Name of Company: **Lallemand Pharma International AG**	**Individual Study Table**	**(For National Authority Use Only)**
Name of finished product: **ISMIGEN®**	Referring to Module 5 of the Dossier: **N/A**	
Name of active substance: **Polyvalent Mechanical Bacterial Lysate (PMBL)**	Vol.: **N/A** Page: **N/A**	

Title of study:	**The influence of Polyvalent Mechanical Bacterial Lysate (ISMIGEN®) on clinical course of asthma and related immunological parameters in asthmatic children (EOLIA Study): randomized, double blind, placebo-controlled multicentre, parallel-group study**	
Coordinating Investigator: **Investigators:**	**Pr. A. EMERYK** Dr M. BARTKOWIAK-EMERYK Dr Z. RAUS Dr L. DYMEK	
Study centre(s):	-Children University Hospital- Pneumology and Rheumatology Dept, 20-095 Lublin , Poland -Alergotest s.c. Medical Centre, 20-095 Lublin, Poland -Lasermed Diagnosis and Treatment Centre, 22-100 Chelm, Poland -Medical Centre Lucyna and Andrzej Dymek, 47-120 Zawadzkie, Poland	
Publication (reference):	Not applicable	
Studied period : **(date of first enrolment)** **(date of last completed)**	36 weeks 14 July 2014 8 June 2015	**Phase of development: III**
Objectives: **Primary:** **Secondary:**	To assess the efficacy of Ismigen® versus Placebo to improve the asthma control level measured by ACT (Asthma Control Test)/P -ACT (Paediatric Asthma Control Test) score after 3 months as add-on treatment to routine asthma therapy. - To assess the decrease (versus Placebo) in number of respiratory tract infections during the observation period (12, 24, 36 weeks and all study period) - To assess the reduction (versus Placebo) of number of asthma exacerbations, times to first, second and third exacerbations after treatment with Ismigen® - To compare the cumulative number of days of short acting beta-2 agonists use for exacerbation over the total study period - To compare mean daily doses of ICS in both groups over all study period -To assess the improvement in the quality of life of patients and caregivers -To assess the specific changes occurring in a panel of immunological markers as the result of Ismigen® treatment (biological subset of patients).	

Name of Company: **Lallemand Pharma International AG**	**Individual Study Table**	**(For National Authority Use Only)**
Name of finished product: **ISMIGEN®**	Referring to Module 5 of the Dossier: **N/A**	
Name of active substance: **Polyvalent Mechanical Bacterial Lysate (PMBL)**	Vol.: **N/A** Page: **N/A**	

Methodology:	Randomised, double blind, placebo-controlled, multicentre, parallel-group study. The protocol included a total of 5 visits: Screening / Randomization visit (Baseline; V1), Biology visit (3 weeks; V2 only for patients enrolled in the biological assessments), End-of-treatment visit (12 weeks; V3), follow-up visits (24 weeks; V4 and 36 weeks; V5). Two phone contacts with parents/patients were performed at 6 weeks (TC1) and at 18 weeks (TC2). A subset of 48 patients participated to the biological and immunological assessments.
Number of patients (planned and analysed):	**Planned**: 150 (75 Ismigen®, 75 Placebo) **Randomized**: 152 (75 Ismigen®, 77 Placebo) **Analysed**: -Safety population: 151 (75 Ismigen®, 76 Placebo) -Full Analysis Set: 150 (74 Ismigen®, 76 Placebo); biology subset: (21 Ismigen®, 28 Placebo) -Per Protocol set: 144 (70 Ismigen®, 74 Placebo)
Diagnosis and main criteria for inclusion:	**Main inclusion criteria** 1. Children of both genders aged 6 to 16 years 2. Written informed consent obtained from the parents/legally authorized representatives and the subject assent (as appropriate) 3. Allergic asthma diagnosis with at least one perennial allergen according to the Global Strategy for Asthma Management and Prevention (GINA 2012 guidelines) prior to screening 4. Patient shows clinical characteristics of partly controlled or uncontrolled asthma according to the Global Strategy for Asthma Management and Prevention (GINA 2012 guidelines) prior to screening visit 5. Already treated with SABA prn and ICS or ICS + LABA during the previous 3 months 6. Patient shows antigen-specific IgE against HDM ≥ class 2 (RAST) or positive skin prick test for at least one perennial allergen 7. Patient who had at least 2 exacerbations of asthma within the 12-mo period 8. Patient not treated with PMBL (Ismigen®) with the previous 6 months prior to visit **Main exclusion criteria** 1. Patient received mechanical or any other bacterial lysate immunostimulation within the previous 6 months before Visit 1 2. Patient received oral / subcutaneous allergen-immunotherapy within

	the previous 6 months before Visit 1 3. History of near fatal asthma (e.g. brittle asthma, hospitalization for asthma exacerbation in intensive care unit) 4. Pregnant or breastfeeding woman
Test product: **Dose:** **Mode of administration:** **Batch number:**	Biological vaccine PMBL, Ismigen®: tablets (48 billion organisms / tablet); 1 tablet daily, over ten days of each month followed by 20 days of rest. This sequence of treatment is repeated during three months The drug is applied under the tongue until completely melted N°130189 (clinical batch)
Duration of treatment:	10 days per month, 3 months A patient was considered compliant to treatment if ≥80% of the tablets dispensed were taken at the 12 weeks visit.
Reference therapy: **Dose:** **Mode of administration:** **Batch number:**	Placebo: matched tablets without any active substance 1 tablet daily, over ten days of each month followed by 20 days of rest during three months The drug is applied under the tongue until completely melted N°130189 (clinical batch)
Criteria for evaluation: **Efficacy:**	**Primary efficacy variable**: Evaluation of the efficacy of Ismigen® versus Placebo to improve asthma control level as measured by the ACT/P-ACT (Asthma Control Test/Paediatric Asthma Control Test) score after a 3-month treatment as add-on to routine asthma therapy. **Secondary efficacy variables**: **Respiratory infections**: Mean number of respiratory infections, % of patients with at least one infection leading to exacerbation at 12, 24 and 36 weeks and over the total study period. **Asthma exacerbations:** -% of patients without any exacerbation, mean number of asthma exacerbations, mean number of days with asthma exacerbations, mean number of school days lost due to asthma exacerbation, mean duration per event, number of patients with at least one exacerbation related to infection / unrelated to infections, mean number of asthma exacerbations related / unrelated to infection at 12, 24 and 36 weeks and over the total study period. -Times to onset for first, second, and third asthma exacerbations. -Cumulative number of days of short acting beta-2 agonists use for

	exacerbation over the total study period. -Mean daily dose of inhaled corticosteroid used during the whole study (in equivalent dose of beclomethasone). **Quality of Life Questionnaires:** PAQLQ (Paediatric Asthma Quality of Life questionnaire) and PACQLQ (Paediatric Asthma Caregivers Quality of life Questionnaire) at the 36-week visit as compared with baseline. **Biological and immunological parameters:** Blood cells counts (at 12 weeks), serum non-specific immunoglobulins (at 12 weeks), serum Ismigen® specific immunoglobulins (at 3 and 12 weeks), lymphocytes phenotypes (at 12 weeks) were compared with baseline (biological subset).
Safety:	- Reporting of adverse events and serious adverse events (all the visits). - Vital signs and physical exams (all the visits). - Blood cells count (at baseline and 12 weeks; biological subset).
Statistical methods:	-Between groups comparisons of the mean changes for the questionnaires scores (ACT / P-ACT, PAQLQ / PACQLQ), for each laboratory parameter between baseline and values at subsequent study times were performed using an ANCOVA with baseline as covariate. In case of non-normal distribution (assessed by Shapiro-Wilk test), a non-parametric analysis of covariance based on ranks was used. -The comparisons of mean number of respiratory infections / asthma exacerbations, mean duration per exacerbation, mean number of days with asthma exacerbation, mean number of school days lost due to asthma exacerbation, mean days number of SABA use, mean daily dose of ICS, were performed using Student's t test or Wilcoxon's test in case of non-normality. -The between groups comparisons of the numbers of children with at least one infection / exacerbation, children with one infection leading to exacerbation, were analysed using Chi2 or Fisher exact test. -The times to first, second and third asthma exacerbations: the cumulative survival rates (period without exacerbation) were estimated using a survival analysis (Kaplan–Meier).

Summary - Conclusions:

Efficacy results

<u>Main criterion</u>: At baseline, the mean overall score of P-ACT/ACT was similar (16.8±2.4 in both groups). The absolute changes in P-ACT/ACT scores between baseline and 12 weeks were similar in both groups (FAS and PP) after the 3 months treatment period. The asthma status for most of the patients could be categorized as "controlled" according to the mean score values (mean overall score 21.0±3.5 and 21.1±3.0 in Ismigen® and Placebo groups respectively). Similar results were obtained at 24 and 36 weeks and asthma of most patients was controlled at the end of study (23.0±2.7 versus

22.3±3.3 at 36 weeks in Ismigen® and Placebo groups respectively).

Secondary criteria:

- **Number of respiratory infections**: the mean number of infections and the percentage of patients with at least one infection were not significantly different between groups, whatever the study time point. The number of patients with at least one infection leading to exacerbation was significantly lower in the Ismigen® group at 12 weeks (10 versus 21, p=0.002) and over the total study period (22 versus 38, p=0.003) in the Ismigen® and Placebo groups respectively.
- **Number of asthma exacerbations**: the percentage of patients without asthma exacerbation was significantly higher at 12 weeks in the Ismigen® group as compared to the Placebo group (75% versus 57.9%, p=0.028). The mean number of asthma exacerbations was significantly lower in the Ismigen® group as compared to the Placebo group at 12 weeks (0.3±0.6 versus 0.8±1.1, p=0.009) and over the total study period (1.1±1.3 versus 1.9±2.0, p=0.011). Consistently, the mean number of days with exacerbation per patient was significantly decreased in the Ismigen® group (13.3±11.2 versus 19.8±15.7 over the total study period, p=0.009). Surprisingly, the number of school days lost due to asthma exacerbation was not significantly different between groups. This apparent discrepancy is most likely related to the high variability of the reported data (4.7±5.5 versus 7.1±9.1 days over the whole study, NS). The mean duration per event was not significantly different between groups at all study time points. The exacerbations related to infection were more impacted by Ismigen® treatment than those unrelated to infection. Indeed, the mean number of asthma exacerbations related to infection was significantly reduced at 12 weeks and over the total study period in the Ismigen® group as compared to the Placebo group (0.2±0.5 versus 0.4±0.8, p=0.034 and 0.5±0.9 versus 0.9±1.2, p=0.005 respectively) whereas a trend to reduce exacerbations unrelated to infection was observed in the Ismigen® group at 12 weeks only (0.1±0.3 versus 0.3±0.7, p=0.057).

The time to onset of first asthma exacerbation (event-free time) was not significantly different between groups although a trend to a longer event-free time was observed for the Ismigen® group. This trend was confirmed for the times to onset of second and third asthma exacerbations which are significantly longer for the Ismigen® group (p=0.0024 and p= 0.0004 respectively).

- Asthma treatments:

The daily dose of ICS was not significantly different between groups.

Consistently with the positive effect of Ismigen® on asthma exacerbations, the mean number of days of SABA use for exacerbation relief during the study was significantly lower (11.5±11.2 in the Ismigen® group versus 15.6±10.2 in the Placebo group, p=0.016). In addition, a lower cumulative number of days of SABA use for exacerbation relief was reported in the Ismigen® group (426 days versus 732 days).

- **PAQLQ and PACQLQ:** No difference was observed between groups at any time points for both questionnaires and the quality of life scores were increased to a similar level at the end of the study.

- Laboratory measurements:

Blood cells count: with exception of an increase of lymphocytes in the Ismigen® group (+0.12±0.49 versus -0.30±0.86, p=0.0141), the evolution of blood cells counts between baseline and 12 weeks was not significantly different between groups.

Phenotypic analysis of T, B and NK cells subsets:

- Among peripheral blood CD3+ T cells, significant decreases of T cell activation markers were observed in the Ismigen group, as both CD3+CD69+ cells (early activated) and CD3+CD25+ cells (late activated) expressed as % of CD3+ were decreased, whereas increases occurred in

the Placebo group (respectively -2.2±5.2% versus +2.5±15.3%, p=0.0140 and -0.6±5.3% versus +0.7±4.1%, p=0.0243).

- The absolute number of T regulatory cells (Treg) expressing CD4, FoxP3 and high level of CD25 was increased in the Ismigen® group as compared to the Placebo group (+6.5±37.3 versus -10.8±24.8, p=0.0395 respectively).
- The NK cells absolute count was increased in the Ismigen® group and decreased in the Placebo group (+19.1±114.8 versus -35±164.0 respectively, p=0.0463).
- No effect was observed on B cells.

Serum specific and non-specific immunoglobulins: the evolution of specific and non-specific immunoglobulins was not significantly different between groups whatever the experimental conditions.

Safety results

Four serious adverse events (1 in the Ismigen® group and 3 in the Placebo group) have been reported during the study. The investigator considered that none was related to study product intake.

Two adverse events related to Ismigen® intake have been reported: drowsiness (3 occurrences scored as mild for the same patient during the treatment period) and drug intolerance (scored as severe by the investigator because the two concerned patients withdrew the study).

No treatment-associated emergent adverse event relative to either abnormal value of individual laboratory measurement or abnormal vital sign has been reported.

Conclusion

This study showed a strong and parallel increase of the ACT/P-ACT scores in both Ismigen®- and Placebo-treated patients at all visits. The asthma control status improvement based on ACT/P-ACT scores in both groups is not really surprising. Indeed, it is recognised that participation in clinical trials improves asthma control. Furthermore the incapacity of the tests to discriminate between Ismigen® and Placebo groups could be explained because, as recently published, ACT and P-ACT do not take into account exacerbations if not in the recall window of the questionnaires.

Basically, this trial has not been designed for demonstration of an effect of Ismigen® on respiratory tract infections (c.a. 70% of patients did not present recurrent respiratory infections at baseline), but the objective of this study was to investigate for the first time the efficacy of Ismigen® on allergic asthma paediatric patients.

The efficacy of Ismigen® on rate and times to the onset of asthma exacerbations as well as the lower mean number of days of SABA used for exacerbation relief are new and important findings.

Possible explanation could be that Ismigen® reduces the locoregional inflammatory status secondary to infections or aeroallergen exposure. This hypothesis is supported by the observed decrease of activation markers in T cells (CD25 and CD69) and the increase of regulatory T cells.

The results of the study also demonstrated no safety concerns with Ismigen® use for the allergic asthma paediatric population.

In conclusion, the EOLIA study clearly demonstrates the efficacy of Ismigen® to reduce exacerbations number in allergic asthma children. The benefit/risk balance associated with Ismigen® use is highly favourable in this population.