Usage of Omics Technology for Identification of Critical Mediators and Pathways in Patients with Hidradenitis Suppurativa

Sponsor: University College Dublin

Samples from the DERMMARK HS trial were analyzed in tandem with samples from two other studies - Atopic Dermatitis (AD) and Psoriasis (PS) - and results from data analysis of all three studies are presented alongside each other below. Results from the DERMMARK trial are indicated by 'HS' in results tables.

PRINCIPAL OR COORDINATING INVESTIGATOR(S) SIGNATURE(S)

STUDY TITLE: DERMMARK

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I have read this report and confirm that to the best of my knowledge it accurately describes the conduct and results of the study

INVESTIGATOR: Brian Kirby SIGNATURE(S) _____

AFFILIATION: University College Dublin

DATE: 28/10/23

Study Information

Study title: Usage of Omics Technology for Identification of Critical Mediators and Pathways in Patients with Hidradenitis Suppurativa

Study Acronym: DERMMARK

Test drug/investigational product: Adalimumab will be supplied as Humira 40 mg/0.4 ml concentration in Pre-filled Pen, Pre-filled Syringe and Vial. The total treatment period is 12 weeks.

Indication studied: Hidradenitis Suppurativa

Study design: Investigator led, open label, single-arm progression study

Sponsor: University College Dublin, Belfield, Dublin 4, Ireland

Protocol identification: UCDCRC/16/002

EudraCT number: 2016-001566-28

Development phase of study: IV

Clinical Sites: St Vincent's University Hospital, Dublin

Study initiation date: 29-Aug-2016 (start of recruitment)

Early termination date: March 2020 (recruitment stopped early due to COVID-19 pandemic)

Chief Investigator: Prof Brian Kirby

Sponsor representative: Prof Peter Doran

Statement: This study was conducted in compliance with Good Clinical Practice (GCP), including the archiving of essential documents.

Report date(s): 25/07/23 (content final)

Earlier reports from the same study: None

Credentials

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ABBREVIATIONS

AE	Adverse event
CRF	Case report form
CRO	Contract research organisation
CRP	C-Reactive Protein
CXR	Chest x-ray
DLQI	Dermatologic Life Quality Index
ECG	Electrocardiogram
EU	European Union
GCP	Good Clinical Practice
GP	General Practitioner
HiSCR	Hidradenitis Suppurativa Clinical Response
HS	Hidradenitis suppurativa
ICH	International Conference on Harmonisation
EC	Ethics Committee
IMP	Investigational medicinal products
HPRA	Health Products Regulatory Authority
HSE	Health Service Executive
PI	Principal investigator
PIL	Patient/subject information leaflet
REC	Research ethics committee
ROI	Republic of Ireland
SAE	Serious adverse event
SmPC	Summary of product characteristics
SOP	Standard operating procedure

SFI-AbbVie Collaboration
Scientific Final Report

Usage of Omics technology for Identification of Critical Mediators and Pathways in Patients with Atopic Dermatitis, Psoriasis and Hidradenitis Suppurativa

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Contents

1. Overview of the study

This study sought to identify key pathological pathways in Hidradenitits Suppurativa (HS) by comparing the lesional and non-lesional skin of HS with that of Psoriasis (Pso), Atopic Dermatitis (AD) and healthy controls. We analysed whole-cell RNA sequencing data from skin biopsies in these groups. In addition, we completed mass spectrometry (proteomics) and multiplex analysis of microdialysates from lesional skin, a novel technique in HS. We also used these techniques to identify key mediators of inflammation in HS prior to and during treatment with adalimumab and to identify any predictive biomarkers of therapeutic efficacy, as measured using HiSCR and IHS4.

1.1 Aims

The aims and objectives of the study of the study were:

- Identification of key mediators of inflammation in patients with Hidradenitis Suppurativa (HS) prior to and during treatment with Adalimumab
- Identification of key mediators in the pathogenesis of Atopic Dermatitis (AD) by prospective analysis of acute AD lesions over 12 weeks.
- Identification of key critical mediators in Psoriasis (Pso) pathogenesis by prospective analysis of early lesions over 12 weeks.

1.2 Work Packages

<u>WP1: Defining the molecular mediators of response to Adalimumab in HS</u>

- Identify key cytokines/chemokines and other mediators from patients with HS prior to and during treatment with adalimumab.
- Examine the biological profiles of patients who fail to respond to treatment with adalimumab, with a view to identifying alternative disease pathways and potentially novel therapeutic targets.
- Identify predictive biomarkers of therapeutic efficacy so that patients can be stratified into the appropriate treatment arms at the earliest time point.

<u>WP2&3: Identifying Mediators of Psoriasis pathogenesis (WP2) and key signalling networks in patients with AD (WP3)</u>

- Identify key cytokines/chemokines and in lesional skin as compared to non-lesional skin in Pso and AD patients and healthy controls

Identify key cytokines/chemokines in lesional skin as compared to non-lesional skin in Pso and AD patients and healthy controls

2. Methods

2.1 Patients recruitment

DERMMARK (Investigator led, open label, single-arm progression study) and OMICS study (Non-interventional, case control study with longitudinal biological sampling) were approved by the Research Ethics Committee of St Vincent's Healthcare Group (SVHG). Patients and controls were recruited primarily from the dermatology department in St Vincent's University Hospital (SVUH). Patients with HS, Psoriasis and atopic dermatitis (AD) were assessed for suitability when attending routine outpatient appointments and phototherapy. Posters were placed in the dermatology department with information about the studies and contact details. In addition, advertisements were placed in local newspapers inviting interested patients and healthy controls to contact the research team. Patients and controls who contacted the research team were reviewed in the Clinical Research Centre in SVUH. Those who were deemed ineligible after assessment did not undergo full screening procedures. Inclusion and exclusion criteria are listed in Appendix 1.

Recruitment was stopped in March 2020 due to the Covid-19 pandemic.

2.2 Samples collection

Skin microdialysis (SMD) and skin biopsies were collected from lesional and non-lesional skin at week 0 (W0) and week 12 (W12) of the study for the HS, Pso and AD cohorts. SMD and skin biopsies from healthy Volunteers (HV) were collected at one time point as controls.

Skin biopsy

6mm punch skin biopsy was performed after local anaesthesia. Immediately after collection, samples were cut in half. One half was snap frozen for further RNA isolation. The other half was frozen in OCT for further immunohistochemistry staining.

Skin microdialysis

A SMD semi-permeable polycarbonate membrane catheter (with a nominal 20kDa molecular weight cut-off as specified by the manufacturer) was inserted intradermally after local

anesthesia (Xylocaine 2% with Adrenaline) by a dermatologist. Ringer's solution was perfused at a rate of 5µl.min^{-1} for 30 minutes. The collected eluates were analyzed by mass-spectrometry based proteomics and multiplexed immunoassay.

2.3 RNA sequencing

RNA isolation

RNA isolation from skin biopsy was optimised to obtain high quality RNA. Skin biopsies were cut in half and snap frozen in liquid nitrogen less than five minutes after collection. Samples were first pulverised using a BioPulverizer and homogenised in lysis buffer using a Precellys homogenizer (CK14 beads, three runs of 23 seconds at 6300rpm). RNA isolation was then performed using a Fibrous RNA isolation kit (Qiagen) following the manufacturer's instruction. RNA concentration and RIN score were assessed by Nanodrop and Bioanalyzer.

RNA sequencing

RNA sequencing was performed by AbbVie. Three batches of high-quality RNA were sent to Chicago, IL, USA (n=196). >50 million 2x50 bp reads were generated per sample.

Reads alignment and quantification

The reads were aligned using the align function of the R implementation of the Rsubread package under its default parameters. The fragments were assigned to exons and quantified using the featureCounts function of Rsubread with default parameters (Liao et al., 2019).

Normalization and Differential Expression

The raw data was normalized using the DESeq2 package in R (Love et al., 2014). This algorithm assumes that the counts $K_{i,j}$ for gene i and sample j follow a negative binomial distribution with mean $\mu_{i,j}$ and dispersion α_i. The mean is assumed to be proportional to the concentration of cDNA fragments from the gene in the sample $q_{i,j}$ multiplied by sample specific scaling factor s_j. The normalization process consists of estimating the values for these parameters. The differential expression between conditions is then assessed by building linear models with the form $log2(q_{i,j}) = \sum_{i=1}^{r} x_{j,,r}\beta_{r,i}$, where $x_{j,.} = [x_{j,1}, \dots, x_{j,r}]$ is the experiment design matrix for sample j with r conditions and $\beta_{.,i} = [\beta_{1,i}, \dots, \beta_{r,i}]$ the coefficients estimates.

To determine if a gene i is differentially expressed, DESeq2 uses a Wald test on the regression coefficient $\beta_{.,i}$ to see if it is statistically significantly different from zero (Love et al., 2014). We set statistical significance at 0.01 after multiple comparisons adjustment. Pathway and gene ontology enrichment was done in the DAVID platform (Huang et al., 2009).

Data Visualization

The normalized gene expression levels were transformed using the variance stabilization transformation (VST) in the DESeq2 package (Love et al., 2014). The data were visualized using the first two components of the principal components analysis (PCA) plot (plotPCA function in DESeq2) and, independently, the uniform manifold approximation and projection (UMAP) from the UMAP package.

2.4 Mass spectrometry

Protein digestion

Proteins from SMD were digested following the Single-pot solid-phase-enhanced sample preparation (SP3) protocol as previously described (Hughes et al., 2019), as this is the most efficient method for samples with low protein content (Sielaff et al., 2017).

Reversed-phase nano-LC-MS/MS

Peptide separation was carried out on an ACQUITY UPLC M-Class System (Waters) coupled to an Orbitrap Fusion Lumos Mass Spectrometer (Thermo Scientific). 40% of purified peptides were trapped on a nanoEase M/Z Symmetry C18 column at a flow rate of 15 uL/min. Peptide separation was done using a nanoEase M/Z HSS C18 T3 column using a flow rate of 300nL/min. For DDA experiments full MS resolution was set to 120,000 at m/z 200 and full MS AGC target was 4E5 with an IT of 50 ms, covering the mass range 375–1500. AGC target value for fragment spectra was set to 5E4 with a resolution of 30,000 and 54 ms as injection time using a cycle time of 3 sec. Isolation width was set at 1.2 m/z and a fixed first mass of 110 m/z was used. NCE was set to 30%. For DIA experiments full MS resolution was set to 120,000 at m/z 200 and full MS AGC target was 2E5 with an IT of 50 ms, covering the mass range 400–1000. AGC target value for fragment spectra was set at 5E4. 50 windows of 12 Da were used. Resolution was set to 30,000, IT to 54 ms, and NCE to 30 %. The spectral library was generated using pools consisting of nine or ten individual samples.

Peptide identification and quantification

Hybrid spectral libraries using DDA and DIA files were built using Spectronaut Pulsar (14.0.200601) with default settings. Spectronaut default settings were used for DIA file processing with PTM localization activated and site confidence score cutoff set to 0.75 (for library generation), data filtering set to Q-value and Normalization Strategy set to Global Normalization using the median.

2.5 Multiplex array

The multiplex immunoassay (IFNγ, IL-1β, -2, -4, -5, -6, -8, -10, -17A, -17F, -22 and TNFα) was performed following the manufacturer's instructions (GeniePlex Multiplex Immunoassay).

3. Results

3.1 Clinical data

Recruitment

Twenty-six patients underwent full screening for DERMMARK and 20 commenced the study. Nineteen patients completed the study. One patient withdrew voluntarily due to a perceived lack of effect and personal circumstances.

All Psoriasis patients (n=24) and AD patients (n=17) who met inclusion and exclusion criteria and underwent full screening commenced the OMICS study. Eighteen Psoriasis patients and ten AD patients completed visits at week 12.

21 healthy controls (HCs) participated in the study.

Sociodemographic results

Parameter	**HS (n=20)**		**Psoriasis (n=24)**		**AD (n=17)**		**Healthy Controls (n=21)**	
Gender	**Count**	**%**	**Count**	**%**	**Count**	**%**	**Count**	**%**
Male	3	15	18	75	10	58.8	9	42.9
Female	17	85	6	25	7	41.2	12	57.1
Age at Screening Visit (years)	**Mean ± SD** 37 ± 9.1	**Range** 22-57	**Mean ± SD** 49 ± 15	**Range** 21-78	**Mean ± SD** 45.8 ± 17.7	**Range** 19-68	**Mean ± SD** 33.4 ± 9.6	**Range** 20-58
Race	**Count**	**%**	**Count**	**%**	**Count**	**%**	**Count**	**%**
White	19	95	24	100	17	100	17	81
Black or Black Irish	1	5	0	0	0	0	0	0
Asian or Asian Irish	0	0	0	0	0	0	3	14.3

Other including mixed background	0	0	0	0	0	0	1	4.8
Smoking Status	**Count**	**%**	**Count**	**%**	**Count**	**%**	**Count**	**%**
Current Smoker	13	65	5	20.8	1	5.9	5	23.8
Smoker in the past	5	25	10	41.7	8	47.1	4	19
Non Smoker	2	10	9	37.5	8	47.1	12	57.1
Duration of Disease	**Count**	**%**	**Count**	**%**	**Count**	**%**	**Count**	**%**
≤5 Years	3	15	1	4.2	0	0	-	-
5-10 Years	8	40	3	12.5	2	11.8	-	-
10-20 Years	6	30	8	33.3	3	17.6	-	-
≥20 Years	3	15	12	50	12	70.6	-	-

Table 1. Baseline Demographics All Groups

The mean age in the HS group was 37 years, compared with 49 years in the Psoriasis group, 46 in the AD group and 33 in the HC. Females represented 85% of HS patients, 25% of Psoriasis, 41% of AD and 57% of HCs.

The race of all groups was predominantly white. 65% of the HS group were current smokers and 25% ex-smokers. This was compared with 20.8% and 41.7% respectively in the Psoriasis group, 5.9% and 47.1% respectively in the AD group and 23.8% and 19% respectively in the HC group (Table 1).

Additional demographic data was collected from the HS group including marital status, education and employment details, and unemployment / sick leave details (Table 2). Of 8 patients who were on sick leave or unemployed, 50% reported that this was due to their HS.

Marital Status (n=20)	**Count**	**%**
Single	12	60
Married / Co-habiting	7	35
Divorced / Separated	1	5
Highest Education Attained (n=20)		
Secondary School	9	45
College / University <4 Years	10	50
College / University >4 Years	5	5
Work Status (n=20)		
Student / Employed / Self Employed	11	55
Unemployed	3	15
Retired	2	10
Sick Leave	4	20
Is unemployment or sick leave due to HS? (n=8)		
Yes	4	50
No	4	50
Duration of sick leave or unemployment (n=8)		
<6 months	2	25
6-12 months	2	25
>12 months	2	25
Unknown	2	25

Table 2. Social Demographics HS group

Co-morbidities

At the screening visit, patients were asked about co-morbidities from a set list of pre-specified conditions. 85% of the HS group reported at least 1 co-morbidity, compared with 100% of the Psoriasis and AD groups and 57.1% of the HCs (Table 3).

Parameter	HS (n=20)		Psoriasis (n=24)		AD (n=17)		Healthy Controls (n=21)	
Co-morbidity Reported	**Count**	**%**	**Count**	**%**	**Count**	**%**	**Count**	**%**
Yes	17	85	24	100	17	100	12	57.1
No	3	15	0	0	0	0	9	42.9
Number of Co-morbidites reported	**Count**	**%**	**Count**	**%**	**Count**	**%**	**Count**	**%**
0	3	15	0	0	0	0	8	38.1
1	5	25	7	29.2	1	5.9	8	38.1
2	6	30	6	25	5	29.4	5	23.8
3	1	5	5	20.8	6	35.3	0	0
4	2	10	1	4.2	4	23.5	0	0
5	3	15	5	20.8	1	5.9	0	0
Co-morbidity Type	**Count**	**%**	**Count**	**%**	**Count**	**%**	**Count**	**%**
Arthritis	4	20	5	20.8	1	5.9	1	4.8
Ankylosing Spondylitis	0	0	0	0	0	0	0	0
Other Inflammatory	0	0	1	4.2	1	5.9	0	0
Osteoarthritis	3	15	2	8.3	0	0	0	0
Other Arthritis	2	10	2	8.3	0	0	1	4.8
Asthma/Bronchitis/ COPD	1	5	4	16.7	10	58.8	1	4.8
Autoimmune Disease	0	0	0	0	0	0	0	0
Cancer	0	0	1	4.2	0	0	0	0
Cardiovascular Disease	0	0	5	20.8	6	35.3	0	0
Cardiac Disease	0	0	2	8.3	1	5.9	0	0
Vascular Disease	0	0	1	4.2	1	5.9	0	0
Hypertension	0	0	5	20.8	5	29.4	0	0
Chronic Pain	4	20	2	8.3	1	5.9	0	0
Diabetes Mellitus Type 1 or 2	0	0	1	4.2	1	5.9	0	0
Dyslipidaemia	1	5	6	25	4	23.5	1	4.8
Inflammatory Bowel Disease	4	20	0	0	0	0	0	0
Crohn's Disease	1	5	0	0	0	0	0	0
Ulcerative Colitis	0	0	0	0	0	0	0	0
Undifferentiated	3	15	0	0	0	0	0	0
Inflammatory Eye Disease	0	0	0	0	2	11.8	0	0
Liver Disease	0	0	1	4.2	1	5.9	0	0
Metabolic Syndrome	0	0	0	0	0	0	0	0
Obesity	5	25	5	20.8	0	0	0	0
Osteoporosis	0	0	1	4.2	1	5.9	0	0
Polycystic Ovarian Syndrome	1	5	0	0	0	0	1	4.8
Psychiatric Disease	5	25	3	12.5	7	41.2	6	28.6
Anxiety	2	10	1	4.2	3	17.6	2	9.5
Depression	5	25	3	12.5	5	29.4	3	14.3

Other	0	0	0	0	0	0	1	4.8
Renal Disease	0	0	0	0	2	11.8	0	0
Skin Disease	9	45	1	4.2	5	29.4	3	14.3
Acne	3	15	0	0	1	5.9	2	9.5
Skin Cancer	0	0	1	4.2	0	0	0	0
Psoriasis	3	15	-	-	0	0	0	0
Other	6	30	0	0	4	23.5	1	4.8
Sleep Disorder	0	0	1	4.2	3	17.6	0	0
Other	9	45	16	66.7	6	35.3	5	23.8

Table 3 Co-morbidities All Groups

Family History of HS

45% of the HS group reported a family history of HS, 45% reported no family history and 10% were unknown. Five reported a family history in a parent (25%), three in a sibling (15%), one in their children (5%) and two in another relative, not specified (10%).

Clinical findings

BMI and Waist Circumference

The mean BMI was highest in the HS group at 33.5kg/m^2, followed by 28.1kg/m^2 in the Psoriasis group, 26.6kg/m^2 in the AD group and 25.2kg/m^2 in the HV group. When classified into groups as per the World Health Organisation, 20% of HS were in the "normal weight" range, compared with 29.2% of Psoriasis, 35.5% of AD and 42.9% of HC. 25% of HS patients were classified as "Obese Class 3" compared with 8.3% of Psoriasis patients and zero of AD and HC. Waist circumference was highest in the HS group at 106.4cm, followed by Psoriasis (99.6cm), AD (94.6cm) and HCs (85cm) (Table 4).

Parameter	**HS (n=20)**		**Psoriasis (n=24)**		**AD (n=17)**		**Healthy Controls (n=21)**	
BMI (kg/m^2)	**Mean ± SD**	**Range**	**Mean ± SD**	**Range**	**Mean ± SD**	**Range**	**Mean ± SD**	**Range**
	33.5 ± 8.6	21.4-47.9	28.1 ± 5.6	19.4-40.8	26.6 ± 4.5	19.4-36.4	25.2 ± 3.9	17.9-36
BMI Category (WHO)	**Count**	**%**	**Count**	**%**	**Count**	**%**	**Count**	**%**
Underweight (<18.5)	0	0	0	0	0	0	1	4.8
Normal (18.5-24.9)	4	20	7	29.2	6	35.3	9	42.9
Overweight (25-29.9)	4	20	10	41.7	7	41.2	7	33.3
Obese Class 1 (30-24.9)	4	20	4	16.7	2	11.8	2	9.5
Obese Class 2 (35-39.9)	3	15	1	4.2	1	5.9	1	4.8
Obese Class 3 (≥40)	5	25	2	8.3	0	0	0	0
Not available	0	0	0	0	1	5.9	1	4.8
	HS (n=18)		**Psoriasis (n=23)**		**AD (n=15)**		**Healthy Controls (n=20)**	
Waist Circumference	**Mean ± SD**	**Range**	**Mean ± SD**	**Range**	**Mean ± SD**	**Range**	**Mean ± SD**	**Range**
(cm)	106.4 ± 18.3	75-138	99.6 ± 14.2	77-129.5	94.6 ± 13.8	71-120	85 ± 11.8	68-116

Table 4. BMI and waist circumference all groups

HS: Hurley Stage and Clinical Findings (Table 5)

60% (n=12) had Hurley Stage 2 HS and 40% had Hurley Stage 3 HS. The mean number of abscesses at baseline was 2.3 (range 0-15), mean number of nodules 5 (1-28) and draining fistulas 1.1 (0-8). The mean AN count was 7.3 (3-34). 5% (n=1) were classified as mild according to IHS4, 40% (n=8) moderate and 50% (n=10) severe.

35% (n=7) reported involvement of the intermammary region, 75% (n=15) involvement of the axillae, 55% (n=11) involvement of the buttocks, 95% (n=19) involvement of the groin, 30% (n=6) involvement of the perianal region and 100% (n=20) involvement of the perineal region. The mean DLQI at baseline was 16.8 (range 3-30) and mean pain VAS was 6.6cm (0-9).

HS: Response to Treatment with Adalimumab (Table 5)

The response to treatment with Adalimumab was measured at 12 weeks. 19 of the 20 patients who commenced treatment finished the trial. Ten patients achieved HiSCR at 12 weeks (52.6%).

21.1% of patients (n=4) improved 2 IHS4 categories (from severe to mild), 36.8% (n=7) improved 1 category, 36.8% (n=7) did not change category and 5.3% (n=1) deteriorated by 1 category.

The mean improvement in DLQI and pain VAS at week 12 was 5.5 (range -9 – 24) and 1.5 (-7 – 9) respectively.

	Baseline (n=20)		Week 4 (n=20)		Week 12 (n=19)	
HiSCR	**Count**	**%**	**Count**	**%**	**Count**	**%**
≥50% reduction in AN	-	-	13	65	12	63.2
No increase in abscesses	-	-	20	100	17	89.5
No increase in fistula	-	-	16	80	14	73.7
HiSCR achieved	-	-	11	55	10	52.6
IHS4	**Mean ± SD**	**Range**	**Mean ± SD**	**Range**	**Mean ± SD**	**Range**
	14.2 ± 15.4	3-69	6.1 ± 5.5	0-18	6.1 ± 7.3	0-25
	Count	**%**	**Count**	**%**	**Count**	**%**
Mild	1	5	8	40	7	35
Moderate	8	40	7	35	10	50
Severe	11	55	5	25	2	10
DLQI	**Mean ± SD**	**Range**	**Mean ± SD**	**Range**	**Mean ± SD**	**Range**
	16.8 ± 8	3-30	10.5 ± 8.2	2-29	10.8 ± 9.1	1-29
Pain VAS (cm)	**Mean ± SD**	**Range**	**Mean ± SD**	**Range**	**Mean ± SD**	**Range**
	6.6 ± 2.5	0-9	4.4 ± 3	0-10	5 ± 3.6	0-10

Table 5. HS Response to Treatment with Adalimumab.

Psoriasis

Patients in the Psoriasis group were assessed at 0, 4 and 12 weeks. The mean PASI at baseline was 11.1 and mean DLQI was 10.3 (Table 6).

	Baseline (n=23)		Week 4 (n=19)		Week 12 (n=17)	
Clinical Examination	**Mean ± SD**	**Range**	**Mean ± SD**	**Range**	**Mean ± SD**	**Range**
PASI	11.1 ± 4.5	7-25.3	9.0 ± 6.0	1.2-26.9	4.8 ± 3.2	0-12.8
DLQI	10.3 ± 6.3	1-22	9.2 ± 6.3	0-23	6.1 ± 8.1	0-26
Change in PASI	**Count**	**%**	**Count**	**%**	**Count**	**%**
PASI 50	-	-	3	15.8	10	58.8
PASI 75	-	-	0	0	1	5.9
PASI 90	-	-	0	0	1	5.9
PASI 100	-	-	0	0	1	5.9

Table 6. Clinical Findings Psoriasis Group

Atopic Dermatitis

Patients in the AD group were assessed at 0, 4 and 12 weeks. The mean EASI at baseline was 14.5 and mean DLQI was 7.4. The mean BSA involved at baseline was 19.8% (Table 7).

	Baseline (n=17)		Week 4 (n=9		Week 12 (n=9)	
Clinical Examination	**Mean ± SD**	**Range**	**Mean ± SD**	**Range**	**Mean ± SD**	**Range**
EASI	14.5 ± 8.1	5.8-36.1	7.7 ± 4.4	1.4-14	11.1 ± 13.0	1.7-44.4
BSA involved (%)	19.8 ± 14.2	4-70	12.6 ± 5.1	5-22	16.1 ± 17.0	2-58
DLQI	7.4 ± 3.7	3-19	7.7 ± 3.3	4-13	4.6 ± 3.0	0-12
Change in EASI	**Count**	**%**	**Count**	**%**	**Count**	**%**
EASI 50	-	-	2	22.2%	6	66.7%
EASI 75	-	-	1	11.1%	0	0.0%
EASI 90	-	-	0	0.0%	0	0.0%
EASI 100	-	-	0	0.0%	0	0.0%

Table 7. Clinical Findings AD Group

Adverse Events (DERMMARK trial)

Adverse events were documented in 18 out of 20 HS patients. These were mostly considered mild (1 was recorded as moderate in severity) and the majority were self-limiting and unrelated to treatment with adalimumab. There were 7 treatment-related adverse events occurring in 6 subjects. Adalimumab was not discontinued in any patient due to adverse events but was interrupted in one instance (subject with stomach bloating/GI pain).

Three serious adverse events (SAEs) occurred during the trial, in 3 subjects (15% of enrolled subjects). These were described as: infected abscess and hospitalization, throad infection/tonsillitis, RIF + right-sided chest pain. A complete listing of adverse events and associated attributes is included in Appendix 7.

The most frequently reported adverse event was hypertension, which was reported in 10 patients during the study. Two patients had a urinary tract infection and one had tonsillitis. One patient reported a sore throat and another a high temperature, but no diagnosis of a specific

infection was made. One developed a flare of their HS requiring oral antibiotics (flucloxacillin for 1 week) and one developed an infected abscess requiring hospitalisation.

Three patients developed wound infections at their biopsy sites and one had a wound dehiscence.

One patient experienced injection site redness on one occasion and an injection site infection on a separate occasion.

Other adverse events reported included backpain (n=4); gastrointestinal: vomiting (n=2), constipation (n=2) and nausea / "stomach upset" (n=2); headache (n=2); fatigue (n=2); itch (n=2), mouth ulcers (n=1), other pain / discomfort: joint pains (n=1), tingling in legs (n=1); low mood (n=1); new diagnosis of hypothyroid (n=1); laboratory abnormalities: elevated white cell count (n=1), elevated γGT (n=1).

Adverse Events (OMICS study)

Seventeen out of 24 in the Psoriasis group had at least one adverse event reported, most commonly hypertension (n=15). One patient had an infected biopsy site and one reported swelling at the biopsy site. Others included chest infection (n=1), flu / upper respiratory tract infection (n=1), low vitamin D level (n=1).

Eleven out of 17 in the AD group had at least one adverse event document. Hypertension in six, infected biopsy site in three, a chest infection and anaemia, each in one patient.

Hypertension was also reported in 4 of the HC and wound dehiscence in 2.

3.2 RNA sequencing

Samples

A total of three batches of samples were sent to AbbVie (n=228). Numbers of samples for each cohort are shown in table 8.

Cohort	LS W0	NLS W0	LS W12	NLS W12	Full set (W0 and W12)	Total
HS	16	15	14	14	14	73
Pso	21	21	14	14	14	84
AD	16	16	7	6	6	51
HV	20	-	-	-	-	20

Batch Effect

After normalizing the data with the DESeq2 package, we performed a PCA plot on the VST-transformed values to assess whether there were differences between the three batches (Figure 1). The results show that there is no clear grouping between the three batches, i.e. no batch effects.

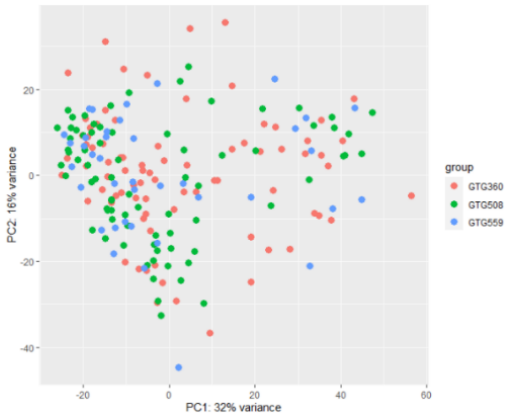

Figure 1. PCA Plot for inspection of batch effect. It shows a PCA plot form the VST-transformed data. The results do not show grouping between batches. The PCA plot was done in the DESeq2 package which only uses the top 500 genes with the highest variance.

Unsupervised Analysis

Similarly, we used a PCA plot to test whether the tissue of origin (lesional or non-lesional) and the disease of the participants could explain differences in gene expression (Figure 2). The results show that it in linear PCA, most of the variance does not come from the tissue of origin or disease. However, PCA uses a linear transformation of the data that might fail to capture other forms of relationships between the data. For this reason, we used the UMAP approach. In UMAP, the distance between samples is used to reconstruct a lower dimensional representation of the data that respects the relationship that samples might have between each other. The results are shown in Figure 3. The UMAP plot is quite different form the PCA plot in this case. There are two clear groupings in the data. One contains mostly lesional samples from patients with Psoriasis, while the other contains all the other samples. Interestingly, the

organization within the second, larger cluster seems to divide samples with hidradenitis suppurativa (HS) (especially lesional samples) from the rest.

Figure 2. PCA plot from the VST-transformed data with disease labels. The labels are organized as disease:site. LS stands for lesional site and NL for non-lesional. AD stands for atopic dermatitis, HV for healthy volunteers, Pso for Psoriasis and HS for hidradenitis suppurativa. The results do not show clear clusters between samples.

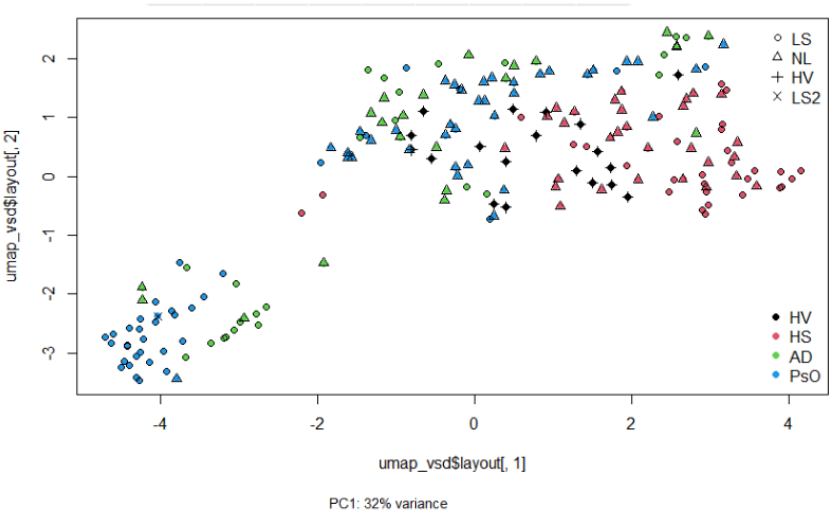

Figure 3. UMAP plot form the VST-transformed data with disease labels. The colour indicates the disease while the shape indicates the site. In this case, the results show two clusters. On the bottom left corner, are samples coming mostly from Psoriasis lesions, while the rest of samples lie on the top right corner. Interestingly, the HS samples are located together. HV, healthy volunteer; LS, lesional; NLS, non-lesional.

Differential Expression Analysis and Enrichment

Given the results from the unsupervised analysis we examined the differences between lesional and non-lesional HS. The results show close to 8,000 differentially expressed genes. Table 8 shows the top 10 differentially expressed genes.

Gene Entrez	Symbol	log2FoldChange LS vs NLS	P-value	P-Adjusted	Comments
9487	PIGL	0.505763	7.53E-23	1.72E-18	Homozygous PIGL mutation causes nonsyndromic erythrodermic ichthyosis PMID: 31535386
2515	ADAM2	-29.2452	1.42E-22	1.72E-18	Metallo-protease expressed in spermatids; plays a role in fertility, muscle development and neurogenesis
6318	SERPINB4	-4.02838	1.11E-21	8.96E-18	Protease inhibitor; inhibits granzyme. Found overexpressed in HS lesions (gene arrays) https://doi.org/10.1111/jdv.16147
339883	C3orf35	0.75224	4.81E-21	2.91E-17	Associated with Muir–Torre syndrome, a rare hereditary, subtype of HNPCC. Patients develop cancers of the colon, genitourinary tract, and skin,

					such as keratoacanthomas and sebaceous tumours.
22806	IKZF3	-0.97122	1.92E-20	9.31E-17	IKAROS; Transcription factor that regulates B-cell differentiation, proliferation and maturation to an effector state.
338324	S100A7A	-4.09201	7.61E-20	3.07E-16	Associated with Psoriasis & found overexpressed in HS lesions: https://doi.org/10.1371/journal.pone.0216249, https://doi.org/10.1111/jdv.16147
6280	S100A9	-3.40468	1.09E-18	3.76E-15	A calcium- and zinc-binding protein which stimulates inflammatory processes and the immune response. Overexpressed in HS lesions. Refs. as above
55764	IFT122	0.285358	2.77E-18	8.37E-15	component of the IFT complex A (IFT-A), a complex required for retrograde ciliary transport and entry into cilia of G protein-coupled receptors (GPCRs)
92797	HELB	-0.65037	4.54E-18	1.10E-14	DNA helicase functioning in S-phase. Replication forks are altered in HS hair follicle stem cells doi: 10.1172/JCI131180
147727	ILF3-DT	0.354374	4.38E-18	1.10E-14	lncRNA associated with ILF3-DT include Cervical Squamous Cell Carcinoma and Melanoma.

Table 8. Genes differentially expressed in HS between lesional and non-lesional sites.

To assess what biological information was contained in this large number of genes, we performed a gene ontology and KEGG pathway enrichment (tables 9 and 10). Interestingly, this analysis revealed very significant changes in immune cell functions.

Gene Ontology Term	**Gene count**	**%**	**P-Value**	**Benjamini-Hochberg Adjustment**
immune response	157	3.7	8.30E-21	5.00E-17
regulation of immune response	83	2	6.10E-18	1.80E-14
complement activation	51	1.2	2.10E-16	4.50E-13
complement activation, classical pathway	55	1.3	4.20E-16	6.70E-13
Fc-gamma receptor signalling pathway involved in phagocytosis	58	1.4	3.10E-12	3.80E-09
Fc-epsilon receptor signalling pathway	67	1.6	1.60E-09	1.60E-06
receptor-mediated endocytosis	66	1.6	3.20E-08	2.70E-05
T cell differentiation	19	0.5	4.00E-07	3.00E-04
T cell activation	24	0.6	1.50E-06	9.70E-04

transcription, DNA-templated	432	10.3	1.90E-06	1.10E-03

Table 9. Gene Ontology Enrichment from Genes differentially expressed in HS lesions

KEGG Pathway	**Gene Count**	**%**	**P-Value**	**Benjamini-Hochberg Adjustment**
NF-kappa B signaling pathway	36	0.9	2.30E-07	6.80E-05
Primary immunodeficiency	20	0.5	3.20E-07	4.60E-05
Cytokine-cytokine receptor interaction	72	1.7	1.30E-06	1.20E-04
T cell receptor signaling pathway	33	0.8	1.80E-04	1.30E-02

Table 10. KEGG Pathway Enrichment from Genes differentially expressed in HS lesions

Next, we investigated if there were differentially expressed genes in the HS cohort between week 0 and 12 (lesional skin). To isolate the patient specific and tissue of origin specific effect we performed two sets of paired t-tests on every gene. The multiple comparison adjusted p-value had no significant results.

Treatment Response Prediction

To see if we could predict treatment response for HS patients, we performed first a differential expression analysis between non-lesional and lesional sites of HS patients. The list of differentially expressed genes was selected as an intrinsic gene list, analogous to the intrinsic gene list for the breast cancer molecular subtypes (Parker et al., 2009). From this list, we used a logistic regression to see if the expression levels of this genes could predict treatment output using lesional samples from the first visit (the VST-transformed levels were standardised). The top three genes with the highest logistic model fit according to the Akaike information criterion were DCLK3, IRF1 and ADCY2 (Figure 4).

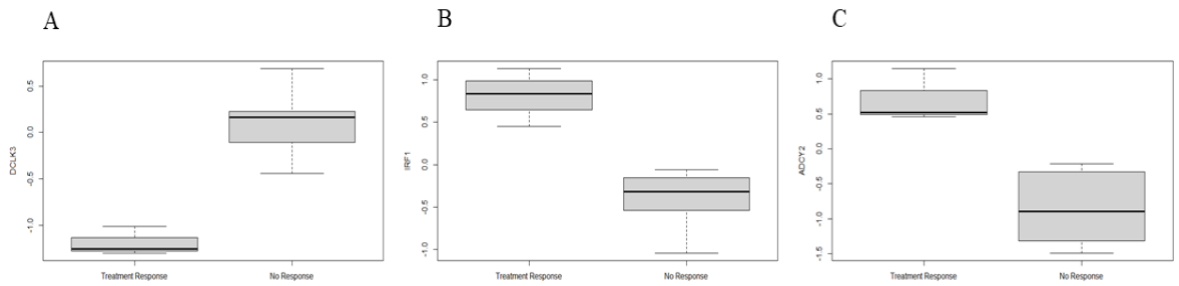

Figure 4. Boxplots Treatment Response vs. No Treatment Response. Shows a boxplot of the expression of DCLK3 (A), IRF1 (B) and ADCY2 (C) in week 1, for treatment responders and non-responders.

DCLK3 (Doublecortin Like Kinase 3) is a Protein Coding gene. Diseases associated with DCLK3 include Congenital Lactase Deficiency and Congenital, Autosomal Recessive 11, Ichthyosis.

IRF1 (Interferon Regulatory Factor 1) is transcriptional regulator and tumour suppressor, serving as an activator of genes involved in both innate and acquired immune responses. The type I IFN pathway is upregulated in HS skin (Byrd et al., 2019). Hair follicle cells from HS patients have an increased number of proliferating progenitor cells and a reduction of quiescent stem cells. Alteration of replication forks in HS leads to activation of the ATR/CHK1 DNA damage pathway and an increased number of micronuclei and with the presence of cytoplasmic ssDNA. This activates the IFI16/STING pathway and the production of type I IFNs.

ADCY2 (Adenylate-Cyclase 2) catalyses the formation of the signalling molecule cAMP in response to G-protein signalling. Down-stream signalling cascades mediate changes in gene expression patterns and lead to increased IL6 production. ADCY2 expression is downregulated in HS (Teng et al., 2020). IL-6 is pro-inflammatory and upregulated in the serum of HS patients (Xu et al., 2017). HS lesions overexpress IL-1β, and IL-1β treatment of keratinocytes induces a characteristic transcriptomics signature including IL-6 (Witte-Händel et al., 2019).

To corroborate the results, we used a t-test between responders and non-responders at week 1. The top 10 results are shown in table 11.

Symbol	coeff	deviance	AIC	Paired-t-test P-value	Benjamini-Hochberg Adjustment
LOC101929777	380.8774647	2.24E-10	4	0.001325493	0.9322067
AICDA	-599.3875966	2.30E-10	4	0.013194102	0.9322067
DNMBP-AS1	727.6558786	2.42E-10	4	0.004231517	0.9322067
ADCY2	462.9273223	2.66E-10	4	0.007336274	0.9322067
IRF1	491.0975907	2.69E-10	4	0.006759302	0.9322067
GNGT2	897.3949548	2.90E-10	4	0.00235485	0.9322067
CYTL1	483.9454153	2.90E-10	4	0.016132385	0.9322067
DCLK3	-631.4137234	2.92E-10	4	0.011551878	0.9322067
LOC105379282	445.2167933	2.97E-10	4	0.115505682	0.9322067
KIAA0556	-842.6788993	3.10E-10	4	0.042127839	0.9322067
SNX16	611.7406215	3.12E-10	4	0.015697406	0.9322067
PDLIM4	-335.8831952	3.15E-10	4	0.041377627	0.9322067
PSMB9	470.4131633	3.19E-10	4	0.026170662	0.9322067

ODAPH	-530.1618564	3.26E-10	4	0.004703808	0.9322067
OPN4	-515.0986712	3.33E-10	4	0.005619301	0.9322067
MAP3K5	624.9930041	3.45E-10	4	0.000153391	0.9322067
CTXN1	-463.1916895	3.47E-10	4	0.021265357	0.9322067
AKR1B10	-223.0649584	3.54E-10	4	0.026330691	0.9322067
MYBPHL	-815.5314548	3.74E-10	4	0.040515594	0.9322067
FZD10-AS1	349.0802446	3.76E-10	4	0.005582139	0.9322067

Table 11. Genes that Predict Treatment Response

We used STRING to assess if the top 20 predictors sorted by deviance contained genes which interact with TNF. The results showed that PSMB9, IRF1 and MAP3K5 are direct interactors of TNF (Figure 5).

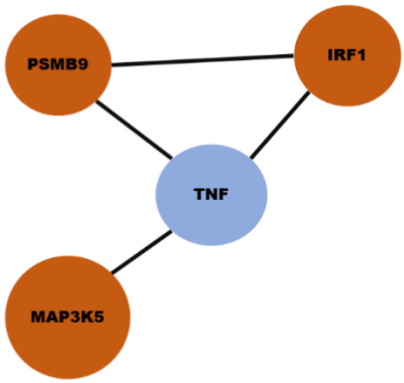

Figure 5. Several genes that can predict patient responses directly interact with TNF.

Change in Treatment Prediction Biomarkers After Treatment

We used a paired-t-test to check if these genes changed their expression after treatment. Interestingly only MAP3K5 expression changed after treatment in patients who responded (table 12). For non-responders the expression levels of these genes remained the same.

Gene Symbol	Entrez Id	statistic	p-value
IRF1	3659	0.136525	0.903909
PSMB9	5698	-0.11027	0.922266
MAP3K5	4217	4.704229	0.042339

Table 12. Change in TNF interactor expression in treatment responders

PSMB9 (Proteasome 20S Subunit Beta 9) is a part of the core proteasome. Diseases associated with PSMB9 include Proteasome-Associated Autoinflammatory Syndrome 3. This is an autosomal recessive syndrome with onset in early infancy. Affected individuals present with nodular dermatitis, recurrent fever, myositis, panniculitis-induced lipodystrophy, lymphadenopathy, and dysregulation of the immune response, particularly associated with abnormal type I interferon-induced gene expression patterns (Kniffin, 2018).

MAP3K5 (ASK1, MEKK5) is a serine/threonine kinase which is required for the innate immune response. It mediates signal transduction of various stressors like oxidative stress as well as by receptor-mediated inflammatory signals, such as the tumour necrosis factor (TNF) or lipopolysaccharide (LPS). Once activated, acts as an upstream activator of the MKK/JNK signal transduction cascade and the p38 MAPK signal transduction cascade through the phosphorylation and activation of several MAP kinase kinases like MAP2K4/SEK1, MAP2K3/MKK3, MAP2K6/MKK6 and MAP2K7/MKK7. These MAP2Ks in turn activate p38 MAPKs and c-jun N-terminal kinases (JNKs). Both p38 MAPK and JNKs control the transcription factors activator protein-1 (AP-1).

ASK1/2 signalling promotes inflammation in a mouse model of neutrophilic dermatosis. Mechanistically, ASK regulates Ptpn6spin (SHP1 mutant)-mediated disease by controlling proinflammatory signaling in the neutrophils (Tartey et al., 2018).

3.3 Mass spectrometry

Samples

Digested proteins from 72 SMD samples were analysed at the Functional Genomics Centre Zurich (FGCZ), Switzerland, which is part of the European Proteomics Infrastructure (EPIC-XS), a EU funded research infrastructure for shared use by EU scientists (https://epic-xs.eu/). Samples for each cohort are shown in Table 14.

Cohorts	Week 0	Week 12	Total
HS	10	10	20
Pso	11	8	19
AD	9	4	13
HV	20	-	20

DDA analysis on healthy volunteers

294 proteins were identified by mass-spectrometry in at least one SMD sample. Similar LFQ (Label Free Quantification) intensity profiles were observed between donors (Figure 6a).

Because of interindividual heterogeneity, a majority vote analysis was applied, where proteins identified in at least 60% of samples are counted as detected. This analysis approach is especially suited for samples with high variability and missing data due to low protein abundance (Chen et al., 2012). 68 proteins were identified in at least 4 of the 6 donors (67%). Cell component (CC) and biological process (BP) gene ontology (GO) analysis were used to identify the functions of these proteins using the Database for Annotation, Visualization and Integrated Discovery (DAVID). The top 5 CC-GO terms were extracellular exosome, extracellular space, blood microparticle, extracellular region and matrix (Figure 6b), and the top 5 BP-GO terms were epidermis development, platelet degranulation, innate immune response, intermediate filament organization and fibrinolysis (Figure 6c). To refine the analysis by including network context, we used the search tool for the retrieval of interacting genes/proteins (STRING) to generate a protein-protein interaction (PPI) network. A total of 58 nodes and 205 edges were enriched in PPI network with an average node degree (number of connections) of 7.07 and a PPI enrichment p-value < 1.0e-16. This indicates that the identified proteins present a biologically highly interconnected group (Figure 6d). Proteins involved in epidermis development, platelet degranulation and innate immune response are represented in green, blue and red respectively.

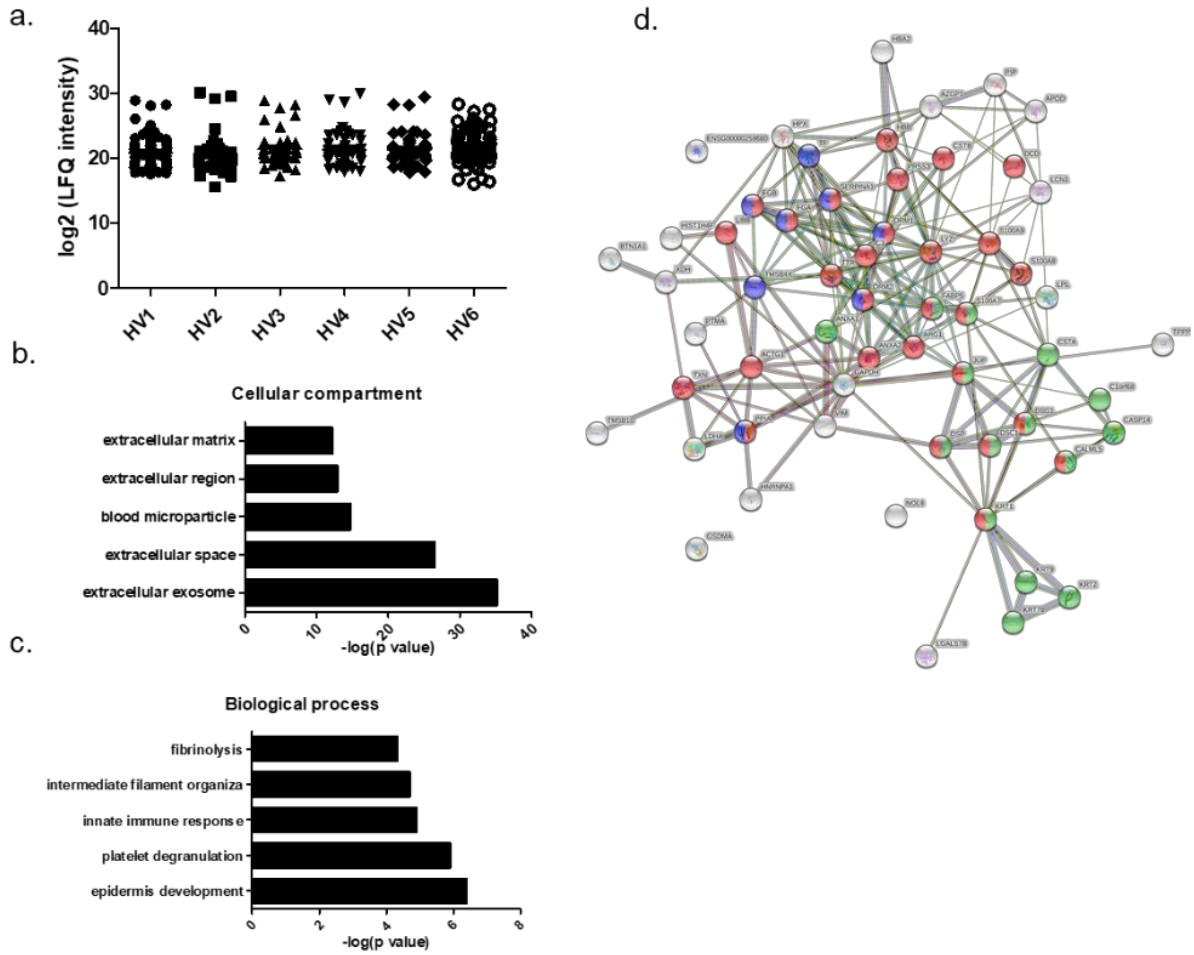

Figure 6. Mass-spectrometry based proteomics and Multiplex ELISA array on SMD. (a) Log2(LFQ intensity) of proteins identified in 6 healthy volunteers (HV). (b,c) Histograms representing the top 5 results of the GO analysis for cellular compartment and biological processes. Analysis was done on the 68 proteins identified after majority vote analysis. (d) STRING protein-protein interaction network of the 68 proteins. Proteins involved in epidermis development, platelet degranulation and innate immune response are represented in green, blue and red, respectively.

Data Independent Acquisition (DIA) analysis

DIA is a mass spectrometry technique that allows a better coverage and hence can reduce the number of missing values, which was an issue with the SMD samples. DIA requires a special instrument setup that is available through the EPIC-XS infrastructure, which we used for this purpose.

The following analysis carried out with DIA aimed to assess if there are reproducibly measured differences on protein level between Visit 1 (Week 0) and Visit 1 (Week 12) (SamplingTime) in the HS group of patients. Figure 7 shows the heatmap of protein intensities clustered by sample and row. The heatmap shows only proteins with a small number of missing observations

were selected. When looking at the grouping of the samples (hierarchical clustering), we do not see a clear correlation between the cluster structure either with individual or SamplingTime.

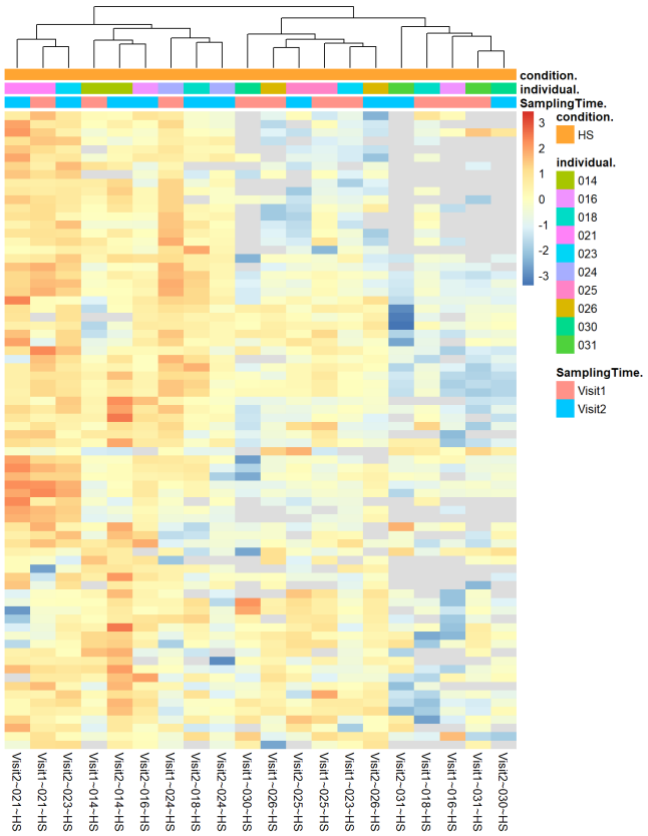

Figure 7. Heatmap of protein intensities

The same result was obtained when looking at the presence (white) or absence (black) of protein measurements (Figure 8.)

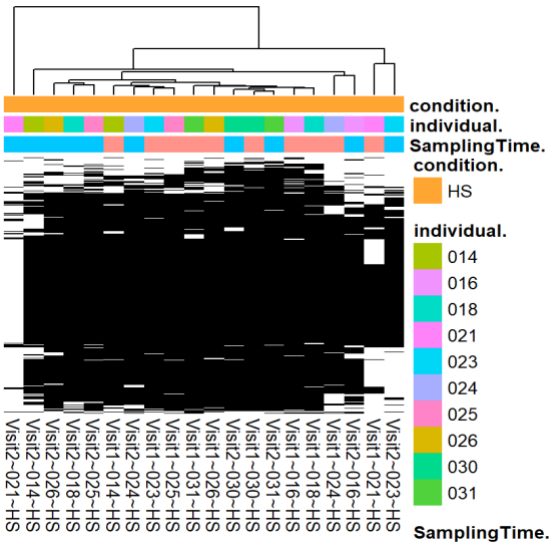

Figure 8. Heatmap of protein presence

In total, 313 proteins were differentially expressed between Week 0 and Week 12. Table 15 shows the top 10 differentially expressed proteins in SMDs.

Protein ID	Protein name	p.value	Comments
P09429	High mobility group protein B1	0.016591	Multifunctional redox sensitive protein. is involved in the coordination and integration of innate and adaptive immune responses.
P49908	Selenoprotein P	0.020248	Might be responsible for some of the extracellular antioxidant defence properties
P09651	Heterogeneous nuclear ribonucleoprotein A1	0.026458	Involved in the packaging of pre-mRNA into hnRNP particles
P06576	ATP synthase subunit beta, mitochondrial	0.028774	produces ATP from ADP
P11171	Protein 4.1	0.031988	plays a key role in regulating membrane physical properties
P42357	Histidase	0.03503	involved in step 1 of the subpathway that synthesizes N-formimidoyl-L-glutamate from L-histidine.
P68104	EF-1-alpha-1	0.043839	involved importantly in Th1 cytokine production.
O60240	Perilipin-1	0.047258	Modulator of adipocyte lipid metabolism
P08123	Collagen alpha-2(I) chain	0.051804	extracellular matrix constituent

Q6ZVX7	F-box only protein 50	0.053369	Promotes cell proliferation

Table 15. Proteins differentially expressed in HS between Week 0 and Week 12.

We then performed a gene set enrichment analysis (GSEA). We used the t-statistic to rank the proteins. To run the GSEA analysis, we use Webgestalt.org and the Gene Ontology (GO) gene signature database, with the subsets Biological Process (Figure 9) and Molecular Function (Figure 10). The only significantly enriched gene set, upregulated at Week 12, is phospholipid binding in the GO Molecular function signature database. Several gene sets linked to immune function were enriched although not at a statistically significant level (Figure 9)

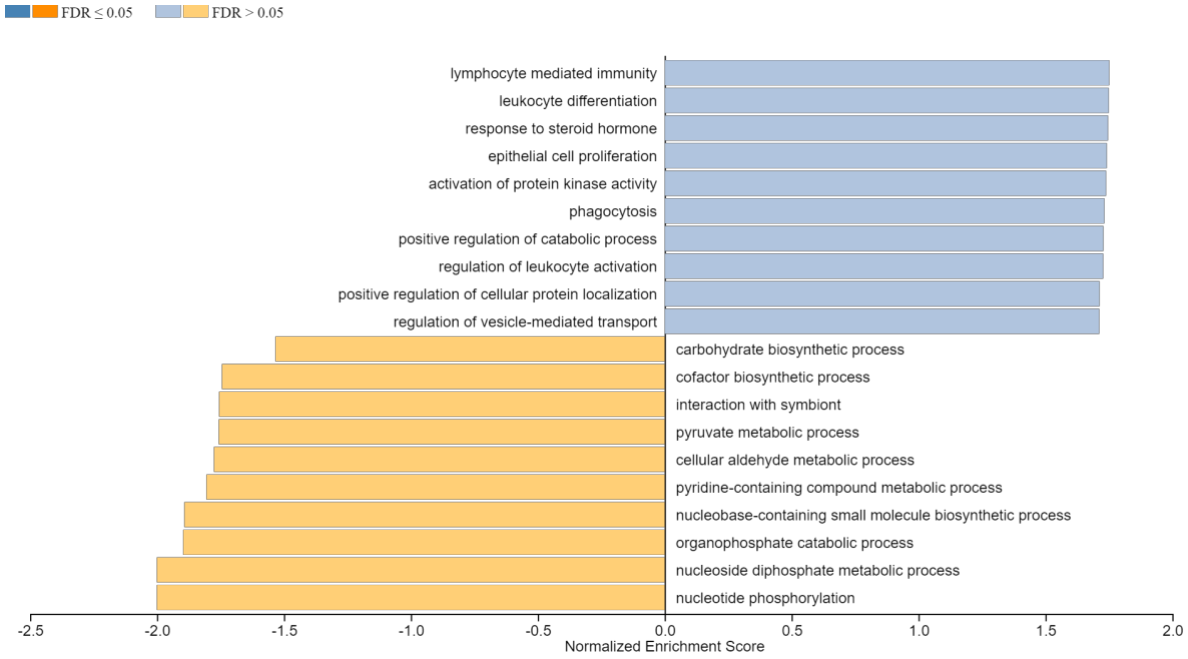

Figure 9: GO analysis for Biological process

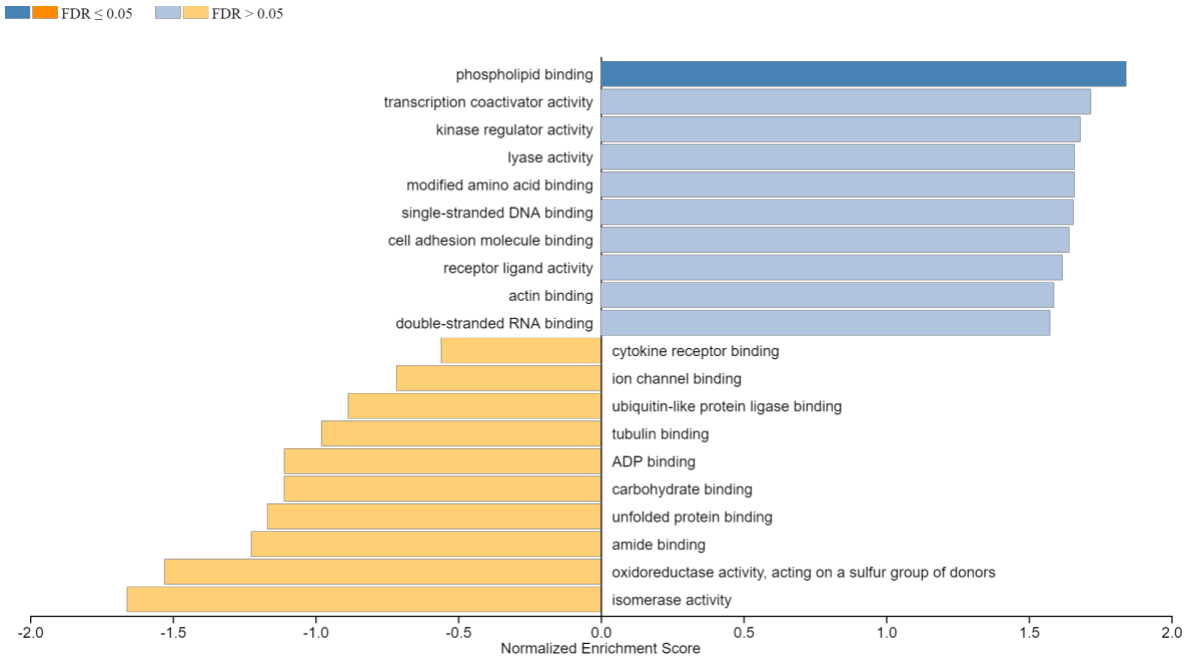

Figure 10: GO analysis for Molecular Function

Interestingly, when comparing samples from the HS cohort at W0 and W12, a cluster is potentially enriched in patients that are non obese and non responders to treatment (Figure 11).

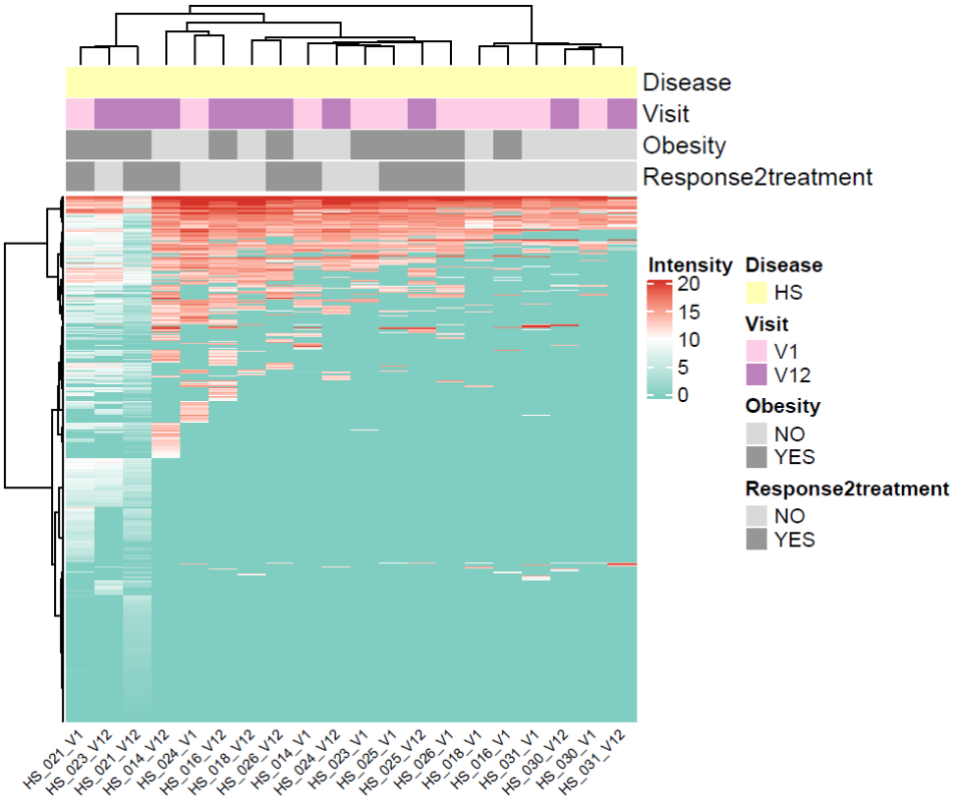

Figure 11. Clustering HS cohort (before/after treatment)

3.4 Multiplex array

As cytokines are of very low abundance in HV, and thus typically escape detection by mass-spectrometry, we also performed a multiplex immunoassay on 5 of the 6 SMD samples. 11 out of the 12 cytokines tested (IFNγ, IL-1β, -4, -5, -6, -8, -17A, -17F, -22, TNFα) were above the sensitivity threshold and quantifiable showing consistent expression across HV (Figure 12). No statistical difference was observed between cohort at week 0 except for IL-4, which was increased in the AD cohort, and IL-17F, which was decreased in the HS cohort compared to the controls. Similarly, we observed a statistically significant increase of IL-2 after treatment with adalimumab in the HS cohort.

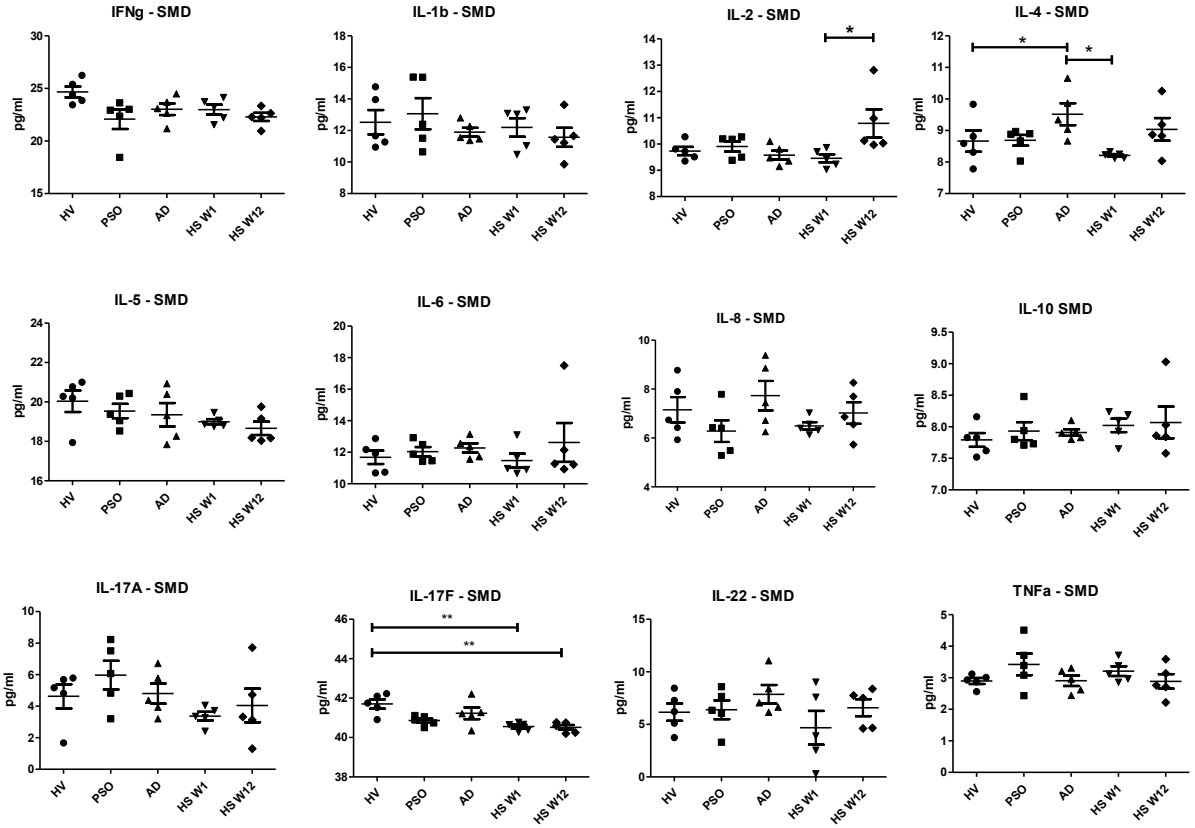

Figure 12. Multiplex immunoassay on SMD samples. Concentration of cytokines identified in SMD of HV by multiplex array (n=5)

4. Appendix

Appendix 1: Inclusion and Exclusion Criteria

DERMMARK

Inclusion criteria

Subjects meeting all of the criteria below may be included in the study:

1. Patients must be over 18 years of age.
2. Diagnosis of Hidradenitis suppurativa (HS) for at least 1 year prior to Baseline.
3. Diagnosis of HS Hurley stage II or III made by a consultant dermatologist.
4. Capable of giving informed consent.
5. Naïve to previous anti-TNF treatment
6. Patients must have an AN (total count of abcesses and inflammatory nodules) count of 3 or greater and a draining fistula count ≤ 20
7. Female subjects of child bearing potential and male subjects whose partner is of child bearing potential must be willing to ensure that they or their partner use effective contraception during the study. Women of child bearing potential should use effective birth control methods throughout the trial and for at least 5 months afterwards.
8. Subject must have stable HS for at least 7 days prior to screening visit and at Baseline visit.
9. Patients should have an adequate indication for receiving Humira treatment for hidradenitis suppurativa as per current SmPC.
10. Clinically acceptable laboratory findings imply that blood results are within the expected normal reference range for the laboratories in both St Vincent's Hospital and the Mater Hospital.
11. ECGs should show no abnormalities /no new changes compared with previous ECGs
12. CXR findings should be non- significant/ stable compared with previous CXRs.

Exclusion criteria

Any candidates meeting any of the following exclusion criteria at screening/ baseline will be excluded from study participation:

1. Allergy/sensitivity to study medications or their ingredients
2. Previously treated with Humira or another anti-TNF therapy
3. Pregnancy/breast feeding
4. <18 years of age
5. Major Psychiatric illness
6. Contraindication to anti-TNF treatment
7. History of moderate to severe congestive heart failure (New York Health Association [NYHA] class III or IV), recent cerebrovascular accident and any other condition which, in the opinion of the investigator would put the subject at risk by participation in the protocol;
8. History of demyelinating disease (including myelitis) or neurologic symptoms suggestive of demyelinating disease;
9. History of invasive infection (e.g., listeriosis, histoplasmosis), human immunodeficiency virus (HIV);
10. Subject has an active systemic viral infection or any active viral infection that based on the investigator's clinical assessment makes the subject an unsuitable candidate for the study
11. Hepatitis B: HBsAg positive (+) or detected sensitivity on the HBV-DNA PCR qualitative test for HBcAb/HBsAb positive subjects

12. Chronic recurring infections or active TB;
13. Female subjects who are pregnant or breast-feeding or considering becoming pregnant during the study.
14. Subjects who have participated in another study and received any other investigational agent within 1 month
15. Subjects unable to provide written informed consent
16. Subjects who have any other significant disease or disorder (including uncontrolled diabetes, unstable ischemic heart disease, moderate to severe congestive heart failure, recent cerebrovascular accident) which, in the opinion of the investigator, may either put the subject at risk by participation in the study, or may influence the result of the study.
17. Subjects who have a history of drug or alcohol use that, in the opinion of the investigator, would interfere with adherence to study requirements.
18. Prior or concurrent malignancy (except non-melanoma skin cancer).
19. AST or ALT ≥ 3 x ULN
20. Creatinine clearance (CrCl) < 60 mL/min measured by 24-hour urine collection or estimated by the Cockcroft and Gault formula
21. Subjects have clinically significant ECG findings as judged by the investigator
22. Scheduled for procedures requiring general anaesthesia during the study
23. Latex allergy

OMICS Study of Psoriasis and Atopic Dermatitis

Atopic dermatitis:

Inclusion Criteria

1. Patients with severe atopic dermatitis: >10% body surface area
2. Capable of giving informed consent
3. Established diagnosis of AD
4. Consent to give skin biopsy
5. Patients previously on systemic treatment must have undergone washout period (4 weeks for systemic treatment and 8 weeks for phototherapy).
6. Topical emollients and steroids/calcineurin inhibitors are allowed throughout the study.
7. Patients should stop topical steroids on the study site and for 10cm around this site.
8. Greater than 18 years of age

Exclusion Criteria

1. Aged less than 18 years of age
2. Other co morbid inflammatory skin diseases
3. Patients on systemic treatment (4 weeks washout) or phototherapy (8 weeks washout)

Psoriasis:

Inclusion Criteria

1. Patients with chronic plaque Psoriasis
2. PASI > or equal to 7
3. Capable of giving informed consent
4. Consent to give skin biopsy
5. Patients previously on systemic or biologic treatment must have undergone washout period (4 weeks for systemic treatment, 12 weeks for biologics, 8 weeks for phototherapy)
6. Greater than 18 years of age

Exclusion Criteria

1. Aged less than18 years of age
2. Other co morbid inflammatory skin diseases
3. Active Psoriatic arthritis

Healthy controls:

Inclusion Criteria

1. No history of AD, Psoriasis, HS or systemic inflammatory disease
2. Capable of giving informed consent
3. Consent to give skin biopsy
4. Healthy adults aged between 18 and 70 years of age

Exclusion Criteria

1. Aged less than18 years of age
2. History of AD, Psoriasis, HS or systemic inflammatory disease
3. Other co morbid inflammatory skin diseases
4. Patients on systemic treatment (4 weeks washout) or phototherapy (8 weeks washout)
5. Significant chronic medical illness

Appendix 2: Schedule of visits

DERMMARK

	Screening Visit	Visit 1	Telephone Check-up	Visit 2	Visit 3	Follow Up Visit
		Week 0	Week 3	Week 4	Week 12	Week 20
Informed Consent, Inclusion / Exclusion criteria, Demographics, Medical History, Concomitant Meds, BP and HR, ECG, Pregnancy test, Biologic screening tests (CXR, Quantiferon, FBC, Renal, Liver profile, HIV, Hepatitis C antibody, Hepatitis B antigen and core antibody, Varicella zoster titre)	X					
AE Assessment			X	X	X	X
Dispense Humira		X		X		
Humira Accountability and Check				X	X	
Photography		X*		X*	X*	
HS Lesion Counts (Abscesses, Inflammatory Nodules and Draining Fistulas)	X			X	X	
DLQI and Pain VAS score		X		X	X	X
Skin Biopsy, Microdialysis, Blood sampling		X**			X**	
Vital signs	X	X		X	X	X
Physical Examination	X	X		X	X	X
Clinical Laboratory Assessments	X	X		X	X	X

*Photographs taken for 5 patients only
**Microdialysis from peri-lesional and lesional skin in 5 patients only and lesional for 27 patients.

OMICS Study of Psoriasis and Atopic Dermatitis

Psoriasis and Atopic Dermatitis Groups

Procedures	**Visit 1** Screening	**Visit 2** Week 1	**Visit 3** Week 4	**Visit 4** Week 12
Core Study Procedures				
Inclusion/Exclusion Criteria	X			
Informed consent	X			
Medical/surgical history	X			
Smoking and Alcohol use history	X			X
Physical examination and weight	X	X	X	X
Height	X			
Vital signs	X	X	X	X
Demographics	X			
Photography		X	X	X
DLQI		X	X	X
Skin Biopsy		X		X
Microdialysis		X		X
Record Concomitant Treatments	X	X	X	X
Working Group Specific Assessments				
Atopic Dermatitis Group only: Body Surface Area	X	X	X	X
Atopic Dermatitis Group only: EASI	X	X	X	X
Psoriasis Group only: PASI	X	X	X	X

Healthy Control Group

Procedures	**Visit 1** Screening
Eligibility	X
Informed consent	X
Medical history	X
Smoking and Alcohol use history	X
Physical Exam & Weight	X
Height	X
Vital signs	X
Demographics	X
Skin Biopsy	X
Microdialysis	X
Record any treatments	X

Appendix 3: Ethics and Informed Consent

Ethics for the DERMMARK trial

Before initiating this study the Study Protocol, Summary of Product Characteristics (SmPC), Patient Information Leaflet and Informed Consent Form, applicable advertising, and any other written
information to be given to participants was reviewed and approved by a properly constituted Ethics Committee (EC) and Regulatory Authority, as applicable. This study was conducted in accordance with Good Clinical Practice (GCP), as defined by the International Conference on Harmonisation (ICH) and in accordance with the ethical principles underlying European Union Directive 2001/20/EC and 2005/28/EC. The sponsor and site investigator also ensured that this study was conducted in accordance with the ethical principles that have their origins in the Declaration of Helsinki.

Informed Consent in the DERMMARK trial

The patients received a patient information leaflet prior to consent being obtained where the investigator or his/her delegate informed each participant of the objectives, benefits, risks and requirements imposed by the study, as well as the nature of the study drug. The participants were provided with an information and consent form in clear, simple language. He/she was allowed ample time to enquire about details of the study and to decide whether or not to participate in the study. Any questions the patient may have regarding the study were answered prior to taking consent. Three original patient information leaflet and consent forms were completed, dated and signed personally by the participant and by the person responsible for collecting the informed consent, one copy of which was kept in the clinical chart. The participant was also given one signed original information and consent form, the final original was kept by the investigator.

Appendix 4: Study treatment

Description of study treatment for the DERMMARK trial

Adalimumab was supplied as Humira 40 mg/0.4 ml concentration in Pre-filled Pen, Pre-filled Syringe and Vial. Humira is a clear colourless solution injected subcutaneously. Participants were required to take the study drug as the per the following schedule:
1. 160mg loading dose week 0
2. 80 mg on week 2
3. 40mg weekly starting at week 4 for 8 further weeks.

The treatment period was a total of 12 weeks. Patients were provided with an alert card which detailed their use of the medication as part of the clinical trial and their GPs were also informed of their participation in the trial.

Formulation, packaging and handling

The Humira provided for participants was supplied by Marketing Authorisation Holder Abbvie Limited UK with packaging and handling by the following sites in accordance with its marketing authorisation and was not modified by the sponsor:

Manufacturer :
Vetter Pharma-Fertigung GmbH & Co. KG,
Schützenstraße 87,
88212 Ravensburg,
Germany.

Site of clinical trial packaging and labelling :
AbbVie Deutschland GmbH & Co. KG,
67061 Ludwigshafen,
Germany.

QP release of clinical supplies :
AbbVie Deutschland GmbH & Co. KG,
67061 Ludwigshafen,
Germany

The study drug contained labelling in line with Annex 13 – Good Manufacturing Practice requirements for Investigational Medicinal Product as part of a clinical trial, in addition to the existing labelling on the Humira.

Appendix 5: Protocol Deviations

There were no deviations of significance from the DERMMARK study protocol during conduct or analysis of the study.

Appendix 6: Safety Monitoring Processes

Described here are the safety monitoring processes followed during the DERMMARK trial.

All AEs occurring during the study observed by the investigator or reported by the subject, whether or not attributed to the study medication, were recorded on the CRF. The following information was recorded: description, date of onset and end date, severity, assessment of relatedness to the study medication and action taken. Follow-up information was provided as necessary.

AEs considered related to the study medication as judged by an investigator or the sponsor were followed until resolution or until the event was considered stable. All related AEs that resulted in a subject's withdrawal from the study or were present at the end of the study, were followed up until a satisfactory resolution occurred.

It was left to the investigator's clinical judgment whether or not an AE is of sufficient severity to require the subject's removal from treatment. A subject could also voluntarily withdraw from treatment due to what he or she perceives as an intolerable AE. If either of those occurred, the subject underwent an end-of-study assessment and was given appropriate care under medical supervision until symptoms ceased or the condition became stable.

Appendix 7: Adverse event listings for the DERMMARK trial

The table below summarize all adverse events data collected in the database of the DERMMARK trial.

	Frequency (%)
Number of subjects enrolled	20
Number of adverse events	68
Subjects with AEs**	18 (90.0%)
Serious adverse events (SAEs)*	3 (4.4%)
Number of Subjects with SAEs **	3 (15.0%)
Number of AEs by Severity*	0 (0.0%)
Mild	66 (97.0%)
Moderate	1 (1.5%)
Severe	0 (0.0%)
N-Miss	1 (1.5%)
Number of treatment related AEs*	7 (11.1%)[1]
Subjects with treatment related AEs**	6 (30.0%)
Action taken*	
None	63 (92.6%)
Drug withdrawn/stopped	0 (0.0%)
Drug interrupted	1 (1.5%)
Not Applicable	3 (4.4%)
N-Miss	1 (1.5%)
Number of AEs by Outcome*	
Ongoing	36 (52.9%)
Resolved without sequalae	23 (33.8%)
Resolved with sequalae	6 (8.8%)
Death/Fatal	0(0.0%)
N-Miss	3(4.4%)

N-Miss denotes number of missing values on described variable
*** Percentages are based on number of AEs reported**
**** Percentages are based on number of subjects enrolled**
[1]Denominator excludes 5 events with missing information on relatedness

Below is a complete listing of all adverse events occurring during the DERMMARK trial and associated attributes.

Listing of Serious Adverse Events occurring during the DERMMARK trial							
Record ID	**Event Name**	**Start Date**	**Stop Date**	**Severity**	**Study Drug Action Taken**	**Outcome**	**Relationship to Study Drug**
SVH005	Infected abscess and hospitalization	15/04/2018	16/04/2018	Mild	None	Resolved without Sequelae	Not Related
SVH007	THROAT INFECTION/ TONSILLITIS	18/06/2018	17/07/2018	Moderate	None	Resolved without Sequelae	Related
SVH013	RIF + RIGHT SIDED CHEST PAIN	23/05/2019	24/05/2019	Mild	None	Resolved without Sequelae	Not Related
Listing of Non-serious Adverse Events occurring during the DERMMARK trial							
Record ID	**Event Name**	**Start Date**	**Stop Date**	**Severity**	**Study Drug Action Taken**	**Outcome**	**Relationship to Study Drug**
SVH003	High Blood pressure 149/96	29/01/2018	23/02/2018	Mild	None	Resolved without Sequelae	Not Related
SVH003	Vomiting and Headache	19/03/2018	23/03/2018	Mild	None	Resolved without Sequelae	Not Related
SVH003	headache x 1 day	16/03/2018	17/03/2018	Mild	None	Resolved without Sequelae	Not Related
SVH003	Pressure in chest	16/03/2018	23/03/2018	Mild	None	Resolved without Sequelae	Not Related
SVH003	WCC Elevated 13.0, Neurophils 8.9	18/05/2018	05/12/2018	Mild	None	Ongoing	Not Related
SVH004	Hypertension 150/85	23/02/2018	23/03/2018	Mild	None	Resolved without Sequelae	Not Related
SVH004	headaches/ heaviness	23/03/2018	24/03/2018	Mild	None	Resolved without Sequelae	Not Related
SVH004	Swollen R Cheek	23/03/2018	24/03/2018	Mild	None	Resolved without Sequelae	Not Related
SVH004	Hypertension 152/96	18/05/2018		Mild	None	Ongoing	Not Related
SVH004	Hypertension 141/98	12/07/2018		Mild	None	Ongoing	Not Related
SVH005	back pain	21/03/2018		Mild	None	Ongoing	Not Related
SVH005	Vomiting	12/03/2018	14/03/2018	Mild	None	Resolved without Sequelae	Not Related
SVH005	Tingling sensation in knees	29/03/2018	30/03/2019	Mild	None	Resolved without Sequelae	Not Related
SVH007	Elevated Blood Pressure 146/64	12/02/2018	06/03/2018	Mild	None	Resolved without Sequelae	Not Related

SVH009	Sore Throat	02/05/2018	04/05/2018	Mild	None	Resolved without Sequelae	Not Related
SVH012	Hypertension 158/98	04/04/2018		Mild	None	Ongoing	Not Related
SVH012	?SEPSIS, HIGH TEMP	28/04/2018	04/05/2018	Mild	None	Resolved without Sequelae	Not Related
SVH012	HYPERTENSION 152/96	09/07/2018		Mild	None	Ongoing	Not Related
SVH012	HYPERTENSION 159/102	08/08/2018		Mild	None	Ongoing	Related
SVH013	Nausea/ Constipation/Abdominal cramps	14/03/2019		Mild	None	Ongoing	Not Related
SVH013	Wound Dehiscence	16/03/2019	16/03/2019	Mild	None	Resolved without Sequelae	Not Related
SVH013	Pain in R under arm similar to abscess coming up	02/04/2019	09/04/2019	Mild	None	Ongoing	Not Related
SVH013	Stomach bloating/ GI Pain	09/05/2019	16/05/2019	Mild	Drug Interrupted	Resolved with Sequelae	Not Related
SVH014	Nerve block for compression of disc C3-C7	26/09/2018	27/09/2018	Mild	None	Resolved with Sequelae	Not Related
SVH014	Wound infection	12/10/2018	22/10/2018	Mild	None	Resolved with Sequelae	Not Related
SVH014	worsening of condition	Nov-18		Mild	None	Resolved with Sequelae	Not Related
SVH016	HTN / 151/ 77	18/10/2018		Mild	None	Ongoing	Not Related
SVH016	HTN	16/01/2019		Mild	None	Ongoing	Not Related
SVH016	Generalised Itch	19/01/2019	20/01/2019	Mild	Not Applicable	Resolved without Sequelae	Not Related
SVH016	Urinary Tract Infection	31/01/2019	07/02/2019	Mild	None	Resolved without Sequelae	Related
SVH016	HTN 143/81	10/04/2019		Mild	None	Ongoing	Not Related
SVH016	HTN 140/92	17/06/2019		Mild	None	Ongoing	Not Related
SVH016	Bowel Impaction	20/05/2019	21/05/2019	Mild	None	Resolved with Sequelae	Not Related
SVH016	Bowel Impaction	07/06/2019	08/06/2019	Mild	None	Resolved without Sequelae	Not Related
SVH018	Itching/puritis	Jan-19	Jan-19	Mild	None	Resolved with Sequelae	
SVH018	flare in back pain	08/02/2019	Feb-19	Mild	None		
SVH018	fatigue	08/02/2019	Feb-19	Mild	None		
SVH018	Low Mood	14/03/2019		Mild	None	Ongoing	Not Related
SVH018	Increased Pain	14/03/2019		Mild	None	Ongoing	Not Related

SVH018	Infected Wound - Fluclox given 1/52	04/04/2019	12/04/2019	Mild	Not Applicable	Ongoing	Not Related
SVH021	Infected Biopsy Site	21/01/2019		Mild	Not Applicable	Ongoing	Not Related
SVH023	HTN 142/96	24/01/2019	20/03/2019	Mild	None	Ongoing	Not Related
SVH023	Injection site redness	10/04/2019	12/04/2019	Mild	None	Resolved without Sequelae	Related
SVH023	Local infection at Injection Site	04/06/2019	11/06/2019	Mild	None	Resolved without Sequelae	Not Related
SVH024	HTN 141/80	08/03/2019	28/05/2019	Mild	None	Ongoing	Not Related
SVH024	HTN 150/92	30/04/2019	28/05/2019	Mild	None	Ongoing	Not Related
SVH024	HTN 145/103	19/08/2019		Mild	None	Ongoing	Not Related
SVH024	Tonsillitis	01/09/2019	15/09/2019	Mild	None	Resolved without Sequelae	Related
SVH025	Flare - Fluclox given 500mg TDS 1/52	08/09/2019	15/09/2019	Mild	None	Ongoing	Not Related
SVH025	Urinary Tract Infection	01/10/2019	07/10/2019	Mild	None	Resolved without Sequelae	Not Related
SVH027	HTN 145/95	10/06/2019		Mild	None	Ongoing	Not Related
SVH027	HTN 144/82	11/07/2019		Mild	None	Ongoing	Not Related
SVH027	HTN 176/85	09/08/2019		Mild	None	Ongoing	Not Related
SVH027	Aches and Pains in Wrists and Ankles	11/07/2019		Mild	None	Ongoing	
SVH027	Gum boil	02/08/2019		Mild	None	Ongoing	Not Related
SVH027	Under active Thyroid	31/08/2019		Mild	None	Ongoing	Not Related
SVH027	Occasional Mouth Ulcers that drain	30/09/2019		Mild	None	Ongoing	Not Related
SVH028	gamma gt 110	10/06/2019		Mild	None	Ongoing	Not Related
SVH028	Fatigue	31/07/2019	01/08/2019	Mild	None	Ongoing	Related
SVH028	Nausea	31/07/2019	01/08/2019	Mild	None	Ongoing	Related
svh030	htn 153/100	26/08/2019		Mild	None	Ongoing	Not Related
svh030	HTN 160/98	16/09/2019		Mild	None	Ongoing	Not Related
SVH031	HTN 147/86	20/11/2019		Mild	None	Ongoing	Not Related
SVH031	Stomach upset	12/12/2019		Mild	None	Ongoing	Not Related
SVH021	Backpain						

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