# Endolysin treatment against Staphylococcus aureus in adults with atopic dermatitis: a randomized controlled trial

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*To the Editor:*

*Staphylococcus* (*S.) aureus* density is increased in many patients with atopic dermatitis (AD) and is thought to contribute to the disease pathogenesis, interacting with an altered skin barrier and immunological changes.[1] *S. aureus* might induce or aggravate inflammation via different mechanisms, for example through excretion of virulence factors, even if the *S. aureus* overgrowth is primarily caused by other factors.[2] Current guidelines only recommend antimicrobial therapy directed against *S. aureus* in clinically infected AD based on a Cochrane review where no clinical benefit of short-term antimicrobial treatment in noninfected AD was found.[3]

Arguably, long-term anti-staphylococcal treatment, such as antibiotics, might reduce symptoms in AD.[4] However, this is undesired because antibiotics can affect the commensal microbiota and could induce bacterial resistance.[5] In contrast, long-term treatment of AD with an endolysin that targets only *S. aureus* is feasible. It might improve AD symptoms and reduce the use of corticosteroids consecutively.[2,6] Therefore, we aimed to determine the topical corticosteroid (TCS)-sparing effect and safety of 12 weeks of endolysin treatment against *S. aureus* in patients with AD.

We performed a double-blind, vehicle-controlled superiority trial (MAAS trial, ClinicalTrials.gov NCT02840955) in 100 adult patients with TCS-treated, not clinically infected, moderate-to-severe AD, which was defined by an Eczema Area and Severity Index (EASI) of 7.1 to 50.0. After a two-week run-in period to standardize the TCS treatment with triamcinolone acetonide 0.1% cream, patients were randomly assigned 1:1 to a 12-week intervention with either a topical endolysin against *S. aureus* or a vehicle twice daily, followed by an eight-week follow-up period. The vehicle and recombinant chimeric endolysin, Staphefekt™ SA.100, were provided by Micreos Human Health (Bilthoven, The Netherlands) and topically applied in a cetomacrogol cream. Details on patient inclusion, randomization, and study procedures at six assessments are described in a previously published study protocol (Figure S1).[2]

The primary outcome, the TCS-sparing effect of endolysin treatment against *S. aureus*, was evaluated by the patients, who registered daily use of a TCS (yes/no) over 12 weeks. Secondary outcomes included differences in TCS use measured in grams, clinical efficacy, quality of life using the Skindex-29, *S. aureus* load on the skin, and safety and tolerability of endolysin treatment. Clinical efficacy was measured using the EASI, Investigators Global Assessment, Patient-Oriented Eczema Measure, pruritus Numeric Rating Scale, and by registration of the number of flares. The *S. aureus* load on the skin was assessed by a semi-quantitative culture and quantitative polymerase chain reaction (qPCR, Appendix 1).

Generalized linear mixed-effect models for repeated measurements were used to analyze the primary and dichotomous secondary outcomes, and linear mixed-effect models were used for continuous secondary outcomes. Data were analyzed as intention-to-treat and per-protocol. Furthermore, a subgroup analysis was performed in patients with a positive *S. aureus* skin culture at two time points before start of the intervention.

Eighty-eight (88.0%) patients completed the intervention, and 87 (87.0%) completed follow-up (13% dropout rate, Figure S2). Patients' characteristics were comparable between the endolysin and vehicle groups (Table 1). Over the 12-week intervention period (corresponding to 8400 days for 100 patients), patients in the endolysin group used a TCS for 1889 (45.0%) days compared with 1566 (37.3%) days in the vehicle group. There was no statistically significant difference in the probability of TCS use per day between the groups in the intention-to-treat analysis, per-protocol analysis, and in the subgroup of *S. aureus*-positive patients ($p$=0.97, $p$=0.40 and $p$=0.08, respectively, Table 2). Sensitivity analyses showed no differences in the odds ratio of TCS use per assessment day. Except for the number of doctor-reported AD flares during the intervention period (per-protocol, n=2 in endolysin group vs. n=10 in vehicle group, $p$=0.03), no statistically significant differences were found in the secondary outcomes after both intention-to-treat and per-protocol analyses (Table S1 and S2). At baseline, 62 (64.6%) patients had positive results for *S. aureus* based on skin culture and 24 (24.7%) by qPCR. Both methods showed no significant difference in *S. aureus* reduction (Table S3 and S4). During the study, one serious adverse event occurred in the endolysin group eight weeks after the last application of endolysin cream (pleural effusion with hospitalization), which was considered unlikely to be related to the study intervention (Table S5).

Our results are in accordance with data from a Cochrane review showing no significant effect of short-term anti-*S. aureus* therapy in patients with noninfected AD.[3] We cannot confirm the positive results of other longer-term studies. However, these studies used broad-spectrum antimicrobials and mainly included patients with signs of bacterial infection.[4] Patients with clinically infected AD were excluded from our study, and a possible effect of anti-*S. aureus* endolysins in this patient group should be determined in future studies.

Several hypotheses could explain our results. First, use of triamcinolone in the run-in phase resulted in a decrease in AD severity (Table 1), which might have masked a possible benefit of endolysin treatment.

Second, daily use of an emollient and good compliance with the treatment could have resulted in a reduction of triamcinolone use in both the endolysin and vehicle groups.[7]

**Table 1.** Baseline characteristics

| | Total (n=100) | Staphefekt (n=50) | Vehicle (n=50) |
|---|---|---|---|
| **Age** | | | |
| years; median (IQR) | 33.5 (25.5-47.5) | 36.5 (25.0-51.0) | 32.5 (24.0-44.0) |
| **Sex (male)** | | | |
| n (%) | 55 (55.0) | 24 (48.0) | 31 (62.0) |
| **Race,** n (%) | | | |
| American Indian or Alaska Native | 5 (5.0) | 2 (4.0) | 3 (6.0) |
| Asian | 10 (10.0) | 2 (4.0) | 8 (16.0) |
| Black or African American | 8 (8.0) | 5 (10.0) | 3 (6.0) |
| White | 77 (77.0) | 41 (82.0) | 36 (72.0) |
| **Atopic disease,** n (%) | | | |
| Food allergy | 43 (43.0) | 18 (36.0) | 25 (50.0) |
| Rhinoconjunctivitis | 63 (63.0) | 28 (56.0) | 35 (70.0) |
| Asthma | 47 (47.0) | 25 (50.0) | 22 (44.0) |
| **EASI,** median (IQR) | | | |
| Screening (V1) | 12.9 (9.2-19.0) | 13.7 (8.9-19.1) | 12.5 (9.2-19.0) |
| Baseline (V2) | 8.0 (5.0-13.5)[4] | 8.3 (5.0-14.7)[5] | 8.0 (4.9-12.9)[6] |
| **IGA,** median (IQR) | | | |
| Baseline (V2) | 2.0 (2.0-3.0)[4] | 2.0 (2.0-3.0)[5] | 2.0 (2.0-3.0)[6] |
| **POEM**, mean (SD) | | | |
| Baseline (V2) | 12.9 (6.2)[4] | 14.5 (8.3-17.0)[5] | 13.0 (8.0-15.0)[6] |
| **Pruritus NRS,** median (IQR) | | | |
| Baseline (V2) | 3.0 (2.0-4.0)[4] | 3.0 (2.0-4.0)[5] | 3.0 (2.0-4.0)[6] |
| **Skindex-29,** mean (SD) | | | |
| Baseline (V2) | 35.1 (17.1)[4] | 37.3 (15.3)[5] | 32.9 (18.7)[6] |
| **Use of topical corticosteroids at screening,** n (%) | | | |
| Class 1 | 3 (3.0) | 1 (2.0) | 2 (4.0) |
| Class 2 | 13 (13.0) | 6 (12.0) | 7 (14.0) |
| Class 2-3 | 11 (11.0) | 3 (6.0) | 8 (16.0) |
| Class 3 | 44 (44.0) | 22 (44.0) | 22 (44.0) |
| Class 3-4 | 3 (3.0) | 2 (4.0) | 1 (2.0) |
| Class 4 | 18 (18.0) | 11 (22.0) | 7 (14.0 |
| Unknown | 8 (8.0) | 6 (12.0) | 4 (8.0) |
| ***Staphylococcus aureus* skin culture*, n (%)** | | | |
| Positive | 56 (56.0) | 32 (64.0) | 24 (48.0) |
| Intermediate | 20 (20.0) | 7 (14.0) | 13 (26.0) |
| Negative | 20 (20.0) | 9 (18.0) | 11 (22.0) |
| Missing | 4 (4.0) | 2 (4.0) | 2 (4.0) |

Abbreviations: EASI, Eczema Area and Severity Index; IGA, Investigators Global Assessment; IQR, interquartile range; NRS, Numeric Rating Scale; POEM, Patient Oriented Eczema Measure; SD, standard deviation. Missings: [1]n=15 (15.0%), [2]n=8 (16.0%), [3]n=7 (14.0%), [4]n=3 (3.0%), [5]n=2 (4.0%), [6]n=1 (2.0%). *Positive is defined as having a positive culture at visit 1 and visit 2a; intermediate is defined as having one positive culture and one negative culture at visit 1 and visit 2a; negative is defined as having two negative cultures at visit 1 and visit 2a.

**Table 2.** Generalized Linear Mixed-Effect model results for the difference in topical corticosteroid use per day during the 12-week intervention period

| Analysis | Time period or time point in days from baseline | Patients included in analysis | | Topical corticosteroid use 'yes', n (%) | | OR (95% CI) | *P*-value[1] |
|---|---|---|---|---|---|---|---|
| | | **Staphefekt**, n | **Vehicle**, n | **Staphefekt**, n (%) | **Vehicle**, n (%) | | |
| **Intention-to-treat** | Intervention | | | | | | 0.97 |
| | 14 | 40 | 37 | 26 (65.00) | 20 (54.05) | 0.99 (0.95 – 1.03) | 0.49 |
| | 42 | 37 | 37 | 26 (70.27) | 22 (59.46) | 0.99 (0.78 – 1.24) | 0.91 |
| | 84 | 30 | 34 | 18 (60.00) | 21 (61.76) | 1.04 (0.87 – 1.23) | 0.68 |
| **Per-protocol** | Intervention | | | | | | 0.40 |
| | 14 | 22 | 21 | 14 (63.64) | 10 (47.62) | 1.09 (0.98 – 1.21) | 0.10 |
| | 42 | 17 | 18 | 12 (70.59) | 11 (61.11) | 1.36 (0.84 – 2.20) | 0.21 |
| | 84 | 13 | 13 | 7 (53.85) | 5 (38.46) | 1.69 (1.14 – 2.51) | **0.01** |
| ***Staphylococcus aureus* positive**[2] | Intervention | | | | | | 0.08 |
| | 14 | 26 | 16 | 17 (65.38) | 7 (43.75) | 0.87 (0.78 – 0.96) | **0.01** |
| | 42 | 22 | 17 | 16 (72.73) | 8 (47.06) | 0.85 (0.49 – 1.48) | 0.56 |
| | 84 | 18 | 15 | 11 (61.11) | 11 (73.33) | 1.29 (0.84 – 1.96) | 0.24 |

NOTE: Given the low number of patients using escape medication (n=5), we did not correct for its use. [1]Overall effect of endolysin treatment during intervention period was calculated with a Likelihood-Ratio test, the effect per time point using a Wald test with t-distribution. [2]Defined as having a positive culture both at visit 1 and visit 2a (endolysin n=32, vehicle n=24). Sensitivity analyses, performed by adding/subtracting 0.25 times the standard deviation to/of the odds, showed no differences in the odds ratio of topical corticosteroid use per assessment day.

Because AD is a heterogeneous disease, anti-*S. aureus* treatment might not be suitable for all patients with AD, indicating the need for subphenotyping. Because only 56% of our study population had two consecutive positive *S. aureus* skin cultures (indicating persistent colonization) before start of the intervention, the target population that would probably benefit the most from endolysin treatment was small.

Our data suggest that endolysin treatment has no effect on *S. aureus in vivo.* However, patients might have been recolonized with *S. aureus* from the nose because 73% of them were nasal carriers (data not shown). Alternatively, cetomacrogol as the basis of the endolysin cream might have created a barrier on the skin that prevented the endolysin to reach and subsequently kill *S. aureus*. However, some reduction in *S. aureus* load would have been expected in both treatment groups because of the use of TCSs and emollients in this study, which both have been shown to reduce the *S. aureus* load on the skin.[8,9] Nonetheless, it is unclear whether complete eradication of *S. aureus* is required for clinical improvement because a case series showed a clear clinical improvement without *S. aureus* reduction using a qualitative culture in *S. aureus*-related dermatoses.[6] In addition, the discrepant results between culture and qPCR indicate the complexity of the interpretation

of *S. aureus* testing. Despite the limitations and outcome, this study provides estimates of AD symptoms, use of TCSs, and the percentage of persistent *S. aureus* carriers that can be used for future clinical studies.

In conclusion, long-term targeted endolysin treatment against *S. aureus* in this study was well tolerated but had no TCS-sparing effect in patients with AD. However, an effect cannot be excluded because good compliance with the treatment and concurrent application of TCSs, emollients, or both might have masked a clinical benefit.

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**Table S1.** Results for the difference in secondary clinical outcomes – intention-to-treat analysis

| Outcome | Time period or visit | Staphefekt Endolysin | Vehicle | Difference in change (95% CI)[1] | OR (95% CI)[2] | *P*-value[3] |
|---|---|---|---|---|---|---|
| **Mean grams/week topical corticosteroid use,** median (IQR) | Intervention[4] | 4.5 (2.2-8.7) | 3.6 (1.9-7.4) | | | 0.66 |
| | Intervention + follow-up[5] | 5.1 (1.9-8.9) | 4.1 (2.3-10.4) | | | 0.91 |
| **Proportion of patients who indicated to have used less corticosteroids compared with baseline,** n (%) | Intervention + follow-up | | | | | 0.19 |
| | 3 | 36 (76.60) | 34 (72.34) | | 0.77 (0.45 – 1.29) | 0.32 |
| | 4 | 30 (65.22) | 27 (60.00) | | 0.79 (0.51 – 1.22) | 0.29 |
| | 5 | 22 (50.00) | 20 (45.45) | | 0.91 (0.61 – 1.38) | 0.67 |
| | 6 | 23 (53.49) | 13 (29.55) | | 0.32 (0.21 – 0.51) | <0.001† |
| **Change in EASI from baseline,** mean (SD) | Intervention + follow-up | | | | | 0.57 |
| | 3 | -0.61 (4.88) | -1.63 (5.09) | 1.03 (-1.30 – 3.36) | | 0.38 |
| | 4 | 0.11 (6.40) | -0.88 (6.22) | 1.08 (-1.41 – 3.57) | | 0.39 |
| | 5 | 0.29 (7.40) | -1.08 (6.16) | 1.28 (-1.48 – 4.04) | | 0.36 |
| | 6 | 0.43 (7.83) | -1.71 (7.26) | 2.61 (-0.53 – 5.75) | | 0.10 |
| **IGA corrected for baseline** | Intervention + follow-up | | | | | 0.78 |
| **Proportion of patients with a reduction of ≥2 points in IGA from baseline,** n (%) | 3 | 0 (0.00) | 2 (4.26) | | | 0.46[6] |
| | 4 | 1 (2.17) | 2 (4.44) | | | 0.62[6] |
| | 5 | 1 (2.27) | 3 (6.82) | | | 0.62[6] |
| | 6 | 1 (2.33) | 5 (11.36) | | | 0.20[6] |
| **Change in POEM from baseline,** mean (SD) | Intervention + follow-up | | | | | 0.98 |
| | 3 | -3.15 (4.64) | -2.94 (5.52) | -0.23 (-2.55 – 2.09) | | 0.84 |
| | 4 | -2.93 (5.80) | -2.09 (5.43) | -0.54 (-2.87 – 1.78) | | 0.64 |
| | 5 | -2.43 (6.13) | -2.02 (6.76) | -0.57 (-3.01 – 1.88) | | 0.65 |
| | 6 | -1.79 (6.32) | -1.09 (6.68) | -0.79 (-3.57 – 1.99) | | 0.57 |

**Table S1.** Results for the difference in secondary clinical outcomes – intention-to-treat analysis (continued)

| Outcome | Time period or visit | Staphefekt Endolysin | Vehicle | Difference in change (95% CI)[1] | OR (95% CI)[2] | *P*-value[3] |
|---|---|---|---|---|---|---|
| **Change in pruritus NRS from baseline,** mean (SD) | Intervention + follow-up | | | | | 0.56 |
| | 3 | 0.26 (1.57) | 0.21 (1.64) | 0.03 (-0.63 – 0.69) | | 0.92 |
| | 4 | 0.20 (1.77) | -0.09 (1.43) | 0.37 (-0.31 – 1.05) | | 0.28 |
| | 5 | 0.23 (1.85) | -0.05 (1.70) | 0.32 (-0.43 – 1.07) | | 0.39 |
| | 6 | -0.05 (2.23) | 0.14 (1.98) | -0.09 (-0.97 – 0.79) | | 0.84 |
| **Proportion of patients with a reduction of ≥2 points in pruritus NRS from baseline,** n (%) | Intervention + follow-up | | | | | 0.65 |
| | 3 | 14 (29.79) | 10 (21.28) | | 0.56 (0.24 – 1.28) | 0.17 |
| | 4 | 16 (34.78) | 11 (24.44) | | 0.57 (0.26 – 1.23) | 0.15 |
| | 5 | 14 (31.82) | 17 (38.64) | | 1.44 (0.71 – 2.94) | 0.31 |
| | 6 | 16 (37.21) | 15 (34.09) | | 0.84 (0.42 – 1.68) | 0.62 |
| **Proportion of patients with a reduction of ≥3 points in pruritus NRS from baseline,** n (%) | Intervention + follow-up | | | | | 0.71 |
| | 3 | 7 (14.89) | 3 (6.38) | | 0.41 (0.11 – 0.53) | 0.18 |
| | 4 | 5 (10.87) | 4 (8.89) | | 0.69 (0.17 – 2.77) | 0.60 |
| | 5 | 7 (15.91) | 6 (13.64) | | 0.83 (0.32 – 2.18) | 0.71 |
| | 6 | 11 (25.58) | 8 (18.18) | | 0.50 (0.22 – 1.13) | 0.09 |
| **Change in Skindex-29 from baseline,** mean (SD) | Intervention + follow-up | | | | | 0.71 |
| | 3 | -7.15 (9.83) | -4.86 (9.32) | -2.63 (-6.81 – 1.54) | | 0.21 |
| | 5 | -7.35 (10.54) | -4.82 (12.96) | -2.35 (-7.22 – 2.51) | | 0.34 |
| | 6 | -8.38 (12.98) | -4.66 (13.87) | -3.35 (-8.97 – 2.26) | | 0.24 |
| **Number of doctor reported flares from baseline,** n (%) | Intervention[7] | 14 (10.22) | 17 (12.50) | | | 0.55 |
| **Number of patient reported flares from baseline,** n (%) | Intervention[7] | 14 (10.22) | 17 (12.50) | | | 0.58 |

**Table S1.** Results for the difference in secondary clinical outcomes – intention-to-treat analysis (continued)

| Outcome | Time period or visit | Staphefekt Endolysin | Vehicle | Difference in change (95% CI)[1] | OR (95% CI)[2] | *P*-value[3] |
|---|---|---|---|---|---|---|
| **Mean time (days) to doctor reported flare from baseline,** median (IQR) | Intervention[8] | 44.50 (35.25-84.00) | 43.00 (30.50-84.50) | | | 0.95 |
| **Mean time (days) to patient reported flare from baseline,** median (IQR) | Intervention[8] | 40.50 (20.00-72.25) | 30.00 (15.00-64.00) | | | 0.71 |
| | Follow-up[9] | 30.50 (16.75-50.50) | 22.00 (8.50-43.50) | | | 0.52 |
| **Number of patients with at least one (serious) AE,** n | Intervention + follow-up | 40 | 40 | | | 1.00 |
| **Number of (serious) AEs,** n | Intervention + follow-up | 82 | 74 | | 1.14 (0.83 – 1.56)[10] | 0.42 |

Abbreviations: CI, confidence interval; EASI, Eczema Area and Severity Index; IGA, Investigators Global Assessment; IQR, interquartile range; NRS, Numeric Rating Scale; POEM, Patient Oriented Eczema Measure; OR, odds ratio; SD, standard deviation. Patients included in analyses per visit in endolysin and vehicle group: visit 3, n=47 and n=47; visit 4, n=46 and n=45; visit 5, n=44 and n=44; visit 6, n=43 and n=44. NOTE: Given the low number of patients using escape medication (n=5), we did not correct for its use in the analysis of the mean grams/week topical corticosteroid use. [1]Differences in change presented for analyses using a Linear Mixed-Effect model. [2]OR presented for analysis using a Generalized Linear Mixed-Effect model. [3]*P*-values were calculated using a Chi-Square test or Fisher's Exact test for categorical data, where appropriate. A non-parametric Mann-Whitney U Test for independent samples was used for continuous variables. The overall effect of endolysin treatment during the intervention and follow-up period ((Generalized) Linear Mixed-Effect models) was analyzed with a Likelihood-Ratio test and per visit using a Wald test with t-distribution. [4]Endolysin group n=32, vehicle group n=34. [5]Endolysin group n=32, vehicle group n=32. [6] Since the number of patients per cell are ≤5 a Fisher's Exact test per visit was used instead of a Generalized Linear Mixed-Effect model. A *P*-value of 0.0125 will be considered significant after Bonferroni correction. [7]For every visit from baseline through week 12, 137 visits in endolysin group and 136 visits in vehicle group, it was registered if a flare occurred yes/no. [8]Endolysin group n=14, vehicle group n=17. [9]Endolysin group n=8, vehicle group n=5. [10]Rate ratio with 95% CI. [†]Significant result.

**Table S2.** Results for the difference in secondary clinical outcomes – per-protocol analysis

| Outcome | Time period or visit | Endolysin | Vehicle | Difference in change (95% CI)[1] | OR (95% CI)[2] | *P*-value[3] |
|---|---|---|---|---|---|---|
| **Mean grams/week topical corticosteroid use,** median (IQR) | Intervention[4] | 6.0 (3.3-10.9) | 4.1 (2.6-7.9) | | | 0.44 |
| | Intervention + follow-up[5] | 6.1 (4.2-7.3) | 5.0 (2.6-14.7) | | | 1.00 |
| **Proportion of patients who indicate to have used less corticosteroids compared with baseline**, n (%) | Intervention + follow-up | | | | | 0.40 |
| | 3 | 19 (76.00) | 21 (72.41) | | 0.91 (0.41 – 2.00) | 0.81 |
| | 4 | 8 (42.11) | 11 (52.38) | | 1.52 (0.70 – 3.34) | 0.30 |
| | 5 | 7 (43.75) | 11 (73.33) | | 3.17 (1.06 – 9.48) | 0.04 |
| | 6 | 8 (50.00) | 5 (33.33) | | 0.50 (0.18 – 1.39) | 0.18 |
| **Change in EASI from baseline,** mean (SD) | Intervention + follow-up | | | | | 0.81 |
| | 3 | -1.17 (5.70) | -0.64 (4.99) | -0.45 (-3.64 – 2.73) | | 0.78 |
| | 4 | -0.58 (7.19) | 1.73 (6.14) | -1.95 (-5.55 – 1.65) | | 0.28 |
| | 5 | -0.59 (7.79) | -0.71 (4.16) | -0.77 (-4.83 – 3.30) | | 0.71 |
| | 6 | -0.94 (6.44) | -0.20 (5.61) | -1.45 (-5.92 – 3.03) | | 0.52 |
| **Change in IGA from baseline** | Intervention + follow-up | | | | | 0.86 |
| **Proportion of patients with a reduction of ≥2 points in IGA from baseline,** n (%) | 3 | 0 (0.00) | 1 (3.45) | | | 1.00[6] |
| | 4 | 0 (0.00) | 1 (4.76) | | | 1.00[6] |
| | 5 | 1 (6.25) | 1 (6.67) | | | 1.00[6] |
| | 6 | 1 (6.25) | 2 (13.33) | | | 0.60[6] |
| **Change in POEM from baseline,** mean (SD) | Intervention + follow-up | | | | | 0.77 |
| | 3 | -4.04 (4.72) | -3.14 (5.78) | -0.91 (-4.11 – 2.30) | | 0.57 |
| | 4 | -3.63 (5.56) | -2.76 (6.31) | -0.78 (-4.25 – 2.70) | | 0.66 |
| | 5 | -3.06 (5.95) | -2.93 (7.44) | -0.14 (-3.83 – 3.54) | | 0.94 |
| | 6 | -3.00 (3.93) | -0.87 (6.55) | -2.13 (-5.82 – 1.55) | | 0.25 |

**Table S2.** Results for the difference in secondary clinical outcomes – per-protocol analysis (continued)

| Outcome | Time period or visit | Endolysin | Vehicle | Difference in change (95% CI)[1] | OR (95% CI)[2] | *P*-value[3] |
|---|---|---|---|---|---|---|
| **Change in pruritus NRS from baseline,** mean (SD) | Intervention + follow-up | | | | | 0.50 |
| | 3 | 0.08 (1.53) | 0.34 (1.88) | -0.24 (-1.13 – 0.65) | | 0.59 |
| | 4 | -0.16 (1.34) | -0.10 (1.38) | -0.29 (-1.29 – 0.71) | | 0.56 |
| | 5 | 0.19 (1.56) | 0.00 (1.69) | 0.11 (-1.09 – 1.31) | | 0.85 |
| | 6 | -0.06 (2.41) | 0.80 (1.86) | -0.89 (-2.38 – 0.60) | | 0.24 |
| **Proportion of patients with a reduction of ≥2 points in pruritus NRS from baseline,** n (%) | Intervention + follow-up | | | | | 0.58 |
| | 3 | 8 (32.00) | 7 (24.14) | | 0.63 (0.22 – 1.78) | 0.39 |
| | 4 | 7 (36.84) | 3 (14.29) | | 0.32 (0.07 – 1.38) | 0.13 |
| | 5 | 5 (31.25) | 5 (33.33) | | 1.06 (0.23 – 5.01) | 0.94 |
| | 6 | 8 (50.00) | 5 (33.33) | | 0.42 (0.10 – 1.81) | 0.24 |
| **Proportion of patients with a reduction of ≥3 points in pruritus NRS from baseline,** n (%) | 3 | 3 (12.00) | 3 (10.34) | | 0.41 (0.11 – 0.53) | 1.00[6] |
| | 4 | 0 (0.00) | 2 (9.52) | | 0.69 (0.17 – 2.77) | 0.49[6] |
| | 5 | 2 (12.50) | 3 (20.00) | | 0.83 (0.32 – 2.18) | 0.65[6] |
| | 6 | 5 (31.25) | 4 (26.67) | | 0.50 (0.22 – 1.13) | 1.00[6] |
| **Change in Skindex-29 from baseline,** mean (SD) | Intervention + follow-up | | | | | 0.66 |
| | 3 | -6.62 (9.39) | -5.41 (10.26) | -1.53 (-7.33 – 4.27) | | 0.60 |
| | 5 | -6.14 (9.67) | -5.00 (15.72) | -2.21 (-10.58 – 6.16) | | 0.60 |
| | 6 | -8.41 (14.56) | -2.99 (14.05) | -6.60 (-16.48 – 3.27) | | 0.19 |
| **Number of doctor reported flares from baseline,** n (%) | Intervention[7] | 2 (3.33) | 10 (15.38) | | | 0.03† |
| **Number of patient reported flares from baseline,** n (%) | Intervention[7] | 5 (8.33) | 6 (9.23) | | | 0.86 |
| **Mean time (days) to doctor reported flare from baseline,** median (IQR) | Intervention[8] | 22.50[11] | 42.00 (11.75-53.25) | | | 0.76 |

**Table S2.** Results for the difference in secondary clinical outcomes – per-protocol analysis (continued)

| Outcome | Time period or visit | Endolysin | Vehicle | Difference in change (95% CI)[1] | OR (95% CI)[2] | *P*-value[3] |
|---|---|---|---|---|---|---|
| **Mean time (days) to patient reported flare from baseline,** median (IQR) | Intervention[9] | 39.00 (15.00-53.50) | 29.00 (5.00-47.00) | | | 0.66 |
| | Follow-up[9] | 14.00[11] | 32.00[11] | | | NA |
| **Number of patients with at least one (serious) AE,** n[12] | Intervention + follow-up | 33 | 33 | | | 1.00 |
| **Number of (serious) AEs,** n[12] | Intervention + follow-up | 61 | 53 | | 1.20 (0.84 – 1.72)[13] | 0.31 |

Abbreviations: CI, confidence interval; EASI, Eczema Area and Severity Index; IGA, Investigators Global Assessment; IQR, interquartile range; NA, not available; NRS, Numeric Rating Scale; POEM, Patient Oriented Eczema Measure; OR, odds ratio; SD, standard deviation. Patients included in analyses per visit in Staphefekt and placebo group: visit 3, n=25 and n=29; visit 4, n=19 and n=21; visit 5, n=16 and n=15; visit 6, n=16 and n=15. NOTE: Given the low number of patients using escape medication (n=5), we did not correct for its use in the analysis of the mean grams/week topical corticosteroid use. [1]Differences in change presented for analyses using a Linear Mixed-Effect model. [2]OR presented for analysis using a Generalized Linear Mixed-Effect model. [3]*P*-values were calculated using a Chi-Square test or Fisher's Exact test for categorical data, where appropriate. A non-parametric Mann-Whitney U Test for independent samples was used for continuous variables. The overall effect of endolysin treatment during the intervention and follow-up period ((Generalized) Linear Mixed-Effect models) was analyzed with a Likelihood-Ratio test and per visit using a Wald test with t-distribution. [4]Endolysin group n=14, vehicle group n=10. [5]Endolysin group n=15, vehicle group n=10. [6]Since the number of patients per cell are ≤5 a Fisher's Exact test per visit was used instead of a Generalized Linear Mixed-Effect model. A *P*-value of 0.0125 will be considered significant after Bonferroni correction. [7]For every visit from baseline through week 12, 60 in endolysin group and 65 in vehicle group, it was registered if a flare occurred yes/no. [8]Endolysin group n=2, vehicle group n=10. [9]Endolysin group n=5, vehicle group n=6. [10]Endolysin group n=1, vehicle group n=2. [11]No IQR because n≤2. [12]AEs that occurred until 8 weeks (follow-up period in intention-to-treat analysis) after the first protocol deviation, i.e. no use of endolysin or vehicle on total skin surface twice daily, were analyzed. [13]Rate ratio with 95% CI. [†]Significant result.

**Table S3.** Results for the difference in reduction of *Staphylococcus aureus* 0.5 hour after first application determined by semi-quantitative culture

| Analysis | Decrease in semi-quantitative culture of *S. aureus*[1] | Endolysin, n (%)[2] | Vehicle, n (%)[3] | *P*-value[4] |
|---|---|---|---|---|
| **Intention-to-treat** | Yes | 6 (12.5) | 4 (8.3) | 0.74 |
| | No | 42 (87.5) | 44 (91.7) | |

Abbreviations: *S. aureus*, *Staphylococcus aureus*. [1]Decrease is defined as a decrease of at least 1 point on semi-quantitative scale (scale ranges from 0-4). [2]Patients included in analysis in endolysin group: n=49. [3]Patients included in in analysis in vehicle group: n=48. [3]*P*-values were calculated using a Fisher's Exact test for categorical data.

**Table S4.** Results for the difference in reduction of *Staphylococcus aureus* from baseline determined by qPCR

| Analysis | Time period | Log10 reduction in qPCR for *S. aureus* | | *P*-value[1] |
|---|---|---|---|---|
| | | **Endolysin**, n (%) | **Vehicle**, n (%) | |
| **Intention-to-treat**[2] | Visit 2a to visit 3 | 3 (6.5)[6] | 4 (8.5)[6] | 1.00 |
| | Visit 2a to visit 5 | 3 (6.8)[7] | 8 (18.2)[7] | 0.20 |
| **Per-protocol**[3] | Visit 2a to visit 3 | 2 (7.1) | 3 (9.4) | 1.00 |
| | Visit 2a to visit 5 | 1 (6.3) | 5 (35.6)[8] | 0.07 |
| ***S. aureus* positive**[4-6] | Visit 2a to visit 3 | 3 (33.3) | 4 (30.8) | 1.00 |
| | Visit 2a to visit 5 | 3 (33.3) | 9 (66.7) | 0.20 |

Abbreviations: *S. aureus*, *Staphylococcus aureus*; qPCR, quantitative polymerase chain reaction. [1]*P*-values were calculated using a Fisher's Exact test for categorical data. [2]Patients included in endolysin and vehicle group: visit 2a to visit 3, n=47 and n=47; visit 2a to visit 5, n=44 and n=44. [3]Patients included in endolysin and vehicle group: visit 2a to visit 3, n=25 and n=29; visit 2a to visit 5, n=16 and n=15. [4]Patients included in endolysin and vehicle group: visit 2a to visit 3, n=9 and n=13; visit 2a to visit 5, n=9 and n=12. [5]Analysis additionally to analyses described in the study protocol. [6]*Staphylococcus aureus* positive is defined as having a positive qPCR at visit 2a (endolysin group n=10, vehicle group n=14). Missings: [6]n=1 (2.1%), [7]n=1 (2.3%), [8]n=1 (6.7%).

**Table S5.** Incidence of (non-) Treatment Emergent Adverse Events – Overall and per study phase

| | Total (n=100) | | Endolysin (n=50) | | Vehicle (n=50) | |
|---|---|---|---|---|---|---|
| | **Patients**, n (%) | **Events**, n | **Patients**, n (%) | **Events**, n | **Patients**, n (%) | **Events**, n |
| **Overall (V1-V6)** | 83 (83.0) | 183 | 43 (86.0) | 96 | 40 (80.0) | 87 |
| **Run-in (V1-V2)** | | | | | | |
| At least 1 non-TEAE | 21 (21.0) | 27 | 11 (22.0) | 14 | 10 (20.0) | 13 |
| At least 1 serious non-TEAE | 0 (0.0) | 0 | 0 (0.0) | 0 | 0 (0.0) | 0 |
| At least 1 non-TEAE leading to study discontinuation | 0 (0.0) | 0 | 0 (0.0) | 0 | 0 (0.0) | 0 |
| At least 1 non-TEAE leading to death | 0 (0.0) | 0 | 0 (0.0) | 0 | 0 (0.0) | 0 |
| **Intervention (V2-V5)** | | | | | | |
| At least 1 TEAE | 73 (73.0) | 125 | 36 (72.0) | 67 | 37 (74.0) | 58 |
| At least 1 serious TEAE | 0 (0.0) | 0 | 0 (0.0) | 0 | 0 (0.0) | 0 |
| At least 1 TEAE leading to study discontinuation | 3 (3.0) | 3 | 1 (2.0) | 1 | 2 (4.0) | 2 |
| At least 1 TEAE leading to death | 0 (0.0) | 0 | 0 (0.0) | 0 | 0 (0.0) | 0 |
| **Follow-up (V5-V6)** | | | | | | |
| At least 1 TEAE | 25 (25.0) | 31 | 15 (30.0) | 15 | 10 (20.0) | 16 |
| At least 1 serious TEAE | 1 (1.0) | 1 | 1 (2.0) | 1 | 0 (0.0) | 0 |
| At least 1 TEAE leading to study discontinuation | 1 (1.0) | 1 | 1 (2.0) | 1 | 0 (0.0) | 0 |
| At least 1 TEAE leading to death | 0 (0.0) | 0 | 0 (0.0) | 0 | 0 (0.0) | 0 |

Abbreviations: TEAE, Treatment Emergent Adverse Event (adverse events after the first endolysin or vehicle administration); V, visit.

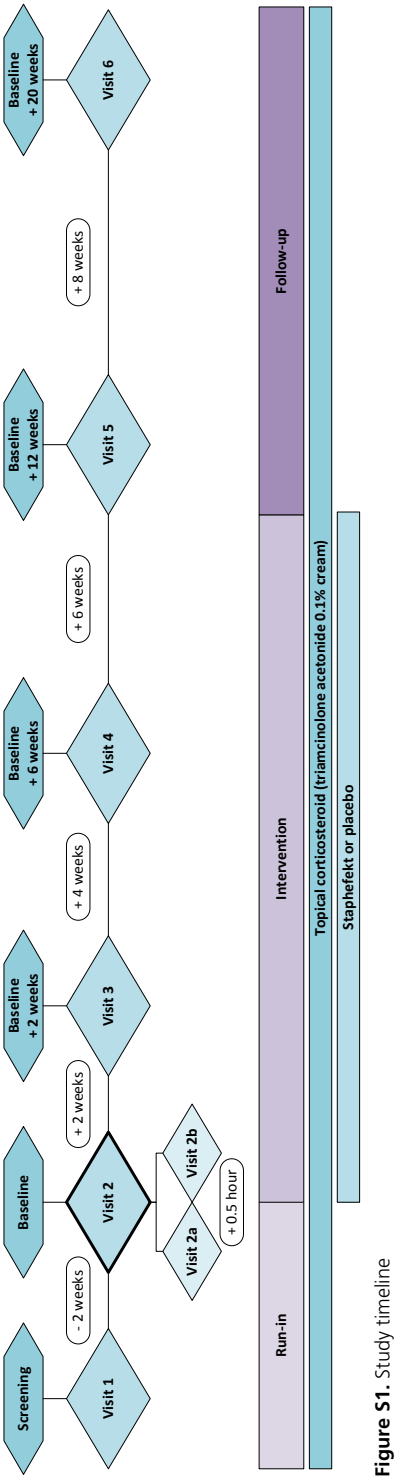


**Figure S1.** Study timeline

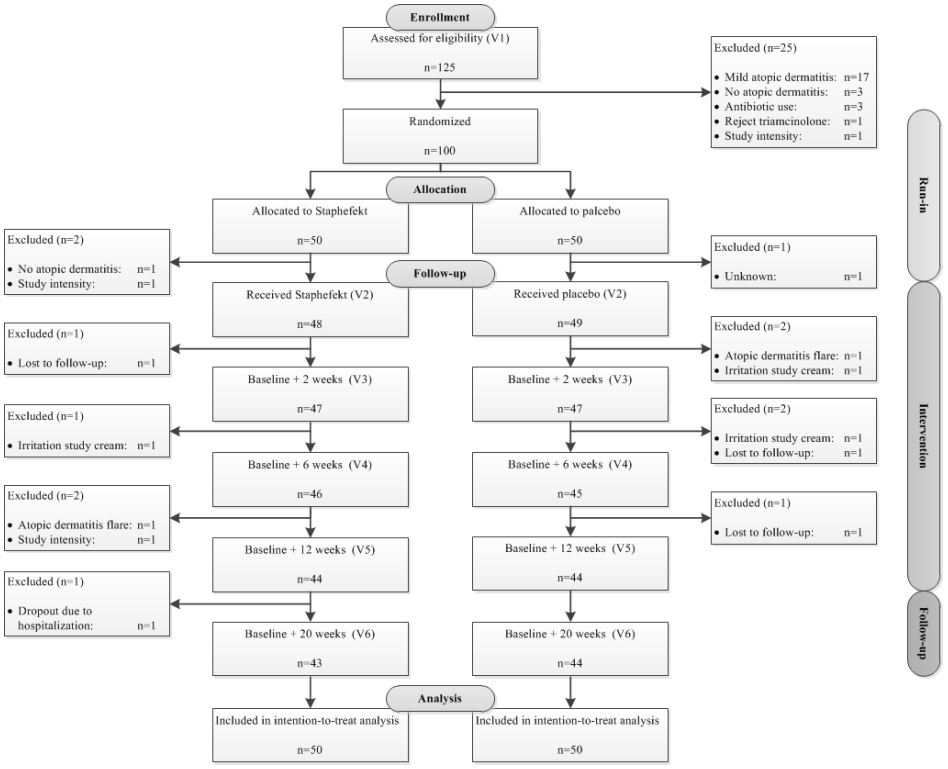


**Figure S2.** Flowchart of the study design

## Appendix 1. Microbial methods

*a. Microbial sampling methods*

Samples of the skin were collected using sterile Copan 490CE.A swabs for culture analysis and skin scrubs for qPCR analysis. Skin swabs were collected from a skin lesion at the first visit, preferably located at the antecubital fold or the popliteal fold. For all consecutive visits the swab was collected from the location chosen at the first visit. The scrubs were collected from the lower arm, adjacent to the antecubital fold according to methods described previously.[23] Scrub samples were stored at -80 °C.

*b. Semi-quantitative culture and qPCR for Staphylococcus aureus*

Bacterial cultures were performed using routine diagnostic culture procedures, using blood agar plates and specific S. aureus culture plates (ChromID S. aureus Elite agar (SAIDE), Biomérieux, France) for overnight incubation and subsequent species determination by MALDI-TOF (Bruker Daltonics, Bremen, Germany). For DNA isolation 150 uL from the sample was added to 350 uL lysis buffer, 500 uL Phenol (Tris pH 8) and 500 uL 0.1 mm zirconium beads. This mixture was mechanically disrupted by bead beating twice for 2 minutes, followed by centrifuging for 10 minutes at 1690 RCF to separate the aqueous and phenolic phases. The aqueous phase was purified using AGOWA mag Mini DNA isolation kit. After elution, we used qPCR to determine the total load of S. aureus with the following primers and probes: 16S-S.aur-F1 (5'-GCG AAG AAC CTT ACC AAA TCT TG-3'), 16S-S.aur-R1 (5'-TGC ACC ACC TGT CAC TTT GTC-3'), 16S-S.aur MGB Taqman® probe (5'-CAT CCT TTG ACA ACT CT-3') with NED™ label.