

Bacterial Mucosal Immunotherapy with MV130 Prevents Recurrent Wheezing in Children

A Randomized, Double-Blind, Placebo-controlled Clinical Trial

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Abstract

Rationale: Recurrent wheezing in children represents a severe public health concern. Wheezing attacks (WA), mainly associated with viral infections, lack effective preventive therapies.

Objectives: To evaluate the efficacy and safety of mucosal sublingual immunotherapy based on whole inactivated bacteria (MV130) in preventing WA in children.

Methods: A Phase 3 randomized, double-blind, placebo-controlled, parallel-group trial including a cohort of 120 children <3 years old with ≥3 WA during the previous year was conducted. Children with a positive skin test to common aeroallergens in the area where the clinical trial was performed were excluded from the trial. Subjects received MV130 or placebo daily for 6 months. The primary

endpoint was the number of WA within 1 year after the first dose comparing MV130 and placebo.

Measurements and Main Results: There was a significant lower number of WA in MV130 versus the placebo group, 3.0 (interquartile range [IQR], 2.0–4.0) versus 5.0 (IQR, 3.0–7.0) ($P < 0.001$). As secondary outcomes, a decrease in the duration of WA and a reduction in symptoms and medication scores in the MV130 versus placebo group were found. No adverse events were reported related to the active treatment.

Conclusions: Mucosal bacterial immunotherapy with MV130 shows safety and clinical efficacy against recurrent WA in children.

Clinical trial registered with www.clinicaltrials.gov (NCT 01734811).

Keywords: clinical trial; wheezing attacks; mucosal vaccination; MV130

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Data collected for the study will be made available for researchers whose proposed use of the data has been approved for specified purposes and on a signed data access agreement. Inmunotek will take all necessary measures to ensure that patient privacy is safeguarded. Request for this information must be sent to the corresponding author.

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At a Glance Commentary

Scientific Knowledge on the Subject:

Wheezing attacks constitute a worldwide health threat for children. Almost 50% of children during their first 6 years of life present episodes of wheezing attacks. The etiology of wheezing attacks is diverse, although rhinovirus and respiratory syncytial virus are the main causes of wheezing episodes in young children. Although wheezing attacks can be treated with antiinflammatory drugs, the lack of vaccines remains the major concern in the prevention of the disease. Based on the evidence to date, bacterial immunostimulants provide clinical benefit in patients with recurrent respiratory tract infections, including pediatric wheezing.

What This Study Adds to the Field:

For the first time, in this Phase 3 randomized, double-blind, placebo-controlled clinical trial, the safety and efficacy of the sublingual heat-inactivated whole-cell bacterial formulation MV130 were demonstrated. An important reduction in the number and duration of wheezing attacks in the MV130 group compared with the placebo group is shown. The heterologous immunity conferred by MV130 provides persistent protection without the limitations of antigen specificity being useful in case of coinfection or against pathogens with high mutation rates.

Wheezing attacks (WA) or bronchospasms are defined as episodes of cough with difficult breathing and/or wheezing, lasting at least 6 hours, with or without fever and without chest radiographic abnormalities (1). WA constitute an important health threat in children worldwide (2), affecting a high percentage (30–50%) of them within their first 6 years of life. Recurrent WA show a high (17%) prevalence (3, 4). Although most of the affected children with bronchiolitis eventually outgrow this disorder, recurrent WA constitute a

public health problem, with a considerable social, quality of life, and economic impact (2, 5).

Most wheezing episodes in young children have a viral etiology, mainly rhinovirus and respiratory syncytial virus (2, 6). The prevention of recurrent WA is a major concern, as vaccines targeting these viruses are not yet available (7) and effective antiviral therapies are still lacking (6). Current prevention strategies with antiinflammatory drugs are far from being optimal (2). Some studies propose the use of daily inhaled corticosteroid therapy among preschool children with recurrent wheezing and high-dose intermittent inhaled corticosteroids among preschool children with viral-triggered wheezing (8). The use of oral corticosteroids for acute wheeze exacerbation in preschool children has also been suggested, but the level of clinical benefit demonstrated remains to be low (9). Azithromycin has been also postulated as a therapeutic option in children with recurrent wheezing in the context of lower respiratory tract infections for acute exacerbations or to prevent severe exacerbations (9, 10); however, potential benefits must be weighed against further selection of antimicrobial-resistant microorganisms and possible detrimental effects on the commensal or beneficial airway or gut microbiota. Montelukast has been also studied as a potential alternative in the prevention of preschool recurrent wheezing with controversial results (11, 12). All in all, the lack of clearly effective therapeutic interventions and preventive strategies are a reality.

An increasing number of studies indicate that certain microbial stimuli acting on innate immune cells can promote a quite long-lasting nonspecific protection against different pathogens, a phenomenon termed trained immunity (13–15).

MV130 is a sublingual polybacterial preparation able to stimulate innate immune cells and to enhance certain T cell responses to related and unrelated (bystander) antigens (16, 17). In previous studies in adults, MV130 was effective in preventing recurrent infections (18, 19), including some of viral origin (17). Here, we have addressed the efficacy and safety of MV130 in children with recurrent WA in a double-blind, placebo-controlled (DBPC) study. Some of the results of this study have been previously reported in the form of abstracts (20, 21).

Methods

Study Design, Randomization, and Masking

A Phase 3 randomized, DBPC, parallel-group clinical trial was performed in two hospitals in Spain (La Fe University Hospital and Manises Hospital, both in Valencia) to assess the safety and efficacy of MV130. From October 2012 through May 2015, subjects were enrolled in the clinical trial. The study included 120 children <3 years old with recurrent WA, defined as three or more episodes of WA during the previous year (2). WA were defined as episodes of cough with difficult breathing and/or wheezing, lasting at least 6 hours, with or without fever and without chest radiographic abnormalities in the case that a radiograph was made (1). All children were referred by their primary care pediatrician because of recurrent wheezing during the previous year. The exclusion criteria were other chronic respiratory diseases, malnutrition, positive skin tests to common aeroallergens in the area where the clinical trial was performed, or treatment with γ -globulin, immunostimulants, or immunosuppressants in the previous 12 months. The WA were confirmed by the review of the medical records. Aeroallergen sensitization at baseline was used as an exclusion criterion to avoid eventual WA due to allergic asthma (2, 22, 23). Details on the characteristics of the study and inclusion and exclusion criteria are described in Table 1.

The clinical trial was conducted within the ethical and legal framework established by the Spanish Agency for Medicines and Health Products (RD 223/2004) following good clinical practice (ICH E6: Good Clinical Practice: Consolidated Guideline, CPMP/ICH/135/95) in accordance with European Union Directive 2005/28/CE. Parents or legal guardians provided written informed consent. Regulatory approvals and consent procedures are described in the online supplement.

Participants were allocated by means of a list generated by Random software (Random Software Ltd.) in a 1:1 ratio to receive either MV130 or placebo. Both participants and physicians (who enrolled the patients) were masked to treatment assignments. The active product (MV130) (Bactek; Immunotek; S.L.) consists of a suspension of heat-inactivated, whole-cell gram-positive (90%) and gram-

Table 1. Design Characteristics of the Trial

Type of study	Prospective, two-center, randomized, double-blind, placebo-controlled, parallel-group	
Patients	Children <3 yr old with recurrent wheezing	
Exclusion criteria	Other chronic respiratory diseases Malnutrition Positive skin tests to any of <i>Dermatophagoides pteronyssinus</i> , <i>Dermatophagoides farinae</i> , <i>Alternaria alternata</i> , dog dander, cat dander, olive tree pollen, and/or grass pollen Treatment with γ -globulin, immunostimulants, or immunosuppressants in the previous 12 mo	
Sample size	$N = 120$; difference in expected number of WA: 1.7; SD: 2.55 α -error: 0.05, β -error (two tailed): 0.1 estimated drop-out rate: 20%	
Intervention	Active treatment (MV130): Glycerol Sodium chloride Artificial pineapple flavoring 300 FTU/ml ($\sim 10^9$ whole-cell inactivated bacteria/ml): <i>Streptococcus pneumoniae</i> (60%) <i>Staphylococcus aureus</i> (15%) <i>Staphylococcus epidermidis</i> (15%) <i>Klebsiella pneumoniae</i> (4%) <i>Moraxella catarrhalis</i> (3%) <i>Haemophilus influenzae</i> (3%)	Placebo: Glycerol Sodium chloride Artificial pineapple flavoring
Dose and duration of treatment	Two puffs sublingually daily for 6 mo (10^8 bacteria/100 μ l per puff)	Two puffs sublingually daily for 6 mo
Allocation randomization	Allocation (1:1) by simple randomization Randomization list generated using Random software	
Blinding	Parents, physicians involved in recruiting and follow-up, and any other care providers were blinded	
Treatment concealment	Identical bottles with active treatment or placebo were supplied. Each bottle was marked with the individual patient numeric exclusive code generated by the randomization list	
Treatment adherence	The volume of medication remaining was measured from the bottles returned at each scheduled visit	
Code breaking	For analysis, after the last visit of the last patient	
Main outcome	Number of WA during a whole year (6 mo of treatment + 6 additional mo of follow-up)	
Other outcomes	Duration and severity of WA, symptom and medication scores, use of health and social resources, time to first WA	
Statistical analysis	Shapiro-Wilk test. Chi-square or Fisher's exact tests. Student's t test and Mann-Whitney U test. Hodges-Lehmann estimator. The number needed to treat. Kaplan-Meier estimator and Cox model. $P < 0.05$ was considered significant	
Type of analysis	Per intention-to-treat. Sensitivity analysis using the "worst-case scenario"	
Ethics	Approved by Ethics Committee and Spanish health authorities Run according to the Helsinki declaration, the European laws, and the rules of good clinical practice Written information. Informed consent signed by parents	
Trial registration	EudraCT number 2012-002450-24 Clinicaltrials.gov identifier NCT01734811	

Definition of abbreviations: FTU = formazin turbidity units; WA = wheezing attacks.

negative (10%) bacteria (300 formazin turbidity units/ml $\sim 10^9$ bacteria/ml) in glycerol, sodium chloride, and artificial pineapple flavoring with the following formula: *Streptococcus pneumoniae* (60%), *Staphylococcus aureus* (15%), *S. epidermidis*

(15%), *Klebsiella pneumoniae* (4%), *Moraxella catarrhalis* (3%), and *Haemophilus influenzae* (3%). The placebo preparation contained all excipients without bacteria. The treatment was administered sublingually by spraying under the tongue two puffs of 100

μ l each daily for 6 months. To check the correct administration of the medication, the first dose was administered in the hospital; the following doses were administered at home. To assess the degree of compliance to the trial medication, the volume of medication

remaining was measured from the bottles returned at each scheduled visit. The whole study lasted 12 months, with 6 months of treatment plus 6 additional months of follow up.

Patients could receive additional medication as needed according to the Global Initiative for Asthma guidelines. Visits to primary care pediatricians or an emergency department were allowed as needed for the child's clinical condition. A symptom score and medication score were rated, calculated, and recorded. WA that occurred during the trial were diagnosed by the primary care pediatrician or in emergency room visits. Parents or legal guardians were duly instructed to record symptom and medication scores in daily diary cards. These were carefully reviewed in a blinded manner by the investigators who defined for each patient the initiation and the end of each wheezing attack. In these cards, health and social resources during the study (including complementary tests and unscheduled visits to a pediatrician) were also recorded.

Outcomes

The primary endpoint was to compare the mean number of WA between active (MV130) and placebo groups during a follow up of 12 months after receiving the first dose. A reduction in the active group as compared with the placebo group will indicate that MV130 may prevent WA in children at risk. Secondary endpoints included the number of days with wheezing during the study, duration (days) of WA, time (days) until the appearance of the first WA, number of patients with recurrent WA during the study, symptom score and medication score and their combination related to WA, and, during the whole study period, the use of health and social resources and days with fever. Patients were visited at the clinics every 3 months, and diaries were reviewed together with the parents. Chest symptoms (bronchial mucus secretion, cough, difficult breathing, and wheezing), nose symptoms (nasal mucus secretion), and discomfort were rated as follows: 0 = absent (no sign/symptom evident), 1 = mild (sign/symptom clearly present but easily tolerated), 2 = moderate (definite awareness of sign/symptom that is bothersome but tolerable), and 3 = severe (sign/symptom that is hard to tolerate and causes interference with activities of daily life and/or sleeping). Fever was scored as the number of days with axillary temperature over 37°C. The total symptom score was calculated as the sum of all individual scores.

We did not address specifically whether some symptoms, like cough, were mainly due to upper or lower tract infections, as they can be shared in many cases and therefore difficult to distinguish. The medication was rated according to Dreborg and colleagues (24) with slight modifications: two points were given for one tablet of 4 mg of Montelukast, for one puff of 50 µg budesonide equivalent, or for one dose of oral prednisolone, and three points were given for the inhalation of one puff of 200 µg budesonide equivalent. In the case of antibiotics and antipyretic and/or antiinflammatory drugs, the number of daily doses was recorded.

The evaluation of the worst-case scenario, which assumes that all missing participants have a poor outcome in the MV130 group and a favorable outcome in the placebo group, was further performed as described in the online supplement.

Safety was assessed via the recording of all adverse events that occurred during the trial. Adverse events were individually examined to evaluate their severity and to classify them as probably related or unrelated to the study medication. Adverse events were coded with the use of the Medical Dictionary for Regulatory Activities. Respiratory tract infections, the objective of the study, were not considered an adverse event.

Statistical Analysis

The sample size for the clinical trial was calculated using data from Gutiérrez-Tarango and colleagues related to the number of acute respiratory tract infections in children (25). In this study, we found a difference of means for episodes of bronchospasm of 2.96 (95% confidence interval [CI], -4.22 to -1.70). To detect a difference of 1.7 (the lowest 95% CI limit) or higher in episodes of bronchospasm at 12 months between both groups, assuming an α -error of 0.05 and a power of 90% in a two-sided comparison, a minimum of 59 subjects per group was required. A loss to follow up of 20% was assumed.

All randomized patients were included in our analysis (i.e., an intention-to-treat analysis). We present summary statistics as frequency (percent) for categorical data and median (interquartile range [IQR]) or 95% CI for continuous data, according to the normal distribution analyzed by the Shapiro-Wilk test. Chi-square or Fisher's exact tests and paired Student's *t* tests were used to analyze the significant differences of the variables. When data did not fit a normal distribution, a nonparametric Mann-Whitney *U* test and

Hodges-Lehmann estimator were used. The Kaplan-Meier estimator was used to compare time until the appearance of the first WA after the initiation of treatment and after the discontinuation of treatment 6 months later. A Cox model was adjusted to evaluate the real effect expressed as a hazard ratio and the 95% CI. The Hodges-Lehmann estimator was used to measure the effect size of the differences between MV130 and placebo groups. The number needed to treat was calculated based on the number of patients to be treated to prevent one case of recurrent wheezing (three or more WA) during the study. In any case, a level of significance of $P < 0.05$ was established for all tests performed.

For those patients who stopped study medication prematurely, the values recorded at the last visit postbaseline (last observation carried forward) were used to maintain the intention to treat.

Statistical analyses were performed using the STATA 12.0 (StataCorp) software or Prism Software 6.01 (GraphPad). Outliers were identified and removed by means of Tukey's range test.

Results

Trial Participants

Of the 373 children (age range 6–35 mo) who underwent screening, 120 were enrolled. A total of 62 patients were assigned to MV130 (active) and 58 to the placebo. Seven patients abandoned the study (three active, four placebo): five cases (three active, two placebo) could not be followed up and two placebo requested withdrawal (one placebo because of an epileptic seizure at the beginning of the study, and the other one due to vomiting and diarrhea on the third day of treatment). The flow chart of patients is shown in Figure 1. The groups appear balanced at baseline.

Efficacy

The median of WA before the study was 8.0 (IQR, 7.0–10.0) and 9.0 (IQR, 7.0–11.3) for the MV130 and placebo groups, respectively ($P = 0.102$) (Table 2). Notably, the median of WA per child during the 12-month clinical trial period (primary outcome) was 3.0 (IQR, 2.0–4.0) versus 5.0 (IQR, 3.0–7.0) for MV130 and placebo, respectively ($P < 0.001$). Thus, a significant reduction in the number of WA was observed in the subjects who received MV130 when compared with the placebo. The total number of WA was 176 in the MV130 group

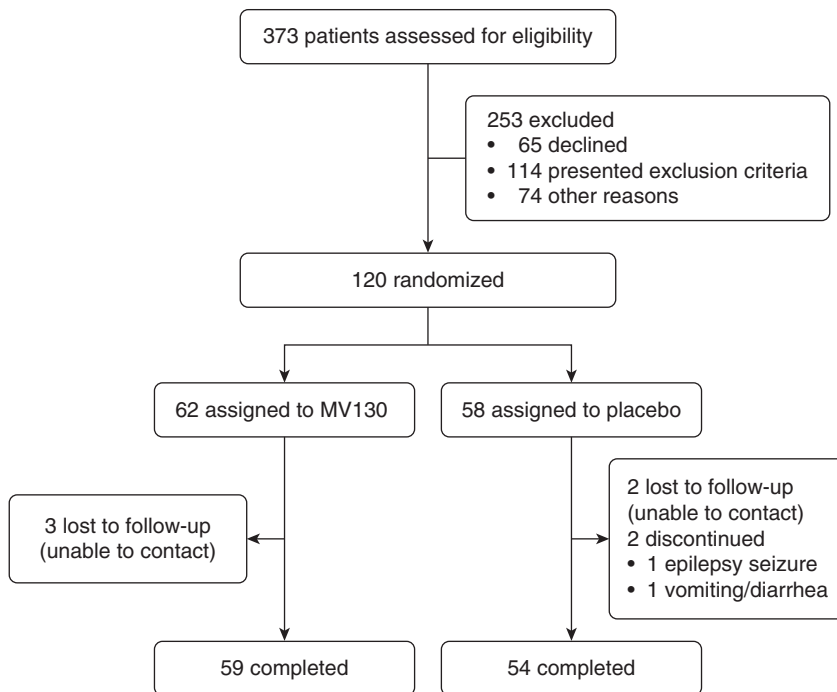


Figure 1. Trial profile.

and 299 in the placebo group (Figure 2A and see Table E1 in the online supplement). The total median number of days with WA (19.0 [IQR, 8.5–35.0] vs. 42.0 [IQR, 17.0–57.5]) and the median duration in days of WA (6.0 [IQR, 4.0–9.5] vs. 7.9 [IQR, 6.2–11.0]), both secondary efficacy endpoints, were also significantly lower in the MV130 group ($P < 0.001$ and $P = 0.005$, respectively) (Figures 2B and 2C). These data are shown for the whole year study and for the different time points (Table E1). A *post hoc* statistical analysis showed that the age (as a covariate) of the population included in the study has no effect ($P = 0.749$) in the highly significant clinical improvement ($P < 0.001$) obtained with MV130 when compared with placebo. Because the number of children younger than 18 months was low (13 in the placebo and 16 in the active group), we analyzed the model adjusted with age as a categorical variable (younger or older than 18 mo) and its interaction with the group; the effect of the intervention on the number of WA remains highly significant ($P < 0.001$), the effect of the age (as a categorical variable) on the number of WA between groups is not significant ($P = 0.114$), and the P value of the interaction of age–group is 0.048, suggesting that the effect of MV130 compared with the placebo is different between those younger than 18 months and those older.

Patients free of new WA were 6 in the MV130 and 0 in the placebo group ($P = 0.029$) (Figure 2D), whereas children continuing to have recurrent WA (≥ 3 WA/yr) were 36 (58%) in the MV130 and 45 (80%) in the placebo group ($P = 0.009$). The number needed to treat to prevent one case of recurrent wheezing was five (95% CI, 2.6–16.1). Among the participants, the median time until the appearance of the first WA after the initiation of treatment was significantly delayed in the MV130 group compared with the placebo (41.0 [15.0–94.5] vs. 5.0 [2.0–28.8] d, respectively). Likewise, during the observational posttreatment period, there were differences in the same outcome (180 [48.5–180.0] vs. 44 [20.0–121.5] d, respectively) (Figures 2D and 2E).

Other secondary efficacy endpoints were the symptom score, medication score, and their combination considered during the WA and throughout all the study periods. These were also significantly lower in the MV130 group when considering global combinations (Figure 3 and Table 3). In addition, in the evaluation of the worst-case scenario, the MV130 group still showed a highly significant difference in the analyzed outcomes (Table E2). The results of additional secondary endpoints including health and social resources are detailed in Table E3.

Safety

Neither local nor systemic reactions occurred during the clinical trial. One patient in the placebo group had a serious adverse event (epileptic seizure), and this subject withdrew on the recommendation of his/her neuropsychiatrist. A total of 166 adverse events were registered, 81 in the MV130 group and 85 in the placebo group, and none of them were considered to be related to the active product. These adverse events were common pathologies in infants, including gastroenteritis (21 [12.7%] of 166), conjunctivitis (20 [12.0%] of 166), diarrhea (17 [10.2%] of 166), and dermatitis (17 [10.2%] of 166). The adverse reactions were classified as mild (155 [93.3%]), moderate (10 [6.0%]), and severe (1 [0.6%]). The most frequent adverse events are shown in Table 4.

Discussion

Based on the available clinical data, there is evidence supporting the benefit of bacterial mucosal immunotherapy in the prevention of recurrent respiratory tract infections in children, including WA. However, the diverse trial results, together with poor methodological quality, give rise to a need to conduct randomized controlled trials to generate high-quality data (26, 27). Herein, we have shown in a randomized DBPC parallel-group clinical trial that MV130 is safe and reduces WA in infants and toddlers by 40% over a placebo. No adverse reactions related to the active product were reported. In addition, the period without respiratory symptoms until the next WA were statistically different between groups, even when MV130 was discontinued (second half-year), pointing to a long-lasting effect inferring clinical significance. The robustness of our study was further supported by a “worst-case scenario” analysis (28). Finally, in the active group, there was a significant reduction in social resources, including daycare, absenteeism, and caregivers, that might have a direct impact on the economy of families.

The current clinical trial is, to the best of our knowledge, the first one in which a heat-inactivated, whole-cell bacterial, sublingual preparation is evaluated in a DBPC study for efficacy against WA in children. Compared with gastrointestinal administration, sublingual and intranasal delivery induce a superior mucosal immune response in a range of tissues, including the airways, and present similar magnitude and anatomic

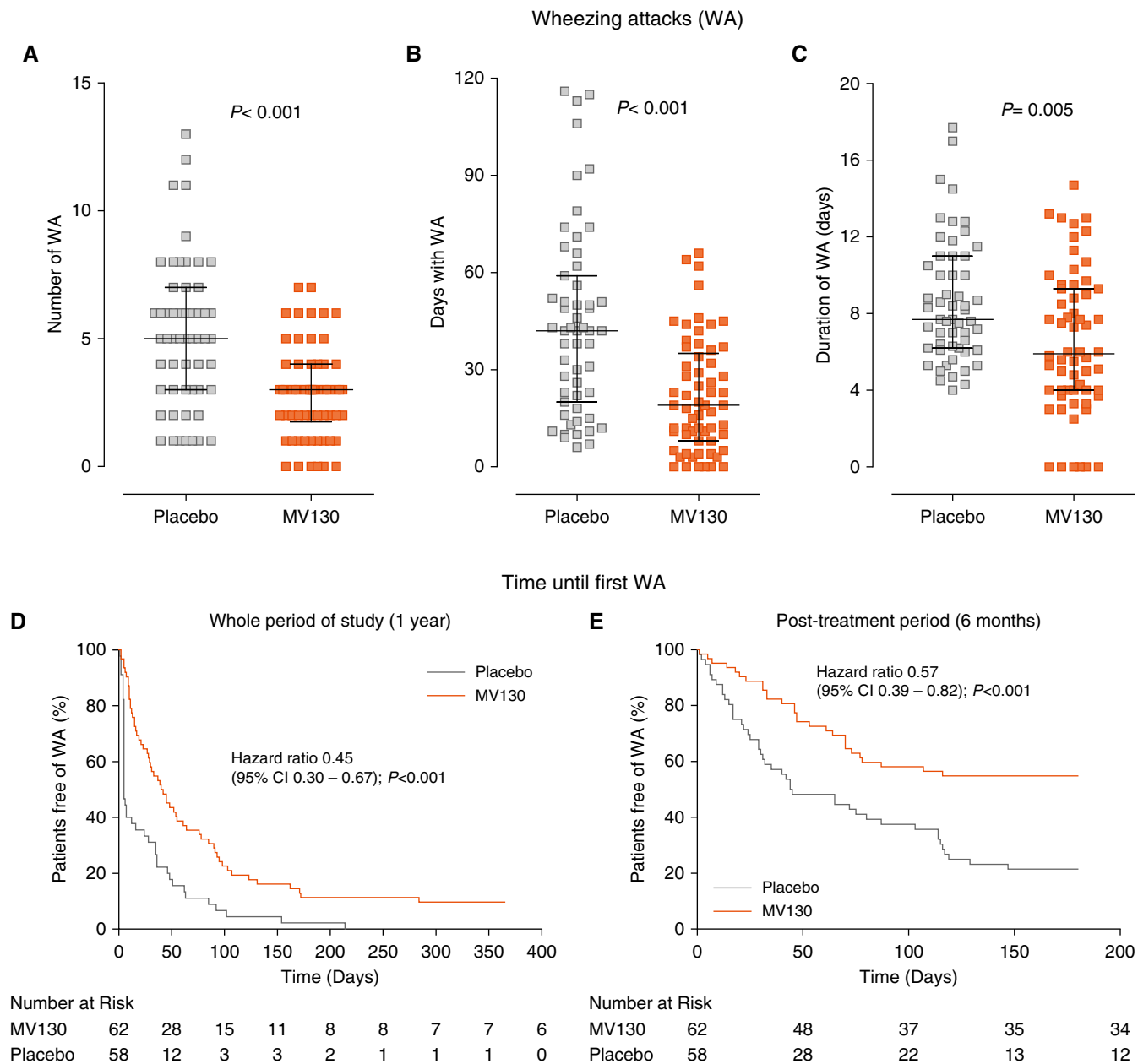


Figure 2. MV130 reduces the number and duration of wheezing attacks (WA). (A–C) WA episodes during the whole study: (A) number of WA, (B) days with WA, and (C) duration of WA. The results in the scatter plots represent values from single patients; the horizontal lines indicate medians, and error bars show the interquartile range. The *P* values were calculated using a Mann-Whitney *U* test. (D and E) A survival analysis (Kaplan-Meier estimator) representing the number of days until the appearance of the first WA (D) during the whole study period (1 yr) and (E) during the posttreatment period of the study (6 mo). The *P* values were calculated using log-rank (Mantel-Cox) test. CI = confidence interval.

dissemination of the induced immune responses (29). Moreover, the sublingual route is effective for vaccine delivery, inducing both systemic and mucosal immunity (29, 30) for MV130 (16–18) and other whole-cell bacterial formulations (31–33). Improving mucosal immunity is an important aspect when considering pathogens that infect through mucosal tissues (34, 35). Our results are in line

with a previous study of Razi and colleagues in preschool children who received oral capsules of bacterial lysates, showing a reduction of 38% of WA over placebo (36). Despite similar efficacy in preventing WA, no reduction in systemic steroids was achieved in that study (36), in contrast to our results showing reduced medication score (including inhaled budesonide and oral

prednisolone). In a more recent study, the same product used by Razi and colleagues appeared to be effective in preventing severe lower respiratory illness in at-risk infants during the first winter (37) yet without any carryover protection, in contrast to the previous results (36) and current study with MV130, which might be related to the younger age of the children studied (37). In

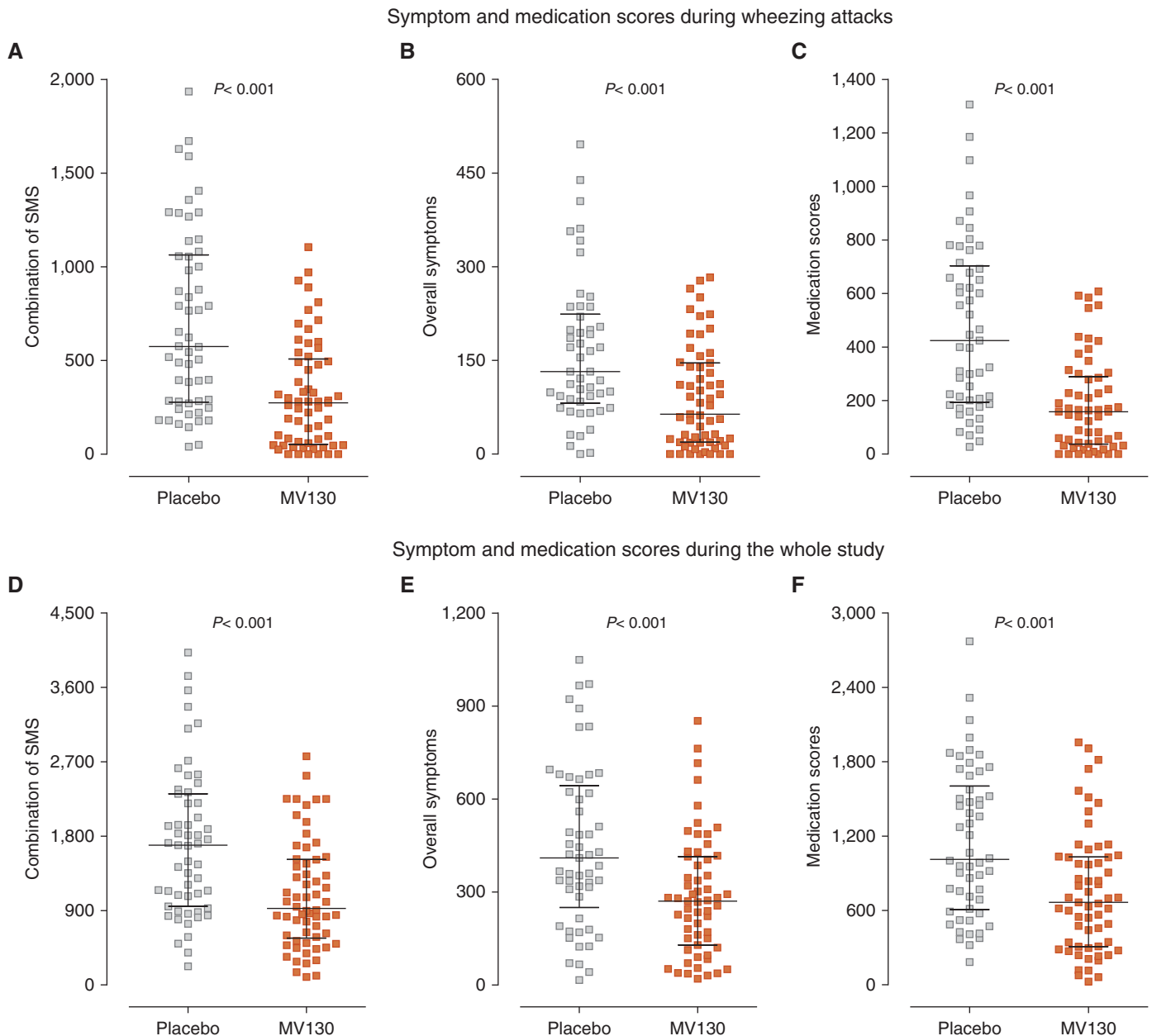


Figure 3. MV130 decreases symptom and medication scores (SMS). (A–C) The combination of SMS (A), overall symptom scores (B), and medication scores (C) during wheezing attacks. (D–F) The combination of SMS (D), overall symptom scores (E), and medication scores (F) throughout the study. Data are displayed in scatter dot plots in which values from single patients are represented. The line indicates the median, and error bars show the interquartile range. The *P* values were calculated using Mann-Whitney *U* test.

our study, a *post hoc* analysis suggested that the effect of MV130 is different between those younger than 18 months and those older. However, this finding would need further confirmation because only 24% of the participants in our study were younger than 18 months, as the main inclusion criterion (at least three WA during the previous year) was more difficult to achieve in the youngest children.

Allergen-sensitized children have an increased risk of recurrent and severe wheezing in slightly older ages (4, 6). In our study, children with positive skin tests were excluded to avoid enrolling subjects whose recurrent wheezing might be triggered by aeroallergens instead of viral infection. Moreover, although the inability of patients with asthma to generate an adequate type I IFN response during viral infection leading to greater or prolonged

infection is well established (38), this could interfere with the correct interpretation of the results. Although some of these children eventually develop allergic sensitization, we assume that they were equally represented in our two groups; however, this could be considered as a limitation of the study. Confirmation of this would require longer follow up, and the opportunity to initiate treatment would be lost.

Table 2. Demographic and Clinical Characteristics of the Study Populations at Baseline

Characteristics	MV130 (n = 62)	Placebo (n = 58)	P Value
Sex, M/F	37/25	33/25	0.757
Median age, mo (IQR)	24.0 (17.3–28.0)	24.0 (16.5–29.0)	0.475
<1 yr	2 (3.2%)	7 (12.1%)	0.060
Between 1 and 2 yr	30 (48.4%)	23 (39.7%)	0.335
>2 yr	30 (48.4%)	28 (48.3%)	0.990
Mean weight, kg (95% CI)	12.6 (12.0–13.2)	12.2 (11.6–12.7)	0.398
Mean height, cm (95% CI)	85.1 (83.3–87.0)	84.9 (82.8–87.0)	0.878
Median previous WA (IQR)	8.0 (7.0–10.0)	9.0 (7.0–11.3)	0.102
Median previous monthly WA (IQR)	0.67 (0.58–0.83)	0.79 (0.67–1.00)	0.053
Atopic dermatitis	22 (35.5%)	25 (43.1%)	0.393
Smoking during pregnancy	11 (17.8%)	15 (25.9%)	0.280
Gestational age			
<37 wk	9 (14.5%)	11 (19.0%)	0.513
37–41 wk	52 (83.9%)	45 (77.6%)	0.382
>42 wk	1 (0.02%)	2 (0.03%)	0.517

Definition of abbreviations: CI = confidence interval; IQR = interquartile range; WA = wheezing attacks.

As observed in previous trials (36), the number of WA in the placebo group declined during the whole study period compared with the baseline. Viral-induced wheezing has a

good prognosis and spontaneous improvement is expected, but our study was designed to assess the saving effect of MV130 while the condition is present, both in clinical

symptoms and medication consumption, and it was significantly better than the placebo in children with similar risk characteristics at baseline.

Table 3. Symptom and Medication Scores Alone and in Combination during the Whole Period of the Study (1 yr)

	MV130 (n = 62)	Placebo (n = 58)	P Value (Mann-Whitney U Test)	Hodges-Lehmann
Symptoms				
Overall symptom score	276.5 (151.0 to 426.0)	421.0 (303.0 to 673.0)	0.001	−147 (−248 to −63)
Respiratory symptoms	115.0 (53.8 to 165.5)	157.0 (110.0 to 295.3)	0.002	−53 (−97 to −21)
Other symptoms	152.0 (73.3 to 262.0)	253.5 (175.3 to 353.8)	0.001	−84 (−151 to −38)
Score of individual symptoms and days with symptoms				
Days with nasal mucus secretion	71.0 (32.0 to 101.3)	100.5 (61.8 to 146.8)	0.005	−33 (−55 to −10)
Score of nasal mucus secretion	82.0 (42.0 to 147.8)	123.0 (83.8 to 199.5)	0.004	−44 (−75 to −12)
Days with bronchial mucus secretion	21.0 (8.0 to 42.5)	41.0 (14.8 to 73.0)	0.008	−14 (−28 to −4)
Score of bronchial mucus secretion	29.0 (11.0 to 59.0)	58.5 (24.0 to 102.5)	0.005	−21 (−41 to −6)
Days with cough	54.5 (26.0 to 87.0)	78.5 (53.0 to 113.0)	0.013	−22 (−37 to −4)
Score of cough	83.0 (35.5 to 119.5)	104.5 (72.0 to 153.5)	0.020	−30 (−54 to −5)
Days with difficult breathing	8.5 (1.3 to 18.8)	19.5 (6.8 to 43.3)	0.003	−8 (−16 to −2)
Score of difficult breathing	11.0 (2.0 to 26.5)	25.5 (10.0 to 62.8)	0.001	−11 (−21 to −3)
Days with wheezing	5.0 (0.0 to 14.0)	19.0 (7.0 to 44.0)	<0.001	−11 (−18 to −6)
Score of wheezing	11.0 (2.0 to 33.5)	25.5 (8.5 to 61.5)	<0.001	−15 (−24 to −6)
Days with discomfort	11.0 (3.3 to 32.3)	20.0 (13.8 to 42.3)	0.010	−10 (−16 to −2)
Score of discomfort	14.5 (4.0 to 47.5)	31.0 (18.8 to 59.5)	0.010	−14 (−23 to −3)
Fever	9.5 (5.0 to 14.0)	11.5 (7.0 to 20.5)	0.068	−3 (−6 to 0)
Medication				
Overall medication	618.5 (318.3 to 1,035.5)	1,043.5 (669.5 to 1,696.0)	<0.001	−399 (−642 to −188)
Antibiotics	22.5 (6.0 to 44.5)	41.5 (18.8 to 77.3)	0.013	−16 (−28 to −2)
Antipyretic and antiinflammatory drugs	31.0 (18.0 to 50.5)	38.5 (19.5 to 98.3)	0.064	−12 (−29 to 0)
Salbutamol	92.0 (40.3 to 224.0)	252.5 (120.0 to 387.0)	<0.001	−130 (−193 to −65)
Inhaled budesonide	38.0 (0.0 to 272.0)	323.0 (80.0 to 539.0)	<0.001	−185 (−282 to −62)
Oral prednisolone	0.0 (0.0 to 8.0)	7.5 (0.0 to 18.0)	0.002	−3 (−7 to 0)
Montelukast, 4 mg	212.5 (129.8 to 254.0)	206.0 (141.5 to 257.0)	0.957	0 (−30 to 34)
Other medications*	58.0 (129.8 to 254.0)	103.0 (19.0 to 194.5)	0.334	−11 (−58 to 14)
Combination of symptom and medication scores	1,092.2 (925.8 to 1,258.5)	1,760.9 (1,505.9 to 2,016.0)	0.001	−508 (−828 to 238)

*Includes antihistamines and mucolytics.

Table 4. Adverse Events

Adverse Event(s)	MV130 (n = 62) Events (n = 81)	Placebo (n = 58) Events (n = 85)
Most frequent adverse events*		
Gastroenteritis	5 (8%)	16 (28%)
Conjunctivitis	14 (23%)	6 (11%)
Diarrhea	6 (10%)	11 (19%)
Dermatitis	6 (10%)	11 (19%)
Head injury	0 (0%)	4 (7%)
Chickenpox	4 (6%)	1 (2%)
General malaise	3 (5%)	2 (3%)
HFMD	5 (8%)	1 (2%)
Iron-deficiency anemia	2 (3%)	3 (5%)
Oral candidiasis	3 (5%)	0 (0%)
Scarlet fever	3 (5%)	3 (5%)
Synovitis	3 (5%)	2 (3%)
Urticaria	2 (3%)	3 (5%)
Vomiting	2 (3%)	3 (5%)
Severe adverse event(s) [†]	0 (0%)	1 (2%) [†]

Definition of abbreviation: HFMD = hand-foot-and-mouth disease.

Data are n (%) of patients with one or more such events in each group. No variable showed significant differences ($P > 0.05$).

*Adverse events reported in more than 5% of patients in either treatment group are shown.

[†]Epileptic seizure; the patient withdrew from the clinical trial.

Despite the effect on WA, some secondary variables on health resource consumption did not differ between the active treatment and the placebo. This might mean that parents learn to cope with their child's symptoms, use rescue medication, and limit the use of those resources. However, there was a significant reduction in some social resources that might have a direct impact on the economy of families.

The safety data showed a good general profile. Adverse events were not different between the active and placebo preparations. They were mild (except for epileptic seizures in a child with the placebo) and subsided during the treatment; the only two dropouts because of possible side effects appeared in the placebo group. In this line, the most common adverse event in the active group was conjunctivitis, with no obvious explanation. The patients fully recovered, and although it was not statistically different from placebo and causality assessment did not estimate any relation with the medication, there was a numerical difference that would need further assessment with larger groups.

We did not directly address a mechanistic evaluation of MV130 on samples from the children included in the DBPC by assessing immunological parameters that might correlate with the clinical outcome (39). This would have shed further light on the

mechanism of protection of MV130 (16, 40, 41). On the other hand, whether immunotherapy with MV130 may impact the airway microbiome would have been very interesting to address, as it may influence susceptibility versus resilience to viral infection (42). These are limitations of our study because both approaches would have raised new insights about how mucosal bacterial immunotherapy may modulate the airway response against viral infections. Further studies will need to be performed to clarify these issues.

Although the beneficial role of bacterial preparations in the protection against recurrent respiratory tract infections, including those of viral origin, has been already evaluated in different studies (27, 43, 44), their mechanism of action remains unclear. Although MV130 does not affect oral epithelial cell activation (41), it modulates the function of human dendritic cells (16). In addition, it enhances both specific and nonspecific T-cell responses *in vitro* and *in vivo*, including Th1 and Th17 responses, known to be involved in host resistance against intracellular and extracellular pathogens, respectively. Furthermore, MV130 promotes the generation of IL-10-producing T cells (16), which are essential for pathogen clearance and to keep tissue homeostasis (45). In this sense, induction of IL-10 by regulatory T cells has been proposed

as an antiinflammatory mechanism of bacterial lysates having a role in preventing wheezing-asthma in childhood (46).

In experimental respiratory virus infection models, MV130 has recently been shown to confer protection by inducing trained immunity (21). Trained immunity endows innate immunity with immunological memory based on stable epigenetic modifications (47, 48). As a result, nonspecific innate immune responses are enhanced during a relatively long time, so the host responds rapidly and robustly in a nonspecific way to subsequent challenges, providing cross-protection against different infections (14, 40). In this regard, it has been postulated that the underlying mechanism behind the heterologous immunity induced by mucosal bacterial vaccines (35), bacillus Calmette-Guérin, and other vaccines can be mediated by trained immunity (49, 50). Thus, the term "trained immunity based vaccines" has been proposed for those formulations containing trained immunity inducers that confer broad and durable protection against infections far beyond the nominal microorganism antigens they may contain (40). Of note, the protection achieved by MV130 in the current clinical trial was also observed in the following 6 months after treatment discontinuation, in line with a memory ascribed to a trained immunity-mediated mechanism (50–54). Whether MV130 may be acting this way in humans is currently under investigation.

In summary, our study reveals, for the first time, that mucosal immunotherapy with a sublingual polybacterial preparation formulated with a defined composition of heat-inactivated, whole-cell bacteria (MV130) prevents wheezing episodes in young children and reduces symptom and medication scores, which together with a good safety profile makes it an alternative to other treatments currently used. A further understanding of its underlying mechanism of action may support the development of prophylactic and therapeutic options, particularly in conditions associated with recurrent infections or new emerging pathogens, for which conventional vaccines are not available yet. ■

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