

Accepted Manuscript

Methotrexate versus azathioprine in patients with atopic dermatitis: two years follow-up data

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PII: S0091-6749(17)31657-3

DOI: [10.1016/j.jaci.2017.09.033](https://doi.org/10.1016/j.jaci.2017.09.033)

Reference: YMAI 13078

To appear in: *Journal of Allergy and Clinical Immunology*

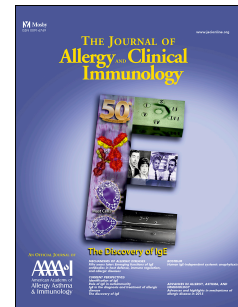
Received Date: 15 March 2017

Revised Date: 3 August 2017

Accepted Date: 13 September 2017

Please cite this article as: Roekevisch E, Schram ME, Geertruida Leeftang MM, Dorothée Brouwer MW, Andrea Gerbens LA, Bos JD, Spuls PI, Methotrexate versus azathioprine in patients with atopic dermatitis: two years follow-up data, *Journal of Allergy and Clinical Immunology* (2017), doi: 10.1016/j.jaci.2017.09.033.

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TITLE PAGE

Letter to the Editor

Methotrexate versus azathioprine in patients with atopic dermatitis: two years follow-up data

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Funding: none

Disclosure of potential conflict of interest:

P.I. Spuls had consultancies in the past for Leopharma, Anacor, AbbVie and Novartis and received independent research grants in the past from Schering Plough and Leopharma. She was involved in performing clinical trials with many pharmaceutical industries that manufacture drugs used for the treatment of psoriasis and atopic dermatitis.

E. Roekevisch, M.E. Schram, M.M.G. Leeftang, M.W.D. Brouwer, L.A.A. Gerbens, J. D. Bos, MD, have declared that they have no conflict of interest.

This trial was registered in the Dutch Trial Register (NTR1916)

Word count: 1000

Number of Tables: 2

Number of Figures: 0

Online repository:

Methods section, word count: 1049

Number of Tables: 5

Number of Figures: 5

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Clinical implications

Methotrexate and azathioprine seem to be effective and safe long-term therapies for adult patients with severe atopic dermatitis.

Capsule summary

Patients randomized to methotrexate or azathioprine maintained a significant reduction in SCORAD after 2 years compared to baseline. No important differences were found in type, number and severity of the adverse events between the groups.

Key words

Atopic dermatitis

Azathioprine

Methotrexate

Off-label

RCT

Trial

Long-term study

Open-label extension study

List of Abbreviations

AD: Atopic dermatitis

AE: Adverse event

AZA: Azathioprine

CsA: Cyclosporin A

EASI: Eczema Area and Severity Index

FLG: Filaggrin gene

FMG: Filaggrin Mutation Group

HOME: Harmonising Outcome Measures for Eczema

IGA: Investigator Global Assessment

ITT: Intention-to-treat

LOCF: Last observation carried forward

MTX: Methotrexate

PGA: Patient Global Assessment

PP: Per-protocol

POEM: Patient-Oriented Eczema Measure

RCT: Randomized controlled trial

SAE: Serious adverse event

SD: Standard deviation

SCORAD: SCORing Atopic Dermatitis index

VAS: Visual Analogue Scale

w: weeks

m: months

y: years

Methotrexate versus azathioprine in patients with atopic dermatitis: two years follow up data

To the Editor:

Long-term data of methotrexate (MTX) and azathioprine (AZA) in atopic dermatitis (AD) is lacking. Previously, we published the results of a randomized controlled trial (RCT) of 42 adults with severe AD treated with MTX or AZA for 12 to 24 weeks. Both MTX and AZA induced significant reductions in SCORing Atopic Dermatitis (SCORAD) index at 12 and 24 weeks after baseline, without a significant difference between treatments.¹ To assess long term efficacy and safety of MTX versus AZA, all 42 patients were asked to participate in an observational follow-up study to be evaluated 3 months for 2 years. After 12 weeks of treatment with MTX or AZA, treatments were continued, stopped or switched, reflecting normal clinical practice. The same outcomes as in the original RCT were investigated which correspond to the 4 core outcome domains for AD suggested by the Harmonising Outcome Measures for Eczema (HOME) initiative. Patients were scored by trained blinded investigators. One of the primary outcomes was the difference in mean absolute and relative change of SCORAD after 2 years compared to baseline. An intention-to-treat (ITT) and per protocol analysis (PP) were performed. Adverse events (AEs) were coded according to Medical Dictionary for Regulatory Activities (MedDRA)/ Common Terminology Criteria for Adverse Events (CTCAE).²

In addition, the effect of filaggrin gene (*FLG*) mutation status on treatment outcome (R501X, 2282del4, R2447X, S3247X, 3321delA) was evaluated by collecting buccal mucosa swabs. Patients were divided into a *FLG* mutation group (FMG) and non-FMG.

For detailed methods on dosing and monitoring we refer to the online repository at www.jacionline.org and previous publication.¹

For the MTX and AZA group, an overview of primary and secondary outcomes after 2 years compared to baseline is presented in Table IA (ITT, n=35) and Table IB (PP, n=18).

In the online repository we present in Table EI the baseline characteristics. Treatment and dosage overview are presented in Table EIIA, EIIB and Figure EI. Figure EIIA (ITT) and Figure EIIB (PP) show the mean (SD) total SCORAD. Table EIIIA (ITT) and Table EIIBB (PP) show AEs and other key safety data. Figure EIIIA (ITT) and Figure EIIBB (PP) show the mean SCORAD after 2 years compared to baseline for the FMG and non-FMG.

Thirty-five patients, 17 primary randomized to MTX and 18 to AZA, participated in this follow-up study. Two years after baseline, 10 patients still used allocated MTX and 11 allocated AZA, of which 8 continuously since randomization. Reasons for patients in the ITT analysis to discontinue treatment (17/35) were controlled AD (7/35), inefficacy (7/35) or AEs (3/35). These AEs concerned fatigue, nausea, recurrent herpes infections in the MTX group(2/35) and increased liver enzyme values and worsening of pre-existing mild anemia in the AZA group(1/35) both reversible after stopping AZA.

Two years after baseline, improvement was found for all primary and secondary outcomes. ITT analysis showed a mean absolute reduction in total SCORAD of 37.8 (SD 13.8) and 32.6 (SD 17.4) in the MTX and AZA group respectively, representing a relative reduction of 63% ($P < 0.01$) and 53% ($P < 0.01$), respectively. There was no significant difference in SCORAD between groups ($P = 0.33$). PP analysis showed a mean absolute reduction in total SCORAD of 41.7 (SD 10.6) and 43.6 (SD 18.5) in the MTX and AZA group respectively, representing a relative reduction of 67% ($P < 0.01$) and 65% ($P < 0.01$), respectively. There was no significant difference in SCORAD between groups ($P = 0.79$). Also for other outcome measures, there was no significant difference between treatments, except for the Patient Global Assessment (PGA) in the PP analysis, which showed a favorable effect for the AZA group. As the PGA gives insight in the patient's own health experience

this may be an important finding. Interestingly, although not significant, the Investigator Global Assessment (IGA) in the PP analysis, also showed a trend of more efficacy ($P = 0.07$) in the AZA group.

Amounts of high potent (class III-IV, scale I-IV) topical immunosuppressive treatment (total gram, $P = 0.17$) and oral corticosteroids needed as rescue medication ($P = 0.70$) were comparable between groups during the course of 2 years ($P = 0.17$). Oral antibiotics used for bacterial skin infections were more often used in the MTX than in the AZA group ($P = 0.03$).

Concerning safety, 3 serious adverse events (SAEs) occurred, 2 exacerbations of AD and 1 hospitalization because of psychiatric comorbidity. No life-threatening SAEs and occurred. No malignancies were observed. Most AEs were found in the group of infections and infestations disorders. Common cold and liver enzyme disorders were most frequently seen in the MTX group. Influenza was significantly more present in the AZA group.

Concerning *FLG* mutation status, one patient declined to perform DNA testing, therefore information was gathered from 34 patients. Previously, we showed a better response of systemic treatment in the non-FMG over the course of 24 weeks.³ However, in this study we observe that this difference in effect fades away over time, and after 2 years the response in both groups is comparable. In the non-FMG there is a steep curve in reduction of SCORAD in the first 3 months, in other words not much further improvement is to be expected after 3 months of therapy (absolute SCORAD reduction of only 4 points), while in the FMG still significant effect can be expected (absolute SCORAD reduction of 25 points). *FLG* mutation status may therefore be important in relation to the duration of systemic treatment.

In conclusion, because AD is a chronic disease, it is imperative that any proposed therapy has persistent efficacy and acceptable side effects during years of use. This study demonstrates that MTX and AZA seem to be appropriate and safe options for the treatment of adult patients with severe AD. As with most observational prospective studies, a limitation is the interpretation of the ITT analysis as patients stop treatment for various reasons over the course of 24 weeks. Patients with a *FLG* mutation seemed to have slower but prolonged effects of therapy compared to patients without a mutation.

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Acknowledgment

We like to thank all patients who participated in this study, all fellow dermatologists for referring patients, Mandy Schram for initiating the study, Jochen Schmitt for contribution on the design of the study, Gabrielle Appel, Anna- Christa de Vries and Stef Menting for being efficacy investigators, Marleen van der Stok for monitoring the study.

We like to thank Pina Middelkamp-Hup, Sanja Kezic, Linda Campbell and Irwin McLean for their contribution on filaggrin gene mutations.

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1 TABLE IA. Clinical response after 2 years compared to baseline (intention-to-treat analysis)

Variable	MTX (n=17)	AZA (n=18)	Mean Difference (95% Confidence Interval of the Difference)	P Value
Baseline values of SCORAD [‡]				
SCORAD				
- Total SCORAD, mean (SD)	59.4 (11.4)	59.7 (11.0)	-0.3 (-8.0 to 7.4)	0.93[*]
- Objective SCORAD, mean (SD)	46.6 (10.3)	46.3 (10.1)	0.3 (-6.7 to 7.3)	0.93[*]
- Subjective SCORAD, mean (SD)	12.8 (4.0)	13.4 (3.4)	-6.2 (-2.9 to 1.7)	0.59[*]
Primary outcomes, after 2 years compared to baseline				
SCORAD				
Absolute reduction				
- Total SCORAD, mean (SD)	37.8 (13.8)	32.6 (17.4)	5.3 (-5.6 to 16.1)	0.33[*]
- Objective SCORAD, mean (SD)	29.0 (12.7)	25.0 (13.8)	3.9 (-5.2 to 13.0)	0.39[*]
- Subjective SCORAD, mean (SD)	8.8 (4.1)	7.5 (5.5)	1.3 (-2.1 to 4.7)	0.43[*]
Relative reduction				
- Total SCORAD, mean (SD)	63% (17%)	53% (24%)	9.9 (-4.3 to 24.2)	0.16[*]
- Objective SCORAD, mean (SD)	61% (19%)	52% (23%)	8.3 (-6.2 to 22.7)	0.25[*]
- Subjective SCORAD, mean (SD)	49% (33%)	37% (41%)	12.6 (-13.2 to 38.4)	0.33[*]
SCORAD50, no (%)	15 (88%)	11 (61%)	-	0.12[^]
Reduction IGA, median (range)	2 (0-3)	2 (-1-4)	-	0.86[#]
Cleared, minimal or mild disease (IGA <3), no (%)	15 (88%)	14 (78%)	-	0.66[^]
Secondary outcomes, after 2 years compared to baseline				
Reduction in PGA, median (range)	2(0-4)	1(-2-4)	-	0.27[#]
Reduction in EASI, mean (SD)	24.9 (13.0)	26.4 (14.7)	-1.5 (-11.1 to 8.1)	0.75[*]
EASI50, no (%)	17 (100%)	17 (94%)	-	1.0
EASI75, no (%)	14 (82%)	14 (78%)	-	1.0
Reduction in POEM, mean (SD)	10.6 (5.4)	10.2 (9.1)	0.4 (-4.8 to 5.5)	0.89[*]
Reduction in VAS pruritus, mean (SD)	3.1 (2.1)	2.4 (3.3)	0.7 (-1.3 to 2.6)	0.49[*]
Reduction in VAS sleeplessness, mean (SD)	4.2 (2.1)	4.1 (2.8)	0.1 (-1.6 to 1.8)	0.88[*]
Reduction in Skindex-17				
- Total Skindex, mean (SD)	8.8 (6.9)	7.4 (7.4)	1.4 (-3.5 to 6.3)	0.56[*]
- Symptoms, mean (SD)	2.4 (2.1)	1.7 (2.5)	0.6 (-0.9 to 2.2)	0.43[*]
- Psychosocial, mean (SD)	6.5 (5.6)	5.7 (5.5)	0.8 (-3.0 to 4.6)	0.67[*]

2 Table includes all patients who participated in the observational follow-up study (n=35). Last available observation value was carried
3 forward (ITT analysis).

4 EASI, Eczema Area and Severity Index; EASI50, Improvement in EASI with at least 50%; EASI75, Improvement in EASI with at least 75%;
5 IGA, Investigator Global Assessment; LOCF, last observation carried forward; PGA, Patient Global Assessment; POEM, Patient-Oriented
6 Eczema Measure; SCORAD, SCORing Atopic Dermatitis; SCORAD50, Improvement in SCORAD with at least 50%; SD, Standard
7 Deviation; VAS, Visual Analogue Scale.

8 [‡] Baseline SCORAD values are values at the first visit of the original study before starting MTX or AZA; * T-test for independent groups;

9 [#] Fisher's exact test; [^] Pearson Chi-Square.

1 TABLE IB. Clinical response after 2 years compared to baseline (per-protocol analysis)

Variable	MTX (n=10)	AZA (n=8)	Mean Difference (95% Confidence Interval of the Difference)	P Value
Baseline values of SCORAD [‡]				
SCORAD				
- Total SCORAD, mean (SD)	61.9 (9.4)	67.0 (8.3)	-5.0 (-14.0 to 3.9)	0.25*
- Objective SCORAD, mean (SD)	48.5 (8.2)	52.7 (9.0)	-4.1 (-12.7 to 4.5)	0.83*
- Subjective SCORAD, mean (SD)	13.4 (3.5)	14.4 (3.4)	-1.0 (-4.4 to 2.5)	0.56*
Primary outcomes, after 2 years compared to baseline				
SCORAD				
Absolute reduction				
- Total SCORAD, mean (SD)	41.7 (10.6)	43.6 (18.5)	-1.9 (-16.6 to 12.8)	0.79*
- Objective SCORAD, mean (SD)	32.4 (9.5)	33.8 (14.9)	-1.3 (-13.6 to 10.9)	0.82*
- Subjective SCORAD, mean (SD)	9.3 (4.7)	9.9 (5.9)	-0.6 (-5.8 to 4.7)	0.82*
Relative reduction				
- Total SCORAD, mean (SD)	67% (8%)	65% (25%)	1.9 (-16.1 to 19.3)	0.83*
- Objective SCORAD, mean (SD)	66% (10%)	63% (24%)	2.5 (-15.5 to 20.5)	0.77*
- Subjective SCORAD, mean (SD)	45% (30%)	39% (36%)	5.6 (-27.5 to 38.8)	0.72*
SCORAD50, no(%)	10 (100%)	6 (75%)	-	0.18[^]
Reduction IGA, median (range)	2 (1-3)	3 (-1-4)	-	0.07[#]
Cleared, almost clear or mild disease (IGA <3), no (%)	9 (90%)	7 (88%)	-	1.00[^]
Secondary outcomes, after 2 years compared to baseline				
Reduction in PGA, median (range)	1.5 (0-4)	3 (-2-3)	-	0.01[#]
Reduction in EASI, mean (SD)	28.1 (12.6)	34.9 (13.8)	-6.8 (-20.0 to 6.5)	0.29*
EASI50, no (%)	10 (100%)	8 (100%)	-	-
EASI75, no (%)	10 (100%)	6 (94%)	-	0.18
Reduction in POEM, mean (SD)	12.9 (4.4)	13.0 (10.1)	-0.1 (-7.6 to 7.4)	0.98*
Reduction in VAS pruritus, mean (SD)	3.2 (2.5)	2.6 (3.2)	0.6 (-2.2 to 3.4)	0.68*
Reduction in VAS sleeplessness, mean (SD)	4.6 (2.0)	5.0 (3.3)	-0.4 (-3.0 to 2.2)	0.75*
Reduction in Skindex-17				
- Total, mean (SD)	11.1 (7.8)	10.0 (8.6)	1.1 (-7.1 to 9.3)	0.78*
- Symptoms, mean (SD)	2.8 (2.3)	2.6 (2.1)	0.2 (-2.0 to 2.8)	0.77*
- Psychosocial, mean (SD)	8.3 (6.5)	7.4 (6.7)	0.9 (-5.7 to 7.6)	0.87*

2 Table includes all patients who were continuously on the randomized treatment till 2 years after baseline (n=18).

3 EASI, Eczema Area and Severity Index; EASI50, Improvement in EASI with at least 50%; EASI75, Improvement in EASI with at least 75;

4 IGA, Investigator Global Assessment; IGA <2 = Cleared or minimal disease; LOCF, last observation carried forward; PGA, Patient Global
5 Assessment; POEM, Patient-Oriented Eczema Measure; SCORAD, SCORing Atopic Dermatitis; SCORAD50, Improvement in SCORAD with
6 at least 50%; SD, Standard Deviation; VAS, Visual Analogue Scale.7 [‡] Baseline SCORAD values are values at the first visit of the original study before starting MTX or AZA; * T-test for independent groups;8 [#] Fisher's exact test; [^] Pearson Chi-Square.

9

MATERIALS AND METHODS

The methods of the original RCT have been published previously.¹

Study design

This is an open-label observational follow-up study of patients who participated in a single blind RCT evaluating efficacy and safety of MTX versus AZA over a 12-week period. This RCT was conducted between September 2009 and May 2010 at the Academic Medical Center (AMC) of Amsterdam, the Netherlands.¹ Eligible patients had severe AD, graded at baseline by the Rajka and Langeland⁴ criteria and defined by the millennium and UK Working Party Criteria.⁵ They were unresponsive, contraindicated or intolerant to CsA and were not treated with MTX or AZA before. Concealment of MTX or AZA was done by a computerized program. Trained investigators and the epidemiologist who did the statistics were blinded for the allocated treatments. Patients and treating physician were not blinded for allocation.

All 42 randomized patients who used MTX or AZA were offered the opportunity to enroll in the observational follow-up study in which they would be evaluated 3 monthly over a total period of 2 years (and eventually 5 years) after baseline. Patients had the option to remain under treatment at the AMC or to be referred back to their non-academic dermatologist. The last patient had data point 2 year check in April 2012.

The local medical ethics committee (institutional review board) approved the observational follow-up study protocol, which was performed in accordance with the Good Clinical Practice Guidelines of the International Conference of Harmonization, Declaration of Helsinki. The trial was registered in the Dutch Trial Register (NTR1916). Written informed consent was obtained from all patients for this observational follow-up study before study-related procedures were commenced. This study report followed the Consolidated Standards of Reporting Trials (CONSORT) guidelines.

Treatment regimens

Twelve weeks after randomization, patients could continue, switch or stop the treatment they were randomized to, reflecting normal practice. MTX dose increments was restricted to a maximum of 22.5 mg/week in combination to 5 mg of folic acid the following day, once a week. AZA dosage was restricted to a maximum of 2.5 mg/kg/day. There was no limitation in the use of concomitant topical medication or oral treatment with corticosteroids (if needed as rescue medication). Oral antibiotics were giving according to standard clinical practice usually when the eczema seemed to be infected.

Treatment outcomes

Primary outcomes were differences in mean absolute and relative change of SCORAD⁶ and Investigator Global Assessment (IGA; six point scale, range 0-5, relative change IGA data not shown)

between groups after 2 years compared to baseline. Also the amount of patients with a SCORAD reduction of 50% or more (SCORAD50) and the difference in amount of patients with an IGA score of < 3 (clear / almost clear / mild disease) were compared between groups after 2 years compared to baseline. Deviating from the study protocol, the SCORAD was subdivided in objective and subjective SCORAD.

Secondary outcomes were the absolute and relative change of Eczema Area And Severity Index (EASI)⁷, Patient-Oriented Eczema Measure (POEM)⁸, Patient Global Assessment (PGA, 5 grades), pruritus and sleeplessness scores on a Visual Analogue Scale (VAS), quality of life assessed by the Skindex-17⁹ and the frequency of remissions (PGA <2, clear / almost clear), between groups after 2 years compared to baseline. Deviating from the study protocol, the Skindex-17 was subdivided in symptomatic and psychosocial subscale.⁹

Reason of discontinuation of treatment was subdivided in: (1) controlled AD; (2) inefficacy; (3) adverse event (AE); (4) relapse; (5) miscellaneous.

Controlled AD was defined as improvement of AD (in the opinion of the patient and/or physician) leading to discontinuation of the oral immunosuppressive drug. Inefficacy was registered as reason for discontinuation when patient and/or physician were not satisfied with the clinical response and the treatment was stopped. AEs leading to discontinuation of treatment, included both subjective and objective (such as laboratory abnormalities) AEs. A relapse was defined as a worsening of AD (in the opinion of the patient and/or physician) which resulted in (re)starting an oral immunosuppressive drug. Any other reasons for discontinuation were registered as miscellaneous.

Safety

AEs (type, frequency, duration, severity and relation to MTX or AZA) were determined. AEs that were transient and easily tolerated were considered mild. Moderate AEs were defined as causing discomfort and interrupting the subject's usual activities. AEs were severe if the event caused considerable interference with the subject's usual activities and could be incapacitating or life-threatening. A serious adverse event (SAE) was defined as life-threatening event, death, prolonged or initial hospitalization, disability or permanent damage. AEs were retrospectively coded according to the standardized and recognized Medical Dictionary for Regulatory Activities (MedDRA)/ Common Terminology Criteria for Adverse Events (CTCAE).²

Data collection

Treating physicians checked 3 monthly all prescribed concomitant medication (i.e. topical calcineurin inhibitors, topical and/or oral antibiotics, corticosteroids (rescue medication)) and AEs. When indicated routine laboratory evaluations were done (i.e. hematology, biochemistry and urinalysis (including serum pregnancy test)). Blood chemistry included procollagen-3 N-terminal peptide (P3NP) while on MTX and thiopurine methyltransferase (TPMT) before starting AZA. A yearly FibroScan to quantify liver fibrosis was performed when on MTX (FibroScan and P3NP data not shown). Treatment regimens were adjusted according to daily practice. Trained investigators evaluated 3 monthly the objective SCORAD, IGA and EASI. Patients evaluated the subjective SCORAD, PGA, POEM, pruritus and sleeplessness VAS and Skindex-17. Patients and treating physician were not blinded for allocation, the trained investigators were blinded for the allocated treatments.

Filaggrin

During this observational follow-up study a buccal swab was taken to investigate the *FLG* mutation status (R501X, 2282del4, R2447X, S3247X and 3321delA mutations). Patients were divided into a *FLG* mutation group (FMG) and non-FMG to assess the effect of *FLG* mutations on primary and secondary outcomes.

Statistical analysis

In the intention-to-treat (ITT) analysis we included all patients who participated in this observational follow-up study (35 patients), with the last available observation carried forward method between baseline and 2 years. In the per-protocol (PP) analysis we included patients who were continuously on the allocated systemic treatment for 2 years.

We used the T-test for normally distributed continuous outcome measures and Fisher's exact test for dichotomous outcome measures. AEs were analyzed by using the Chi-square test. Two-sided *P* values of less than .05 were considered to indicate statistical significance. Analyses were done in SPSS 22.0 for Windows (SPSS, Inc., Chicago, Ill).

1 **TABLE E1. Baseline characteristics of the included and non-included participants**

	MTX	AZA	Total	P-Value	Total	P- Value
	(n=17)	(n=18)	(n=35)	(of MTX vs AZA)	(n=7)	(of total included vs total not included)
Age (y), mean (SD)	43.3 (15.8)	39.5 (14.4)	41.3 (15)	0.46	32.4 (9.6)	0.14*
Male sex (%)	10 (59%)	11 (61%)	21 (60%)	1.00 [#]	1 (14%)	0.04[#]
Presence of asthma or allergic rhinitis (%)	16 (94%)	17 (94%)	33 (94%)	1.00 [#]	4 (57%)	0.03[#]
Duration of AD (y), mean (SD)	39.6 (17.5)	34.8 (18.1)	37.1 (17.7)	0.43*	31.9 (9.7)	0.45*
Outcomes						
SCORAD, mean (SD)						
-Total SCORAD, mean (SD)	59.4 (11.4)	59.7 (11.0)	59.5 (11.0)	0.93*	49.2 (5.8)	0.02*
-Objective SCORAD, mean (SD)	46.6 (10.3)	46.3 (10.1)	46.4 (10.1)	0.93*	38.5 (5.2)	0.052*
-Subjective SCORAD, mean (SD)	12.8 (4.0)	13.4 (3.4)	13.1 (3.6)	0.59*	10.7 (4.2)	0.10*
IGA, median (range)	4.0 (3.0-5.0)	4.0 (3.0-5.0)	4.0 (3.0-5.0)	0.60 [#]	3.0 (3.0-4.0)	0.82 [#]
PGA, median (range)	4.0 (3.0-5.0)	4.0 (2.0-5.0)	4.0 (2.0-5.0)	0.84 [#]	4.0 (3.0-4.0)	0.56 [#]
EASI, mean (SD)	29.2 (12.8)	31.1 (14.6)	30.2 (13.6)	0.67*	24.4 (10.8)	0.30*
POEM, mean (SD)	20.6 (5.2)	20.3 (3.5)	20.4 (4.36)	0.84*	15.7 (4.4)	0.01*
Skindex-17, mean (SD)	20.4 (6.2)	20.9 (5.1)	19.9 (5.5)	0.78*	20.7 (5.6)	0.72*

2 These baseline values apply to patients included in the observational follow-up study (n=35) and are values at the first visit of the original
3 study before starting MTX or AZA.

4 * T-test for independent groups; [#] Fisher's exact test.

1 **TABLE EIIA. Two years treatment and dosage overview: Methotrexate group**

Patient	Weight at BL	2w	4w	8w	3m	6m	9m	1y	15m	18m	21m	2y
1	73 kg	M10	M15	M20	M22.5	M20	M22.5	M22.5	M20	M20	M20	M17.5
2	87 kg	M10	M15	M20	M22.5	M22.5	M20	M20	M20	M20	M20	M17.5
3	59 kg	M10 ³	T	T	T	T	T	T	T	T	T	T
4	143 kg	M10	M15	M20	M22.5	M22.5	M20	M20	M20	M20	M20	M20
5	100 kg	M10	M15	M20	M22.5	M22.5	M10	M12.5	M12.5	M12.5	M12.5	M10
6	71 kg	M10	M15	M20	M22.5	M22.5	M20	M12.5	M12.5	M12.5	M12.5	M10
7	85 kg	M10	M15	M20	M22.5	M10	M12.5	M12.5	M15	M15	M15	M15
8	67 kg	M10	M15	M20	M22.5	M22.5	M12.5	M12.5	M15 ²	A50	A50	A50
9	55 kg	M10	M15	M15	M20	M20	M15	M15	M15	M12.5	M12.5	M10
10	94 kg	M10	M15	M20	M22.5	M20	M17.5	M10	M10	M10	M10 ²	T
11	76 kg	M10	M15	M15	M20	M15	M15 ³	T	T	T	T	T
12	64 kg	M10	M15	M15	M15	M15	M15	M15	M15	M12.5	M12.5	M12.5
13	65 kg	M10	M15	M20	M22.5	M22.5	M22.5	M15 ¹	T	T	T	T
14	85 kg	M10	M15	M20	M20	M20 ²	A150	A150 ⁴	T	T	T	T
15	85 kg	M10	M15	M20	M22.5	M22.5	M17.5	M15	M15	M15	M15	M12.5
16	52 kg	M10	M15	M20	M22.5	M17.5	M20	M20	M7.5 ¹	T	T	T
17	65 kg	M10	M15	M20	M22.5	M22.5	M22.5	M22.5	M17.5	M20	M20	M20
Mean dosis MTX												14.5 mg

2 A150, Azathioprine 150 mg; m, months; MTX, Methotrexate; M10, Methotrexate 10 mg; T, topical; w, week;; y, year.

3 Reasons for discontinuation of systemic treatment: 1) controlled AD, 2) inefficacy, 3) adverse event (AE), 4) miscellaneous.

4 Reasons to start systemic treatment: 5) relapse.

5 **SAE** Framed-months stands for the months in which the patient experienced a SAE.

1 **TABLE EIIB. Two years treatment and dosage overview: Azathioprine group**

Patient	Weight at BL	2w	4w	8w	3m	6m	9m	12m	15m	18m	21m	24m
1	75 kg	A125	A150	A175	A175	A175	A150	A150 ¹	T	T	T ⁵	A125
2	78 kg	A125	A150	A200	A200	A200 ¹	T	T	T ⁵	A100	A100	A100
3	55 kg	A50	A75 ³	M10	M15	M17.5	M17.5	M12.5	M12.5	M12.5	M10	M10
4	70 kg	A100	A150	A175	A200	A200 ¹	T	T	T	T	T	T
5	74 kg	A125	A150	A175	A175	A175	A125	A150	A175	A175	A175	A200
6	63 kg	A100	A125	A125	A125	A125 ²	MTX? ²	P ¹	T	P	P	P
7	87 kg	A125	A175	A175	A175	A200	A175	A175	A150	A150	A150	A175
8	73 kg	A100	A150	A175	A175 ²	M15 ²	C ²	T	T	T	T	T
9	89 kg	A125	A175	A225	A225	A200	A200	A200	A200	A200	A175	A100
10	68 kg	A100	A125	A150	A150	A150	A125	A125	A125	A150	A150	A150
11	81 kg	A125	A150	A200	A125	A125	A125 ¹	T	T	T	T ⁵	A100
12	79 kg	A125	A150	A200	A200	A200	A175	A200	A200	A200	A200	A200
13	105 kg	A150	A200	A275	A275	A250	A225	A250 ²	T	T	T	T
14	87 kg	A125	A150	A200	A200	A200	A200	A150	A175	A150	A125	A125
15	95 kg	A150	A125	A175	A175	A150	A150	A150	A125	A125	A150	A100
16	65 kg	A100	A125	A125	A125 ²	T	T	T	T	T	T	T
17	68 kg	A100	A100	A100	A100	A75	A50	A25 ¹	T	T	T	T
18	90 kg	A125	A175	A175	A175	A175	A175	A175	A150	A150	A125	A125
Mean dosis AZA												136.4 mg

2 AZA, Azathioprine; A150, Azathioprine 150 mg; C, Cellcept; m, months; MTX?, Methotrexate unknown dose; M10,
3 Methotrexate 10 mg; P, Prednisolone; T, topical; w, week.

4 Reasons for discontinuation of systemic treatment: 1) controlled AD, 2) inefficacy, 3) adverse event (AE), 4) miscellaneous.

5 Reasons to start systemic treatment: 5) relapse.

6 **SAE** Framed-months stands for the months in which the patient experienced a SAE.

1 **TABLE EIIIA Adverse events and other key safety data up to 2 years after baseline in patients (intention-to-treat analysis)**

	Total AEs	Total AEs/pt	Total AEs MTX group	Total AEs MTX group/pt	Total AEs AZA group	Total AEs AZA group/pt	P-value
	(n=35)	(n=35)	(n=17)	(n=17)	(n=18)	(n=18)	
AE, total (events)*, no. of pt	422	1.06	220	12.94	202	11.22	0.14
- Mild, no. of pt	372	10.63	198	11.65	174	9.67	0.07
- Moderate, no. of pt	45	1.29	21	1.24	24	1.33	0.80
- Severe, no. of pt	5	0.14	1	0.06	4	0.22	0.20
SAE, total (events)*, no. of pt	3	0.09	2	0.12	1	0.06	0.53
Treatment-related AE [§] , no. of pt	252	7.20	133	7.82	119	6.61	0.18
AE requiring dose adjustment, no. of pt	6	0.17	4	0.24	2	0.11	0.38
Type of AE coded according to MedDRA, no. of pt							
Blood and lymphatic system disorders	34	0.97	11	0.65	23	1.28	0.058
Cardiac disorders	9	0.26	4	0.24	5	0.28	0.80
Eye disorders	7	0.20	5	0.29	2	0.11	0.23
Gastrointestinal disorders	44	1.26	26	1.53	18	1.00	0.16
General disorders and administration site conditions	46	1.31	22	1.29	24	1.33	0.92
Hepatobiliary disorders	20	0.57	11	0.65	9	0.50	0.57
Immune system disorders	1	0.03	0	0.00	1	0.06	0.33
Infections and infestations	98	2.80	52	3.06	46	2.56	0.37
• Ear and Labyrinth disorders	2	0.06	1	0.06	1	0.06	0.97
• Eye disorders	2	0.06	2	0.12	0	0.00	0.15
• General disorders and administration site conditions	11	0.31	2	0.12	9	0.50	0.04
• Musculoskeletal and connective tissue disorders	9	0.26	3	0.18	6	0.33	0.36
• Neoplasms benign, malignant and unspecified **	4	0.11	4	0.24	0	0.00	0.04
• Renal and urinary disorders	5	0.14	4	0.24	1	0.06	0.16
• Reproductive system and breast disorders	1	0.03	0	0.00	1	0.06	0.33
• Respiratory, thoracic and mediastinal disorders	27	0.77	15	0.88	12	0.67	0.46
• Skin and subcutaneous tissue disorders	37	1.06	21	1.24	16	0.89	0.31
- Bacterial	19	0.54	11	0.65	8	0.44	0.41
- Viral	10	0.29	5	0.29	5	0.28	0.93
- Fungal	6	0.17	3	0.18	3	0.17	0.94
Injury, poisoning and procedural complications	8	0.23	4	0.24	4	0.22	0.94
Metabolism and nutrition disorders	1	0.03	0	0.00	1	0.06	0.33
Musculoskeletal and connective tissue disorders	28	0.80	16	0.94	12	0.67	0.36
Neoplasms benign, malignant and unspecified **	9	0.26	3	0.18	6	0.33	0.36
Nervous system disorders	34	0.97	20	1.18	14	0.78	0.23
Psychiatric disorders	2	0.06	0	0.00	2	0.11	0.17
Renal and urinary disorders	11	0.31	6	0.35	5	0.28	0.69
Reproductive system and breast disorders	4	0.11	2	0.12	2	0.11	0.95
Respiratory, thoracic and mediastinal disorders	16	0.46	10	0.59	6	0.33	0.27
Skin and subcutaneous tissue disorders	47	1.34	26	1.53	21	1.17	0.36
Surgical and medical procedures	3	0.09	2	0.12	1	0.06	0.53
Type of SAE coded according to MedDRA, no. of pt							
Injury, poisoning and procedural complications	1	0.03	1	0.06	0	0.00	0.30
Skin and subcutaneous tissue disorders	2	0.06	1	0.06	1	0.06	0.97

2 *Adverse events were classified as mild, moderate, severe or serious according to predefined definitions (see the Methods section) . **No
3 malignant neoplasm were found in both groups. [§] Treatment-related adverse events are those classified as possibly, probably, or definitely
4 related to the study drug by the safety assessor.

5 AE, Adverse Event; AZA, Azathioprine; MedDRA, Medical Dictionary for Regulatory Activities; MTX, Methotrexate; SAE, Serious adverse
6 events; pt, patient.

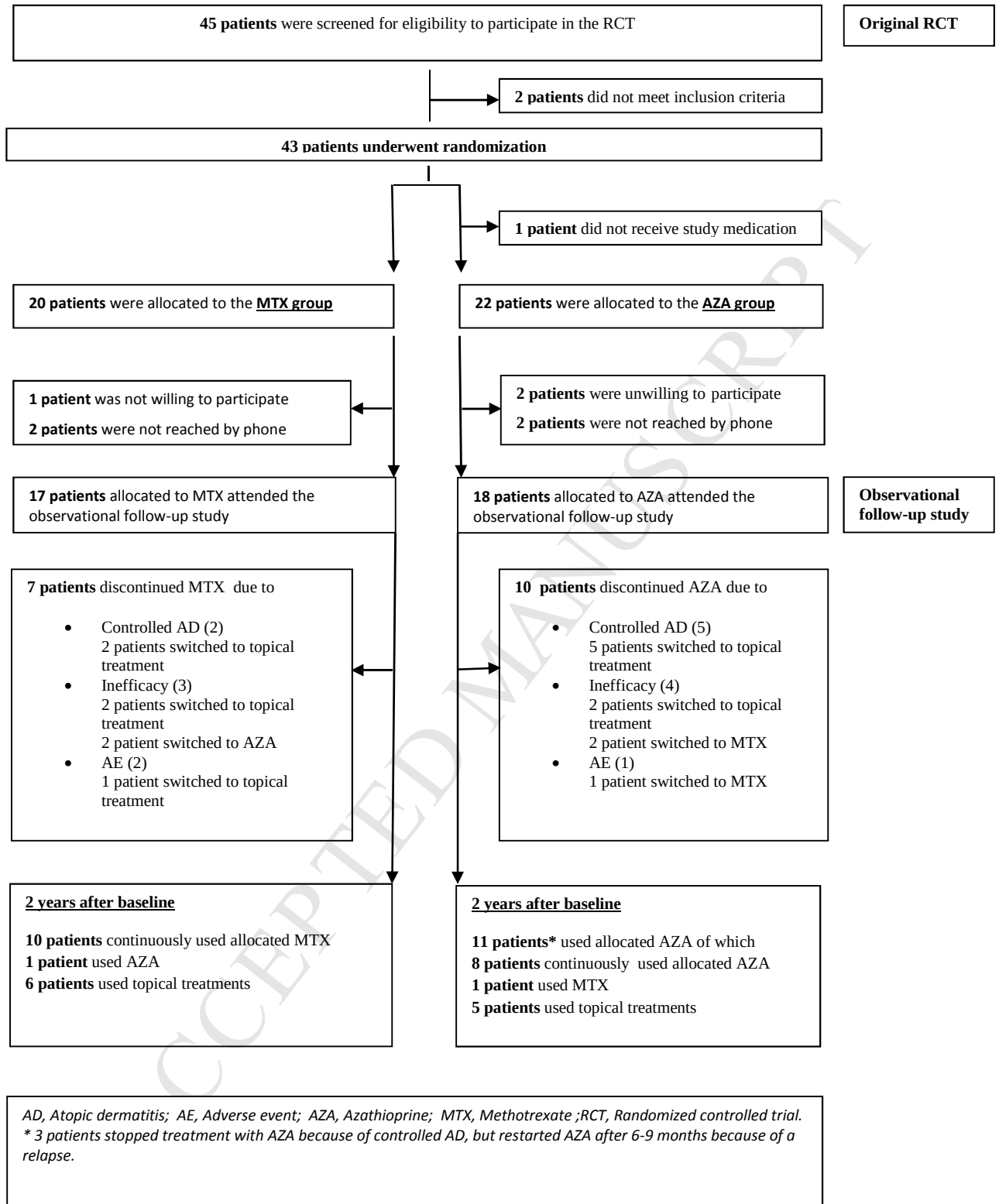
TABLE EIIIB. Adverse events and other key safety data up to 2 years after baseline in patients who were continuously on allocated treatment (per-protocol analysis)

	Total AEs (n=18)	Total AEs/pt (n=18)	Total AEs MTX group (n=10)	Total AEs MTX group/pt (n=10)	Total AEs AZA group (n=8)	Total AEs AZA group/pt (n=8)	P-value MTX vs AZA
Total AE*, no. of pt	203	11.3	122	12.2	81	10.1	0.19
• Mild, no. of pt	183	10.2	107	10.7	76	9.5	0.43
• Moderate, no. of pt	18	1	15	1.5	3	0.4	0.02
• Severe, no. of pt	2	0.1	0	0	2	0.3	0.11
Total serious AE*, no. of pt	2	0.1	1	0.1	1	0.1	0.87
Treatment-related AE §, no. of pt	131	7.3	76	7.6	55	6.9	0.57
AE requiring dose adjustment, no. of pt	4	0.2	3	0.3	1	0.1	0.43
Type of AE coded according to MedDRA, no. of pt							
Blood and lymphatic system disorders	13	0.7	5	0.5	8	1	0.22
Cardiac disorders	4	0.2	1	0.1	3	0.4	0.22
Eye disorders	4	0.2	3	0.3	1	0.1	0.43
Gastrointestinal disorders	23	1.3	16	1.6	7	0.9	0.18
General disorders and administration site conditions	24	1.3	9	0.9	15	1.9	0.08
Hepatobiliary disorders	13	0.7	11	1.1	2	0.3	0.04
Immune system disorders	1	0.1	0	0	1	0.1	0.26
Infections and infestations	44	2.4	29	2.9	15	1.9	0.17
• Eye disorders	1	0.1	1	0.1	0	0	0.37
• General disorders and administration site conditions	7	0.4	1	0.1	6	0.8	0.03
• Musculoskeletal and connective tissue disorders	2	0.1	0	0	2	0.3	0.11
• Neoplasm benign, malignant and unspecified **	1	0.1	1	0.1	0	0	0.37
• Renal and urinary disorders	3	0.2	3	0.3	0	0	0.12
• Reproductive system and breast disorders	1	0.1	0	0	1	0.1	0.26
• Respiratory, thoracic and mediastinal disorders	14	0.8	12	1.2	2	0.3	0.02
• Skin and subcutaneous tissue disorders	15	0.8	11	1.1	4	0.5	0.17
- Bacterial	11	0.6	7	0.7	4	0.5	0.59
- Viral	3	0.2	3	0.3	0	0	0.12
Injury, poisoning and procedural complications	5	0.3	4	0.4	1	0.1	0.27
Metabolism and nutrition disorders	1	0.1	0	0	1	0.1	0.26
Musculoskeletal and connective tissue disorders	12	0.7	7	0.7	5	0.6	0.85
Neoplasms benign, malignant and unspecified **	8	0.4	3	0.3	5	0.6	0.30
Nervous system disorders	18	1	11	1.1	7	0.9	0.64
Renal and urinary disorders	7	0.4	4	0.4	3	0.4	0.93
Respiratory, thoracic and mediastinal disorders	10	0.6	7	0.7	3	0.4	0.36
Skin and subcutaneous tissue disorders	14	0.8	10	1	4	0.5	0.23
Surgical and medical procedures	2	0.1	2	0.2	0	0	0.21
Type of SAE coded according to MedDRA, no. of pt							
Injury, poisoning and procedural complications	1	0.06	1	0.1	0	0	0.37
Skin and subcutaneous tissue disorders	1	0.06	0	0	1	0.1	0.26

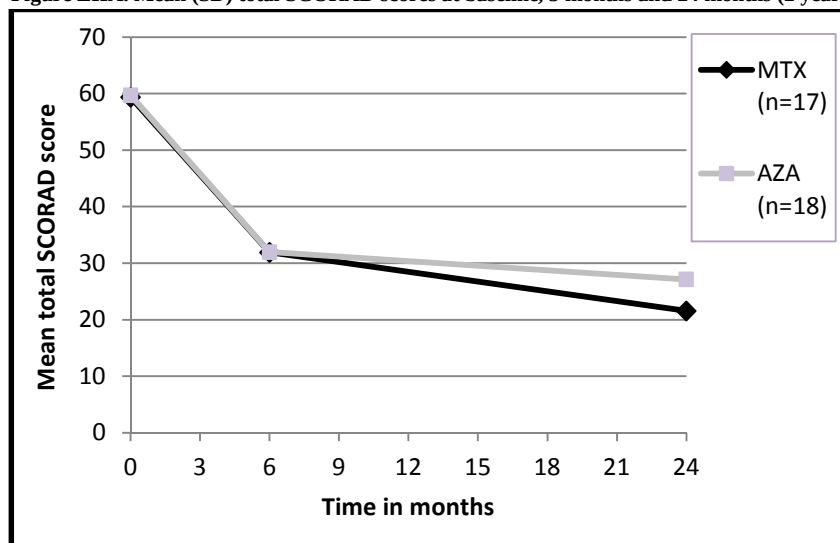
*Adverse events were classified as mild, moderate, severe or serious according to predefined definitions (see the Methods section). **No malignant neoplasm were found in both groups. § Treatment-related adverse events are those classified as possibly, probably, or definitely related to the study drug by the safety assessor.

AE, Adverse event; AZA, Azathioprine; MedDRA, Medical Dictionary for Regulatory Activities; MTX, Methotrexate; SAE, Serious adverse event; pt, patient.

Figure E1. Flowchart

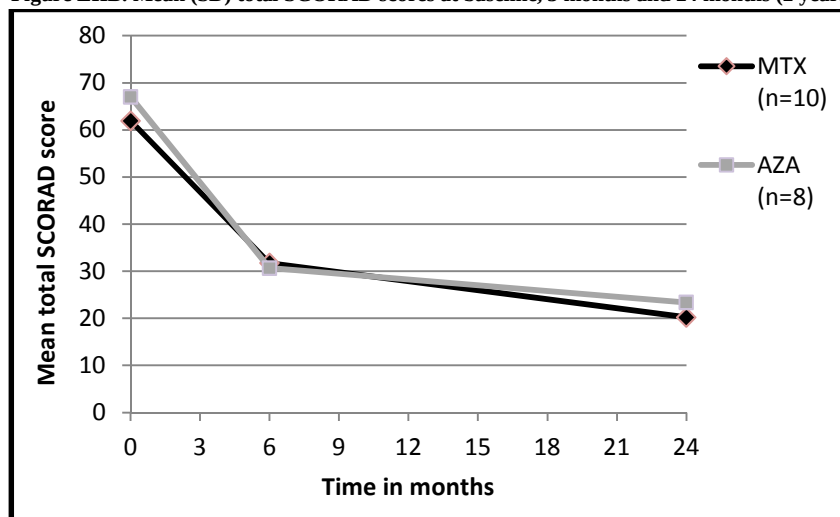


1 Figure EIIA. Mean (SD) total SCORAD scores at baseline, 3 months and 24 months (2 years) (intention-to-treat analysis)



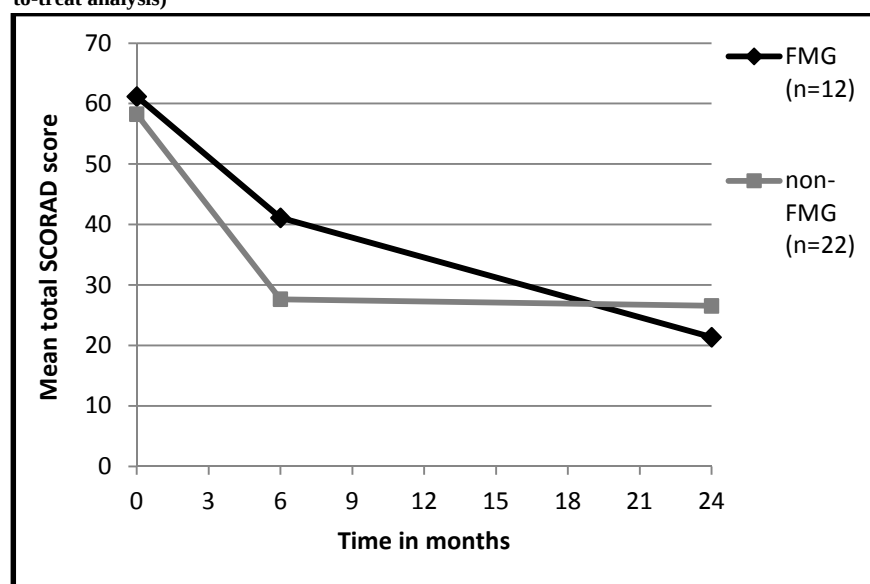
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3 AZA, Azathioprine; MTX, Methotrexate.

1 Figure EIIB. Mean (SD) total SCORAD scores at baseline, 3 months and 24 months (2 years) (per-protocol analysis)



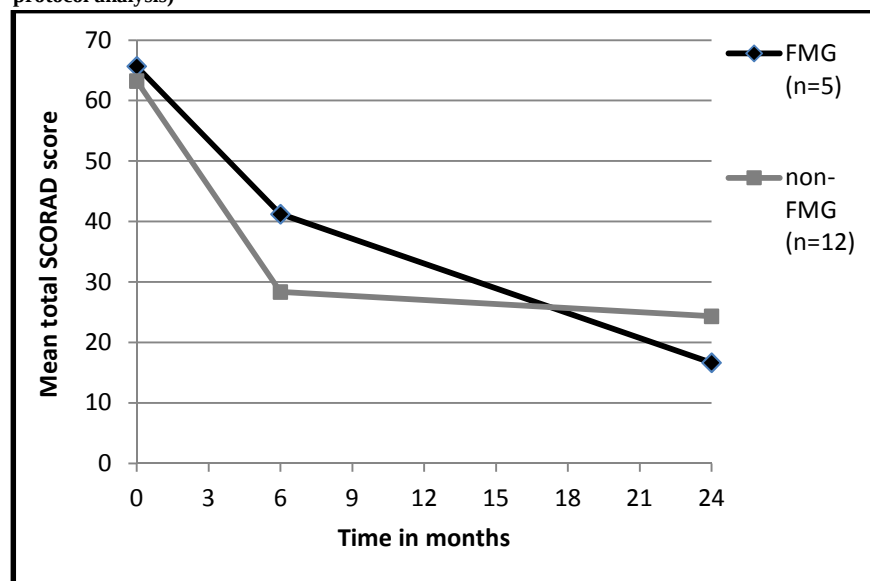
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3 AZA, Azathioprine; MTX, Methotrexate

Figure EIIIA. Mean (SD) SCORAD scores up to 2 years after baseline in patients with and without a filaggrin mutation (intention-to-treat analysis)



FMG, filaggrin mutation group; non-FMG, non filaggrin mutation group.

Figure E111B. Mean (SD) SCORAD scores up to 2 years after baseline in patients with and without a filaggrin mutation (per-protocol analysis)



FMG, filaggrin mutation group; non-FMG, non filaggrin mutation group.