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ORIGINAL ARTICLE

Food Allergy and Gastrointestinal Disease

The step-down approach in children with cow's milk allergy: Results of a randomized controlled trial

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Abstract

Background: The **S**tep-**D**own **A**pproach for **C**ow's **M**ilk **A**llergy (SDACMA) trial evaluated the tolerability and the rate of immune tolerance acquisition in CMA children starting dietary treatment with amino acid-based formula (AAF) and then switching to EHCF containing the probiotic *Lacticaseibacillus rhamnosus* GG (EHCF + LGG).

Methods: Randomized controlled trial involving IgE-mediated CMA children receiving AAF from at least 4 weeks. EHCF + LGG tolerance was evaluated by the results of double-blind placebo-controlled food challenge (DBPCFC). Subjects tolerating EHCF + LGG were randomly allocated to remain on AAF, or to switch to EHCF + LGG. Immune tolerance acquisition to cow's milk proteins was evaluated with DBPCFC after 12 months of treatment. Allergy screening tests and body growth were also monitored.

Results: Sixty IgE-mediated CMA children were enrolled. The proportion of children treated with AAF who resulted tolerant to the first exposure of EHCF + LGG was 0.98 (exact 95% CI 0.91–0.99). The rate of the immune tolerance acquisition to cow milk proteins after 12 months treatment was higher in the EHCF + LGG (0.48, 95% exact CI 0.29–0.67, $n/N = 14/29$) than in the AAF group (0.03, 95% exact CI 0.001–0.17, $n/N = 1/30$). There was an absolute benefit increase (ABI) of tolerance rate equal to 0.45 (95% CI 0.23–0.63, Newcombe method 10) for EHCF + LGG versus AAF, corresponding to a NNT of 2 (2–4, Bender's method). A normal body growth pattern was observed in the two study groups.

Conclusion: In IgE-mediated CMA children the step-down from AAF to EHCF + LGG is well tolerated and could facilitate the immune tolerance acquisition.

KEYWORDS
diet therapy, food allergy, hypoallergenic formula, immune tolerance, probiotics

Abbreviations: AAF, amino acid-based formula; CMA, cow's milk protein allergy; CRF, case report form; DBPCFC, double-blind, placebo-controlled food challenge; EHCF, extensively hydrolyzed casein formula; IgE, immunoglobulin E; LAZ, length-for-age *z*-score; LGG, *L. rhamnosus* GG; MPAT, multidisciplinary pediatric allergy team; NNT, number needed to treat; OFC, oral food challenge; RT, research team; SD, standard deviation; SPT, skin prick test; ST, statistical team; WAZ, weight-for-age *z*-score; WHO, World Health Organization.

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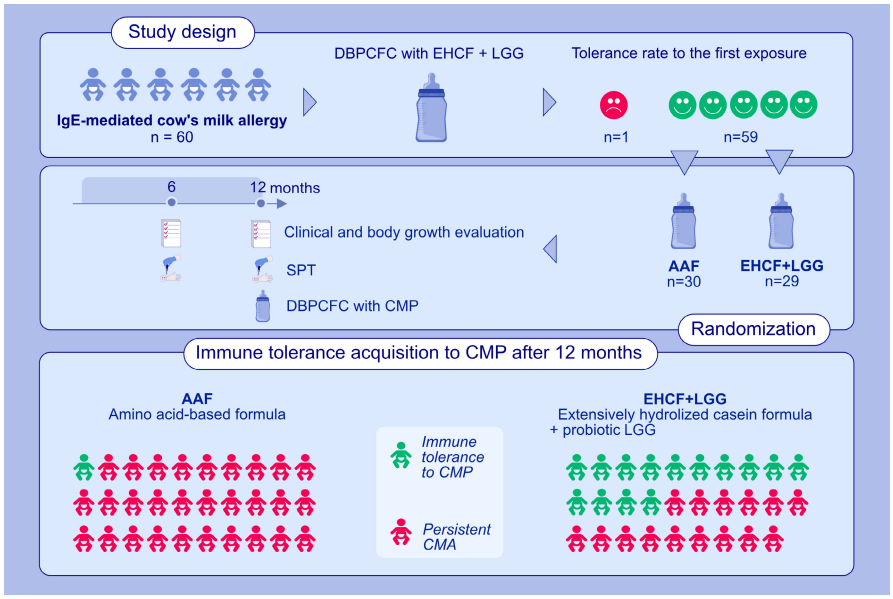

GRAPHICAL ABSTRACT

The SDACMA trial evaluated the tolerability and the rate of immune tolerance acquisition in CMA children starting dietary AAF treatment and then switching to EHCF + LGG. Subjects tolerating EHCF + LGG were randomly allocated to remain on AAF, or to switch to EHCF + LGG. In IgE-mediated CMA children the step-down from AAF to EHCF + LGG is well tolerated and could facilitate the immune tolerance acquisition. Abbreviations: AAF, amino acid-based formula; CMP: cow's milk protein; DBPCFC, double-blind placebo-controlled food challenge; EHCF+LGG, extensively hydrolyzed casein formula plus *Lactobacillus rhamnosus* GG; IgE, immunoglobulin E; SDACMA, Step-Down Approach in children with Cow's Milk Allergy; SPT, skin prick test

1 | INTRODUCTION

Cow's milk allergy (CMA) is one of the most common food allergies and one of the most expensive allergic diseases in the pediatric age with significant cost for the families and health care systems.[1–5] The avoidance of cow milk proteins is the hallmark of the management.[6] While breastmilk remains the ideal nutrient source in children with CMA, if breastfeeding is not available, the patient must be fed a special formula adapted to CMA dietary management, during infancy and later, if the disease persists. The most used are the following: extensively hydrolyzed whey or casein formulas, soy formulas, hydrolyzed rice formulas, or amino acid-based formulas (AAF). Special formula use is the most relevant cost driver in CMA.[5,7–9]

Extensively hydrolyzed casein formula (EHCF) is considered an example of extensively hydrolyzed formula as first-line approach in most formula-fed infants with CMA.[10–13] Whereas, AAF has been indicated for severe forms of CMA, or in patients with multiple food allergies and growth faltering.[10,12–20]

Amino acid-based formula could also be considered as second-line strategy in children reacting to EHCF. Previous studies reported that children with CMA may react to residual allergens in EHCF, but data are still conflicting.[7,21–26] This aspect could be relevant because AAF is considered the safest dietary strategy for severe CMA children, but it is also the most expensive and preclinical and clinical data suggest that this formula is unable to promote immune tolerance , substantially due to the absence of peptides.[27,28] On the contrary, several experimental and clinical evidence suggest that EHCF, supplemented with the probiotic *L.rhamnosus* GG (EHCF + LGG), could promote the acquisition of immune tolerance in children affected by CMA.[29–34]

The **S**tep-**D**own **A**pproach for **C**ow's **M**ilk **A**llergy (SDACMA) project was designed to evaluate in a prospective clinical trial the rate of tolerability of EHCF + LGG in children with IgE-mediated CMA treated with AAF, and to investigate the potential effect on immune tolerance acquisition of switching to EHCF + LGG in CMA pediatric patients previously treated with AAF.

2 | METHODS

2.1 | Trial design and ethics

The SDACMA study was a randomized, double-blind, parallel-arm trial performed at a tertiary center for pediatric allergy between February 2018 and December 2020. The trial was approved by Ethics Committee of the University Federico II of Naples and was performed in accordance with the Helsinki Declaration (Fortaleza revision, 2004), the Good Clinical Practice Standards (CPMP/ICH/135/95), and with the pertinent European and Italian regulations about privacy. Written informed consent to participate in the study was obtained by the parents of the children. The trial was part of the SDACMA project and was registered on clinicaltrials.gov as NCT03449537. The design of the study is depicted in Figure 1.

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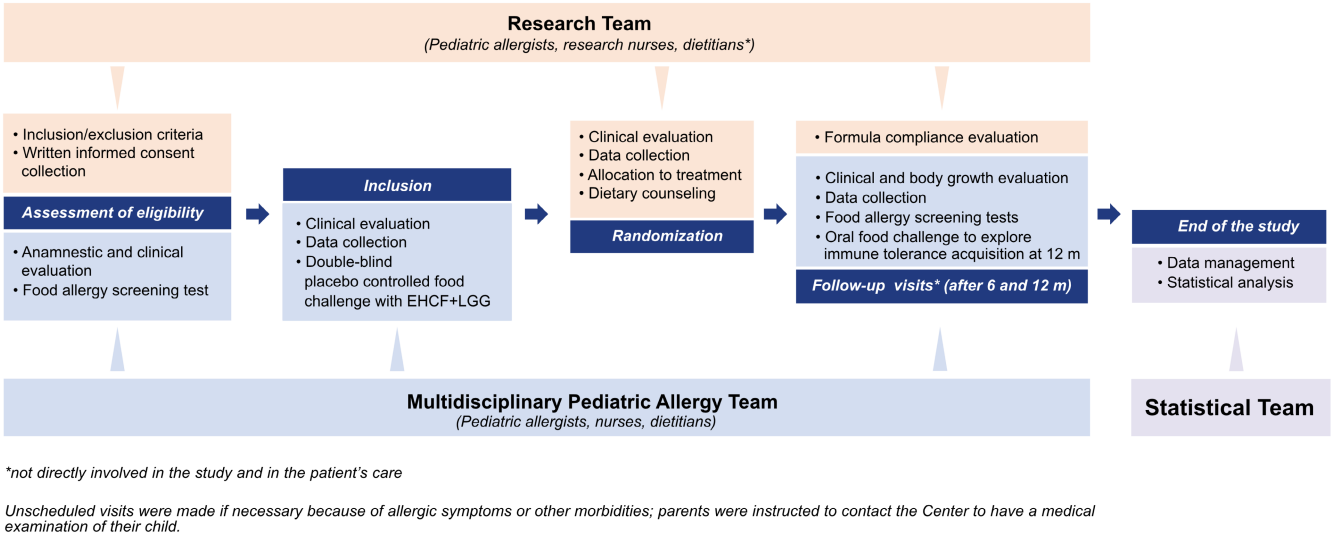

FIGURE 1 Study design.

2.2 | Participants and intervention

Non-breastfed infants (aged <6 months) with IgE-mediated CMA, previously placed on AAF by their family pediatricians or physician because their clinical history, symptoms, and a positive skin prick test for cow's milk, were considered for the study. These patients were referred to the center as part of the standard care procedures and follow-up for CMA.

At enrollment all subjects were in stable clinical condition without CMA-related symptoms, following a strict cow milk protein elimination diet with the use of AAF from ≥4 weeks prior to recruitment.

The exclusion criteria were: treatment with pre−/pro−/synbiotics or antibiotics in the previous 4 weeks; previous history of CMA-induced anaphylaxis or EHF intolerance; food protein-induced enterocolitis syndrome; concomitant presence of other allergic diseases, chronic systemic diseases, infectious diseases, autoimmune diseases, immunodeficiencies, malformations, malignancies, genetic and metabolic diseases; history of gastrointestinal tract surgery; participation in other studies; investigator's uncertainty about the willingness or ability of the subject to comply with the protocol requirements.

The trial involved three parallel teams: the Multidisciplinary Pediatric Allergy Team (MPAT), the Research Team (RT), and the Statistical Team (ST).

The MPAT was composed by pediatric allergists, registered dietitians, and pediatric nurses. It performed a full anamnestic and clinical evaluations, including the diagnostic oral food challenge of all patients. The RT was composed by pediatric allergists, research nurses, and registered dietitians. It was dedicated to the evaluation of the inclusion criteria, the collection of the informed consent from the parents/tutors of each child and then to the randomization of participants, but it was not involved in the patient's care. The ST was composed by biostatisticians who performed all procedures for data analysis.

All subjects with a challenge-proved diagnosis of IgE-mediated CMA were invited to participate to the study by the RT.

In a dedicated visit, after the collection of the informed consent from the parents/tutors of each child, skin prick tests (SPT) to cow's milk proteins, raw cow's milk, and EHCF + LGG were performed in all study subjects. Briefly, SPT was performed with the EHCF + LGG reconstituted according to the manufacturer's instructions. Allergens and EHCF + LGG were applied to the patient's volar forearm. SPTs were performed using a 1-mm single peak lancet (ALK) with histamine dihydrochloride (10 mg/mL) and an isotonic saline solution (NaCl 0.9%) as positive and negative controls, respectively. Reactions were recorded based on the largest diameter (in millimetres) of the wheal-and-flare reaction at 15 min. The SPT result was considered "positive" if the wheal was 3 mm or larger, without reaction to the negative control.

After SPT, all patients underwent the double-blind, placebo-controlled challenge (DBPCFC) with EHCF + LGG or the placebo formula (namely, the AAF previously used). A computer-generated randomization list of participant numbers indicating the order in which each study formula was adopted for all oral food challenges (OFC). Randomization and preparation of the challenges were performed by experienced registered dietitians. In addition, bottles were covered by a paper sheet so that they were not distinguishable. The investigator, the nursing staff, and the family were therefore not informed of what formula the child was being fed. Before each OFC day, the investigator ensured that the child was not presenting any clinical abnormalities and had stopped all medications, including antihistamines, that could interfere with the results of the OFC. All subjects were fasted from at least 3 h before the procedure. Briefly, increasing doses of formula (corresponding to 3, 10, 30, 100, 300, 1000, and 3000 mg of food protein) were administered in a blinded fashion under medical supervision at intervals of 20 min. The infants were observed for 2 h after the final dose and then discharged. In the case of a positive OFC, at any testing dose, the patient was treated as deemed necessary by the investigator and remained under observation until symptom resolution. If patients did not show any symptoms within the first 24 h, to assess long-term tolerance and

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reveal any false-negative results to the challenges, parents administered one single top dose of the tested formula (EHCF + LGG or AAF) to the patients every day at home for 7 days (7-day home feeding period), and parents were instructed not to introduce any new foods. In addition, an emergency treatment plan and prescriptions for emergency medications were provided to the parents. If any symptoms occurred during this period, the subjects returned to the outpatient clinic on the same day. During the 7-day home feeding period, parents were invited to record daily the following data: the total amount of formula ingested by the subject; the types of foods eaten; the occurrence and severity of vomiting, diarrhea, rash, runny nose, wheezing, or any other symptoms (rated as mild, moderate, or excessive); the number of bowel movements and stool color, consistency and odor; any adverse or serious adverse events; and the formula acceptability by their child, from very unsatisfied to very satisfied. After a 7-day home feeding period of EHCF + LGG or AAF administration, the patients were examined, and the parents were interviewed at the center. To rule out a false-negative challenge result, parents contacted the center if any symptoms occurred in the following 7 days after the OFC procedures. The challenge was considered negative if the patient tolerated the entire challenge, including the observation period. All objective and subjective symptoms were assessed simultaneously by the MPAT and were registered using a standardized symptom score.[35,36]

All patients with negative OFC to EHCF + LGG were randomized by the RT to two study groups: Group #1, who remained on AAF (Puramino®; Mead Johnson Nutrition) and Group #2, who switched to EHCF + LGG (EHCF + LGG, Nutramigen LGG®; Mead Johnson NutritionUSA), according to a computer-generated randomization list.

Children in Group 2 received formula 2 until 12 months of age and then formula 3 after this age.

The composition of the study formulas is reported in the Table S1.

The MPAT, parents and patients were blinded to the allocated treatment. The parents were instructed on how to follow an adequate cow milk protein-free diet with an oral and written instruction about the preparation, use and about how to weigh formula and solid foods, and how to record daily formula and solid foods intake in the diary. Specifically, it was recommended that patients follow a normocaloric diet (daily energy intake was based on the patient's age and gender), consisting of protein (Population Reference Intake: 1.32–1.00 g/kg/die), carbohydrates (45%–60% of energy intake [En]), fat (35%–40% En; <10% En from saturated fatty acids, 5%–10% En from polyunsaturated fatty acids), and fiber (8.4 g/1000 kcal). Furthermore, supplementation with calcium and vitamin D was evaluated in case of deficiency and/or insufficient dietary intake.[37]

The study products were provided in tins containing 400 g of powder and were stored at room temperature in a dry environment. The packages and contents of treatments were indistinguishable. At each visit, the study formula was dispensed to the parents free of charge. Formula adherence was evaluated by counting and weighing the returned tins and by reviewing the notes on the diary recorded by parents. Compliance was judged acceptable in the presence of >80% recommended formula intake.

Then, two visits after 6 and 12 months were planned. During these visits, the MPAT assessed clinical status, the compliance to the cow's milk protein-free diet, the compliance to the formula previously assigned (operationally defined as the consumption of at least 80% of the formula used), the SPT to cow's milk protein and to raw milk, and body growth.

Anthropometric measurements were collected following standardized procedures. Briefly, naked subjects were weighed twice on calibrated an electronic scale (Seca 834; Seca). Supine length of infants was measured twice using a standard measuring board (Seca 210 Mobile Measuring mat). If the anthropometric measurements differed substantially (>100 g for weight and >5 mm for length), a third measurement was obtained. Weight-for-age *z*-score (WAZ) and length-for-age *z*-score (LAZ) were calculated based upon the World Health Organization (WHO) child growth standards[38] using the WHO Anthro Software (Available at http://www.who.int/childgrowth/software/en/).

After 12 months of dietary treatment, a new DBPCFC with cow's milk protein was performed as previously described, with the aim to evaluate the possible acquisition of immune tolerance to cow's milk proteins.

Unscheduled visits were made if necessary, because of allergic symptoms or other morbidities. Whenever allergic symptoms or other comorbidities occurred, parents were instructed to contact the Center.

Adverse events, serious and non-serious, during the 12-month study period were notified by the investigators and coded by diagnosis, severity, date of onset, and resolution. They were reported and classified as related (definitely, probably, or possibly related) or unrelated (unlikely or not related) based on a relationship to study formula intake according to the study investigators.

All data were collected in the specific clinical chart.

2.3 | Outcome

The main outcome of the trial was twofold: to explore the tolerance rate to EHCF + LGG and to evaluate the immune tolerance acquisition in CMA children starting dietary treatment with an AAF and then switching to EHCF + LGG.

Allergy screening tests and a body growth assessment were also performed during the study period.

2.4 | Sample size

We hypothesized that 5% of the subjects in the placebo group and 35% of those in the EHCF + LGG group would develop immune tolerance at the end of the study. A sample size of 27 subjects per arm was estimated to detect an absolute difference of 30% in immune tolerance at an alpha level of 0.05 with a power of 0.80 (Pearson's Chi-square test; Stata 14.0; Stata Corp). Based on previous studies, we expected that up to 5% of the subjects would be

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resulted intolerant to the EHCF + LGG formula, and 5% of them could be lost to the follow-up. Thus, we enrolled 30 (27 + 3) subjects per arm.

2.5 | Randomization

Treatment (AAF or EHCF + LGG) was assigned in 1:1 ratio using a randomization list with block sizes of 2 that was produced using the *ralloc* command (Stata 14.0; Stata Corp).

2.6 | Allocation concealment

Amino acid-based formula and EHCF + LGG were packaged in tins containing 400 g of powder, which were consecutively numbered according to the randomization without any reference to the group assignment. Group assignment was known only to independent registered dietitian not directly involved in the study and in the patient's care who prepared the packages.

2.7 | Blinding

The outcome assessors, the parents of the infants, and the researchers who performed data entry were blinded to the treatment. The independent registered dietitian and the statistician who performed data analysis were not blinded to the treatment.

2.8 | Data collection

A clinical trial monitor reviewed the clinical forms for completeness, clarity, consistency, and accuracy. All the data were recorded anonymously and entered into the study database using a single data-entry method by the RT. The study database underwent data cleaning according to standard procedures and was locked before statistical analysis by the ST.

2.9 | Compliance

Compliance was assessed by asking the parents to return the tins containing the powder and by analyzing 3-day food records administered by the study dietitian.

2.10 | Statistical analysis

The Kolmogorov–Smirnov test was used to determine whether continuous variables were normally distributed, in which case they were reported as mean (SD). Continuous variables not normally distributed are reported as median (50th percentile) and interquartile range (IQR, 25th and 75th percentiles). Discrete variables were reported as the number and proportion of subjects with the characteristic of interest. Exact 95% confidence intervals were calculated for tolerance rates, those of the between-group difference in tolerance rate were calculated using Newcombe method 10, and those of number needed to treat (NNT) using Bender's formula. Growth changes were evaluated using a generalized linear regression model (GLM) with a Gaussian family, an identity link, and the infant as random intercept. Individual growth trajectories were plotted and inspected to gain a better insight into the inter-individual variability of growth in the two trial arms.

The level of significance for all statistical tests was two-sided, $p < .05$. Statistical analysis was performed using Stata 17.0 (Stata Corporation).

3 | RESULTS

The flow of the subjects throughout the study is reported in Figure S1.

From April 2018 to December 2020, a total of 77 subjects were evaluated for eligibility. Seventeen subjects were excluded because the presence of at least one exclusion criteria, 60 were enrolled and underwent SPT and DBPCFC for EHCF + LGG. Baseline anamnestic, demographic, and clinical features of the study population are reported in Table S2.

At baseline, all subjects were weaned and had already introduced complementary food into their diet and were receiving AAF previously prescribed by their family pediatrician or physician. The SPT with EHCF + LGG was negative in all study subjects.

The DBPCFC for EHCF + LGG was positive in only one patient (0.02, exact 95% CI 0.0004–0.09) who presented vomiting 2 min after the administration of the last testing dose; the corresponding proportion of children tolerant to EHCF + LGG was 0.98 (exact 95% CI 0.91–0.99). The patient with positive DBPCFC for EHCF + LGG was excluded from randomization.

Of the remaining 59 infants, 30 were randomly allocated to AAF (Group 1) and 29 switched to EHCF + LGG (Group 2). The Table 1 shows that the children randomized to Group 1 and Group 2 had similar baseline features.

No patients abandoned the study after randomization and all patients completed the study without any protocol violations. All children were compliant (i.e., the treatments were well accepted and they consumed at least 80% of the assigned formula, as determined by the returned tins and by the evaluation of 3-day food diary analyzed by dietitians. No case of misunderstanding of formula use was reported).

Figure 2 plots the immune tolerance acquisition rate to cow milk proteins after 12-m dietary treatment, that is, the proportion of children passing the DBPCFC to cow's milk proteins. The tolerance acquisition rate was higher in the EHCF + LGG (0.48, 95% exact CI 0.29–0.67, $n/N = 14/29$) than in the AAF group (0.03, 95% exact CI 0.001–0.17, $n/N = 1/30$). There was therefore an absolute benefit

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TABLE 1 Main features of the study population at randomization.

	AAF	EHCF + LGG
	N = 30	*N* = 29
Male	16 (53.3%)	17 (58.6%)
Spontaneous delivery	8 (26.7%)	12 (41.4%)
Born at term	30 (100%)	29 (100%)
Weight at birth, kg (SD)	3.25 (0.42)	3.23 (0.48)
Breastfed for ≥2 months	14 (46.7%)	17 (58.6%)
Weaning age, months (SD)	5 (0.72)	5.2 (0.76)
Siblings, *n* (IQR)	1 (0)	1 (1)
Familial risk of allergy	21 (70%)	18 (62.1%)
Allergic first-degree relatives, *n* (IQR)	1 (1)	1 (1)
Exposure to passive smoking	7 (23.3%)	11 (37.9%)
Maternal smoking during pregnancy	12 (40%)	13 (44.8%)
Exposure to pets	11 (36.7%)	9 (31%)
Age, months (SD)	5.3 (0.48)	5.5 (0.51)
Weight, kg (SD)	6.7 (1)	6.9 (0.9)
Length, cm (SD)	65.4 (3.7)	64.8 (3.4)
Positive prick by prick test for raw milk	30 (100%)	29 (100%)
Positive skin prick test for α-lactalbumin	27 (90%)	21 (72.4%)
Positive skin test positive for β-lactoglobulin	21 (70%)	21 (72.4%)
Positive skin prick test positive for casein	19 (63.3%)	15 (51.7%)
Duration of treatment with AAF before inclusion, weeks (SD)	4.7 (0.6)	5 (0.7)
Gastrointestinal symptoms at CMA onset	20 (66.7%)	22 (75.9%)
Cutaneous symptoms at CMA onset	24 (80%)	19 (65.5%)
Respiratory symptoms at CMA onset	4 (13.3%)	3 (10.3%)

Abbreviations: AAF, amino acid-based formula; CMA, Cow's milk allergy; EHCF, extensively hydrolyzed casein formula; LGG, *L.rhamnosus* GG; SD, standard deviation.

increase (ABI) of tolerance rate equal to 0.45 (95% CI 0.23–0.63, Newcombe method 10) for EHCF + LGG versus AAF, corresponding to a NNT of 2 (2–4, Bender's method).

Figure S2 plots the SPT negativization rate and the difference in negativization rate between the AAF and EHCF + LGG groups to alpha-lactalbumin (panel A), beta-lactoglobulin (panel B), casein (panel C), and raw milk (panel D) during the study period. There was an increase in the negativization rate for the EHCF + LGG versus AAF group which however did reach statistical significance only for raw milk. 95% CIs are nonetheless wide for all differences suggesting that higher group sizes are needed to detect this effect with precision.

Figure 3 plots the mean (95% CI) of weight (panel A) and length (panel B) at 0, 6, and 12 months for the two study groups (random-effect linear GLM). A normal body growth pattern was observed, and no difference was detected between the two study groups at any time point.

Figure S3 plots the individual changes of the SDS of weight (panel A) and length (panel B) at 0, 6, and 12 months for the two study groups. Already after 6 months from the enrolment, no infant had a weight or length <−1 SDS, confirming that subjects early reached normal growth and maintained this condition throughout the study.

For all study subjects, sex- and age-related energy intake was assessed at each study visit by independent experienced registered dietitians not directly involved in the study and in the patient's care.

Coherently with anthropometric data, energy intake was found to be within normal limits for age and sex at each study visit. There were no differences between the two study groups during the study.

Total daily energy intake was within the recommended energy requirements for sex and age for all study subjects without difference between the two study groups during the study (data not shown; https://sinu.it/larn/, fourth revision).[39]

Regarding safety data, there were 15 non-serious AEs due to acute gastroenteritis (*n* = 2), respiratory infections (*n* = 6), and febrile illness/viral infections (*n* = 7). All AEs were considered to be unrelated to the study formulas.

4 | DISCUSSION

This is the first randomized, double-blind trial evaluating the safety and the efficacy of formula switching from AAF to EHCF + LGG. We found that EHCF + LGG could be tolerated by the vast majority of CMA children, and that the step-down approach from AAF to EHCF + LGG could promote a faster acquisition of immune tolerance. We observed that EHCF + LGG can be tolerated by >98% of CMA children, a rate much higher than that reported by previous small case-series, where up to 35% of CMA children were described as reacting to EHCF.[7,21–26] This discrepancy could be due to several factors including the study design, the simultaneous evaluation of different extensively hydrolyzed cow's milk proteins formulas, the patients feature, and the evaluation procedures adopted in these studies. In the SDACMA trial we adopted a validated DBPCFC-based procedure to evaluate the ECHF + LGG tolerance, we evaluated just one extensively hydrolyzed formula available for CMA treatment, and that we adopted strict exclusion criteria, including CMA-related anaphylaxis, eosinophilic gastrointestinal disorders, and multiple food allergies. Our results support guidelines suggesting the use of EHCF as a first-line treatment for CMA, except for patients with CMA-related anaphylaxis.[10–12,40]

Conversely, according to recent evidence, the results of this RCT confirmed that EHCF + LGG has a greater potential in reducing disease duration if compared with AAF.[29,32,34,41–43]

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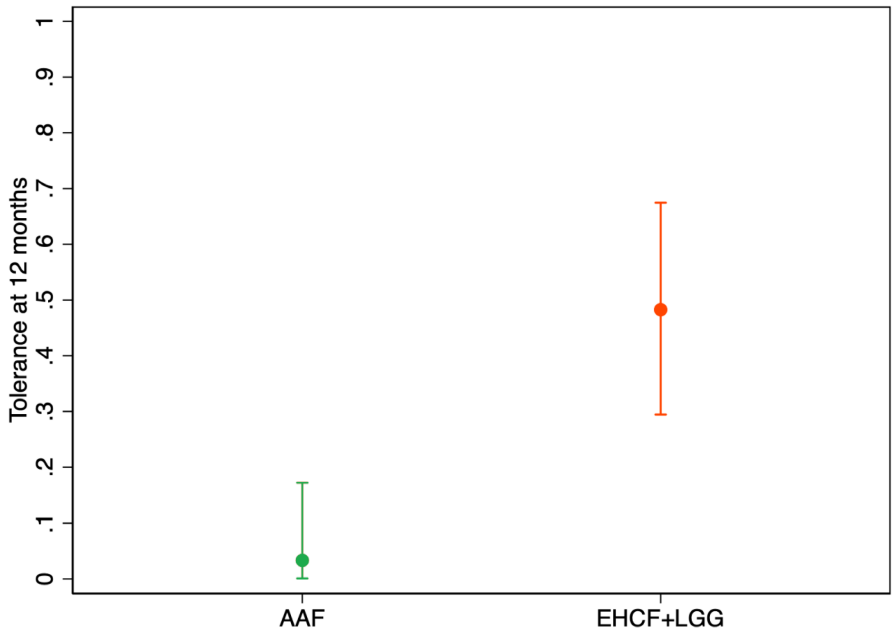

FIGURE 2 The immune tolerance acquisition rate to cow's milk proteins in the study groups after 12 months of dietary treatment.

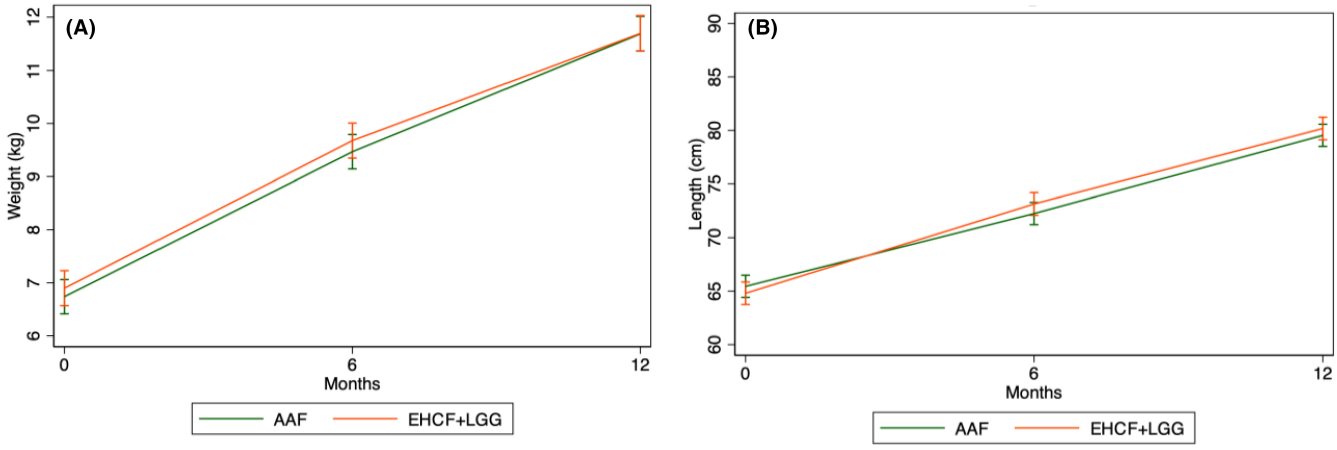

FIGURE 3 Mean (95% CI) of weight (panel A) and length (panel B) at 0, 6, and 12 months (random-effect linear generalized linear regression model) for the study groups.

These data are relevant considering the most recent evidence suggesting that the natural history of CMA has changed over time, with slower rates of resolution and a higher proportion of children with disease persisting into school age and older.[44–46]

The supportive evidence of the potential beneficial role of EHCF + LGG may be due to multiple mechanisms including a positive epigenetic regulation of forkhead box P3, Th1/Th2 cytokine genes and microRNAs expression. In addition, it has been demonstrated that EHCF + LGG exerts a positive modulation of gut microbiome structure and function increasing the number of healthy bacteria strains with an increased production of the short chain fatty acid (SCFA) butyrate that is considered one of the most active gut microbiome-derived metabolites able to drive immune tolerance.[47–55]

In addition, the results of this study are well in line with other evidence demonstrating that infants with CMA fed with an AAF or EHCF + LGG presented adequate body growth.[56–63]

This study has several strengths. First, it was performed on an adequate number of children with an IgE-mediated CMA followed at a tertiary pediatric allergy center with a high follow-up rate. Second, the methodology adopted in this study was rigorous: only the independent registered dietitian not directly involved in the study and in the patient's care was aware of the assigned treatment while the other researchers were blinded to group assignment, and diet and formula intake were assessed systematically.

Nonetheless, this study has some limitations. The main limitation is that our data cannot be generalized to children with conditions that were reasons for exclusion from the study, children with non-IgE-mediated CMA, or in children tolerating baked milk. In fact, recent evidence suggested that the consumption of baked milk and the administration of single dose of milk at the ED_{05} immediately after diagnosis of CMA can accelerate the acquisition of tolerance to whole cow's milk in young children.[64] In this light, additional studies

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combining different treatments are advocated to further enhance the CMA outgrowth.

Furthermore, another limitation is that our results are limited by the lack of data on gut microbiome and Th1/Th2 cytokines.

In summary, this was the first randomized, double-blind, parallel-arm trial performed in a well-characterized population of pediatric patients affected by IgE-mediated CMA showing that EHCF + LGG could be tolerated by the vast majority of children and that in more severe cases AAF could be the first-line strategy for the CMA dietary management, but when a full resolution of symptoms is achieved the step-down approach with EHCF + LGG could promote faster acquisition of immune tolerance.

AUTHOR CONTRIBUTIONS

RN and RBC conceived the study, participated in its design and coordination and wrote the manuscript. RN, SC, LC, AFDGDSS, FO, RDM, IDS, AM cared for the patients and collected data. RN and GB performed the statistical analysis. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

In the past 36 months, Dr. R. Berni Canani received fees as speaker or for research activities from the following companies: Biocodex, Ch. Hansen, Danone, DVB, Humana, Kraft Heinz, iHealth, Mead Johnson Nutriton, Nestlè, Novalac, Sanofi. The authors have no conflicts of interest that are directly relevant to the content of this paper, which remains their sole responsibility.

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SUPPORTING INFORMATION

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