

Supplementary Online Content

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This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods. Immunohistofluorescence Analyses and Assessment of Serum Cytokine Profiles by Luminex

1. Immunohistofluorescence analyses

Paraffin-embedded 5 μm -thick skin sections were available from the patients, originally taken for diagnostic reasons, prior to the start of secukinumab treatment. These skin sections were processed for immunohistofluorescence analyses after heat-induced epitope retrieval in citrate buffer pH 6. After blocking in PBS containing 10% BSA, sections were incubated with a primary anti-IL-17A antibody (goat polyclonal, 1:20, AF-317-NA, R&D Systems) at 4°C overnight. Sections were then incubated for 1 h with Alexa Fluor® 546-conjugated secondary antibody (1:800, A11056, Molecular Probes). Slides were mounted in ready-to-use medium with DAPI (Fluoromount-G®, SouthernBiotech 0100-20). Laser scanning confocal microscopy was performed with a LEICA TCS SP8 SMD microscope with a 40x objective. Stacks of images were processed with Fiji software.

2. Assessment of serum cytokine profiles by Luminex

Cytokine concentrations were measured in sera by Luminex (Bio-Plex Pro Human Th17 and Bio-Plex Pro Human Chemokines, Biorad) according to manufacturer's instructions. Briefly, sera were diluted 4 times and incubated for 1 hour with a mix of beads coupled to antibodies specific for each measured cytokine. After several washing steps, a mix of secondary streptavidin-coupled antibodies was added for 30 min followed by phycoerythrin (PE)-conjugated biotin. The signal intensity was measured by PE fluorescence using a Bio-Plex 200 (Biorad). In parallel, a standard curve of recombinant cytokine was performed to determine the absolute concentration of each cytokine in the patients' sera. Statistical significance was tested with a Mann Whitney test only if each group contained more than 3 independent values. Statistical analyses were performed using Prism software.

eFigure 1. Clinical Response to Secukinumab Therapy in the 2 Adult Patients With Netherton Syndrome

Patient 1 before and after 3 months of treatment with secukinumab

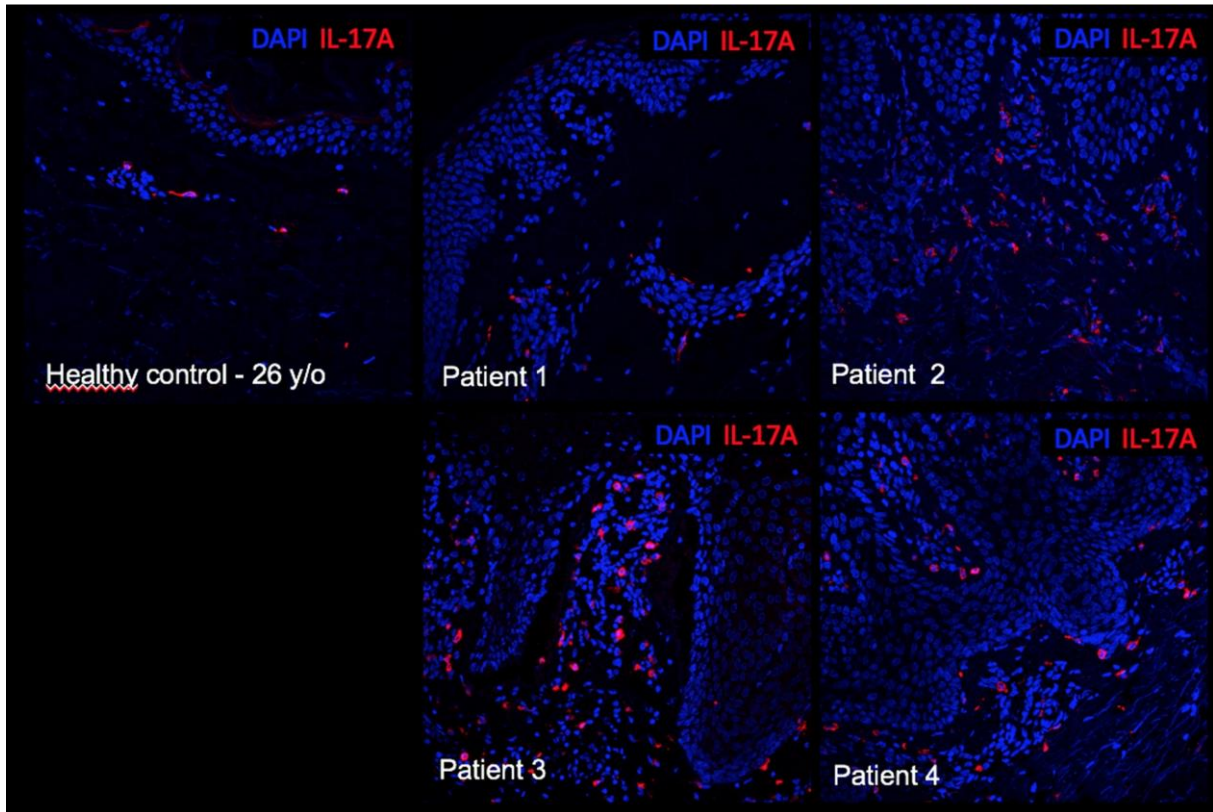


Patient 2 before and after 6 months of treatment with secukinumab



eFigure 2. Immunostaining of Lesional Skin

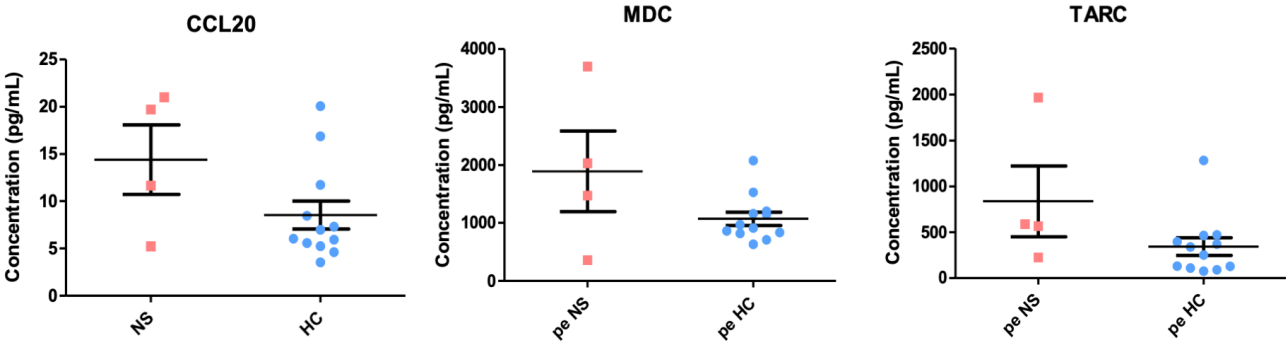
IL-17A expression in NS patients lesional skin was assessed by immunohistochemistry using primary anti-IL17A antibody (goat polyclonal, 1:2, AF-317-NA, R&D Systems). However, positive cells were not recognized by a CD3 antibody, but a majority of them were positively stained by an anti-MPO antibody, demonstrating cross reaction with MPO (data not shown).



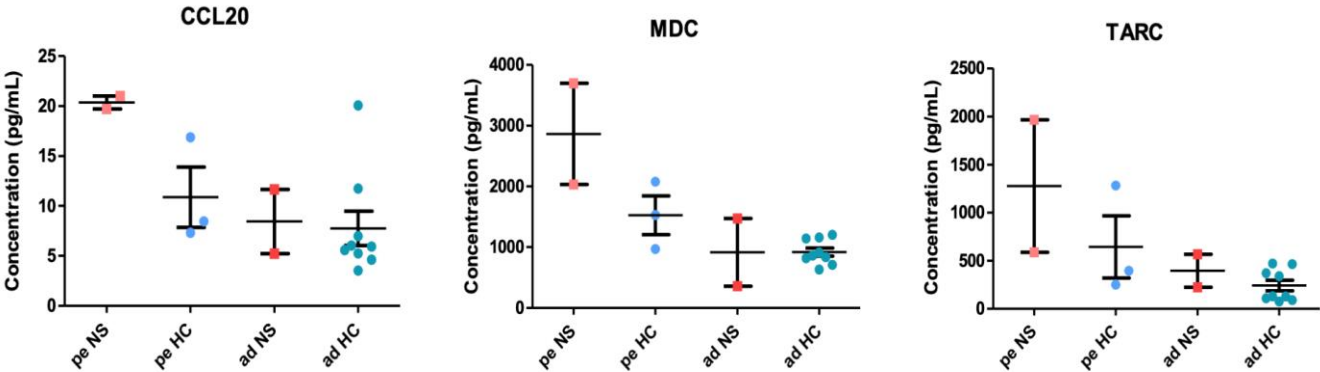
eFigure 3. Cytokine Concentrations of CCL20, MDC and TARC in the Serum Samples of NS

Cytokine concentrations of CCL20, MDC and TARC in the sera of NS patients measured by Luminex before the start of secukinumab treatment. Each dot represents one patient and the lines correspond to the mean \pm SEM. The results for MDC and TARC, which are cytokines related to Th2 response, revealed also increased levels in the affected patients, with a large range of variations. Serum cytokine analysis by age groups showed a 2-fold increase of CCL20 in the two pediatric patients

Results for all 4 NS patients (NS) compared to healthy controls (HC)

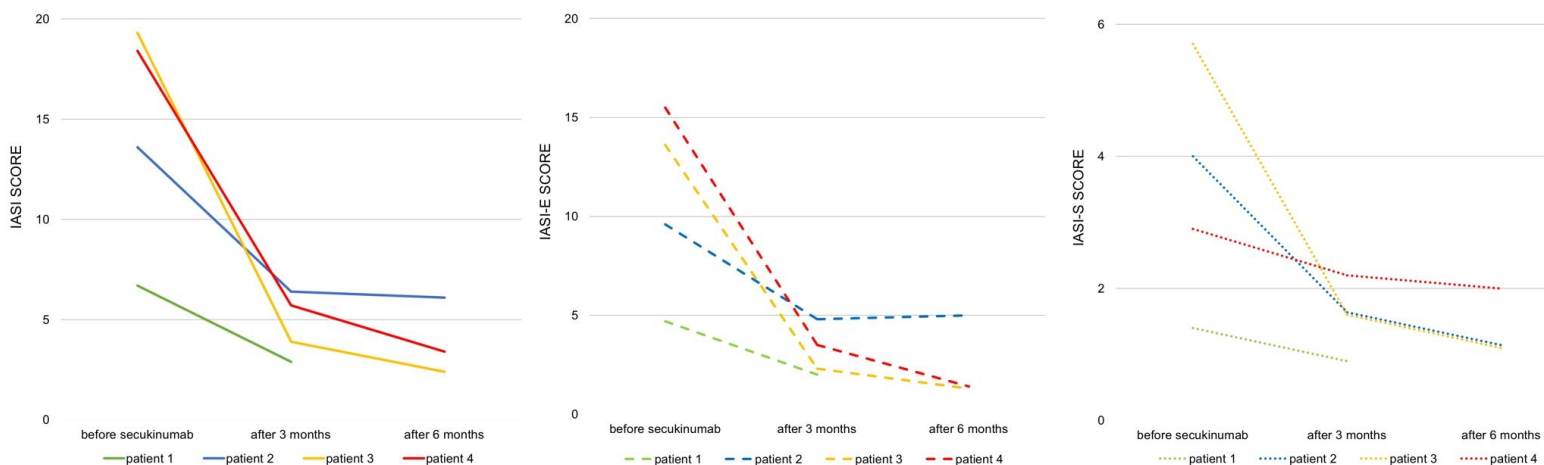


Results for pediatric (pe NS) and adult (ad NS) patients compared to pediatric (pe HC) and adult (ad HC) healthy controls



eFigure 4. IASI Total Score in Comparison With IASI- E and IASI-S Score Before and After 3 and 6 Months of Treatment With Secukinumab

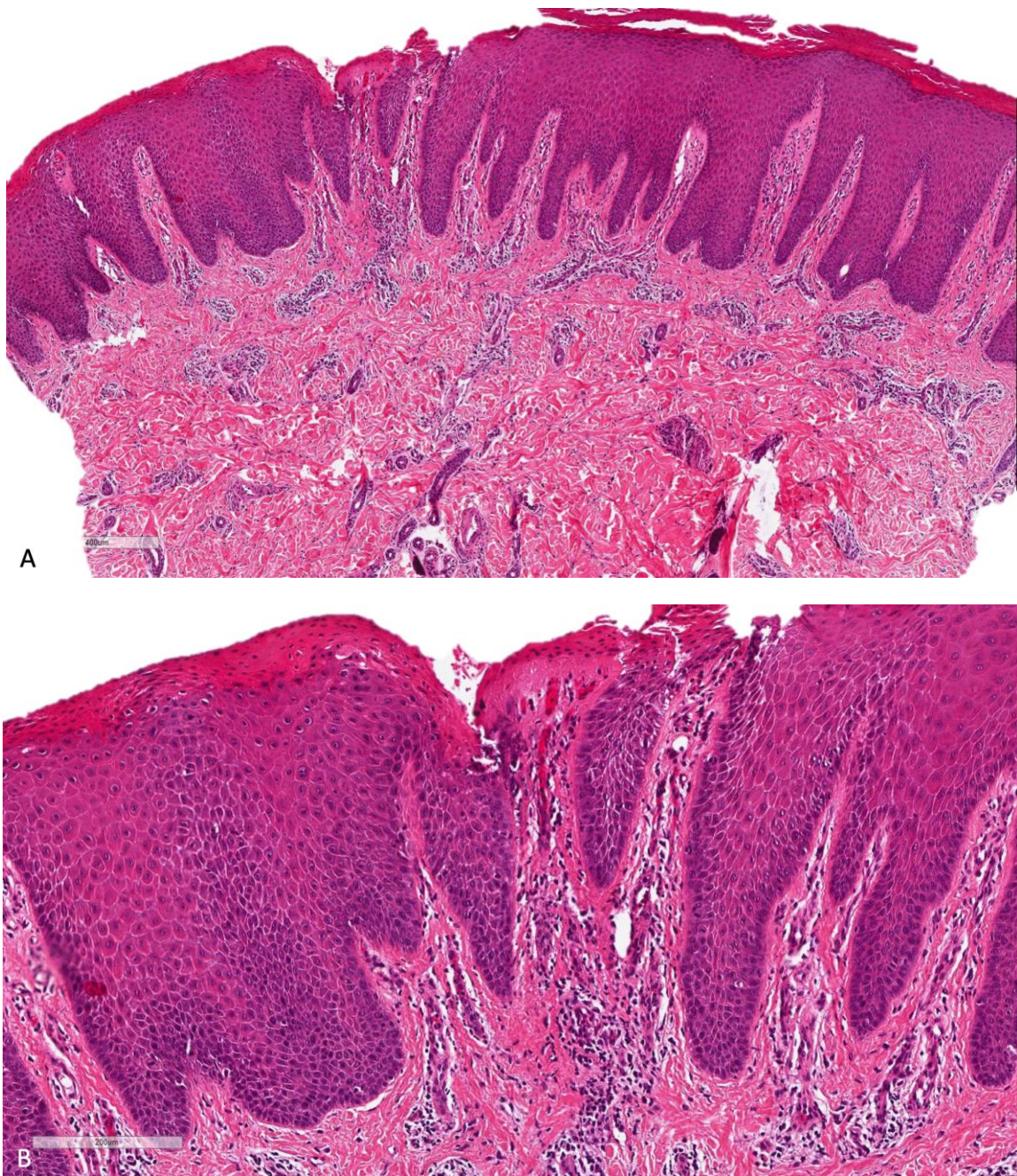
The Ichthyosis Area and Severity Index (IASI) measures the severity of the erythema (Ichthyosis Area Severity Index–Erythema [IASI-E]) and scaling (Ichthyosis Area Severity Index–Scaling [IASI-S]), adding them together to a total IASI score.⁸



eFigure 5. Palmoplantar Eczematous Reaction Under Secukinumab Therapy in Patients 3 and 4



eFigure 6. Histopathologic Findings of a Skin Biopsy of the Palmoplantar Eczematous Eruption in Patient 3



A, Psoriasiform hyperplasia of the epidermis with parakeratosis and a mild, perivascular inflammatory infiltrate in the superficial dermis (Hematoxylin and eosin, x100 magnification). **B**, Prominent spongiosis associated with hyperparakeratosis and foci of serum exudate. Note the perivascular lymphocytic infiltrate in the upper dermis and the absence of intra- or subcorneal collections of neutrophils. (Hematoxylin and eosin, x200 magnification).