

ORIGINAL ARTICLE

Apremilast for moderate hidradenitis suppurativa: no significant change in lesional skin inflammatory biomarkers

A.R.J.V. Vossen,^{1,*}  H.H. van der Zee,¹ N. Davelaar,² A.M.C. Mus,² M.B.A. van Doorn,¹ E.P. Prens¹

¹Department of Dermatology, Erasmus University Medical Center, Rotterdam, The Netherlands

²Laboratory of Rheumatology & Dermatology, Erasmus University Medical Center, Rotterdam, The Netherlands

*Correspondence: A.R.J.V. Vossen. E-mail: a.vossen@erasmusmc.nl

Abstract

Background Treatment with apremilast has recently demonstrated clinically meaningful improvement in moderate hidradenitis suppurativa (HS).

Objective To evaluate the change in expression of inflammatory markers in lesional skin of HS patients receiving apremilast 30 mg twice daily ($n = 15$) for 16 weeks compared with placebo ($n = 5$).

Methods At baseline, 5-mm punch biopsies were obtained from an index lesion (HSL) and non-lesional (HSN) skin in the same anatomical area. Subsequent HSL samples were taken as close as possible to the previously biopsied site at week 4 and week 16. After sampling, biopsies were split; one half was processed for *in vivo* mRNA analysis using real-time quantitative PCR; the other half was cultured for *ex vivo* protein analysis using a proximity extension assay (Olink). Linear mixed effects models were calculated to compare the levels of inflammatory markers in HSL skin between apremilast and placebo over time.

Results At baseline, 17 proteins with a fold change >2 in HSL vs. HSN skin were identified in 20 patients. The top five were IL-17A (5), S100A12, CST5, IL-12/23p40, CD6 (1) with fold changes ranging from 6.6 to 1638, respectively (FDR <0.044). Linear mixed effects models for 75 assays were calculated. Protein levels of S100A12 decreased during treatment in the apremilast group compared with the placebo group ($p = 0.014$, FDR = 0.186). None of the 14 genes exhibited significant changes in expression over time. However, an evident downward trend in relative mRNA expression of IL-17A and IL-17F was demonstrated in patients receiving apremilast.

Conclusion We did not detect statistically significant changes in inflammatory markers in HSL skin of HS patients receiving apremilast compared with placebo, despite clinical improvement in the apremilast group. Nonetheless, S100A12 and IL-17A were significantly elevated in HSL skin and showed a decrease in response to apremilast. The translational model in clinical trials involving HS clearly needs further improvement.

Received: 23 August 2018; Accepted: 6 November 2018

Conflicts of interest

A.R.J.V.V., N.D., A.M.C.M.: no conflicts of interest to declare. H.H.v.d.Z.: advisory board member for AbbVie, InflaRX and Galderma. M.B.A.v.D.: consultant, speaker, principal investigator or received grants from Abbvie, Celgene, Eli Lilly, Janssen-Cilag, Novartis, Pfizer, Cutanea Life Sciences, Idera Pharmaceuticals. E.P.P.: consultant, speaker, principal investigator or received grants from Abbvie, Amgen, Biogen, Celgene, Eli Lilly, Janssen-Cilag, Novartis, Pfizer and UCB.

Funding sources

This study was sponsored by Celgene (Summit, NJ, USA).

Introduction

Hidradenitis suppurativa (HS) is a chronic, recurrent, auto-inflammatory skin disorder with limited effective treatment

options.¹ Recently, treatment with apremilast for 16 weeks has demonstrated clinically meaningful improvement in moderate HS.² In this study (NCT03238469; EudraCT 2016-000859-27), twenty patients with moderate HS (HS-PGA score of 3) were randomized in a 3 : 1 ratio to receive blinded treatment of apremilast 30 mg twice daily or placebo, respectively. Demographic and disease characteristics were similar between

Ethical review board approval: IRB of the Erasmus University Medical Center (NL.57003.078.16, MEC-2016-377)

Trial registration number: NCT03049267 on ClinicalTrials.gov, registered 25 October 2016.

treatment arms. The mean age of the study population was 35 years (range: 21–64), most patients were female (17/20) and current smoker (16/20), the mean BMI was $32.7 \pm$ standard deviation (SD) 6.2, and the mean abscess and inflammatory nodule (AN) count was 6.0 ± 1.8 . The objective of this translational study was to evaluate the change in expression of inflammatory markers in lesional skin of HS patients receiving apremilast 30 mg twice daily for 16 weeks compared with placebo.

Material and methods

Ethical statement

This investigator-initiated trial was approved by the medical ethics committee of the Erasmus University Medical Center Rotterdam, The Netherlands (NL.57003.078.16), and conducted according to the Declaration of Helsinki principles. All patients provided written informed consent for study participation and use of their skin biopsies.

Biopsy procedures and sample processing

At baseline, two 5-mm punch biopsies were taken: one was obtained from an erythematous, indurated, inflamed lesion, i.e. the index lesion (HSL), and one from the normal-appearing unaffected non-lesional (HSN) skin in the same anatomical area (Fig. 1). Subsequent HSL skin samples were taken as close as possible to the previously biopsied index site at week 4 (early response) and week 16 (end of study). After sampling, biopsies were split whereby one half was processed for mRNA analysis of the biopsy, representing the *in vivo* gene expression, and the other half was cultured for *ex vivo* protein analysis. The supernatants and the biopsies in 250 μ L lysis buffer containing 1% β -mercaptoethanol were transferred to a polypropylene tube and stored at -20°C until further analysis.

Ex vivo skin culture and protein quantification

After sampling, half of the 5-mm biopsy was cultured for 24 h at 37°C in an atmosphere of 5% CO_2 and 98% humidity in a transwell system (Netwell; Costar, Cambridge, MA, USA) as previously described.^{3,4} In short, samples were placed in a 12-well plate with the epidermis exposed to the air and the dermis immersed in 1 mL Iscove's modified Dulbecco's medium (Gibco, Paisley, UK) containing 0.5% human AB serum, penicillin (100 U/mL) and streptomycin (100 U/mL). A broad spectrum of 92 inflammatory markers was measured in the supernatant using the Olink Proseek Multiplex Inflammation panel (Olink Proteomics, Uppsala, Sweden).

In vivo gene expression

Total RNA was isolated using the GenElute Mammalian Total RNA Miniprep Kit (Sigma-Aldrich, St. Louis, MA, USA), treated with 0.1 U/ μ L DNase (Invitrogen, Carlsbad, CA, USA) and cDNA was synthesized with SuperScript II reverse transcriptase, random hexamer primers (Invitrogen) and oligo(dT)15 (Promega, Madison, WI, USA). Primers and probes were designed and chosen using ProbeFinder Software and the Universal Probe Library (Roche Applied Science, Indianapolis, IN, USA). *ABL1* was used as housekeeping gene. Real-time quantitative PCR for 14 genes (*IFN- γ* , *TNF- α* , *IL-1 β* , *IL-6*, *IL-8*, *IL-10*, *IL-23p19*, *IL-12/23p40*, *IL-17A*, *IL-17F*, *IL-31*, *CXCL-10*, *CCL-5* and *MPO*) was performed with the ViiA7 System and using the QuantStudio Real-Time PCR Software version 1.3 (Applied Biosystems, Waltham, MA, USA).

Statistical analysis

Protein data are presented as normalized protein expression (NPX), which are log₂-transformed arbitrary units. Relative mRNA expression levels for week 4 and week 16 were calculated

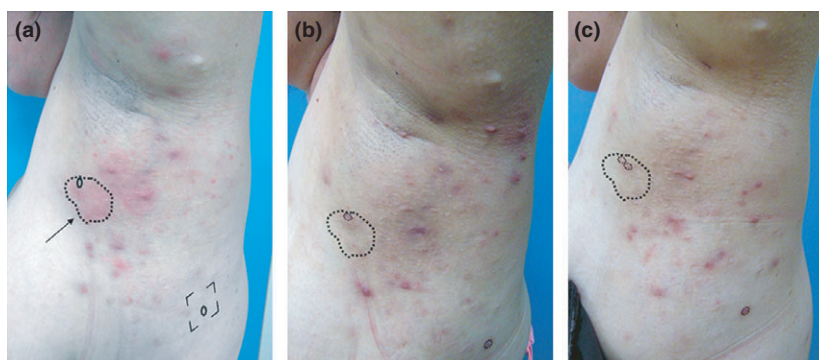


Figure 1 Biopsy procedure in a patient with a good clinical response to apremilast. Note that all deep-seated lesions have resolved. Erythematous lesions seen at week 16 are scars and superficial folliculitis. Panel (a; left) Baseline. A 5-mm punch biopsy was obtained from an erythematous, indurated, inflamed lesion (arrow pointing to index lesion). An additional 5-mm punch biopsy of the non-lesional skin was taken from the same anatomical area to serve as control (circle within the square). Panel (b; middle) Week 4 and Panel (c; right) Week 16. Subsequent skin samples were taken as close as possible to the previously biopsied index site (small circles within dashed line).

using the $2^{-\Delta\Delta CT}$ method using a baseline value of 1 for each individual sample.⁵

First, differentially expressed proteins at baseline were explored by calculating a fold change with the median NPX of HSL skin divided by the median NPX of HSN skin. If the fold change was more than 2, a Wilcoxon signed rank test was subsequently used to pairwise compare protein levels between HSL and HSN skin. Second, linear mixed effects models and associated ANOVA for repeated measures were calculated to compare inflammatory protein and mRNA expression levels between apremilast and placebo over time. Fixed effects were treatment, time and treatment \times time. Third, the Spearman's rho was used to assess translational linkage, i.e. correlation between the levels of inflammation and the AN count.

A two-sided *P*-value below 0.05 was considered significant. *P*-values were adjusted for multiple testing within each test using the Benjamini-Hochberg approach. Statistical analyses were conducted in R Statistical Software version 3.5 (R Foundation for Statistical Computing, Vienna, Austria) using the lmerTest package.

Results

At baseline, 17 proteins with a fold change >2 were identified (Table 1). The top-five were IL-17A (5), S100A12, CST5, IL-

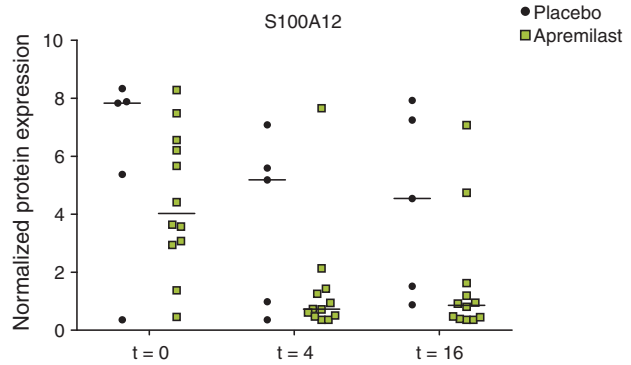


Figure 2 Protein levels of S100A12 in patients receiving apremilast (*n* = 15) and placebo (*n* = 5), linear mixed effect model: *P* = 0.014, FDR = 0.186. *t*: week. Data at week 16 for two patients receiving apremilast are missing as these two patients discontinued after respectively week 4 and week 8.

12p40, CD6 (1) with fold changes ranging from 6.6 to 1638, respectively (FDR \leq 0.044). Out of 92 Olink assays, 75 were included in the linear mixed effects models. The assays removed had less than 25% detectability including TNF- α and IFN- γ . Only the protein levels of S100A12 decreased over time in the apremilast vs. placebo group, statistically non-

Table 1 Proteins with a fold change >2 in HSL vs. HSN skin at baseline

| # | Protein | Fold change HSL/HSN | NPX HSL <i>n</i> = 20 | | NPX HSN <i>n</i> = 20 | | Unadj. <i>P</i> -value | Adj. <i>P</i> -value FDR | Missing data % |
|----|--------------|---------------------|--------------------------|---------|--------------------------|---------|------------------------|-----------------------------|-------------------|
| | | | Median | IQR | Median | IQR | | | |
| 1 | CD6 | 1638 | 3.5 | 2.0–4.1 | 0.002 | 0.0–0.2 | 3.4 e-07 | 0.018 | None |
| 2 | IL-12p40 | 38 | 0.9 | 0.5–1.8 | 0.024 | 0.0–0.1 | 7.2 e-06 | 0.038 | 7.5 |
| 3 | CST5 | 23 | 0.9 | 0.6–1.1 | 0.041 | 0.0–0.1 | 6.1 e-07 | 0.024 | 1.1 |
| 4 | S100A12 | 11 | 4.5 | 3.1–6.3 | 0.4 | 0.4–0.8 | 2.7 e-05 | 0.041 | 20.0 |
| 5 | IL-17A | 6.6 | 3.1 | 1.8–3.8 | 0.5 | 0.5–0.6 | 2.9 e-05 | 0.044 | 26.3 |
| 6 | TNFSF14 | 4.5 | 3.4 | 2.5–4.1 | 0.8 | 0.4–1.0 | 1.0 e-06 | 0.029 | 6.3 |
| 7 | AXIN1 | 4.4 | 1.9 | 1.7–2.1 | 0.4 | 0.4–0.5 | 8.8 e-07 | 0.026 | 8.8 |
| 8 | β -NGF | 3.2 | 1.1 | 0.6–1.4 | 0.3 | 0.3–0.6 | 5.8 e-04 | 0.047 | 15.0 |
| 9 | TNFSF11 | 3.1 | 2.2 | 1.2–3.3 | 0.7 | 0.7–0.7 | 4.6 e-07 | 0.021 | 28.8 |
| 10 | CD244 | 3.0 | 2.7 | 2.3–3.1 | 0.9 | 0.9–0.9 | 1.1 e-07 | 0.012 | 23.8 |
| 11 | CD5 | 3.0 | 7.3 | 5.9–7.6 | 2.5 | 2.2–2.6 | 1.3 e-08 | 0.003 | None |
| 12 | TNFRSF9 | 2.9 | 6.4 | 5.2–7.6 | 2.2 | 1.8–2.7 | 1.3 e-08 | 0.006 | 1.3 |
| 13 | OSM | 2.9 | 6.4 | 6.0–7.8 | 2.2 | 1.3–3.4 | 4.0 e-06 | 0.035 | 3.8 |
| 14 | SIRT2 | 2.8 | 5.0 | 4.5–5.4 | 1.8 | 1.5–1.9 | 1.9 e-07 | 0.015 | 5.0 |
| 15 | GDNF | 2.7 | 2.9 | 2.0–3.8 | 1.1 | 0.8–1.2 | 1.6 e-06 | 0.032 | 5.0 |
| 16 | IL-18R1 | 2.3 | 4.2 | 3.6–5.2 | 1.8 | 1.7–2.0 | 1.3 e-08 | 0.009 | None |
| 17 | ST1A1 | 2.1 | 1.5 | 0.8–1.8 | 0.7 | 0.5–1.2 | 1.3 e-02 | 0.050 | 10.0 |

Missing data: samples with NPX-values below background level throughout all samples, i.e. HSN and HSL baseline, HSL week 4 and HSL week 16. NPX: a high NPX value corresponds with a high protein concentration. Unadj. *P*-value: unadjusted *P*-value by Wilcoxon signed rank, NPX HSL vs. NPX HSN. Adj. *P*-value (FDR): adjusted *P*-value Benjamini Hochberg test, NPX HSL vs. NPX HSN.

FDR: false discovery rate; HS: hidradenitis suppurativa; HSL: HS lesional; HSN: HS non-lesional; IQR: interquartile range; NPX: normalized protein expression.

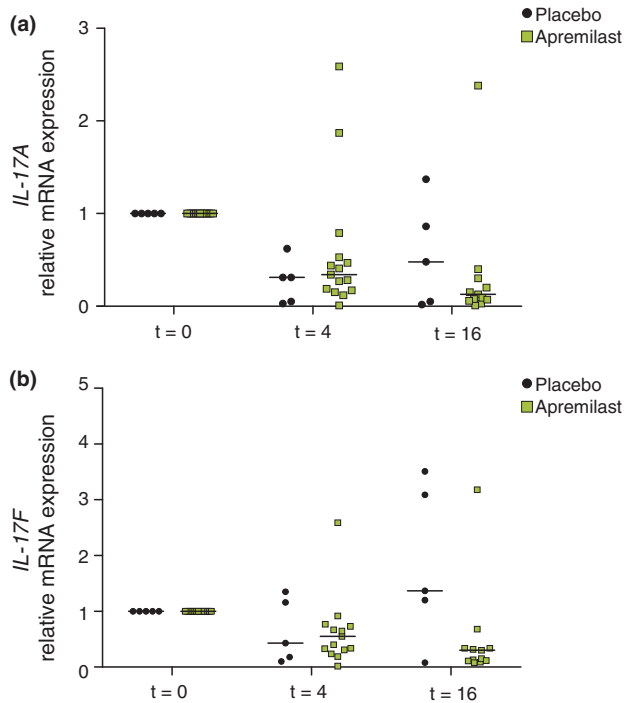


Figure 3 Relative mRNA expression levels of *IL-17A* and *IL-17F* in patients receiving apremilast ($n = 15$) and placebo ($n = 5$). IL: interleukin; t : week. Panel (a; above) *IL-17A*. At $t = 16$ in the apremilast group: one data point is out of the y -axis range. Linear mixed effect model: $P > 0.05$. Panel (b; below). *IL-17F*. At both $t = 4$ and $t = 16$ in the apremilast group, one data point is out of the y -axis range. Linear mixed effect model: $P > 0.05$. Data at week 16 for two patients receiving apremilast are missing as these two patients discontinued after respectively week 4 and week 8.

significant after correction for multiple testing ($P = 0.014$, FDR = 0.186; Fig. 2).

None of the 14 genes analysed by linear mixed effects models yielded significant differences. Only the relative mRNA expression of *IL-17A* and *IL-17F* demonstrated an evident but non-significant downward trend in patients receiving apremilast vs. placebo ($P > 0.05$; Fig. 3). In addition, there was no correlation between S100A12 protein levels or *IL-17A/F* mRNA expression and the favourable clinical response in the apremilast group as measured by the AN count (data not shown).

Discussion

This translational study aimed to identify inflammatory skin markers in response to apremilast 30 mg twice daily for 16 weeks in patients with moderate HS, which showed positive clinical results and have been published previously.² However, S100A12 protein levels and *IL-17A* and *IL-17F* mRNA expression showed a clear but non-significant decrease in patients receiving apremilast compared with placebo.

S100A12 (calgranulin C, EN-RAGE) is a pro-inflammatory, calcium-binding antimicrobial protein that interacts with the receptor for advanced glycation end products (RAGE) and is overexpressed in various inflammatory skin diseases, e.g. psoriasis.^{6,7} Previously, S100A8 and S100A9 (calprotectin or calgranulin A/B) have been demonstrated to be elevated in the serum and lesional skin of HS patients.^{8,9} It is known that IL-17 is a potent inducer of S100A8 and S100A9 in keratinocytes.^{10,11} The other way around, S100A12 stimulates T cells to produce IL-17A.¹² Thus, elevated levels of IL-17A and S100A12 in HSL skin at baseline and the responsiveness of these inflammatory markers to apremilast treatment is not surprising. Moreover, apremilast's impact on the Th17 pathway was recently demonstrated by the significant reduction of IL-17A and IL-17F protein levels in the plasma of psoriasis patients.¹³

The non-significant findings using a broad panel of inflammatory markers may be explained by the regression to the mean phenomenon, particularly at the individual level.¹³ The highly inflammatory index lesions could have spontaneously improved over time in the context of the fluctuating nature of the disease, especially in the placebo group. The effect of regression to the mean increases with larger measurement variability.¹⁴ Expanding the number of biopsies from index lesions, representing several degrees of inflammation, would theoretically be a solution. However, taking more biopsies would not be feasible for ethical reasons.

Another explanation for the decreased levels of inflammation over time in both groups could be that the inflammatory infiltrate of the index lesion was gradually reduced by successively taking biopsies from the same nodule. Moreover, the substrate for the foreign body inflammation such as hair fragments and keratin fibres, may have also been (partially) removed by the biopsy procedure.¹⁵ In addition, this exploratory study might be underpowered which may also have resulted in non-significant results.

Previously, two prospective uncontrolled studies investigating infliximab and ustekinumab in HS were also unable to link translational data to the clinical response.^{16,17} However, in these studies, only inflammatory markers in serum were investigated. Strengths of our study include the placebo-controlled trial design, the use of skin (target organ) instead of serum or plasma, and the standardized biopsy procedure of an index lesion. A limitation is the small study population.

In conclusion, this translational study investigating apremilast in moderate HS did not detect statistically significant changes in important inflammatory markers in lesional skin compared with placebo over 16 weeks of treatment, despite positive clinical findings. Nonetheless, related S100A12 and IL-17A were significantly elevated in HSL skin and showed a decline only in the apremilast group. In addition, our

findings highlight the challenge of assessing pharmacodynamics in the skin in a highly fluctuating inflammatory disease. A better translational model in clinical trials involving HS has yet to be developed.

Acknowledgements

We thank Eddy Florencia and Patrick Asmawidjaja for providing technical assistance in processing the skin samples.

References

- Zouboulis CC, Bechara FG, Dickinson-Blok JL *et al.* Hidradenitis suppurativa/acne inversa: a practical framework for treatment optimization - systematic review and recommendations from the HS ALLIANCE working group. *J Eur Acad Dermatol Venereol* 2018. <https://doi.org/10.1111/jdv.15233>
- Vossen ARJV, van Doorn MBA, van der Zee HH, Prens EP. Apremilast for moderate hidradenitis suppurativa: results of a randomized controlled trial. *J Am Acad Dermatol* 2018. pii: S0190-9622(18)32202-3.
- Wei L, Debets R, Hegmans JJ *et al.* IL-1 beta and IFN-gamma induce the regenerative epidermal phenotype of psoriasis in the transwell skin organ culture system. IFN-gamma up-regulates the expression of keratin 17 and keratinocyte transglutaminase via endogenous IL-1 production. *J Pathol* 1999; **187**: 358–364.
- Companjen AR, van der Wel LI, Wei L *et al.* A modified ex vivo skin organ culture system for functional studies. *Arch Dermatol Res* 2001; **293**: 184–190.
- Rao X, Huang X, Zhou Z *et al.* An improvement of the 2^{Δ(-ΔΔCT)} method for quantitative real-time polymerase chain reaction data analysis. *Biostat Bioinforma Biomath* 2013; **3**: 71–85.
- Eckert RL, Broome AM, Ruse M *et al.* S100 proteins in the epidermis. *J Invest Dermatol* 2004; **123**: 23–33.
- Wilmann-Theis D, Wagenpfeil J, Holzinger D *et al.* Among the S100 proteins, S100A12 is the most significant marker for psoriasis disease activity. *J Eur Acad Dermatol Venereol* 2016; **30**: 1165–1170.
- Lima AL, Karl I, Giner T *et al.* Keratinocytes and neutrophils are important sources of proinflammatory molecules in hidradenitis suppurativa. *Br J Dermatol* 2016; **174**: 514–521.
- Wieland CW, Vogl T, Ordelman A *et al.* Myeloid marker S100A8/A9 and lymphocyte marker, soluble interleukin 2 receptor: biomarkers of hidradenitis suppurativa disease activity? *Br J Dermatol* 2013; **168**: 1252–1258.
- Cho KA, Suh JW, Lee KH *et al.* IL-17 and IL-22 enhance skin inflammation by stimulating the secretion of IL-1beta by keratinocytes via the ROS-NLRP3-caspase-1 pathway. *Int Immunol* 2012; **24**: 147–158.
- Bai B, Yamamoto K, Sato H *et al.* Complex regulation of S100A8 by IL-17, dexamethasone, IL-4 and IL-13 in HaCat cells (human keratinocyte cell line). *J Dermatol Sci* 2007; **47**: 259–262.
- Kessel C, Lippitz K, Weinhage T *et al.* Proinflammatory cytokine environments can drive interleukin-17 overexpression by gamma/delta T cells in systemic juvenile idiopathic arthritis. *Arthritis Rheumatol* 2017; **69**: 1480–1494.
- Garcet S, Nogales K, Correa da Rosa J *et al.* Synergistic cytokine effects as apremilast response predictors in patients with psoriasis. *J Allergy Clin Immunol* 2018; **142**: 1010–1013.e6.
- Barnett AG, van der Pols JC, Dobson AJ. Regression to the mean: what it is and how to deal with it. *Int J Epidemiol* 2005; **34**: 215–220.
- van der Zee HH, de Ruiter L, Boer J *et al.* Alterations in leucocyte subsets and histomorphology in normal-appearing perilesional skin and early and chronic hidradenitis suppurativa lesions. *Br J Dermatol* 2012; **166**: 98–106.
- Blok JL, Li K, Brodmerkel C *et al.* Ustekinumab in hidradenitis suppurativa: clinical results and a search for potential biomarkers in serum. *Br J Dermatol* 2016; **174**: 839–846.
- Montaudie H, Seitz-Polski B, Cornille A *et al.* Interleukin 6 and high-sensitivity C-reactive protein are potential predictive markers of response to infliximab in hidradenitis suppurativa. *J Am Acad Dermatol* 2017; **76**: 156–158.