Non-invasive biomarkers for inflammatory skin diseases: towards systems dermatology

Rousel, J.

CHAPTER 7

GUSELKUMAB TREATMENT ALLEVIATES BARRIER DYSFUNCTION AND NORMALIZES THE ALTERED STRATUM CORNEUM CERAMIDE PROFILE IN LESIONAL PSORIASIS: RESULTS OF A RANDOMIZED, PLACEBO-CONTROLLED TRIAL

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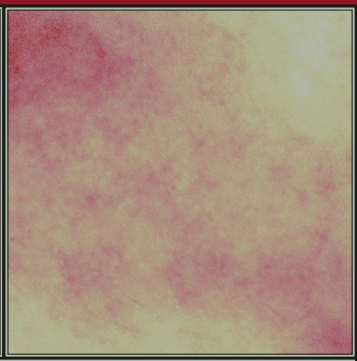


Jannik Rousel,[1,2,*] Catherine Mergen,[2,*] Menthe E. Bergmans,[1,3] Lisa J. Bruijnincx[1], Marieke L. de Kam,[1] Naomi B. Klarenbeek,[1] Tessa Niemeyer-van der Kolk,[1] Martijn B.A. van Doorn,[1,4] Joke A. Bouwstra,[2] and Robert Rissmann,[1,2,3] on behalf of the Next-Generation ImmunoDermatology (NGID) Consortium. **These authors contributed equally.*
1. Centre for Human Drug Research, Leiden, NL / 2. Leiden Academic Centre for Drug Research, Leiden University, Leiden, NL / 3. Department of Dermatology, Leiden University Medical Center, Leiden, NL / 4. Department of Dermatology, Erasmus Medical Centre, Rotterdam, NL

Abstract

BACKGROUND: The epidermal inflammation associated with psoriasis drives skin barrier perturbations. The skin barrier function is dependent on the stratum corneum lipid matrix of which ceramides constitute important components. Changes in the ceramide profile are directly related to barrier function. In this study, we set out to characterize the barrier function and ceramide profile of psoriatic skin before and during treatment with guselkumab.

METHODS: A double-blind, randomized controlled trial was performed in which the barrier function and ceramide profile of 26 patients was assessed before and after 3:1 randomization to 100mg guselkumab or placebo for 16 weeks. Barrier function was measured by trans-epidermal Water loss measurements. Ceramide profiling was performed using liquid chromatography-mass spectrometry after stratum corneum was harvested using tape-stripping.

RESULTS: Barrier function was significantly decreased and the ceramide profile significantly different in lesional skin compared to non-lesional skin and that of healthy controls. Barrier function and the ceramide profile of lesional skin normalized to that of controls during treatment with guselkumab, but not placebo. This resulted in significant differences compared to placebo at the end of treatment. Changes in the lesional ceramide profile during treatment correlated with barrier function and the target lesion severity. Non-lesional skin was similar to controls at baseline and did not change during treatment.

CONCLUSION: Skin barrier function and the ceramide profile are altered in lesional psoriatic skin. Alterations in the ceramide profile normalize in response to guselkumab treatment and correlate with clinical severity. Monitoring the ceramide profile might be exploited as an objective biomarker for treatment responses.

Introduction

Psoriasis is a chronic inflammatory skin disease characterized by erythematous, indurated, and scaly lesions. Psoriasis can be increasingly well-managed in clinical practice, but the presence of any residual lesions can still remain a significant burden to patients.[1] While being primarily an inflammatory condition, cutaneous barrier perturbations allows exogeneous substances to cross the skin barrier and enhance epidermal dysregulation.[2] Indeed, barrier dysfunction is observed in lesional skin and, although not observed consistently throughout all studies, also in non-lesional skin.[3–6]

Proper barrier function is in part regulated by the specific composition of ceramides present in the stratum corneum (SC).[7] Ceramides are incorporated in a highly organized lipid matrix together with mainly fatty acids and cholesterol. This matrix presents the only continuous penetration pathway for substances crossing the intact SC. The ceramide fraction is composed of many different species which can be categorized into as many as 25 different subclasses (figure S1).[8] Alterations in the ceramide composition and concomitant decreases in skin barrier function have been observed in psoriasis and other inflammatory dermatological conditions.[9] In atopic dermatitis (AD), these appear correlated to barrier function.[10–13] Additionally, in vitro studies have highlighted the potential of inflammation to affect the ceramide lipid biosynthesis and thereby aggravate barrier dysfunction.[14,15] Understanding barrier dysfunction in psoriasis might provide further targets for therapy and prevent the formation of new lesions.[16]

Pathomechanistic insights have identified the interleukin (IL)-23 and -17 axis as main driver of the aberrant immunological responses in psoriasis which enabled the use of targeted immunosuppressing therapies to great success.[17] Guselkumab is an ANTI-IL23P19 monoclonal antibody which has shown high and lasting efficacy in clinical practice.[18,19] Besides its pronounces anti-inflammatory effect, ANTI-IL-23 therapy has shown to also improve barrier function in a preclinical psoriasis mouse model.[20]

Although studies have already characterized barrier dysfunction and the lipid composition in psoriasis, their integration and association with measures for disease severity are lacking and warrant further investigation.[21–23] In this study, we set out to characterize the ceramide profile in psoriasis and investigate the dynamics of barrier function and ceramide profile in response to biologic therapy. Differences between healthy and (non-)lesional

psoriatic skin were evaluated at baseline, after which barrier function and ceramide composition were monitored during disease regression induces by guselkumab in a randomized, placebo-controlled trial.

Patients and methods

Extended methods are present in the supplementary information.

STUDY DESIGN

The SKINERGY-PP study was an exploratory, single-center, double-blinded and placebo controlled randomized trial conducted from September 2020 to January 2023 at the Centre for Human Drug Research (Leiden, the Netherlands) and registered under NCT04394936. The study included plaque psoriasis patient that were not undergoing active treatment, or with appropriate wash-out, and had at least one active lesion of sufficient size to allow for sampling and with a minimal lesion severity score $^{3}6$. The use of medication prior and during the study was prohibited, including the application of any topical products on lesions subjected to assessment. Control subjects without any dermatological conditions and otherwise healthy were included and matched for average sex, age and tape-stripping location to the patient group. Baseline measurements were obtained from patients and controls 2 weeks apart. Afterwards, 26 patients were randomized 3:1 to subcutaneous injections at week 0, 4 and 12 with guselkumab 100 mg or placebo, respectively. The full in- and exclusion criteria, demographics of the study population at baseline and locations of the assessments are presented in the supplementary information.

TRANS-EPIDERMAL WATER LOSS

Assessments were performed under controlled environmental conditions of <60% humidity and 22±2 °C. Cutaneous water loss was determined using an AquaFlux AF200 (Biox Systems Ltd., London, United Kingdom) over 180 seconds or until steady state was reached.

TAPE-STRIPPING

Subsequently, a total of 8 tapes (Nichiban, Tokyo, Japan) were pressed onto the skin using a D500-pressure instrument (D-squame, Cuderm Corporation, Dallas, TX, USA) and measured using a SquameScan 850A (Heiland

Electronic, Wetzlar, Germany) to obtain the SquameScan value (SQV) as a measure for SC removed.24 A 16 mm diameter circle was punched out and stored in chloroform:methanol (2:1) awaiting extraction.

LIPID EXTRACTION AND ANALYSIS

Tapes were extracted using a modified 4-step Bligh and Dyer extraction at elevated temperature as described by Boiten *et al.* (2016).[25] Extracts from tape strips 5-8 were pooled for analysis. Lipidomics was performed using Liquid Chromatography-Mass Spectrometry as described in the supplemental materials. The amount of ceramide per sample was normalized by the cumulative SQV of the extracted tapes after correction with the average signal of 2 blank tapes to obtain absolute amounts of ceramides in pm/SQV value.

CLINICAL SCORING OF PSORIASIS SEVERITY

Severity was scored using the Psoriasis Area and Severity Index (PASI).[26] Additionally, the lesion severity score (LSS) of the target lesion was obtained by summing erythema, scaling and induration graded on a 5-point scale (0; clear-4; severe).[27,28]

STATISTICS

Statistical testing at baseline was performed using one or two-way ANOVA with Tukey's test for multiple comparisons in PRISM 9.0 (GraphPad, Software, Boston, United States). Longtitudinal analysis was performed using a least-squares method in SAS 9.4 (SAS Institute Inc., Cary, North Carolina, United States). Repeated Measures Correlation was performed using Rmcorr in R Statistical Software (version 4.1.2, R Core Team 2021).[29] Statistical significance is shown as: $P<0.05$: *, $P<0.005$: – and $P<0.005$: -*.

Results

A total of 26 patients with plaque psoriasis and 10 heathy controls were included in the study (figure s2). Demographics are listed in table s1 and the adverse events associated with guselkumab or placebo treatment have been reported previously.[30,31]

THE CERAMIDE PROFILE IS ALTERED IN LESIONAL PSORIATIC SKIN

Correcting with the sqv as a measure for the amount of SC removed, quantitative analysis showed a significant decrease in total saturated ceramides in lesional skin compared to non-lesional skin and controls (figure 1A). On the level of subclass composition, a significantly decreased CER[NDS], CER[NP], CER[NH], CER[AP], CER[OS] and CER[OH] abundance is observed in lesional skin compared to non-lesional skin and controls (figure 1B). The abundances of CER[ADS] and CER[AH] were additionally decreased in lesional skin compared to non-lesional skin. Contrarily, non-lesional skin shows a significantly increased CER[NP] abundance compared to control skin. There were no significant differences in abundances within the CER[EO] fraction.

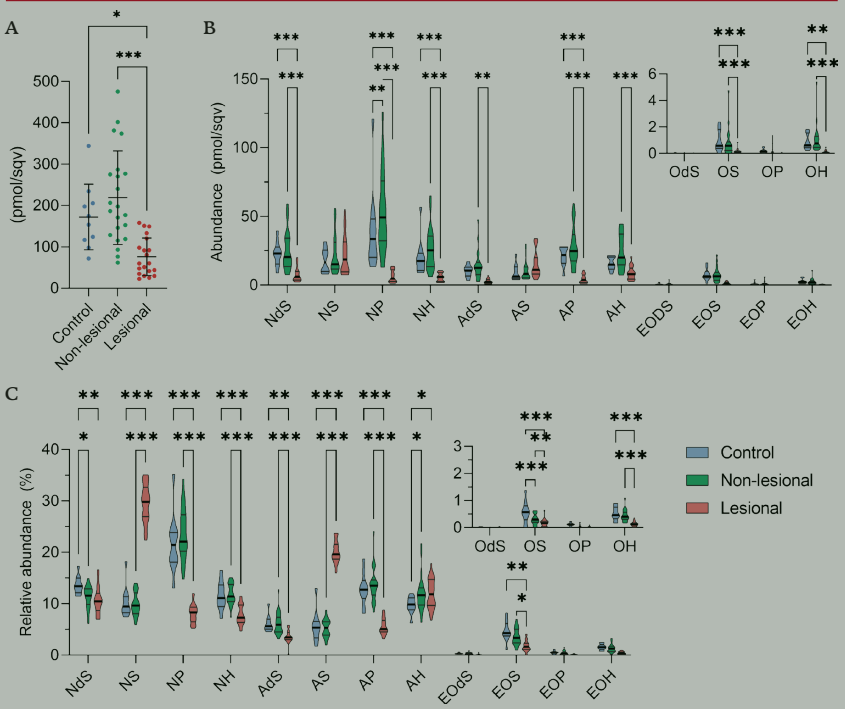
Compositional display of the data shows that decreased CER[NDS], CER[NP], CER[NH], CER[ADS], CER[AP], CER[OS] and CER[OH] abundances are maintained in lesional skin compared to control skin (figure 1C). However, significant increases in CER[NS], CER[AS], CER[AH] and CER[EOS] were observed which were not observed for the absolute profile.

Trans-epidermal water loss (TEWL) represents a surrogate marker for barrier integrity and was significantly higher in lesional compared to non-lesional skin and controls (figure 2A). Skewing of ceramide subclass synthesis is reiterated by the significantly increased CER[NS]:CER[NP] ratio in lesional skin (figure 2B). Additionally, the fraction of monounsaturated ceramides (MUCER) in CER[NS], as an indication for the degree of unsaturation throughout the entire profile, was significantly higher in lesional skin (figure 2C).

Ceramides can be divided in three groups based on their average ceramide chain length (CCL): the CER[A] and CER[N] (CER[N,A]) fraction, the CER[EO] fraction which contains an additional linoleic acid moiety, and the CER[O] fraction which resemble CER[EO] without linoleic acid moiety. Ceramide elongation appears impacted as the average ceramide chain lenght (CCL) in the CER[N,A], CER[EO] and CER[O] fraction is significantly decreased in lesional compared to non-lesional and control skin (figure 2E). However, the decrease in the CCL of CER[O] in lesional skin was small and only significant compared to non-lesional skin. Additionally, the abundance of short-chain CER[NS]-species totaling 34 carbons, CER[NSC34], was increased significantly in lesional skin (figure 2D). When the ceramide profile was measured two weeks before the initiation of treatment, an small but significant increased

in the relative abundance of CER[NDS] and CER[NS] was observed compared to baseline of controls (Supplemental Figure s3). No other differences were observed in the relative and absolute profile, nor in TEWL, CER[NS]:CER[NP] ratio, percentage MUCER[NS], abundance CER[NSC34] nor average CCL of control and patients (Supplemental Figure s4), indicating the ceramide profile in psoriasis was stable in the two weeks prior to baseline.

Figure 1 The ceramide profile of the stratum corneum in controls, non-lesional psoriasis and lesional psoriasis. The total sum of the detected saturated ceramides (A) and the ceramide profile (B) are shown with absolute display of the data using the squamescan value (SQV) as a measure for stratum corneum removed. Additionally, the ceramide profile is shown relative to the total detected ceramides (C).



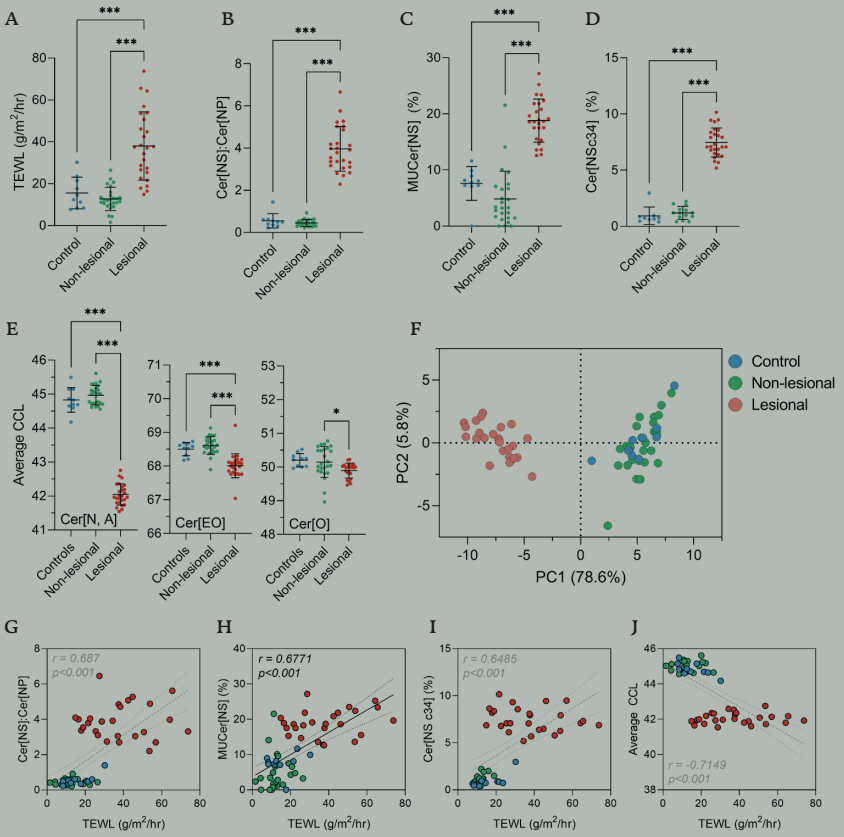
Using dimension reduction analysis to visualize the full ceramide profile, lesional skin forms a separately defined cluster compared to the overlapping non-lesional and control datapoints (figure 2F). This indicates a high degree of difference between the ceramide profile of lesional skin compared to non-

lesional and control skin. A high degree of similarity between non-lesional and control skin is supported by the aforementioned features (figure 2B-E) which do not appear significantly altered in non-lesional skin compared to controls.

Although the study included mild and moderate-to-severe patients, as defined by a PASI ≤5 or ≥10 at screening, no evident differences in the ceramide profile were observed between these two groups. However, a slight but significant decreased relative CER[NP] abundance and increased relative CER[NH] abundance in the moderate-to-severe group was present compared to mild patients (figure s5). This might have resulted in the significantly increased CER[NS]:CER[NP] ratio observed in the moderate-to-severe patients. Additionally, a significant increase in the average CCL of the CER[O] fraction is observed in moderate-to-severe patients compared to mild patients. However, these changes did not result in a markedly different ceramide profile as seen by the overlap with mild patients from principal component analysis (figure s6)

Ceramide parameters were correlated to barrier function (figure 2G-J). TEWL correlated positively with CER[NS]:CER[NP] (r=0.0687), fraction MUCER[NS] (r=0.6771) and abundance CER[NSC34] (r=0.6485) and negatively with the average CCL in CER[N,A] (r=-0.7149). However, linearity appears limited as lesional skin is markedly different compared to control and non-lesional skin in terms of both TEWL and ceramide composition.

Figure 2 ***(next page)*** Specific features of the stratum corneum ceramide profile in lesional psoriasis, non-lesional psoriasis and control skin. Skin barrier functionality is determined by the Trans-epidermal Water Loss (TEWL) (A). Compositional changes of the ceramide profile are summarized by calculating the ratio between CER[NS] and CER[NP] abundances as a measure for subclass synthesis skewing (B), fraction of monounsaturated ceramide (MUCER) in CER[NS] (C), the percentage of very short-chain ceramide CER[NSC34] (D) and the ceramide chain length (CCL) of CER[N] and CER[A] (CER[N,A]), CER[EO] and CER[O] separately (E). For the CCL per subclass, see figure s7. Graphs show mean and standard deviation. The (dis)similarity of the different ceramide profiles is visualized by Principal Component Analysis (PCA) of the full profile, containing the relative abundance of all individually detected saturated ceramides, with the amount of variance explained by the respective components on either axis (F). The relationship between barrier function as measured by TEWL and CER[NS]:Cer[NP] (G), fraction MUCER[NS] (H), percentage of CER[NSC34] (I) and CCL in CER[N,A] (J) are demonstrated with a line representing the best fit of a linear regression through all data points with its 95% confidence intervals shown together with the Pearson correlation coefficient (*r*) and associated p-value. Lines and values in grey indicate linear regression is unsuitable for those parameters.



ALTERATIONS IN THE CERAMIDE PROFILE NORMALIZE DURING TREATMENT WITH GUSELKUMAB BUT NOT PLACEBO

After establishing differences in untreated lesional and non-lesional skin compared to controls, their dynamics under guselkumab or placebo treatment were investigated during a 16-week treatment period. As reported previously in this cohort,[30,31] treatment with guselkumab effectively induced disease remission compared to placebo based on the PASI score (figure s8).

All subclasses that were decreased compared to controls at baseline normalized during guselkumab treatment (figure 3). Of note, the abundance of CER[AH] appeared to exceed that observed in controls. In the placebo group,

abundances of CER[OH] also appeared to recover to the level observed in controls as no significant difference was present when comparing the abundance of CER[OH] in controls with that of patients receiving placebo at week 16. However, the apparent variation in the CER[OH] abundance in controls was substantial and might have contributed to this as the abundance of CER[OH] remained low. All other subclasses remained significantly decreased.

As total-body PASI scoring does not necessarily correspond to target plaque severity, the LSS is used as an alternative to allow correlation between barrier characteristics and severity, and demonstrates a significant decrease compared to placebo in line with PASI scores (figure 4A). Complete skin clearance was not obtained based on PASI and LSS scores >0 after 16 weeks of guselkumab treatment. Barrier function of lesional skin improved significantly in the guselkumab group but remained comparable to baseline for patients receiving placebo (figure 4B). In line with the reduction in TEWL, ceramide parameters distilled from the overall profile ameliorated (figure 4C-F).

Figure 3 The absolute ceramide subclass profile in lesional skin of psoriasis patients after 16 weeks of guselkumab or placebo treatment compared to controls at baseline. The ceramide subclass profile determined at baseline for controls and patients, and for patients after 16 weeks of guselkumab or placebo treatment. Pairwise comparisons are only reported between controls at baseline and patients at week 16. Grey pairwise comparisons indicate no significant difference was observed between lesional skin and control skin at baseline. NS indicates there is no significant difference (P>0.05).

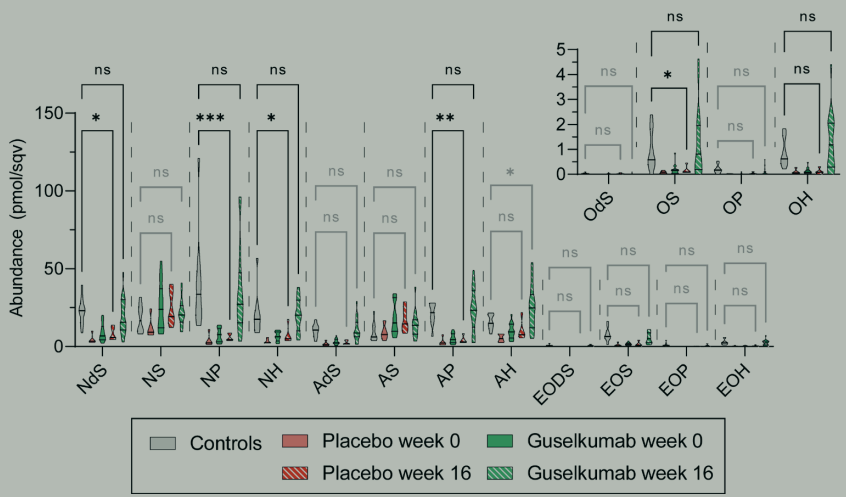
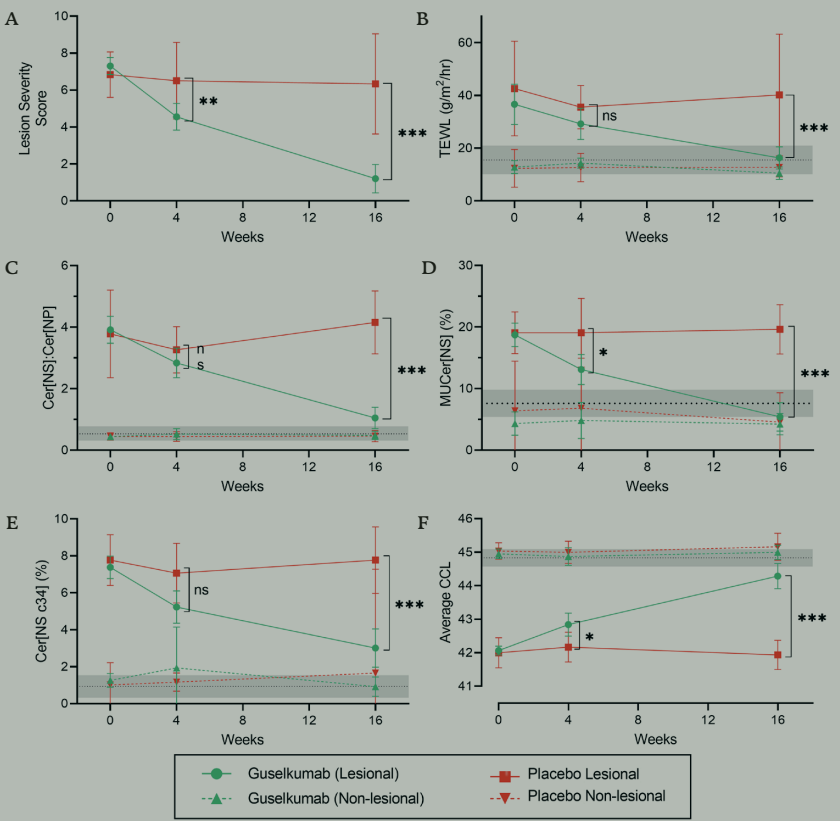


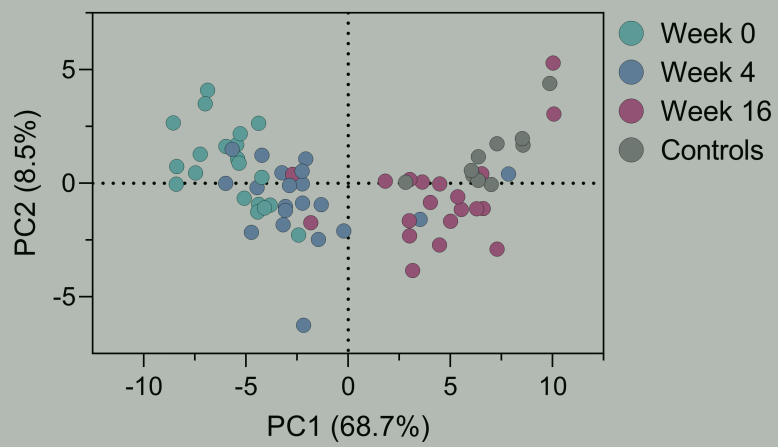
Figure 4 The effect of guselkumab treatment on disease severity and ceramide parameters over time. Non-lesional and non-lesional skin is followed over time. Disease severity of the target plaque is determined using the lesion severity score (LSS) (A). The same plaque was monitored using the Trans-epidermal Water Loss (TEWL) as a measure for barrier function (B) and subsequently assessed with ceramide profiling yielding the the CER[NS]:CER[NP] ratio (C), percentage monounsaturated CER[NS] (MUCER[NS]) of total CER[NS] (D), percentage CER[NSC34] of total CER[NS] (E) and the average ceramide chain length (CCL) of the CER[A] and CER[N] fraction (F) as representative parameters of the altered ceramide profile at baseline. Graphs represent the mean and 95% Confidence Interval. The dotted black line and grey band represents the mean and 95% confidence interval of the control group at baseline. P-values are indicated when comparing lesional skin of the guselkumab group with the lesional skin of the placebo group. No significant changes were present when comparing non-lesional skin in the placebo group with non-lesional skin in the guselkumab group.



Compared to placebo, a significant decrease in the CER[NS]:CER[NP] ratio, fraction MUCER[NS] and percentage CER[NSC34] was observed to the level observed in healthy controls and non-lesional skin. The CCL in the CER[N,A] fraction increased as well but remained slightly lower compared to controls and non-lesional skin. No significant changes between the placebo and guselkumab group was observed in the CCL of the CER[EO] and CER[O] fractions (figure s9). Parameters in non-lesional skin remained consistent over time and comparable to those observed in controls at baseline.

Visualization of the ceramide profile using dimension reduction analysis indicates normalization of the ceramide profile over time to that of healthy controls (figure 5). The ceramide profile of lesional skin is markedly different from controls at baseline but shifts towards that of healthy volunteers already after 4 weeks of treatment. After 16 weeks, all lesional samples but two have become separated from those obtained at baseline and overlap fully with those from healthy controls indicating a high degree of similarity between samples of lesional and control skin.

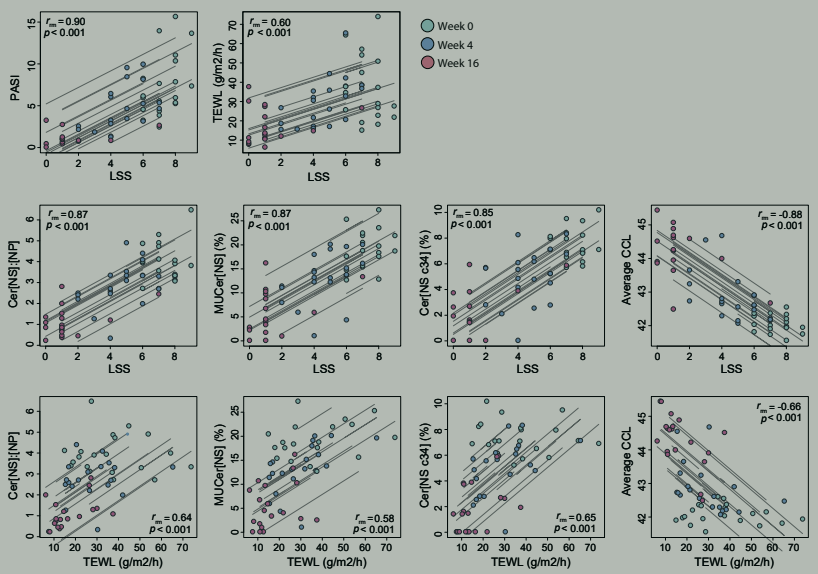
Figure 5 The lesional ceramide profile of patients during guselkumab treatment and the ceramide profile of healthy controls at baseline visualized by Principal Component Analysis (PCA). The relative composition of all detected saturated ceramides is used for each datapoint. Profiles of healthy controls are added as a reference for a normal ceramide profile, and a high proximity to these datapoints indicates a high degree of similarity. The amount of variance explained per principal component (PC) is displayed on the axis.



BARRIER FUNCTION AND CERAMIDE PARAMETERS CORRELATE WITH DISEASE SEVERITY

The high similarity in LSS before treatment disqualifies its use in correlation analysis at baseline. However, the repeated assessment of disease severity, barrier function and the ceramide profile during guselkumab treatment allows for the use of repeated measures correlations (figure 6). Local LSS and total-body PASI correlated strongly (r_{rm}=0.90). Repeated measures correlations between LSS and the CER[NS]:Cer[NP] ratio (r_{rm}=0.87), abundance MUCER[NS] (r_{rm}=0.87), CER[NSC34] (rrm=0.85) and average CCL (r_{rm}=-0.88) were high. However, coefficients of TEWL with LSS (r_{rm}=0.60), CER[NS]:CER[NP] ratio (r_{rm}=0.64), abundance MUCER[NS] (r_{rm}=0.58), CER[NSC34] (r_{rm}=0.65) and average CCL in CER[N,A] (r_{rm}=-0.66) were generally lower.

Figure 6 Correlation between disease severity and barrier function or ceramide parameters from patients receiving guselkumab treatment. Repeated measures correlation coefficients (r_{rm}) between local lesion severity (LSS) score and the psoriasis area and severity index (PASI) and Trans-epidermal Water Loss (TEWL) are plotted. Additionally, the CER[NS]:Cer[NP] ratio, monounsaturated CER[NS] (MUCER[NS]), percentage CER[NSC34] and the average ceramide chain length (CCL) of CER[A] and CER[N] are contrasted with the LSS. Lastly, these ceramide parameters are correlated with Trans-epidermal Water Loss (TEWL). Lines indicate the common within-individual association per subject and directly relate to the r_{rm} coefficient.



Discussion

In this study, we expand the knowledge on the SC ceramide profile in psoriasis by the in-depth profiling of lesional and non-lesional skin compared to controls and were able to correlate these alterations in the ceramide profile to barrier function. Additionally, we demonstrate how barrier impairment is alleviated during guselkumab treatment and how the observed alterations in the ceramide profile normalize throughout a randomized controlled trial.

The ceramide profile of lesional psoriasis is defined by altered subclass composition, increased unsaturation and decreased average ceramide chain lengths

The baseline characterization of this cohort is in good agreement with previously published work where relative increased CER[S] and decreased amount of CER[P] and CER[EO] were observed in lesional skin compared to healthy controls, resulting in a skewed CER[S]:Cer[P] ratio.[21,22,32–34] However, absolute values indicates an decrease in total ceramides with a major decrease in CER[P] instead of increased CER[S]. Reflecting on the ceramide composition of lesional AD skin which has been described more extensively, increased levels of CER[S] were observed in relative profiles[35,36] rather than absolute profiles.[37–39] It should therefore be considered that increased relative CER[S] abundances merely reflect the interdependency of compositional data and results from strong reductions in absolute CER[P] abundances. However, the molar ratio between lipid classes dictates the organization of the extracellular lipid matrix and subsequently affects the barrier function.[7]

Decrease ceramide synthesis in lesional psoriasis has been observed, which is in line with the lower abundance of ceramides observed in the present study.[40] Although alterations in the activity of dihydroceramide desaturase 2 might explain the skewed CER[NS]:CER[NP] ratio as it mediates the final step in CER[P] synthesis and is downregulated in SC of lesional AD,[41,42] an overall decrease in ceramides, including precursor CER[NDS], indicates a more upstream inhibition. Indeed, a psoriasis mouse model indicated other enzymes might differentially contribute to CER[P] synthesis as CER[P] was still present despite knocking out dihydroceramide desaturase 2.[43] Presumably, decreases in the expression of acid sphingomyelinase and its activators as observed in psoriatic skin prevents sufficient sphingomyelin to be converted to ceramide.[44] Aberrant acid sphingomyelinase expression and activity has previously been observed in AD.[35,36]

Furthermore, the amount of CER[OS] and CER[OH] is decreased in lesional skin. CER[O] is a precursor of CER[EO] but may also represent CER[EO] after loss of its linoleic acid moiety. This linoleic acid moiety is required for covalent binding of ceramides to the cornified envelope.[45–47] However, the relevance of decreased CER[OS] and CER[OH] abundances might be questioned as their abundance is already low in controls and even their complete absence did not lead to increased permeability of model membranes.[48] Their relation to the bound lipid fraction might warrant its analysis in future studies.

In addition, the fraction MUCER[NS] was increased in lesional skin as has also been observed in Netherton syndrome, AD and seborrheic dermatitis.[49–51] A causative reason for this increase remains unknown, but might relate to increased Steoryl-CoA-9-desaturase activity.[41,52] Although the degree of unsaturation is only determined for CER[NS], increased MUCER has been linked to increased monounsaturated fatty acids which are incorporated in ceramides regardless of subclass and appear especially prevalent in CER[EO].[8] Therefore, it might be expected that increased unsaturation extents past CER[NS] and is present in other subclasses.

Besides compositional changes, ceramide elongation is impaired in lesional skin with an evident reduction in CCL observed in CER[N] and CER[A] with a more limited reduction in the CCL of CER[EO] and CER[O] fractions. An murine psoriasis model showed decreases in fatty acid elongases ELOVL1, -3 and -4, as well as in Ceramide Synthases 3 which are all involved in the synthesis of ceramides exceeding 24 carbons.[21] These findings are supported by studies in AD where comparable changes were observed in patients and after inducing inflammation in models.[36,38,53] Of note, the presence of short-chain CER[NSC34] is increased in lesional skin as indicated priorly in AD, ichthyosis and psoriasis.[30] These specific ceramides are associated with cellular stress and might therefore reflect the dysregulated homeostasis in psoriasis instead of impaired lipid elongation.[54,55] The high similarity in the ceramide profile of psoriasis and AD might reflect more general state of epidermal dysregulation rather than being primarily related to distinct pathogenic mechanisms.

Association of the altered ceramide profile with barrier dysfunction

In this study, alterations in the ceramide composition, unsaturation and CCL of lesional skin are paired with decreased barrier function. Correlations between changes in ceramide composition and permeability have been demonstrated using model systems that mimic the lipid matrix of inflamed skin

with a skewed CER[NS]:CER[NP],[56,57] decreased CCL58,[59] and increased unsaturation[60] resulting in a decreased barrier function. Moreover, the altered ceramide profile is implicated in barrier impairment of in-vitro cultured human skin equivalents.[61,62] Despite these associations, linearity between ceramide alterations and barrier dysfunction within lesional skin at baseline appears limited in this study and might indicate co-occurrence rather than correlation. In AD and seborrheic dermatitis, alterations in the ceramide profile and barrier function follow a more gradual transition from non-lesional to lesional skin.[33,35,37,49,63] However, the lesions assessed in this study are all moderate in baseline severity which might explain the marked differences between lesional and non-lesional skin. Indeed, monitoring TEWL and lesion severity during treatment suggest a more gradual normalization instead of strict dichotomy.

Altogether, in-depth characterization of lesional psoriatic ceramide profile yields a markedly different profile compared to non-lesional and control skin. The non-inflammatory phenotype of uninvolved psoriasis is reiterated by a high similarity to control skin as reported priorly.[34,64] This differentiates psoriasis from AD were non-lesional barrier defects are cemented in its pathogenesis.[65] Variation in the study is controlled by matching the controls with patients for approximate age, sex, assessment location and recruitment throughout seasons. The robustness of the results is substantiated by their consistency when measured two weeks apart, which exceeds the turnover time of the SC in psoriasis.[66]

Correlation of the altered ceramide profile with disease severity during guselkumab treatment

We performed a double-blind, randomized, placebo-controlled trial to solidly affirm the relationship between alterations in the ceramide profile, barrier dysfunction and psoriasis. Priorly, a smaller cross-sectional study linked barrier dysfunction to alterations in the ceramide profile but did not evaluate the same patients barring direct correlations.[23] Guselkumab treatment effectively decreased disease severity as expected based on several studies including real-world evidence.[18,19,67] Concurrently, barrier dysfunction and alterations in the ceramide profile associated with lesional skin resolved. The ceramide profile was similar in mild and moderate-to-severe patients (PASI≤5 or PASI≥10 at screening), in line with pathomechanistic studies that demonstrated a high degree of similarity in the lesional transcriptomic profiles

between these groups.[68,69] Instead, the ceramide profile primarily relates to the target lesion severity. The narrow range of lesion severity at baseline, as a direct result of the inclusion criteria, precluded any correlation to severity pre-dose. However, variability in local severity increased during guselkumab treatment and allowed for its association with barrier parameters which were strongly correlated. Of note, the association between local severity and barrier parameters was affirmed by two patients who showed incomplete remission despite a decrease in PASI score and maintained a ceramide profile closer to baseline despite receiving guselkumab.

Ceramide profiling as a biomarker for treatment monitoring

The relationship between treatment effect and ceramide profiles might allow for exploitation in a biomarker capacity. Indeed, CER[NSC34] constitutes a single biomarker that is able to discern lesional from non-lesional skin and gradually decreases during treatment. Traditional clinical endpoints, such as the PASI, have become commonplace but are associated with poor sensitivity, bias and interrater variability.[26,70,71] Expanding clinical endpoints with objective measures might increase confidence in these results.[72] Earlier, normalization of the ceramide profile was observed in a more concise analysis during dupilumab treatment in AD patients.[73,74] Considering the similarity of the ceramide profile in psoriasis and AD, together with its comparable response to effective treatment, ceramide profiling might also be applied to other indications where similar alterations are observed such as ichthyosis.[9] However, treatment responses of plaques within the patient might differ which should be considered when only selected lesions are monitored.[75] Although TEWL and ceramides are both markers of skin barrier function, ceramide readouts from lesional skin seem less variable. TEWL can be impacted by personal factors, environmental factors and differ between measurement locations.[76,77] Although these factors can also impact the ceramide profile, their influences might be more limited.[78–82] Tape-stripping has already been applied to obtain protein and transcriptome-based biomarkers that are able to reflect the specific inflammatory component of specific diseases.[83,84] In contrast, ceramides represent more distal biomarkers that might be more primarily related to overall epidermal differentiation.[85] Although this disconnect might invalidate their use in a diagnostic manner, their more general relationship to disease provide a more holistic view of the response to treatment.

Conclusion

In this study, we show that guselkumab therapy alleviates skin barrier dysfunction and normalizes the underlying ceramide profile in psoriasis patients. We showed barrier dysfunction and concurrent alterations in the ceramide profile are limited to the lesional skin of patients. Correlations between the ceramide profile and barrier function underline their interdependency, and the effective clinical response towards guselkumab therapy further establishes their relationship with disease severity. Ceramide profiling might represent a suitable biomarker for treatment monitoring.

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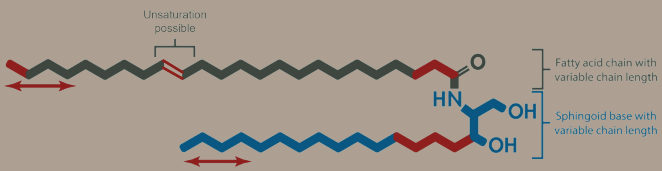
SUPPLEMENTAL MATERIAL

Figure s1 An overview of the variety in ceramides Adapted from Janssens *et al.* (2012). A general structure with the structural differences highlighted such as differences in headgroup architecture, chain length and degree of unsaturation. Additionally, it presents the structure of all ceramide classes used in this study according to the nomenclature by Motta *et al.* (1993).



	Non-hydroxy fatty acid [N]	α-hydroxy fatty acid [A]	ω-hydroxy fatty acid [O]	Esterified ω-hydroxy fatty acid [EO]
Dihydrosphingosine [dS]	[NdS]	[AdS]	[OdS]	[EOdS]
Sphingosine [S]	[NS]	[AS]	[OS]	[EOS]
Phytosphingosine [P]	[NP]	[AP]	[OP]	[EOP]
6-hydroxy sphingosine [H]	[NH]	[AH]	[OH]	[EOH]

Figure s2 Consort flow chart of the study population.

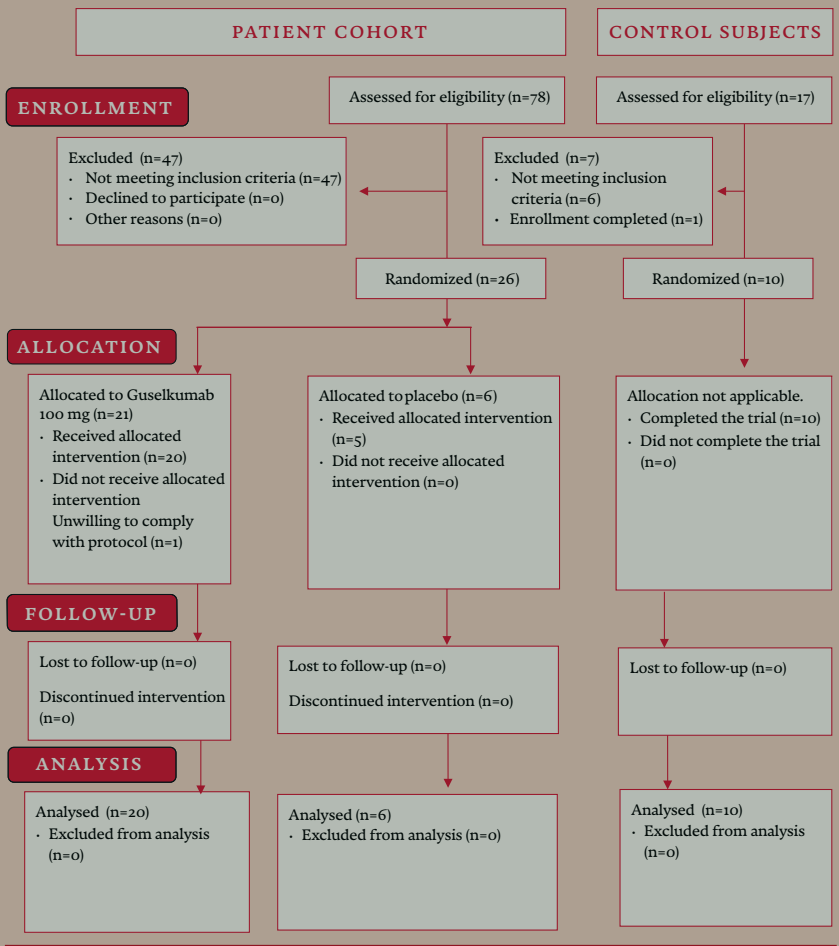


Table s1 Baseline demographics.

		Controls	Placebo	Guselkumab
Total number of patients		10	6	20
Age at first dose	≤ 18 years	0 (0%)	0 (0%)	0 (0%)
	18-65 years	10 (100%)	6 (100%)	19 (95%)
	≥ 65 years	0 (0%)	0 (0%)	1 (5%)
	Average age (years ± SD)	42.6±9.8	44.4±12.5	40.2±6.9
Gender	Female	7 (70%)	1 (17%)	5 (25%)
	Male	3 (30%)	5 (83%)	15 (75%)
Race	White	9 (90%)	5 (83%)	17 (85%)
	Asian	0 (0%)	1 (17%)	0 (0%)
	Hispanic	1 (10%)	0 (0%)	1 (5%)
	More than one	0 (0%)	0 (0%)	2 (10%)
Fitzpatrick	I	0 (0%)	0 (0%)	1 (5%)
	II	5 (50%)	3 (50%)	8 (40%)
	III	4 (40%)	2 (33%)	10 (50%)
	IV	1 (10%)	0 (0%)	0 (0%)
	V	0 (0%)	0 (0%)	0 (0%)
	VI	0 (0%)	1 (17%)	1 (5%)

OVERVIEW OF THE IN- AND EXCLUSION CRITERIA FOR THIS STUDY

Eligible healthy controls must meet all of the following inclusion criteria at screening:

1. Male or non-pregnant female subjects, 18 to 75 years of age (inclusive); during COVID-19 pandemic this is set to 18 to 69 year of age (inclusive)
2. Healthy as defined by the absence of any uncontrolled active or uncontrolled chronic disease following a medical and surgical history, documentation of general symptoms, and a symptom-directed physical examination including vital signs;
3. Willing to give written informed consent and willing and able to comply with the study protocol;

And eligible healthy controls must meet none of the following exclusion criteria at screening:

1. History or symptoms of any uncontrolled, significant disease including (but not limited to), neurological, psychiatric, endocrine, cardiovascular, respiratory, gastrointestinal, hepatic, or renal disorder that may interfere with the study objectives, in the opinion of the Investigator;
2. History of immunological abnormality (e.g., immune suppression, severe allergy or anaphylaxis) that may interfere with study objectives, in the opinion of the Investigator;
3. Known infection requiring antibiotic therapy within the last three months prior to the study;

4 Immunosuppressive or immunomodulatory treatment within 30 days prior to the study;
5 Body mass index (BMI) ≤ 18.0 or ≥ 40.0 kg/m^2; during COVID-19 pandemic only ≤ 18.0 or > 33.0 kg/m^2
6 Participation in an investigational drug study within 3 months prior to screening or more than 4 times a year;
7 Previous participation in an investigational drug study involving the dosing of an investigational compound targeting an immune pathway within one year prior to screening;
8 Loss or donation of blood over 500 mL within three months prior to screening;
9 The use of any medication or vitamin/mineral/herbal/dietary supplement within less than 5 half-lives prior to study participation, if the Investigator judges that it may interfere with the study objectives. The use of paracetamol (up to 4 g/day) is allowed;
10 History of alcohol consumption exceeding 5 standard drinks per day on average within 3 months of screening. Alcohol consumption will be prohibited from at least 12 hours preceding each study visit;
11 Any other condition that could interfere with the conduct of the study or the study objectives, in the opinion of the Investigator.
12 During COVID-19 pandemic: presence of high risk comorbidities: such as cardiovascular, respiratory or immune system disorders

Eligible psoriasis patients must meet all of the following inclusion criteria at screening:

1 Male or non-pregnant female sub jects, 18 to 75 years of age (inclusive); during COVID-19 pandemic this is set to 18 to 69 year of age (inclusive)
2 Diagnosed with plaque psoriasis at least 6 months prior to study participation
3 Willing to discontinue any psoriasis therapy other than emollients.
4 Having mild (PASI ≥1 and ≤ 5) or moderate-to-severe (PASI ≥ 10) plaque psoriasis;
5 Currently not using psoriasis medication and ≥ 2 plaques suitable for repeated biopsies and target lesion assessments. At least one of these lesions must be located on the extremities, preferably on the elbow or knee, with a minimal target lesion score between 6 and 9. Or, when currently using psoriasis medication and insufficient lesional skin is present, willing to discontinue treatment awaiting rescreening (see also exclusion criteria 3 for psoriatic patients);
6 Willing to give written informed consent and willing and able to comply with the study protocol;

And none of the following exclusion criteria at screening:

1 Having primarily erythrodermic, pustular or guttate psoriasis;
2 Having medication-induced psoriasis;
3 Having previously failed on ANTI-IL23 therapy;
4 Having received treatments for psoriasis within the following intervals prior to the start of the study:
   a < 2 weeks for topical treatment, e.g. retinoids, corticosteroids, vitamin D analogs
   b < 4 weeks for phototherapy, e.g. PUVA, PDT

c < 4 weeks for non-biologic systemic treatment, e.g. retinoids, methotrexate, cyclosporine, fumaric acid esters

d < 4 weeks for etanercept

e < 8 weeks for adalimumab

f < 3 months for anti-IL17, anti-IL12(/23) and anti-IL23 treatments

5 History or symptoms of any significant uncontrolled disease including (but not limited to), neurological, psychiatric, endocrine, cardiovascular, respiratory, gastrointestinal, hepatic, or renal disorder that may interfere with the study objectives, in the opinion of the Investigator, excluding psoriasis and conditions that are related to psoriasis;

6 History of immunological abnormality (e.g., immune suppression, severe allergy or anaphylaxis) that may interfere with study objectives, in the opinion of the Investigator;

7 Known infection requiring antibiotic therapy within the last 3 months prior to the study, including latent tuberculosis;

8 Systemic immunosuppressive or immunomodulatory treatment within 30 days prior to the study;

9 Body mass index (BMI) ≤ 18.0 or ≥ 40.0 kg/m^2; during COVID-19 pandemic only ≤ 18.0 or > 33.0 kg/m^2

10 Participation in an investigational drug study within 3 months prior to screening or more than 4 times a year;

11 Loss or donation of blood over 500 mL within three months prior to screening;

12 The use of any medication or vitamin/mineral/herbal/dietary supplement within less than 5 half-lives prior to study participation, if the Investigator judges that it may interfere with the study objectives. The use of paracetamol (up to 4 g/day) and is allowed;

13 History of alcohol consumption exceeding 5 standard drinks per day on average within 3 months of screening. Alcohol consumption will be prohibited from at least 12 hours preceding each study visit;

14 Any other condition that could interfere with the conduct of the study or the study objectives, in the opinion of the Investigator.

15 During COVID-19 pandemic: presence of high risk comorbidities: such as cardiovascular, respiratory or immune system disorders other than psoriasis and psoriasis arthritis.

SUPPLEMENTAL MATERIALS AND METHODS

Chemicals

HPLC-grade chloroform (Honeywell, Charlotte, North Carolina, United States), UPLC grade ethanol (Biosolve, Valkenswaard, the Netherlands), UPLC grade heptane (LiChorSolv, Merck, Darmstadt, Germany), UPLC grade isopropyl alcohol (Biosolve, Valkenswaard, the Netherlands), UPLC grade Methanol (Biosolve, Valkenswaard, the Netherlands) were used. The 0.25M potassium chloride solution was prepared by dissolving reagent grade potassium chloride (Sigma Aldrich, Saint-Louis, Missouri, USA) in ultrapure water from a Milli-Q Advantage A10 system (Merck, Darmstadt, Germany). Deuterated internal standard and ceramide standards were bought from Avanti Polar Lipids (Alabaster, Alabama, United States) or received from Evonik (Darmstadt, Germany). Polyphenylene sulfide tape (Nichiban, Tokyo, Japan) was used for tape stripping.

Tape stripping was performed after TEWL was determined and at the same location. Tapes 5, 6, 7, 8 were measured using a SquameScan 850A (Heiland Electronic, Wetzlar, Germany) to obtain the squame scan value (SQV) before a 16 diamter circle was punched out. The circles of tape were stored in in a 20 ml glass vial with chloroform:methanol (2:1) at <-40 °C prior to extraction. Tapes were extracted in three batches. Tapes were agitated by rotary shaker using an IKA S4000 at 120 rounds per minute at 40 °C for one hour. The solvent was collected and shaking repeated with 1 ml of chloroform:methanol:water (1:2:0.5), chloroform:methanol (1:1) and heptane:isopropylalcohol (1:1), sequentially, pooling the solvent of the 4 tapes per assessment together between each round of shaking. A liquid-liquid extraction was performed by the addition of 4 ml 0.25M KCL and the samples stored overnight at 4 °C to allow for phase separation. The organic layer was collected and the aqueous layer washed with 4 ml of chloroform. Organic layers were combined and filtered through 0.45 µm PVDF syringe filters (Grace, Deerfiel, Illinois, Unites States). After filtration, a aliquot of the samples were dried and reconstituted in 60 µl heptane:chloroform:methanol (95:2.5:2.5) with containing 10 µM CER[N(24DEU)S(18)] for analysis by ultra-performance liquid chromatography-mass spectrometry on a Acquity UPLC H-class (Waters, Milford, Massachusetts, Unites States) with a PVA-silica column (5 µm particles, 100 × 2.1 mm i.d.) (YMC, Kyoto, Japan) hyphenated to a XEVO TQ-S mass spectrometer (Waters, Milford, MA, USA) operating with atmospheric pressure chemical

ionization in positive mode. Samples were analyzed in three runs and each contained quality control samples from the same from combined stratum corneum extract pool and calibration curves. All patient samples at baseline showed sufficient responses and were used for relative analysis. Peak picking was performed using TargetLynx V4.1 (Waters, Milford, Massachusetts, Unites States). The monoisotopic response of ceramides corrected with the internal standard was used for corrections including adduct formation as determined from quality control samples and calibration curves, 13C isotope abundance, difference in response based on molecular weight determined from calibration curves and, if applicable, the SQV. 6 samples did not show any analytes. Absolute quantification using the SQV resulted in 2 non-lesional samples and 3 lesional baseline samples identified as outliers (ROUT method) which were excluded from analysis. Full details and validation of the method is described by Boiten, *et al.* (2016).

Statistical analysis

Analysis of baseline data, including pearson correlations, between groups was performed using PRISM 9.0 (GraphPad, Software, Boston, Massachusetts, United States). Profiles of controls, non-lesional psoriasis and lesional psoriasis were compared using One or Two-Way Analysis of Variance (ANOVA). Multiple comparisons were conducted with Tukey's test. Longitudinal analysis were performed in SAS 9.4 (SAS Institute Inc., Cary, North Carolina, United States). A repeated measures mixed effects model with treatment, time and the interaction as fixed factors, and time as a repeated factor is used. The baseline measurement is used as covariate. Any log-normal distributed data is log transformed before analysis, whereby ana are treated as missing. Analysis results are back transformed. Treatment graphs show the mean and 95% Confidence Interval and are reported by p-value. Common within-individual associations for paired measures were determined using Rmcorr in R Statistical Software (version 4.1.2, R Core Team 2021). Statistical significance is shown as: P<0.05: *, P<0.005:-and P<0.005: -*.

Figure s3 Stability of the ceramide profile over two weeks. The relative ceramide profile (A) and absolute ceramide profile (B) from lesional psoriasis, non-lesional psoriasis and matched locations of controls. Displayed data shows the ceramide profile at baseline (week 0) and two weeks before baseline (week -2). Statistical testing is performed with a two-way ANOVA comparing week -2 and week 0. Significance is only indicated for comparisons yielding $p<0.05$.

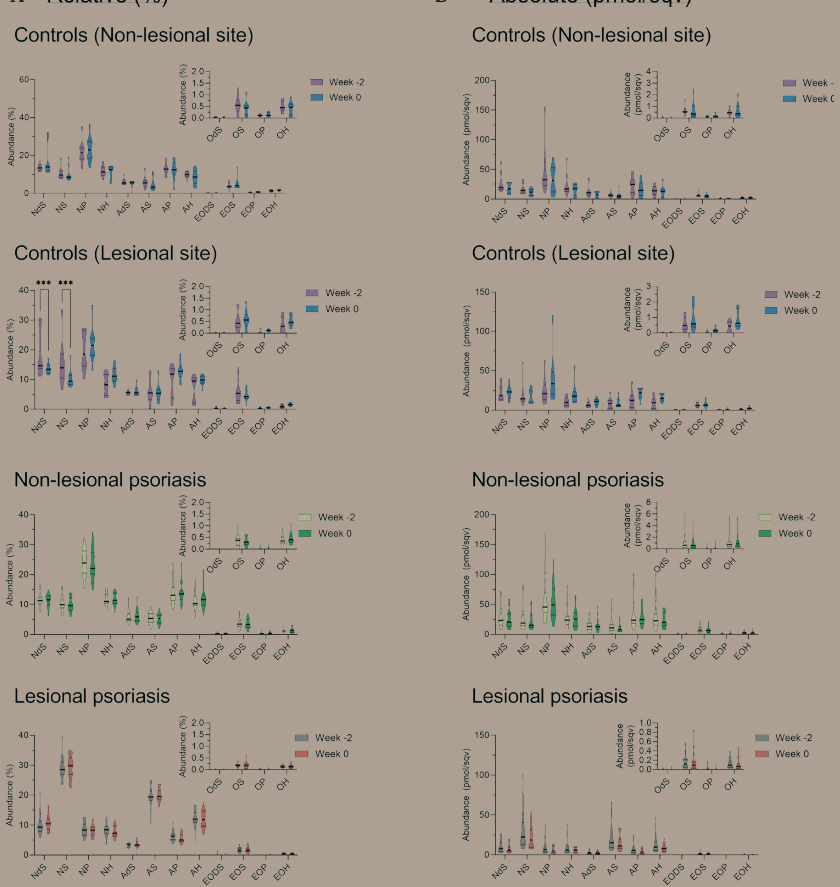


Figure s4 Stability of barrier function (A) and selected characteristics of the ceramide profile from lesional psoriasis, non-lesional psoriasis and matched locations of controls. The ratio between the abundance of CER[NS] and CER[NP] (B) and abundance of monounsaturated MUCER[NS] from the entire CER[NS] fraction (C) are shown. Specifics on the ceramide chain length (CCL) are shown with the abundance of CER[NSC34] of total CER[NS] (D), the average CCL of the CER[N] and CER[A] fraction (Cer[N,A]) (E), the CER[EO] fraction (F) and the CER[O] fraction (G). Displayed data shows the features at baseline (week 0) and two weeks before baseline (week -2) of the same location. Statistical testing is performed with a two-way ANOVA with "NS" indicating p>0.05.

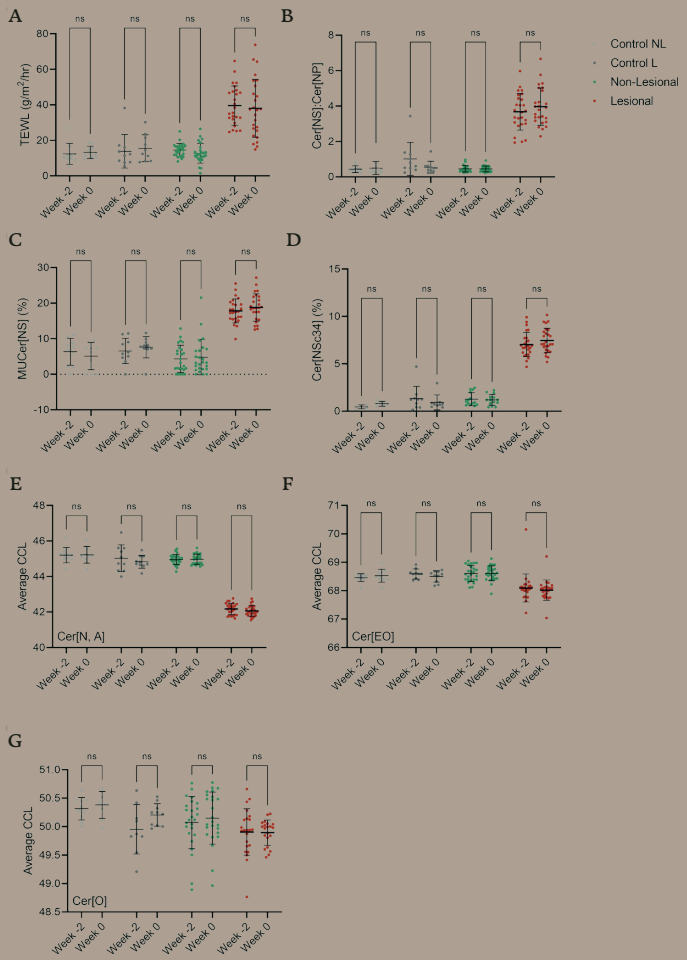


Figure s5 Stratification of the patient cohort based on PASI scores at baseline. Patients are assigned to the mild or moderate-to-severe group based on a PASI score of ≤5 or ≥10, respectively. Comparisons of disease severity based on PASI score (A) or Lesion Severity Score (B) showing target lesion severity is scored similarly despite a higher overall PASI in the moderate-to-severe group. Additionally, the ceramide characteristics in lesional skin are shown with the total ceramides after correction with the squamescan value (C), relative ceramide subclass profile (D), abundance of CER[NS C34] of total CER[NS] (E), ratio between the abundance of CER[NS] and CER[NP] (F), the fraction of monounsaturated ceramides in CER[NS] (G) and the average ceramide chain length (H) of CER[N, A], CER[EO] and CER[O], respectively. Statistical testing is performed with a two-way ANOVA, "NS" indicated p>0.05 and is not shown for panel (D).

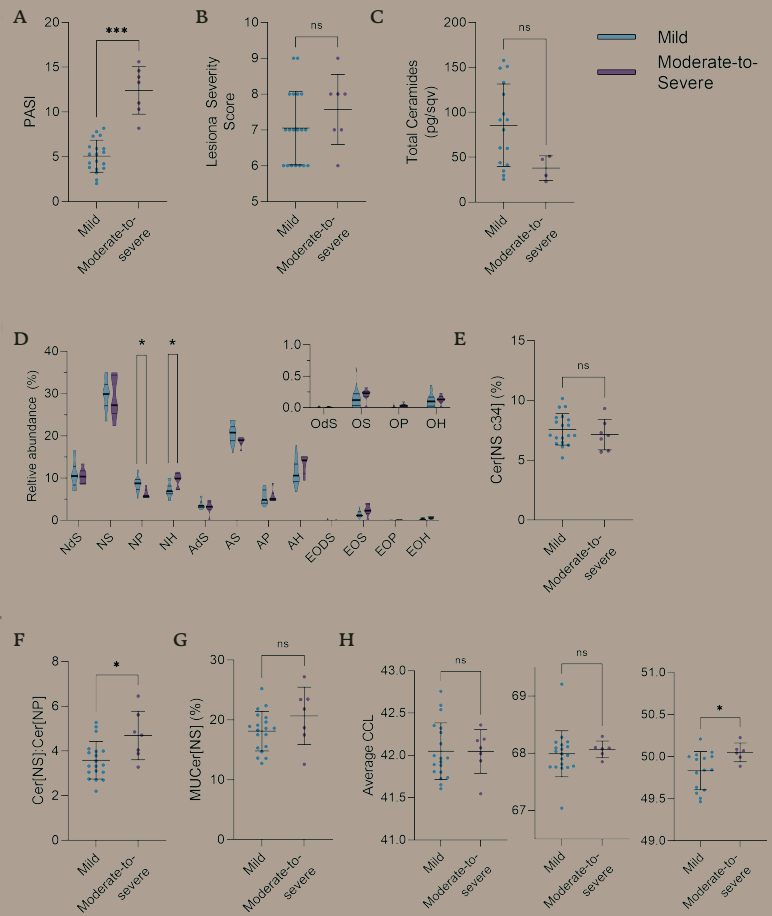


Figure s6 Principal component analysis of the ceramide profile at baseline of mild and moderate-to-severe patients as defined by a PASI score of ≤5 or ≥10, respectively. The high degree of overlap between the two severity groups indicates the ceramide profile is similar.

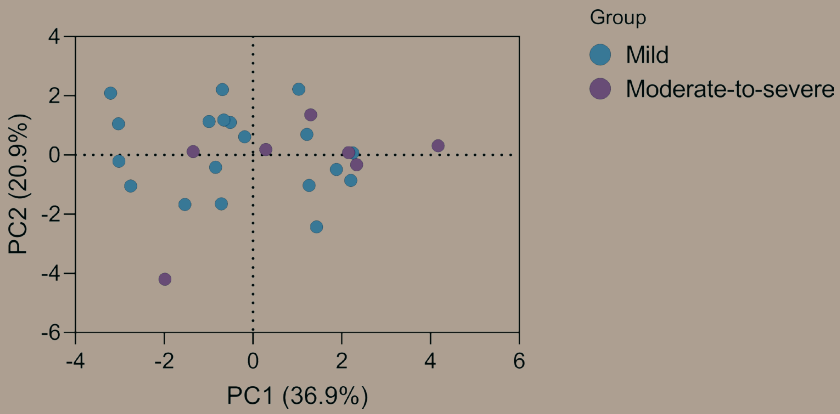


Figure s7 The ceramide chain length per subclass indicates that the decrease in Ceramide Chain Length (CCL) in lesional skin is seen throughout all subclasses, except for CER[EOP], CER[EOH] and CER[OH]. Of note, decreased CCLs in lesional skin are maintained when ceramides of ≤34 carbons in lengths are excluded from the analysis (data not shown).

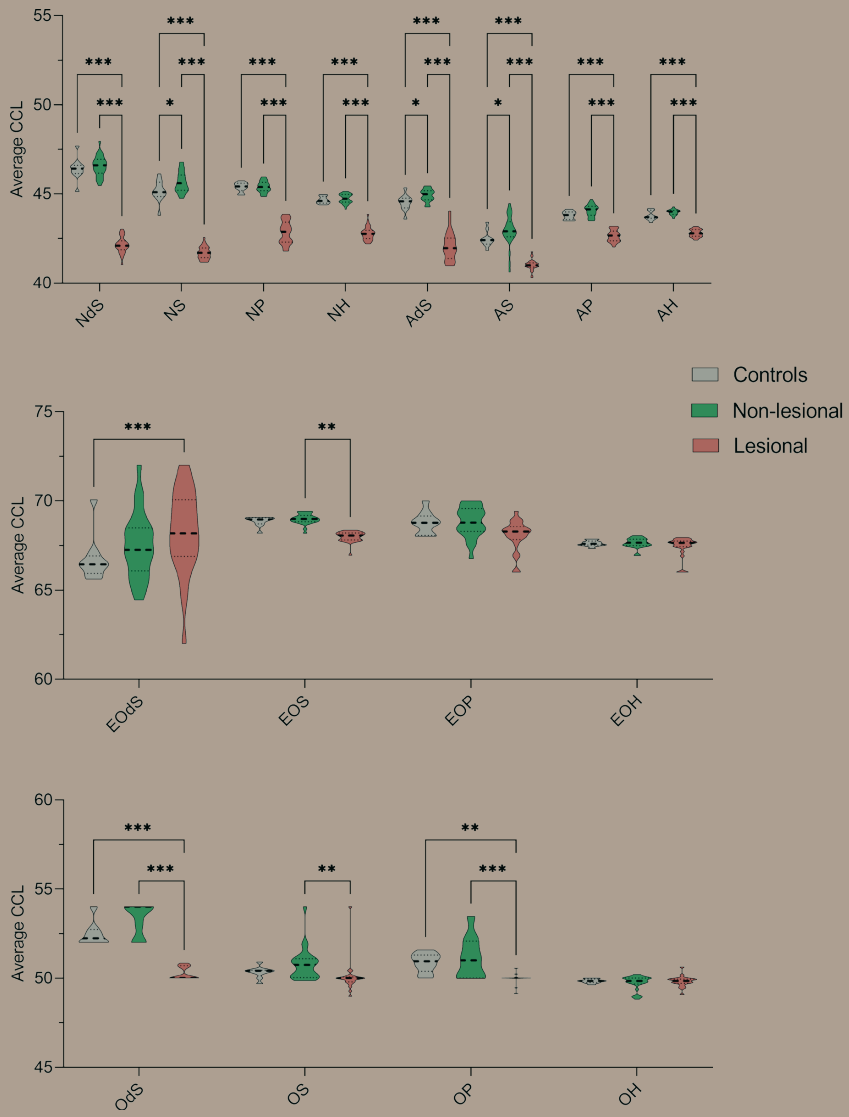


Figure s8 The Psoriasis Area and Severity Index (PASI) score over time in the guselkumab and placebo group.

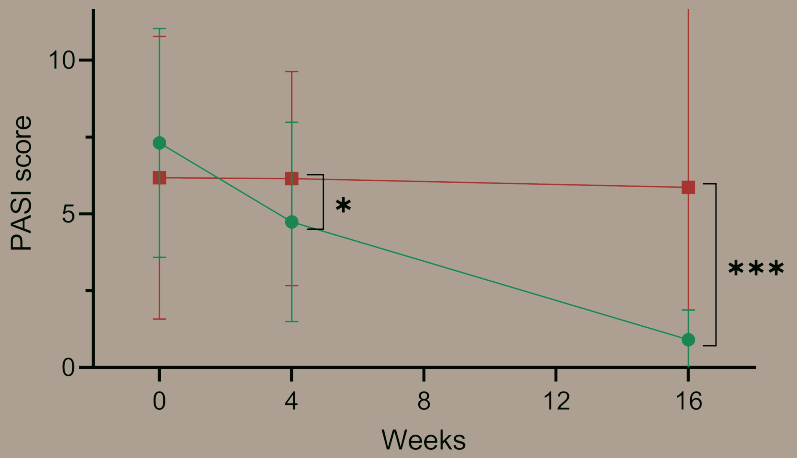


Figure s9 Ceramide chain length (CCL) over time during the treatment period. No significant differences are observed in the CCL of the CER[EO] fraction (left) nor CER[O] fraction (right) during treatment with guselkumab and placebo.