USTEKINUMAB IN HIDRADENITIS SUPPURATIVA:
A CLINICAL OPEN LABEL STUDY WITH ANALYSES OF THE PROTEIN EXPRESSION PROFILE IN SERUM

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ABSTRACT

Introduction
Treatment of hidradenitis suppurativa (HS) is difficult and the search for effective therapies continues.

Objectives
To evaluate the efficacy of ustekinumab in HS.
To discover a potential biomarker.

Material and methods
Seventeen patients were included in this open label study and treated with 45 to 90 mg ustekinumab at weeks 0, 4, 16 and 28. Proteomic technology and enzyme-linked assay analysis (ELISA) was applied on serum.

Results
Twelve patients completed the protocol. Moderate to marked improvement of the modified Sartorius Score was achieved in 82% of patients at week 40 and the hidradenitis suppurativa clinical response (HiSCR-50) in 47%. There was significant modulation in the expression of 54 serum proteins in patients at baseline compared to normal controls. Involved pathways were related to inflammation, immune cell signaling, and tissue morphology and development. Four of these (FSH, LH, HCG and LTA4H) were significant modulated at the end of treatment. Good responders had milder disease and lower expression of leukotriene A4-hydrolase (LTA4H). IL-2R, TNF-α, IL17A and IL-17F were not elevated in patient serum and did not change during treatment.

Conclusion
The majority of patients showed improvement with ustekinumab. Although no biomarker was discovered, low LTA4H concentrations with mild disease severity may be predictive for the effectiveness of ustekinumab.
INTRODUCTION

Hidradenitis suppurativa (HS) is an inflammatory skin disease characterized by recurrent abscesses and sinus tract formation. The clinical severity of HS is commonly classified according to the Hurley stages. HS is painful and disfiguring, impairing quality of life to a great extent. Genetic susceptibility, smoking and obesity are important risk factors for the development of HS.

A dual approach is usually adopted in the treatment of HS. First, the inflammatory reaction is inhibited with systemic anti-inflammatory or immunosuppressive agents. Lesions resistant to systemic therapy, like sinus tracts, are subsequently surgically removed. Commonly used immunosuppressive agents are tumor-necrosis-factor-α (TNF-α) inhibitors, like infliximab and adalimumab. However, in a substantial number of patients TNF-α inhibitors are ineffective or cause adverse events requiring discontinuation of therapy. Therefore, there is still a need for new effective immunosuppressive agents in HS.

The pathogenesis of HS has not been clarified yet. It has been suggested that follicular plugging followed by the release of follicular material into the dermis are primary events that activate the immune system. Unraveling what cytokines are involved has been the main objective of several studies. Recently, it has been shown that the interleukin-23/ T-helper 17 cells (IL-23/Th17) and IL-12/Th1 pathways come to expression in HS skin. Ustekinumab is a human IgG1κ monoclonal antibody that binds with high affinity to the p40 subunit of IL-12 and IL-23. Thereby, these cytokines are prevented from interacting with their IL-12Rβ1 receptor protein that is expressed on the surface of T-cells and natural killer cells. Certain genetic variations within the gene encoding for the common IL-12β1R1 subunit of the IL-12/IL23 receptor have shown to be associated with a more severe course of HS. Ustekinumab has been approved for the treatment of psoriasis. The effectiveness of ustekinumab in HS has been retrospectively studied in a total of seven patients with conflicting results. With this open label prospective study we investigated the efficacy and safety of ustekinumab in a group of 20 patients with moderate to severe HS. To identify candidate biomarkers for HS we performed proteomics and immunoassays on serum of patients and healthy volunteers.
MATERIAL AND METHODS
The manuscript was approved by the institutional review board.

Study patients
Subjects were recruited from May 2012 until March 2013. Patients with moderate to severe HS (Hurley stage II-III) with a treatment history of at least one systemic anti-inflammatory/immunosuppressive agent or surgery were eligible for participation. The diagnosis of HS was made by a dermatologist.

Design and intervention
The trial had a prospective uncontrolled open-label design. The washout period for systemic immunosuppressive medication was at least 3 months. All patients received ustekinumab according to the psoriasis dosing regimen. Subcutaneous injections were administered at weeks 0 and 4 (induction phase) and week 16 and 28 (maintenance phase). Each injection contained 45 mg ustekinumab, with subjects weighing >100 kg receiving 90 mg per injection. The intervention period was set to 40 weeks, consisting of the treatment phase (weeks 0-28) followed by a post-treatment phase of 12 weeks. Topical resorcinol 15% cream or incision and drainage of acutely painful were the only allowed escape treatments. Assessments were performed by the same investigator at baseline (week 0) and weeks 4, 10, 16, 22, 28, 34 and 40. The study was approved by the local institutional review board and registered with ClinicalTrials.gov (NCT01704534).

Assessments of disease severity
At every visit, the modified Sartorius scale (mSS) and modified hidradenitis suppurativa-lesion area and severity index (mHSLASI) were assessed. The mSS is a dynamic scoring system for HS and includes the number of involved anatomical regions, the type and number of lesions, the extent of involvement and the Hurley stage. The mHSLASI reflects the degree of inflammatory activity by assessing the level of experienced pain, redness, edema and lesional discharge. A ≥50% reduction in these scores was considered as a marked response; 25-<50% reduction as moderate; >0-<25% reduction as mild and ≤0 as non-response. A visual analogue scale (VAS) to determine the degree of experienced pain ranging from 0 mm (no pain) to 100 mm (maximum pain), the Dermatology Life Quality Index (DLQI) and Skindex-29 questionnaires were used to investigate patient-reported outcomes.
Outcomes and follow-up
The primary endpoint was the proportion of patients with marked improvement (≥50% reduction) of the mSS and mHSLASI scores at week 40. Secondary outcomes included the mean change in patient's reported pain, Skindex-29 and DLQI. Regarding the Skindex-29, the cut-off scores as proposed by Prinsen et al. were applied. Improvement of the Skindex-29 was considered as clinically meaningful if the score of a specific domain went at least one scale down compared to week 0. A DLQI of 0-1 corresponds to no effect on patient's quality of life, an index of 2-5 to a small effect, 6-10 to a moderate effect, 11-20 to a very large effect and 21-30 to an extremely large effect on patient's quality of life. A reduction of ≥5 points on the DLQI was considered as clinically meaningful improvement.

We performed a post-hoc analysis using the Hidradenitis Suppurativa Clinical Response (HiSCR), which is a recently validated endpoint for assessing HS treatment effectiveness. Responders (HiSCR achievers) are defined as patients with at least 50% reduction in the number abscesses or inflammatory nodules, without an increase in draining fistulas (HiSCR-50). This endpoint was added to support our clinical scores as the mSS and mHSLASI may not be optimal in assessing inflammatory manifestations.

Sample collection and analyses
Protein analyses from serum:
A total of 1129 proteins were measured in serum by somalogics (high content proteomics) using “SOMAscan” (SomaLogic Inc. Boulder, CO) at baseline and at week 40.

Pathway analyses:
Pathway analyses were conducted with QIAGEN's Ingenuity Pathway Analysis (IPA®, www.qiagen.com/ingenuity).

Immunoassays:
Enzyme-linked Immunosorbent Assay (ELISA) was performed on serum at baseline, week 4, 16, 28 and 40 for TNF-α (MesoScale Discovery, Rockville, MD) and the soluble IL-2 receptor (sIL2R) (Alpco, Salem, NH) according to the manufacturer's instructions. Serum IL17A and IL17F were measured by the Erenna® Immunoassay system according to the manufacturer's
instructions (Singulex, Alameda, CA). Based on the protein Somalogics data measured at week 0 and 40, additional ELISA analyses were performed for leucotriene A4-hydrolase (LTA4H), follicle stimulating hormone (FSH), luteinizing hormone (LH) and human chorionic gonadotropin (HCG) at weeks 0, 4, 16 28 and 40.

**Safety assessments**

Serious adverse events were reported to the competent Health Authorities and the ethics committee within 24 hours in addition to completion of a Suspected Unexpected Serious Adverse Reaction form. A Data Safety Monitoring Board gathered every six months.

**Statistical analysis**

Descriptive statistics (mean, percentages, minimum and maximum) were performed for the outcome variables. Efficacy analyses were conducted on the intention-to-treat (ITT) population that included all patients who received at least one dose of study medication. If a patient dropped out it was attempted to obtain further patient assessments for the primary outcomes, if not the missing data were handled by carrying the last observation forward. The Wilcoxon rank test was used to analyze the efficacy of treatment. The criterion for statistical significance was $p<0.05$. Analyses were performed with SPSS Statistics 22.

Array Studio software version 8.0 (OmicSoft Corp., St. Morrisville, NC) was used to analyze proteomics data. Blood samples from healthy volunteers were used as controls for serum proteomics. Expression modulation was analyzed using a general linear model. A $>1.5$ fold change and an FDR-adjusted $p$-value $< 0.05$ were considered significant. Scatter plots were made with Matlab version 8.3.0.532 (R2014a).
RESULTS

Patients and drop-outs
A total of 26 patients were screened for entering the study. Seventeen (four men, 13 women) instead of the initially planned 20 patients were included, as the trial medication expired due to a slower recruitment rate than expected. Patient’s characteristics are described in table 1. Five subjects dropped out prematurely. The mean age was 35 years (range 20-53), mean BMI 28.3 kg/m² and mean disease duration was 18 years.

Primary endpoints (mSS and mHSLASI)
At week 40 improvement of the mSS was marked in six of 17 patients (35%), moderate in eight patients (47%), mild in one patient (6%) and in two patients (12%) there was no change or worsening. The mean mSS of the ITT population significantly reduced from 112.12 at baseline to 60.18 at week 40 (46.33% improvement; p=0.001) (fig. 1a). Improvement of the mHSLASI at week 40 was marked in three of 17 patients (18%), moderate in six patients (35%), mild in three patients (18%) and in five patients (29%) there was no change or worsening. The mean mHSLASI of the ITT population significantly reduced from 26.29 at baseline to 19.59 at week 40 (25.5% improvement; p=0.011) (fig. 1a).

Secondary endpoints (Skindex-29, VAS, DLQI)
At week 40 clinically meaningful improvement on the Skindex-29 overall domain was observed in six of 17 subjects (35%), on the functioning domain in eight subjects (47%), on the emotions domain in four subjects (24%) and on the symptoms domain in three subjects (18%) (fig. 1c). At baseline, the DLQI indicated that HS had a very large or extremely large effect on daily life in 71% of subjects, at week 40 this was 59% (fig. 1d). At week 40 clinically meaningful improvement of the DLQI had occurred in seven patients (41%). The mean reported pain on a VAS was 5.8 out of 10 at the start of treatment and 4.6 at week 40.

Post-hoc analysis with HiSCR
The number of HiSCR-50 achievers increased from the induction phase through to week 22. At week 40, eight out of 17 patients (47%) were HiSCR50 achievers (fig. 1b).
**Escape treatments**
Resorcinol 15% cream was used for troublesome inflammatory lesions by four patients and incision and drainage in one patient. The protocol was violated in two patients who received intraleisional corticosteroids for painful lesions that were not suitable for incision and drainage.

**Safety assessments**
The most common adverse events were headache, fatigue and upper respiratory tract infections. All events were mild and temporary.

**Protein expression in the serum**
Fifty-four proteins were significantly differentially expressed in serum of patients (n=17) at baseline compared to healthy controls (n=10) (fig. 2a). The top involved dysregulated pathways were related to inflammation, immune cell signaling, as well as tissue morphology and development (fig. 2b). Significant modulation of the leukotriene A4-hydrolase (LTA4H), follicle stimulating hormone (FSH), luteizing hormone (LH) and human chorionic gonadotropin (HCG) were identified at week 40 in the four best responders (subjects 6, 12, 14, 15).

**Serum ELISA for LTA4H, FSH, LH and HCG**
As LTA4H, FSH, LH and HCG were identified as potential biomarkers with protein microarray analysis, these were further analyzed with ELISA. No linear correlation between clinical disease severity and serum concentrations was identified, making these proteins not suitable as biomarkers for treatment effectiveness. However, good responders showed a trend towards having a relatively less severe clinical phenotype as well as lower concentrations of LTA4H (fig. 3).

**Serum ELISA for sIL-2R, TNF-α, IL17A and IL-17F**
There were no significant differences in serum concentrations of sIL-2R, TNF-α, IL17A and IL-17F between patients and controls, nor did we identify a significant decrease in any of these cytokines during ustekinumab treatment (data not shown).
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<th>Gender</th>
<th>Age</th>
<th>History</th>
<th>BMI (kg/m²)</th>
<th>Smoking status</th>
<th>Disease duration**</th>
<th>mSS</th>
<th>Treatment history</th>
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<td>18</td>
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<td>Former</td>
<td>16</td>
<td>79</td>
<td>I.l. corticosteroids, antibiotics, incision/drainage, deroofing</td>
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</table>

*at the start of the study in years; **in years

IPL-EPI = Intense pulse-light epilation; mSS = modified Sartorius Score; T. = topical; i.l.=intralesional

Table 1. Patient characteristics
Figure 1. Clinical scores during ustekinumab treatment

(a) Percentual reduction of the mSS and mHSLASI during ustekinumab treatment.
(b) The number of patients achieving >50% improvement of HiSCR during ustekinumab treatment.
(c) Improvement in Skindex-29 occurred for each domain with worsening of the scores during the post-treatment phase.
(d) The impact of HS on daily life as measured by the DLQI. The number of patients is presented within the bars.

Arrows represent ustekinumab administration points.
Figure 2. Disease profile in serum

(A) The heat-map of the HS disease profile in serum based on somalogics. The expression of 54 proteins was significantly different between HS (red boxes) and healthy controls (grey boxes) at baseline.

(B) The top 10 involved canonical pathways in serum of HS patients.

- Natural Killer Cell Signaling
- Fc Epsilon RI Signaling
- Insulin Receptor
- Ephrin Receptor
- T Cell Receptor Signaling
- HGF Signaling
- Pancreatic Adenocarcinoma Signaling
- Role of Tissue Factor in Cancer
- Ovarian Cancer Signaling
- PI3K Signaling in B Lymphocytes

- log (p-value)
Figure 3. Scatter plot for the correlation between LTA4H and the absolute HiSCR. There is no linear correlation between the concentration of LTA4H and the HiSCR. Note that good responders (green circle) have lower HiSCR combined with lower LTA4H concentrations compared to non-responders (red circle).
DISCUSSION

This is the first prospective study investigating the efficacy of ustekinumab in HS. Both primary outcomes (mSS and mHSLASI) showed a significant reduction of the mean at week 40. Additionally, moderate to marked improvement of the mSS and mHSLASI was seen in the majority (82% and 53% respectively) of patients. This suggests that ustekinumab may be applied for the treatment of HS as the reduction in mSS is comparable to studies on adalimumab and infliximab.\textsuperscript{8,28}

The mSS reduced >50% in 35% of patients. Other studies considered reductions of 30% to 40% as therapeutic responses.\textsuperscript{29,30} The HiSCR-50 was achieved in 47%. Considering ≥50% improvement of the mSS as clinically meaningful in HS is therefore probably too strict. The psoriasis dosing regimen may not have been sufficient for HS, further explaining why only a minority met the primary endpoint. Indeed, inflammatory marker levels are higher in HS than in psoriasis.\textsuperscript{10} Increasing the administration frequency, as has been shown to improve the results of adalimumab in HS, as well as increasing the dosage, which has been safely applied in Crohn’s disease, may therefore improve the effectiveness of ustekinumab.\textsuperscript{8,31}

Almost half of the patients showed clinically meaningful improvement on the functioning domain of the Skindex-29 at week 40. Quality of life scores worsened at the post-treatment phase, suggesting that longer treatment duration may lead to prolonged effectiveness.\textsuperscript{12}

Upper respiratory tract infections were one of the most common adverse events. The increased infection risk during ustekinumab treatment is caused by suppression of Th-17 cell differentiation, as IL-17 provides immunity against microbes like staphylococcus and candida. We could not confirm the potential of sIL2R as a serum biomarker in HS.\textsuperscript{32,33} Neither could we put IL-17A, IL-17F and TNF-\textalpha forward as potential biomarkers. However, HS patients had a significant different serum protein profile compared to controls, indicating that several proteins are secreted towards the circulation by cells present in inflammatory skin. The significant modulation in LH, FSH and HCG after treatment in the best responders, who were all female, is difficult to interpret as these hormones naturally fluctuate during the menstrual cycle. In psoriasis no correlation was found between disease severity and concentrations of FSH and LH in male patients.\textsuperscript{34} However, estradiol concentrations were associated with milder disease severity, confirming the potential protective role of estrogen in inflammatory diseases. Therefore, the role of sex hormones in HS also remains an interesting topic for future studies.

We show significant modulation in the expression of LTA4H in the best ustekinumab responders. LTA4H is an intracellular enzyme released by epithelial cells and macrophages. It converts leukotriene A4 (LTA4) into leukotriene B4 (LTB4), a pro-inflammatory mediator capable of recruiting and activating a wide range of immune cells, including neutrophils.
The subsequent LTA4H release from neutrophils counteracts the inflammatory reaction by degradation of neutrophilic attractant peptides.\textsuperscript{15} This dual effect may be responsible for the lack of a clear linear correlation between LTA4H serum concentration and clinical disease severity. However, the LTA4H concentration in combination with disease severity (HiSCR) may be used to predict the effect of ustekinumab: low concentrations with a relatively mild clinical disease severity may be associated with a good response.

Limitations of this study include the small number of patients and the lack of a control group. Furthermore, there was a relatively high dropout rate, increasing the risk of bias. This was restricted to a minimum by analyzing the ITT population.

In summary, this study shows that ustekinumab is well tolerated and that the standard psoriasis dosing schedule improved HS in the majority of patients. The dosing schedule may need to be intensified for HS to achieve sufficient immune suppression. Modulated pathways in serum of HS patients were related to inflammation, immune cell signaling, as well as tissue morphology and development. A biomarker for measuring treatment effects in HS was not identified. However, low LTA4H serum concentrations in combination with relatively mild disease severity may be predictive for the efficacy of ustekinumab.
REFERENCES


