

Appendix S1

SUPPLEMENTARY MATERIALS AND METHODS

Source of samples and associated data

Microbiological test results of skin swabs, along with selected clinical data from the placebo group of 2 randomized, double-blind, placebo-controlled mono-centre phase 2 clinical trials conducted at the Centre for Human Drug Research (Leiden, The Netherlands) between June 2015 and December 2017, were used in this evaluation. Both clinical trials were approved by the independent Medical Ethics Committee ("Evaluation of Ethics in Biomedical Research", Assen, The Netherlands) and were designed to assess the pharmacodynamics of omiganan in patients with mild to moderate AD. The Declaration of Helsinki was the guiding principle for trial execution. Written informed consent was obtained from all patients.

Data from 250 samples obtained in the initial clinical trial (ClinicalTrials.gov: NCT03091426) were used to determine the variability of the skin microbiota. Briefly, each patient administered the vehicle gel (hydroxyethyl cellulose, sodium benzoate, glycerin, purified water) without the active compound twice daily for 28 consecutive days on all AD lesions. At the start of this treatment period (Day 0), the severity of the lesional skin was assessed clinically. Two skin swabs were collected for bacterial culture and molecular methods using an ESwab and a sterile cotton swab (Puritan, Guilford, ME, USA), respectively. Swabs were dipped in a NaCl-Tween solution, before rubbing the tip of the swab firmly over 4 cm² of the target lesion 5 times. Hereafter the swab material was placed in a vial containing 1 ml NaCl-Tween solution. The skin swabs were obtained from a predefined part of an AD lesion (preferably the antecubital fossa) and from a predefined part of non-lesional skin (preferably the contralateral site). Both clinical assessment and sample collection were repeated each week during a period of 42 days. During the treatment period, patients were allowed to use bland emollients (unguentum leniens) as maintenance therapy. The patients were not allowed to wash the selected sites 6 h prior to the clinical assessment and sample collection and had to avoid prolonged exposure of their involved skin to sunlight during the complete study period. Incomplete datasets or data of samples obtained after concomitant use of corticosteroids were excluded from the analysis.

Data of 76 skin swabs obtained in a separate clinical trial (ClinicalTrials.gov: NCT02456480) were used for verification purposes. This clinical trial differed in study design as: (i) the vehicle gel without the active compound was administered once daily on only the predefined AD lesion on the antecubital fossa; (ii) only lesional skin was sampled each week; (iii) clinical assessment of lesional skin was not measured at day 35 and 42; and (iv) bacterial culture was not performed.

Clinical assessment of lesional skin

The severity of the lesional skin was assessed clinically based on objective-SCORing Atopic Dermatitis (oSCORAD) system, calculated as: $A/5+7B/2$ (S1). "A" in the calculation was defined as the extent of AD, which was assessed as a percentage of each defined body area and reported as the sum of all areas, with a maximum score of 100%. "B" in the calculation was defined as the severity of 6 specific symptoms of AD (erythema, excoriation, swelling, oozing/crusting, lichenification and dryness), which were scored 0–3 and reported as the sum of all symptoms, with a maximum score of 18. A total score of 0–7.9 was categorized as clear skin, 8.0–23.9 as mild AD, 24.0–37.9 as moderate AD, and 38.0–83.0 as severe AD.

Bacterial culture

Skin swabs were inoculated on blood agar (Becton, Dickinson and Co., Franklin Lakes, NJ, USA) and incubated at 35°C in a 5% CO₂ incubator for 24 h. Species identification was performed by MALDI-TOF (Bruker Corp., Billerica, MA, USA) and colony-forming units (CFU) were calculated for *S. aureus* after dilution if necessary.

DNA extraction

Each skin swab was diluted by addition of 50 µl 10× phosphate-buffered saline (PBS) to 450 µl swab in NaCl-Tween solution. DNA was extracted and eluted in a final volume of 100 µl with the MagNA Pure 96 instrument using the MagNA Pure 96 DNA and Viral NA Large Volume Kit and the Pathogen universal 500 protocol (Roche Diagnostics, Meylan, France).

Microbiota analysis

Microbiota analysis was performed as described elsewhere (S2). Briefly, a fragment of approximately 464 bp of the V3–V4 regions of the 16S ribosomal RNA (rRNA) gene was amplified and sequenced with the MiSeq desktop sequencer (Illumina, San Diego, CA, USA). Sequencing data was processed using the QIIME pipeline and a pre-clustered version of the Augustus 2013 GreenGenes database. High-quality sequences (> 100 bp in length; quality score > 20) were clustered into operational taxonomic units using an open reference-based approach that implements reference-based clustering following by *de novo* clustering at a 97% similarity level. No low abundance filtering was used. For the bar charts, a limited number of genera were selected, representing the microbiota composition of each sample. Only genera with a relative abundance ≥ 1% of the total reads were included. The remaining genera formed the other genera category.

Quantitative real-time PCRs

S. aureus was detected by quantitative real-time PCRs (qPCRs) aimed at the *nuc* gene, using primers and a probe described elsewhere (S3). The total bacterial DNA load (16S rRNA gene) was established using a primer set (Fw 5'-CGAAAGCGTGGG-GAGCAA-3', Rv1 5'-CCGTACTCCCCAGGCGG-3' and Rv2 5'-GTCGTACTCCCCAGGCGG-3') based on Bogaert et al. (S4) and 20× EVA green (Biotium, Inc., Fremont, CA, USA). Both qPCRs were carried out in a total volume of 10 µl, containing 5 µl (2×) LC480 Probes Master mix (Roche) and 2 µl extracted DNA. Amplification reactions were performed using a LightCycler 480 II Instrument (Roche) under the following conditions: 5 min at 95°C followed by 45 cycles of 95°C for 10 s, 60°C for 50 s and 72°C for 1 s (*nuc* gene) or 5 min at 95°C followed by 45 cycles of 95°C for 10 s, 60°C for 15 s and 72°C for 1 s (16S rRNA gene). For quantification, a 10-fold dilution series of a plasmid was included in each run and the second derivative analysis method was used for data analysis.

The total load of human DNA (RNaseP gene) was determined using primers and a probe, described elsewhere (S5). Each qPCR was carried out in a total volume of 25 µl, containing 12.5 µl (2×) IQ Supermix (Bio-Rad Laboratories Inc., Hercules, CA, USA) and 5 µl extracted DNA. Amplification reactions were performed using a CFX96 instrument (Bio-Rad Laboratories Inc.) under the following conditions: 3 min at 95°C followed by 45 cycles of 95°C for 15 s and 60°C for 50 s. For quantification, a 10-fold dilution series of MOLT cell line DNA was included in each run. For data analysis, the threshold was set on 850 relative fluorescence units.

Statistical analysis

The statistical software package SPSS was used for statistical analysis. Clinical data for patient groups were compared using 1-way analysis of variance (ANOVA) and χ^2 tests.

SUPPLEMENTARY REFERENCES

- S1. Chopra R, Vakharia PP, Sacotte R, Patel N, Immaneni S, White T, et al. Severity strata for Eczema Area and Severity Index (EASI), modified EASI, Scoring Atopic Dermatitis (SCORAD), objective SCORAD, Atopic Dermatitis Severity Index and body surface area in adolescents and adults with atopic dermatitis. *Br J Dermatol* 2017; 177: 1316–1321.
- S2. van den Munckhof EHA, de Koning MNC, Quint WGV, van Doorn LJ, Leverstein-van Hall MA. Evaluation of a stepwise approach using microbiota analysis, species-specific qPCRs and culture for the diagnosis of lower respiratory tract infections. *Eur J Clin Microbiol Infect Dis* 2019; 38: 747–754.
- S3. Pichon B, Hill R, Laurent F, Larsen AR, Skov RL, Holmes M, et al. Development of a real-time quadruplex PCR assay for simultaneous detection of nuc, Panton-Valentine leucocidin (PVL), mecA and homologue mecALGA251. *J Antimicrob Chemother* 2012; 67: 2338–2341.
- S4. Bogaert D, Keijser B, Huse S, Rossen J, Veenhoven R, van Gils E, et al. Variability and diversity of nasopharyngeal microbiota in children: a metagenomic analysis. *PLoS One* 2011; 6: e17035.
- S5. Luo W, Yang H, Rathbun K, Pau CP, Ou CY. Detection of human immunodeficiency virus type 1 DNA in dried blood spots by a duplex real-time PCR assay. *J Clin Microbiol* 2005; 43: 1851–1857.