



Sub-lingual administration of a polyvalent mechanical bacterial lysate (PMBL) in patients with moderate, severe, or very severe chronic obstructive pulmonary disease (COPD) according to the GOLD spirometric classification: A multicentre, double-blind, randomised, controlled, phase IV study (AIACE study: Advanced Immunological Approach in COPD Exacerbation)



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ABSTRACT

Polyvalent mechanical bacterial lysates (PMBLs) have been shown to reduce the number of infectious episodes in patients with recurrent infections of the respiratory tract. Some previous investigations have also shown the effectiveness of PMBLs in reducing exacerbations of chronic obstructive pulmonary disease (COPD). The AIACE study, which was developed according to criteria of evidence-based medicine, evaluated whether the administration of PMBLs to COPD patients, in addition to the recommended treatment, was able to reduce the number of exacerbations by 25%. Two hundred eighty-eight patients with moderate to very severe COPD were recruited and randomly assigned to either placebo or PMBLs. The placebo or PMBLs were administered according to the standard scheme. The primary outcome of the study was not achieved. However, the number of days with fever (21 days per year versus 40.15; $p < 0.001$), the days of hospitalisation (65 days vs 162 days; $p < 0.001$), the interval between the first and second exacerbations (123.89 days vs 70.36; $p = 0.03$) and the number of days in poor health (109 days/year vs 171 days/year; $p < 0.001$) were significantly better in the PMBL group than in the placebo group. In conclusion, the results of this trials showed that Ismigen, in addition to guideline-suggested treatment, could not significantly reduce the number of exacerbations in the considered population; nevertheless, the secondary outcome results demonstrated potential benefits of this compound for relevant clinical outcomes.

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1. Introduction

Chronic obstructive pulmonary disease (COPD) is characterised by airflow limitation that is not fully reversible, is progressive and is associated with an inflammatory response in the lungs [1]. An

increasing number of COPD diagnoses are expected in the coming years, and COPD is already considered a major cause of death [1]. The available COPD treatments are effective in reducing symptoms and exacerbations and in improving lung function, exercise capacity and health-related quality of life. In addition to anti-

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inflammatory therapies and bronchodilators, other drugs seem to be effective in modulating the immune responses of COPD patients. For example, bacterial lysates (BLs) and, in particular, polyvalent mechanical bacterial lysates (PMBLs) focus on potentiating the immune response at the systemic and/or mucosal level, and these lysates have been suggested to have a beneficial impact on the number and the severity of COPD exacerbations and on recurrent respiratory tract infections (RRTIs). [2]. Over the last 10 years, the mechanism of action of PMBLs, which consist of a mixture of lyophilised bacterial fragments derived from the mechanical lysis of strains commonly involved in respiratory infections, such as *Staphylococcus aureus*, *Streptococcus viridans*, *pneumoniae* and *Streptococcus pyogenes*, *Klebsiella pneumoniae* and *Klebsiella ozaenae*, *Moraxella catarrhalis* and *Haemophilus influenzae*, has been extensively studied. PMBLs have the capacity to induce the maturation of dendritic cells [3], to recruit B and T lymphocytes, to increase the number of circulating NK cells [4] and to induce the secretion of specific IgA, which is directed at PMBL antigens and is characterised by an opsonising activity [5]. The PMBL-mediated capacity to induce a specific immune response as well as control recurrent bacteria- and virus-mediated infections was directly linked to the clinical results [6,7]. In recent years, a potential effect of PMBLs on prophylaxis against RRTIs has been demonstrated in different studies [8–13]. However, a recent meta-analysis [14] showed that the role of PMBLs should be further demonstrated in COPD patients. Notably, all of the results except those of one study [13] were published when long-acting bronchodilators and inhaled corticosteroids were unavailable. For this reason, this clinical study focused on the role of a PMBL compound (Ismigen®) in the prophylaxis of COPD exacerbations in patients treated with the best treatments available today.

2. Materials and methods

2.1. Study design

The AIACE (Advanced Immunological Approach in COPD Exacerbation) study was a multicentre, double-blind, randomised, controlled, phase IV study (EudraCT 2007-000006-67). The primary objective of the trial was to demonstrate the clinical efficacy of Ismigen®, defined as reducing the number of exacerbations by 25% in patients with moderate, severe and very severe COPD (M/S/VS-COPD) and determined by the GOLD 2006 classification guidelines over a 12-month observation period. COPD exacerbations were defined as events during the natural course of the disease characterised by worsening of the patient's baseline dyspnoea, coughing and/or sputum production, beyond day-to-day variability, sufficient to warrant a change in management according to the ATS/ERS [15].

The secondary objectives of the trial were time from the randomisation to the first exacerbation, the average interval between the first and second exacerbation, the effects of Ismigen® on symptoms (fever, coughing, dyspnoea, days in poor health, pulmonary findings, sputum characteristics and sputum quantity), reductions in the use of other drugs (e.g., antibiotics, anti-inflammatory drugs, bronchodilators, mucolytics), days of absence from work, and days of hospitalisation. Adverse drug effects and the impact on quality of life were also assessed using validated tools (SF-12, CCIQ).

The study was conducted in accordance with good clinical practices and was approved by the Ethical Committee of the University Hospital S. Martino Genova. The study was conducted in 13 different Italian centres that were representative of the different Italian regions and climates.

The study enrolled men and women equal to or older than 40 years old. The inclusion criteria required a documented diagnosis of moderate, severe or very severe COPD, according to the GOLD 2006 guidelines and based on the WHO performance grading of 0, 1 or 2, and adequate haematological, renal and liver function 14 days prior to study randomisation. The female patients were required to be non-lactating and to be of non-child bearing potential (either surgically sterile or using effective contraception). Smokers and ex-smokers were included, and the smoking status was recorded. Written informed consent was signed by the participants according to ICH/GCP and national/local regulations before patient randomisation.

Patients were excluded from the study if they had received any prior antineoplastic drug therapy or immunosuppressive drugs, were under continuous treatment with systemic steroids, exhibited severe cardiac disease, including uncontrolled angina pectoris or myocardial infarction, within 6 months of enrolment, or had uncontrolled high blood pressure or any other uncontrolled severe medical condition, including active gastroduodenal ulcer, alcohol disorders (hepatitis, Korsakoff syndrome), and diabetes. Additionally, they were excluded if there was an active or uncontrolled infection or evolutive intracranial hypertension. Patients who were pregnant or nursing at the beginning of the study or who were suffering from any psychological, familial, sociological or geographical condition potentially hampering compliance with the study protocol and follow-up schedule were also excluded.

Randomisation was performed by the CRO (SPRIM Italy/ALS GCP), using a validated system that automated the random assignment of treatment groups by randomised numbers in a 1:1 ratio.

Both the drug and the placebo were produced by Bruschetti Srl, Genova, Italy and were then sent to Doppel Farmaceutici which labelled the boxes and blisters with sequential numbers, according to the randomisation list. The treated group received Ismigen® (Lallemand Pharma International, Bahnhofstrasse 7, 6301 ZUG, Switzerland) in tablet form. The active substance in Ismigen® (batch number: TFR08181; expiration date: 4/2011; 357PC0018 expiration date: 12/2013) was constituted of a mixture of lyophilised bacterial fragments derived from *S. aureus*, *S. viridans*, *S. pneumoniae* (6 strains), *S. pyogenes*, *K. pneumoniae*, *K. ozaenae*, *M. catarrhalis* and *H. influenzae*. There were a total of 6 billion bacteria for each strain in glycine solution. The drug excipients were cellulose microcrystalline, calcium hydrogen phosphate dehydrate, colloidal hydrated silica, magnesium stearate, ammonium glycyrrhizinate and essence of meat powder.

The placebo tablets (batch number: TFR08181; expiration date: 4/2011; 357PC0018 expiration date: 12/2013) contained the same excipients as the Ismigen® tablets (including glycine and microcrystalline cellulose) but no active, bacteria-derived substances. Ismigen® and the placebo were packed in carton boxes containing three blisters made of white-opaque PVC, each containing 10 tablets, sealed with an aluminium foil and stored at room temperature (25 °C). No differences between the active drug and the placebo were evident on the packaging.

The treatment schedule was based on two three-month cycles. One cycle consisted of sublingual consumption of one tablet per day for 10 consecutive days, followed by 20 days of standard treatment for three consecutive months. After three months without any PMBL treatment, a second cycle of therapy (as described above) was undertaken. At the end of the second treatment period, a second three-month period without PMBL was observed.

According to the intention-to-treat criterion, the subjects who withdrew from the study were followed up until month 12 of the study when possible. All of the subjects were followed according to the protocol.

The permitted treatments were bronchodilators, mucolytic and anti-inflammatory drugs as maintenance treatment, in accordance

with the GOLD 2006 guidelines. Antibiotics were added in the event of acute exacerbations, in Type I Anthonisen exacerbation, in Type II Anthonisen exacerbation, when increased purulence of sputum was one of the two cardinal symptoms and in patients requiring invasive or non-invasive mechanical ventilation.

Acute COPD exacerbations that occurred during the observation period of the patients were specifically recorded in the CRF with the specific indication of the primary cause of exacerbation (e.g., severe pneumonia, aggravation of COPD symptoms), the need for associated treatments, including bronchodilators, antibiotics, anti-inflammatory drugs, and mucolytics, the need for hospitalisations and duration of hospital stay and the need for oxygen therapy. Depending on the severity of the COPD exacerbation, the patients were treated at home and/or were hospitalised.

2.2. Statistical analysis

All of the randomised patients were included in the statistical analyses, based on their assignments to the treatment or the placebo group and independent of the patient's eligibility and the treatment actually received (the intention-to-treat principle).

All adverse events, defined by the Good Clinical Practice Guideline, were recorded on the CRF. An adverse event (AE) was defined as any untoward medical occurrence in a patient that occurred following the administration of the trial medication, regardless of the dose or causal relationship. An adverse drug reaction (ADR) was a response to a drug that was noxious and that occurred at the doses normally used in humans. An unexpected adverse drug reaction was any adverse reaction the nature or severity of which was not consistent with the applicable product information. A serious adverse event (SAE) was defined as any undesirable experience occurring in a patient, whether considered related to the protocol treatment or not. An SAE that was considered related to the protocol treatment was defined as a serious adverse drug reaction (SADR).

The sample size was calculated based on the number of exacerbations during the 12-month observation period, which followed a Poisson distribution and was appropriate for rare events. This mean was 2.5 in the control group. For power of 90% against the hypothesis of a relative reduction of at least 25% in the average number of exacerbations in the experimental group (mean equal to or less than 1.875) and a 5% false positive rate against the hypothesis of no difference between the treatment groups, 120 patients had to be enrolled in each arm. This number was thus increased to 144 patients for each arm to prevent Type I and II errors from being caused when subjects dropped out of the study.

The effectiveness of the experimental drug was estimated using the exacerbation incidence rate ratio. The exacerbation incidence rate ratio was calculated using the log-linear Poisson model, which is a generalised linear model with Poisson error and a link log. The crude estimate of the exacerbation incidence rate ratio and its 95% confidence interval were calculated. Moreover, a multivariate adjusted estimate of the exacerbation incidence rate ratio was calculated, introducing the appropriate covariate terms for institutions and COPD severity in the log-linear Poisson model. All of the secondary analyses were conducted according to the probability distribution followed by the involved variable. Monitoring activities were performed by SPRIM Italy/ALS GCP Clinical Monitors and by a Lallemand clinical monitor (coordinating centre).

3. Results

The study enrolment started on July 31, 2009 and was completed on June 16, 2012. The study closed on July 4, 2013. The

clinical and statistical data were available in December 2013. Two hundred eighty-eight patients (142 in the placebo group and 146 in the treatment group) were included in the study. The patients' baseline characteristics are reported in Table 1, and the study flowchart is shown in Fig. 1. All of the patients who took at least one dose of the study medication or placebo were included in the intention to treat (ITT) population. Ninety-nine of the patients randomised to the placebo and 110 patients randomised to the active treatment completed the study. No significant differences were found in the dropout number (X^2 test, $p = 0.28$), the study period completed ($p = 0.99$) or the number of treatment cycles completed ($p = 0.91$). Two hundred nine patients completed the study. Among these patients, 205 consumed more than 80% of the expected tablets. No significant differences in compliance were observed between the treatment and placebo groups. The reasons for study discontinuation were loss to follow-up (21%), adverse events (9%) and withdrawal of consent (65%). The remaining patients did not complete the study due to protocol violation (2%) or death (3%). No differences between the active and placebo groups were observed (X^2 test, $p = 0.28$).

The primary outcomes were analysed only according to the ITT population. Thus, 101 of 142 (71.13%) of placebo patients and 103 of 146 (70.55%) of treated patients did not experience exacerbation during the study period. These results corresponded to 0.52 exacerbations/patient/year in the placebo group, compared to 0.51 in the PMBL group ($p = 0.93$).

The intervals between each exacerbation are outlined in Fig. 2. The mean numbers of days between randomisation and the first exacerbation were 149.34 and 149.12 days for the placebo and PMBL groups (log-rank test, $p = 0.79$), respectively. The mean numbers of days between the first and second exacerbations were 70.36 and 123.89 days for the placebo and PMBL groups (log-rank test, $p = 0.03$), respectively.

The mean numbers of days with fever were 0.06 for the treatment group (corresponding to 21.9 days per year) and 0.11 for the placebo group (corresponding to 40.15 days per year) ($t = -3.29$, $p < 0.001$). Similarly, the numbers of days in poor health were 0.3 ± 0.026 (109 days/year) for the PMBL group and 0.47 ± 0.021 (171 days/year) for the placebo group ($t = -0.578$, $p < 0.001$).

Concerning hospitalisations, although the overall number of days of hospitalisations, independent of cause, was not statistically significantly different (p value = 0.21) between the study arms, the number of days of hospitalisation due to COPD exacerbation was significantly smaller in the PMBL group (65 days vs 162 days; $X^2 = 38.17$, $p < 0.001$).

The rate of adverse events potentially related to the drug was low (2.99% with placebo and 0% with PMBLs), with no significant difference between the groups. Table 2 outlines the adverse events that occurred during the study. Notably, 2.99% were considered possibly related to the placebo control, and 4.48 were "unlikely to be related" to the placebo control, whereas a large majority (>80%) were considered not related to the treatment. Antibiotics were used in 24% of the patients, non-steroid anti-inflammatory drugs in 21%, bronchodilators in more than 80%, and mucolytics in 7.5%, and combinations of local corticosteroids and bronchodilators were used in 50% of the treated patients, with no significant difference between the treatment and placebo groups. Finally, no significant differences were detected in quality of life between the active and placebo groups (data not shown).

4. Discussion

This phase IV, randomised, multicentre, double-blind, placebo-controlled study was designed to evaluate the ability of Ismigen to

Table 1
Patients' baseline characteristics.

Characteristics	Subclasses	Placebo	Active
Number		142	146
Mean age		68.6 (SD ^a = 9.4)	69.3 (SD = 8.6),
Sex	Males	64%	73%
	Females	36%	27%
Mean number of exacerbations over the previous 2 years		2.87 (SD = 3.36)	2.87 (SD = 3.32)
Mean number of COPD-related infections over the previous 2 years		1.7 (SD = 1.92)	1.66 (SD = 1.99),
COPD Severity	Stage 1: mild COPD	0 (0%)	0 (0%)
	Stage 2: moderate COPD	84 (59.15%)	84 (57.53%)
	Stage 3: severe COPD	46 (32.39%)	50 (34.25%)
	Stage 4: very severe COPD	12 (8.45%)	12 (8.22%)
Duration of COPD (years)		10.1 (SD = 9.70)	9.2 (SD = 7.70)
Smoking status	Current smoker	24 (16.90%)	23 (15.75%)
	Ex-smoker	105 (73.94%)	113 (77.40%)
	Never smoked	24 (16.90%)	23 (15.75%)
Chest X-ray	Normal	105 (73.9%)	103 (70.55%)
	Abnormal	35 (24.6%)	43 (29.45%)
	Missing	2 (1.4%)	0
FEV1 (%)		52.66 (SD = 16.26)	52.18(SD = 18.99)
Concomitant diseases	Hypertension	51	51
	Diabetes	10	19

^a SD = Standard deviation.

reduce exacerbations in patients with moderate, severe and very severe COPD.

COPD exacerbations are considered an event in the natural course of the disease and are characterised by worsening of the patient's baseline dyspnoea, cough and/or sputum production beyond day-to-day variability that is sufficient to warrant a change in management, according to the GOLD definition. Using this definition, the primary endpoint was not achieved. Some explanations of this result could be postulated. First, the study sample size was calculated based on the assumption that the mean number

of exacerbations per year was 2.5 per patient in the population enrolled. For a power of 90% against the hypothesis of a relative reduction of at least 25% in the average number of exacerbations in the experimental group and a 5% false positive rate against the hypothesis of no difference between the treatment groups, 120 patients had to be enrolled in each arm, but this number was increased to 144 patients for each arm to prevent type I and II errors resulting from subjects dropping out of the study. The frequency of exacerbations in the years preceding the beginning of the study was declared by the patients to be almost 1.5 exacerbation/year,

AIACE study: Consort Flow Diagram

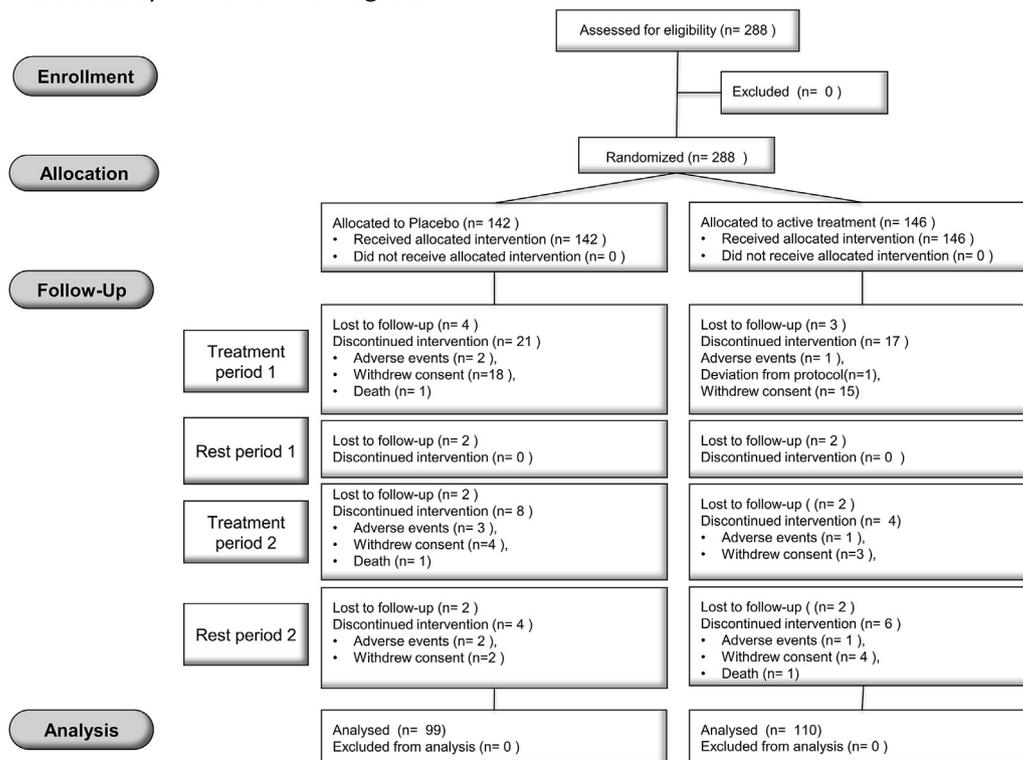


Fig. 1. The CONSORT flowchart of the AIACE study.

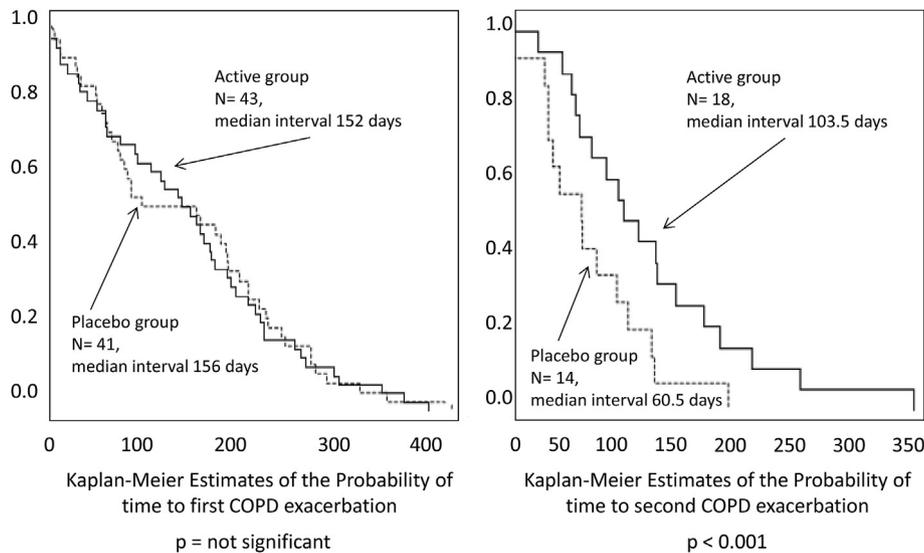


Fig. 2. Kaplan–Meier estimate of the time (in days) to first and (in days) to second exacerbations. Days were calculated from the beginning of the treatment to the first exacerbation and from the first to the second exacerbation. Although no differences were observed in the first period, the time to the second exacerbation was significantly longer in the PMBL-treated group.

which was approximately three-fold greater than the frequency of exacerbations observed during the study in both the treatment and control groups. Additionally, more than 70% of the enrolled patients did not have an exacerbation during the study period, whereas only patients with very severe COPD had exacerbation frequencies similar to the mean for the eligibility criteria. Finally, of the 288 patients recruited, only 209 patients completed the study (110 in the treatment and 99 in the placebo groups).

Although the frequencies of COPD exacerbations in the treatment and placebo groups were not significantly different when the entire period of the study was considered, a significant difference was observed in treatment period 2. Notably, no differences were observed in the efficacy of the treatment regarding different degrees of spirometric severity (moderate, severe and very severe).

In the AIACE study, the standard treatment available to both the treatment group and the placebo group was provided according the GOLD guidelines; the administration of Ismigen was the only difference between the treatment and placebo groups. Ismigen and the planned follow-up induced a strong reduction in the mean number of exacerbations compared with the two years prior to

enrolment (2.87 vs 0.52 exacerbations per subject per year). Thus, the primary endpoint was not met because the placebo group experienced improvement with the baseline treatments. Similarly, it should be noted that a large majority of the previous studies on the effects of BLs on COPD exacerbation were conducted prior to the introduction of inhaled steroids and long-acting bronchodilators. These therapies have significantly modified the natural history of COPD in treated patients. Furthermore, COPD exacerbation, as defined by the GOLD guidelines, might not have been adequate for these BL studies. In fact, a recent study, based on an ancillary AIACE study protocol, used a slightly different approach in defining exacerbations, and a powerful effect of PMBL treatment was observed [7].

Considering the aforementioned limits of the study, some relevant inferences could be drawn. In fact, although the “time to first exacerbation” was not statistically significant, the time to second exacerbation was significantly longer in the PMBL group. This finding could be explained by the already known effects of BLs on immune-competent cells [3,4]. Indirect evidence of bacterial lysate demonstrated that PMBLs improved the number and duration of hospitalisations. Indeed, although no differences were observed in the number of hospitalisation periods, a –249% change in hospital days related to COPD exacerbation was recorded for the PMBL group compared with the placebo group.

Further associated with the aforementioned results were the positive effects of PMBLs in reducing the patients' infection-related symptoms, such as fever.

These data broadened the perspective regarding the use of PMBLs as an add-on treatment for COPD. In fact, although bronchodilators and inhaler steroids could reduce but not eliminate the rate of exacerbations, they act by restoring lung function, decreasing dyspnoea, improving airway clearance and reducing airway inflammation. It is evident that these drugs cannot improve the host immune-response. In contrast, several reports [16,17] have shown an increased rate of pneumonia in COPD patients treated with inhaled steroids.

In conclusion, the results of this trial showed that Ismigen, beyond the guidelines for suggested treatment, could not significantly reduce the number of exacerbations in the considered population; nevertheless, the secondary outcome results indicated

Table 2
Adverse event summary by treatment group.

	Placebo	PMBL
	Number (%)	Number (%)
Subjects with events	36 (27.46%)	39 (24.66%)
Subjects with serious events	21 (14.79%)	14 (9.59%)
Deaths	3 (2.11%)	2 (1.36%)
Number of individual events		
Total individual non-serious events	67	67
Total serious events	25	21
Total events	92	88
Intensity of individual events		
Mild	18 (27.87%)	17 (25.37%)
Moderate	48 (71.64%)	46 (68.66%)
Severe	1 (1.49%)	4 (5.97%)
Relationships of individual events		
Likely related	2 (2.99%)	0 (0%)
Possibly related	0 (0%)	2 (2.99%)
Unlikely to be related	3 (4.48%)	0 (0%)
Not related	54 (80.60%)	58 (86.57%)
Not assessable	8 (11.94%)	7 (10.45%)

the potential benefits of this compound on relevant clinical outcomes. To show clear benefits of adding PMBLs to standard treatment, trials focused on patients with consistently high rates of exacerbations despite ongoing treatment or trials designed for long-term prospective evaluation are needed.

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