue to be unreachable, he/she will be considered to have withdrawn from the study.

8 STUDY ASSESSMENTS AND PROCEDURES

- Study procedures and their timing are summarized in the SoA (Section 1.3 and Section 1.4). Protocol waivers or exemptions are not allowed.
- At the first contact during screening, the study will be explained to the patient's parents or legal guardian (hereinafter the "parent"). The parent will receive verbal information concerning the aims and methods of the study, its constraints and risks, and the study duration. Written informed consent must be signed by the parent prior to any investigations. In addition, provision of assent form will be signed by minor patients or ICF will be signed by emancipated or mature minors (defined by local laws).
- Adherence to the study design requirements, including those specified in the SoA (Section 1.3 and Section 1.4), is essential and required for study conduct.
- All screening evaluations must be completed and reviewed to confirm that potential participants meet all eligibility criteria. The Investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.
- Procedures conducted as part of the participant's routine clinical management and obtained before signing of the ICF may be utilized for screening or baseline purposes provided the procedures met the protocol-specified criteria and were performed within the time frame defined in the SoA (Section 1.3 and Section 1.4). The participants may be rescreened once during the open screening period and all the screening procedures will be repeated; a different patient identification number will be issued. There is no requirement for a waiting period between the screen failure date and the rescreening date. Participants that are rescreened must sign a new consent form and all Visit 1 procedures must be repeated.
- The type, amount (number of fingertip units), and frequency of topical products (see also Section 6.5) used during the study will be recorded in a medication log provided by the study site. The medication log might look different for AD patients and healthy volunteers.
- The maximum amount of blood collected from each participant, if any, over the duration of the study, including any extra assessments that may be required, will not exceed 50 mL. Repeat or unscheduled samples may be taken for safety reasons or for technical issues with the samples.
- In light of the public health emergency related to COVID-19 (or in case of any other public health emergency), the continuity of clinical study conduct and oversight may require implementation of temporary or alternative mechanisms, such as phone contact, virtual visits, online meetings, use of local clinic or laboratory locations, and home visits by skilled staff. Implementation of such mechanisms may differ country by country, depending on country regulations and local business continuity plans. Additionally, no waivers to deviate from protocol enrollment criteria due to COVID-19 (or any other pandemic) will be granted. All temporary mechanisms utilized, and deviations from planned study procedures are to be documented as being related to COVID-19 (or any other public health emergency) and will remain in effect only for the duration of the public health emergency.

Procedures Performed at the Screening/Baseline Visit

Assessments performed at the screening and/or baseline visit include informed consent/assent form, medical/surgical history, medication history, IGA, demographics, prior/concomitant medication/procedure, confirm emollient and washing compliance, and diagnosis of chronic AD. Atopic dermatitis disease characteristics are assessed by clinical evaluations and PROs. Baseline skin barrier function and structure evaluated by TEWL, STS, OCT, and FTIR.

8.1 EFFICACY ASSESSMENTS

8.1.1 Transepidermal water loss assessment and skin tape stripping

8.1.1.1 Transepidermal water loss assessment

TEWL is a skin barrier function test that measures perspiration or water loss through the skin. TEWL is generally higher in AD skin compared with normal skin as the barrier is disrupted allowing water to evaporate more readily. If the barrier improves with dupilumab treatment, then TEWL will be expected to decrease.

TEWL assessment will be conducted in a quiet environment with temperature and humidity being documented. TEWL measurements will be conducted in the same room throughout the study where possible. Participants will be acclimated to the room for a minimum of 20 minutes prior to TEWL measurements. Room temperature will be set to be within 19°C to 23°C. Humidity will be set to be below 60% as the maximum allowed humidity. To further control TEWL readout, moisturizers will be standardized for all study participants starting from Day -7 to Week 28 (EoS). The first TEWL assessment at each visit will be conducted each of the 3 × 2 spots in predefined lesional and non-lesional areas. After start of STS all subsequent TEWL assessments at each visit will be done on the single lesional and non-lesional spot respectively (see also Figure 3 and Figure 4).

8.1.1.2 Skin tape stripping and collection of skin tapes

In addition to TEWL measurements at the skin surface, TEWL measurements can be combined with STS to measure skin barrier function in the predefined skin areas. With STS, the uppermost layers of the skin are peeled away using adhesive discs. Skin with compromised skin barrier exhibits greater changes in TEWL. The AUC for TEWL measurements over a defined number of STS reflects the overall function of the stratum corneum.

This procedure involves the defined, non-invasive application of a commercially available adhesive sheet on the surface of the skin at predefined areas and then peeling it off the skin (= the STS method). This procedure will be repeated up to 20 times sequentially, interrupted every 5 STS by TEWL to collect samples from the stratum corneum. The samples will be collected for lipidomics, proteomics, and transcriptomics.

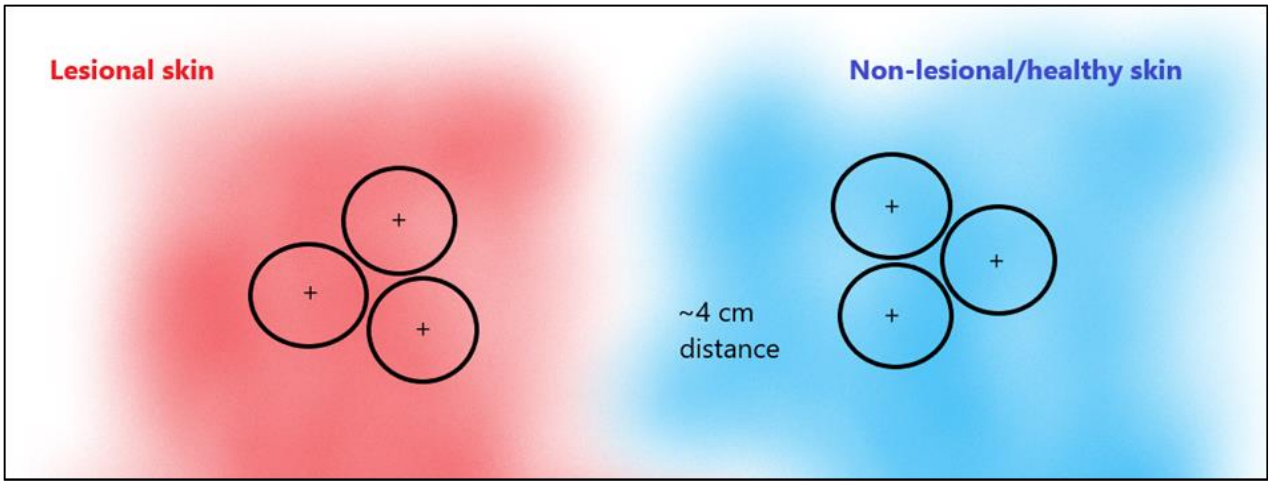
Lesional and non-lesional areas of skin will be tested. Patients will undergo skin barrier function tests at time points according to Section 1.3 before, and after STS.

Healthy volunteers will undergo skin barrier function tests at time points according to Section 1.4 before, and after STS.

Specifically, lipidomics, proteomics, and transcriptomics samples will be collected by STS at baseline, and Weeks 2, 4, 8, 12, 16, 22, and 28. A total 20 skin tapes will be collected in lesional, non-lesional, and normal skin at each STS visit.

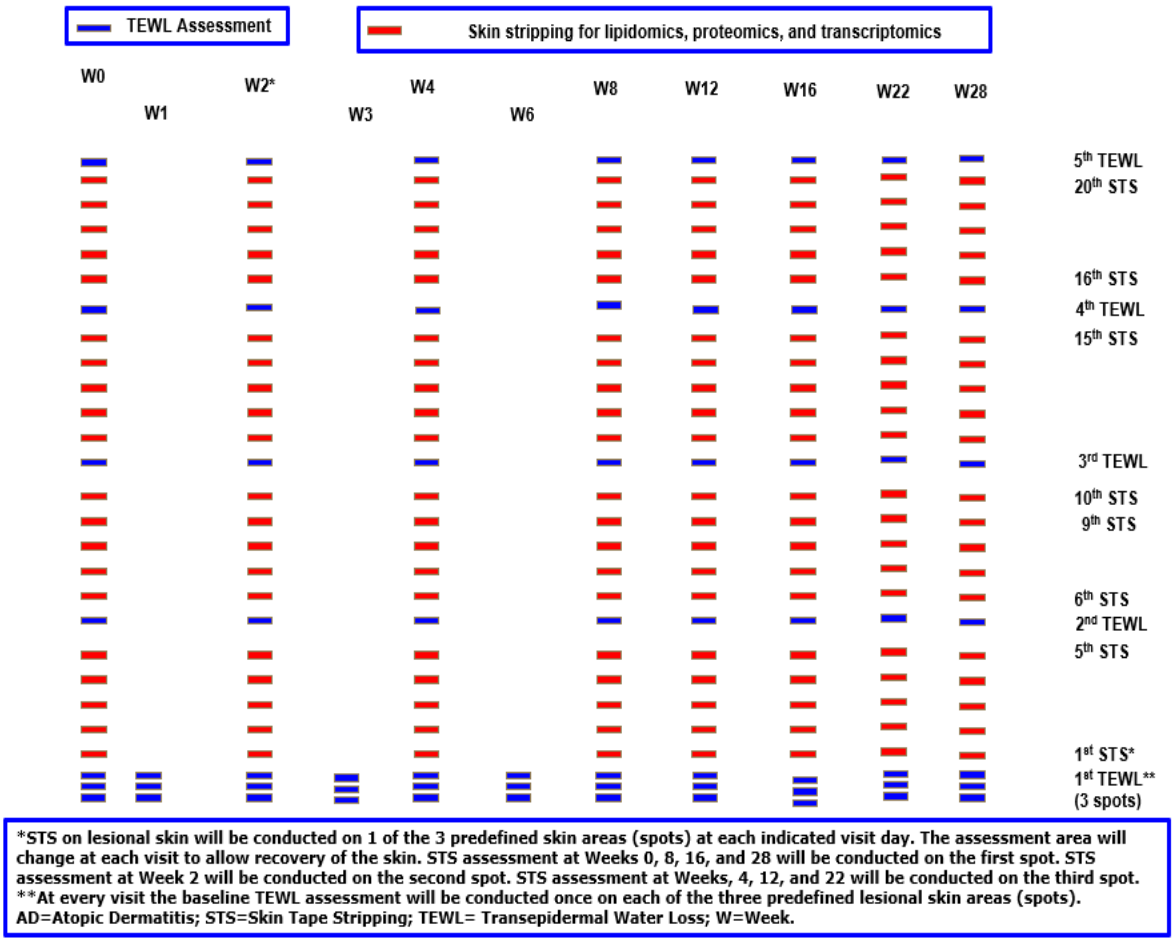
In order to allow the skin to recover from STS, 3 closely adjacent spots (without an overlap for the skin tapes) will be identified for subsequent skin barrier function assessment within lesional, non-lesional, and normal skin area (Figure 2). STS assessments from the same spot will be separated by at least 8 weeks. In case of STS assessment at an unscheduled visit or at a premature end of treatment visit the assessment should be conducted at that spot of the skin area, for which the time period passed since the last STS assessment is the longest.

Figure 2 - Example of selection of skin spots within lesional and non-lesional/healthy skin



TEWL assessment in lesional, non-lesional, and normal skin (healthy subject) will be conducted before, and after STS (Figure 3 and Figure 4). A total of 20 tape strips for lesional and non-lesional skin in AD patients and normal skin in healthy subjects will be collected, stored, and analyzed for lipidomics, proteomic, and transcriptomic analyses.

Figure 3 - TEWL assessment and STS in lesional skin of AD patients



*STS on lesional skin will be conducted on 1 of the 3 predefined skin areas (spots) at each indicated visit day. The assessment area will change at each visit to allow recovery of the skin. STS assessment at Weeks 0, 8, 16, and 28 will be conducted on the first spot. STS assessment at Week 2 will be conducted on the second spot. STS assessment at Weeks, 4, 12, and 22 will be conducted on the third spot.
**At every visit the baseline TEWL assessment will be conducted once on each of the three predefined lesional skin areas (spots).
AD=Atopic Dermatitis; STS=Skin Tape Stripping; TEWL= Transepidermal Water Loss; W=Week.

Figure 4 - TEWL assessment and STS in non-lesional skin of AD patients and in normal skin of healthy volunteers

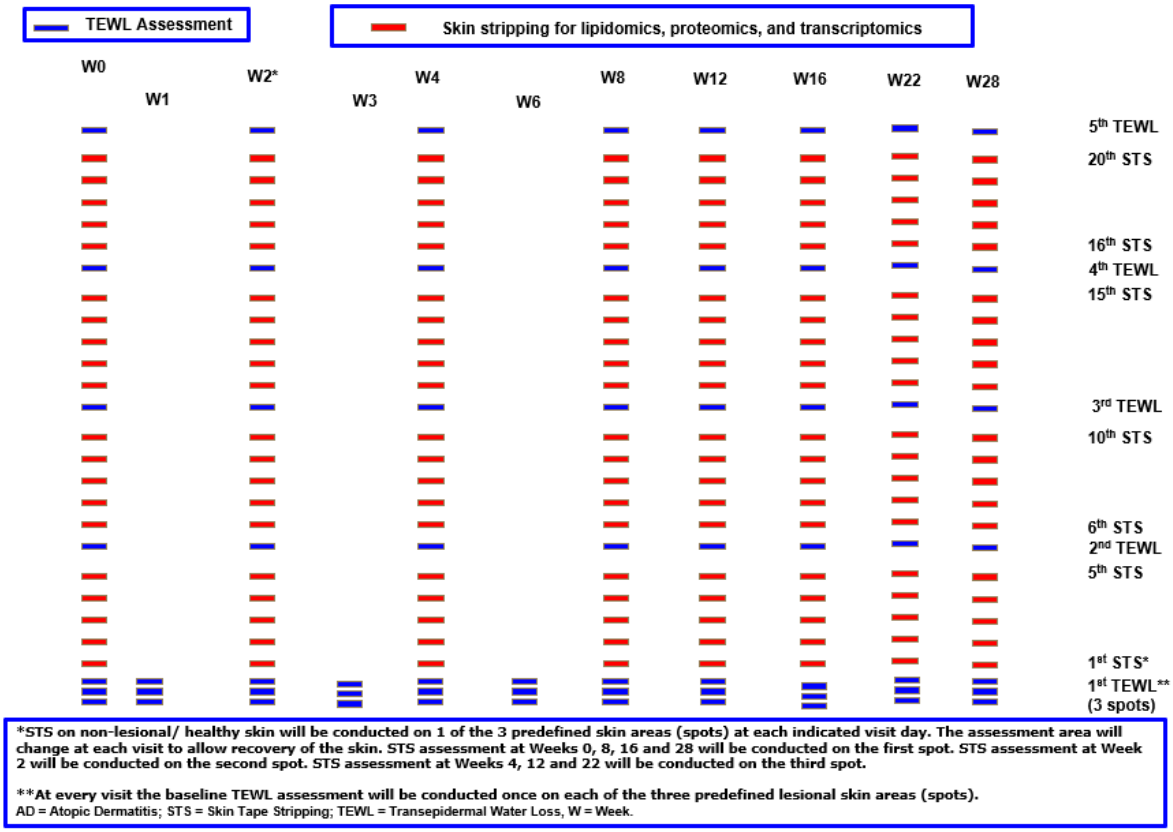


Figure 5 - STS assessment in 3 spots within the predefined lesional and non-lesional skin area in AD patients and in normal skin area in healthy children

AD = Atopic Dermatitis; STS = Skin Tape Stripping.

The detailed procedure for TEWL, STS, and collecting and storing skin tapes for lipidomic, proteomic, and transcriptomic analyses will be provided in the study reference manual. Details about lipidomic, proteomic, and transcriptomic analyses by time point will be provided in the statistical analysis plan (SAP).

8.1.2 Standardized photographs

In AD patients, photographs will be taken of a representative area of AD involvement (the lesional area used for TEWL assessments) as well of a representative area without AD involvement (the non-lesional areas used for TEWL assessments) on Day 1/baseline (predose). Subsequent photographs of the same area will be taken at each visit (except Weeks 10 and 14) until Week 16, and at follow-up visits at Week 22 and Week 28 and premature EoT.

In healthy volunteers photographs will be taken of a skin area corresponding to the lesional area of the AD patients, to which this healthy volunteer is matched on Day 1 at baseline. Subsequent photographs of the same area will be taken at each visit until Week 28 in healthy volunteers.

For each image of each targeted lesional, non-lesional and healthy skin area morphologic parameters will be assessed by a validated method. The parameters may include size, color, and other parameters. In addition, each image will be scored by a blinded expert reader to grade the severity of skin findings.

Full body photographs will be taken for documentation purposes.

All photographs should be taken before the first TEWL assessment at each visit. Further instructions for taking the photographs and additional information on the parameters to be analyzed will be provided in the study reference manual.

8.1.3 Clinician-reported outcome assessments (ClinROs) and patient reported outcome assessments (PROs)

Patient reported outcome (PRO) assessments will only be conducted in AD patients. Patient reported outcome assessments will be collected in an appropriate form, for which the respective device, diary and/or instruction is handed out to the patient at the screening/and or baseline visit. The electronic diary (eDiary) will be only used for PRO data collection. These PRO measures (questionnaires and Numerical Rating Scale [NRS]) should be completed by the patients before the TEWL assessment at each visit and in a quiet place. The questionnaires should be completed by the patients themselves, independently from their physician, the study nurse or any other medical personnel and without any help from friends or relatives. For the questionnaires completed by caregivers (eg, Patient Oriented Eczema Measure [POEM]), to ensure consistency in perception over longitudinal administration and to minimize respondent variability, it is important that the same caregiver complete the questionnaire at each time point. This should be communicated to caregivers during consent and prior to the first completion of the questionnaire.

8.1.4 Eczema area and severity index (EASI)

The Eczema Area and Severity Index (EASI) is a validated ClinRO measure used in clinical practice and clinical trials to assess the severity and extent of AD (37). The EASI is a composite index with scores ranging from 0 to 72. Four AD disease characteristics (erythema, thickness [induration, papulation, and edema], scratching [excoriation], and lichenification) will be assessed for severity by the Investigator or designee on a scale of “0” (absent) through “3” (severe). In addition, the area of AD involvement will be assessed as a percentage by body area of head and neck, trunk, upper limbs, and lower limbs, and converted to a score of 0 to 6. In each body region, the area is expressed as 0, 1 (1% to 9%), 2 (10% to 29%), 3 (30% to 49%), 4 (50% to 69%), 5 (70% to 89%), or 6 (90% to 100%). The EASI will be collected at time points according to Section 1.3.

The EASI assessment tool will be provided in the study reference manual.

8.1.5 Individual signs score

The individual signs score (ISS) also called Global Individual Signs Score (GISS), is ClinRO assessment scale used to evaluate individual targeted AD lesions (erythema, edema/papulation, excoriations, and lichenification) by the Investigator based on a 4-point scale ranging from 0 (none) to 3 (severe). The ISS score on erythema and edema/papulation will be collected at time points according to Section 1.3.

The ISS will be provided in the study reference manual.

8.1.6 Worst itch numerical rating scale

The worst itch NRS includes 2 items asking about the "worst itching" experienced by the child: one that addresses the worst itching "last night" and one that addresses the worst itching "today." Both items are rated by the child with AD using an 11-point NRS in which, 0 indicates no itching and 10 indicates the worst itching possible, with figures depicting the 0 and 10 anchors on each scale.

The worst itch NRS will be administered via eDiary, at time points according to Section 1.3.

8.1.7 Sleep disturbance numerical rating scale

The Sleep disturbance numerical rating scale (NRS) consists in one item asking the patients to rate how their sleep has been affected last night, using a scale from 0 (Best possible sleep) to 10 (Worst sleep possible).

Patients will complete the rating scale daily from Day-7 until Week 4, and then until Week 28 (EoS) at the week before the visits as indicated at Section 1.3.

The sleep disturbance NRS will be provided in the study reference manual.

8.1.8 Skin pain numerical rating scale

Skin pain NRS includes 2 items asking about the "worst skin pain" experienced: one that addresses the worst skin pain "last night" and one that addresses the worst skin pain "today." Both items are rated by the child with AD using an 11-point NRS in which 0 indicates "not at all" and 10 indicates "worst pain possible".

The skin pain NRS will be administered via eDiary, at time points according to Section 1.3.

8.1.9 Patient oriented eczema measure (POEM)

The POEM is a 7-item, validated questionnaire used in clinical practice and clinical trials to assess disease symptoms in children and adults (38). The format is a response to 7-items (dryness, itching, flaking, cracking, sleep loss, bleeding, and weeping) based on frequency of these disease symptoms during the past week (ie, 0 = no days, 1 = 1 to 2 days, 2 = 3 to 4 days, 3 = 5 to 6 days, and 4 = all days). Total scores range from 0 to 28, with higher scores indicating greater frequency of AD symptoms and sleep disturbance.

Charman et al. (2013) proposed the following severity score bands for POEM scores, derived from analysis of data collected from 1000 children and adults with AD: 0 to 2 for clear or almost clear, 3 to 7 for mild AD, 8 to 16 for moderate AD, 17 to 24 for severe AD, and 25 to 28 for very severe AD (39). The proxy version will be used, ie, the questionnaire asks the caregiver to report their perception of patient's AD symptoms. It is therefore completed by the caregiver.

The questionnaire will be administered at time points according to Section 1.3.

8.1.10 Children's dermatology life quality index (CDLQI)

Children's dermatology life quality index (CDLQI) is the respective, validated version of DLQI to be used in adolescents and younger children (cartoon version) (40). Waters et al. (2010) proposed the following severity bands for CDLQI scores: 0 to 1 for no effect on a child's life, 2 to 6 for small effect on a child's life, 7 to 12 for moderate effect on a child's life, 13 to 18 for very large effect on a child's life, and 19 to 30 for extremely large effect on a child's life (41).

The questionnaire will be administered at time points according to Section 1.3.

8.2 SAFETY ASSESSMENTS

Planned time points for all safety assessments are provided in the SoA (Section 1.3 and Section 1.4).

8.2.1 Physical examinations

- A complete physical examination will include, at a minimum, assessments of the skin, the cardiovascular, respiratory, and neurological systems. Height and weight will also be measured and recorded.
- Signs and symptoms of hypersensitivity reaction (if any) will be documented.
- A skin focused physical examination will be performed prior to TEWL assessments at time points according to Section 1.3 and Section 1.4.
- Investigators should pay special attention to clinical signs related to previous serious illnesses.
- Any new finding or worsening of previous finding should be reported as a new AE.

8.2.2 Vital signs

Vital signs, including heart rate, blood pressure, pulse rate, body temperature, and respiratory rate will be collected prior to TEWL assessments at time points according to Section 1.3 and Section 1.4. Heart rate, blood pressure, and respiratory rate will be measured with the patient in sitting position, after the patient has rested comfortably for at least 5 minutes.

8.2.3 Laboratory testing

Urinalysis for pregnancy testing will be performed for all female of childbearing potential. Urine pregnancy testing will be performed at time points according to Section 1.3 and Section 1.4. Please also refer to Section 8.7 for any laboratory testing.

8.2.4 Electrocardiograms

No electrocardiogram (ECG) assessments are required in this study.

8.2.5 Clinical safety laboratory assessments

No clinical safety laboratory assessments are required in this study.

8.2.6 Suicidal ideation and behavior risk monitoring

Not applicable for this study.

8.3 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

Adverse events will be reported by the participant (or, when appropriate, by a caregiver, surrogate, or the participant's legally authorized representative).

The Investigator and any qualified designees are responsible for detecting, documenting, and recording events that meet the definition of an AE or SAE and remain responsible for following up AEs that are serious, considered related to the study intervention or study procedures, or which cause the participant to discontinue the study intervention (see Section 7).

8.3.1 Time period and frequency for collecting AE and SAE information

All SAEs will be collected from the signing of the ICF until the follow-up visit at the time points according to the SoA (Section 1.3 and Section 1.4).

All AEs will be collected from the signing of the ICF at the time points specified in the SoA (Section 1.3 and Section 1.4).

All SAEs and AEs of special interest (AESI) will be recorded and reported to the Sponsor or designee immediately and under no circumstance should this exceed 24 hours, as indicated in Appendix 3 (Section 10.3). The Investigator will submit any updated SAE data to the Sponsor within 24 hours of it being available.

Investigators are not obligated to actively seek AE or SAE after conclusion of the study participation. However, if the Investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and he/she considers the event to be reasonably related to the study intervention or study participation, the Investigator must promptly notify the Sponsor.

8.3.2 Method of detecting AEs and SAEs

The method of recording, evaluating, and assessing causality of AEs and SAEs and the procedures for completing and transmitting SAE reports are provided in Appendix 3 (Section 10.3).

Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and non-leading verbal questioning of the participant is the preferred method to inquire about AE occurrences.

8.3.3 Follow-up of AEs and SAEs

After the initial AE/AESI/SAE report, the Investigator is required to proactively follow each participant at subsequent visits/contacts. At the pre-specified study end-date, all SAEs, AESI (as defined in Section 8.3.8), will be followed until resolution, stabilization, the event is otherwise explained, or the participant is lost to follow-up (as defined in Section 7.3). Further information on follow-up procedures is provided in Appendix 3 (Section 10.3).

8.3.4 Regulatory reporting requirements for SAEs

- Prompt notification within 24 hours by the Investigator to the Sponsor of an SAE is essential so that legal obligations and ethical responsibilities toward the safety of participants and the safety of a study intervention under clinical investigation are met.
- The Sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study intervention under clinical investigation. The Sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, Institutional Review Boards (IRBs)/Independent Ethics Committees (IECs), and Investigators.
- Adverse events that are considered expected will be specified in the reference safety information.
- Suspected unexpected serious adverse reactions (SUSARs) are reported to regulatory authorities, Investigators, and IRBs/IECs as follows:
  - For SUSARs that are life-threatening or result in death, reporting is no later than 7 days after first knowledge by the Sponsor, with all relevant follow-up information subsequently reported within an additional 8 days
  - For SUSARs, other than those that are life-threatening or result in death, reporting is no later than 15 days after first knowledge by the Sponsor
- An Investigator who receives an Investigator safety report describing an SAE, SUSAR, or any other specific safety information (eg, summary or listing of SAEs) from the Sponsor will review and then file it along with the Investigator's Brochure and will notify the IRB/IEC, if appropriate according to local requirements. It is the responsibility of the Sponsor to assess whether an event meets the criteria for a SUSAR, and therefore, is expedited to regulatory authorities.

8.3.5 Pregnancy

Details of all pregnancies in female participants will be collected after the start of study intervention and until 12 weeks after EoS.

If a pregnancy is reported, the Investigator should inform the Sponsor within 24 hours of learning of the pregnancy and should follow the procedures outlined in Appendix 4 (Section 10.4).

Abnormal pregnancy outcomes (eg, spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered as SAEs.

8.3.6 Cardiovascular and death events

Not applicable.

8.3.7 Disease-related events and/or disease-related outcomes not qualifying as AEs or SAEs

Not applicable.

8.3.8 Adverse event of special interest

An AESI is an AE (serious or nonserious) of scientific and medical concern specific to the Sponsor's product or program, for which ongoing monitoring and immediate notification by the Investigator to the Sponsor is required. Such events may require further investigation in order to characterize and understand them. Adverse events of special interest may be added, modified, or removed during a study by protocol amendment.

The following events are AESIs and require reporting to the Sponsor within 24-hours of learning of the event:

- Anaphylactic reactions.
- Systemic hypersensitivity reactions.
- Helminthic infections.
- Any severe type of conjunctivitis or blepharitis.
- Keratitis.
- Clinically symptomatic eosinophilia (or eosinophilia associated with clinical symptoms).

Patients who experience AESI related to eye disorders will also be referred to an ophthalmologist (preferably with expertise in treating pediatric patients or Cornea and External Eye Disease ["front-of-the-eye"] subspecialty expert). Further evaluation of these AESIs will be performed including any additional tests, if applicable, as per the discretion of the ophthalmologist.

The following events also require reporting to the Sponsor within 24 hours of learning of the events:

- Pregnancy of a female subject entered in the study as well as pregnancy occurring in a female partner of a male subject entered in the study.
    - Pregnancy occurring in a female participant entered in the clinical trial or in a female partner of a male participant entered in the clinical trial. It will be qualified as an SAE only if it fulfills 1 of the seriousness criteria.
    - In the event of pregnancy in a female participant, investigational medical product (IMP) should be discontinued.
    - Follow-up of the pregnancy in a female participant or in a female partner of a male participant is mandatory until the outcome has been determined.

- Abnormal pregnancy outcomes (eg, spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered SAEs.

- Significant alanine transaminase (ALT) elevation.
  - ALT >5 × the upper limit of normal (ULN) in participants with baseline ALT ≤2 × ULN;

    or
  - ALT >8 × ULN if baseline ALT >2 × ULN.
- Symptomatic overdose (serious or nonserious) with IMP.
  - An overdose (accidental or intentional) with the IMP is an event suspected by the Investigator or spontaneously notified by the participant and defined as at least twice the intended dose during an interval of less than 11 days. The circumstances (ie, accidental or intentional) should be clearly specified in the verbatim and symptoms, if any, entered on separate AE forms.

The definitions of an AE or SAE can be found in Appendix 3 (Section 10.3).

AE will be reported by the participant (or, when appropriate, by a caregiver, surrogate, or the participant's legally authorized representative).

The Investigator and any qualified designees are responsible for detecting, documenting, and recording events that meet the definition of an AE or SAE and remain responsible for following up AEs that are serious, considered related to the study procedures, or that caused the participant to discontinue the study.

8.3.9 Guidelines for reporting product complaints

Any defect in the IMP must be reported as soon as possible by the Investigator to the monitoring team that will complete a product complaint form within required timelines.

Appropriate information (eg, samples, labels, or documents like pictures or photocopies) related to product identification and to the potential deficiencies may need to be gathered. The Investigator will assess whether or not the quality issue has to be reported together with an AE or SAE.

8.4 TREATMENT OF OVERDOSE

Sponsor does not recommend specific treatment for an overdose.

In the event of an overdose, the Investigator should:

1. Contact the Medical Monitor immediately.
2. Closely monitor the participant for any AE/SAE and laboratory abnormalities until dupilumab can no longer be detected systemically (at least 28 days).
3. Document appropriately in the eCRF.

Decisions regarding dose interruptions or modifications will be made by the Investigator in consultation with the Medical Monitor based on the clinical evaluation of the participant.

8.5 PHARMACOKINETICS

Pharmacokinetic parameters are not evaluated in this study.

8.6 PHARMACODYNAMICS

Pharmacodynamics parameters are evaluated using TEWL assessments as described in Section 8.1.1.

8.7 GENETICS

A saliva swab sample for DNA isolation will be collected at baseline from all participants for FLG gene sequencing analysis. Saliva swab samples for DNA isolation should be collected on Day 1/Baseline (predose), but may be collected at any study visit.

Optional broad genetic analysis may include, but is not limited to, whole exome or whole genome sequencing. Participants will be required to separately agree to this optional genetic research within the ICF to consent to broad genetic analysis. Participants who do not wish to participate in optional broad genetic research may still participate in the study.

In the event of DNA isolation failure, a replacement genetic sample may be requested from the participant. Signed informed consent will be required to obtain a replacement sample unless replacement collection was included in the original consent.

Details on processes for collection, storage, shipment, and destruction of these samples can be found in the study reference manual. See Appendix 5 (Section 10.5) for information regarding genetic research.

DNA samples may be stored for a maximum of 15 years following the last participant's last visit in the study at a facility selected by the Sponsor.

8.8 BIOMARKERS

Collection of skin samples for lipidomics, proteomics, and transcriptomics analyses is an important part of this study.

8.8.1 Lipidomics assessment

Samples for skin lipidomic assessment are required and will be collected from all participants in this study as specified in the SoA (Section 1.3 and Section 1.4).

Targeted skin lipidomics will be performed by means of a validated mass spectrometry method from skin tapes that have been collected during tape stripping at the TEWL assessment.

Skin lipidomics samples may be stored for a maximum of 5 years following the last participant's last visit in the study at a facility selected by the Sponsor.

8.8.1.1 Filaggrin breakdown products

Filaggrin is a structural protein that plays an important role in controlling water retention in the skin, and the hygroscopic properties of FLG breakdown products have an important role as natural moisturizing factor ingredients (42). Filaggrin deficiency leads to impaired lipid profile and altered acidification pathways (43). Lipid abnormalities have been reported in patients with FLG mutations (44).

Filaggrin breakdown products, cis/trans-urocanic acid (total UCA) and pyrrolidone carboxylic acid (PCA), also known as pyroglutamic acid, will be quantified via a liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) approach in the same tape strips that are used for lipidomics assessment.

8.8.1.2 Analysis of stratum corneum lipids

It has been reported that lesional and non-lesional skin of AD patients has decreased EOS CER and other CER with very long-chain fatty acids (C22-C30), and increased short-chain fatty acids NS CER (C16-C22) (17). Highly hydrophobic omega-esterified acid sphingosine CER (EOS CER) and NS CER will be analyzed as follows.

STS processing for lipid extraction

STS will be processed through a Bligh and Dyer procedure (17). The bottom chloroform layer from the skin tape extraction will be used for lipid analyses while upper water-methanol phase will be used for polar component analyses by the Liquid Chromatography with tandem Mass Spectrometry (LC-MS/MS). All mass spectrometric data will be normalized to the total amount of hydrolyzed protein determined.

EOS CER and NS CER will be identified and quantified using a targeted ultra-high performance liquid chromatography electrospray ionization tandem mass spectrometry (UHPLC-ESI-MS/MS).

8.8.1.3 Quantification of protein bound ceramides

Protein bound ceramides are formed from EOS CER and represent a portion of EOS CER without linoleic acid, thus having a terminal hydroxyl group N-linked fatty acid. Protein-bound ceramides are released from proteins during protein hydrolysis. After protein determination in hydrolysates, freed omega-OH CER will be extracted and omega hydroxy fatty acid containing CER will be detected by the UHPLC-ESI-MS/MS. Values obtained will be normalized against protein content in the samples.

Detailed guidelines and requirements for samples preparation, handling of skin tapes after stripping, and storage and shipment of such skin tapes will be provided in the study reference manual.

8.8.2 Proteomics assessment

Protein extracts will be prepared from STS samples by LC-MS/MS.

The following 3 major functional groups of proteins associated with skin barrier function will be examined:

- Keratin intermediate filaments
- Proteins associated with inflammatory response (S100 proteins, alarmins, protease inhibitors)
- Glycolysis and oxidative stress response proteins (glycolytic enzymes, oxidative stress response enzymes)

Skin proteomics samples may be stored for a maximum of 5 years following the last participant's last visit in the study at a facility selected by the Sponsor.

Detailed sample preparation for proteomic analysis will be provided in a separate study reference manual.

8.8.3 Skin tape transcriptome

Skin tapes will be collected and stabilized in RLT buffer with DTT and stored frozen at -80°C for transcriptomic analysis. Skin tape transcriptome samples will be shipped to National Jewish Health, where total RNA will be isolated and RNA sequencing will be performed using qualified methods (3, 13) to support whole transcriptome analysis and data generation.

Skin transcriptomics samples and any RNA isolated from the samples may be stored for a maximum of 5 years following the last participant's last visit in the study at a facility selected by the Sponsor.

Transcriptome sample preparation and analysis details will be provided in a separate study reference manual.

8.8.4 Optical coherence tomography (OCT)

Structural and Angiographic OCT scans using the Vivosight OCT machine

Optical coherence tomography (OCT) is a non-invasive imaging modality conceptually similar to ultrasound but uses near-infrared radiation rather than sound. It has a 2 to 10 µ depth resolution compared with 100 to 1 000 µ typical for clinical ultrasound; and 1 to 2 mm imaging depth vs. 10 to 100 mm for clinical ultrasound. It is thus ideal for imaging the surface layers of accessible tissues such as the skin. It is attracting interest throughout the medical community as a scanning tool and for diagnosis of illnesses such as epithelial cancer, connective tissue disorders, and atherosclerosis.

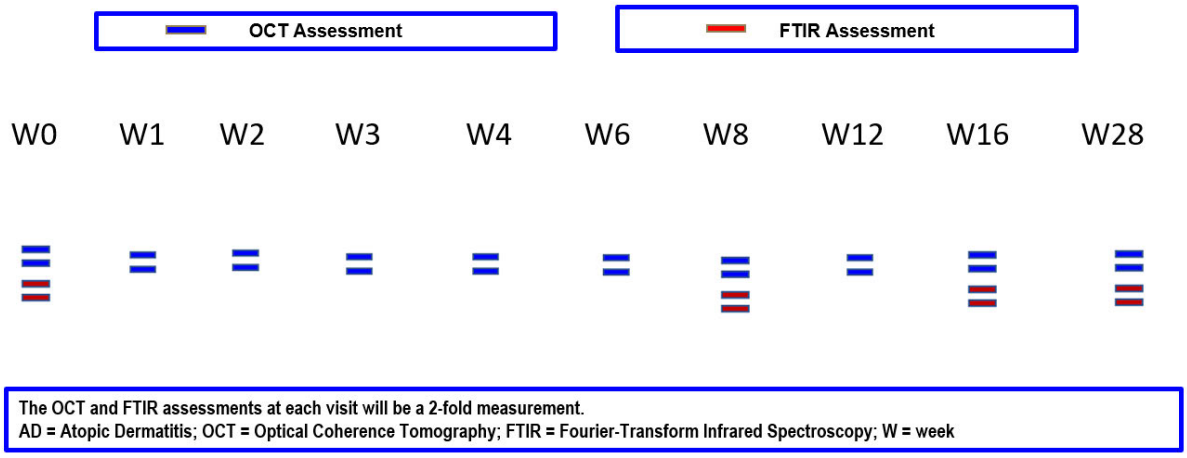
The Vivosight OCT device will be used to provide information for both structural and angiographic OCT outcomes.

1. *Structural* OCT: With a depth focus of 1.0 mm and optical resolution of 7.5 × 5.0 µm the Vivosight provides structural images of the skin comparable to histology sections. From these images the thickness of the epidermis, the suprapapillary epidermis, and the papillary region can be extrapolated. Epidermal thickness has been identified using histology, as a useful biomarker of disease activity and treatment effects in AD.
2. *Angiographic* OCT: Structural OCT performed at high image frame rates can map areas of temporal decorrelation due to moving red blood cells. Based on this, demonstrated that the Vivosight can acquire high-quality maps of the superficial vasculature in living participants and is an ideal tool to monitor and quantify erythema, hyperplasia, and TCS-induced tissue remodeling.

The OCT assessments will be performed before STS/TEWL assessments at each designated visit from Week 0 to Week 28 (see Section 1.3, Section 1.4 and Figure 6). Two scans will be taken from each target area (separate, adjacent, sites for each scan) at each designated visit. All images will be checked for quality upon collection, and images with visible imaging artifacts will be re-captured. Any part of the device in contact with human skin (the stand-off) will be decontaminated between uses.

Details of OCT operation and OCT data analysis will be provided in a separate study reference manual.

Figure 6 - OCT and FTIR assessments in lesional and non-lesional skin of AD patients and in normal skin of healthy volunteers



8.8.5 Attenuated total reflectance (ATR) Fourier-transform infrared (FTIR) spectroscopy

Attenuated Total Reflectance (ATR) FTIR spectroscopy is a form of molecular spectroscopy useful for the analysis of surfaces, including the lipid, water, and carboxylate content of the skin barrier and for analyzing lipid arrangement/structure (23, 24, 25, 26, 27).

In this study the Agilent 4300 handheld FTIR spectrometer will be used. Measurements will be performed by gently but firmly placing the probe in contact with the skin. Duplicate measurements will be collected from each target site at the visits indicated in the schedule of events. The quality of spectra will be assessed at the point of collection, and any that do not meet the established quality parameters (signal to noise ratio) will be discarded. After use in each participant the ATR (probe head) will be decontaminated with 70% alcohol wipe.

The FTIR assessments will be performed before and during the STS assessments at each designated visit (see Section 1.3, Section 1.4 and Figure 6).

Detailed measurement and data collection from FTIR will be provided in a separate study manual.

8.8.6 Optional samples for biomarker research

The following optional serum and plasma samples for biomarker research should be collected from participants in the study, where possible:

- Venous blood samples (not more than 12 mL in total) will be collected at baseline and EoT/end of observation at Week 16 to obtain serum and plasma to be stored for possible future research related to response, disease activity, safety, the Type 2 inflammation pathway, and for assessing the effects of the study intervention on modulation of IL-4 receptor and on atopic disease processes, as well as to study biomarkers that may have predictive utility for response to dupilumab treatment.
- The results of exploratory research testing will not be included in this Clinical Study Report (CSR).

Details on collection, storage, and shipment of these samples will be provided in the study reference manual.

Plasma and serum samples may be stored for a maximum of 5 years following the last participant's last visit in the study at a facility selected by the Sponsor.

8.9 IMMUNOGENICITY ASSESSMENTS

No antibodies to dupilumab will be evaluated in this study.

8.10 HEALTH ECONOMICS

Not applicable.

9 STATISTICAL CONSIDERATIONS

The statistical methodology presented in this section constitutes the SAP for the study. However, biomarkers' analyses: ie, lipidomics, FLG genotyping, proteomics, and transcriptomics, will be detailed in a specific SAP. If the SAP in the present document needs revision during the study to provide further details or adapt to unexpected issues in study execution and data that affect planned analyses, a SAP or an appropriate statistical technical document (STD) reflecting the changes will be issued prior to database lock or any interim analysis.

If no major changes to the statistical analysis planned in the present document are needed, a STD will be issued to document only technical conventions used for the analyses (and minor modifications from the planned analyses, if any).

There will be 2 analyses, 1 for the treatment phase and 1 for the follow-up period. The analysis of the treatment phase will be based on all patients and performed on data collected until the EoT visit (Week 16). This will be done once the last evaluable participant has completed his/her EoT visit. The analysis of the follow-up period will be based on all participants and all data.

The following definitions will be used in this section:

- Cohort: the study includes 2 cohorts: patients with moderate-to-severe AD cohort and a healthy volunteers cohort.
- Group: 3 groups are identified: patients' lesional skin area group, patients' non-lesional skin area group, and healthy volunteers' normal skin group.

9.1 STATISTICAL HYPOTHESES

No hypothesis testing is predefined in this exploratory study.

9.2 SAMPLE SIZE DETERMINATION

Sample size for this exploratory study was based on medical/clinical judgment and is consistent with the sample size from similar studies in the literature (4). No formal sample size calculation was performed. TEWL data collected in similar settings as planned for this study were not available: ie, TEWL values after 5 STS, and pre- and post-dupilumab treatment are unknown.

Allowing for drop-out rate of 15%, a total of approximately 24 pediatric patients with moderate-to-severe AD will be enrolled to achieve 20 evaluable patients, ie, patients with no major or critical deviations related to IMP and/or TEWL measurements, for whom the TEWL data for primary analysis: ie, TEWL at baseline and Week 16, are considered sufficient and interpretable. An approximately equal number of age, gender, location of targeted lesion area and site matched healthy volunteers serving as a reference comparator cohort will be enrolled. Drop-out rate in healthy volunteers is expected to be minimal. Any drop-out healthy volunteer for whom the matched patient is considered as evaluable will not be replaced.

9.3 POPULATIONS FOR ANALYSES

For purposes of analysis, the following populations are defined (Table 3):

Table 3 - Populations for analyses

Population	Description
Enrolled	All participants who sign the informed consent form (ICF)
Intent-to-treat (ITT)	The ITT population includes all enrolled patients, who received at least 1 dose of investigational medicinal product (IMP) and all enrolled healthy volunteers who have at least 1 transepidermal water loss (TEWL)/ skin tape stripping (STS) assessment performed, irrespective of compliance with the study protocol and procedures.
Modified Intent-to-treat (mITT)	The mITT population includes all ITT participants. If prohibited therapies for atopic dermatitis (AD) (see Section 6.5.1) are used and assessed by the study team as having a significant impact on skin barrier only visits prior to rescue treatment use are considered.
Efficacy	The mITT and ITT populations
Safety	The safety population includes all patients, who actually received at least 1 dose of IMP or had at least 1 TEWL/STS assessment and all healthy volunteers who have at least 1 TEWL/STS assessment performed.

9.4 STATISTICAL ANALYSES

9.4.1 Subject description

9.4.1.1 Disposition of subjects

A detailed description of participant accountability including count of participants by analysis populations (Table 3), screen failure participants with reasons for screen failure, participants who did not complete the study observation period along with the main reason for permanent treatment discontinuation, and participants who requested permanent treatment discontinuation, will be generated by cohorts.

All withdrawals from the study, taking place on or after study intervention intake, will be fully documented in the body of the CSR.

A listing of subjects with treatment discontinuation will be provided.

9.4.2 Protocol deviations

During the review of the database, compliance with the protocol will be examined with regard to inclusion and exclusion criteria, treatment compliance, prohibited therapies, and timing and availability of planned assessments. Protocol deviations will be identified by the study team before database lock and listed in the Data Review and Surveillance Report, including missing data and study drug discontinuations, and classified as critical, major, or minor deviations.

Individual deviations to inclusion and exclusion criteria as reported by the Investigator will be listed.

If any major and critical deviations other than those involving inclusion/exclusion will be listed by participant and/or described in the body of the CSR.

9.4.3 Analysis population

The number of participants included in each study population (safety population and efficacy populations (Intent-to-treat and modified-Intent-to-treat) will be provided. All exclusion from any analysis population will be fully documented in the CSR.

9.4.4 Demographic and baseline characteristics

9.4.4.1 Subject demographic characteristics, medical history, and diagnoses

Continuous variables (age, weight) and qualitative variables (gender, race, and body mass index [BMI]) will be summarized by cohorts, by descriptive statistics (summary tables) for the modified intent-to-treat (mITT) population and for additional population if relevant (eg, if many participants from the mITT population are not part of the safety population).

Specific medical/surgical history will be listed. Other baseline characteristics will be listed.

Disease characteristics at baseline: ie, clinical and PROs, and skin barrier function, will be presented along with the on-treatment summary statistics in the Section 9.4.7.

9.4.4.2 Baseline efficacy parameters

Baseline is defined as the last available and evaluable value before and closest to the first dose of the IMP: ie, at Day 1 (Week 0), for patients and as the last available and evaluable value at Day 1 (Week 0) for healthy volunteers. For TEWL data, the average of the values of the 3 spots within each predefined skin area will be used as baseline, unless a large difference between the spots is observed, if so, the 3 spots will be analyzed separately.

9.4.4.3 Baseline safety parameters

Baseline for safety parameters will be defined as the last available and evaluable value before and closest to the first dose of IMP for patients and as the last available and evaluable value at Day 1 (Week 0) for healthy volunteers, for vital sign parameters.

Baseline safety values will be presented along with subsequent safety values assessed during the study.

9.4.5 Extent of study treatment exposure and compliance

The extent of study treatment exposure and compliance will be assessed and summarized for patients' cohort within the safety population.

The following listings will be provided:

- Patients receiving IMP from specified batch; (patient, IMP, and IMP batch number) will be sorted by patient.

9.4.5.1 Extent of investigational medicinal product exposure

Duration of IMP exposure is defined regardless of unplanned intermittent discontinuations as:

- Dose regimen 1: the last dose date - first dose date + 28 days
- Dose regimen 2: the last dose date - first dose date + 14 days.

If the patient's date of last dose is unknown, his/her last IMP dispensing date will be used in its place.

Duration of exposure will be summarized in patients' cohort using descriptive statistics such as mean, standard deviation (SD), median, minimum, and maximum.

9.4.5.2 Compliance

A given administration will be considered noncompliant if the patient did not take the planned dose of treatment as required by the protocol. No imputation will be made for patients with missing or incomplete data.

Percentage of compliance for a patient will be defined as the number of administrations the patient was compliant divided by the total number of administrations the patient was planned to take (the number of doses missed due to interruptions at Investigators' judgment will not be subtracted) on or before the last IMP administration date.

Treatment compliance, above-planned and under-planned dosing percentages will be summarized descriptively (N, mean, SD, median, minimum, and maximum). The percentage of patients with compliance <80% will be summarized. In addition, number and percentage of patients with at least 1 above-planned dosing administration will be given, as well as the number and percentage of patients with 0, (0, 20%), and >20% under planned dosing administrations.

9.4.6 Prior/Concomitant medication/therapy

Medications will be coded according to the World Health Organization Drug Dictionary (WHO Drug Dictionary, last available version before database lock). Concomitant medications with the IMP will be listed separately by participants.

9.4.7 Efficacy analyses

The efficacy evaluation will be based upon the review of the individual values (graphics), descriptive statistics (summary tables, graphics) and, where applicable, exploratory statistical analysis.

Due to the small sample size and the exploratory characteristics of the study, missing, or incomplete data of efficacy marker will not be imputed. In case a significant number of patients using rescue therapy are observed during the study treatment phase, visit values removed due to use of rescue therapy will be imputed. The imputation methods applied will be described in the SAP. Rescue medication may be allowed during the study follow-up period at the discretion of the Investigator.

9.4.7.1 Description of efficacy variable(s)

Efficacy parameters are described in Section 8.1. The derivation of baseline results is described in Section 9.4.4.2.

The analysis of the efficacy marker will be based on the mITT population. As a sensitivity analysis, the primary endpoint will also be analyzed based on the intent-to-treat (ITT) population. Secondary endpoints analysis might be run on the ITT population as sensitivity analysis in case an important number of patients using rescue therapy is observed.

Sub-analysis might be done, if deemed appropriate (eg, by FLG mutation status).

TEWL data collected at the same visit from without and before STS measurement on each of the 3 spots within the predefined skin areas will be averaged, unless a large difference between spots is observed.

Analysis

Table 4 - Efficacy analyses

Endpoint	Statistical analysis methods
Primary	
Percent change from baseline in TEWL after 5 STS assessed on lesional skin at Week 16 in AD patients.	Raw data and change from baseline; ie, absolute and percentage changes, will be summarized with descriptive statistics (such as mean, median, SD, minimum, and maximum) based on the mITT and ITT populations. Profiles for baseline and Week 16 time points will be generated for individual values: eg, spaghetti plot and boxplot, and patients' cohort means. For information purpose as study is not powered (no multiplicity correction will be applied): Difference between baseline and Week 16 values will be tested through 1-sided paired t-test at a Type 1 error level of alpha=0.05 (H_0: $0 \leq TEWL_{Week\ 16}$-$TEWL_{baseline}$, H_a: $0 > TEWL_{Week\ 16}$-$TEWL_{baseline}$). Point estimate, 2-sided 90% confidence interval and corresponding 1-sided p-value will be reported. If assumption of normal distribution is strongly violated, non-parametric methods: eg, Wilcoxon signed-rank test, will be used; normality assumption will be assessed through quantile-quantile plot and Shapiro-Wilk test at 5% Type I error.

Endpoint	Statistical analysis methods
Secondary	
Change (percent and absolute) from baseline in TEWL after 20 STS assessed on lesional skin at Week 16 in AD patients Change (percent and absolute) from baseline in TEWL after 20 STS assessed on non-lesional skin at Week 16 in AD patients. Change (percent and absolute) from baseline in TEWL after 20 STS assessed on normal skin at Week 16 in healthy volunteers. Change (percent and absolute) from baseline in TEWL after 15 STS assessed on lesional skin at Week 16 in AD patients. Change (percent and absolute) from baseline in TEWL after 15 STS assessed on non-lesional skin at Week 16 in AD patients. Change (percent and absolute) from baseline in TEWL after 15 STS assessed on normal skin at Week 16 in healthy volunteers. Change (percent and absolute) from baseline in TEWL after 10 STS assessed on lesional skin at Week 16 in AD patients. Change (percent and absolute) from baseline in TEWL after 10 STS assessed on non-lesional skin at Week 16 in AD patients. Change (percent and absolute) from baseline in TEWL after 10 STS assessed on normal skin at Week 16 in healthy volunteers. Change (percent and absolute) from baseline in TEWL after 5 STS assessed on non-lesional skin at Week 16 in AD patients. Change (percent and absolute) from baseline in TEWL after 5 STS assessed on normal skin at Week 16 in healthy volunteers.	The similar approach as for the analysis of primary endpoint will be used. In addition, percentage change relative to the healthy volunteers' cohort will be calculated as a ratio of means by number of STS, at baseline and Week 16 for lesional and non-lesional skin groups.
Change (percent and absolute) in TEWL before STS on lesional skin in AD patients over time. Change (percent and absolute) in TEWL before STS on non-lesional skin in AD patients over time. Change (percent and absolute) in TEWL before STS on normal skin in healthy volunteers over time. Change (percent and absolute) in TEWL after STS assessed on lesional skin in AD patients over time. Change (percent and absolute) in TEWL after STS assessed on non-lesional skin in AD patients over time. Change (percent and absolute) in TEWL after STS assessed on normal skin in healthy volunteers over time.	Raw data and change from baseline; ie, absolute and percentage changes, will be summarized with descriptive statistics (such as mean, median, SD, minimum, and maximum) by groups, number of STS and time point. For follow-up period data, change from Week16 (or last visit prior to use of rescue treatment) will also be reported.Profiles over study days will be generated for individual values and group means for raw data and absolute change from baseline/Week 16. Percentage change relative to the healthy volunteers' cohort will be calculated as a ratio of means by number of STS and time point for lesional and non-lesional skins groups. Additional analyses using linear mixed model to explore the evolution of TEWL over time in the different conditions might be performed. As an exploratory analysis, the time to improvement of skin barrier function for lesional and non-lesional skin area measured by TEWL will be summarized for the treatment phase. Improvement is defined as time to the first post-baseline day for which at least 1 TEWL measure of a patient is equal or lower than the median, of the corresponding TEWL measured in his/her matched heathy volunteer over time. Results might be reported through Kaplan-Meier plot.

Endpoint	Statistical analysis methods
Change (percent and absolute) in TEWL AUC (a composite measure before and after 5, 10, 15, and 20 STS) for skin barrier function in lesional skin in AD patients over time. Change (percent and absolute) in TEWL AUC (a composite measure before and after 5, 10, 15, and 20 STS) for skin barrier function in non-lesional skin in AD patients over time. Change (percent and absolute) in TEWL AUC (a composite measure before and after 5, 10, 15, and 20 STS) for skin barrier function in normal skin in healthy volunteers over time.	As all TEWL values are expected to be positive, area under the TEWL curve will be calculated using the trapezoidal method over the 20 STS (measurement at every 5 STS) at each visit. Raw data and change from baseline; ie, absolute and percentage changes, will be summarized with descriptive statistics (such as mean, median, SD, minimum, and maximum) by groups and time points. For follow-up period data, change from Week16 (or last visit prior to use of rescue treatment) will also be reported. Profiles over study days will be generated for individual values and group means for raw data and absolute change from baseline/Week 16. Percentage change relative to the healthy volunteers' cohort will be calculated as a ratio of means by time point for lesional and non-lesional skins groups. As exploratory analysis, time to improvement of skin barrier function for lesional and non-lesional skin area measured by TEWL AUC will be summarized for the treatment phase. Improvement is defined as time to the first post-baseline day for which TEWL AUC of a patient is equal or lower than the median, of the corresponding TEWL AUC computed in his/her matched heathy volunteer over time. Results might be reported through Kaplan-Meier plot.
Tertiary/exploratory	
Change (percent and absolute) in lipidomics parameters in lesional and non-lesional skin including the ratio of EOS CER and NS CER, and FLG breakdown products of UCA and PCA concentrations over time. Global characterization of protein-bound CER over time.	Lipidomics data will be summarized with descriptive statistics. Multivariate unsupervised and supervised analyses will be performed as appropriate.
Changes (percent and absolute) in the expression of proteins associated with skin barrier function including keratin intermediate filaments, proteins associated with inflammatory response, and glycolysis and oxidative stress response proteins in STS protein extracts over time.	Proteomics data will be summarized with descriptive statistics. Multivariate unsupervised and supervised analyses will be performed as appropriate.
Change (percent and absolute) in epidermal hypertrophy parameters including epidermal thickness (µm), superficial plexus depth (µm), blood vessel diameter (µm) and density (segments/mm^2) measured by OCT over time.	Raw data and change from baseline; ie, absolute and percentage changes, will be summarized with descriptive statistics (such as mean, median, SD, minimum, and maximum) by groups and time points. For follow-up period data, change from Week16 (or last visit prior to use of rescue treatment) will also be reported.
Change (percent and absolute) in stratum corneum lipid structure measured by FTIR spectroscopy in conjunction with tape-stripping over time.	Profiles over study days will be generated for individual values and group means for raw data and absolute change from baseline/Week 16.
Changes in expression of genes associated with epidermal differentiation, barrier and lipid metabolism, and Type 2 inflammation over time.	Skin transcriptome phenotype data will be summarized with descriptive statistics. Multivariate unsupervised and supervised analyses will be performed as appropriate.

Endpoint	Statistical analysis methods
Change (percent and absolute) in CDLQI over time. Change (percent and absolute) in EASI over time. Change (percent and absolute) in ISS for target lesion over time. Change (percent and absolute) in POEM over time. Change (percent and absolute) in assessment of worst itch NRS over time. Change (percent and absolute) in sleep disturbance NRS over time. Change (percent and absolute) in skin pain NRS over time. Change (percent and absolute) in photograph outputs (eg, severity score) obtained from skin imaging over time.	Raw data and change from baseline; ie, absolute and percentage changes, will be summarized with descriptive statistics (such as mean, median, SD, minimum, and maximum) by groups and time points. For follow-up period data, change from Week16 (or last visit prior to use of rescue treatment) will also be reported.
Correlation between baseline values of TEWL before and after STS, and TEWL AUC in lesional and non-lesional skin of AD patients with the following baseline measures: • EASI • Targeted lesion ISS • PRO measures (POEM, CDLQI, worst itch NRS, sleep disturbance NRS, and skin pain NRS) • Lipidomics in STS (ratio of EOS CER to NS CER) • FLG breakdown products of UCA and PCA concentrations in STS • Image-derived severity score in targeted lesional skin • Key components of skin proteomics in STS (expression of proteins associated with skin barrier function) • Quantified epidermal hypertrophy and structure changes in OCT and FTIR measures • Key components of gene expression from transcriptomics	Scatterplot to visualize the form of the relationship between variables will be produced for patients' cohort at specific time points: ie, baseline, Week 8, Week 16 and Week 28, based on the mITT population. Pearson correlation will be assessed at each defined time points for EASI, ISS, POEM, CDLQI, worst itch NRS, sleep disturbance NRS, skin pain NRS, photograph outputs, OCT outputs:ie, epidermal thickness, supervidial plexus depth, blood vessel diameter and density, FTIR measures and key components of protein and gene expression. Pearson correlation coefficient and p-value will be reported. Spearman's rank correlation will be assessed at each defined time points for ISS. Spearman correlation coefficient and p-value will be reported. As an exploratory analysis repeated measures correlation method might be used to assess the overall correlation pooling data from baseline, Week 8, Week 16 and Week 28 (45).
Correlation between percent change from baseline in TEWL before and after STS, and TEWL AUC in lesional and non-lesional skin of AD patients at Week 8,Week 16 and Week 28 with corresponding change from baseline in the following measures: • EASI • Targeted lesion ISS • PRO measures (POEM, CDLQI, worst itch NRS, sleep disturbance NRS, and skin pain NRS)	

Endpoint	Statistical analysis methods
• Lipidomics in STS (ratio of EOS CER to NS CER) • FLG breakdown products of UCA and PCA concentrations in STS • Image-derived severity score in targeted lesional skin • Key components of skin proteomics in STS (expression of proteins associated with skin barrier function) • Quantified epidermal hypertrophy and structure changes in OCT and FTIR measures • Key components of gene expression from transcriptomics	

AD = Atopic dermatitis; AE = Adverse Events; AD = Atopic Dermatitis; AUC = area under curve; CDLQI = Children's Dermatology Life Quality Index; EASI = Eczema Area and Severity Index; EoT = End of Treatment Phase; EoS = End of Study; EOS CER = E for esterified/O for omega-hydroxy/S for sphingosin ceramides; FLG = Filaggrin; FTIR = Fourier-transform infrared spectroscopy; IGA = Investigator Global Assessment; ISS = Individual Signs Score; mITT = modified intention-to-treat; NRS = Numerical Rating Scale; NS CER = non-hydroxy fatty acid sphingosine ceramides; OCT = optical coherence tomography; PCA = pyrrolidone carboxylic acid; PRO = patient reported outcome; POEM = Patient Oriented Eczema Measure; SAE = Serious Adverse Events; SC = Subcutaneous; SD = standard deviation; STS = Skin Tape Stripping; TEWL = Transepidermal Water Loss; UCA = urocanic acid; UNSCH = unscheduled; V = Visit; W = Week

9.4.8 Safety analyses

The safety evaluation will be based on the review of vital signs parameters and reported AEs. The safety analysis will be conducted according to the Sponsor's document "Analysis and reporting of safety data from Clinical Trials through the CSR" (BTD-009536).

All the safety analyses will be performed using the safety population. When applicable, results will be by cohorts and overall.

9.4.8.1 Adverse events

9.4.8.1.1 Definitions

Adverse events will be coded to a "Preferred Term (PT)" and "High Level Group Term (HLGT)", "High Level Term (HLT)" and primary "System Organ Class (SOC)" using the Medical Dictionary for Regulatory Activities (MedDRA, version currently in use by the Sponsor at the time of database lock).

For patients' cohort, they will be classified into predefined standard categories according to chronological criteria:

- Pre-treatment AEs: AEs that occurred, worsened or became serious during the pre-treatment period defined as the time between informed consent signature and the first IMP administration.

- Treatment emergent AEs (TEAEs): AEs that occurred, worsened or became serious during the TEAE period defined as the time from the first IMP administration up to the EoT visit (EoT included).
- Post-TEAEs: AEs that occurred, worsened or became serious during the post-TEAE period defined as the time starting after the TEAE period (including the follow-up period).

Treatment-emergent AEs will be assigned to the treatment received at the time of the AE onset.

If the onset date (or time) of an AE (occurrence, worsening or becoming serious) is incomplete or missing, then the AE will be considered as a TEAE unless a partial date (or time) shows it as a pre- or post-treatment event.

For all healthy volunteers, safety data will be considered as part of a single period starting with the signature of the informed consent and ending with the EoS visit (EoS included).

All AEs reported in the study will be listed, sorted by subject/patient, onset date and time.

9.4.8.1.2 Treatment-emergent adverse events

The following TEAEs summaries will be provided for the patients' cohort of the safety population:

Overview of TEAEs: number and percentage of subjects with any TEAE, any serious TEAE, any TEAE leading to death (if any occurred), any TEAE leading to permanent treatment discontinuation, and any TEAE of special interest.

- Summary of TEAEs by primary SOC and PT:
  - Number and percentage of patients with at least 1 TEAE;
  - Number of occurrences of TEAEs.

Patients presenting TEAEs will be listed sorted by primary SOC and PT. By definition, no TEAEs summary will be displayed for healthy volunteers.

AEs that occur outside the treatment-emergent period or in healthy volunteers will be summarized separately.

9.4.8.1.3 Deaths, serious, and other significant adverse events

Any deaths, serious and other significant AEs will be listed.

9.4.8.1.4 Adverse events leading to treatment discontinuation

Any AEs leading to permanent treatment discontinuation will be listed.

9.4.8.1.5 Adverse events of special interest (AESI)

Number (%) of subjects experiencing treatment emergent AESI will be presented by AESI category and PT, sorted by decreasing incidence of PT within each AESI category.

9.4.8.2 Vital signs

Heart rate, systolic and diastolic blood pressures (SBP and DBP), body temperature and respiratory rate will be analyzed as raw parameter value and change from baseline.

Body weight will be analyzed as raw parameter value and percent change from baseline. BMI will be analyzed as raw parameter value.

Baseline definition

The values to be used as baselines will be the Day 1 (Visit 02, Week 0) predose assessment. If any of the scheduled baseline tests are repeated for any subject/patients, the last rechecked values will be considered as baselines, provided, for patients, they were done before the IMP administration of the respective treatment phase, and in the same condition.

Abnormalities analyses

For vital sign parameters, analysis will be performed using all post-baseline assessments done during the TEAE period/safety period, including all unplanned and rechecked values. Counts of subjects with PCSAs will be presented by cohort, regardless of the baseline status. PCSA values are defined as abnormal values considered medically important by the Sponsor according to predefined criteria/thresholds based on literature review and defined by the Sponsor.

A listing of individual data from subjects/patients with post-baseline PCSAs will be provided; values will be flagged when reaching the PCSA criteria.

Descriptive statistics and plots

For heart rate, blood pressures, body temperature and respiratory rate, raw data and absolute changes from baseline will be summarized in descriptive statistics (such as mean, median, SD, minimum, and maximum) for patients' cohort and scheduled time of measurement.

For body weight, raw data and percent change from baseline will be summarized in descriptive statistics (such as mean, median, SD, minimum, and maximum) by cohort and scheduled time of measurement.

9.5 INTERIM ANALYSES

A data quality control interim analysis for data quality purposes will be conducted after 10 patients are included and have completed the TEWL assessments at Day 1.

The interim analysis will be performed using the safety population for safety parameters, the mITT and ITT populations for TEWL parameters. Only primary and secondary objectives for which data are available will be analyzed as described in Section 9.

As it is an open-label study with no predefined hypothesis testing there is no issue regarding blinding or multiplicity adjustment.

There will be 2 analyses, 1 for the treatment phase and 1 for the follow-up period. None of these will be considered as interim analysis.

9.6 DATA MONITORING COMMITTEE (DMC) OR OTHER REVIEW BOARD

No DMC or other review board is planned for this study.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 APPENDIX 1: REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1.1 Regulatory and ethical considerations

- This study will be conducted in accordance with the protocol and with the following:
  - Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and the applicable amendments and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines
  - Applicable ICH GCP Guidelines
  - Applicable laws and regulations (eg, data protection law as General Data Protection Regulation [GDPR])
- The protocol, protocol amendments, ICF, Investigator Brochure, and other relevant documents (eg, advertisements) must be submitted to an IRB/IEC by the Investigator and reviewed and approved by the IRB/IEC before the study is initiated.
- Any amendments to the protocol will require IRB/IEC approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants.
- The Investigator will be responsible for the following:
  - Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/IEC Determining whether an incidental finding should be returned to a participant and, if it meets the appropriate criteria, to ensure the finding is returned (an incidental finding is a previously undiagnosed medical condition that is discovered unintentionally and is unrelated to the aims of the study for which the tests are being performed). The following should be considered when determining the return of an incidental finding:
    - The return of such information to the study participant (and/or his/her designated healthcare professional, if so designated by the participant) is consistent with all applicable national, state, or regional laws and regulations in the country where the study is being conducted, and
    - The finding reveals a substantial risk of a serious health condition or has reproductive importance, AND has analytical validity, AND has clinical validity.
    - The participant in a clinical study has the right to opt out of being notified by the Investigator of such incidental findings. In the event that the participant has opted out of being notified and the finding has consequences for other individuals, eg, the finding relates to a communicable disease, Investigators should seek independent ethical advice before determining next steps.

- In case the participant has decided to opt out, the Investigator must record in the site medical files that she/he does not want to know about such findings.

- Notifying the IRB/IEC of SAEs or other significant safety findings as required by IRB/IEC procedures.
- Providing oversight of the conduct of the study at the site and adherence to requirements of 21 CFR, ICH guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations.

As applicable, according to Directive 2001/20/EC, the Sponsor will be responsible for obtaining approval from the Competent Authorities of the EU Member States and/or Ethics Committees, as appropriate, for any amendments to the clinical trial that are deemed as "substantial" (ie, changes which are likely to have a significant impact on the safety or physical or mental integrity of the clinical trial participants or on the scientific value of the trial) prior to their implementation.

10.1.2 Financial disclosure

Investigators and sub-Investigators will provide the Sponsor with sufficient, accurate financial information as requested to allow the Sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the course of the study and for 1 year after completion of the study.

10.1.3 Informed consent process

- The Investigator or his/her representative will explain the nature of the study to the participant or his/her legally authorized representative and answer all questions regarding the study.
- Participants and the parents, or guardians of participants must be informed that their participation is voluntary. The parents, or guardians of participants will be required to sign a statement of informed consent that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act (HIPAA) requirements, where applicable, and the IRB/IEC or study center.
- For the children participation, local law must be observed in deciding whether 1 or both parents/guardians consent is required. If only 1 parent or guardian signs the consent form, the Investigator must document the reason for only 1 parent or guardian's signature.
- The medical record must include a statement that written informed consent was obtained before the participant was enrolled in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.
- The parents, or guardians of participants must be re-consented to the most current version of the ICF(s) during their participation in the study.
- A copy of the ICF(s) must be provided to the participant or the participant's legally authorized representative.

The ICF will contain a separate section that addresses new extra samples for optional exploratory research. The Investigator or authorized designee will explain to each participant the objectives of the exploratory research. Participants will be told that they are free to refuse to participate and may withdraw their consent at any time and for any reason during the storage period. A separate consent will be required to document a participant's agreement to allow any remaining specimens to be used for exploratory research. Participants who decline to participate in this optional research will not provide this separate consent.

10.1.4 Data protection

All personal data collected related to participants, Investigators, or any person involved in the study, which may be included in the Sponsor's databases, shall be treated in compliance with all applicable laws and regulations including the GDPR.

Data collected must be adequate, relevant and not excessive, in relation to the purposes for which they are collected. Each category of data must be properly justified and in line with the study objective.

Participant race and ethnicity will be collected in this study because TEWL may be influenced by ethnicity (46) and could therefore substantially influence the results of this study.

- Participants will be assigned a unique identifier by the Sponsor. Any participant records or datasets that are transferred to the Sponsor will contain the identifier only; participant names or any information which would make the participant identifiable will not be transferred to the Sponsor.
- The participant and the parents, or guardians of participants must be informed that his/her personal study-related data will be used by the Sponsor in accordance with local data protection law. The level of disclosure must also be explained to the participant as described in the informed consent.
- The participant and the parents, or guardians of participants must be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the Sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.
- When archiving or processing personal data pertaining to the Investigator and/or to the participants, the Sponsor shall take all appropriate measures to safeguard and prevent access to this data by any unauthorized third party.
- Participant data are intended to be used for the whole drug development program from collection to reimbursement.

10.1.5 Dissemination of clinical study data

Sanofi shares information about clinical trials and results on publically accessible websites, based on company commitments, international and local legal and regulatory requirements, and other clinical trial disclosure commitments established by pharmaceutical industry associations. These websites include clinicaltrials.gov, EU clinicaltrialregister (eu.ctr), and sanofi.com, as well as some national registries.

In addition, results from clinical trials in patients are required to be submitted to peer-reviewed journals following internal company review for accuracy, fair balance and intellectual property. For those journals that request sharing of the analyzable data sets that are reported in the publication, interested researchers are directed to submit their request to clinicalstudydatarequest.com.

Individual participant data and supporting clinical documents are available for request at clinicalstudydatarequest.com. While making information available we continue to protect the privacy of participants in our clinical trials. Details on data sharing criteria and process for requesting access can be found at this web address: clinicalstudydatarequest.com.

10.1.6 Data quality assurance

- All participant data relating to the study will be recorded on printed or eCRF unless transmitted to the Sponsor or designee electronically (eg, laboratory data). The Investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the eCRF.
- The Investigator must maintain accurate documentation (source data) that supports the information entered in the eCRF.
- The Investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents.
- Monitoring details describing strategy (eg, risk-based initiatives in operations and quality such as Risk Management and Mitigation Strategies and Analytical Risk-Based Monitoring), methods, responsibilities and requirements, including handling of noncompliance issues and monitoring techniques (central, remote, or on-site monitoring) are provided in separate study documents.
- The Sponsor or designee is responsible for the data management of this study including quality checking of the data.
- The Sponsor assumes accountability for actions delegated to other individuals (eg, Contract Research Organizations).
- Study monitors will perform ongoing source data verification to confirm that data entered into the eCRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of participants are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.
- Records and documents, including signed ICFs, pertaining to the conduct of this study must be retained by the Investigator for 25 years after the signature of the final study report unless local regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of the Sponsor. No records may be transferred to another location or party without written notification to the Sponsor.

10.1.7 Source documents

- Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the Investigator's site.
- Data reported on the eCRF 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 study. Also, current medical records must be available.
- Definition of what constitutes source data can be found in the study reference manual.

10.1.8 Study and site start and closure

The signature of the informed consent by the first participant is considered the first act of recruitment and will be the study start date.

The Sponsor or designee reserves the right to close the study site or terminate the study at any time for any reason at the sole discretion of the Sponsor. Study sites will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a study-site closure visit has been performed.

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

Reasons for study termination by the Sponsor, as well as reasons for the early closure of a study site by the Sponsor or Investigator may include but are not limited to:

- For study termination:
  - Information on the product leads to doubt as to the benefit/risk ratio
  - Discontinuation of further study intervention development
- For site termination:
  - Failure of the Investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the Sponsor's procedures, or GCP guidelines
  - Inadequate or no recruitment (evaluated after a reasonable amount of time) of participants by the Investigator
  - Total number of participants included earlier than expected

If the study is prematurely terminated or suspended, the Sponsor shall promptly inform the Investigators, the IECs/IRBs, the regulatory authorities, and any contract research organization(s) used in the study of the reason for termination or suspension, as specified by the applicable regulatory requirements. The Investigator shall promptly inform the subject and should assure appropriate subject therapy and/or follow-up.

10.1.9 Publication policy

- The results of this study may be published or presented at scientific meetings. If this is foreseen, the Investigator agrees to submit all manuscripts or abstracts to the Sponsor before submission. This allows the Sponsor to protect proprietary information and to provide comments.
- The Sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the Sponsor will generally support publication of multicenter studies only in their entirety and not as individual site data. In this case, a coordinating Investigator will be designated by mutual agreement.
- Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

10.2 APPENDIX 2: CLINICAL LABORATORY TESTS

Pregnancy testing

Refer to Section 5.1 (Inclusion criteria) for screening pregnancy criteria.

Table 5 - Protocol-required laboratory assessments

Laboratory assessments	Parameters
Urine pregnancy test	Highly sensitive urine human chorionic gonadotropin (hCG) pregnancy test (as needed for female of childbearing potential)[a]

a Local urine testing will be standard for the protocol unless serum testing is required by local regulation or IRB/IEC.

Investigators must document their review of each laboratory safety report.

10.3 APPENDIX 3: ADVERSE EVENTS: DEFINITIONS AND PROCEDURES FOR RECORDING, EVALUATING, FOLLOW-UP, AND REPORTING

10.3.1 Definition of AE

AE definition

- An AE is any untoward medical occurrence in a patient or clinical study participant, temporally associated with the use of study intervention, whether or not considered related to the study intervention.
- NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of study intervention.

Events meeting the AE definition

- The signs, symptoms, and/or clinical sequelae resulting from lack of efficacy will be reported as AE or SAE if they fulfill the definition of an AE or SAE. Also, "lack of efficacy" or "failure of expected pharmacological action" also constitutes an AE or SAE."
- Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (eg, electrocardiogram, radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the Investigator (ie, not related to progression of underlying disease), eg:
  - Symptomatic and/or
  - Requiring either corrective treatment or consultation, and/or
  - Leading to IMP discontinuation or modification of dosing, and/or
  - Fulfilling a seriousness criterion, and/or
  - Defined as an AESI
- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition.
- New conditions detected or diagnosed after study intervention administration even though it may have been present before the start of the study.
- Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction.
- Signs, symptoms, or the clinical sequelae of a suspected overdose of either study intervention or a concomitant medication.
- "Lack of efficacy" or "failure of expected pharmacological action" per se will not be reported as an AE or SAE. Such instances will be captured in the efficacy assessments. However, the signs, symptoms, and/or clinical sequelae resulting from lack of efficacy will be reported as AE or SAE if they fulfill the definition of an AE or SAE.

Events NOT meeting the AE definition

- Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with the underlying disease, unless judged by the Investigator to be more severe than expected for the participant's condition.
- The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the participant's condition.
- Medical or surgical procedure (eg, endoscopy, appendectomy): the condition that leads to the procedure is the AE.
- Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

10.3.2 Definition of SAE

If an event is not an AE per definition above, then it cannot be an SAE even if serious conditions are met (eg, hospitalization for signs/symptoms of the disease under study, death due to progression of disease).

A SAE is defined as any untoward medical occurrence that, at any dose:

A) Results in death

B) Is life-threatening

The term "life-threatening" in the definition of "serious" refers to an event in which the participant 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.

C) Requires inpatient hospitalization or prolongation of existing hospitalization

In general, hospitalization signifies that the participant has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious.

Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.

D) Results in persistent disability/incapacity

- The term disability means a substantial disruption of a person's ability to conduct normal life functions.
- This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (eg, sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.

E) Is a congenital anomaly/birth defect

F) Other situations:

- Medical or scientific judgment should be exercised in deciding whether SAE 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 participant or may require medical or surgical intervention to prevent 1 of the other outcomes listed in the above definition. These events should usually be considered serious.
- Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

10.3.3 Recording and follow-up of AE and/or SAE

AE and SAE recording

- When an AE/SAE occurs, it is the responsibility of the Investigator to review all documentation (eg, hospital progress notes, laboratory reports, and diagnostics reports) related to the event.
- The Investigator will then record all relevant AE/SAE information in the eCRF.
- It is not acceptable for the Investigator to send photocopies of the participant's medical records in lieu of completion of the AE/SAE eCRF page.
- There may be instances when copies of medical records for certain cases are requested. In this case, all participant identifiers, with the exception of the participant number, will be redacted on the copies of the medical records before submission.
- The Investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.

Assessment of intensity

The Investigator will make an assessment of intensity for each AE and SAE reported during the study and assign it to 1 of the following categories:

- Mild: An event that is easily tolerated by the participant, causing minimal discomfort and not interfering with everyday activities.
- Moderate: An event that causes sufficient discomfort and interferes with normal everyday activities.
- Severe: An event that prevents normal everyday activities. An AE that is assessed as severe should not be confused with a SAE. Severe is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe.

An event is defined as "serious" when it meets at least 1 of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.

Assessment of causality

- The Investigator is obligated to assess the relationship between study intervention and each occurrence of each AE/SAE.
- A "reasonable possibility" of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.
- The Investigator will use clinical judgment to determine the relationship.
- Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study intervention administration will be considered and investigated.

- The Investigator will also consult the Investigator's Brochure and/or Product Information, for marketed products, in his/her assessment.
- For each AE/SAE, the Investigator **must** document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality.
- There may be situations in which an SAE has occurred and the Investigator has minimal information to include in the initial report. However, **it is very important that the Investigator always makes an assessment of causality for every event before the initial transmission of the SAE data.**
- The Investigator may change his/her opinion of causality in light of follow-up information and send a SAE follow-up report with the updated causality assessment.
- The causality assessment is 1 of the criteria used when determining regulatory reporting requirements.

Follow-up of AEs and SAEs

- The Investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by the Sponsor to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.
- If a participant dies during participation in the study or during a recognized follow-up period, the Investigator will provide Sponsor with a copy of any post-mortem findings including histopathology.
- New or updated information will be recorded in the originally completed eCRF.
- The Investigator will submit any updated SAE data to the Sponsor within 24 hours of receipt of the information.

10.3.4 Reporting of SAEs

SAE reporting to Sponsor via an electronic data collection tool

- The primary mechanism for reporting an SAE to Sponsor will be the electronic data collection tool.
- If the electronic system is unavailable, then the site will use the paper SAE data collection tool in order to report the event within 24 hours.
- The site will enter the SAE data into the electronic system as soon as it becomes available.
- After the study is completed at a given site, the electronic data collection tool will be taken off-line to prevent the entry of new data or changes to existing data.
- If a site receives a report of a new SAE from a study participant or receives updated data on a previously reported SAE after the electronic data collection tool has been taken off-line, then the site can report this information on a paper SAE form or to the Sponsor by telephone.
- Contacts for SAE reporting can be found in the study reference manual.

10.4 APPENDIX 4: CONTRACEPTIVE GUIDANCE AND COLLECTION OF PREGNANCY INFORMATION

DEFINITIONS:

Woman of childbearing potential (WOCBP)

A female in this study is considered fertile following menarche.

If fertility is unclear and a menstrual cycle cannot be confirmed before first dose of study intervention, additional evaluation should be considered.

Females in premenarchal are not considered WOCBP

CONTRACEPTION GUIDANCE:

Recommended contraceptive methods are listed below in Table 6.

Table 6 - Highly effective contraceptive methods

Highly effective contraceptive methods that are user dependent[a] *Failure rate of <1% per year when used consistently and correctly*
Combined (estrogen- and progestogen-containing) hormonal contraception associated with inhibition of ovulation oral intravaginal transdermal
Progestogen-only hormone contraception associated with inhibition of ovulation oral injectable
Highly effective methods that are user independent
Implantable progestogen-only hormonal contraception associated with inhibition of ovulation Intrauterine device (IUD) Intrauterine hormone-releasing system (IUS) Bilateral tubal occlusion
(Vasectomized partner is a highly effective contraceptive method provided that the partner is the sole sexual partner of the woman of childbearing potential and the absence of sperm has been confirmed. If not and less than 1 year after vasectomy, additional highly effective method of contraception should be used. Spermatogenesis cycle is approximately 90 days.)
Sexual abstinence *Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatment. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the participant.*

a Typical use failure rates may differ from those when used consistently and correctly. Use should be consistent with local regulations regarding the use of contraceptive methods for participants participating in clinical studies.

COLLECTION OF PREGNANCY INFORMATION:

Male participants with partners who become pregnant

- The Investigator will attempt to collect pregnancy information on any male participant's female partner who becomes pregnant while the male participant is in this study. This applies only to male participants who receive dupilumab.
- After obtaining the necessary signed informed consent from the pregnant female partner directly, the Investigator will record pregnancy information on the appropriate form and submit it to the Sponsor within 24 hours of learning of the partner's pregnancy. The female partner will also be followed to determine the outcome of the pregnancy. Information on the status of the mother and child will be forwarded to the Sponsor. Generally, the follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any termination of the pregnancy will be reported regardless of fetal status (presence or absence of anomalies) or indication for the procedure.

Female participants who become pregnant

- The Investigator will collect pregnancy information on any female participant who becomes pregnant while participating in this study. The initial information will be recorded on the appropriate form and submitted to the Sponsor within 24 hours of learning of a participant's pregnancy.
- The participant will be followed to determine the outcome of the pregnancy. The Investigator will collect follow-up information on the participant and the neonate and the information will be forwarded to the Sponsor. Generally, follow-up will not be required for longer than 6 to 8 weeks beyond the estimated delivery date. Any termination of pregnancy will be reported, regardless of fetal status (presence or absence of anomalies) or indication for the procedure.
- Any pregnancy complication or elective termination of a pregnancy for medical reasons will be reported as an AE or SAE.
- A spontaneous abortion (occurring at <22 weeks gestational age) or still birth (occurring at >22 weeks gestational age) is always considered to be an SAE and will be reported as such.
- Any post-study pregnancy related SAE considered reasonably related to the study intervention by the Investigator will be reported to the Sponsor as described in Section 8.3.5.
- While the Investigator is not obligated to actively seek this information in former study participants, he or she may learn of an SAE through spontaneous reporting.
- Any female participant who becomes pregnant while participating in the study will discontinue study intervention.

10.5 APPENDIX 5: GENETICS

Use/Analysis of DNA

- Genetic variation may impact a participant's response to study intervention, susceptibility to, and severity and progression of disease. Variable response to study intervention may be due to genetic determinants that impact drug absorption, distribution, metabolism, and excretion; mechanism of action of the drug; disease etiology; and/or molecular subtype of the disease being treated. Therefore, where local regulations and IRB/IEC allow, a saliva swab sample will be collected for DNA analysis from consenting participants.
- For all participants, a saliva swab sample will be collected for FLG gene sequencing analysis.
- For participants, who consent to optional genetic research, a saliva swab sample will be collected and isolated DNA will be stored for broad genetic analysis, which may include, but is not limited to, whole genome or exome sequencing.
- The samples may be analyzed as part of a multi-study assessment of genetic factors involved in the response to dupilumab or study interventions of this class to understand study disease or related conditions.
- The results of genetic analyses may be reported in the CSR or in a separate study summary.
- The Sponsor will store the DNA samples in a secure storage space with adequate measures to protect confidentiality.
- The samples will be retained while research on dupilumab or study interventions of this class continues but no longer than 15 years or other period as per local requirements.

10.6 APPENDIX 6: COUNTRY-SPECIFIC REQUIREMENTS

Not applicable.

10.7 APPENDIX 7: ABBREVIATIONS

AD:	atopic dermatitis
AE:	adverse events
AESI:	adverse events of special interest
ALT:	alanine transaminase
ATR:	attenuated total reflectance
AUC:	area under the curve
BMI:	body mass index
CER:	ceramides
COVID-19:	coronavirus disease 2019
CSR:	clinical study report
EASI:	eczema area and severity index

eCRF:	electronic case report form
eDiary:	electronic diary
EoS:	end of study
EOS:	E for esterified/O for omega-hydroxy/S for sphingosin
EoT:	end of treatment
FLG:	filaggrin
FTIR:	fourier transform infrared spectroscopy
GCP:	Good Clinical Practice
ICF:	informed consent form
ICH:	International Council for Hormonisation
IEC:	Independent Ethics Committees
IGA:	Investigator Global Assessment
IL:	interleukin
IMP:	investigational medicinal product
IRB:	Institutional Review Board
ISS:	individual signs score
ITT:	intent-to-treat
LC-ESI-MS/MS:	liquid chromatography electrospray ionization tandem mass spectrometry
LC-MS/MS:	liqulid chromatography with tandem mass spectrometry
mITT:	modified intent-to-treat
NRS:	numerical rating scale
NS:	non-hydroxy fatty acid sphingosine
OCT:	optical coherence tomography
PCA:	pyrrolidone carboxylic acid
PCSA:	potentially clinically significantly abnormalities
POEM:	patient oriented eczema measure
PRO:	patient reported outcome
PT:	preferred term
QOL:	quality of life
SAE:	serious adverse event
SAP:	statistical analysis plan
SC:	subcutaneous
SD:	standard deviation
SoA:	schedule of activities
SOC:	system organ class
STD:	statistical technical document
SUSAR:	suspected unexpected serious adverse reactions
TCS:	topical corticosteroid
TEAE:	treatment emergent adverse events
TEWL:	transepidermal water loss
UHPLC-ESI-MS/MS:	ultra-high performance liqulid chromatography electrospray ionization mass spectroscopy
ULN:	upper limit of normal

10.8 APPENDIX 8: PROTOCOL AMENDMENT HISTORY

Not applicable.

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