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Supplementary appendix

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SUPPLEMENTARY APPENDIX

Anti–Interleukin-17A Monoclonal Antibody Secukinumab in Ankylosing Spondylitis: A Randomized, Double-Blind, Placebo-Controlled Trial

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Key words: ankylosing spondylitis; anti-IL-17; autoimmune disease; biologic therapy; rheumatology; secukinumab; spondylarthropathy

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METHODS

STUDY DESIGN

The study was sponsored by Novartis Pharma AG. Academic advisors and Novartis personnel designed the study. ClinBay (Genappe, Belgium) conducted the data analyses, under the direction of the sponsor, and all authors had access to the data and vouch for the completeness and accuracy of the data and data analyses. The first draft of the manuscript was written by Novartis personnel, with inputs from all authors. All authors reviewed and provided feedback on the subsequent versions and agreed to submit the manuscript for publication.

STATISTICAL ANALYSIS

The predefined criterion for declaring a positive "Proof of Concept" (PoC) required a posterior probability of at least 95% that the ASAS20 (20% response according to Assessment of SpondyloArthritis international Society criteria) response rate for secukinumab patients would be higher than that for placebo patients. Sample size calculations were based on the number of subjects required to meet the above PoC criterion at the final analysis when assuming true underlying response rates of 25% and 60% for placebo and secukinumab, respectively, and were done by simulation. With data from 20 patients on secukinumab and 5 patients on placebo, the study provided at least a 90% probability of achieving PoC under the above assumptions. An additional 5 subjects were included in the study to allow for dropouts and incomplete data. A 4:1 randomization ratio was chosen to reduce the number of placebo-treated patients while maintaining a double-blinded study design and to allow a limited study size in the absence of knowledge of the risk/benefit of secukinumab in ankylosing spondylitis (AS).

The primary analysis of the study was a Bayesian analysis of the ASAS20 response rate after 6 weeks of treatment. An informative prior was used for the proportion of responders in the

placebo treatment group. The prior distribution was a Beta distribution with parameters 11 and 32. The prior for the active treatment group was also a Beta distribution with parameters 0.5 and 1. The derivation of these prior distributions was based on a meta-analysis of historical data as described below.

From a review of anti–tumor necrosis factor (TNF)-α treatment in ankylosing spondylitis,[1] historical data were available from eight randomized placebo-controlled clinical trials in ankylosing spondylitis patients. The earliest timepoint assessed in this review was 12 weeks after dosing. Assuming a stable placebo response rate between weeks 6 and 12, these data were used in the derivation of the historical data prior.

A random effects meta-analysis[2] of the 8 historical trials was performed assuming exchangeable placebo response rates on the logit scale. Using this model, the predictive distribution for the proportion of responders on placebo in a new study was derived, leading to an estimated response rate of 25% (and a 95% credible interval of 13% to 40%). For ease of use and interpretation, this predictive distribution was approximated by a Beta density with matching mean and standard deviation. The result of this process was the Beta(shape1=11, shape2=32) distribution, which was used as the historical data prior for the placebo group in the primary analysis. The information content of this prior was equivalent to observing 11 responders in a sample of size 43. In other terms, the equivalent sample size of this prior was 43 patients.

The prior distribution for the proportion of responders in the active group was also a Beta distribution. One of the parameters was set to 1. The other parameter was chosen such that there was an approximately 50:50 chance that the responder rate on active treatment would be

greater than the responder rate on placebo (based on the prior distributions only). Thus a Beta(shape1=0.5, shape2=1) distribution was chosen.

An exploratory analysis of genetic data was performed on a subgroup of consenting secukinumab patients (N=22). Thirteen genetic polymorphisms previously shown to be either associated with AS[3-64] or in the target IL-17A gene region were selected to be tested for association with response to secukinumab. All genotyping was performed by Novartis; single nucleotide polymorphism (SNP) genotyping was done using TaqMan, and human leukocyte antigen (HLA) genotyping was done using sequence-specific oligonucleotide (SSO). The 13 polymorphisms were tested individually for association with ASAS20 and ASAS40 at week 6 using logistic regression models and with Bath Ankylosing Spondylitis Disease Activity Index (BASDAI**)** scores using a linear regression model, with the efficacy endpoint as the dependent variable, the number of copies of the minor (i.e., less common) allele carried by the individual as the predictor, and baseline BASDAI score as a covariate. All statistical tests were two-tailed, with no imputation for missing data. A permutation test was performed to account for small sample size and multiple comparisons of 13 polymorphisms, with genotypes randomly permuted 250,000 times.

ERAP1 gene transcript levels in whole blood were determined using Affymetrix DNA microarrays (Human Genome 133 Plus 2.0). The Affymetrix '.cel' files were normalized using the RMA procedure[7] and analyzed with the Partek Genomics Suite 6.6 beta software.

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Supplementary Table 1. Participating sites and Principal Investigators for secukinumab Study CAIN457A2209 (NCT00809159).

Participating Site	Principal Investigator	No. Patients
Germany		
Universitaetsklinikum Benjamin Franklin Med. Klinik und Poliklinik I/Rheumatologie Hindenburgdamm 30 12203 Berlin	Joachim Sieper	10
Rheumazentrum Ruhrgebiet St.-Josefs-Krankenhaus Landgrafenstr. 15 44652 Herne	Jürgen Braun	4
Klinikum Eilbek - Schön Kliniken Dehnhaide 120 22081 Hamburg	Jürgen Wollenhaupt	3
Netherlands		
EULAR & FOCIS Center of Excellence Academic Medical Center University of Amsterdam, F4-2181100 22700 Amsterdam	Dominique Baeten	10
Maastricht University Medical Centre Department of Medicine, Division of Rheumatology Maastricht University Medical Centre 6202 AZ 5800 Maastricht	Robert Landewé	1
United Kingdom		
Institute of Cellular Medicine School of Clinical Medical Sciences Newcastle University 4th Floor, Catherine Cookson Building The Medical School, Framlington Place NE2 4HH Newcastle upon Tyne	Jacob M. van Laar	2

Supplementary Table 2. Pharmacogenetic analysis: association with clinical response.

Variant	Gene	Alleles (minor/ major)	Position	Week 4 P-values			Week 6 P-values			Week 24 P-values		
				ASAS20	ASAS40	BASDAI	ASAS20	ASAS40	BASDAI	ASAS20	ASAS40	BASDAI
rs11209032	*IL23R*	A/G	Downstream	0·40	0·45	0·41	0·27	0·81	0·29	0·98	0·55	3·65E-03
rs2201841	*IL23R*	G/A	Intronic	0·26	0·78	0·58	0·24	0·39	0·46	0·56	0·45	8·52E-03
rs11209026	*IL23R*	A/G	Nonsynonymous	0·45	0·83	0·73	0·71	0·97	0·77	0·90	0·20	0·69
rs10865331	—	A/G	Genomic	0·89	0·49	0·86	0·64	0·75	0·24	0·67	1·00	0·41
rs2310173	*IL-1R2*	A/C	Downstream	0·29	0·59	0·75	0·18	0·46	0·70	0·019	0·42	0·37
rs4333130	*ANTXR2*	C/T	Intronic	0·21	0·99	0·45	0·69	0·56	0·71	0·60	0·59	0·080
rs2242944	—	A/G	Genomic	0·89	0·30	0·41	0·12	0·34	0·28	0·32	0·55	0·95
rs27434	*ERAP1*	A/G	Synonymous	0·33	0·083	0·67	0·21	4·75E-03	0·47	0·54	0·71	0·44
rs30187	*ERAP1*	T/C	Nonsynonymous	0·029	4·45E-03	0·26	0·22	8·14E-05	0·22	0·46	0·44	0·61
rs1974226	*IL-17A*	T/C	3’ Utr	0·20	0·90	0·23	0·56	0·87	0·64	0·60	0·14	0·20
rs7747909	*IL-17A*	A/G	3’ Utr	0·60	0·20	0·24	0·40	0·36	0·68	0·68	0·64	0·74
HLA-DRB1*04	*HLA-DRB1*			0·74	0·65	0·36	0·25	0·62	0·49	0·94	0·22	0·61
HLA-B*27	*HLA-B*			0·31	0·58	0·96	0·61	0·10	0·53	0·74	0·55	0·83

ASAS denotes Assessment of SpondyloArthritis international Society criteria, BASDAI denotes Bath Ankylosing Spondylitis Disease Activity Index.
B*27 confers the greatest known risk for ankylosing spondylitis.

Supplementary Table 3. Secondary efficacy analysis, all timepoints.

Visit	ASAS20		ASAS40		ASAS5/6		BASDAI		ASQoL	
	Secukinumab* (N=23) n (%)	Placebo (N=6) n (%)	Secukinumab (N=23) n (%)	Placebo (N=6) n (%)	Secukinumab (N=23) n (%)	Placebo (N=6) n (%)	Secukinumab (N=23) n, mean ±SD	Placebo (N=6) n, mean ±SD	Secukinumab (N=23) n, mean ±SD	Placebo (N=6) n, mean ±SD
Day 8	9/23 (39)	2/6 (33)	4/23 (17)	0/6 (0)	5/23 (22)	1/6 (17)	23, 5·27±2·31	6, 5·97±2·90		
Day 15	9/23 (39)	1/5 (20)	4/23 (17)	0/5 (0)	5/23 (22)	0/5 (0)	23, 5·19±2·29	5, 6·48±2·48		
Day 29	11/23 (48)	0/6 (0)	8/23 (35)	0/6 (0)	8/23 (35)	0 / 6 (0)	23, 4·75±2·63	6, 6·28±2·19	23, 9·0±5·13	6, 9·70±6·53
Week 6	14/23 (61)	1/6 (17)	7/23 (30)	1/6 (17)	8/23 (35)	0/6 (0)	22, 5·19±2·40	3, 4·49±1·99		
Week 8	8/23 (35)	1/6 (17)	5/23 (22)	0/6 (0)	7/23 (30)	0/6 (0)	21, 5·62±2·18	3, 4·39±0·54		
Week 10	9/23 (39)	0/6 (0)	5/23 (22)	0/6 (0)	6/23 (26)	0/6 (0)	20, 5·43±2·24	3, 4·84±0·44		
Week 12	9/23 (39)	1/6 (17)	3/23 (13)	0/6 (0)	3/23 (13)	0/6 (0)	20, 5·65±2·26	3, 4·37±1·78	20, 9·60±3·85	3, 8·30±0·58
Week 16	8/23 (35)	1/6 (17)	3/23 (13)	1/6 (17)	4/23 (17)	0/6 (0)	20, 5·58±2·23	3, 4·34±2·18		
Week 20	6/23 (26)	1/6 (17)	2/23 (9)	1/6 (17)	2/23 (9)	1/6 (17)	18, 5·69±1·20	3, 4·18±1·78		
Week 24	7/23 (30)	0/6 (0)	3/23 (13)	0/6 (0)	3/23 (13)	0/6 (0)	17, 5·58±2·33	3, 4·54±0·63		
Week 28/end of study	7/23 (30)	1/6 (17)	2/23 (9)	0/6 (0)	1/23 (4)	0/6 (0)	23, 6·26±2·31	5, 5·53±1·72	22, 10·6±4·80	4, 9·30±5·91

* Secukinumab: 2x10 mg/kg.

ASAS denotes Assessment of SpondyloArthritis international Society criteria, BASDAI denotes Bath Ankylosing Spondylitis Disease Activity Index, ASQoL denotes Ankylosing Spondylitis Quality of Life.

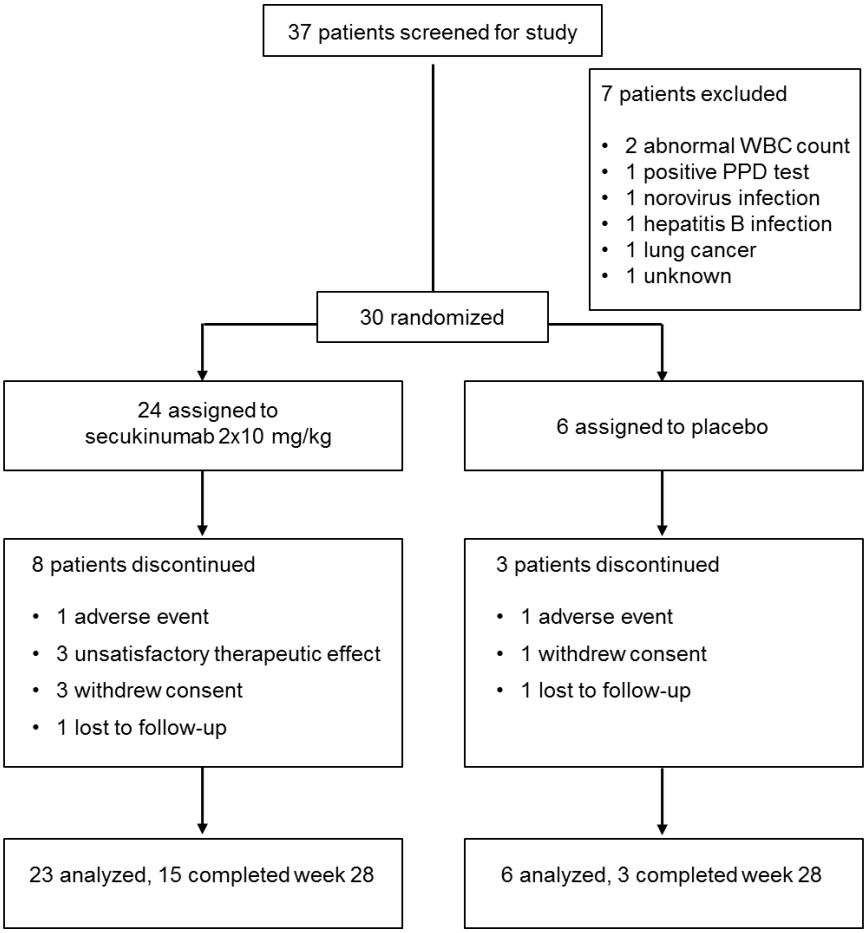
Supplementary Table 4. MRI scores at baseline, week 6, and week 28/end of study.

	Secukinumab 2x10mg/kg			Placebo		
	Baseline	**Week 6**	**Week 28***	**Baseline**	**Week 6**	**Week 28**
Number of patients	22	22	16	5	3	5
ASAS20 responders (n)	—	14	6	—	1	1
Mean Berlin score†	9·2±8·87	6·6±6·56	5·7±6·20	20·6±20·18	21·0±24·56	19·0±19·33
P value (vs. baseline)	—	0·10	0·16	—	0·50	0·25

* Data from 6 patients who discontinued prior to week 28 because of lack of response were not available for analysis.

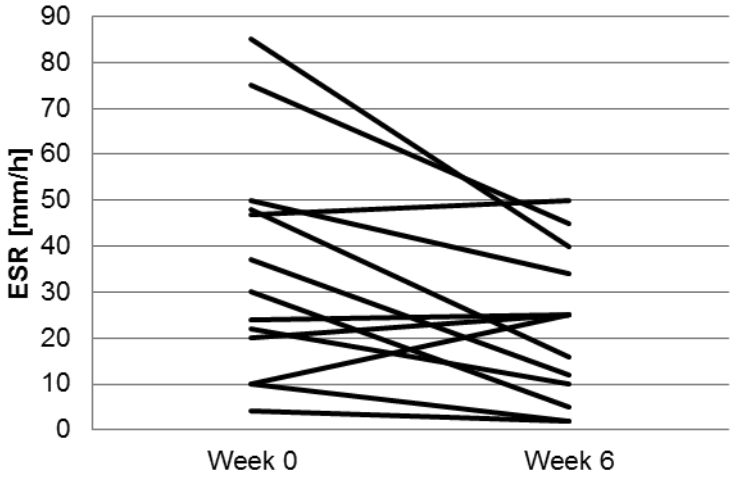
† Plus−minus values are means ±SD.

Supplementary Figure 1. Patient disposition. **PPD denotes purified protein derivative; WBC denotes white blood cell.**

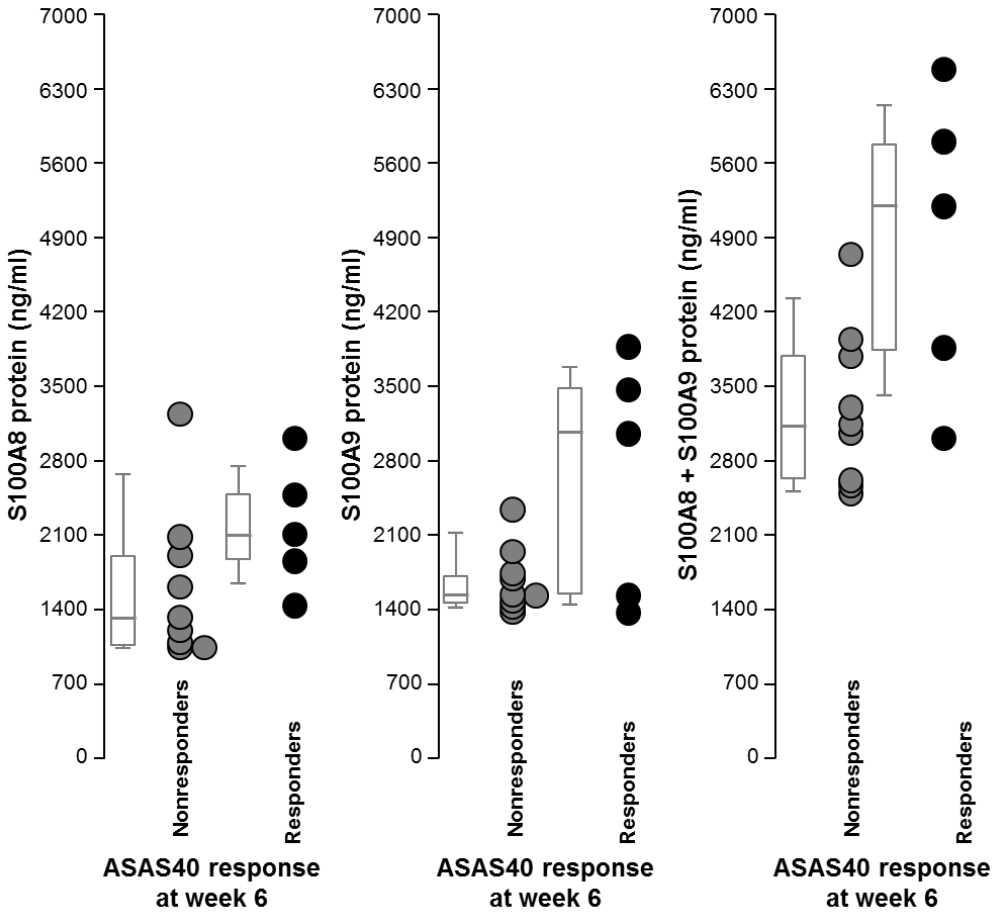


Supplementary Figure 2. Panel A shows erythrocyte sedimentation rate (ESR) at baseline (Week 0) and Week 6 following intravenous secukinumab 2x10 mg/kg at Weeks 0 and 3. Panel B shows baseline serum concentrations of S100A8 (left), S100A9 (center), and S100A8 and S100A9 combined (right). Measurements are shown for individual patients and grouped by their week 6 ASAS40 response. Responders are depicted as dark circles, nonresponders as lighter circles. ASAS denotes Assessment of SpondyloArthritis international Society criteria.

Suppl. Fig. 2A

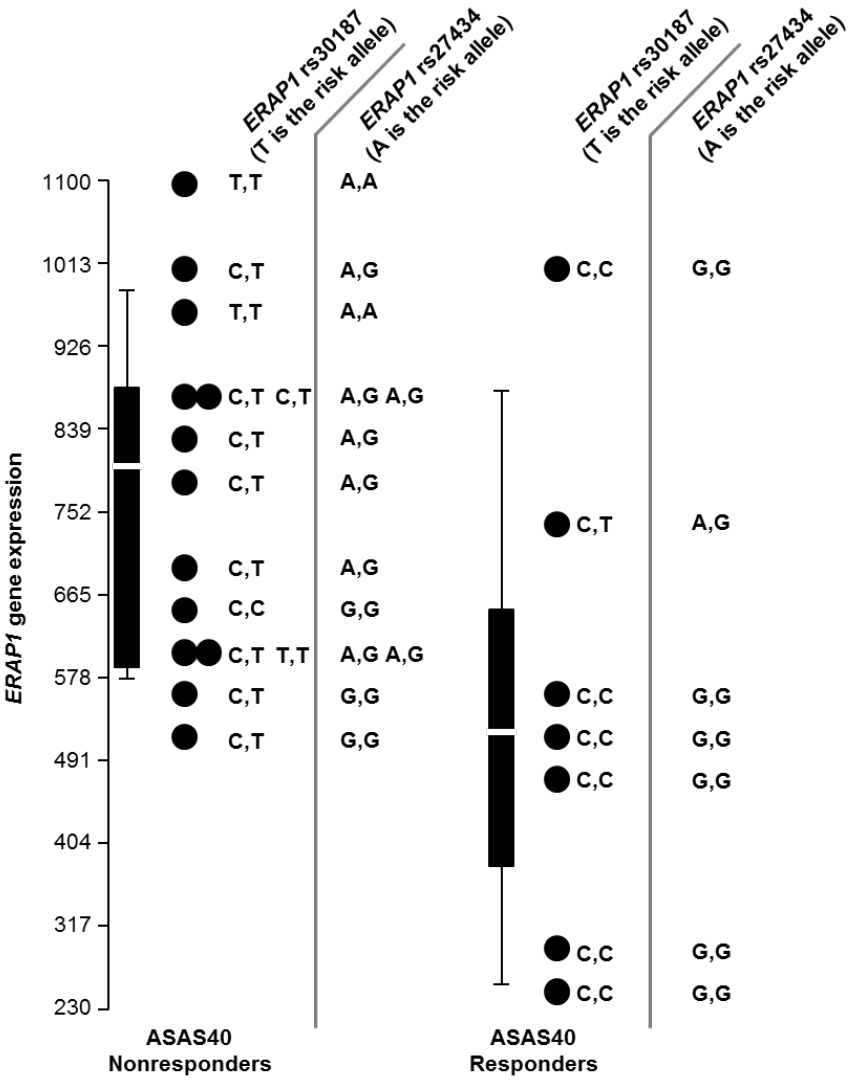


Suppl. Fig. 2B

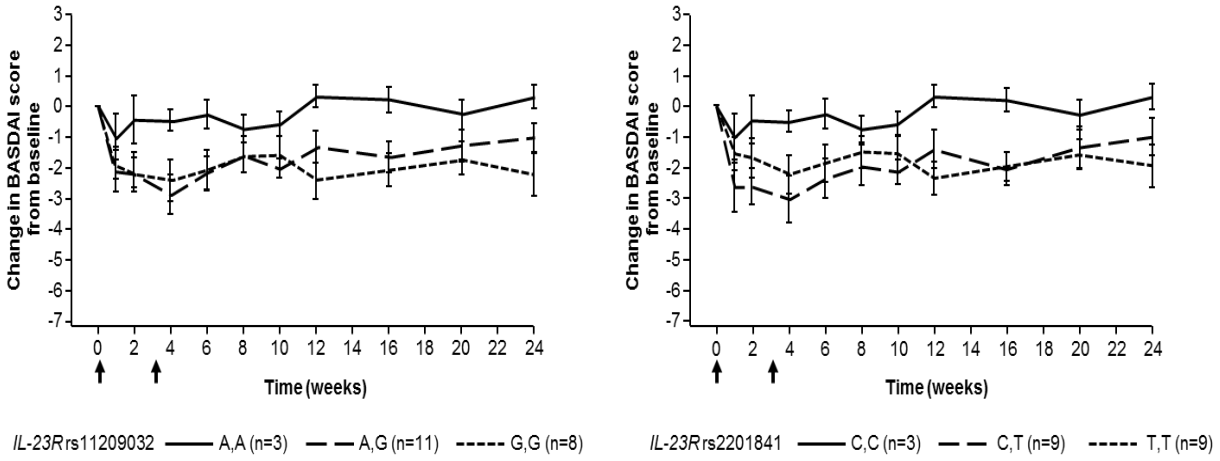

S100A8 protein (ng/ml)
S100A9 protein (ng/ml)
S100A8 + S100A9 protein (ng/ml)
7000
6300
5600
4900
4200
3500
2800
2100
1400
700
0
Nonresponders
Responders
ASAS40 response at week 6

Supplementary Figure 3. Panel A shows *ERAP1* gene expression and genotype and association with clinical response at week 6 in ASAS40 responders and nonresponders. Representations of *ERAP1* gene expression levels and *ERAP1* ankylosing spondylitis risk allele genotypes (rs30187 and rs27434) are combined. Dots represent individual patients from the secukinumab arm at baseline (pre-treatment). Week 6 ASAS40 nonresponders are represented on the left and responders on the right. Panel B shows the association of rs11209032 (left panel; minor allele/AS risk allele=A, major allele=G) and rs2201841 (right panel; minor allele/AS risk allele=C, major allele=T) genotypes in the *IL23R* gene with mean BASDAI change from baseline (±SD) at different time points from first dosing of secukinumab. The polymorphisms were tested individually for association with BASDAI at week 24 using a linear regression model, with number of copies of the minor (less common) allele carried by the individual as the predictor and baseline BASDAI score as a covariate. ASAS denotes Assessment of SpondyloArthritis international Society criteria; BASDAI denotes Bath Ankylosing Spondylitis Disease Activity Index; SD denotes standard deviation.

Suppl. Fig. 3A


ERAP1 rs30187 (T is the risk allele)
ERAP1 rs27434 (A is the risk allele)
ERAP1 gene expression
1100
1013
926
839
752
665
578
491
404
317
230
T,T A,A
C,T A,G
T,T A,A
C,T C,T A,G A,G
C,T A,G
C,T A,G
C,T A,G
C,C G,G
C,T T,T A,G A,G
C,T G,G
C,T G,G
C,C G,G
C,T A,G
C,C G,G
C,C G,G
C,C G,G
C,C G,G
C,C G,G
ASAS40 Nonresponders
ASAS40 Responders

Suppl. Fig 3B


Change in BASDAI score from baseline
3
2
1
0
-1
-2
-3
-4
-5
-6
-7
0 2 4 6 8 10 12 14 16 18 20 22 24
Time (weeks)
IL-23R rs11209032 A,A (n=3) A,G (n=11) G,G (n=8)
Change in BASDAI score from baseline
Time (weeks)
IL-23R rs2201841 C,C (n=3) C,T (n=9) T,T (n=9)