

Effect of Oral Administration of a Mixture of Probiotic Strains on SCORAD Index and Use of Topical Steroids in Young Patients With Moderate Atopic Dermatitis

A Randomized Clinical Trial

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 [Supplemental content](#)

IMPORTANCE Oral intake of new probiotic formulations may improve the course of atopic dermatitis (AD) in a young population.

OBJECTIVE To determine whether a mixture of oral probiotics is safe and effective in the treatment of AD symptoms and to evaluate its influence on the use of topical steroids in a young population.

DESIGN, SETTING, AND PARTICIPANTS A 12-week randomized, double-blind, placebo-controlled intervention trial, from March to June 2016, at the outpatient hospital Centro Dermatológico Estético de Alicante, Alicante, Spain. Observers were blinded to patient groupings. Participants were children aged 4 to 17 years with moderate atopic dermatitis. The groups were stratified and block randomized according to sex, age, and age of onset. Patients were ineligible if they had used systemic immunosuppressive drugs in the previous 3 months or antibiotics in the previous 2 weeks or had a concomitant diagnosis of intestinal bowel disease or signs of bacterial infection.

INTERVENTIONS Twelve weeks with a daily capsule containing freeze-dried powder with 10^9 total colony-forming units of the probiotic strains *Bifidobacterium lactis* CECT 8145, *B longum* CECT 7347, and *Lactobacillus casei* CECT 9104 and maltodextrin as a carrier, or placebo (maltodextrin-only capsules).

MAIN OUTCOMES AND MEASURES SCORAD index score and days of topical steroid use were analyzed.

RESULTS Fifty children (26 [50%] female; mean [SD] age, 9.2 [3.7] years) participated. After 12 weeks of follow-up, the mean reduction in the SCORAD index in the probiotic group was 19.2 points greater than in the control group (mean difference, -19.2; 95% CI, -15.0 to -23.4). In relative terms, we observed a change of -83% (95% CI, -95% to -70%) in the probiotic group and -24% (95% CI, -36% to -11%) in the placebo group ($P < .001$). We found a significant reduction in the use of topical steroids to treat flares in the probiotic arm (161 of 2084 patient-days [7.7%]) compared with the control arm (220 of 2032 patient-days [10.8%]; odds ratio, 0.63; 95% CI, 0.51 to 0.78).

CONCLUSIONS AND RELEVANCE The mixture of probiotics was effective in reducing SCORAD index and reducing the use of topical steroids in patients with moderate AD.

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Atopic dermatitis (AD) is a chronic recurrent inflammatory skin disease characterized by intense pruritus, inflammation, and skin barrier disruption. The prevalence of AD is approximately 3% to 10% in adults and up to 20% in children worldwide.^{1,2} The first symptoms usually develop during childhood, and approximately 50% of cases are diagnosed in the first year of life. Atopic dermatitis significantly reduces the quality of life of patients and their families. Moreover, patients with AD have an increased risk of other atopic disorders, including asthma, allergic rhinitis, and chronic sinusitis.^{3,4}

As occurs in other atopic disorders, a predominance of T helper 2 cells rather than T helper 1 causes an imbalance that might also aggravate the pathogenesis of AD, increasing IgE and activating interleukins.^{5,6} Another point to consider is skin barrier integrity. Filaggrin interacts with intermediate filaments, particularly keratins, causing their aggregation into macrofibrils. Defects in filaggrin cause dysfunctions in the skin barrier, resulting in decreased protection from microbes and allergens.⁷

Clinically, cutaneous manifestations related to AD include erythema, edema and/or papules, exudate, excoriation, and lichenification, as well as the resulting symptoms (pruritus and loss of sleep).^{3,8} Topical corticosteroids have been the keystone of pharmacological treatments for mild to moderate AD. Alternatively, cases of moderate-to-severe AD are treated with long-term applications of topical corticosteroids,⁹ with topical calcineurin inhibitors providing second-line effective agents. However, long-term data for these medications are lacking for pediatric patients.¹⁰ In cases of severe refractory disease, patients might benefit from a short course of systemic therapy with immunosuppressants such as corticosteroids, cyclosporine, and azathioprine, but these drugs have a potentially more severe adverse effect profile and risks of rebound after treatment discontinuation.^{11,12}

During recent years, many authors have suggested an association between a disruption in intestinal barrier function and the origin of AD, mediated by immunological activation leading to a type 2 dominant inflammation.¹³ In this respect, gut microbiota may play an important immunomodulatory role in the development of normal immune tolerance.^{14,15} Recently an analysis of the gut microbiota of patients with AD has shown an intraspecies compositional change in *Faecalibacterium prausnitzii* that reduces the number of high butyrate and propionate producers.¹⁶ Butyrate and propionate are microbial-produced short-chain fatty acids with an anti-inflammatory role.¹⁷ Moreover, butyrate has been shown to be a key player in maintaining gut barrier integrity.¹⁸ Therefore, reduced levels in the microbiota of both butyrate and propionate producers may result in a pro-inflammatory state in the gut and a loss of barrier integrity. All these data indicate the potential role of probiotics as microbiota recovery players, and consequently as potential nutritional supplements in AD treatment.¹⁹⁻²¹

The primary goal of the present study was to determine the efficacy of a mixture of probiotics in improving the SCORAD (Scoring Atopic Dermatitis) index²² and in reducing the percentage of days with topical steroid treatment during flares in patients with moderate AD.

Key Points

Question Can treatment with an oral probiotic reduce the SCORAD index and the use of topical steroids in children with moderate atopic dermatitis?

Findings This randomized clinical trial of 50 children treated with a mixture of probiotics or placebo for 12 weeks found that SCORAD and topical steroid use decreased significantly in the probiotic group compared with the placebo group.

Meaning This probiotic is an effective and safe adjuvant treatment to reduce the SCORAD index and topical steroid use in children with moderate atopic dermatitis.

Methods

Study Design

The study design was a double-blind, 2-arm placebo-controlled trial with stratified randomization by baseline variables (ratio, 1:1). The study received approval from the Ethics Committee for Clinical Research of the Hospital General Universitario de Alicante, and the Spanish Medicines Agency. There was no change to the trial protocol ([Supplement 1](#)) after it commenced.

Study Population

Inclusion criteria of the protocol were as follows: children between 4 and 17 years old, with a diagnosis of AD that meets the Hanifin and Rajka criteria (eTable 1 in [Supplement 2](#)) and moderate SCORAD index (from 20 to 40)²² who had been prescribed topical steroids for the treatment of AD. Participants were also required to be currently consuming a high-quality Mediterranean diet with a Mediterranean Diet Quality Index (KIDMED)²³ score more than 7. Written informed consent was obtained from parents or a legal representative (and the child if >12 years).

Among the exclusion criteria, patients were ineligible for the study if they had used systemic corticosteroids, methotrexate, cyclosporine, or anti-tumor necrosis factor drugs in the previous 3 months, antibiotics in the previous 2 weeks, or had a concomitant diagnosis of intolerance to gluten and/or lactose or signs of bacterial infection (the Trial Protocol in [Supplement 1](#) provides a complete list of exclusion criteria).

Recruitment and Randomization of Participants

Children aged 4 to 17 years were recruited between March and June 2016 from a single outpatient dermatological clinic where 3 dermatologists (A.R.-B. and 2 others) with expertise in pediatric dermatology evaluated their AD and SCORAD index. After informed consent was obtained from their legal representatives, participants completed the dietary screener KIDMED short questionnaire for quality of the Mediterranean diet (eTable 2 in [Supplement 2](#)).²³

Each child was classified into 1 of the 8 possible strata combining 3 binary variables at baseline: sex, age (4-12 vs >12 years), age of onset (0-4 vs >4 years). Patients were then assigned to 1 of the 2 trial arms (probiotics or control) following a comput-

erized randomization list that been previously prepared for each stratum by the principal investigator of the study (V.N.-L.).

Interventions

All patients received treatment during the 12-week study period with topical methylprednisolone aceponate, moisturizer, and 1 oral antihistamine, according to the guidelines for the management of AD.⁹ Participants in the probiotic group received daily a pill containing 10^9 colony-forming units (CFUs) of a mixture of the 3 probiotic strains in a 1:1:1 ratio, freeze-dried powder with maltodextrin as a carrier, and participants in the placebo group received a pill containing only maltodextrin. The probiotic and placebo pills were matched for size, shape, and volume of contents (gelatin capsules of 9.85×16.4 mm) and were dispensed by the pharmacy department staff.

The product was designed not as a single strain but as a bacteria mixture with lactobacilli and bifidobacteria because previous AD clinical trials with probiotics have obtained better results with mixed products.²⁴ The mixture was composed of *Bifidobacterium lactis* CECT 8145, *B longum* CECT 7347, and *Lactobacillus casei* CECT 9104. The blend was selected on the basis of previous data: oxidative stress has been associated with AD,²⁵ and strain *B lactis* CECT 8145 has previously shown antioxidant properties in the *Caenorhabditis elegans* model.²⁶ Strain *B longum* CECT 7347 has an anti-inflammatory profile, previously demonstrated in both preclinical and clinical studies,^{27,28} and it modulates the gut microbiota.²⁸ Finally, strain *L casei* CECT 9104 has in vitro activity against certain gut pathogens (E.C.-C., S.G.-M., D.R.-V., unpublished data, June 2009).

Outcome Measurements

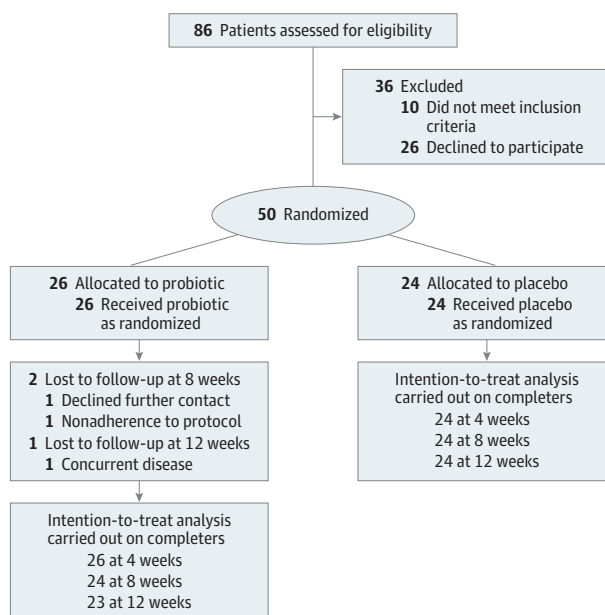
Two primary outcomes were compared between the trial arms to assess the treatment effect: (1) change in SCORAD index between baseline and 12 weeks of follow-up and (2) the proportion of days of topical steroid use during flares within the 12 weeks of follow-up. The SCORAD index was measured at the time of inclusion and every 4 weeks until the end of the 12-week follow-up period. The number of days of topical steroid use during flares was recorded biweekly until week 12. A flare was defined as a worsening of the disease leading to use of topical corticosteroids for at least 3 consecutive days (so steroid use on only 1 or 2 isolated days was not considered a flare).

Secondary outcomes were laboratory values. Peripheral blood samples were collected at baseline and after 12 weeks of treatment and analyzed for routine biochemical laboratory values and for interleukin 4 (IL-4), IL-5, IL-10, and IL-13 levels.

Sample Size

According to the trial protocol, 25 patients per group would be needed to detect a difference of clinical improvement as evaluated by the SCORAD index, assuming 5% loss to follow-up, type I error of .05, and 80% power to detect a difference of 4.13 units on the SCORAD index with a standard deviation of 5 units between the 2 groups on a 2-sided *t* test.

Figure 1. CONSORT Diagram



Statistical Analysis

Data were analyzed as intention to treat, and the last available value was not carried forward for missing observations. Quantitative variables were summarized as means and standard deviations or median and interquartile range, and categorical variables were summarized with proportions.

We used linear mixed-effects models with a group-by-week interaction term to compare the mean SCORAD score between groups at weeks 4, 8, and 12. Participant-level random intercepts accounted for the correlation due to repeated measurements. The model was adjusted for variables used to stratify randomization (age at recruitment, sex, and age at onset). Residuals were examined visually and tested for normality with the Kolmogorov-Smirnov test. To analyze the proportion of days of topical steroid use, we used logistic regression to model the odds per individual with treatment group as the main explanatory variable and adjusted for the variables used for stratified randomization.

Laboratory determinations from blood samples were log-transformed to achieve more normally distributed variables and avoid extreme values. They were then analyzed with linear mixed-effects models (as for the SCORAD although with only 2 time observations, baseline and end of study).

We followed CONSORT recommendations for reporting of randomized clinical trials (Figure 1). All statistical analyses were performed using IBM SPSS statistics, version 22 (SPSS Inc), and R, version 3.2.3.

Results

From March to June 2016, 50 children with a diagnosis of AD were enrolled in the study. All met the criteria for a high-quality Mediterranean diet (KIDMED score >7). There were no

Table 1. Baseline Clinical Characteristics of Study Participants in the Placebo and Probiotic Groups

Characteristic	Probiotic Group (n = 26)	Placebo Group (n = 24)
Female sex, No. (%)	13 (50)	13 (54)
Age, mean (SD), y	9.35 (3.58)	8.96 (3.94)
Race/ethnicity, No. (%)		
White	25 (96)	24 (100)
Black, No. (%)	1 (4)	0
Hispanic, No. (%)	26 (100)	23 (96)
Weight, mean (SD), kg	34.80 (14.77)	38.33 (14.54)
Height, mean (SD), cm	136.31 (19.69)	137.58 (22.72)
KIDMED index, mean (SD)	8.6 (0.66)	8.7 (0.63)
SCORAD, mean SD		
Total	33.58 (3.38)	31.64 (5.05)
Extension	15.58 (5.65)	14.25 (5.49)
Intensity	24.5 (2.62)	22.75 (3.50)
Subjective symptoms	5.96 (2.34)	6.04 (2.22)
Investigator Global Assessment index, median (IQR)	3 (2-3)	3 (2-3)
Eosinophil count, mean (SD), / μ L	300 (545)	390 (465)
Serum IgE, mean (SD), μ g/L	989 (1714)	773 (1528)
Lactate dehydrogenase, mean (SD), U/L	537.42 (123.56)	544.91 (140.34)
C-reactive protein, median (IQR), mg/L	0.25 (0.25-0.25)	0.25 (0.25-0.25)
Mean (SD), pg/mL		
IL-4	32.19 (8.35)	30.11 (4.56)
IL-5	4.35 (0.68)	4.34 (0.45)
IL-10	13.68 (5.08)	12.31 (3.41)
IL-13	85.52 (12.48)	81.82 (13.24)

Abbreviations: IL, interleukin; IQR, interquartile range; SCORAD, Scoring Atopic Dermatitis.

SI conversion factors: To convert c-reactive protein to nanomoles per liter, multiply by 9.524; to convert eosinophil count to billions per liter, multiply by 0.001; to convert IgE to milligrams per liter, multiply by 0.001; to convert lactate dehydrogenase to microkatal per liter, multiply by 0.0167.

significant differences in the baseline characteristics between the 2 groups (Table 1). Three of the patients in the probiotic group failed to provide data in some of the visits. Two of them declined contact in the second and third visit due to a work-related problem of the parents. Another patient had a concurrent disease unrelated to the treatment and could not provide data on the third visit. All reasons for missing data were unrelated to the treatment or the outcome, so we do not expect effect bias. Because missing data were only 2.5% of follow-up measurements, we decided not to use it and we used only observed data. Patients completed the KIDMED test at inclusion and repeated the test at the end of the intervention. The data show no relevant differences in the KIDMED score between the trial arms at baseline (Table 1) or end of the study (mean [SD] score, 8.7 [0.63] vs 8.6 [0.66] for the placebo and treatment groups, respectively).

Effects on Disease Severity

Twenty-two of 23 (96%) patients in the probiotic group and 11 of 24 (46%) in the placebo group improved in the SCORAD in-

dex. The mean reduction and 95% confidence interval for the SCORAD index and its components (eczema extension, eczema intensity, and subjective symptoms) for different weeks are shown in Figure 2, whereas the estimated changes in each trial arm and the differences between the 2 arms (intervention effect) are presented in Table 2 for the total SCORAD index, eTable 4 in Supplement 2 for the subcomponents, and eFigure 1 in Supplement 2 for all. These eTables also give the changes as percentages over baseline values of the variable. At baseline, there were no large differences between arms in any of the variables. As time passed after starting treatment, the differences between arms increased, with better improvements in the probiotic group for total SCORAD, eczema intensity, and eczema spread (with CI excluding the null) but not for subjective symptoms. After 12 weeks of follow-up, the mean change in the SCORAD index (main outcome) was, in relative terms, -83% (95% CI, -95% to -70%) in the probiotic group and -24% (95% CI, -36% to -11%) in the placebo group. This is a difference in effectiveness of -19.2 (95% CI, -23.4 to -15.0) SCORAD points, or -59% (95% CI, -72% to -46%; $P < .001$), in favor of the probiotic. Patients in both probiotic and placebo groups showed improvement in atopic dermatitis disease activity as measured with Investigator Global Assessment (IGA) over the 12-week study period. Comparison between groups showed significant differences in the number of patients with response to treatment: 21 patients (91%) in the probiotic group achieved IGA scores of 0 or 1 (95% CI, 72%-99%) and 5 patients (21%; 95% CI, 7%-42%) achieved these scores in the placebo group. The percentage of patients with IGA score less than 2, measured at each checkup in weeks 4, 8, and 12, is included in eFigure 2 in Supplement 2.

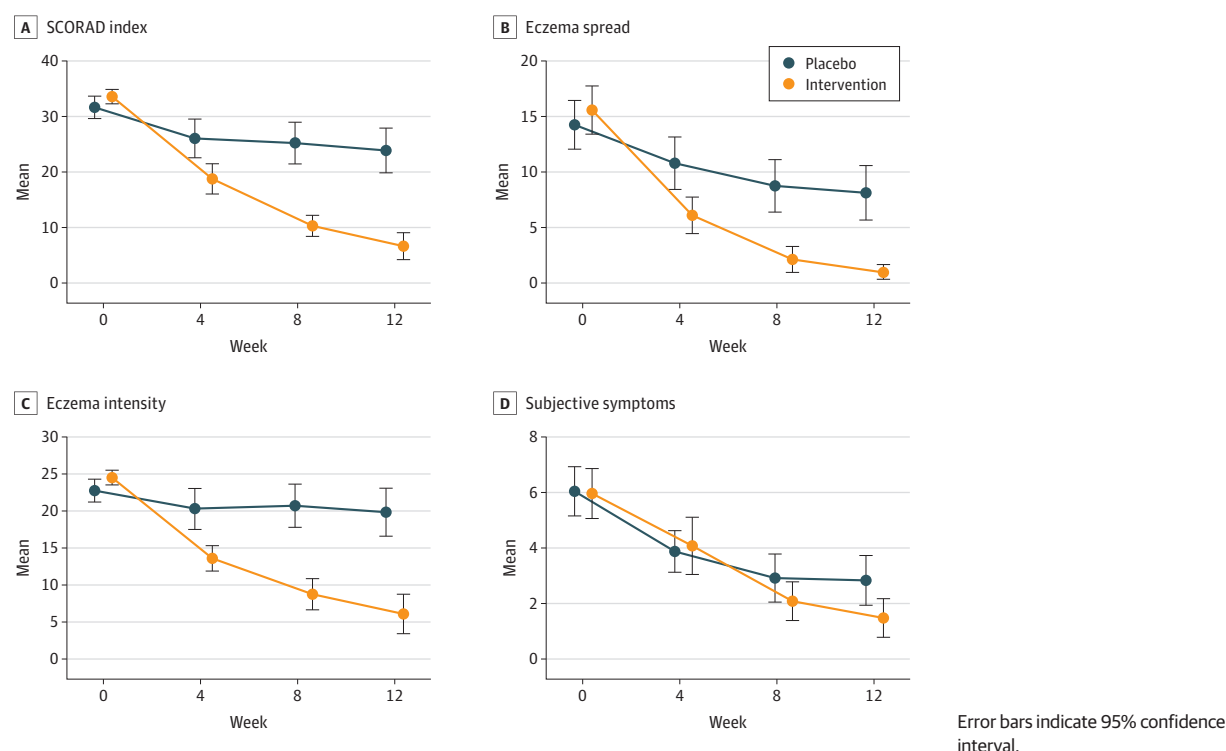
Effect on Topical Use of Steroids

At the end of follow-up, there were 2032 patient-days of observation in the placebo group and 2084 patient-days in the probiotic group. Steroids were used to treat flares on 220 (10.8%) and 161 (7.7%) patient-days, respectively. The distribution of number of days per individual did not seem to follow a Poisson distribution (eFigure 3 in Supplement 2). Considering that follow-up was approximately 84 days, some individuals used steroids for a large proportion of them. The logistic regression adjusted for baseline stratification variables estimated an odds ratio of 0.63 (95% CI, 0.51-0.78; $P < .001$) for the effect of the treatment on the reduction of use of corticosteroids. We also did a sensitivity analysis comparing the total number of days of steroid use (including nonflare use). The placebo arm had 336 patient-days of use (16.5%) whereas the probiotic arm had 291 patient-days of use (14.0%). The adjusted logistic regression model produced an odds ratio of 0.77 (95% CI, 0.65-0.91; $P < .003$), a statistically significant finding of less corticosteroid use in the probiotic group.

Comparison of Blood Marker Levels

Baseline blood marker levels of patients in the probiotic and placebo groups are given in Table 1. During the 12-week intervention period, no significant differences were observed between the 2 groups in the changes in blood levels of IL-4, IL-5, IL-10, IL-13, eosinophils, IgE, and lactate dehydrogenase (eTable 3 in Supplement 2).

Figure 2. Change in SCORAD Index and Its Components

Table 2. Estimation of the Effect of Intervention on the Scoring Atopic Dermatitis (SCORAD) Scale^a

Parameter	Baseline		Change From Baseline					
	Placebo (n = 24)	Probiotic (n = 26)	Week 4 (n = 24)	Probiotic (n = 26)	Week 8 (n = 24)	Probiotic (n = 24)	Week 12 (n = 24)	Probiotic (n = 23)
SCORAD score	32.2 (27.4 to 37.0)	33.8 (29.0 to 38.5)	-5.6 (-9.8 to -1.4)	-14.8 (-18.8 to -10.8)	-6.4 (-10.6 to -2.3)	-23.2 (-27.3 to -19.1)	-7.8 (-11.9 to -3.6)	-27.0 (-31.1 to -22.8)
Proportional effect, % ^b	N/A	N/A	-17 (-30 to -4)	-45 (-58 to -33)	-20 (-32 to -7)	-71 (-84 to -59)	-24 (-36 to -11)	-83 (-95 to -70)
Difference								
SCORAD score	1.5 (-2.3 to 5.3)		-9.2 (-13.4 to -5.1)		-16.7 (-20.9 to -12.6)		-19.2 (-23.4 to -15.0)	
Proportional effect, % ^b	N/A		-28 (-41 to -16)		-51 (-64 to -39)		-59 (-72 to -46)	

Abbreviation: N/A, not applicable.

^a Effects are estimated using linear mixed-effects models with a group-by-week interaction, participant-level random intercepts, and adjusted for variables used to stratify randomization.

^b These rows are the effects on the rows above divided by baseline SCORAD mean of all participants (32.6) and multiplied by 100. This yields the proportional effect, or percentage effectiveness.

Adverse Events

No relevant adverse events were associated with drug or placebo intake.

Discussion

Several important studies have explored the efficacy of certain probiotics in the prevention and treatment of AD. Overall, the current evidence suggests that probiotics could be an option to improve moderate and severe AD recovery rates in children and adults; however, to date, there is no strong experimental evidence supporting their effectiveness and safety

in clinical practice.²⁹ Importantly, evidence and clinical trials demonstrating strain-specific effects are lacking.³⁰⁻³³

The clinical trial reported herein explores the role of a mixture of probiotics administered to patients with moderate AD. Several variables, such as antibiotic use, diet, and other concomitant allergic diseases were controlled for, and, to avoid bias, all cases included in the study were matched for these variables. Our results suggest that administration of this mixture of probiotics, as adjuvant treatment, may be effective in reducing the SCORAD index and, subsequently, decreasing the use of steroids during AD flares. The response rate was significant when the reduction in baseline SCORAD index was compared between groups (Table 2). The clinical response docu-

mented in the probiotic group was greater than that obtained with other probiotics tested in a previous placebo-controlled clinical trial.³²⁻⁴³ This study showed better response in SCORAD (83% relative reduction) than previously communicated results by Farid et al⁴⁴ (68% of response), Iemoli et al⁴⁵ (63%), or Yeşilova et al (64%).⁴⁶ Several factors may influence the response to probiotic treatment in AD, and they were taken into account when the protocol of this clinical study was designed: treatment longer than 8 weeks might condition the positive effect of probiotic use,⁴⁷ patients older than 1 year have a greater response to probiotics,^{35,36,38,47} patients with moderate to severe AD have a better response,²⁰ and a mixture of probiotics has better beneficial effects than a single probiotic, especially when lactobacilli and bifidobacteria are included in the mixture.^{20,44,45} The final blend used in the study was selected on the basis of published results and internal unpublished data, as described in the Methods.²⁴⁻²⁸

Two of 3 subcomponents of the SCORAD index (eczema spread and intensity) showed a clear improvement in favor of the probiotic group compared with the placebo group (Figure 2, B and C). In contrast, there was no statistically significant difference in subjective symptoms (eTable 4 in Supplement 2 and Figure 2D). This finding was not due to a lack of effect in the probiotic group, which showed a clinically relevant proportional reduction of 77%, but rather because there was also a reduction in the placebo group of 53%. A possible explanation is that the placebo group was able to reduce symptoms such as pruritus by using more corticosteroids.

After 12 weeks, we saw a slightly greater reduction of IL-4, IL-5, and IL-13 in the probiotic arm compared with placebo (eTable 3 in Supplement 2, last column). This may suggest decreased activity of the T helper 2 cells in the probiotic arm. We

did not find statistical significance in any of these differences, but lack of statistical significance does not mean evidence of no difference, especially when the trial was not powered to test differences in cytokines. An investigation of the biological mechanism will require a specifically designed study.

Limitations

The limitations of the study should be considered and clarified through further research. These limitations include the applicability of our results to patients consuming a different diet in different geographic areas, and whether the results can be extended to other population groups such as newborns (<1 year) or adults older than 17 years. In addition, the short follow-up of 12 weeks, the fact that topical corticosteroid dose was not recorded, and the inclusion of patients from a single center should also be considered limitations of this clinical trial. Finally, questions should be answered about adequate dosage, the duration of probiotic administration, and at what age the use of probiotics would be most efficacious. Future trials will be necessary and should consider all these questions to assess the probiotic mixture used in this clinical trial and other specific probiotic strains.

Conclusions

The results of our study indicate a strong positive effect in reducing the SCORAD index and use of topical corticosteroids in the group treated with the probiotic mixture. This evidence supports the efficacy of administering this probiotic mixture to patients with moderate AD and suggests that it could be used more extensively in clinical practice.

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**RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED
CLINICAL TRIAL TO EVALUATE THE EFFICIENCY OF A
PROBIOTIC IN REDUCING THE SYMPTOMS AND THE USE OF
TOPICAL CORTICOSTEROIDS IN 4 TO 17 YEAR-OLD PATIENTS
WITH MODERATE ATOPIC DERMATITIS**

Protocol atop/pro version 1

Alicante, April 2015

VNavarro / ARamirez

1 IDENTIFICATION OF THE TRIAL

Protocol code: pso/pro-1

Registration number NCT02585986

TRIAL TITLE: Randomized, double-blind, placebo-controlled trial to evaluate the efficacy and safety of using probiotic CBP-004024 in reducing the symptoms and use of topical corticosteroids in 4 to 17 year-old patients with moderate atopic dermatitis

2 TRIAL TYPE AND DESIGN

Single-center, randomized, double-blind clinical research trial.

3 DESCRIPTION OF THE TRIAL PHARMACEUTICAL PRODUCT

Experimental pharmaceutical product:

Probiotic:

Bifidobacterium lactis, *Bifidobacterium longum*, *Lactobacillus casei*

(Maltodextrin-based formula CBP-004024 containing 5×10^{10} cfu per gram)

Pharmaceutical format:

Capsule containing 30 mg of tapioca maltodextrin, sugar, *Bifidobacterium lactis*, *Bifidobacterium longum*, *Lactobacillus casei*
(5×10^{10} cfu per gram)

Control pharmaceutical product:

Placebo masked with the same format as the experimental drug containing 30 mg of tapioca maltodextrin per capsule.

Entity responsible for preparing the samples (drug and placebo)

Biopolis S.L. Parc Científic Universitat de València; C/ Catedrático Agustín Escardino Benlloch 9, edificio B; 46980-Paterna; Valencia; España

Encapsulation of the samples (medication and placebo)

Korott SL. Polígono industrial Santiago Payá, 184. Alcoi

4 TRIAL SPONSORS

Biopolis SL,

Korott SL,

5 RESEARCHERS UNDERTAKING THE TRIAL

-**Dr. Vicente Navarro López**; (Principal Investigator responsible for trial design and with access to all phases of the project including data analysis, writing the final report and verification of all documentation)

-**Dra. Ana Ramírez Boscá** (Principal Investigator responsible for trial design and with access to all phases of the project, including drafting the final report and verification of all documentation)

-Dr. **Miguel Ángel Carrion Gutiérrez**; (data verification and review of the final report)

-D^a **Beatriz Ruzafa Costas**, (responsible for data collection, data verification and review of the final report)

-Dra. **Asuncion Martínez Andrés**; (responsible for data collection, data verification and review of the final report)

-RGN: **Leticia Sánchez Aguilar**. Responsible for extraction and custody of samples and data from the research trial.

-RGN: **Encarna Espejo Luna**. Responsible for extraction, and for custody of samples and data pertaining to the research trial.

6 PARTICIPATING CENTRES

Centro Dermatológico Estético de Alicante. c/ Alonso Cano, 51; 03014 Alicante. España

7 ESTIMATED DURATION OF THE CLINICAL TRIAL

Recruitment period: 3 months.

Follow-up period: 12 weeks

Start date: October 2015

Estimated completion date: December 2016

Trial schedule:

Start of patient selection/inclusion	October 2015
End of patient selection/inclusion	December 2015
Expected completion date	March 2016
Analysis of trial data	April 2016
Final results of trial	June 2016

8 JUSTIFICATION

Atopic dermatitis is a chronic inflammatory skin disease characterized by intense itching, dry skin, and inflammatory exudate, frequently associated with asthma, allergic rhinitis, food allergy and recurrent skin infections secondary (1,2). The first symptoms usually develop during childhood, and about 50% of cases are diagnosed in the first year of life. Atopic dermatitis is a chronic disease, and at least one third of patients continue to suffer from the disease during adulthood (1,2,3). In developed countries, it affects approximately 10-15% of children under 5 years old at some time. The prevalence in children aged 6-7 years has been estimated at between 1% and 18% in an international trial, and prevalence in childhood is 10% to 20% (2,4). Although the disease, often family related, is not usually serious, and treatment may be simple, atopic dermatitis significantly alters the quality of life of children and adults, having a greater impact on family and economy than psoriasis, and according to some reports, equivalent to other serious diseases such as early onset diabetes mellitus (1,2,4,5).

It has been suggested that atopic dermatitis is a cutaneous manifestation of a systemic disorder, which also leads to other diseases such as asthma, food allergy and allergic rhinitis. Many atopic dermatitis patients have high blood serum levels of IgE and eosinophils, although early-onset cases usually occur without detectable IgE mediated sensitization (3). Immunological mechanisms leading to the symptoms of atopic dermatitis are currently being investigated in depth. The most recent findings, although they require further support, indicate, on the one hand, involvement of antigen-presenting cutaneous dendritic cells (Langerhans cells and inflammatory dendritic epidermal cells, with increased surface expression of FcεRI, the high affinity receptor for IgE); and on the other hand, various disorders regulating cytokines and inflammatory signaling regulated by TH2 lymphocytes, with overexpression of various types of skin cytokines; and finally compromised immunosuppressive capacity of regulatory T cells CD4 + CD25 + (Treg cells) (2,3,6,7)

The disease has a wide range of clinical symptoms, ranging from minor ones such as pityriasis alba or hand eczema, to more severe forms such as erythrodermic rash (2,7). There are no specific microscopic or laboratory alterations, so the diagnosis of atopic dermatitis is clinical (2,8). The criteria proposed by Hanifin-Rajka (9) remain the standard for the diagnosis of atopic dermatitis, and are followed in Spain and in most other countries. Other criteria have been developed and published, which may be less laborious and more practical, but are not yet properly validated in Spain (2,6,8,10,11). The Hanifin-Rajka criteria validated in Spain are listed in Annex II.

Another aspect besides undertaking diagnosis is the degree of severity of the process in these patients. To this end, various methods have been developed and criteria used to assess severity and monitor response to treatment, both in clinical trials and observational studies, and even in clinical practice when the patient's progress should be monitored more accurately than that provided by subjective perception. In this respect, SCORAD (**Scoring Atopic Dermatitis**) is one of the most tested, most widely accepted, most commonly used, most recommended and best validated methods is the scale called the SCORAD index (ScoringAtopicDermatitis), developed by the European Task Force on Atopic Dermatitis group in 1993. Comparative studies have concluded this is the best method of assessing the severity of atopic dermatitis and the most widely supported by conclusive evidence for its validity, reproducibility, sensitivity and acceptability (11, 12, 13, 14, 15, 16). The SCORAD is a scoring system that takes into account the extent and intensity of five types of basic lesions (erythema, edema/papule, oozing/crusting, excoriation and lichenification) and symptoms (itching and loss of sleep). The SCORAD index and how it is used are provided in Annex III.

There is some disagreement about SCORAD index values that define the different degrees of severity. Some Spanish groups consider the SCORAD index scores <25, 25 to 40, or > 40 as mild, moderate or severe, respectively (8), while other authors, based on laboratory correlates in studies published in international journals have applied scores <20, 20 to 40, or > 40 to define mild, moderate and severe symptoms, respectively (15). In conducting this clinical trial, internationally accepted criteria will be followed.

There is relative consensus regarding the treatment of atopic dermatitis (1,2,17), and this consensus (8,11) is followed in Spain. Overall, the objectives of treatment are to prevent itching, remove exudate, cure infection, eradicate inflammatory lesions, and prevent relapse. H1 antihistamines are used to eradicate itching and the consequent scratching, which increases infections and inflammatory reaction, although H1 effectiveness is not clearly demonstrated. Traditional H1 antihistamines are used in the acute phase as they seem to produce better results by causing sedation (hydroxyzine, clemastine, dexchlorfeniramina), whereas, the latest generation of H1 antihistamines (dexfenfluramine, loratadine, cetirizine and derivatives) can be used after the main outbreak, as they are less sedative; however, their results sometimes have no statistically significant differences compared to a placebo (18). Nonetheless, in practice, last generation antihistamines are often used

since the acute phase, as traditional antihistamines may have adverse effects on the CNS that interfere with daily activities: sedation (ranging from drowsiness to sleep), depression (incoordination, dizziness, lassitude, decreased concentration), and occasionally agitation. Although H1 antihistamines are considered to have a low risk of these adverse effects, the data are extrapolated from their use in patients with allergic pathologies (asthma, allergic rhinitis), and it is unclear that the risks in children with other diseases are similar (34). To prevent recurrence, hygiene and dietary-based recommendations are given, to try to avoid contact with triggers and allergens, including food, or skin irritants, as well as heat and excess moisture.

The primary purpose of eczema treatment is to control the inflammatory lesion, which has the secondary effect of controlling itching. For this, topical corticosteroids (1,2) are used with different strengths depending on the severity of the lesions and the patient's condition. In the initial stage or acute exacerbation of atopic dermatitis, topical corticosteroids are used administered daily, whereas weekly administration can be followed during the remission phase.

Adverse effects of topical corticosteroids pose a limitation or restriction to their use. In these patients, adverse reactions may occur which depend on the power of drug prescribed and the duration of treatment (1,2,8,17,19).

- The most common adverse reactions at the administration site include: skin atrophy, petechiae, atrophic striae, hypertrichosis, depigmentation, telangiectasia, folliculitis, and glaucoma if applied periocularly.
 - Also, more serious systemic adverse effects have been described much less frequently: suppression of the hypothalamic-pituitary-adrenal axis, growth retardation or cushingoid manifestations.
 - Approximately 70-80% of parents and caregivers of children with atopic eczema are concerned about the adverse effects of topical corticosteroids, reducing adherence to treatment (about 25% of them do not apply topical corticosteroids due to anxiety) (17).
- The development of new drugs to treat inflammation has been promoted by these adverse effects of corticosteroids, and the lack of response to them in some patients. Among the best studied are calcineurin inhibitors (tacrolimus and pimecrolimus) for topical administration (2,11). These drugs inhibit the activity of cells involved in the immune response, including T cells, and reduce the production of cytokines and inflammatory response, and importantly they do not appear to increase the risk of infection or produce skin atrophy. The potential risk of the absorption of these drugs, and their reaching systemically active concentrations has only been detected with tacrolimus. However, there are still doubts about the safety of these drugs in long-term treatments, such as increased risk of cancer, which have been raised but not confirmed. Consequently, the prospectus of products containing tacrolimus or pimecrolimus for topical treatment of atopic dermatitis state that they should

only be used in children over 2 years old, for whom the use of topical corticosteroids is not recommended, or in patients for whom they have previously failed (20,21). Furthermore:

- The long-term effect on the immune response of the skin locally and on the incidence of malignant skin diseases is unknown;
- Patients who use them have a predisposition to superficial skin infections by the herpes virus or herpes eczema;
- Their efficacy and safety have not been studied in their treatment of clinically infected atopic dermatitis;
- Before starting treatment with these drugs, clinical infections at treatment sites should be cured;
- Cases of malignancies have been reported, including cutaneous and other types of lymphoma, and skin cancer, in patients who have used these products.

Consequently, even though calcineurin inhibitors enjoy growing support and an apparently attractive position, their high costs and the aforementioned information have shed doubts and suggest limitations (18,22).

Probiotics to treat atopic dermatitis. The use of probiotics in the treatment of atopic dermatitis has been studied in recent years in a limited number of pilot studies with varying results depending on the probiotic used and age of the patients in the trial. Recent studies, including a meta-analysis of previous publications, demonstrate the beneficial effect of probiotic use in certain circumstances related to age, type of probiotic used, dosage and combination of probiotics (Ref 23- 28). This trial aims to evaluate the possible beneficial effect of a combination of probiotics, developed by S.L. Biopolis (Paterna; Valencia), which exert anti-inflammatory and immune-modulating activity, with favorable results in various inflammatory (28) and atopic (29-31) processes.

Justification of the trial. There is a clearly established medical need for safe and effective medicines/pharmaceuticals for the long-term treatment of atopic dermatitis. This condition affects a large part of the population, and has notable consequences on patients' quality of life and medical complications. Topical corticosteroids are considered a highly effective treatment, but have high risks of adverse effects. The development of new alternatives to topical corticosteroids (such as calcineurin inhibitors) responds to this need, although the potential adverse effects of these new drugs have yet to be established. By contrast, the safe use and effectiveness of probiotics have already been

demonstrated in other atopic pathologies such as celiac disease. There has been a growing number of studies on the effectiveness of probiotics in atopic dermatitis in recent years, and they support the safety of these substances as well as demonstrating the relationship between digestive and atopic diseases.

9 OBJECTIVES

The purpose of this trial is to evaluate the efficacy of the Probiotic CBP-004024 in reducing, on the one hand, the symptoms of moderate atopic dermatitis (SCORAD index) and, on the other hand, the use of topical corticosteroids, antihistamines and systemic corticosteroids (according to the criteria of Hanifin and Rajka, with index SCORAD 20 to 40, inclusive), in patients aged 4-17 years who require these drugs due to disease outbreaks and who use or are prescribed topical corticosteroids to treat outbreaks of atopic dermatitis.

Trial variables:

Primary variable:

SCORAD index score: baseline and monthly at medical check-ups until the end of the study period (12 weeks).

Evaluate the number of days that each patient requires administration of topical corticosteroids during outbreaks, based on the patient's logbook. Disease outbreak is understood to mean that due to the disease the patient requires the use of topical corticosteroids for at least three consecutive days.

Other variables:

- The number of outbreaks quantified at each check up, based on logbook data and clinical examination.
- The use of antihistamines and the need for new treatments based on the logbook.
- The Investigators' Global Assessment (IGA) score is measured at each check up. IGA <2 is considered a therapeutic success.
- Adverse events are recorded at each check up, based on the patient's logbook and clinical examination

Primary Objective:

The main objective of the trial is dual. On the one hand to assess the percentage of days when topical corticosteroids are used during disease outbreaks during the 12-week treatment period with the probiotic or a placebo, and on the other, to evaluate the variation and differences in the SCORAD index (Annex III) between groups (probiotic versus placebo) at 4, 8 and 12 weeks of the trial period.

Other objectives:

- a) A) Number of outbreaks during the study (use of corticosteroids on three or more consecutive days) comparing the results of the two treatment groups
- b) Quantification of the time between randomization and a new outbreak event
- c) The need to incorporate new treatments, other than those specified, to treat patients that do not respond to the therapeutic trial protocol.
- d) Clinical Global Impression (IGA; Annex IV)
- e) Record of adverse events

10 METHOD AND PATIENTS

10.1 Design:

Double-blind, placebo-controlled trial, on the efficacy of an orally-administered probiotic in patients aged 4-17 years suffering from Moderate Atopic Dermatitis in a 12-week trial treatment.

11 ELEGIBILITY

11.1 Trial subjects:

- Patients diagnosed with atopic dermatitis according to the Hanifin and Rajka criteria, with a SCORAD index of 20 to 40, inclusive, which met all inclusion criteria and none of the exclusion criteria, who use or have been prescribed topical corticosteroid treatment for outbreaks of atopic dermatitis.
- **INCLUSION CRITERIA**
- Patients 4-17 years of age, inclusive.
- Patients diagnosed with atopic dermatitis according to the criteria of Hanifin and Rajka
- Patients with a SCORAD index 20 to 40, inclusive

- Patients who use or are prescribed topical corticosteroids for the treatment of atopic dermatitis outbreaks
- Patients whose parents or legal representative sign the informed consent
- When the child is 12 or older, he/she must also give consent to participate in the trial

- **EXCLUSION CRITERIA**

- Pregnancy
- Breastfeeding
- Women of childbearing age who do not agree to use some effective contraception, according to the researcher, and who are or become sexually active during the trial
- Patients treated with phototherapy for atopic dermatitis
- Patients treated with systemic corticosteroids in the 2 months prior to the trial.
- Patients treated with immunosuppressive or cytostatic drugs in the 2 months prior to the trial
- Patients who have received treatment with probiotics in the 2 months prior to the trial
- Patients who have been treated with systemic antibiotics in the four days prior to the trial
- Patients with fever (temperature > 37.5 °C axillary or equivalent)
- Patients with severe allergic diseases
- Patients with pathologies associated with immunodeficiency or cancer processes
- Patients with other dermatological diseases that may hinder the evaluation of atopic dermatitis or require the continued use of topical corticosteroids
- Patients for whom any of the trial drugs are contraindicated according to their technical specifications.
- Patients with KIDMED score for Mediterranean Diet under 8 points.
- Patients with intolerance to gluten and / or lactose.
- Patients who have participated in research studies with drugs in the 3 months prior to the trial.

- **Criteria for patient withdrawal from therapy or assessment:**

If during the course of the trial a patient needs to take any kind of concurrent treatment they must notify the trial investigator, who should take note of the dose, reason, route of administration, etc. on an individual monitoring sheet.

Those patients requiring systemic corticosteroid therapy during the trial for over five days if under 10 years of age, or more than 7 days if over 10 years of age, **will be removed from the trial.**

Patients not taking the probiotic for more than 8 consecutive days (10% of treatment time) will also be removed.

11.2 Protocol and statistical analysis

Trial framework

N= 25 Probiotic, 1 capsule/day

N= 25 placebo, 1 capsule/day

	Selection/inclusion patients						End of study
Week	0	2	4	6	8	10	12
Check-up	0/1	2	3	4	5	6	7
	▣		▣ •		▣ •		•

Delivery of probiotic / placebo ▣

Return and tally of capsules •

Usual treatment: emollients, antihistamines and topical corticosteroids

11.3 Sample size estimation

Previous data from studies in adults and children, with similar diagnosis and moderate severity of atopic dermatitis, show that in patients and conditions similar to those in this trial, the placebo group used topical corticosteroids on 50% of the days during the trial period. In these trials, there are no data on the use of topical corticosteroids exclusively in disease outbreaks and therefore this value can not be estimated. Furthermore, in clinical trials in the same conditions described and reproduced in our study, comparing the placebo group with the probiotic group, there was a 30% difference in clinical improvement as evaluated by the SCORAD index (15% vs 45%).

Assuming a similar effect in response to the oral placebo in this clinical trial and a reduction

equal to or greater than 33% compared to that observed in the placebo group, together with an estimated 5% loss in trial subjects, the sample size required for alpha 0.05 and a statistical power of 80% was 25 patients per group of intervention.

11.4 Planned statistical analyses

1. Demographics, baseline characteristics and trial data: Data and descriptive global results are expressed as mean $\pm$ standard deviation for continuous quantitative variables. For categorical variables, the corresponding numbers will be presented with the percentages.
2. Efficacy Analysis: For the main and secondary analyzes, data collected at check-up 1 will be used as baseline data. All analyzes will be conducted according to intention-to-treat (ITT) analysis for patients enrolled in the trial, and per protocol analysis, for patients who have completed the different monitoring stages. For the ITT analysis, patients who left the trial will be carried forward to the end, to the last assessment made.
3. Primary Efficacy Analysis:
 - The percentage of days when topical corticosteroids are used is calculated as the number of days of topical corticosteroid treatment divided by the number of days in the trial. The mean values + standard deviation will be calculated for the global data at 4, 8 and 12 weeks of treatment. The trial groups will be compared using the Wilcoxon test with a statistical significance level of 5%.
 - Other efficacy analysis:
 - SCORAD Index: means + standard deviation values will be calculated for global data at 4, 8 and 12 weeks of treatment. At each of these time points, the values will be compared with the patient's values taken at the beginning of the trial by the Wilcoxon test. Corrections for multiple comparisons will be performed using the Bonferroni method.
 - Disease-free time interval until a new outbreak: Final data are expressed as median and range.
 - For safety and tolerability assessment, the appropriate specifications will be made for each and every variable that appears (according to adverse events). These will be tabulated by treatment group, age, sex,

and issues that may influence the occurrence and / or severity of adverse events. Also a descriptive analysis will be made of the changes in health indicator variables (obtained from tests performed in the clinical check up). The safety parameters will be expressed individually and as means $\pm$ standard deviation values

- Exposure to treatment and therapeutic compliance
- The consumption antihistamines and antibiotics will be evaluated in the same way as described for the analysis of topical corticosteroids consumption.
- A descriptive analysis will be conducted per group for the atopic dermatitis treatments administered during the trial.
- A descriptive analysis will be conducted of therapeutic compliance to the masked product used in the trial. Data will be tabulated by treatment group, age, sex, and aspects considered of interest.

11.5 Trial population and homogeneity analysis:

1. Primary Efficacy Analysis

- a. **The percentage of days when topical corticosteroids are used during outbreaks** will be calculated as the number of days on topical corticosteroid therapy in outbreaks, divided by the number of days in the trial. Mean values $\pm$ standard deviation will be calculated for the global data at 4, 8 and 12 weeks of treatment. The two study groups will be compared using the Wilcoxon test with a level of statistical significance of 5%.
- c. SCORAD Index: mean values $\pm$ standard deviation will be calculated for global data on check-ups at 0 and 4, 8 and 12 weeks of treatment. At each of these intervals, the values will be compared with those recorded for the patient at the beginning of the trial, using the Wilcoxon test. Corrections for multiple comparisons will be performed using the Bonferroni method.

2. Other efficacy analysis

- a. **Exposure to other treatment:** Antihistamine and systemic corticosteroids consumption will be evaluated in the same way as described for the analysis topical corticosteroids, as pre-specified secondary variable.
- b. The trends and values of Investigator Global Assessment (IGA) scale will be analyzed at

each check-up during the trial.

c. Patients will belong to one of two groups depending on whether their IgE values in blood are higher or lower than 100 IU / ml, and the development of each group will be analyzed separately.

12. ETHICS AND LEGISLATION IN RESEARCH STUDIES

The trial will be run in accordance with the Declaration of Helsinki, as amended in successive world assemblies, and Spanish legislation on Clinical Trials in humans. The trial protocol has been reviewed and approved by the Ethics Committee for Clinical Research (CEIC) of the Hospital General Universitario de Alicante, and the Agencia Española del Medicamento (Spanish Medicines Agency) and registered in the American Registry of Clinical Trials (ClinicalTrials.gov)

In order to recruit patients for the trial, all consecutive patients attending consultations at the center participating in the trial will be informed about it and assessed by the trial investigators. The researcher shall explain to each subject the nature of the trial, its purposes, procedures, expected duration and potential risks and benefits related to participation in the trial as well as any inconvenience this might involve. Each participant will be informed that their participation in the trial is voluntary and that they could leave the trial at any time, without this affecting their future medical treatment, or the relationship with their physician.

Informed consent will be provided in a standard written document, in language easily understood by the participant. The subject/participant shall be given ample time to read and understand explanations before dating and signing the informed consent, and shall receive a copy of the signed document. No subject will be included in the trial without having given written informed consent.

Prior informed consent shall be obtained from a parent or legal representative; the agreement will reflect the presumed willingness of the child. When the child is 12 or older, he/she will also provide his/her consent to participate in the trial. The minor will receive information about the trial, the risks and benefits appropriate to his/her capacity for understanding. In compliance with the provisions of Article 7, paragraph 3 a) 4 of the Royal Decree 223/2004 on clinical trials, the sponsor shall inform the Public Authorities of the authorization for the clinical trial.

13. TRIAL RESEARCHERS AND ADMINISTRATIVE STRUCTURE

13.1 Scientific research coordinator:

Name: Ana Ramírez Bosca and Vicente Navarro López

Address: C/ Grecia Nº 9, 3º-3. CP 03140. Guardamar del Segura. Alicante

Telephone: 695845742

13.2 Collaborating Investigators and Centres where the trial is to be run:

Participating centre: Centro Dermatológico Estético. Address: C/ Alonso Cano nº 51 03014
Alicante, Telephone: 965 140 460

-D^a **Beatriz Ruzafa Costas**
-Dra. **Asuncion Martínez Andrés**
-RGN: **Leticia Sánchez Aguilar**
-RGN: **Encarna Espejo Luna**

13.2 Trial Sponsors/Promoters:

Name: Biopolis SL, Korott SL

1. Daniel Ramón Vidal. Biopolis S.L. Parc Científic Universitat de València; C/ Catedrático Agustín Escardino Benlloch 9, edificio B; 46980-Paterna; Valencia; Spain
2. Jose Manuel Pérez Ortín. Korott SL. Poligono industrial Santiago Payá, 184. Alcoi, Valencia Spain

13.3 Authors of the PROTOCOL:

- Dr. Vicente Navarro López
- Dr. Ana Ramírez Bosca

13.4 Responsible for the statistical analysis:

- Dr. Vicente Navarro López
- Dr Vicente García Román

13.5 Randomization procedure:

Treatment will be assigned by 1:1 randomization blocks, stratified taking into account the following three variables: sex and age of the patient (under 12 years of age or over) and age at disease onset (before or after 4 years of age).

14. Treatment

14.1 Treatment period

The treatment period will be 12 weeks. After the trial inclusion consultation (baseline) and assigned treatment, seven check-ups will be programmed, which will take place at 4, 8 and 12 weeks from the start of treatment with the probiotic or placebo, and SCORAD will be performed, and each patient's Data Collection Logbook (DCL) will be reviewed at weeks 2, 6 and 10.

14.2 Treatment description and definition of exposure

Patients will receive Probiotic or an identical-looking masked placebo (double blind).

Trial pharmaceutical: Probiotic CBP-004024 MADE BY BIOPOLIS (5×10^{10} cfu of *Bifidobacterium lactis*, *Bifidobacterium longum*, *Lactobacillus casei* per gram)

Control pharmaceutical: placebo with a similar appearance to the Probiotic, containing 30mgr of powdered Maltrotexin

Dosage regimen:

- A capsule of the pharmaceutical. This regimen corresponds to 30 mgr/day of Probiotic CBP-004024, or equivalent placebo (maltrotexin)

Topical corticosteroids:

To treat outbreaks of Methylprednisolone aceponate, a thin layer is applied once a day to the affected area and rubbed lightly.

In general, treatment duration should not exceed two weeks. As a general rule, the duration of treatment in children should be limited to a minimum. If excessive dryness of the skin occurs during treatment with topical steroid, the use of an emollient adjunctive therapy is recommended.

Systemic corticosteroids:

Should systemic corticosteroids be required, Deflazacort will be prescribed.

Antihistamine medication

Desloratadine will be used to treat itching, in either syrup or tablets. Children 7 to 11 years: 5 ml of syrup once a day (2.5 mg desloratadine). Children over 12 and adults: one tablet (5 mg of desloratadine) once a day.

Topical antibiotics

Ac-Fusidic antibiotic will be used, an antibiotic commonly prescribed for infected atopic dermatitis.

14.2.1 Delivery of medication

The study drugs will be delivered under the conditions established by the regulations in force in the pharmacy of the center involved. All these medicines shall be used in accordance with the specifications given in the technical specifications of the products. The sponsor/promoter shall provide:

- Probiotic CBP-004024 vs placebo

14.2.2 Data collection.

All medications taken by the patient during the study will be recorded in Data Collection Logbook (DCL) specifying the doses, route of administration and duration of treatment (start-end date).

Information on study medication will be recorded in the corresponding DCL. The investigator and / or trial collaborators will assess fulfillment/adherence at each check up by counting the returned capsules and the information provided by the patient. All medication dispensed and returned during the trial will be recorded in the DCL.

15. RESEARCH PROCEDURE

15.1 Determination of sample size

Previous data from studies in adults and children with similar diagnosis and moderate severity, showed that in the placebo group, topical corticosteroids were used $52 \pm 24.6\%$ of the days during the trial, in patients and conditions similar to those in this study. Assuming a similar effect in response to oral placebo, and a 60% reduction in the number of days of treatment with topical corticosteroids in the group treated with Probiotic, with an estimated 5% of study subjects losses, group of **25 patients per group** would be required to detect such differences as statistically significant with a power of 80%. To carry out the trial 25 patients are randomized to each of the two groups.

15.2 Trial framework

The trial will last a total of 12 WEEKS from the recruitment of patients to the last check-up, and will be run according to the following diagram: TRIAL FRAMEWORK

Check-ups	0 / 1	2	3	4	5	6	7
Week	0	2	4	6	8	10	12
Inclusion/exclusion criteria	X						
Signature of informed consent	X						
Medical records	X						
Physical examination	X		X		X		X
Laboratory analyses	X						X
Other diagnostic tests	X			X			
SCORAD	X		X		X		X
IGA	X		X		X		X
Pharmacological treatment	X		X		X		
DCL dispensed to patients	X						
Daily data collection in DCL		X		X		X	X
Evaluation of adverse events		X	X	X	X	X	X

Prior to inclusion (Check-up 1):

- Explanation of the trial and procurement of written informed consent, from the parent or guardian and the child if over 12 years.
- Inclusion / exclusion criteria
- Evaluation of atopic dermatitis
 - Criteria of Hanifin-Rajka (Annex II)
 - SCORAD (Annex III)
 - IGA (Annex IV)

The following clinical tests will be performed for each subject:

- Medical record that includes personal and family history and anamnesis for appliances.
- Complete physical examination including height, temperature and body weight.
- Routine tests of hematology, biochemistry, IgE and urinalysis. These laboratory tests are considered routine in the diagnosis and monitoring of these patients, and will only be performed at check-ups 1 and 7.
- Determination of bacterial DNA in blood. Interleukins IL-5, IL-10, IL-13, IL-31
- Determination of the microbiota from feces sample.
- Urine-based pregnancy test (women of childbearing age).
- Once included in the study, each subject will receive a numbered box, coded and labeled in accordance with current regulations, with capsules for the first month of treatment. All subjects will receive a greater number of capsules than that required by treatment regimen assigned, in order to enable accurate tally of the consumed units and evaluate patient compliance. The subject and his/her guardian will be clearly informed how to take the capsule every day, ingesting it before meals.

Check-ups 3, 5 and 7:

Assessment will be made of disease outbreaks, treatments and problems

Evaluation of atopic dermatitis

- SCORAD (Annex III)

- IGA (Annex IV)

Adverse events will be assessed following the procedures established in the protocol. All adverse events reported by the patient will be recorded, specifying date of commencement and termination, and treatment received, if applicable.

At check-ups 3, 5 and 7 the subject shall return the box with the leftover medication, and be given a new box with treatment corresponding to the following month, with leftover capsules included. The last box will be returned by the subject at check up No. 7.

Check-ups 2, 4 and 6:

Check-ups will be held within 15 days of each main consultancy to review the patient's DCL and review the patient's progress. SCORAD will not be performed.

Check-up 7: End-of trial check-up

Evaluation of atopic dermatitis

- SCORAD (Annex III)
- IGA (Annex IV)

The assessment of adverse events will be performed following the procedures established in the protocol. All adverse events reported by the patient will be recorded, specifying date of commencement and termination, and treatment received if applicable.

A complete check-up of the patient will be performed, including analytical and other diagnostic examinations, evaluation of adverse events and evolution of atopic dermatitis, information about medications used and number of outbreaks.

15.3 Analytical assessment

Laboratory tests

- 15.3.1 **Clinical hematology:** erythrocytes, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, total and differential leukocyte.
- 15.3.2 **Clinical biochemistry:** SGOT, SGPT, LDH, alkaline phosphatase, GGT, total bilirubin, creatinine, glucose, cholesterol, triglycerides, total protein and albumin.

15.3.3 **Atopic dermatitis-related serology:** IgE

15.3.4 **Biomarkers of immunity:** interleukins IL-4, IL-5, IL-10, IL-13, IL-31

15.3.5 **Bacterial DNA.**

15.3.6 **Faces for determination of microbiota.**

15.3.7 **Pregnancy test,** in the case of women of childbearing age, at the beginning and end of the study. All women are considered potentially fertile from menarche unless there is evidence of infertility.

Variables and measuring instruments. Definition and description of measurements

1. Number of days per month that each patient requires administration of topical corticosteroids: based on the patient's logbook. The patients will record the following in the logbook every day:

- i. If they have used topical corticosteroids, if so the number of times and treated area/s
- ii. If they have used other treatments: treatment and dose
- iii. If there has been a relapse that has led to the use of systemic corticosteroids
- iv. Adverse events

2 Antihistamine consumption and new treatments are both considered.

- i. SCORAD index score: a baseline and monthly performed as indicated in Annex III
- ii. The number of outbreaks will be counted at each check up, based on information from the patient's logbook and the clinical examination. An outbreak is considered as the exacerbation of the disease which is quantified after evaluating the logbook and the exploration and interview with the patient or guardian, with IGA> 4 or the specific application of topical corticosteroids for at least 3 days.
- iii. The IGA is measured at each check up as established in Annex IV.
- iv. Adverse events are recorded at each check up, based on information from the patient's logbook and the clinical examination.

16. STATISTICAL ANALYSIS

16.1 Quality control of the data submitted for analysis:

All the analyzed data will be entered into a database built in ACCESS version 2007 platform using a double data entry procedure. Any doubts about the information provided in the Data

Collection Logbooks will be clarified by sending appropriate consultations to the trial supervisor. The data analyzed will correspond to those resulting after settling disagreements in the double entry and consultations on anomalous data in the logbooks.

A randomization table is included as an additional table in the database.

16.2 Transfer of data from the database to the statistical analysis software SPSS (SPSS20.0 for Windows):

In each of the statistical analyzes carried out, a specific query is performed across data from each check up required for analysis with the treatment assigned in the randomization table. The resulting query is imported directly from Microsoft Access to SPSS using the specific function available for this in SPSS 20.0. Once the data from a query have been uploaded in an SPSS file, parity is found in the data between the two files (1 record in 5 is checked for the parity of each and every one of the variables in the file).

SPSS files in YES / NO variables have been coded as 1/0.

16.3 Primary efficacy analysis

Analysis will be made of the percentage of days when topical corticosteroids are used, calculated as the number of days in treatment with topical corticosteroids divided by the number of days in the study, and global data will be compared at 4, 8 and 12 weeks of treatment using the Wilcoxon test with a level of statistical significance of 5%. An analysis by intention-to-treat and per protocol will be performed in accordance with the provisions of the protocol.

Primary endpoint: the primary objective of the study is to compare the percentage of days when topical corticosteroids are used during 12 weeks of treatment with Probiotic or placebo:

Intention-to-treat analysis:

Percentage of days when topical corticosteroids are used during 12 weeks of treatment: this is analyzed by counting the number of days the patient has taken topical corticosteroids (Metilprednisolona Aceponato) throughout the trial, divided by the number of days of participation in the trial. In those patients whose participation in the trial ends before 12 weeks, the percentage of days that topical corticosteroids are used is calculated for the interval between the penultimate and the last check-ups attended by the patient. This percentage is applied to the unattended check-ups up until the end of time foreseen for participation in the study (3 months = 90 days).

17. MODIFICATIONS TO THE PROTOCOL:

No modifications will be made to the protocol approved by the CEIC and the Spanish Medicines Agency.

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

cm = centimeters

DCL = Data Collection Logbook

SD = standard deviation

ALP= alkaline

phosphatase

HR = heart rate

h = hours

IGA: Investigators' Global

Assessment

kg = kilograms

l = liters

mg = milligrams

ml = milliliters

mm =

millimeters

mm Hg = millimeters of mercury

ms = milliseconds

ng = nanograms

Nº = number

SCORAD: **S**coring **A**topic **D**ermatitis index

T = temperature

BP = blood pressure

SBP = systolic blood pressure

DBP = diastolic blood pressure

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ANNEX I.- FULL LIST OF RESEARCHERS, EMPLOYEES AND CENTRES RUNNING THE TRIAL

-**Dra. Ana Ramírez Bosca** (Researcher primarily responsible for project design and with access to all its phases including drafting the final report and verification of all documentation)

-**Dr. Vicente Navarro López**; (Researcher primarily responsible for the design of the project and with access to all its phases including data analysis, drafting the final report and verification of all documentation)

-**Dr. Miguel Angel Carrion Gutiérrez** (Responsible for monitoring, data verification and reviewing the final report)

-**D^a Beatriz Ruzafa** (Responsible for data collection, data verification and review of the final report)

-**Dra. Asuncion Martínez Andrés** (responsible for data collection, data verification and review of the final report)

-**RGN: Leticia Sánchez Aguilar**. Responsible for extraction and custody of samples and data from the research study. Collection and completion of the DCL

-**RGN: Encarna Espejo Luna**. Responsible for extraction and custody of samples and data from the research study

ANNEX II.- HANIFIN-RAFKA CRITERIA FOR THE DIAGNOSIS OF ATOPIC DERMATITIS.

Major Criteria *(need three or more of the following):*

Pruritus

Typical morphology and distribution

Facial and extensor involvement in infants and children

Flexural lichenification or linearity in adults

Chronic or chronically relapsing dermatitis

Personal or family history of atopy (allergic rhinitis, asthma, atopic dermatitis)

Minor Criteria *(need three or more of the following):*

Anterior neck folds

Anterior subcapsular cataracts

Cheilitis

Course influenced by environmental or emotional factors

Dennie-Morgan infraorbital fold

Early age of onset

Facial pallor or facial erythema

Food intolerance

Keratoconus

Ichthyosis, palmar hyperlinearity, or keratosis pilaris

Immediate skin test reactivity

Intolerance to wool and lipid solvents

Itch when sweating

Nipple eczema

Orbital darkening

Perifollicular accentuation

Pityriasis alba

Raised serum IgE

Recurrent conjunctivitis

Tendency toward cutaneous infections (especially *S. aureus* and herpes simplex)
or impaired-cell immunity

Tendency toward nonspecific hand or foot dermatitis

White dermatographism or delayed blanch

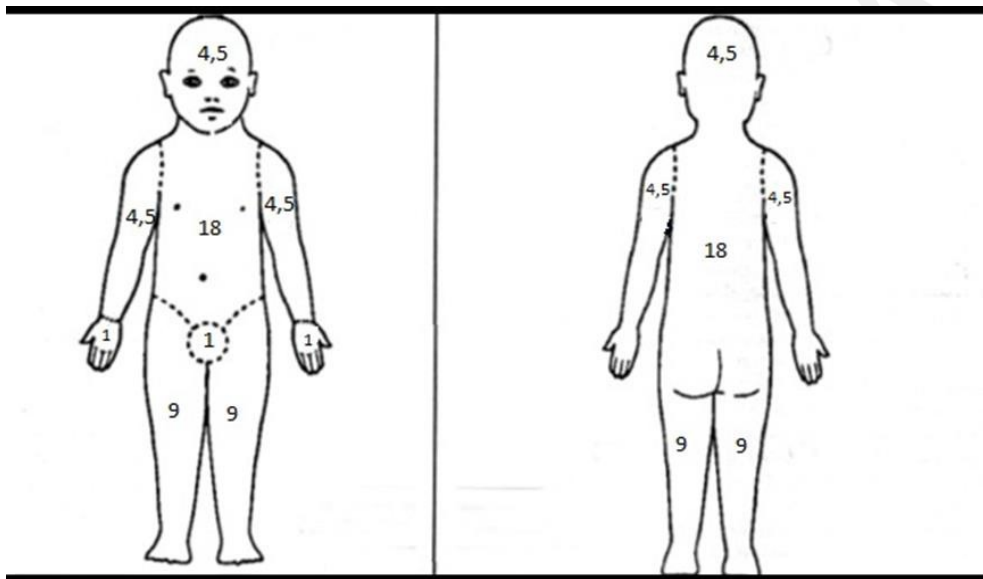
Xerosis

Source: Dermatol Nurs © 2006 Jannetti Publications, Inc.

ANNEX III: SCORAD INDEX TO ASSESS LESION SEVERITY.

Data collection and the formula for calculating the score are as follows:

1.- **EXTENSION:** The body surface is divided into four segments (head and neck, trunk, upper extremities, lower extremities), which are assigned a relative percentage in terms of the surface area they represent. The affected area is calculated according to the diagram below. The surface of the genitals is considered 1%. The palm or the back of the hand are considered 1%.



Extension: Record the sum of the affected areas:

 = Value A

2.- INTENSITY: erythema, edema / papule, oozing / crusting, excoriation and lichenification: clinical signs five basic types of injuries are assessed. Depending on the intensity, each of these is scored from 0 to 3. Skin dryness is assessed in unaffected skin areas.

VARIABLE	INTENSITY (0-3)	METHOD OF CALCULATION
Erythema		Score of intensity (average value of representative area) 0= Absence 1= Mild 2= Moderate 3= Severe
Edema / papule		
Oozing / crusting		
Excoriation		
Lichenification		
Dryness *		* = In unaffected skin areas

Intensity: Record the sum of the intensities:

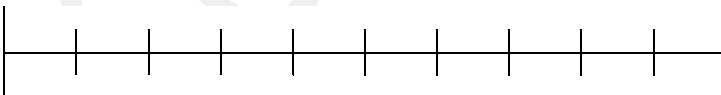
= Value B

3.- SUBJECTIVE SYMPTOMS: Based on the visual analog scale, the presence of itching and loss of sleep are scored from 0 to 10. **The patient or parents indicate the estimated average score for the last 3 days or nights** on the figure. Afterwards it is measured with a ruler and the measured value is

recorded in centimeters



Itching



Loss of sleep
(0 to 10)

= Value C

FINAL SCORAD SCORE = $A/5 + 7B/2 + C$ =

Annex IV: Investigators' Global Assessment (IGA)

The severity of the disease is measured on a scale of 6 points, based on the overall evaluation of skin lesions:

SCORE	EVALUATION OF LESION
0 (clear)	No signs of Atopic dermatitis-related inflammation
1 (almost clear)	Erythema only perceptible and papule / infiltrate only perceptible
2 (mild)	Mild erythema and mild papule / infiltrate
3 (moderate)	Moderate erythema and moderate papule / infiltrate
4 (severe)	Severe disease with severe erythema and severe papule / infiltrate
5 (very severe)	Severe erythema and severe papule / infiltrate with exudate/crusting

ANNEX V. Check-ups and monitoring during the trial.

Check-up number	Date	Objective

Supplementary Online Content

Navarro-López V, Ramírez-Boscá A, Ramón-Vidal D, et al. Effect of oral administration of a mixture of probiotic strains on SCORAD index and use of topical steroids in young patients with moderate atopic dermatitis: a randomized clinical trial. *JAMA Dermatol*. Published online November 8, 2017. doi:10.1001/jamadermatol.2017.3647

eTable 1. Major and minor criteria in the study participants within placebo and probiotic groups

eTable 2. KIDMED test to assess the Mediterranean Diet

eTable 3. Estimation of the effect of the intervention in laboratory parameters

eTable 4. Estimation of the effect of the intervention in SCORAD components

eFigure 1. Estimated differences between the two trial arms

eFigure 2. Percentage of patients with IGA score under 2, measured at each checkup in weeks 4, 8, and 12

eFigure 3. Distribution of number of days using steroids to treat flares

This supplementary material has been provided by the authors to give readers additional information about their work.

eTable 1. Major and minor criteria in the study participants within placebo and probiotic groups

	Probiotic group, n (%)	Placebo group, n (%)
<i>Major criteria</i>		
Pruritus	26 (100)	24 (100)
Typical morphology and distribution	26 (100)	24 (100)
Chronic dermatitis	26 (100)	24 (100)
Personal or family history of atopy	19 (73,1)	16 (66,7)
<i>Minor criteria</i>		
Xerosis	26 (100)	24 (100)
Facial pallor or facial erythema	26 (100)	24 (100)
Pityriasis alba	25 (96,2)	24 (100)
White dermographism	26 (100)	23 (95,8)
Cheilitis	25 (96,2)	23 (95,8)
Perifollicular accentuation	26 (100)	22 (91,7)
Orbital darkening	24 (92,3)	23 (95,8)
Immediate (type 1) skin-test reactivity	24 (92,3)	20 (83,3)
Early age of onset	24 (92,3)	20 (83,3)
Dennie-Morgan infraorbital fold	23 (88,5)	21 (87,5)
Course influenced by environmental or emotional factors	20 (76,9)	19 (79,2)
Itch when sweating	15 (57,7)	13 (54,2)
Raised serum IgE	14 (53,9)	13 (54,2)
Ichthyosis, palmar hyperlinearity, or keratosis pilaris	13 (50)	13 (54,2)
Intolerance to wool and lipid solvents	12 (46,2)	10 (41,7)
Tendency toward non-specific hand or foot dermatitis	8 (30,8)	6 (25)
Anterior neck folds	6 (23,1)	6 (25)
Tendency toward cutaneous infections	4 (15,4)	3 (12,5)
Recurrent conjunctivitis	3 (11,5)	2 (8,3)
Food intolerance	2 (7)	2 (8,3)
Nipple eczema	2 (7,7)	0 (0)
Keratoconus	0 (0)	0 (0)
Anterior subcapsular cataracts	0 (0)	0 (0)

eTable 2. KIDMED test to assess the Mediterranean Diet.

KIDMED test	Scoring
Takes a fruit or fruit juice every day	+1
Has a second fruit every day	+1
Has fresh or cooked vegetables regularly once a day	+1
Has fresh or cooked vegetables more than once a day	+1
Consumes fish regularly (at least 2–3/week)	+1
Goes >1/ week to a fast food restaurant (hamburger)	-1
Likes pulses and eats them >1/week	+1
Consumes pasta or rice almost every day (5 or more per week)	+1
Has cereals or grains (bread, etc) for breakfast	+1
Consumes nuts regularly (at least 2–3/week)	+1
Uses olive oil at home	+1
Skips breakfast	-1
Has a dairy product for breakfast (yogurt, milk, etc)	+1
Has commercially baked goods or pastries for breakfast	-1
Takes two yoghurts and/or some cheese (40 g) daily	+1
Takes sweets and candy several times every day	-1

Adapted from: Serra-Majem L, Ribas L, García A, Pérez-Rodrigo C, Aranceta J. Nutrient adequacy and Mediterranean Diet in Spanish school children and adolescents. Eur J Clin Nutr. 2003 Sep;57 Suppl 1:S35-9. PubMed PMID: 12947450.

eTable 3. Estimation of the effect of intervention in laboratory parameters

Parameters in the log scale	Baseline ⁽¹⁾	Follow-up ⁽²⁾
Log(IL-4)	0.038 (-0.045, 0.113)	-0.068 (-0.176, 0.041)
Log(IL-5)	-0.029 (-0.109, 0.051)	-0.046 (-0.142, 0.049)
Log(IL-10)	0.061 (-0.087, 0.209)	0.006 (-0.127, 0.138)
Log(IL-13)	-0.026 (-0.094, 0.038)	-0.047 (-0.156, 0.064)
Log(Eosinophils (n/μl))	-0.069 (-0.597, 0.459)	0.350 (-0.167, 0.868)
Log(IGE (IU/ml))	-0.019 (-0.129, 0.085)	-0.035 (-0.181, 0.129)
Log(Lactic deshydrogenase)	0.207 (-0.841, 1.256)	-0.311 (-0.808, 0.190)

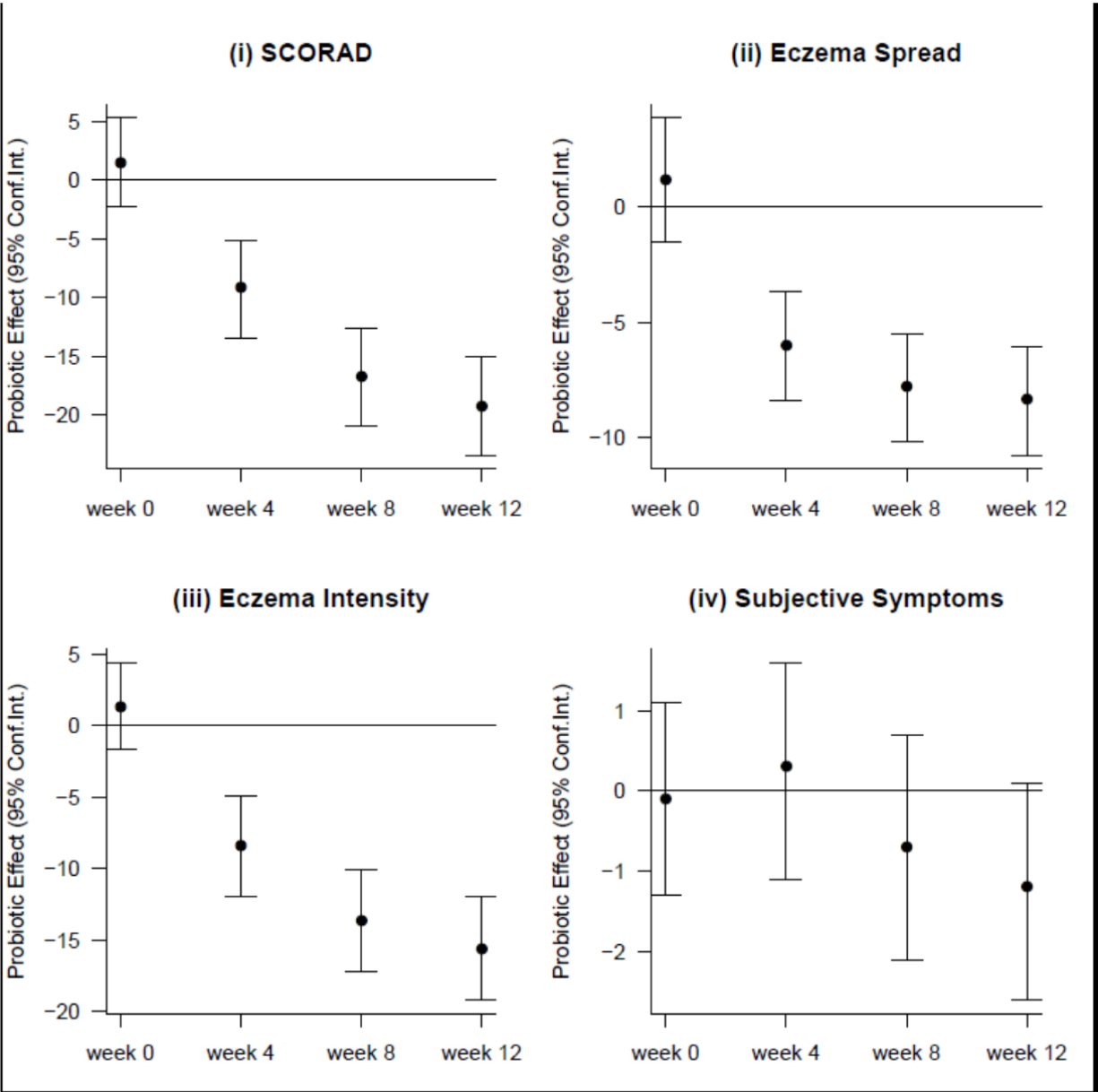
Logarithms were taken on the variables to normalize the data. Effects are estimated using linear mixed-effects models with a group-by-week interaction, participant-level random intercepts and adjusted for variables used to stratified randomization. (1): mean difference between the groups (probiotic minus placebo) at baseline. (2): Differences in change from baseline.

eTable 4. Estimation of the effect of the intervention in SCORAD components

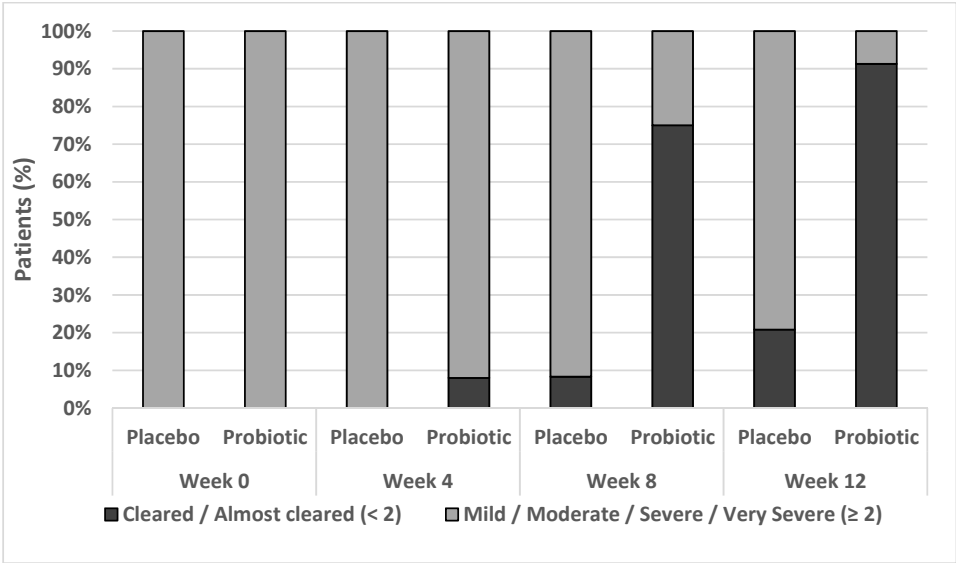
	Group	Baseline	Week 4	Week 8	Week 12
SCORAD subcomponent of EXTENSION					
(1)	Placebo	15.5(11.9, 19.1)	-3.5(-5.8, -1.1)	-5.5(-7.8, -3.2)	-6.1(-8.5, -3.8)
(2)	% of 14.9 ^(A)		-23%(-39%, -7%)	-37%(-52%, -21%)	-41%(-57%, -25%)
(3)	Probiotic	16.7(13.2, 20.2)	-9.5(-11.7, -7.2)	-13.3(-15.6, -11)	-14.6(-16.9, -12.2)
(4)	% of 14.9 ^(A)		-64%(-78%, -48%)	-89%(-104%, -74%)	-98%(-113%, -82%)
(5)	Difference	1.2(-1.5, 3.9)	-6(-8.4, -3.7)	-7.8(-10.2, -5.5)	-8.4(-10.8, -6.1)
(6)	% of 14.9^(A)		-40%(-56%, -25%)	-52%(-68%, -37%)	-56%(-72%, -41%)
	(A) Percentage effectiveness: the rows above divided by baseline mean of all participants (14.9) and multiplied by 100.				
SCORAD subcomponent of INTENSITY					
(7)	Placebo	23.2(19.4, 27.1)	-2.5(-6, 1.1)	-2(-5.6, 1.5)	-2.9(-6.5, 0.6)
(8)	% of 23.7 ^(B)		-11%(-25%, 5%)	-8%(-24%, 6%)	-12%(-27%, 3%)
(9)	Probiotic	24.6(20.8, 28.4)	-10.9(-14.3, -7.5)	-15.7(-19.2, -12.2)	-18.5(-22, -14.9)
(10)	% of 23.7 ^(B)		-46%(-60%, -32%)	-66%(-81%, -52%)	-78%(-93%, -63%)
(11)	Difference	1.3(-1.7, 4.4)	-8.4(-12, -4.9)	-13.7(-17.2, -10.1)	-15.6(-19.2, -12)
(12)	% of 23.7^(B)		-36%(-51%, -21%)	-58%(-73%, -43%)	-66%(-81%, -51%)
	(B) Percentage effectiveness: the rows above divided by baseline mean of all participants (23.7) and multiplied by 100.				
SCORAD subcomponent of SUBJECTIVE					
(13)	Placebo	5.6(4.2, 7)	-2.2(-3.5, -0.8)	-3.1(-4.5, -1.8)	-3.2(-4.6, -1.8)
(14)	% of 6 ^(C)		-37%(-58%, -13%)	-52%(-75%, -30%)	-53%(-77%, -30%)
(15)	Probiotic	5.5(4.1, 6.9)	-1.9(-3.2, -0.6)	-3.8(-5.2, -2.5)	-4.4(-5.8, -3.1)
(16)	% of 6 ^(C)		-32%(-53%, -10%)	-63%(-87%, -42%)	-73%(-97%, -52%)
(17)	Difference	-0.1(-1.3, 1.1)	0.3(-1.1, 1.6)	-0.7(-2.1, 0.7)	-1.2(-2.6, 0.1)
(18)	% of 6^(C)		5%(-18%, 27%)	-12%(-35%, 12%)	-20%(-43%, 2%)
	(C) Percentage effectiveness: the rows above divided by baseline mean of all participants (23.7) and multiplied by 100.				

Effects are estimated using linear mixed-effects models with a group-by-week interaction, participant-level random intercepts and adjusted for variables used to stratified randomization. Columns of weeks contain differences from baseline to this week (and 95% CI). Rows of “difference” are differences of the changes between the two arms (i.e effect of the trial).

eFigure 1. Estimated differences between the two trial arms



eFigure 2. Percentage of patients with IGA score under 2, measured at each checkup in weeks 4, 8, and 12. Comparison between groups



eFigure 3. Distribution of number of days using steroids to treat flares

