Investigations of skin inflammation with a novel dermatology toolbox for early phase clinical drug development

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SECTION III

CLINICAL APPLICATION IN ATOPIC DERMATITIS

CHAPTER VII

PHARMACODYNAMIC EFFECTS OF TOPICAL OMIGANAN IN PATIENTS WITH MILD TO MODERATE ATOPIC DERMATITIS IN A RANDOMIZED PLACEBO-CONTROLLED PHASE II TRIAL

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ABSTRACT

Omiganan is an indolicidin analogue with antimicrobial properties that could be beneficial for patients with atopic dermatitis. This randomized, double-blind, placebo-controlled phase II trial explored efficacy, pharmacodynamics and safety of topical omiganan once daily in 36 patients with mild to moderate AD. Patients were randomized to apply topical omiganan 1%, omiganan 2.5% or vehicle gel to one target lesion once daily for 28 consecutive days. Small but significant improvements of the local OSCORAD index and morning itch were observed in the omiganan 2.5% group compared to the vehicle gel group (-18.5%; 95%CI=-32.9,-1.0; p=0.04 and -8.2; 95%CI=-16.3,-0.2; p=0.05 respectively). A shift from lesional to non-lesional skin microbiota was observed in both omiganan treatment groups, in contrast to the vehicle group. In conclusion, treatment with topical omiganan improved dysbiosis in patients with mild to moderate atopic dermatitis and small but statistically significant improvement of clinical scores were detected. Our findings warrant further exploration in future clinical trials.

INTRODUCTION

Atopic dermatitis (AD) is a common skin disorder with a prevalence up to 3% in adults and up to 20% in children of the Western world.[1] Patients with AD have a xerotic erythematous skin with oozing and crusting that typically occurs on the flexor sites of the body. Severe pruritus is the main and most bothersome symptom for most patients, and can lead to reduced quality of life and reduced quality of sleep.[2] Current topical therapies for AD include bland emollients in combination with anti-inflammatory agents such as corticosteroids and calcineurin inhibitors. Side effects can be serious including HPA-axis suppression with extensive topical corticosteroid therapy. Recently dupilumab, the first monoclonal anti-IL4 antibody treatment for patients with moderate to severe AD was registered, and it is likely that may others will follow. However, for the patients with mild disease, novel therapeutic agents with a favorable side effect profile are wanted.

The pathophysiology of atopic AD is multifactorial and only partially understood. One of the factors involved in the pathogenesis is colonization and infection of the skin with *Staphylococcus aureus* that can produce and secrete toxins and act as super antigens leading to inflammation.(3) Colonization rates in a study of Park and colleagues show that up to 90% of the AD patients is colonized with this pathogen, compared to only 5-30% of the healthy subjects.(4) A deficiency in antimicrobial peptides (AMPs), which are important in the host defense system of the skin, accounts for the susceptibility to this bacterium in AD patients.[5] A dysregulated type 2 T helper (TH2) response in the skin leading to the production of pro-inflammatory cytokines such as IL-4 and IL-13 is most likely responsible for this AMP deficiency.[6,7] Therefore, topical AMPs are new potential therapeutic options for patients with AD.

Omiganan is a novel, synthetic, cationic peptide and an analogue of indolicidin, a short AMP from the cathelicidin family. It has demonstrated antimicrobial activity against a wide variety of gram positive and negative bacteria and fungi.[8-10] Cationic peptides are also suggested to have immunomodulatory roles, in both pro- and anti-inflammatory pathways depending on the context.[11,12] The combination of anti-bacterial and anti-inflammatory properties make omiganan a promising compound for the topical treatment of AD. While omiganan has been investigated before in several other indications, e.g. acne vulgaris and rosacea, this is the first trial to elucidate its effects in patients with AD.

In this study we aimed to explore the clinical efficacy, patient-reported outcomes, pharmacodynamics, safety and tolerability of topical omiganan on one target lesion in patients with mild to moderate AD.

MATERIALS AND METHODS

STUDY DESIGN, RANDOMIZATION AND TREATMENTS We conducted a randomized, double-blind, placebo-controlled mono-center phase 2 study to explore the clinical efficacy, patient-reported outcomes (PROs), pharmacodynamics (PD), safety and tolerability of omiganan in 36 patients with mild to moderate AD. The Declaration of Helsinki was the guiding principle for trial execution, and the study was approved by the independent Medical Ethics Committee 'Medisch Ethische Toetsingscommissie van de Stichting Beoordeling Ethiek Biomedisch Onderzoek' (Assen, the Netherlands) prior to any trial procedure. All patients provided written informed consent before participation. The study was conducted from June 2015 to November 2015 at the Centre for Human Drug Research, Leiden, The Netherlands. Eligible patients were randomized to apply either topical omiganan 1%, omiganan 2.5% or vehicle gel, which served as placebo with an identical appearance, once daily (QD) for 28 consecutive days to one target lesion. This was done 1:1:1 in blocks of three according to a randomization list with codes generated by the unblinded statistician. Next to the active ingredient omiganan pentahydrochloride, the excipients of the compound are glycerin, hydroxyethyl cellulose, benzoic acid, sodium benzoate and water. For safety reasons, only the pre-defined target

lesion (one antecubital fossa) was treated in this first in patient study with drug/placebo but not with emollient. Study drug application was recorded with use of a validated mobile phone e-diary application. Subjects, study personnel and investigators were blinded for the allocated treatment throughout the study. Emollients (unguentum leniens) were handed out to use QD, but not on the target lesion. Patient visits were scheduled at day -14 (run-in period), 0, 1, 3, 7, 14, 21, 28 (end of treatment, EOT), 35 and 42 (end of study, EOS). During the run-in period patients applied emollients to the skin (not on the target lesion) and triamcinolone 0.1% ointment to eczema lesions except for the target lesion, if needed. All study details are provided in the protocol and assessment schedule, Supplemental Material S1 and S2.

PATIENTS ❋ Patients were included if: 1) AD was mild to moderate and (intermittently) present for more than one year, 2) aged between 18 and 65 years, and 3) willing to give written informed consent. Inclusion criteria were an OSCORAD index of 8-40, and an affected body surface area (BSA) of 5-15%. For PD assessments throughout the study, all patients should have at least one target lesion (one antecubital fossa) affected by AD, with a BSA of ≥0.5% and a pruritus numeric rating scale (NRS) score of at least 30 on a 0-100 scale. There was a wash-out period for any type of AD medication; for cyclosporine, mycophenolate mofetil and other systemic immunosuppressive drugs 4 weeks, phototherapy 3 weeks, biologics 5 half-lives of the drug, topical calcineurin-inhibitors 10 days, topical corticosteroids 2 weeks, and any other topical medication (prescription or over the counter) 2 weeks. Patients with other clinically significant (skin) conditions in the treatment area were excluded. Health status of included patients was verified by a detailed medical history, physical examination, vital signs, 12-lead ECG and laboratory test (including hepatic and renal panels, complete blood count, chemistry panel, virology and urinalysis). Patients were evaluated the four most prevalent fillagrin mutations in Europe at screening (2282DEL4, R501X, S3247X AND R2447X).

CLINICAL EFFICACY, PHARMACODYNAMIC ASSESSMENTS AND PATIENT REPORTED OUTCOMES ❋ One target lesion was assigned for treatment and PD assessments in the trial. Another non-treated eczema lesion served as a control lesion for part of the PD measurements (off-target side). Clinical efficacy was assessed by the local OSCORAD index (% of BSA, erythema, edema/papulation, oozing/crusting, excoriation, lichenification and dryness) of the target lesion. Standardized pictures were taken by a 3D stereo-camera system (LifeVizTM QuantifiCare, Valbonne, France) for the assessment of the target lesion size and roughness analysis. The skin barrier status of lesional and non-lesional skin was assessed by trans-epidermal water loss assessment (TEWL, AquaFlux AF200 system, Biox, London, UK). This was done under standardized environmental conditions (temperature 22°C±2°C; relative humidity <60%) and patients were acclimatized under relaxed conditions for at least 15 minutes prior to measurements. All measurements were performed at each study visit. PROs consisted of NRS itch (0-100) and the 5-D itch scale.[13,14] The itch scores were divided in morning and evening itch in order to link the application time with efficacy outcomes.

SWAB COLLECTION FOR MICROBIOLOGY ❋ Patients were instructed not to wash or apply the study drug and keep the target lesion dry for at least 24 hours prior to the study visit. Skin swab samples were collected with sterile swabs that were dipped in a NaCl Tween solution (Puritan Sterile Polyester Tipped Applicators REF 25-806-1PD), before rubbing the tip of the swab firmly over 4cm² of the target lesion for 5 times. Hereafter the swab material was placed in a micro tube (REF 72.694.105, Sarstedt, Numbrecht, Germany) containing 0.9% NaCl and 0.1% Tween 20. Analysis was performed as described by van den Munckhof *et al.*[15]

MICROBIOLOGY ANALYSIS – S. AUREUS QUANTIFICATION ❋ A single-plex quantitative PCR (qPCR) adapted from literature.[16] targeting the nuc gene was applied to quantify the *S. aureus* bacterial load. Quantification of bacterial load was done by comparing the results of the samples with the results of a standard curve with known concentrations. This standard curve was tested in parallel to the samples in each experiment. Samples that were below the limit of quantification (LOQ) were used in the analysis as ½ LLOQ.

MICROBIOME ANALYSIS ❋ The DNA extraction was performed automatically with the Magna pure 96 instrument using the Magna Pure 96 DNA and Viral NA Large Volume Kit and the Pathogen universal 500 (Roche Diagnostics, Basel, Switzerland). An input volume of 500µL sample and an elution volume of 100µl was used. After DNA extraction, the variable regions V3 and V4 of the 16S rRNA gene were amplified using the primers described by Klindworth, *et al.*[17]: Bakt_341F (5′CCTACGGGNGGCWGCAG -3′) and Bakt_805R (5′- GACTACHVGGGTATCTAATCC -3′) with Illumina overhang adaptor sequences added. The generated amplicons of around 460 base pairs were analyzed on a capillary system using a standard protocol, to confirm successful amplification of a PCR fragment of the expected

size. As a next step, PCR products were cleaned up by Ampure XP beads (Beckman Coulter) to remove primer-dimers and small a-specific PCR products and the purified PCR products were quantified using the Quant-iT PicoGreen dsDNA kit (Life Technologies), followed by serial dilution steps to reach the correct amount of input DNA. Index primers (Nextera XT Index kit) were added by limited cycle PCR to the diluted PCR products. Prior to pooling, samples were normalized by using beads with maximum binding capacity (Nextera XT sample preparation kit).

The sequencing was performed on the Illumina MiSeq platform by using the MiSeq v2 sequencing kit with 500 cycles (Illumina). De-multiplexed FASTQ files were generated as output and the sequences of the FASTQ files were analyzed using the Metagenomics workflow of the MiSeq Reporter software of Illumina, resulting in a taxonomy percentage summary of the sequenced bacterial sample. To calculate the relative abundance of microorganisms at genus level the unclassified DNA was excluded, hence the sum of the percentages of the DNA of all microorganisms found was set to 100 %. Furthermore, the microorganisms with an abundance of less than 10% were excluded from the analysis.

BIOPSY BIOMARKERS ❋ Skin punch biopsies (4mm) were collected from lesional skin at day 0 (pre-dose) and day 28 and from non-lesional skin at day 0. Biopsies were placed in RNA later medium directly after harvest of the biopsy and stored and -80ºC. The biopsies were analyzed at DDL Laboratory, Rijswijk, The Netherlands. RNA extraction and real-time quantitative PCR analysis relative to the housekeeping gene GAPDH. Because of limited material a selection of markers in the protocol was made. The final set of markers was chosen based on disease involvement (IL-31, eotaxin, IFN-γ) and expected investigational drug effects (IFN-α, IFN-γ, IL-6).[18]

SAFETY AND TOLERABILITY ❋ Safety and tolerability endpoints were assessed by the frequency of treatment-emergent adverse events (TEAEs), serious adverse events (SAEs), discontinuations due to AEs or deaths occurred, laboratory values (hematology, chemistry, coagulation, and urinalysis), vital signs, electrocardiographic parameters, and physical examination.

TREATMENT COMPLIANCE AND EXPOSURE ❋ Compliance of study drug application was recorded using a mobile e-diary application which entailed a notification and photo capture function enabling the date and time documentation of each gel application.

STATISTICAL METHODS ❋ All calculations were performed using SAS for windows v9.4 (SAS Institute, Inc., Cary, NC, USA). No formal power calculation was performed given the exploratory character of this first in patient study. Clinical efficacy and pharmacodynamic endpoints were analyzed with a mixed model analysis of variance using treatment, time and treatment by time as fixed factors and subject as random factor. Analyses were conducted in the clinical evaluable (CE) population. This population consisted of all subjects that applied the study medication for at least 21 days and completed the EOT visit. The results per variable were generated with estimates of the difference of the different contrasts and a back transformed estimate of the difference in percentage for log transformed parameters, 95% confidence intervals (in percentage for log-transformed parameters) and Least Square Means (geometric means for log transformed parameters), and the p-value of the contrasts. The analyses of the mRNA expressions in the biopsies incorporated normalization for housekeeping gene GAPDH. Moreover, it incorporated the values from non-lesional skin to correct for the high variability. For the organization and visualization of the microbiome data, Python 3.5.2 was used.

RESULTS

PATIENT CHARACTERISTICS ❋ Fifty-nine patients were screened of whom 36 were enrolled in the study. All enrolled patients completed the study (Figure 1). Baseline characteristics were comparable between the treatment groups (Table I). The overall mean BSA of the target lesion was 1.4% (±0.9).

CLINICAL EFFICACY ❋ A reduction of the target lesion OSCORAD index was observed in both active treatment groups compared to vehicle gel, mainly due to a reduction in % of BSA. This reduction was statistically significant for omiganan 2.5% (-18.5%; 95%CI=-32.9,-1.0), but not for omiganan 1% (-13.4%; 95%CI=-28.4,4.6) (see *Figure* 2A), which might indicate a dose dependency. The reduction in the OSCORAD index in the omiganan 2.5% group was accompanied with a trend in reduction of % of BSA of the target lesion (-0.31; 95%CI=-0.64,0.03) (see Figure2B).

PATIENT-REPORTED OUTCOMES ❋ A significant decrease on the 0-100 NRS itch scale was observed during the morning in the omiganan 2.5% group compared to the vehicle gel (-8.2; 95%CI=-16.3,-0.2), but not in the omiganan 1% group (-1.1; 95%CI=-9.5,7.4). Itch during the evening slightly decreased in

both active treatment groups. However, these decreases were not statistically significant (see *Figure* 2C,D).

EXPLORATORY PHARMACODYNAMICS ❋ Skin barrier function as measured by TEWL improved in all treatment groups (-4.5, -8.8 and -12 in the vehicle gel group, omiganan 1% and omiganan 2.5% group respectively) (*Figure* 3A). The 3D photo analysis revealed no significant changes in the roughness of the skin surface (data not shown). Biomarkers demonstrated a high degree of variability in general. At baseline a significant difference between lesional and non-lesional mRNA expression in skin relative to GAPDH was observed for the markers eotaxin, IFN-γ and IL-31 (see Figure3B, C and D). There was no difference between lesional and non-lesional skin for the markers IFN-α and IL-6. No significant reductions of biomarkers were observed in any of the treatment groups. An example of the relative mRNA expression of eotaxin before and after treatment is shown in *Figure* 3E.

MICROBIOLOGY AND MICROBIOME ❋ No significant reductions in total bacterial load were observed in both active treatment groups, i.e. omiganan 1% and omiganan 2.5%, compared to the vehicle gel group (data not shown). For the bacterial load data of *S. aureus* data by qPCR, a high proportion of samples showed results below the limit of quantification. There were no differences between the treatment groups of the quantified samples. Microbiome data demonstrated a high degree of variability between patients in the presence and abundances of the genera. However, in general, the presence of the *Staphylococcus* genus dominated lesional skin compared to non-lesional skin (*Figure* 4). Individual data can be found in Supplemental material S3. After both active treatments *Staphylococcus* abundance decreased significantly from 64% to 37% for omiganan 1% (p=0.05) and from 70% to 42% (p=0.01) for omiganan 2.5% compared to vehicle (*Figure* 4 and *Figure* 5A), while the abundance in the vehicle group remained stable. With the decrease of *Staphylococcus* abundance, the summary of the microbiome diversity (Shannon index) significantly increased up to a total change of 0.11 for omiganan 1% (p=0.03) and 0.08 for omiganan 2.5% (p=0.03 compared to vehicle). At baseline, a moderate correlation was found between the target lesion OSCORAD index and *Staphylococcus* abundance (r=0.409) and between the target lesion OSCORAD index and diversity index (r=0.333). However, the decrease in the target lesion OSCORAD index as seen in both treatment groups did not correlate with the reduction in *Staphylococcus* abundance and increase in diversity (r=0.182 and r=-0.096, respectively) (*Figure* 6).

SAFETY AND TOLERABILITY ❋ One or more TEAEs were experienced by 18 of 36 patients (50%). All TEAEs were of mild (n=24) or moderate severity (n=3), The most frequent TEAEs were headache, upper respiratory tract infections and influenza like illness. They were all self-limiting and were considered unlikely related (n=28) to treatment. No local AEs were reported. No discontinuations due to adverse events occurred. Application did not result in any clinically significant changes in safety laboratory parameters or vital signs. No SAEs, discontinuations due to AEs or deaths occurred.

TREATMENT COMPLIANCE AND EXPOSURE ❋ Treatment compliance was high and comparable in all treatment groups and ranged from 89-100%.[19] The mean usage of study drug per day ranged from 1.2 to 1.3 mg per cm² in all treatment groups.

DISCUSSION

This is the first randomized, double-blind, controlled clinical trial exploring the clinical efficacy, pharmacodynamics and safety of omiganan in patients with AD. Treatment with omiganan 2.5% resulted in a clinically small but statistically significant reduction in the target lesion OSCORAD index and morning itch after 28 days of treatment compared to treatment with a vehicle gel. This reduction was mainly caused by a reduction in BSA. As *proof-of-pharmacology*, a shift from lesional to a non-lesional microbiome profile in terms of a reduction of *Staphylococcus* genus and increase in diversity was seen in both active treatment groups compared to the vehicle gel group.

The shift in microbiome profile observed, was predominantly driven by a reduction in *Staphylococcus* genus. This can be explained by the previously reported activity of omiganan against this genus.[8] The abundance of *Staphylococcus* is known to increase in skin affected by AD. Our study did show a moderate correlation between the clinical score (OSCORAD index) and the disturbed microbiome profile at baseline, which is in concordance with the correlations of around 0.50 found by others.[20–22] The correlation of improvement of the OSCORAD index and the degree of dysbiosis would suggest an important role of the microbiome in the pathogenesis of AD. However, a decrease in abundance of *Staphylococcus*, or an increase of microbial diversity, did not correlate with OSCORAD index improvement in our study. In some patients, this relationship was even reversed. In contrast, in previous studies with other treatments, such as topical corticosteroids and emollients, a correlation of clinical improvement and *Staphylococcus* reduction and/or increase

in diversity index was reported.[20,22] A delay in clinical improvement after a recovery of the microbiome or variable individual responsiveness of the microbiome in the small treatment group in our study, may be underlying mechanisms prohibiting a clear insight in the relation between the microbiome and clinical efficacy. It is also known that some subjects by nature have a higher abundance of *Staphylococcus* up to 30% and/or a greater microbial diversity on the skin than others while this does not appear to contribute to disease activity.[23,24] An alternative explanation could be that recovering the microbiome in mild to moderate AD patients does not lead to an improvement of clinical symptoms, but the effect is the other way around, in which reduction of inflammation leads to normalization of the microbiome. This notion is supported by the fact that there is no evidence for a beneficial effect of antimicrobial interventions in non-infected AD, and the fact that the microbiome can recover with topical corticosteroids alone, which evidently lack antimicrobial properties.[25] More studies are needed to provide full insight in the relationship between the microbiome and inflammation in AD, as the outcomes of our study do not give a clear insight.

The clinical effects on the target lesion OSCORAD and affected BSA might be explained by the immunomodulatory effects of omiganan. Although no data of omiganan on this is available yet, comprehensive data of immunomodulatory potential is available on another member of the cathelicidin family, i.e. LL-37, the only endogenous human cathelicidin. LL-37 has anti-inflammatory properties including the inhibition of AIM2 inflammasome formation and suppression of IFN-γ, TNF-α, IL-4 and IL-12.[11,26,27] IFN-γ expression remained unchanged after treatment (data not shown), the other pro-inflammatory markers were not included in this study. Moreover, LL-37 is involved in skin barrier homeostasis and presumably suppresses itch.[28] On the contrary, LL-37 is also involved in several pro-inflammatory pathways when excessively present, e.g. in the downregulation of IL-10 and mast cell release of inflammatory mediators.[29,30] In summary, there is evidence from AMP family members that the clinical effects of omiganan can rely on the immunomodulatory properties rather than the antimicrobial properties but more *in vitro* and *in vivo* studies with omiganan are needed to draw conclusions.

MICROBIOME AS BIOMARKER ✻ In a previous literature review we described the potential of skin microbiome associated outcomes as biomarker in early phase clinical AD trials.[31] Although the exact relation between the skin microbiome and AD pathophysiology remains debatable based on this review the implementation looked promising with respect to antimicrobial therapies.[31] In a longitudinal observation of AD patients no major dissimilarity and robust microbiome profiles over time of lesional and non-lesional skin were observed.[15] With the current study we observed that the clear improvements in the microbiome only weakly correlated with clinical response. Therefore the microbiome may be considered as disease biomarker in AD to a lesser extent. For this part, since only a single AD lesion was treated and since a high inter-individual microbiome variability was observed, a larger study with total body application is needed to explore the full potential. However, the microbiome in this study provided *proof-of-pharmacology* of topical omiganan in patients with mild to moderate atopic dermatitis by serving as drug mechanistic biomarker when assessing target engagement.

ITCH REDUCTION NOT CLINICALLY MEANINGFUL ✻ Although there was a small statistically significant reduction in the morning itch with omiganan 2.5% treatment compared to vehicle, the minimal clinically important difference (MCID), which is the smallest patient-reported outcome change that is considered clinically meaningful,[32] was not achieved. The mean reduction in NRS morning itch in the 2.5% treatment group was 8.2 (on a scale of 0-100), while the MCID for itch is determined as at least 20 to 30-point reduction.[33] The evening itch did not decrease significantly. When looking at the time of dose administration, most patients applied the gel in the morning (60%), which might explain why the effects on itch in the evening are less apparent. The itch reduction did not correlate with a reduction of IL-31 mRNA expression, a biomarker for itch, in the skin biopsies at EOT which supports the debate about the questionable clinical relevance of the observed reduction.[34]

LIMITATIONS ✻ Only one target lesion was treated with the study drug for safety reasons, therefore potential efficacy when applied on all lesions remains unknown. When treating and trying to recover only one lesion in terms of inflammation and *Staphylococcus* reduction, auto contamination of other lesions may occur, which might be important assuming *Staphylococcus* plays a major role in the pathogenesis. However, our results indicated a clear shift in microbiome profile with a reduction in *Staphylococcus* genus. It remains unclear whether this includes *S. aureus* or other *Staphylococcus* species since analysis on species level was not feasible with our NGS determination method. Unfortunately, the qPCR analysis was not able to perform quantitation in many samples which precluded statistical analysis. Cultures to compare with our NGS method were not performed in this study. Another limitation concerns the scoring of itch of the target lesion only. I may be hard for the patients to discern the itch from

the target lesion from the itch related to other AD lesions and this might have influenced the scoring. Furthermore, study groups were relatively small to make definite conclusions about efficacy, and the clinical relevance of the decrease in target lesion OSCORAD and morning itch is therefore debatable.

In conclusion, in patients with mild to moderate atopic dermatitis, the topical administration of omiganan QD to a limited treatment area for up to 28 days is safe and well tolerated. Pharmacological activity in terms of a significant reduction in the OSCORAD of the predefined target lesion and the patient-reported itch was observed with the highest dose of 2.5%. However, since these reductions were small, clinical relevance of both is debatable. The microbiome showed a significant shift from lesional to non-lesional skin profile with both active treatments, which did only show low correlations with the clinical improvement. Future studies with optimization of the treatment regimen, i.e. dose (exploration of more concentrations), frequency (BID instead of QD), and other sub-indications (such as infected AD) are necessary to explore the true potential of omiganan in patients with AD.

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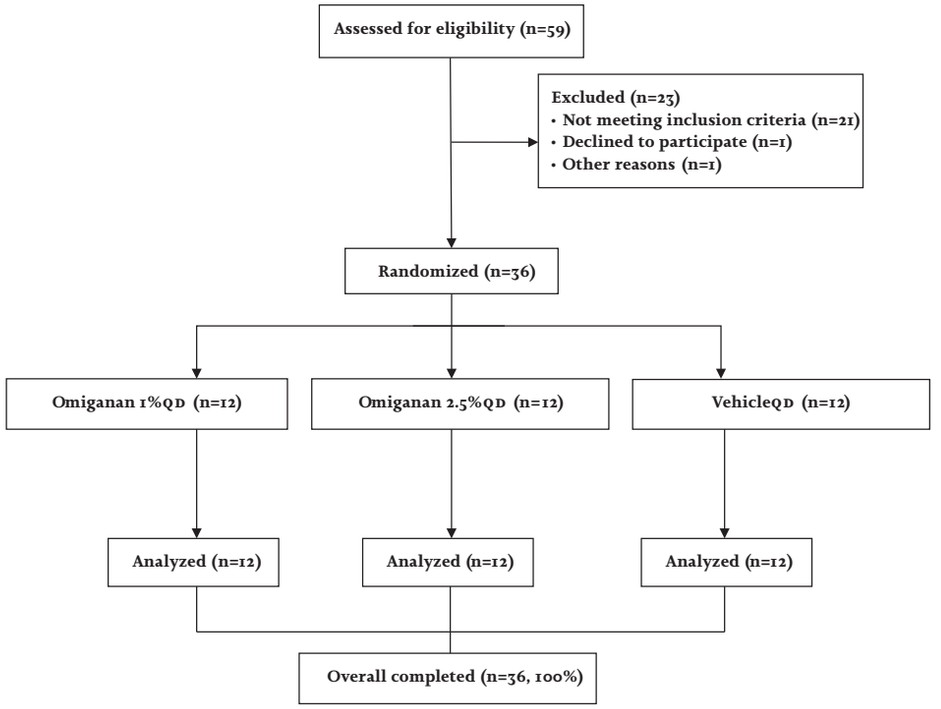
TABLE 1 **Baseline characteristics of the study.**

	Omiganan 1% N = 12	Omiganan 2.5% N = 12	Vehicle gel N = 12	Total N = 36
Sex, n (%)				
Female	9 (75%)	10 (83%)	8 (67%)	27 (75%)
Male	3 (25%)	2 (17%)	4 (33%)	9 (25%)
Age, years (SD)	25.0 (5.2)	25.1 (7.1)	24.7 (10.9)	24.9 (7.8)
Fitzpatrick Skin Type, n (%)				
I	0 (0%)	0 (0%)	0 (0%)	0 (0%)
II	4 (33%)	5 (42%)	5 (42%)	15 (42%)
III	4 (33%)	5 (42%)	3 (25%)	12 (33%)
IV	1 (8%)	1 (8%)	2 (17%)	4 (11%)
V	2 (17%)	0 (0%)	1 (8%)	3 (8%)
VI	1 (8%)	0 (0%)	1 (8%)	2 (6%)
Years since diagnosis – mean (SD)	20.5 (9.4)	19.2 (10.9)	21.1 (12.2)	20.3 (10.6)
Exacerbations per year – mean (SD)	7.3 (6.9)	9.1 (6.4)	11.2 (9.8)	9.2 (7.8)
Subjects with Filaggrin mutation – n (%)	0 (0%)	3 (25%)	4 (33%)	7 (19%)
% BSA - target lesion – mean (SD)	1.5 (0.9)	1.0 (0.5)	1.6 (1.1)	1.4 (0.9)
OSCORAD index - target lesion – mean (SD)	17.6 (7.5)	16.3 (4.5)	18.1 (8.4)	17.3 (6.8)
% BSA - all lesions – mean (SD)	9.1 (5.7)	7.0 (7.2)	8.9 (3.4)	8.3 (5.6)
OSCORAD index - total body – mean (SD)	18.4 (8.4)	18.9 (6.4)	17.8 (5.4)	18.4 (6.7)
Previous treatment with corticosteroids (USA classification)– n (%)				
Class IV corticosteroid	6 (50%)	8 (67%)	6 (50%)	20 (56%)
Class III corticosteroid	6 (50%)	7 (58%)	7 (58%)	20 (56%)
Class II corticosteroid	5 (42%)	7 (58%)	6 (50%)	17 (47%)
Class I corticosteroid	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Calcineurin inhibitor	2 (17%)	7 (58%)	2 (17%)	11 (31%)

SD = standard deviation; BSA = body surface area; OSCORAD = objective SCORAD index

FIGURE 1 Flowchart of the study.



QD = *qualified dose (once daily)*.

FIGURE 2 **Change from baseline in body surface area, OSCORAD index and morning and evening itch in the omiganan 1% and 2.5% treatment groups compared to vehicle gel.** Change from baseline graphs: delta least squares means (LSM) over time of clinical assessments BSA (Panel A.) and OSCORAD (Panel B.) of target lesion. In the lower panels the patient-reported outcomes are depicted, i.e. itch morning (C.) and itch evening (D.).

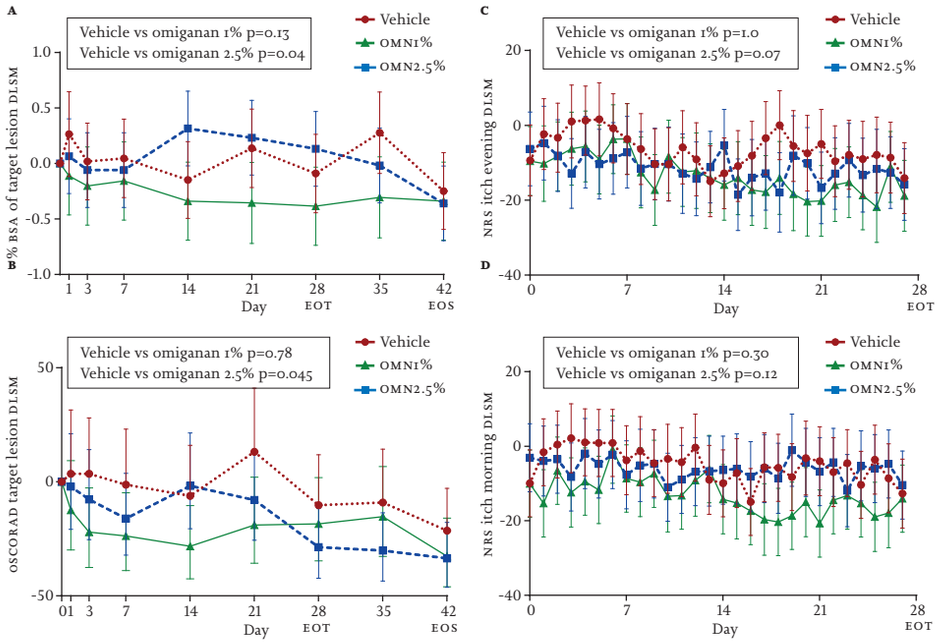


FIGURE 3 Pharmacodynamic effects of topical omiganan in the omiganan 1%, omiganan 2.5% and vehicle gel group. In panel A the trans-epidermal water loss (TEWL) over time is depicted showing improvement in all treatment groups including vehicle. In panels B, C and D relative mRNA expressions in skin punch biopsy of markers IL-31 (B), eotaxin (C) and IFN-γ (D) in lesional versus non-lesional skin in mild to moderate atopic dermatitis patients at baseline, medians with interquartile ranges are presented. Statistical significance is indicated as follows: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, based on a paired t-test on log transformed data. E) relative mRNA expression of eotaxin per treatment group before treatment lesional skin (day 0), after end of treatment lesional skin (day 28) and non-lesional (NL). No treatment effect occurred on this marker.

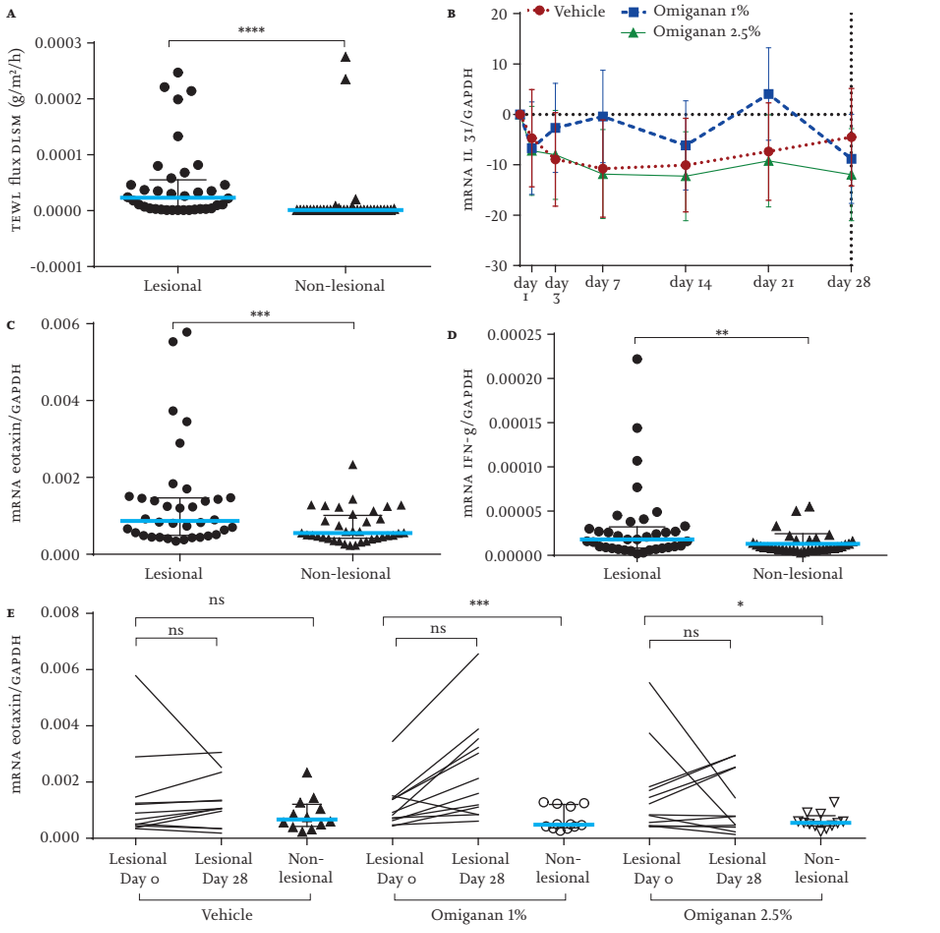


FIGURE 4 Course of cutaneous microbiome after omiganan treatment over time. Relative *Staphylococcus abundance* over time in the omiganan 1% group (panel A), omiganan 2.5% group (panel B.) and vehicle group (panel C) are depicted. A reduction is seen in both active treatment groups while this is not present in the vehicle group. Non-lesional boxes are presented as control on the right side. Above the box plots the values of the median are indicated in blue.

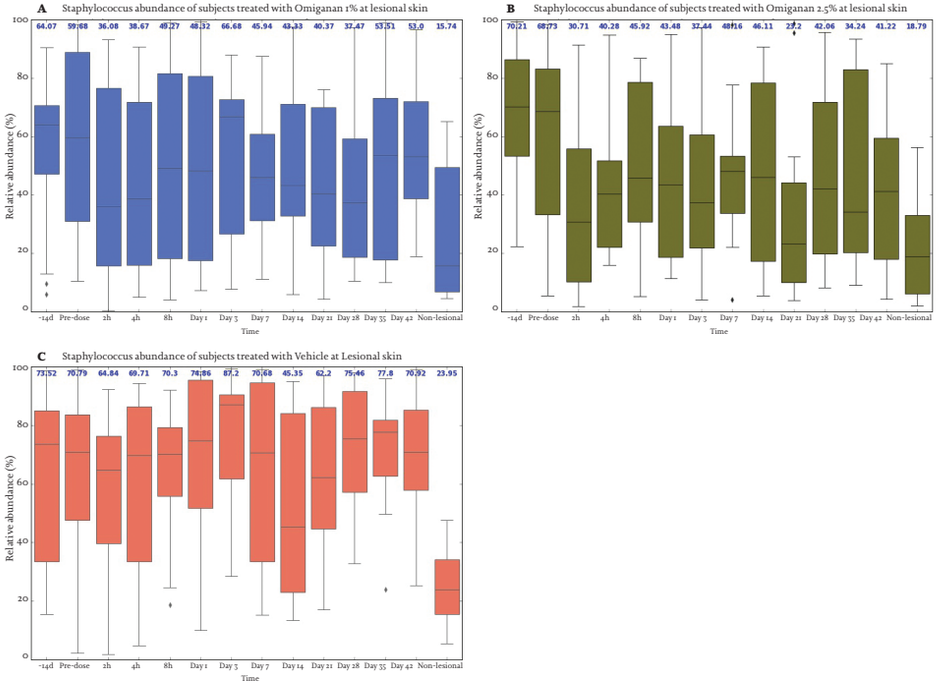


FIGURE 5 Omiganan improves dysbiosis of target lesion. *Staphylococcus abundance* (A.) and diversity index (B.) from baseline to day 28 (end of treatment) per treatment group with p-values of the differences as calculated with a mixed model of data over time.

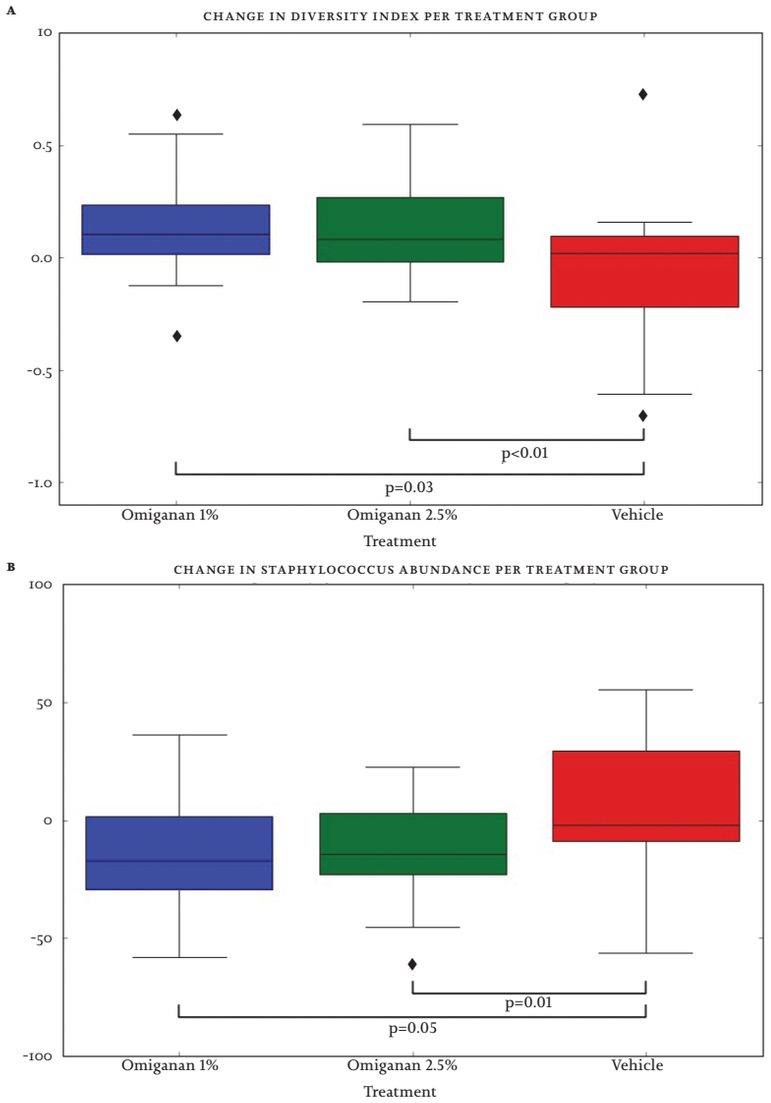
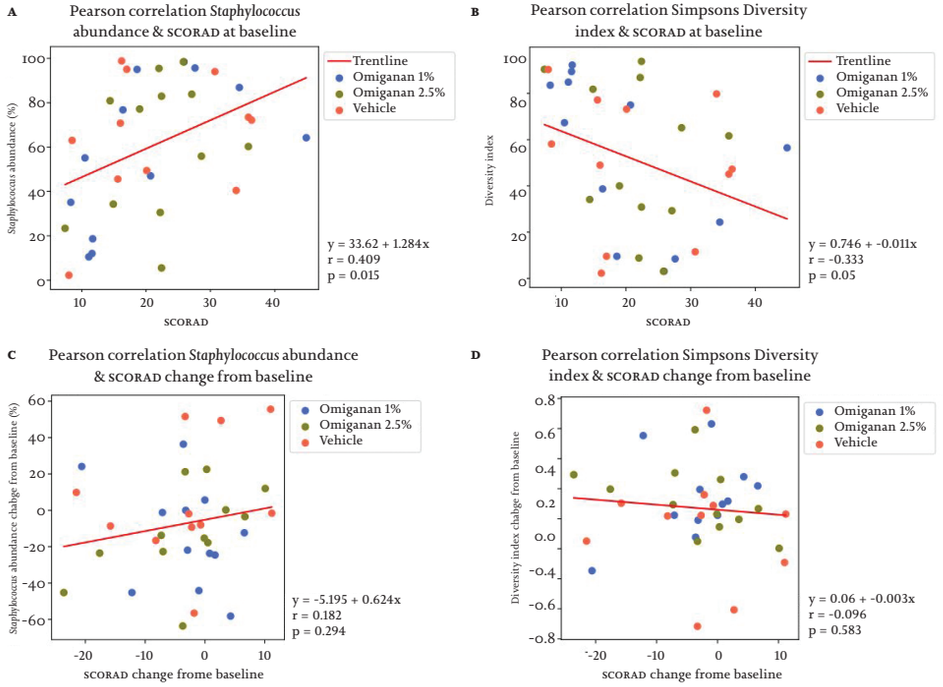


FIGURE 6 Correlation analysis of target lesion OSCORAD index and *Staphylococcus abundance*. The correlation between the local target lesion OSCORAD and *Staphylococcus abundance* in the microbiome is shown in panel A, and for the target lesion OSCORAD and diversity index in B. The delta correlations are presented in panels C and D.



SUPPLEMENTAL FIGURE S1 Assessment schedule of the study.