NCT04447417

AMENDED CLINICAL TRIAL PROTOCOL 01

Protocol title:	**Open label exploratory study to evaluate the effect of dupilumab on skin barrier function in patients with moderate to severe atopic dermatitis**
Protocol number:	**LPS15991**
Amendment number:	**01**
Compound number (INN/Trademark):	**SAR231893/REGN668 dupilumab/Dupixent**
Study phase:	**Phase 4**
Short title:	**Dupilumab skin BArrier function and LIpidomics STudy in Atopic Dermatitis - BALISTAD**
Sponsor name:	**Sanofi-Aventis Recherche & Développement**
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Monitoring Team's Representative Name and Contact Information	[redacted]

Regulatory agency identifier number(s):

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PROTOCOL AMENDMENT SUMMARY OF CHANGES

DOCUMENT HISTORY

Document	Country/Countries impacted by amendement	Date, version
Amended Clinical Trial Protocol 01	All	28 July 2020, version 1 (electronic 1.0)
Original Clinical Trial Protocol	All	17 March 2020, version 1 (electronic 4.0)

AMENDED PROTOCOL 01 (28 July 2020)

This amended protocol 01 is considered to be substantial based on the criteria set forth in Article 10(a) of Directive 2001/20/EC of the European Parliament and the Council of the European Union.

OVERALL RATIONALE FOR THE AMENDMENT

To further characterize the treatment effect of dupilumab in adolescent and adult patients with moderate to severe atopic dermatitis in reference to match healthy controls, proteomics assessment and transcriptomics assessment from skin tape strips has been added.

These assessments will allow to better assess the mechanism of dupilumab in atopic dermatitis. To have comparable data in lesional skin (LS) and non-lesional skin (non-LS) of AD patients as well as in normal skin of healthy volunteers the number of skin tape strips collected has been set to 20 for each skin area.

A statement concerning the risk-benefit assessment due to COVID-19 has been added.

Protocol amendment summary of changes table

Section # and Name	Description of Change	Brief Rationale
Throughout the document	Inconsistencies and clarification were added to increase clarity of the protocol	Correct inconsistencies
1.1 Synopsis, Secondary endpoints	Additional secondary endpoints for TEWL assessment for lesional, non-lesional and normal skin added	Number of STS assessments in lesional skin increased from 10 to 20, therefore more TEWL assessments are to be conducted. Endpoint assessment in lesional, non-lesional and normal skin has been aligned.
1.1 Synopsis, Tertiary objectives and endpoints	“Proteomics in STS” added	Proteomics will provide additional mechanistic insight in treatment-related effect
1.1 Synopsis, Tertiary objectives and endpoints	“Transcriptomics in STS” added	Transcriptomics will provide additional mechanistic insight in treatment-related effect

Section # and Name	Description of Change	Brief Rationale
1.3 Schedule of activities / AD patients	Line added with proteomics assessment schedule	Implementation of specimen sampling for proteomics
1.3 Schedule of activities / AD patients	Line added with transcriptomics assessment schedule	Implementation of specimen sampling for transcriptomics
1.3 Schedule of activities / AD patients	Footnotes and annotations were added and modified	Increase in clarity and precision
1.4 Schedule of activities / Healthy volunteers	Line added with proteomics assessment schedule	Implementation of specimen sampling for proteomics
1.4 Schedule of activities / Healthy volunteers	Line added with transcriptomics assessment schedule	Implementation of specimen sampling for proteomics
1.4 Schedule of activities / Healthy volunteers	Footnotes and annotations were added and modified	Increase in clarity and precision
Multiple locations in protocol	Proteomics and transcriptomics were added, whenever applicable	Adding proteomics and transcriptomics requires adaptation of multiple sections
2.1 Study rationale	Text added for rationale of the study	Proteins and gene expression changes in AD patients as reason for amendment
2.2 Background	Reference to methods for proteomics and transcriptomics methods	Explanation of methods used
2.3.2 Benefit/risk related to COVID-19	Benefit/risk assessment due to COVID-19 pandemic	Specific section concerning COVID-19 added
3 Objectives and endpoints	Adapted objectives and endpoints due to more STS and TEWL timepoints and due to transcriptomics/proteomics assessment	Number of STS increased, proteomics and transcriptomics added
5.1 Inclusion criteria	For several inclusion criteria the specification has been added for which population this would be applicable. The criteria remain unchanged.	Clarity and precision
5.4 Screen failures	Re-screening of participants is allowed once	To reduce risk of not including participants with temporary reasons for ineligibility
6.1 Study interventions administered	A monitoring period of at least 30 minutes after study drug administration added. Self-injection training modified.	Monitoring period for adverse symptoms specified. Self-injection training modified for more flexibility at the study site.

Section # and Name	Description of Change	Brief Rationale
6.2.1 Storage and handling of IMP	AD patients to inform study site in case of issues with storing IMP	Tracking of adequate storage of IMP provided to patients for self-administration
8 Study assessments and procedures	Monitoring of COVID-19 situation and implementation of alternative methods for patient follow-up described	COVID-19 specific text to describe mechanisms by which the sponsor will react to the pandemic or other public health emergencies
8.2.2 Standardized photographs	Separate ICF for photographs mentioned	Study participants will need to separately consent to publication of photos taken during the study
8.2.3 Questionnaires	Sentence added that patients will bring diary to the site at each visit for review	Clarity and precision
8.9.2 Proteomics	Description of proteomics assessment	Proteomics is an additional study objective
8.9.3 Transcriptomics	Description of transcriptomics assessment	Transcriptomics is an additional study objective
8.9.4 Optional samples for biomarker research	Section has been re-numbered	Due to inclusion of new sections for proteomics and transcriptomics
9.4.7.1 Description of efficacy variables	Biostatistical analysis of additional endpoints	To address additional TEWL and STS endpoints. To address addition of proteomics and lipidomics analysis
9.4.8.1.1 Definitions	Grading of adverse events by NCI CTCAE criteria had been deleted	Grading of non-serious adverse events will be in three categories (mild, moderate, severe) and not in five (mild, moderate, severe, life-threatening, death). The latter two would be documented as serious adverse events
10.1.3 Informed consent process	Separate ICF for agreement to publication of photos taken during the study added	Consistency and clarity to Section 8.2.2

1 PROTOCOL SUMMARY

1.1 SYNOPSIS

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

Short title: Dupilumab skin BArrier function and LIpidomics STudy in Atopic Dermatitis - BALISTAD

Rationale:

To assess the effect of dupilumab on skin barrier function measured as transepidermal water loss (TEWL) and on skin lipidomics in conjunction with skin tape stripping (STS) in atopic dermatitis (AD) patients.

Objectives and endpoints

Objectives	Endpoints
Primary	
• Evaluate changes in skin barrier function with transepidermal water loss (TEWL) assessed after skin tape stripping (STS) in pre-defined lesional skin in 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 Week16 in AD patients.
Secondary	
• Evaluate changes in skin barrier function with TEWL assessed after STS in pre-defined lesional and non-lesional skin in 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 Week16 in AD patients. • Change (percent and absolute) from baseline in TEWL after 20 STS assessed on non-lesional skin at Week16 in AD patients. • Change (percent and absolute) from baseline in TEWL after 20 STS assessed on normal skin at Week16 in healthy volunteers. • Change (percent and absolute) from baseline in TEWL after 15 STS assessed on lesional skin at Week16 in AD patients. • Change (percent and absolute) from baseline in TEWL after 15 STS assessed on non-lesional skin at Week16 in AD patients. • Change (percent and absolute) from baseline in TEWL after 15 STS assessed on normal skin at Week16 in healthy volunteers. • Change (percent and absolute) from baseline in TEWL after 10 STS assessed on lesional skin at Week16 in AD patients.

Objectives	Endpoints
	• Change (percent and absolute) from baseline in TEWL after 10 STS assessed on non-lesional skin at Week16 in AD patients. • Change (percent and absolute) from baseline in TEWL after 10 STS assessed on normal skin at Week16 in healthy volunteers. • Absolute change from baseline in TEWL after 5 STS assessed on lesional skin at Week16 in AD patients. • Change (percent and absolute) from baseline in TEWL after 5 STS assessed on non-lesional skin at Week16 in AD patients. • Change (percent and absolute) from baseline in TEWL after 5 STS assessed on normal skin at Week16 in healthy volunteers.
• Evaluate time course of skin barrier function with TEWL assessed before and after STS in pre-defined lesional and non-lesional skin in 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 before STS on lesional skin in AD patients over time. • Change (percent and absolute) from baseline in TEWL before STS on non-lesional skin in AD patients over time. • Change (percent and absolute) from baseline 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) from baseline in TEWL after STS assessed on lesional skin in AD patients over time. • Change (percent and absolute) from baseline in TEWL after STS assessed on non-lesional skin in AD patients over time. • Change (percent and absolute) from baseline 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 pre-defined lesional and non-lesional skin in patients with moderate to severe AD 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 ω-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 at Week 8 and Week 16, respectively. • Global characterization of protein-bound ceramides over time.
• Evaluate dupilumab treatment effect on skin proteomics using STS samples in both predefined lesional and non-lesional skin in patients with moderate-to-severe AD 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 skin transcriptome using STS samples in both predefined lesional and non-lesional skin in patients with moderate-to-severe AD 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 severity assessed by standard AD severity assessments (Eczema Area and Severity Index (EASI), SCORing Atopic Dermatitis (SCORAD), local lesion severity on target lesion), patient reported outcomes (PRO) (Patient Oriented Eczema Measure (POEM), Dermatology Life Quality Index (DLQI) / Children Dermatology Life Questionnaire Index (CDLQI), peak pruritus Numerical Rating Scale (NRS), and quality of sleep NRS), standardized photos, and the biomarker profiles of lipidomics, proteomics, and transcriptomics assessed in the STS sample.	• Change (percent and absolute) from baseline in EASI over time. • Change (percent and absolute) from baseline in SCORAD over time. • Change (percent and absolute) from baseline in Individual Signs Score (ISS) for target lesion over time. • Change (percent and absolute) from baseline in PRO (POEM) over time. • Change (percent and absolute) from baseline in PRO of DLQI in patients 18 years of age and older / CDLQI in patients ≥12 and <18 years of age over time. • Change (percent and absolute) from baseline in PRO of peak pruritus NRS over time. • Change (percent and absolute) from baseline in PRO of quality of sleep NRS over time. • Change (percent and absolute) from baseline in photograph outputs (eg, severity score) obtained from skin imaging over time. • Correlation between baseline values of TEWL before STS and TEWL AUC in lesional and non-lesional skin of AD patients with the following baseline measures: - Local target lesion erythema and edema/papulation; ISS - EASI, SCORAD

Objectives	Endpoints
	- PRO measures (POEM, DLQI, CDLQI, peak pruritus NRS, and quality of sleep NRS) - Lipidomics in STS (ratio of EOS CER to NS CER) - Filaggrin breakdown products of UCA and PCA concentrations in STS - Key components of skin proteomics in STS (expression of proteins associated with skin barrier function) - Key components of gene expression from transcriptomics - Image-derived severity score in targeted lesional skin • Correlation between percent change from baseline in TEWL before STS and TEWL AUC in lesional and non-lesional skin of AD patients at Week 8 and Week 16 with corresponding change from baseline in the following measures: - Local target lesion erythema and edema/papulation; ISS - EASI, SCORAD - PRO measures (POEM, DLQI, CDLQI, peak pruritus NRS, and quality of sleep NRS) - Lipidomics in STS (ratio of EOS CER to NS CER) - Filaggrin breakdown products of UCA and PCA concentrations in STS - Key components of skin proteomics in STS (expression of proteins associated with skin barrier function) - Key components of gene expression from transcriptomics - Image-derived severity score in targeted lesional skin

Overall design:

- Phase IV.
- Two study sites (Dr Leung, Denver, USA; Dr Bissonnette, Innovaderm, Montreal, Canada).
- Open-label, exploratory study.
- Approximately 24 patients with moderate to severe AD will be enrolled to achieve 20 evaluable patients.
- Approximately 24 evaluable healthy volunteers matched for age, gender, location of skin area, and study site will serve as a reference comparator for skin barrier function.

This is a 16-week, open label, exploratory study designed to investigate dupilumab's effect on skin barrier function as measured by TEWL before and after skin tape stripping in approximately 10 adolescent and approximately 10 adult patients with moderate to severe AD (not more than 12 of either group). During the first 2 treatment weeks, patients will have 2 on-site visits/week, followed by one on-site visit/week up to Week 4, one on-site visit every two weeks from Week 4 to Week 8, and one on-site visit every 4 weeks up to Week 16 End of Treatment phase visit (EoT) thereafter.

A follow-up visit by phone 4 weeks after the last study assessment at Week 16 will end the study for each participant (End of Study: EoS). The maximum duration of the study per participant will be 24 weeks.

Lesional and non-lesional skin areas for TEWL assessment and STS will be identified on the upper limbs or lower limbs at baseline ("predefined skin area"). TEWL and STS-assessment at pre-defined non-lesional (normal looking) skin area in AD patients should be made about 4 cm from the edge of the lesional area.

Within the predefined lesional skin (LS) and non-lesional skin (non-LS) areas three closely adjacent spots will be identified for subsequent skin barrier function assessment (3 spots on lesional skin, 3 spots on non-lesional skin). These spots must not overlap with each other once skin tapes are applied.

The skin barrier function without and before STS will be assessed using TEWL measurement on each of the three spots within these pre-defined skin areas at each visit.

Repeated TEWL assessment in pre-defined lesional and non-lesional skin areas of AD patients after STS for lipidomics, proteomics and transcriptomics analysis in STS samples will be conducted at baseline (Week 0, Day 1), Week 2 (Day 15), Week 4 (Day 29), Week 8 (Day 57), Week, 12 (Day 85), and at EoT at Week 16 (Day 113).

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

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

Skin barrier function in approximately twenty healthy volunteers matched for age, gender, location of targeted lesion area, and study site to the AD cases will be assessed in a similar manner at baseline, twice a week during the first two weeks, once a week during Week 3 to Week 4, every two weeks from Week 4 to Week 8, and every four weeks up to Week 16 thereafter, serving as a reference comparator for skin barrier function.

Within the targeted skin area of healthy volunteers -the predefined skin location for TEWL and STS must be identical to the lesional area of the patient to which a healthy volunteer is matched- three 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 three spots at each visit.

On normal skin in healthy volunteers at each visit at baseline, Week 2, Week 4, Week 8, Week 12, and Week 16, 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.

In particular, STS assessment on baseline (Week 0, Day 1), Week 8 (Day 57) and Week 16 (Day 113) will be conducted on the first spot within the location-matched, normal skin. STS assessment on Week 2 (Day 15) will be conducted on the second spot. STS assessment on Week 4 (Day 29) and Week 12 (Day 85) will be conducted on the third spot.

Study drug will be administered on-site by study site staff at baseline, Week 2, 4, 6, 8 and Week 12, after the TEWL assessment has been completed. Study drug will be self-administered at home by AD patients at Week 10 and 14.

Intervention groups and duration:

This is a 16-week, open-label study of dupilumab in moderate to severe AD 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 phone call will be 24 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 subcutaneous injection.
- Route(s) of administration: subcutaneous injection (SC).

Dupilumab 300 mg

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

- For AD patients aged ≥12 to <18 years the dose regimen will be based on body weight on Day 1. No change in dosing will be made if body weight increases or decreases during the study. Dose allocation will be according to the following table:

Body Weight	Loading Dose on Day 1	Subsequent Doses (Q2W) [a]
less than 60 kg	400 mg (two 200 mg injections)	200 mg
60 kg or more	600 mg (two 300 mg injections)	300 mg

a Every second week (Q2W)

- AD patients age 18 years and older will receive a SC loading dose of 600 mg on Day 1, followed by 300 mg Q2W SC through Week 14. Drug will be administered by study site staff at baseline, Week, 2, 4, 6, 8 and 12. Drug will be self-administered by AD patients at home at Week 10 and 14.

Post-trial access to study medication

- After the study is completed, patients will not be provided with any further study medication as part of this protocol.

Duration of study period (per participant)

Study participation for each patient and healthy volunteer will be a total of approximately 24 weeks including:

- Screening period: Up to 4 weeks from signed informed consent.
- Open label dupilumab treatment (AD patients) and observation period (healthy volunteers): 16 weeks from baseline on Day 1.
- Follow-up period: 4 weeks after the EOT visit at Week 16.

Statistical considerations: Analyses will be descriptive and explorative.

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.

- **Sample size calculations**
  - Sample size for this exploratory study was based on medical/clinical judgement and is consistent with the sample size from similar studies in the literature (1). 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.
- **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 subjects will provide normal reference values.

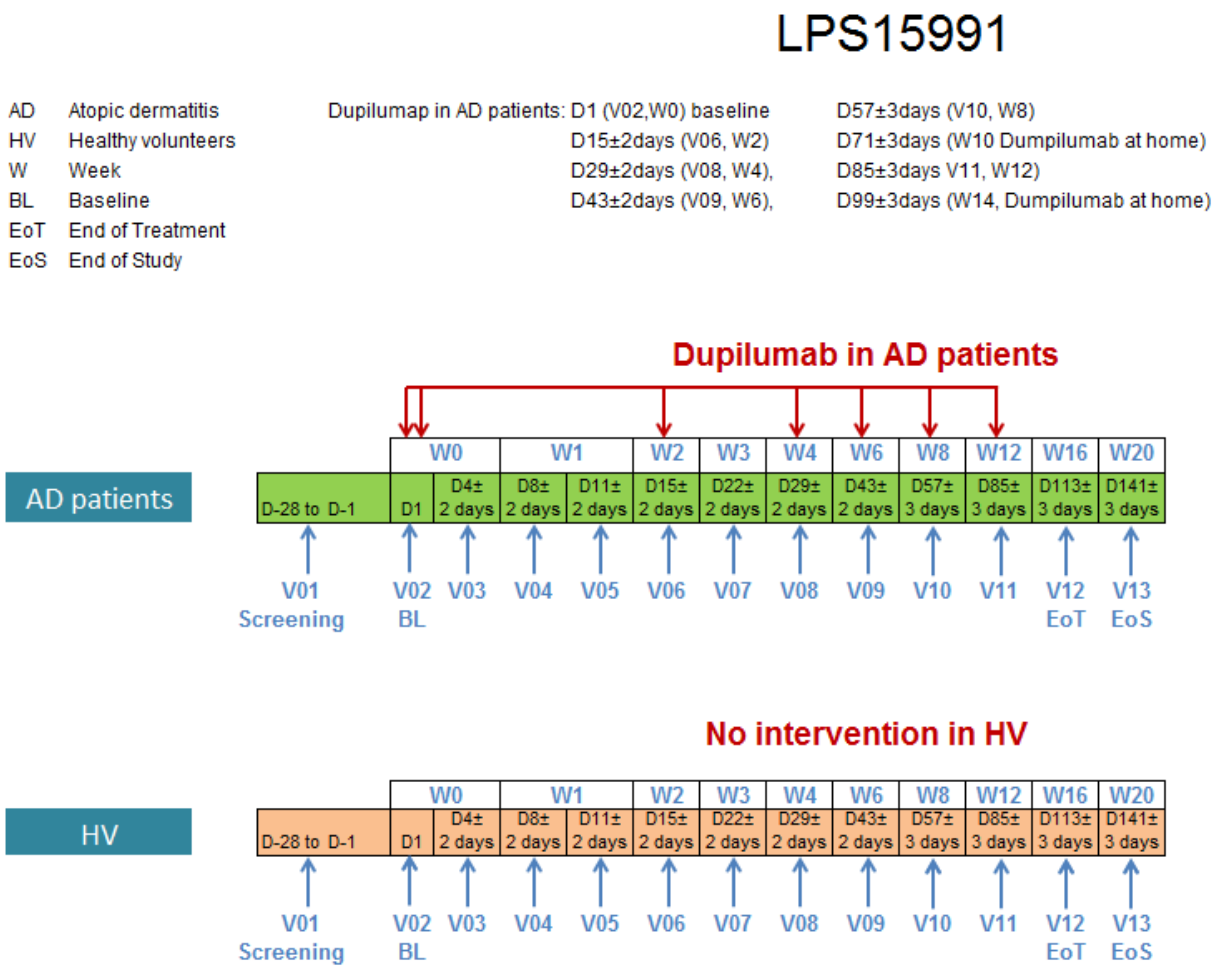
- **Safety/other analysis:**

  - The safety evaluation will be conducted on all AD patients who receive at least one dose of dupilumab or had at least one TEWL/STS assessment performed, and on all healthy volunteers, who had at least one 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 (PCSAs)) and reported adverse event.

Data Monitoring Committee: None

1.2 SCHEMA

Figure 1 - Graphical study design



1.3 SCHEDULE OF ACTIVITIES (SOA) / AD PATIENTS

Phase	Screening	Baseline	Treatment Phase									EoT	EoS[a]	Un-scheduled Visit	Premature EoT
Day	**D-28 to D-1**	**D 1**	**D4 ±2 days**	**D8 ±2 days**	**D11 ±2 days**	**D15 ±2 days**	**D22 ±2 days**	**D29 ±2 days**	**D43 ±2 days**	**D57 ±3 days**	**D85 ±3 days**	**D113 ±3 days**	**D141 ±3 days**	**UNSCH**	
Week		**W0**		**W1**		**W2**	**W3**	**W4**	**W6**	**W8**	**W12**	**W16**	**W20**		
Visits	**V01**	**V02**	**V03**	**V04**	**V05**	**V06**	**V07**	**V08**	**V09**	**V10**	**V11**	**V12**	**V13**		
Informed consent / assent form	X														
Inclusion/exclusion criteria	X	X													
Medical/surgical history / IGA / Demographics	X														
Prior/concomitant medications / procedures	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Confirm emollient and washing compliance[j]	X	X	X	X	X	X	X	X	X	X	X	X		X	X
Study treatment administration															
SC administration of dupilumab[b]		X				X		X	X	X	X			(X)	
Study drug self-administration training by site staff[o]										X				(X)	
Study drug will be handed out at V10 (Week 8) for self-administration at Week 10 and at V11 (Week 12) for self-administration at Week 14										X	X			(X)	
Compliance check of self-administrated study drug at Week 10 and Week 14											X	X		(X)	X

Phase	Screening	Baseline	Treatment Phase									EoT	EoS[a]	Un-scheduled Visit	Premature EoT
Day	D-28 to D-1	D 1	D4 ±2 days	D8 ±2 days	D11 ±2 days	D15 ±2 days	D22 ±2 days	D29 ±2 days	D43 ±2 days	D57 ±3 days	D85 ±3 days	D113 ±3 days	D141 ±3 days	UNSCH	
Week		W0		W1		W2	W3	W4	W6	W8	W12	W16	W20		
Visits	V01	V02	V03	V04	V05	V06	V07	V08	V09	V10	V11	V12	V13		
Skin barrier function test in lesional and non-lesional skin[c]															
TEWL at baseline/ before STS[l,m]		X	X	X	X	X	X	X	X	X	X	X		(X)	X
TEWL after STS assessment[h]		X[i]				X[i]		X[i]		X[i]	X[i]	X[i]		(X)[n]	X[n]
Lipidomics, filaggrin (FLG) breakdown products, proteomics, and transcriptomics assessment from skin tape strip[j]		X[i]				X[i]		X[i]		X[i]	X[i]	X[i]		(X)[n]	X[n]
Standardized photographs of lesional and non-lesional skin areas used for TEWL[l]		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 on lesion erythema and edema/papulation	X	X	X	X	X	X	X	X	X	X	X	X		(X)	X
SCORAD	X	X	X	X	X	X	X	X	X	X	X	X		(X)	X
Patient reported outcomes (PRO)															
POEM		X		X		X	X	X	X	X	X	X		(X)	X
DLQI/CDLQI[f]		X		X		X	X	X	X	X	X	X		(X)	X
Peak pruritus NRS[g]		X	X	X	X	X	X	X	X	X	X	X		(X)	X

Phase	Screening	Baseline	Treatment Phase									EoT	EoS[a]	Un-scheduled Visit	Premature EoT
Day	**D-28 to D-1**	**D 1**	**D4 ±2 days**	**D8 ±2 days**	**D11 ±2 days**	**D15 ±2 days**	**D22 ±2 days**	**D29 ±2 days**	**D43 ±2 days**	**D57 ±3 days**	**D85 ±3 days**	**D113 ±3 days**	**D141 ±3 days**	**UNSCH**	
Week		**W0**		**W1**		**W2**	**W3**	**W4**	**W6**	**W8**	**W12**	**W16**	**W20**		
Visits	**V01**	**V02**	**V03**	**V04**	**V05**	**V06**	**V07**	**V08**	**V09**	**V10**	**V11**	**V12**	**V13**		
Quality of sleep NRS[g]		X	X	X	X	X	X	X	X	X	X	X		(X)	X
Safety															
Adverse event collection	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height		X													
Body weight		X										X			X
Vital signs[d]	X	X								X		X		(X)	X
Physical examination	X	X[k]										X[k]			X[k]
Pregnancy test (urine), WOCBP only[e]	X	X						X		X	X	X			
DNA sampling for filaggrin gene sequencing		X													
Optional assessments															
DNA sampling for future research		X													
Biomarker research samples (serum and plasma)		X										X			X

a EoS safety follow-up by phone.

b Loading dose dupilumab arm 600 mg (300 mg x 2 ie, two 300 mg syringes) for all adult patients and for those adolescents weighing 60 kg and above, and 400 mg (200 mg x 2 ie. two 200 mg syringes) for adolescent patients below 60 kg, followed by bi-weekly SC dosing of 300 mg dupilumab to adults and adolescents weighing 60 kg and above and bi-weekly SC dosing of 200 mg to adolescents weighing below 60 kg. Dupilumap administration on following days: D1 (V02,W0) baseline, D15±2days (V06, W2), D29±2days (V08, W4), D43±2days (V09, W6), D57±3days (V10, W8), D71±3days (W10; self-administration of dupilumab at home), D85±3days V11, W12), D99±3days (W14; self-administration of dupilumab at home).

c Assessments on treatment days are to be conducted before administration of dupilumab.

d Vital signs, including systolic and diastolic blood pressure (mmHg), pulse rate (beats per minute), body temperature (°C), and respiratory rate will be measured at Screening, Baseline at Week 0, Week 8, and Week 16 (EoT). Height (cm) will be measured at Baseline only. Body weight (kg) will be measured at Baseline, and Week 16 (EoT).

e Only for women of childbearing potential. Pregnancy will lead to definitive treatment discontinuation in all cases.

f DLQI will be used for adults; CDLQI will be used for adolescents.

g Peak puritus NRS and quality of sleep NRS will be assessed daily from Day-7 to Day -1; daily from Day 1 to Day 29 and then for the 7 days prior to Week 6, Week 8, to Week 12 and to Week 16 by e-diary. Patients will bring the eDiary to the site at each visit. eDiary will be reviewed for any questionnaires omission and dispensed back to patients at each visit.

h TEWL to be conducted before STS and then after 5, 10, 15, and 20 STS in pre-defined lesional and non-lesional skin. All skin tape strips will be collected and stored.

i At Week 0, 8 and 16 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 Week 4 and 12 STS will be conducted on the third spot within the predefined skin area. TEWL will be assessed in lesional and non-lesional skin before STS and after 5, 10, 15, and 20 STS. All skin tape strips will be collected and stored for lipidomics, proteomics, and transcriptomics analyses.

j Emollients should NOT be applied from Day -7 to the EoT (Week 16, Day 113) to the targeted, pre-defined 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. Compliance must be documented in participant's source data. 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.

k Limited to skin-related physical examination at baseline and EoT visit.

l Pre-defined lesional (3 spots) and non-lesional skin areas (3 spots), whereby the assessment spots on non-lesional skin are located about 4 cm apart from the edge of the AD lesion.

m TEWL will be measured at each of the three closely adjacent spots within the predefined skin areas at all visits as baseline assessment; if there is STS, subsequent TEWL assessment will only be done at the lesional and no-lesional spot, at which STS is performed.

n 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.

o Study participants may by trained for study drug self-administration starting from D1 (Visit 2). Training should be completed and documented at Week 8 (Visit 10). Site staff has to verify patients' injection technique before they are allowed to self-inject at home. On-site injections may be performed by either site staff or AD patient or caregiver/parent/legal representative if previously trained on self-injection technique

EASI = Eczema Area and Severity Index; EoT = End of Treatment Phase; EoS = End of Study; IGA = Investigator Global Assessment; SCORAD = SCORing Atopic Dermatitis; STS = Skin Tape Stripping; TEWL = Transepidermal Water Loss; V = Visit; W = Week; WOCBP = Women of Childbearing Potential

1.4 SCHEDULE OF ACTIVITIES (SOA) / HEALTHY VOLUNTEERS

Phase	Screening	Baseline	Observation Period									EoT	EoS[a]	Un-scheduled Visit	Premature EoT
Day	**D-28 to D-1**	**D 1**	**D4 ±2 days**	**D8 ±2 days**	**D11 ±2 days**	**D15 ±2 days**	**D22 ±2 days**	**D29 ±2 days**	**D43 ±2 days**	**D57 ±3 days**	**D85 ±3 days**	**D113 ±3 days**	**D141 ±3 days**		
Week		**W0**		**W1**		**W2**	**W3**	**W4**	**W6**	**W8**	**W12**	**W16**	**W20**		
Visits	**V01**	**V02**	**V03**	**V04**	**V05**	**V06**	**V07**	**V08**	**V09**	**V10**	**V11**	**V12**	**V13**		
Informed consent/ assent form	X														
Inclusion/exclusion criteria	X	X													
Matching by age and gender[i]	X														
Medical/surgical history / Demographics	X														
Prior/concomitant medications / procedures	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Confirm emollient and washing compliance[f]	X	X	X	X	X	X	X	X	X	X	X	X		X	X
Skin barrier function test in pre-defined normal skin															
TEWL at baseline/ before STS[h]		X	X	X	X	X	X	X	X	X	X	X		(X)	X
TEWL after STS assessment[d]		X[e]				X[e]		X[e]		X[e]	X[e]	X[e]		(X)[j]	X[j]
Lipidomics, FLG breakdown products, proteomics, and transcriptomics assessment from skin tape strip[k]		X[e]				X[e]		X[e]		X[e]	X[e]	X[e]		(X)[j]	X[j]
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

Phase	Screening	Baseline	Observation Period									EoT	EoS[a]	Un-scheduled Visit	Premature EoT
Day	**D-28 to D-1**	**D 1**	**D4 ±2 days**	**D8 ±2 days**	**D11 ±2 days**	**D15 ±2 days**	**D22 ±2 days**	**D29 ±2 days**	**D43 ±2 days**	**D57 ±3 days**	**D85 ±3 days**	**D113 ±3 days**	**D141 ±3 days**		
Week		**W0**		**W1**		**W2**	**W3**	**W4**	**W6**	**W8**	**W12**	**W16**	**W20**		
Visits	**V01**	**V02**	**V03**	**V04**	**V05**	**V06**	**V07**	**V08**	**V09**	**V10**	**V11**	**V12**	**V13**		
Safety															
Adverse event collection	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height		X													
Body weight		X										X			X
Vital signs[b]	X	X								X		X		(X)	X
Physical examination	X	X[g]										X[g]			X[g]
Pregnancy test (urine), WOCBP only[c]	X	X						X		X	X	X			
DNA sampling for filaggrin gene sequencing		X													
Optional assessments															
DNA sampling for future research		X													
Biomarker research samples (serum and plasma)		X										X			X

a EoS safety follow-up by phone.

b Vital signs, including systolic and diastolic blood pressure (mmHg), pulse rate (beats per minute), body temperature (°C), and respiratory rate will be measured at Screening, Baseline at Week 0, Week 8, and Week 16 ("EoT"). Height (cm) will be measured at Baseline only. Body weight (kg) will be measured at Baseline and at Week 16 ("EoT").

c Only for women of childbearing potential. Pregnancy in a healthy volunteer will lead to definitive discontinuation of the study in all cases.

d TEWL to 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 Week 0, 8 and 16 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 Week 4 and 12 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 will be collected and stored for lipidomics, proteomics, and transcriptomics analyses.

f Emollients should NOT be applied from Day -7 to EoT (Week 16, Day 113) to the targeted, pre-defined 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. Compliance must be documented in participant's source data. 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.

g Limited to skin-related physical examination at baseline and end-of-study visit.

h TEWL will be measured at each of the three closely adjacent spots within the predefined skin areas at all visits as baseline assessment; if there is STS, subsequent TEWL assessment will only be done at the spot, at which STS is performed.

i Age- and gender-matched to a selected AD patient by study site. Adolescents aged 12 to 17 years will be matched by puberty status. Adults will be matched by age as close as possible within a range of 10 years above or below the age of the matched AD patient.

j 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.

k First spot to be assessed by STS at Baseline, Week 8 and Week 16, second spot to be assessed by STS at Week 2, third spot to be assessed by STS at Week 4 and Week 12

EoT = End of Treatment Phase; for healthy volunteers this is the end of the "Observation Period"; EoS = End of Study; STS = Skin Tape Stripping; TEWL = Transepidermal Water Loss; V = Visit; W = Week; WOCBP = Women of Childbearing Potential

2 INTRODUCTION

Dupilumab is a human monoclonal antibody (2, 3) 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 (4).

Dupilumab is the first targeted biologic agent approved in the United States of America for subcutaneous administration every 2 weeks (Q2W) for the treatment of patients aged ≥12 years with moderate-to-severe atopic dermatitis (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 (5) demonstrated that dupilumab-mediated inhibition of interleukin-(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 IgE, 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 filaggrin (FLG) , loricrin, claudins, and elongation of very long chain fatty acids protein (ELOVL3), and significantly reduced lesional epidermal thickness (5, 6). 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 an intact skin barrier is critical and its impairment leads to downstream signals that aim to restore barrier homeostasis (7).

2.1 STUDY RATIONALE

The objective of this study is to assess dupilumab treatment effect on skin barrier function measured by TEWL and measurement of lipids, proteins and gene expression associated with skin barrier function from skin tape stripping in patients with moderate to severe atopic dermatitis, and to evaluate the relationship between skin barrier function and disease activity measured by clinical assessments and patient reported outcomes, as well as high definition standard photography. 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 (8). 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 (8, 9). 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 (AD) 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 (10). AD 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 (11). 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 (12).

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. Filaggrin mutations are the most profound single-gene defects involved in AD (13). FLG 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 (14).

Healthy epidermis has lipids that are mostly composed of ceramides (CER), free fatty acids, and cholesterol (15, 16), with very little presence of other lipids. A very specific, highly hydrophobic group of CER, called esterified omega-hydroxy sphingosine (EOS) ceramides, is present only in the skin. Fatty acids are also unique in skin ceramides, 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 ceramides and other ceramides with very long–chain fatty acids (C22–C30), and short-chain non-hydroxy fatty acids sphingosine ceramides (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 transepidermal water loss in AD skin (12). 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 (17).

Leung et al have pioneered novel methods to profile skin through STS analysis combined with lipidomics, proteomics and transcriptomics (17). 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 diagnostic examination of genomic, lipidomic, and proteomic expression profiles in non-lesional and lesional AD skin (10).

2.3 BENEFIT/RISK ASSESSMENT

Dupilumab has demonstrated a positive benefit-risk profile and is approved for treatment in patients with moderate to severe AD aged 12 years and above in the United States, Canada and the European Union. Dupilumab dose in this study is consistent with the approved label. The safety data observed so far in completed and currently ongoing studies in atopic dermatitis has demonstrated a satisfactory safety profile.

More detailed information about the known and expected benefits and risks and reasonably expected adverse events of dupilumab may be found in the Investigator's Brochure.

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 the 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 one localized area of skin, presumably due to the mild mechanical disturbance. The erythema is expected to resolve within 12 hours without sequelae.

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 Health Questionnaires

There is a possibility that participants may find questions too personal. Participants 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 severe AD or severe asthma 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 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 stick. 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 a total of 16 weeks of dupilumab treatment during study, and health volunteer's cohort will receive no treatment during study.

One potential benefit for participants in the AD cohort is that their AD may improve and their itch may be reduced while on dupilumab; 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 are given 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.

Although the results of this study may be of commercial value, it will be explained to participants that they will not have ownership of these results, and will not benefit financially 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 to 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. Information obtained from these studies will improve our understanding of the immune, epidermal, and barrier defects observed in study participants.

2.3.3 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 6-18 year olds) 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 LPS15991 is adult and adolescent patients with moderate-to-severe Atopic Dermatitis (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.

Based on the aforementioned potential benefits to patients participating in LPS15991, 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 adult and adolescent population with moderate-to-severe AD. The efficacy and safety of dupilumab were confirmed based on data that included in pivotal Phase 3 trials in which dupilumab were used alone or in combination with topical corticosteroids (TCS) and compared to placebo or TCS alone in patients with moderate to severe AD. In the trials, patients treated with dupilumab alone or in combined with TCS experienced significant improvements in overall disease severity, skin clearance and quality of life. Therefore, the operation of LPS15991 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).

2.3.4 Overall benefit: risk conclusion

Taking into account the measures taken to minimize risk to participants participating in this study, the potential risks identified in association with dupilumab, TEWL and skin tape stripping are justified by the anticipated benefits that may be afforded to participants with atopic dermatitis. 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	Endpoints
Primary	
• Evaluate changes in skin barrier function with transepidermal water loss (TEWL) assessed after skin tape stripping (STS) in pre-defined lesional skin in patients with moderate to severe 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 pre-defined lesional and non-lesional skin in 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 Week16 in AD patients. • Change (percent and absolute) from baseline in TEWL after 20 STS assessed on non-lesional skin at Week16 in AD patients. • Change (percent and absolute) from baseline in TEWL after 20 STS assessed on normal skin at Week16 in healthy volunteers. • Change (percent and absolute) from baseline in TEWL after 15 STS assessed on lesional skin at Week16 in AD patients. • Change (percent and absolute) from baseline in TEWL after 15 STS assessed on non-lesional skin at Week16 in AD patients. • Change (percent and absolute) from baseline in TEWL after 15 STS assessed on normal skin at Week16 in healthy volunteers. • Change (percent and absolute) from baseline in TEWL after 10 STS assessed on lesional skin at Week16 in AD patients. • Change (percent and absolute) from baseline in TEWL after 10 STS assessed on non-lesional skin at Week16 in AD patients. • Change (percent and absolute) from baseline in TEWL after 10 STS assessed on normal skin at Week16 in healthy volunteers. • Absolute change from baseline in TEWL after 5 STS assessed on lesional skin at Week16 in AD patients. • Change (percent and absolute) from baseline in TEWL after 5 STS assessed on non-lesional skin at Week16 in AD patients. • Change (percent and absolute) from baseline in TEWL after 5 STS assessed on normal skin at Week16 in healthy volunteers.

Objectives	Endpoints
• Evaluate time course of improvement in skin barrier function with TEWL assessed before and after STS in pre-defined lesional and non-lesional skin in 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 before STS on lesional skin in AD patients over time. • Change (percent and absolute) from baseline in TEWL before STS on non-lesional skin in AD patients over time. • Change (percent and absolute) from baseline 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) from baseline in TEWL after STS assessed on lesional skin in AD patients over time. • Change (percent and absolute) from baseline in TEWL after STS assessed on non-lesional skin in AD patients over time. • Change (percent and absolute) from baseline 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 pre-defined lesional and non-lesional skin in patients with moderate to severe AD 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 EOS CER and NS CER, and FLG breakdown products of UCA and PCA concentrations at Week 8 and Week 16, respectively. • Global characterization of protein-bound ceramides over time.
• Evaluate dupilumab treatment effect on skin proteomics using STS samples in both predefined lesional and non-lesional skin in patients with moderate-to-severe AD 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 skin transcriptome using STS samples in both predefined lesional and non-lesional skin in patients with moderate-to-severe AD 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 severity assessed by standard AD severity assessments (Eczema Area and Severity Index (EASI), SCORing Atopic Dermatitis (SCORAD), local lesion severity on target	• Change (percent and absolute) from baseline in EASI over time. • Change (percent and absolute) from baseline in SCORAD over time.

Objectives	Endpoints
lesion), patient reported outcomes (PRO) (Patient Oriented Eczema Measure (POEM), Dermatology Life Quality Index (DLQI) / Children Dermatology Life Questionnaire Index (CDLQI), peak pruritus Numerical Rating Scale (NRS), and quality of sleep NRS), standardized photos, and the biomarker profiles of lipidomics, proteomics, and transcriptomics assessed in the STS sample.	• Change (percent and absolute) from baseline in Individual Signs Score (ISS) for target lesion over time. • Change (percent and absolute) from baseline in PRO POEM over time. • Change (percent and absolute) from baseline in PRO of DLQI in patients 18 years of age and older / CDLQI in patients ≥12 and <18 years of age over time. • Change (percent and absolute) from baseline in PRO of peak pruritus NRS over time. • Change (percent and absolute) from baseline in PRO of quality of sleep NRS over time. • Change (percent and absolute) from baseline in photograph outputs (eg, severity score) obtained from skin imaging over time. • Correlation between baseline values and change from baseline of TEWL before STS and TEWL AUC in lesional and non-lesional skin of AD with the following measures: - Local target lesion erythema and edema/papulation; ISS - EASI, SCORAD - PRO measures (POEM, DLQI, CDLQI, peak pruritus NRS, and quality of sleep NRS) - Lipidomics in STS (ratio of EOS CER to NS CER) - FLG breakdown products of UCA and PCA concentrations in STS - Key components of skin proteomics in STS (expression of proteins associated with skin barrier function) - Key components of gene expression from transcriptomics - Image-derived severity score in targeted lesional skin • Correlation between percent change from baseline in TEWL before STS and TEWL AUC in lesional and non-lesional skin of AD patients at Week 8 and Week 16 with corresponding change from baseline in the following measures: - Local target lesion erythema and edema/papulation; ISS - EASI, SCORAD - PRO measures (POEM, DLQI, CDLQI, peak pruritus NRS, and quality of sleep NRS) - Lipidomics in STS (ratio of EOS CER to NS CER) - Filaggrin breakdown products of UCA and PCA concentrations in STS - Key components of skin proteomics in STS (expression of proteins associated with skin barrier function) - Key components of gene expression from transcriptomics - Image-derived severity score in targeted lesional skin

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 AUC after various number of STS. The stratum corneum (SC) provides skin barrier protection and controls transcutaneous water loss. TEWL measurement, which quantifies water diffusion across the SC, is commonly used for the physiologic assessment of skin barrier function (18). 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 skin tape stripping (STS) to measure skin barrier integrity (7). 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. The area under the curve (AUC) for TEWL measurements (TEWL AUC) done over a defined number of STS is used to reflect the overall integrity of the SC. Skin barrier dysfunction and increased TEWL are major pathologic features of AD (14, 19, 20). TEWL has been shown to correlate with AD severity (21, 22, 23). 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 IV open-label, exploratory study evaluating the effect of dupilumab on skin barrier function in adolescent and adult patients with moderate to severe AD with a healthy volunteer cohort as a reference comparator in two study sites.

The maximum study duration per participant will be 24 weeks. The study will comprise of:

- Screening Period 1 (Day -28 to -1): Subjects will be evaluated according to inclusion and exclusion criteria.
- Baseline Visit (Day 1): Subjects who remain eligible will be enrolled.
- 16-week treatment period for AD patients and a 16-week observation period for healthy volunteers.
- 4-week safety follow-up period.

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

The study design includes two parallel study cohorts: the AD patient's cohort with open-label dupilumab treatment for 16-weeks, and the healthy volunteer's cohort without any treatment, 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, and lipidomics, proteomics and transcriptomics from STS samples, standardized full body photographs and photographs from the targeted lesional and non-lesional areas, and clinical disease severity and patient reported outcomes.

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 skin barrier perturbation using STS to measure skin barrier function and integrity (7). 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 of 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 (24, 25). 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 an age-, gender-, targeted lesion area-, and study site-matched healthy volunteers' cohort assessed at the same time in the same measurement conditions with reference thresholds of skin barrier function evaluation indicating pathological relevance is important in interpreting study assessment results (8, 9).

4.2.1 Participant input into design

Not applicable.

4.3 JUSTIFICATION FOR DOSE

The dose selected in this study is the dose approved for the treatment of moderate to severe AD for Dupixent in US, Canada, 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 end of study visit (EoS) as shown in the Schedule of Activities in Section 1.3.

The end of the study is defined as the date of the last visit of the last participant in the study shown in the Schedule of Activities for the last participant in the trial globally.

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 12 to 65 years, with moderate to severe AD, because dupilumab as approved in patients ≥12 years with moderate to severe AD. An upper age limit has been put on the study population because skin barrier function /structure deteriorates in elderly individuals, and we want to minimize the effect of this confounding variable would have on our study interpretations.

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 12 to 65 years of age (inclusive), at the time of signing the informed consent.

Type of participant and disease characteristics

Atopic dermatitis patients and healthy volunteers:

I 02. Male or female patients.

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

I 04. Investigator Global Assessment (IGA) score of ≥3 at screening (on the 0-4 scale). (AD patients only)

I 05. Patients with moderate to severe atopic dermatitis that are eligible to be treated with dupilumab according to product monograph. (AD patients only)

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

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. (AD patients only)

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

I 09. Willing to refrain taking showers or soaking 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 the EoT visit.

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

Healthy volunteers only:

I 12. Age and gender matched to a selected AD patient by study site. Adolescents aged 12 to 17 years will be matched by post puberty status, and adults aged 18 to 65 years will be matched by age as close as possible within 10 years of age.

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

Weight

Not applicable.

Sex

I 14. Male or Female

Contraceptive use by women should be consistent with local regulations regarding the methods of contraception for those participating in clinical studies. See Section 10.4.

Informed Consent

I 15. 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. For adolescents ≥12 and <18 years of age a specific ICF must also be signed by the participant's legally authorized representative.

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, Netherton syndrome, severe photo damage).

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 dry eye syndrome that require daily use of supplemental lubrication; or individuals with ocular conditions that require the use of ocular corticosteroids or cyclosporine.

E 06. Systemic AD treatment 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 short life expectancy, patients with uncontrolled diabetes (hemoglobin A1c ≥9%), patients with 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, rheumatoid arthritis, 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 forms [eCRF], screening logs, etc).

E 09. History of hypersensitivity reaction to tape or adhesives.

Prior/concomitant therapy

E 10. Treatment with an investigational drug within 8 weeks or within 5 half-lives (if known) prior to Day 1, whichever is longer.

Prior/concurrent clinical study experience

E 11. Current participation in another investigational clinical study.

Diagnostic assessments

Not applicable.

Other exclusions

E 12. Individuals accommodated in an institution because of regulatory or legal order; prisoners or subjects who are legally institutionalized.

E 13. Participants are dependent on the Sponsor or Investigator (in conjunction with section 1.61 of the ICH-GCP Ordinance E6).

E 14. Individuals directly involved in the conduct of the study, or immediate family members of such individuals.

E 15. Any specific situation during study implementation/course that may rise ethics considerations.

E 16. Planned or anticipated major surgical procedure during the patient's participation in this study.

E 17. Pregnant or breast-feeding women, or were planning to become pregnant or breastfeed during the subject's participation in this study.

E 18. Women unwilling to use adequate birth control, if of reproductive potential* and sexually active. Adequate birth control is defined as agreement to consistently practice an effective and accepted method of contraception throughout the duration of the study and for 12 weeks after last dose of study drug. These include condom with spermicide, hormonal contraceptives, intrauterine device, or double barrier contraception (ie, condom + diaphragm) or a male partner with documented vasectomy. Additional requirements for acceptable contraception may apply in certain countries, based on local regulations. Investigators in these countries will be notified accordingly in a protocol clarification letter.

*For females, menopause is defined as at least 12 consecutive months without menses; if in question, a follicle stimulating hormone (FSH) level of ≥25 mU/mL must be documented. Hysterectomy, bilateral oophorectomy, or bilateral tubal ligation must be documented, as applicable; if documented, women with these conditions are not required to use additional contraception.

E 19. Healthy volunteers with a personal history of an atopic condition.

E 20. Healthy volunteers with use of any topical treatment anywhere except Cetaphil, Vanicream, or the preferred moisturizer not containing additives on non-targeted skin areas.

5.3 LIFESTYLE CONSIDERATIONS

- Study participants should not take a shower or soaking in a bath within 6 hours before assessment of TEWL.
- Emollients for use during the study will be provided by the study site to study participants.
- Participants should apply Cetaphil, Vanicream, or preferred same emollients not containing additives except at the pre-defined skin assessment areas up to two times daily from Day -7 to the EoT visit.
- Participants should not apply any emollients on or within 5 cm of the pre-defined skin assessment areas during the entire study period from Day-7 until the EoT visit.
- The type, amount (number of fingertip units), and frequency of topical products used during the study will be recorded at home in a medication log/diary.

5.4 SCREEN FAILURES

Screen failures are defined as participants who consent to participate in the clinical study but are subsequently found not to be eligible to be enrolled 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 adverse event (SAE). Subjects, who do not meet the criteria for participation in this study (screen failure) may be rescreened once. In such a case 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.

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 adolescent and adult moderate to severe AD patients will receive dupilumab.
- For AD patients aged ≥12 to <18 years the dose regimen will follow the table below and based on the body weight on Day 1 (the dose will not be changed during the study):

Body Weight	Loading Dose on Day 1	Subsequent Doses (Q2W)
less than 60 kg	400 mg (two 200 mg injections)	200 mg
60 kg or more	600 mg (two 300 mg injections)	300 mg

- AD patients age 18 years and older will receive a SC loading dose of 600 mg on Day 1, followed by 300 mg Q2W SC through Week 14. Drug will be administered by study site staff at baseline, Weeks 2, 4, 6, 8, 12. Drug will be self-administered by AD patients at Week 10 and 14.

One kit number list by dosage strength is generated centrally by Sanofi and IMP are packaged in accordance with these lists. The treatment will be given to the patient depending on their age and 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	A 150 mg/mL dupilumab solution in a pre-filled syringe to deliver 300 mg in 2 mL
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 14 ±2 or 3 days after an initial loading dose of 600 mg
Route of administration	Subcutaneous[a]	Subcutaneous[a]
Use	Experimental	Experimental
IMP	IMP	IMP

ARM name	Dupilumab 200	Dupilumab 300
Packaging and labeling	One glass pre-filled syringe packed in a patient kit box. Both the glass 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 the glass 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 a trained person (parent/legally authorized representative/caregiver trained by Investigator or Delegate) 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 following clinical procedures and blood collection (if any). AD patients will be monitored for at least 30 minutes after each injection. Patients should monitor themselves for at least 30 minutes after performing self-injection at home.

Study participants may by trained for study drug self-administration starting from D1 (Visit 2). Training should be completed and documented at Week 8 (Visit 10). Site staff has to verify patients injection technique before they are allowed to self-inject at home. On-site injections may be performed by either site staff or AD patient or caregiver/parent/legal representative if previously trained on self-injection technique.

Between the protocol-scheduled on-site visits, participants are allowed to self-inject IMP at home. Patient should also be trained by the site staff to recognize potential signs and symptoms of hypersensitivity reaction in order to self-monitor at home. In case of hypersensitivity symptoms the patient should contact healthcare provider/emergency. The training must be documented in the participant's study file. Participants will be dispensed an adequate amount of IMP for home administration to last through the next scheduled study visit. Participants who prefer to have a healthcare professional administer the IMP may choose to have injections administered at the study site.

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 appropriate 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 participants enrolled in the study (or their legal representatives, parents, or guardians in case of adolescents) may receive study intervention and only authorized site staff may supply

or administer study intervention. 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). Study medication may be dispensed to study participants for self-administration at Week 10 and/or Week 14. In this case study participants need to inform the site is there are any issues with temperature storage during transport, storage at home, or any other issues with IMP.

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 8.4.9).

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 trial protocol, or dispose of IMP in any other manner.

6.3 MEASURES TO MINIMIZE BIAS

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.
- PROs will be collected by electronic data capture.
- Efficacy data (TEWL) 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 to 2 very experienced study centers
- One central laboratory for lipidomics, proteomics and transcriptomics analyses to reduce skin barrier assessment variabilities.
- Standardized study assessment procedures, including TEWL and STS skin barrier function
- Standardized photography for targeted lesional and non-lesional areas, and clinical disease severity and patient reported outcome 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:

- Intervention units are returned by the participant at the next study visit.
- 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 which he/she has retrieved and source documents.

When participants are dosed at the site (see Section 1.3), they will receive study intervention 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 recorded in the 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.

When participants self-administer study intervention(s) at home (see Section 1.3) , they will document compliance on a home dosing diary. Compliance with study intervention will be assessed at each visit. Deviation(s) from the prescribed dosage regimen should be recorded in the eCRF.

6.5 CONCOMITANT THERAPY

Any medication or vaccine (including over-the-counter or prescription medicines, vitamins, and/or herbal supplements) 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 grams/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, or the preferred same 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. 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 HV for TEWL assessments from Day -7 to Day 113 (Week 16, EoT).

6.5.1 Rescue medicine

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. Investigators should make every attempt to conduct efficacy and safety assessments (eg, disease severity scores, safety labs) immediately before administering any rescue treatment. An unscheduled visit may be used for this, if necessary.

6.5.1.1 Prohibited medications and procedures

Treatment with the following concomitant medications and procedures is prohibited until the 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, CsA, AZA, MTX, MMF, 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 16. If a participant requires a vaccination prior to 16 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 is to be avoided. Moisturizers other than Cetaphil, Vanicream, or the preferred same 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 treatment with contraceptives, nasal and inhaled corticosteroids, and oral antihistamines.

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, lipidomics, proteomics and transcriptomics. See the SoA 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 e-CRF 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:

Eg, temporary intervention discontinuation decided by the Investigator corresponds to one 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 discontinuing from the study, if possible, an early discontinuation visit should be conducted, as shown in the SoA. See SoA for data to be collected at the time of

study discontinuation and follow-up and for any further evaluations that need to be completed.

- 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 Investigators should discuss with them key visits to attend. 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.

All study withdrawals should be recorded by the Investigator in the appropriate screens of the e-CRF 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. 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.
- Adherence to the study design requirements, including those specified in the SoA, 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 (eg, blood count) 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.
- 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. The medication log might look different for AD patients and healthy volunteers.
- The maximum amount of blood collected from each participant 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.

8.1 PROCEDURES PERFORMED ONLY AT THE SCREENING/BASELINE VISIT

Assessments performed only at the screening and/or baseline visit include medical history, medication history, IGA, demographics, and diagnosis of chronic AD. AD disease characteristics are assessed by clinical and patient reported outcomes.

8.2 EFFICACY ASSESSMENTS

8.2.1 Transepidermal water loss assessment and skin tape stripping

8.2.1.1 Transepidermal water loss assessment

Transepidermal water loss is a skin barrier function test that measures perspiration or water loss through the skin. Transepidermal water loss is generally higher in AD skin compared to normal skin as the barrier is disrupted allowing water to evaporate more readily. If the barrier improves with dupilumab treatment, TEWL would 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. Subjects will be acclimated to the room for a minimum of 20 minutes prior to TEWL measurements. Room temperature will be set to be within 19.0°C to 23.0°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 until Day 113 (Week 16). The first TEWL assessment at each visit will be conducted at each of the three spots in predefined lesional and non-lesional areas respectively. After start of STS all subsequent TEWL assessments at each visit will be done on the single lesional and non-lesional spot respectively at which the STS is performed (see also Figure 3).

8.2.1.2 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. 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 area under the curve for TEWL measurements over a defined number of STS reflects the overall function of the SC.

This procedure involves the defined, non-invasive application of a commercially available adhesive sheet on the surface of the skin at predefined areas. A dedicated instrument (eg, D-SQUAME Pressure Instrument D500) is used to apply a defined pressure onto the skin tape strip for 5 to 10 seconds. Thereafter the skin tape strip will be peeled off the skin in a ripping motion using a forceps (= the skin tape-stripping 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 assessment.

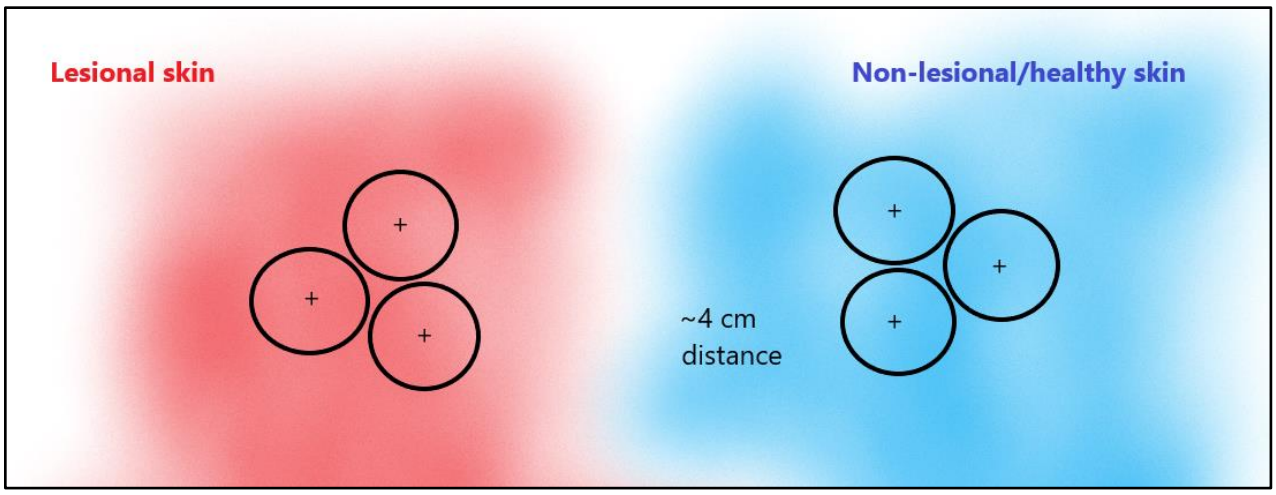
Lesional and non-lesional areas of skin will be tested. Patients will undergo skin barrier function tests at time points according to Section 1.3 before, and after skin tape stripping.

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

Specifically, lipidomic, proteomic and transcriptomics samples will be collected by STS at baseline, Week 2, 4, 8, 12, and 16. A total 2~~1~~0 skin tapes will be collected in lesional, non-lesional or normal skin, respectively.

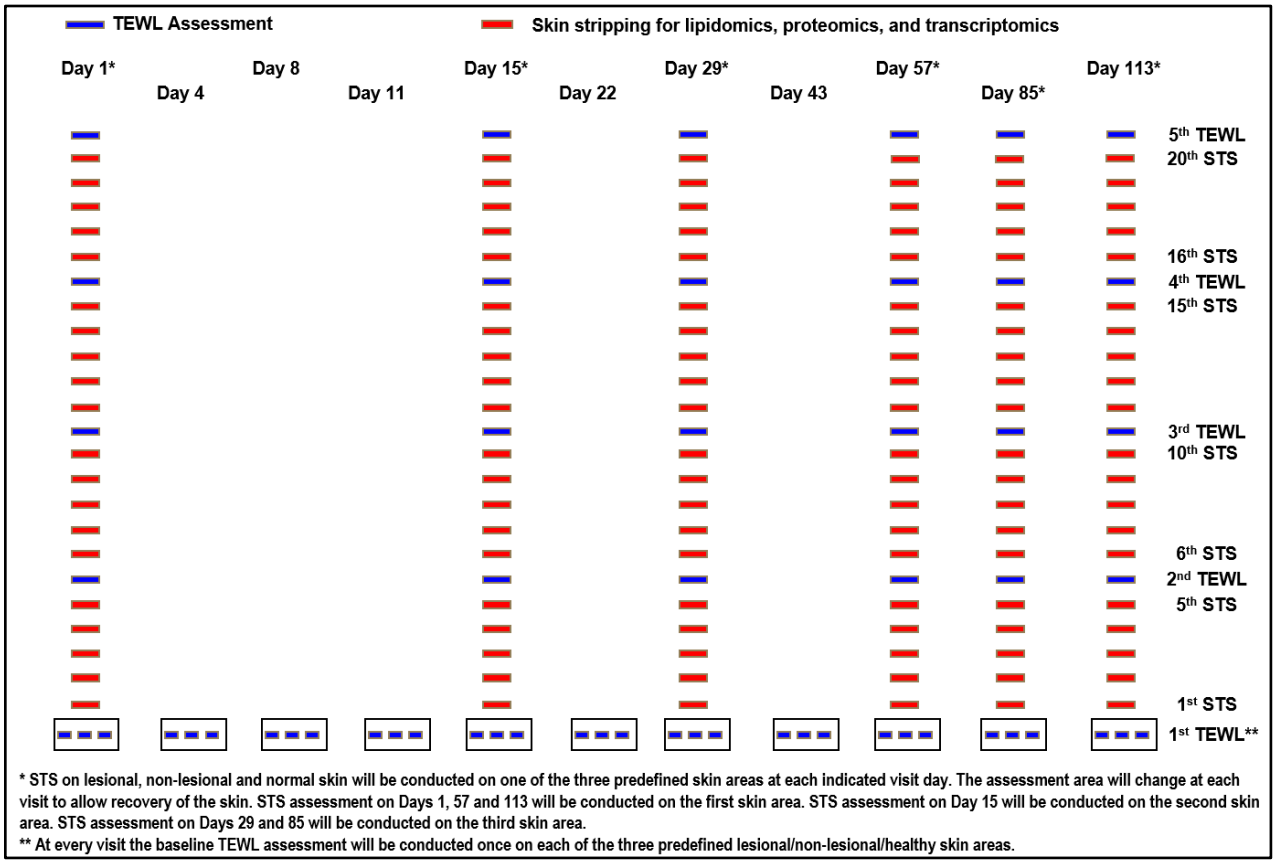
In order to allow the skin to recover from STS three closely adjacent spots (without an overlap for the skin tapes) will be identified for subsequent skin barrier function assessment within lesional, non-lesional and normal skin area (Figure 2). STS assessments from the same spot will be separated by 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 skin



TEWL assessment in lesional, non-lesional and normal skin will be done before, and after skin tape stripping according to the attached Figure 3. All skin tape strips will be collected, stored, and analyzed for lipidomics, proteomics and transcriptomics according to Section 8.9.

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



* STS on lesional, non-lesional and normal skin will be conducted on one of the three predefined skin areas at each indicated visit day. The assessment area will change at each visit to allow recovery of the skin. STS assessment on Days 1, 57 and 113 will be conducted on the first skin area. STS assessment on Day 15 will be conducted on the second skin area. STS assessment on Days 29 and 85 will be conducted on the third skin area.
** At every visit the baseline TEWL assessment will be conducted once on each of the three predefined lesional/non-lesional/healthy skin areas.

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

8.2.2 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 (pre dose). Subsequent photographs of the same area will be taken at each visit until Week 16 in AD patients.

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 16 in HV.

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.

A separate photography ICF will be signed by study participants to confirm agreement to photography publication. Photographs are mandatory to be taken in all subjects, but agreement to their publication is only mandatory for AD patients. Healthy volunteers can decline consent for photography publication but still participate in the study.

Further instructions for taking the photographs and additional information on the parameters to be analyzed will be provided in the study reference manual.

8.2.3 Questionnaires and patient reported outcome assessments (PROs)

Questionnaires and patient-reported assessments will only be conducted in AD patients. Patient reported outcomes 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 (or baseline) visit. The electronic diary will be only used by the subset of patients who speak fluently the language in which adequate translations (ie, validated translations where applicable) are available. These Patient-Reported Outcome (PRO) questionnaires and 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.

Patients will bring the diary to the site at each visit. Diary will be reviewed for any questionnaires omission and dispensed back to patients at each visit.

8.2.4 Investigator's Global Assessment (IGA)

The IGA is an assessment scale used in clinical studies to determine severity of AD and clinical response to treatment based on a 5-point scale ranging from 0 (clear) to 4 (severe). The IGA score will be assessed at time points according to Section 1.3. IGA will be required for inclusion decision and is only be collected at screening.

The IGA will be provided in the study reference manual.

8.2.5 Individual Signs Score (ISS)

The ISS -also called Global Individual Signs Score (GISS)- is an assessment scale used to evaluate individual components of the 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 assessed at time points according to Section 1.3.

The ISS will be provided in the study reference manual.

8.2.6 Eczema Area and Severity Index (EASI)

The EASI is a validated measure used in clinical practice and clinical trials to assess the severity and extent of AD (26). 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 each 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, 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.

8.2.7 Severity Scoring of Atopic Dermatitis (SCORAD)

The SCORAD is a validated tool used in clinical research and clinical practice that was developed to standardize the evaluation of the extent and severity of AD (27). The extent of AD is assessed as a percentage of each defined body area and reported as the sum of all areas, with a maximum score of 100% (assigned as "A" in the overall SCORAD calculation). The severity of 6 specific symptoms of AD is assessed using the following scale: none (0), mild (1), moderate (2), or severe (3) (for a maximum of 18 total points, assigned as "B" in the overall SCORAD calculation). Subjective assessment of itch and sleeplessness is recorded for each symptom by the patient or relative on a visual analogue scale (VAS), where 0 is no itch (or sleeplessness) and 10 is the worst imaginable itch (or sleeplessness), with a maximum possible score of 20. This parameter is assigned as "C" in the overall SCORAD calculation.

The SCORAD is calculated as: A/5 + 7B/2 + C. Patients will undergo this assessment at time points according to Section 1.3.

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

8.2.8 Patient Oriented Eczema Measure (POEM)

The POEM is a 7-item, validated questionnaire used in clinical practice and clinical trials to assess frequency of disease symptoms in children and adults (28). The format is a response to 7 items (dryness, itching, flaking, cracking, sleep loss, bleeding, and weeping) based on frequency 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) with a scoring system of 0 to 28; the total score reflects disease-related severity. The questionnaire will be administered at time points according to SoA (Section 1.3).

The POEM will be provided in the study reference manual.

8.2.9 Dermatology life quality index / Children's dermatology life quality index

The DLQI is a 10-item, validated questionnaire used in clinical practice and clinical trials to assess the impact of AD disease symptoms and treatment on Quality of Life (QOL) (29). The

format is a simple response to 10 items, which assess QOL over the past week, with an overall scoring system of 0 to 30; a high score is indicative of a poor QOL. The questionnaire is adapted to patient age and will be administered at time points according to Section 1.3. CDLQI is the respective, validated version of DLQI to be used in adolescents (30).

The DLQI/CDQLI will be provided in the study reference manual.

8.2.10 Peak Pruritus Numerical Rating Scale (Peak Pruritus NRS)

The Peak Pruritus NRS is a simple assessment tool that patients will use to report the intensity of their pruritus (itch) during a daily recall period. Patients will be asked the following questions:

"On a scale of 0 to 10, with 0 being 'no itch' and 10 being the 'worst itch imaginable', how would you rate your itch at the worst moment during the previous 24 hours?"

Patients will complete the rating scale daily from Day-7 until Day 29, and then until Day 113 (EoT) at the week before the visits as indicated at Section 1.3.

The Peak Pruritus NRS will be provided in the study reference manual.

8.2.11 Quality of Sleep NRS

Quality of Sleep NRS to collect information on patient-reported quality of sleep. Numerical rating scale range is from 0 ("worst possible sleep") to 10 ("best possible sleep").

Patients will complete the rating scale daily from Day-7 until Day 29, and then until Day 113 (EoT) at the week before the visits as indicated at Section 1.3.

The Quality of Sleep NRS will be provided in the study reference manual.

8.3 SAFETY ASSESSMENTS

Planned time points for all safety assessments are provided in the SoA.

8.3.1 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 adverse event.

8.3.2 Vital signs

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

8.3.3 Laboratory testing

Urinalysis for pregnancy testing

Pregnancy testing will be performed for all women of childbearing potential. Urine pregnancy testing will be performed at time points according to Section 1.3 and Section 1.4.

8.3.4 Electrocardiograms

No ECG assessments will be required in this study.

8.3.5 Clinical safety laboratory assessments

No clinical safety laboratory assessments are required in this study.

8.3.6 Suicidal ideation and behavior risk monitoring

Not applicable for this study.

8.4 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

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 intervention or study procedures, or that caused the participant to discontinue the study intervention (see Section 7).

8.4.1 Time period and frequency for collecting Adverse Events (AE) and Serious Adverse Events (SAE) information

All SAEs will be collected from the signing of the informed consent form (ICF) until the follow-up visit at the time points specified in the SoA (Section 1.3 and Section 1.4).

All AE 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 adverse events 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.

8.4.2 Method of detecting AEs and SAEs

The method of recording, evaluating, and assessing causality of AE and SAE 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.

8.4.3 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, AEs of special interest (as defined in Section 8.4.8), 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).

8.4.4 Regulatory reporting requirements for SAEs

- Prompt notification within 24 hours by the Investigator to the Sponsor of a SAE is essential so that legal obligations and ethical responsibilities towards 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 (IRB)/Independent Ethics Committees (IEC), 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 a 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.

8.4.5 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 SAEs.

8.4.6 Cardiovascular and death events

Not applicable.

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

Not applicable.

8.4.8 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).

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

- Pregnancy of a female subject entered in a study as well as pregnancy occurring in a female partner of a male subject entered in a study with IMP.
  - 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 one 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 x the upper limit of normal (ULN) in participants with baseline ALT ≤2 x ULN;

  or
  - ALT >8 x ULN if baseline ALT >2 x 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 adverse event forms.

The definitions of an AE or SAE can be found in the study protocol.

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 (see study protocol).

8.4.9 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.

8.5 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 CRF.

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.

8.6 PHARMACOKINETICS

PK parameters are not evaluated in this study.

8.7 PHARMACODYNAMICS

Pharmacodynamics is evaluated using TEWL assessments as described Section 8.2.1.

8.8 GENETICS

A blood sample for DNA isolation will be collected at baseline from all participants for filaggrin gene sequencing analysis. Blood for DNA isolation should be collected on Day 1/Baseline (pre-dose) but may be collected at any study visit.

Participants who provide a blood sample may agree to broader genetic analysis, in addition to the mandatory filaggrin gene sequencing analysis. 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 informed consent form (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 blood 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 (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 for the study at a facility selected by the Sponsor.

8.9 BIOMARKERS

Collection of serum, plasma and skin samples for potential, future biomarker research is also part of this study.

8.9.1 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.

Lipidomics in skin will be assessed in skin tapes from skin tapes obtained from lesional skin, non-lesional skin and normal skin.

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

8.9.1.1 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 (31). Filaggrin deficiency leads to impaired lipid profile and altered acidification pathways (32). Lipid abnormalities have been reported in patients with FLG mutations (33).

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.

8.9.1.2 Analysis of stratum corneum lipids

It has been reported that lesional and non-lesional skin of AD patients has decreased EOS ceramides and other ceramides with very long–chain fatty acids (C22–C30), and increased short-chain fatty acids NS CER (C16–C20) (12). Highly hydrophobic ω-esterified acid sphingosine ceramides (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 as previously described (12). 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 LC-MS/MS. A fixed amount of internal standards (N-palmitoyl-D-erythro-sphingosine (d7), (d7)NS CER and d18:1/26:0/18:1(d9) or (d9)EOS CER) will be added at the beginning of the extraction process. Protein interphase formed during extraction process will be hydrolyzed with 1N NaOH (80°C x 3h), neutralized with 1N HCl and protein content will be measured using BCA (bicinchonic acid) protein assay. All mass spectrometric data will be normalized to the total amount of hydrolyzed protein determined as described above.

EOS CER and NS CER will be identified and quantified using a targeted UHPLC-ESI-MS/MS.

8.9.1.3 Quantification of protein bound ceramides

Protein bound ceramides are formed from EOS CER and represent a portion of EOS CER without linoleic acid and thus having a terminal hydroxy group in N-linked fatty acid. Protein-bound ceramides are released from proteins during protein hydrolysis procedure (see above). Therefore, after protein determination in hydrolysates, freed omega-OH ceramides will be extracted using Bligh and Dyer procedure as described above with the addition of fixed amount of (d7)NS CER to ensure ceramide quantitation. Omega hydroxy fatty acid containing ceramides will be detected by the UHPLC-ESI-MS/MS as described above using appropriate transitions from corresponding molecular ions to the m/z 264, 292, and 320. As most standards of omega hydroxy fatty acid containing ceramides are not available, quantitation will be performed against standard curves of NS-ceramides. Obtained values will be normalized against protein content in the samples.

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

8.9.2 Proteomics

Proteomics in skin will be assessed in skin tapes obtained from lesional skin, non-lesional skin and normal skin.

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.

8.9.3 Transcriptomics

Transcriptomics in skin will be assessed in skin tapes obtained from lesional skin, non-lesional skin and normal skin.

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 (10, 17) 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.

8.9.4 Optional samples for biomarker research

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

- Venous blood samples will be collected at baseline and end of treatment/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 drug 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 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 for the study at a facility selected by the Sponsor.

8.10 IMMUNOGENICITY ASSESSMENTS

No antibodies to dupilumab will be evaluated in this study.

8.11 HEALTH ECONOMICS

Not applicable.

9 STATISTICAL CONSIDERATIONS

The material in Section 9 of the clinical trial protocol constitutes the statistical analysis plan for the study. However, biomarkers' analyses: ie, lipidomics, proteomics, transcriptomics, and filaggrin genotyping, will be detailed in a specific statistical analysis plan. If the statistical analysis plan 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 statistical analysis plan or an appropriate statistical technical document (STD) reflecting the changes will be issued prior to database lock or any interim analysis.

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

The following definitions will be used in Section 9:

- Cohort: the study includes two cohorts: patients with moderate to severe AD cohort and healthy volunteers cohort.
- Group: three 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 judgement and is consistent with the sample size from similar studies in the literature (1). 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 skin tape strips, and pre- and post-dupilumab treatment are unknown.

Allowing for drop-out rate of 15%, a total of approximately 24 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 Week16, 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 zero. 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 ICF
Intent-to-treat (ITT)	The ITT population includes all enrolled patients, who received at least one dose of IMP and all enrolled healthy volunteers who have at least one TEWL/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 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 one dose of IMP or had at least one TEWL/STS assessment and all healthy volunteers who have at least one 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 drug intake, will be fully documented in the body of the Clinical Study Report (CSR).

A listing of subjects with treatment discontinuation will be provided.

A listing of all comments related to IMP compliance and dosing, safety (AEs, vital signs), or other comments will be provided by cohorts, participants, visit and examination type in chronological order.

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 clinical study report.

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 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 patient reported outcomes, and skin barrier function, will be presented along with the on-treatment summary statistics in the efficacy 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 D1 (V02, W0), for patients and as the last available and evaluable value at D1 (V02, W0) 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.

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 D1 (V02, W0) 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, drug product batch number, drug product) will be sorted by patient.

9.4.5.1 Extent of investigational medicinal product exposure

Duration of IMP exposure is defined as: last dose date – first dose date + 14 days, regardless of unplanned intermittent discontinuations.

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 an important number of patients using rescue therapy is observed, visit values removed due to use of rescue therapy will be imputed. The imputation methods applied will be described in an SAP.

9.4.7.1 Description of efficacy variables

Efficacy parameters are described in Section 8.2. 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 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 for adolescents and adults, if deemed appropriate.

TEWL data collected at the same visit from without and before STS measurement on each of the three 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 one-sided paired t-test at a Type 1 error level of alpha=0.05 (H_0: $0 \leq TEWL_{Week16}$-$TEWL_{baseline}$, H_a: $0 > TEWL_{Week16}$-$TEWL_{baseline}$). Point estimate, two-sided 90% confidence interval and corresponding one-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 HV 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 HV 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 HV Absolute change from baseline in TEWL after 5 STS assessed on lesional skin at Week 16 in AD patients 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 HV	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.

Endpoint	Statistical Analysis Methods
Change (percent and absolute) from baseline in TEWL before STS on lesional skin in AD patients over time Change (percent and absolute) from baseline in TEWL before STS on non-lesional skin in AD patients over time Change (percent and absolute) from baseline in TEWL before STS on normal skin in healthy volunteers over time Change (percent and absolute) from baseline in TEWL after STS assessed on lesional skin in AD patients over time Change (percent and absolute) from baseline in TEWL after STS assessed on non-lesional skin in AD patients over time Change (percent and absolute) from baseline 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. Profiles over study days will be generated for individual values and group means for raw data and absolute change from baseline. 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. As exploratory analysis time to improvement of skin barrier function for lesional and non-lesional skin area measured by TEWL will be summarized. Improvement is defined as time to the first post-baseline day for which at least one 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.
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 area under the curve (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 area under the curve (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 10 or 20 STS (measurement at every five 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. Profiles over study days will be generated for individual values and group means for raw data and absolute change from baseline. 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. 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 at Week 8 and Week 16, respectively Global characterization of protein-bound ceramides over time	Lipidomics data will be summarized with descriptive statistics. Multivariate unsupervised and supervised analyses will be performed as appropriate.

Endpoint	Statistical Analysis Methods
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.
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.
Change (percent and absolute) from baseline in EASI over time Change (percent and absolute) from baseline in SCORAD over time Change (percent and absolute) from baseline in ISS over time Change (percent and absolute) from baseline in POEM over time Change (percent and absolute) from baseline in DLQI in patients 18 years of age and CDLQI in patients ≥12 and <18 years of age over time Change (percent and absolute) from baseline in peak pruritus NRS over time Change (percent and absolute) from baseline in quality of sleep NRS over time Change (percent and absolute) from baseline in photograph outputs (eg, severity score) obtained from skin imaging over time	EASI, SCORAD, POEM, DLQI/CDLQI, peak pruritus NRS, and quality of sleep NRS, photograph outputs: • 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 time points based on patients from mITT population. • Mean plots (mean ± SEM) on raw data and absolute change from baseline over time for patients' cohort will be produced. ISS: • Number and percentage of patients by severity will be reported by time points based on the patients from mITT population. • Barplot (percentages of patients by severity grade) over time for patients' cohort will be produced.
Correlation between baseline values of TEWL before STS and TEWL AUC in lesional and non-lesional skin of AD patients with the following baseline measures: • Local target lesion erythema and edema/papulations; ISS • EASI, SCORAD • POEM, DLQI, CDLQI, peak pruritus NRS, and quality of sleep NRS • Lipidomics in STS (ratio of EOS CER to NS CER) • FLG breakdown products of UCA and PCA concentrations in STS • Key components of skin proteomics in STS (expression of proteins associated with skin barrier function) • Key components of gene expression from transcriptomics • Image-derived severity score in targeted lesional skin	Scatterplot to visualize the form of the relationship between variables will be produced for patients' cohort at specific time points: ie, baseline, Week 8 and Week 16, based on the mITT population. Pearson correlation will be assessed at each defined time points for EASI, SCORAD, POEM, DLQI/CDLQI, peak pruritus NRS, quality of sleep NRS, and photograph outputs. 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 exploratory analysis repeated measures correlation method might be used to assess the overall correlation pooling data from baseline, Week 8 and Week 16 (34).

Endpoint	Statistical Analysis Methods
Correlation between percent change from baseline in TEWL before STS and TEWL AUC in lesional and non-lesional skin of AD patients at Week 8 and Week 16 with corresponding change from baseline in the following measures: • Local target lesion erythema and edema/papulations; ISS • EASI, SCORAD • POEM, DLQI, CDLQI, peak pruritus NRS, and quality of sleep NRS • Lipidomics in STS (ratio of EOS CER to NS CER) • FLG breakdown products of UCA and PCA concentrations in STS • Key components of skin proteomics in STS (expression of proteins associated with skin barrier function) • Key components of gene expression from transcriptomics • Image-derived severity score in targeted lesional skin	

9.4.8 Safety analyses

The safety evaluation will be based on the review of vital signs parameters and reported adverse events. The safety analysis will be conducted according to the sponsor's document "Analysis and reporting of safety data from Clinical Trials through the Clinical Study Report" (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 end of treatment 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.

TEAEs 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 end of study 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 one 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 heathy 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 (HR), 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 D1 (V02, W0) 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 Potentially Clinically Significant Abnormality (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.

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

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.

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 Good Clinical Practice (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 must be informed that their participation is voluntary. Participants or their legally authorized representative 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.
- 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.
- 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 the use of remaining mandatory samples or 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.

A separate consent will be required to document a participant's agreement to allow publication of photos taken during the study. Participants who decline to agree to publication of photos taken during the study 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 (General Data Protection Regulation).

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 (35) 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 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 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 electronic CRF 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 CRF.
- The Investigator must maintain accurate documentation (source data) that supports the information entered in the CRF.
- 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 CRF 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 CRF or entered in 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 women of childbearing potential)[a]

NOTES :
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 fulfil 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, ECG, 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 fulfil the definition of an AE or SAE.
- The signs, symptoms, and/or clinical sequelae resulting from lack of efficacy will be reported as AE or SAE if they fulfil the definition of an AE or SAE. Also, "lack of efficacy" or "failure of expected pharmacological action" also constitutes 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 one 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 CRF.
- It is not acceptable for the Investigator to send photocopies of the participant's medical records in lieu of completion of the AE/SAE CRF 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 (IB) 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 one 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 CRF.
- 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 (see next section) 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 (see next section) 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 woman is considered fertile following menarche and until becoming post-menopausal unless permanently sterile (see below).

If fertility is unclear (eg, amenorrhea in adolescents or athletes) and a menstrual cycle cannot be confirmed before first dose of study intervention, additional evaluation should be considered.

Women in the following categories are not considered WOCBP

1. Premenarchal
2. Premenopausal female with 1 of the following:
   - Documented hysterectomy
   - Documented bilateral salpingectomy
   - Documented bilateral oophorectomy

   For individuals with permanent infertility due to an alternate medical cause other than the above, (eg, Mullerian agenesis, androgen insensitivity), Investigator discretion should be applied to determining study entry.

   Note: Documentation can come from the site personnel's: review of the participant's medical records, medical examination, or medical history interview.
3. Postmenopausal female
   - A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.
     - A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy (HRT). However, in the absence of 12 months of amenorrhea, confirmation with more than one FSH measurement is required.
     - Females on HRT and whose menopausal status is in doubt will be required to use one of the non-estrogen hormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status before study enrollment.

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 *(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.*
NOTES: *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 8.4.4. 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 blood sample will be collected for DNA analysis from consenting participants.
- For all participants, a blood sample will be collected for mandatory filaggrin gene sequencing analysis.
- For participants, who consent to optional genetic research, a DNA sample 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 clinical study report (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 event
AESI:	adverse events of special interest
ALT:	alanine transaminase
AUC:	area under the curve
CDLQI:	children dermatology life quality index
CER:	ceramides
DLQI:	dermatology life quality index
EASI:	eczema area and severity index
EOS:	E for esterified / O for omega-hydroxy / S for sphingosin
EOS CER:	highly hydrophobic omega-esterified fatty acid sphingosine ceramides
EoT:	end of treatment phase
FLG:	filaggrin
ICF:	informed consent form
IGA:	investigator global assessment
IL:	interleukin
IMP:	investigational medical product
ISS:	Individual Signs Score
ITT:	intent-to-treat
LC-MS/MS:	liquid chromatography with tandem mass spectrometry
mITT:	modified intent-to-treat
MKI67:	antigen ki-67
NRS:	numerical rating scale
NS:	non-hydroxy fatty acid sphingosine
NS CER:	non-hydroxy fatty acid sphingosine ceramides
PCA:	pyroglutamic acid, also pyrrolidone carboxylic acid
POEM:	patient oriented eczema measure

PRO:	patient reported outcomes
Q2W:	every second week
QOL:	quality of life
SAE:	serious adverse event
SC:	subcutaneous
SCORAD:	SCORing atopic dermatitis
STS:	skin tape stripping
TEWL:	transepidermal water loss
UCA:	urocanic acid
ULN:	upper limit of normal

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