Clinical and anti-inflammatory effects of ultra-short pre-seasonal vaccine to Parietaria in asthma

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Abstract:	Objective: The ultra-short course pre-seasonal allergy vaccine, containing the adjuvant monophosphoryl lipid A (MPL), is effective in treating allergic symptoms; however, the efficacy in controlling asthmatics symptoms has not been fully demonstrated. We aimed at evaluating whether the ultra-short preseasonal course of immunotherapy contributes to asthma control. Methods: Four subcutaneous injections of the active product (Pollinex Quattro) were administered, before the pollen season, to 20 Parietaria-sensitive asthmatics (M/F: 12/8; age: 38±14 yrs). After the screening visit (Visit 1), asthma control was assessed by the Asthma Control Test (ACT) immediately before the first (Visit 2) and immediately after the last (Visit 5) injections, as well as during the pollen season (Visit 6). Bronchial and alveolar exhaled nitric oxide (eNO) concentrations were also measured. Nine parietaria-sensitive asthmatics (M/F: 3/6; age: 40±12 yrs) served as untreated controls. Results: The ACT remained constant during allergen exposure in SIT-treated asthmatics (Visit 2: 22±3.2, Visit 5: 23±2.8, Visit 6: 22±3.6; ANOVA, p=0.47), whereas it dropped during pollen exposure in controls (Visit 2: 20±2.5, Visit 5: 21±2.8, Visit 6: 16±5.7; ANOVA, p=0.01). The FENO values significantly increased during pollen exposure in both groups; however, the alveolar NO concentrations remained stable in SIT-treated asthmatics (p=0.11), whereas they doubled in controls (p=0.01). Conclusions: The current findings show that the pre-seasonal vaccine adjuvated with MPL contributes to maintain control of asthma during the pollen season.

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Clinical and anti-inflammatory effects of ultra-short pre-seasonal vaccine to *Parietaria* in asthma

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Running head: Pre-seasonal immunotherapy in asthma

Key words: airway inflammation, asthma, immunotherapy, quality of life

ABSTRACT

Objective: The ultra-short course pre-seasonal allergy vaccine, containing the adjuvant monophosphoryl lipid A (MPL), is effective in treating allergic symptoms; however, the efficacy in controlling asthmatics symptoms has not been fully demonstrated. We aimed at evaluating whether the ultra-short preseasonal course of immunotherapy contributes to asthma control.

Methods: Four subcutaneous injections of the active product (Pollinex Quattro) were administered, before the pollen season, to 20 *Parietaria*-sensitive asthmatics (M/F: 12/8; age: 38±14 yrs). After the screening visit (Visit 1), asthma control was assessed by the Asthma Control Test (ACT) immediately before the first (Visit 2) and immediately after the last (Visit 5) injections, as well as during the pollen season (Visit 6). Bronchial and alveolar exhaled nitric oxide (eNO) concentrations were also measured. Nine *parietaria*-sensitive asthmatics (M/F: 3/6; age: 40±12 yrs) served as untreated controls.

Results: The ACT remained constant during allergen exposure in SIT-treated asthmatics (Visit 2: 22±3.2, Visit 5: 23±2.8, Visit 6: 22±3.6; ANOVA, p=0.47), whereas it dropped during pollen exposure in controls (Visit 2: 20±2.5, Visit 5: 21±2.8, Visit 6: 16±5.7; ANOVA, p=0.01). The FENO values significantly increased during pollen exposure in both groups; however, the alveolar NO concentrations remained stable in SIT-treated asthmatics (p=0.11), whereas they doubled in controls (p=0.01).

Conclusions: The current findings show that the pre-seasonal vaccine adjuvated with MPL contributes to maintain control of asthma during the pollen season.

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INTRODUCTION

Bronchial asthma is one of the most common chronic diseases worldwide, characterized by an infiltrate of inflammatory cells such as lymphocytes, eosinophils and mast cells in the bronchial wall. Up to 80% of asthmatics also present with features of rhinitis, which triggers asthma attacks and worsens health-related quality of life. To manage chronic respiratory allergies, international guidelines [GINA 2010] recommend the avoidance of allergens and removal of triggers, together with the pharmacological therapy and specific immunotherapy (SIT). The latter is considered the only treatment capable of modifying the natural course of allergic, preventing (or delaying) the onset of asthma in patients with allergic rhinitis [Bousquet et al. 1998]. In addition, SIT has been demonstrated to improve upper and lower airway symptoms, and to reduce the use of medications [Holt et al. 1998].

To improve the efficacy of SIT, immunological adjuvants have been incorporated in the allergy vaccines. The monophosphoryl lipid A (MPL), a detoxified lipopolysaccharide component extracted and purified from *Salmonella minnesota*, appears to promote and increase the immunological response [Wheeler et al. 2001]. In a recent study from our group [Scichilone et al. 2011], a short course of pre-seasonal vaccine for *parietaria* was demonstrated to reduce the concentration of 8-isoprostane, a marker of bronchial inflammation, as early as the fourth (last) administration, and to maintain low levels of inflammation during the pollen season. The pre-seasonal MPL vaccine has been shown to be effective in relieving nasal and ocular symptoms [Patelet al. 2006]; however, the efficacy of the adjuvated vaccine in controlling asthmatics symptoms has not been demonstrated.

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In the current study, we tested whether, in asthmatics with concomitant rhinitis, a pre-seasonal course of *parietaria* MPL vaccine is effective in: 1) reducing the level of airway inflammation and, 2) maintaining optimal asthma control as well as symptom-related quality of life.

METHODS

Subjects

Individuals attending the Allergy Outpatient Clinic of the Division of Respiratory Diseases of the University of Palermo, Italy, who had previously received the diagnosis of asthma and concomitant rhinitis, were enrolled. To be eligible for the study, subjects had to be allergic only to *parietaria*, with no previous history of any type of specific immunotherapy. Subjects with moderate or persistent asthma, those who were unable to perform pulmonary function tests, and individuals with a family history of fatal asthma were excluded. In addition, current smokers were not allowed to participate to the study. Subjects were tested at least four weeks after their most recent upper respiratory infection. Eligible subjects who refused SIT were invited to participate as untreated controls. All study subjects were allowed to use symptomatic treatment for upper and lower respiratory symptoms as required. The study was performed in accordance with the Good Clinical Practice guidelines recommended by the International Conference on Harmonization of Technical Requirements and was approved by the Ethics Committee of University Hospital of Palermo, and all participants gave written informed consent before inclusion.

Study design

The study included two groups: Group 1 consisted of subjects who underwent pre-seasonal SIT, and Group 2 included individuals who did not perform SIT. All subjects underwent the same functional, clinical and biological assessments.

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The study entailed six visits. At Visit 1, which took place in the months of July and August 2010, all inclusion and exclusion criteria were checked and subjects were enrolled. The screening evaluation included a respiratory questionnaire for both upper and lower airways, allergy skin testing to a panel of 10 common aeroallergens, and lung functional assessment. The presence of features of rhinitis was also diagnosed according to the ARIA recommendations [ARIA 2010]. During the months of September-October (pre-pollen season), each subject visited the Clinic on four separate occasions (Visit 2 to 5). Group 1 received a subcutaneous injection of the active product with increasing strength; the first three 1.0-ml increasing strength injections were administered at one-week intervals, and the last top-strength injection was administered at six weeks from the first injection. At the time of the first (Visit 2) and the last (Visit 5) injections, health-related quality of life (HR-QoL) and the level of asthma control were assessed, and measurements of nitric oxide (NO) concentrations in the exhaled air were obtained. The ultra-short course pre-seasonal allergy vaccine (Allergy Therapeutics, U.K., Ltd, Worthing, U.K.), containing appropriate allergoids with the MPL, was employed in the current study. In details, the vaccine consists of purified extract of *parietaria judaica* pollen allergen (*par j1*: 16.64 µg/ml), which is modified with glutaraldehyde. The MPL adjuvant is adsorbed to the L-tyrosine at 50 µg /ml in all vaccine dose levels. The asthmatics that served as untreated controls also underwent the same clinical, functional and biological assessments. Finally, all subjects (Group 1 and 2) returned to the Clinic during the peak of the pollen season (May 2011) to undergo the same investigations (Visit 6). The pollen count was provided by the Italian Society of Aerobiology, which weekly updates the pollen concentrations in the air by region. The pollen count in Sicily in May 2011 for Urticacee was between 20 and 69.9 grains per cubic metre of air, which is considered high. The protocol of the study is described in **Figure 1**.

CLINICAL ASSESSMENT

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Asthma Control Test (ACT)

Asthma control was assessed by using a self-administered questionnaire (Asthma Control Test, ACT). The ACT is a validated 5-item instrument, each item rated on a five-point scale [Schatz et al. 2007], providing a total score that allows to discriminate between "well-controlled" (total score: 25), "partially controlled" (total score: >20<25), and "not controlled" (total score: <20). The questions address symptoms occurring within the 4 weeks preceding the evaluation.

Rhinasthma

The HRQoL was evaluated by the *Rhinasthma* questionnaire [Baiardini et al. 2003], which consists of 30 items. The questionnaire explores three domains: quality of life related to Upper Airways (UA), Lower Airways (LA), and Respiratory Allergy Impact (RAI). This analysis leads to a composite score, namely the Global Summary (GS) score, which indicates the overall impact of the disease. Answers to the *Rhinasthma* items are converted into a score from 0 to 100: higher scores correspond to worse QoL related to respiratory symptoms.

FUNCTIONAL ASSESSMENT

Spirometry

Functional assessment included conventional spirometry. FEV_1 and FVC, as well as forced expiratory airflows at different lung volumes (FEF_{25}, FEF_{50}, FEF_{75} and FEF_{25-75} of forced vital capacity), were recorded. All spirometric measurements were obtained from a computerized water-sealed spirometer (Biomedin; Padua, Italy), which allowed compliance with criteria for acceptability and reproducibility on-line. Acceptability and reproducibility of FEV_1 and FVC were evaluated based on the recommendations of the European Respiratory Society [Miller et al. 2005].

BIOLOGICAL ASSESSMENT

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Measurement of exhaled nitric oxide

The measurement of the fraction of exhaled NO is a non-invasive method, simple and reproducible, which reflects the degree of inflammation in the airways. The exhaled NO was obtained by means of a chemiluminescence analyzer (FENO Hypa, Medisoft), which detects the light emitted by the photochemical reaction between NO and ozone generated by the tool. The amount of light emitted is proportional to the concentration of NO, in parts per billion (ppb). Subjects performed two acceptable and reproducible maneuvers at expiratory airflow of 50 ml/sec, and the mean of the two observations was used for analysis. In addition, measurements were conducted at expiratory airflow rates of 100, 150 and 350 ml/sec. This allowed to estimate the concentration of NO coming from the peripheral lung (alveolar NO, CANO).

STATISTICAL ANALYSIS

The data obtained are presented as mean±standard deviation (SD). The ANOVA was used for repeated measures. A p-value less than or equal to 0.05 was considered statistically significant.

RESULTS

The study was conducted on a total of 29 asthmatics: Group 1 consisted of 20 subjects (M/F: 12/8, age 38.2±14 years), and Group 2 comprised 9 age-matched subjects (M/F: 3/6, age 40±12 years). Lung function was within the normal range (FEV_1% predicted, for Group 1: 103±15%; for Group 2: 93±21%). The two groups did not differ in terms of baseline lung function characteristics, level of asthma control and HR-QoL (**Table 1 and 2**). In addition, the levels of exhaled NO concentrations were similar between the two groups (Group 1 vs. Group 2; FENO: 42.3±23 ppb vs. 54.6±31 ppb, p=0.24; CANO: 5.1±2.9 ppb vs. 5.1±3.2 ppb, p=0.95). All subjects in Group 1 completed the protocol and none of them reported adverse events associated with the SIT.

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Lung function

Lung function did not change during the study periods in both groups (ANOVA for repeated measures: p>0.05 for both groups). In Group 1, FEV_1% predicted was 103±15% at Visit 2, 104±15% at Visit 5 and 105±14% at Visit 6. In Group 2, FEV_1% predicted was 93±21% at Visit 2, 97±22% at Visit 5 and 97±24% at Visit 6.

Asthma Control Test

The level of asthma control, as assessed by the ACT, remained constant during the vaccine administrations and did not decrease during allergen exposure in Group 1 (Visit 2: 22±3.2, Visit 5: 23±2.8, Visit 6: 22±3.6; ANOVA, p=0.47). Conversely, the ACT scores significantly dropped during pollen exposure in SIT-untreated subjects (Group II, Visit 2: 20±2.5, Visit 5: 21±2.8, Visit 6: 16±5.7; ANOVA, p=0.01) (**Figure 2**).

Rhinasthma

The overall impact of the respiratory diseases on patients' quality of life did not change significantly during pollen exposure both in treated and untreated subjects. Indeed, in Group 1 the *Rhinasthma* global score was 14±11 at Visit 2, 10±9 at Visit 5, and 18±14 at Visit 6 (ANOVA, p=0.08). In Group 2, the *Rhinasthma* global score was 22±15 at Visit 2, 21±15 at Visit 5, and 32±18 at Visit 6 (p=0.11). Similar trends were obtained for the *Rhinasthma* partial scores in both groups (data not shown).

Exhaled nitric oxide

Figure 3.A shows the changes in exhaled NO concentrations for both groups during the study visits. The FENO values significantly increased during pollen exposure in both groups; however,

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the increase was more impressive in SIT-untreated asthmatics (Group 2), in whom FENO concentrations doubled (Visit 6 vs. Visit 2, mean difference: 72 ppb). In particular, in Group 1 FENO was 42±23 ppb at Visit 2, 34±16 ppb at Visit 5, and 63±35 ppb at Visit 6 (p=0.03), whereas in Group 2 FENO was 56±37 ppb at Visit 2, 100±69 ppb at Visit 5, and 128±87 ppb at Visit 6 (p=0.009). Interestingly, the alveolar NO concentrations remained stable in Group 1 throughout the study (5.1±2.9 ppb at Visit 2, 4.1±2.6 ppb at Visit 5, and 5.7±3.6 ppb at Visit 6; p=0.11). On the other hand, the CANO doubled during pollen season with respect to the pre-season values in Group 2 (5.0±3.2 ppb at Visit 2, 5.4±3.4 ppb at Visit 5, and 12.9±9.8 ppb at Visit 6 (p=0.01) (**Figure 3.B**).

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DISCUSSION

The findings of the present study clearly show that, in a selected population of mild asthmatics, an ultra-short course of pre-seasonal immunotherapy contributes to maintain control of asthma symptoms during pollen season. The current observations suggest that the clinical benefit provided by the vaccine could be mediated by anti-inflammatory effects, which mainly occurred at the level of peripheral airways, as indicated by the unchanged alveolar NO concentrations during pollen season in the treated subjects. The finding of preserved HR-QoL both in treated and untreated subjects advocates for larger studies specifically aiming at assessing the role of pre-seasonal SIT on quality of life.

The pre-season vaccine used in this study consists of natural allergens chemically modified with glutaraldehyde and adsorbed on L-tyrosine, to ensure a slow release of active ingredient, to reduce the possibility of having immediate and potentially dangerous reactions, therefore resulting in greater desensitization and higher tolerability. In addition, the immune response is enhanced and

directed by adjuvant monophosphoryl lipid A (MPL), which is derived from the cell wall lipopolysaccharide of Salmonella Minnesota R595 (Gram negative), and chemically detoxified, thus promoting the Th1 response [Hopkins et al. 2001]. Puggioni and colleagues [2005] demonstrated *in vitro* that MPL promotes immune deviation of allergen-induced peripheral Th2-cell responses in favour of protective Th1 responses. We recently showed that, in asthmatics, the ultra-short course of pre-seasonal vaccine for *parietaria* with MPL exerts anti-inflammatory effects, as documented by the significant reduction in the levels of 8-isoprostane in the exhaled breath condensate [Scichilone et al. 2011], which appeared to be maintained during and after pollen season.

The clinical benefit of pre-seasonal vaccine are based on recent observations and mostly related to rhino-congiuntivitis symptoms [Drachenberg et al 2001]. A significant effect of ultra-short course of immunotherapy to grass over placebo in relieving rhino-congiuntivitis allergic symptoms was shown in the largest controlled study of allergen-specific immunotherapy conducted to date [DuBuske et al. 2011]. Subjects receiving the four injections of MPL vaccine achieved a significant 13.4% benefit during the peak of the pollen exposure. Data regarding on the beneficial effect on asthmatic symptoms are scarce and can be extrapolated by the observational study of Musarra and colleagues [Musarra et al. 2010], who followed patients with asthma allergic to *parietaria* and subjected to MPL-SIT. The authors found that, after 5 years of discontinuing the vaccine, symptoms of asthma were still low, whereas they doubled in the control (untreated) group.

Taken together, these studies led us to investigate whether a short course of vaccine adjuvanted with MPL, already shown to be well-tolerated and efficaceous in allergic rhinitics, can also provide beneficial effect on lower airways symptoms. To this aim, we employed the ACT questionnaire, which has been largely used in clinical trials attempting to optimize the level of asthma treatment. The results of the current study clearly demonstrate that asthma control can be maintained by pre-seasonal immunotherapy. In this regard, it should be noticed that our study population consisted of

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asthmatics with mild forms of the disease, and therefore our findings cannot be extrapolated and applied to subjects with more severe asthma.

Interestingly, the clinical benefit paralleled the anti-inflammatory effect of SIT during pollen season, as demonstrated by the lack of increased markers of inflammation in the expired air. Evaluation of NO in exhaled air has been proposed as a valuable clinical tool for asthma diagnosis and monitoring. A relationship between exaled NO and clinical signs and symptoms of asthma has been shown in different study [Alving et al. 1993; Mahut et al. 2011; Caudri et al. 2010; Tseliou et al. 2010; Perez-de-Llano et al. 2010; Sandrini et al. 2010]. Recently, Papakosta and colleagues [2011] performed a study on asthmatic patients in order to evaluate the relationship between ACT and the degree of airway inflammation, by means of exhaled NO concentrations, before and after treatment with inhaled corticosteroids. The study demonstrated an improvement in lung function and ACT scores and a parallel decrease in NO levels in the expired air. With regard to the effect of SIT, Cevit and colleagues [2007] conducted a study of asthmatic children allergic to dust mites and subjected to immunotherapy subcutaneously. The study showed a reduction in plasma levels of NO after one year, possibly reflecting a reduction in systemic allergic reaction. In patients with house dust mite asthma, an incremental schedule of autologous autovaccine with E. Coli showed a significant effect on bronchial inflammation as expressed by a reduced increase of exhaled NO [Rose et al. 2011].

In recent years, the contribution of the alveolar component of NO levels has been investigated [van Veen et al. 2006; Paraskakis et al. 2006; Kelly et al. 2010]. This observation emphasizes the role of alterations in the peripheral bronchial district on the QoL of patients with asthma and rhinitis, which is impaired to varying degrees in absence of traditional physiological or biological functional alterations. As also inferred in several studies, the peripheral airways in asthmatics are the site of an inflammatory infiltrate that is not observed in non-asthmatic controls, and, within asthmatic lungs,

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is more intense than in the central airways [Hamid et al. 1997]. In a study conducted in consecutive asthmatic children [Puckett et al. 2010], increased levels of CANO were related to poor asthma control independent of lung function, atopic status, or use of inhaled corticosteroids. The finding that CANO increases in uncontrolled asthmatics supports the observations that the site of inflammation might change with the disease severity, becoming more prevalent and clinically important in the periphery of the airways. In this context, the effect of SIT on alveolar NO levels is intriguing. The most plausible explanation for this observation is that the anti-inflammatory contribution of SIT, which is administered systemically, occurs primarily in the periphery of the lung. Additional studies, specifically designed to answer this mechanism, are required.

We did not document any adverse effect related to the administration of the vaccine. The safety and tolerability of short-term specific immunotherapy with pollen allergoids adjuvanted by MPL has been largely demonstrated [Baldrick et al. 2002; Baldrick et al. 2002; Rosewich et al. 2010]. In a prospective open study [Rosewich et al. 2010], a large cohort of juvenile patients with rhinitis, conjunctivitis and/or asthma received four pre-seasonal injections with pollen allergoids formulated with MPL over a minimum of 3 weeks. Response to treatment was assessed as good or very good in the majority of patients. The safety of the ultra-short term SIT with pollen allergoids adjuvanted with MPL was recently confirmed in an Italian survey, which was conducted prospectically for a three-year period on a total of 510 adult individuals with seasonal rhinitis and/or asthma [Crivellaro et al. 2011].

The study has some limitation. We acknowledge that the small number of subjects may have influenced the outcomes. For example, the lack of difference between groups with respect to parameters reflecting quality of life could be simply due to this factors. However, the study was exploratory in nature, and was intended as preliminary to larger studies with the attempt to select

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those biological markers and clinical variables that could be employed when assessing the effect of pre-seasonal immunotherapy.

In conclusion, we showed for the first time that an ultra-short course of pre-seasonal immunotherapy provides clinical benefit in mild asthmatics, by maintaining symptom control during pollen season. Our findings suggest that the effect of SIT on asthma control could be mediated by anti-inflammatory effects on peripheral airways. Further studies are needed to specifically explore the mechanism behind these observations.

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COMPETING INTEREST:

The authors declare no conflict of interest.

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REFERENCES

1. GINA Report, Global Strategy for Asthma Management and Prevention. NIH Pub. No 02-3659, January 1995. Updated 2010. Available from: *www.ginasthma.org.*

2. Bousquet J, Lockey R, Malling HJ, Alvarez-Cuesta E, Canonica GW, Chapman MD, et al (1998). Allergen immunotherapy: therapeutic vaccines for allergic diseases. World Health Organization. American academy of Allergy, Asthma and Immunology. *Annals of allergy, asthma & immunology*.;81(5 Pt 1):401-5.

3. Holt PG, Sly PD, Smith W. Sublingual immunotherapy for allergic respiratory disease (1998) *Lancet*. 351(9103):613-4.

4. Wheeler AW, Marshall JS, Ulrich JT. A Th1-inducing adjuvant, MPL, enhances antibody profiles in experimental animals suggesting it has the potential to improve the efficacy of allergy vaccines (2001). I*nternational Archives of Allergy and Immunology* 126(2):135-9..

5. Scichilone N, Minaldi C, Santagata R, Battaglia S, Camarda G, Bellia V (2011) Anti-inflammatory effects of pre-seasonal Th1-adjuvant vaccine to Parietaria judaica in asthmatics. *Journal of Asthma and Allergy* 4:19-25.

6. Patel P, Salapatek AM. Pollinex Quattro: a novel and well-tolerated, ultra short-course allergy vaccine (2006) *Expert Review of Vaccines* 5(5):617-29.

7. Allergic rhinitis and its impact on asthma. Updated 2010. Available from: *www.whiar.org.*

Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines.

8. Schatz M, Mosen DM, Kosinski M, Vollmer WM, Magid DJ, O'Connor E, et al. (2007) Validity of the Asthma Control Test completed at home. *The American Journal of Managed Care* 13(12):661-7.

9. Baiardini I, Pasquali M, Giardini A, Specchia C, Passalacqua G, Venturi S,et al. (2003) Rhinasthma: a new specific QoL questionnaire for patients with rhinitis and asthma. *Allergy* 58(4):289-94.

For Peer Review

10. Miller MR, Crapo R, Hankinson J, Brusasco V, Burgos F, Casaburi R, et al. ATS/ERS Task Force. General considerations for lung function testing. (2005) *European Respiratory Journal* 26(1):153-61.

11. Hopkins M, Lees BG, Richardson DG, Woroniecki SR, Wheeler AW. Standardisation of glutaraldehyde-modified tyrosine-adsorbed tree pollen vaccines containing the Th1-inducing adjuvant, monophosphoryl lipid A (MPL). (2001) *Allergologia et Immunopathologia* 29(6):245-54.

12. Puggioni F, Durham SR, Francis JN. Monophosphoryl lipid A (MPL) promotes allergen-induced immune deviation in favour of Th1 responses. (2005) *Allergy* 60(5):678-84.

13. Drachenberg KJ, Wheeler AW, Stuebner P, Horak F. A well-tolerated grass pollen-specific allergy vaccine containing a novel adjuvant, monophosphoryl lipid A, reduces allergic symptoms after only four preseasonal injections. (2001) *Allergy* 56(6):498-505.

14. DuBuske LM, Frew AJ, Horak F, Keith PK, Corrigan CJ, Aberer W, et al. Ultrashort-specific immunotherapy successfully treats seasonal allergic rhinoconjunctivitis to grass pollen. (2011) *Allergy and Asthma Proceedings* 32(3):239-47.

15. Musarra A, Bignardi D, Troise C, Passalacqua G. Long-lasting effect of a monophosphoryl lipid-adjuvanted immunotherapy to parietaria. (2010) A controlled field study. *European Annals of Allergy and Clinical Immunology* 42(3):115-9.

16. Alving K, Weitzberg E, Lundberg JM. Increased amount of nitric oxide in exhaled air of asthmatics. (1993) *European respiratory Journal* 6(9):1368-70.

17. Mahut B, Delclaux C. Peripheral airway/alveolar nitric oxide concentration in asthma. (2011) *Thorax* 66(7):632-3.

18. Caudri D, de Jongste JC. Exhaled nitric oxide and childhood asthma. (2010) *The Journal of Pediatrics* 156(3):514.

19. Tseliou E, Bessa V, Hillas G, Delimpoura V, Papadaki G, Roussos C, et al. Exhaled nitric oxide and exhaled breath condensate pH in severe refractory asthma. (2010) *Chest* 138(1):107-13.

For Peer Review

20. Perez-de-Llano LA, Carballada F, Castro Anon O, Pizarro M, Golpe R, Baloira A, et al. Exhaled nitric oxide predicts control in patients with difficult-to-treat asthma. (2010) *European Respiratory Journal* 35(6):1221-7.

21. Sandrini A, Taylor DR, Thomas PS, Yates DH. Fractional exhaled nitric oxide in asthma: an update. (2010) *Respirology* 15(1):57-70.

22. Papakosta D, Latsios D, Manika K, Porpodis K, Kontakioti E, Gioulekas D. Asthma control test is correlated to FEV1 and nitric oxide in Greek asthmatic patients: influence of treatment. (2011) *Journal of Asthma* 48(9):901-6.

23. Cevit O, Kendirli SG, Yilmaz M, Altintas DU, Karakoc GB. Specific allergen immunotherapy: effect on immunologic markers and clinical parameters in asthmatic children. (2007) *J Investig Allergol Clin Immunol* 17(5):286-91.

24. Rose MA, Weigand B, Schubert R, Schulze J, Zielen S. Safety, tolerability, and impact on allergic inflammation of autologous E.coli autovaccine in the treatment of house dust mite asthma--a prospective open clinical trial. (2011) *BMC complementary and alternative medicine* 11:45.

25. van Veen IH, Sterk PJ, Schot R, Gauw SA, Rabe KF, Bel EH. Alveolar nitric oxide versus measures of peripheral airway dysfunction in severe asthma. (2006) *European Respiratory Journal* 27(5):951-6.

26. Paraskakis E, Brindicci C, Fleming L, Krol R, Kharitonov SA, Wilson NM, et al. Measurement of bronchial and alveolar nitric oxide production in normal children and children with asthma. *(*2006) *American Journal of Respiratory and Critical Care Medicine* 174(3):260-7.

27. Kelly HW. Alveolar nitric oxide concentration, small airways inflammation, and targeted asthma therapy: are we there yet? (2010) *The Journal of Allergy and Clinical Immunology* 126(4):736-7.

28. Hamid Q, Song Y, Kotsimbos TC, Minshall E, Bai TR, Hegele RG, et al. Inflammation of small airways in asthma. (1997) *The Journal of Allergy and Clinical Immunology* 100(1):44-51.

For Peer Review

29. Puckett JL, Taylor RW, Leu SY, Guijon OL, Aledia AS, Galant SP, et al. Clinical patterns in asthma based on proximal and distal airway nitric oxide categories. (2010) *Respiratory Research* 11:47.

30. Baldrick P, Richardson D, Wheeler AW. Review of L-tyrosine confirming its safe human use as an adjuvant. (2002) *Journal of Applied Toxicology* 22(5):333-44.

31. Baldrick P, Richardson D, Elliott G, Wheeler AW. Safety evaluation of monophosphoryl lipid A (MPL): an immunostimulatory adjuvant. (2002) *Regulatory toxicology and Pharmacology* 35(3):398-413.

32. Rosewich M, Schulze J, Fischer von Weikersthal-Drachenberg KJ, Zielen S. Ultra-short course immunotherapy in children and adolescents during a 3-yrs post-marketing surveillance study. (2010) *Pediatric Allergy and Immunology* 21(1 Pt 2):e185-9.

33. Crivellaro M, Senna GE, Pappacoda A, Vanzelli R, Spacal B, Marchi G, et al. Safety of ultrashort-term sit with pollen allergoids adjuvanted by monophosphoryl lipid A: a prospective Italian survey. (2011) *European Annals of Allergy and Clinical Immunology* 43(2):58-60.

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FIGURE LEGENDS

Figure 1

Schematic of the protocol of the study.

Figure 2

Asthma Control Test (ACT) score in SIT-treated (closed circles) and SIT-untreated (open circles) groups. * : p=0.01 ANOVA for repeated measures. For further explanation of Visit 2, 5 and 6 see Methods.

Figure 3

FENO (Panel A) and CANO (Panel B) concentrations in the expired air in SIT-treated (closed circles) and SIT-untreated (open circles) groups. *: p=0.009; #: p=0.03, §: p=0.01, ANOVA for repeated measures. For further explanation of Visit 2, 5 and 6 see Methods.

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Table 1. Lung function characteristics of the study subjects.

	GROUP 1	GROUP 2	p value
FEV_1 (% pred)	102.6±14.9	93.1±21.1	0.20
FVC (% pred)	105.2±14.8	98.4±16.8	0.30
FEV_1/FVC	0.8±0.08	0.8±0.08	0.54
FEF_{25} (% pred)	85.9±33.9	68.2±39.9	0.23
FEF_{50} (% pred)	93.8±28.8	79.1±30.9	0.22
FEF_{75} (% pred)	97.5±22.3	79.9±20.5	0.06
FEF_{25-75} (% pred)	88.6±26.3	73.3±32.1	0.20

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Table 2. Level of asthma control (ACT) and health-related quality of life (Rhinasthma) in the two study groups. ACT: asthma control test; UA: Upper Airways; LA: Lower Airways; RAI: Respiratory Allergy Impact; GS: Global Summary score. UA, LA and RAI are the three domains of the Rhinasthma questionnaire.

	GROUP 1	GROUP 2	p value
ACT	22±3.2	20±2.5	0.20
UA	22±19	26±21	0.60
LA	10±10	19±18	0.12
RAI	12±10	22±18	0.06
GS	14±11	22±15	0.13

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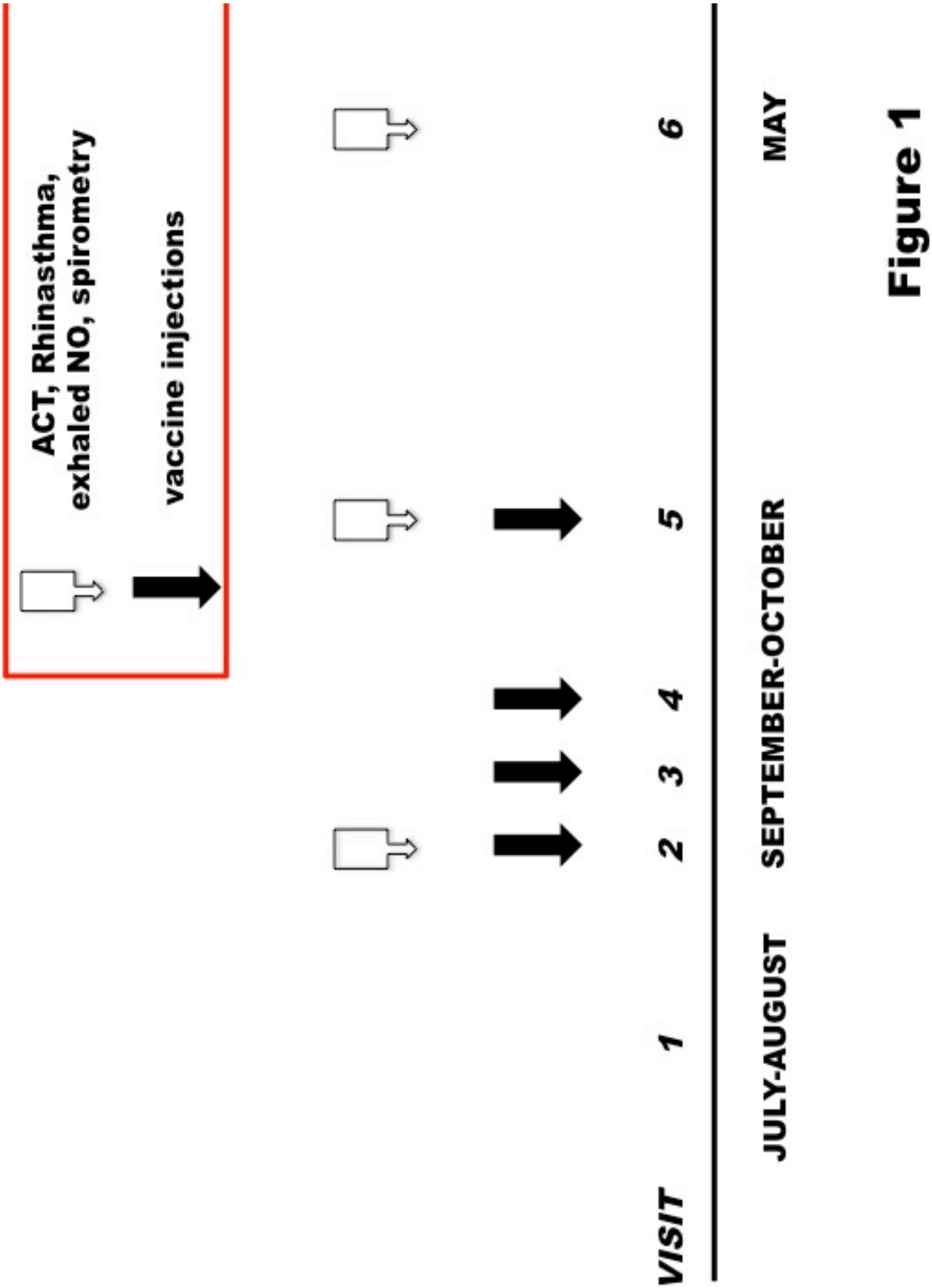
ACT, Rhinasthma, exhaled NO, spirometry
vaccine injections
VISIT
1
2
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4
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JULY-AUGUST
SEPTEMBER-OCTOBER
MAY

Figure 1

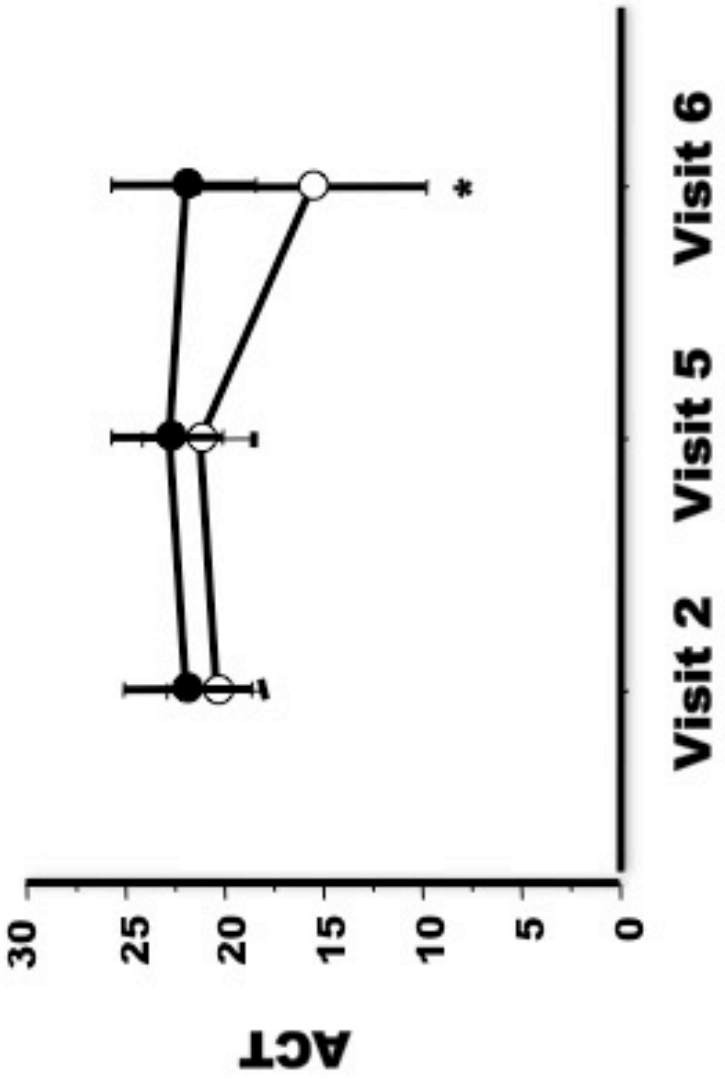

Figure 2

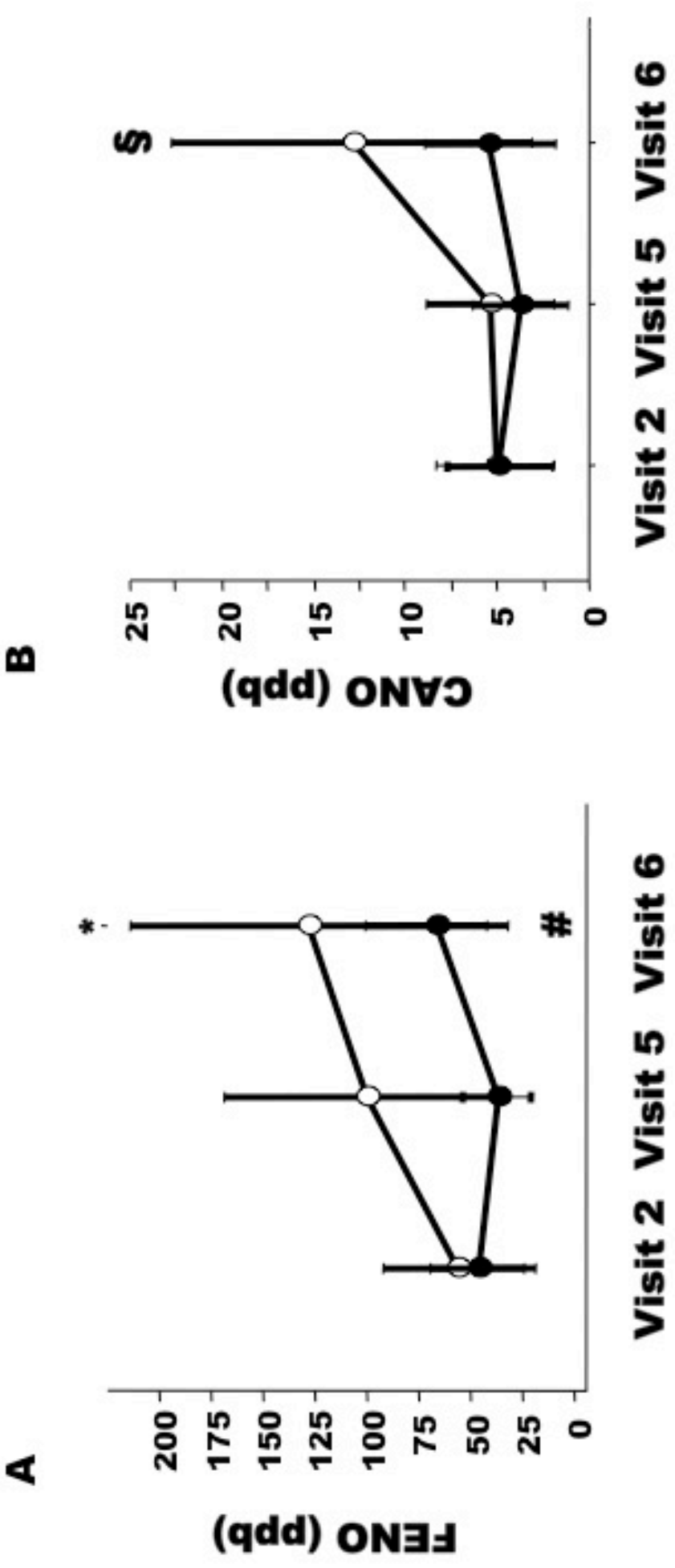
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Figure 3

UNIVERSITÀ DEGLI STUDI DI PALERMO
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December 22, 2012

To the Editor
of the "Therapeutic Advances in Respiratory Diseases" journal

Dear Editor,

We are pleased to submit the article "**Clinical and anti-inflammatory effects of ultra-short pre-seasonal vaccine to *Parietaria* in asthma**" for publication in the Journal.

In this paper, we address the contribution of a short course of pre-seasonal specific immunotherapy to parietaria in maintaining asthma control, and explore the anti-inflammatory effect of the treatment. Our findings show that the pre-seasonal vaccine adjuvated with MPL reduces the degree of airway inflammation, and contributes to maintain control of asthma during the pollen season, as opposed to untreated asthmatics. Finally, we discuss the implications of the distal lung inflammation in asthma control and the response to specific treatment.

We declare that neither the manuscript nor any part of its essential substance, tables or figures has been or will be published or submitted to another scientific journal or is being considered for publication elsewhere.

For Peer Review

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We hope that the topic described in this manuscript can be of high interest for the Journal, and on behalf of my co-authors, I would like to thank you for your kind consideration, and look forward to reading from you.

Yours sincerely,

Nicola Scichilone

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