**On line supplement**

**Title**

Mepolizumab in severe eosinophilic asthma: A two-year Follow-up in specialized asthma clinics in Greece: An interim analysis.

**Methods**

Asthma exacerbations were defined as symptoms deterioration requiring the use of systemic corticosteroids with and/or hospital admission. Inclusion criteria were the following: age ≥18 years, uncontrolled severe (characterized by an inadequate symptom control despite a continuous therapy with high doses of Inhaled corticosteroids (ICS) plus long-acting β2-adrenergic agonists (LABA)), eosinophilic (peripheral blood eosinophils ≥150 cells/μL in a recent measurement or ≥300 cells/μL in any measurement during the previous year) asthma, initiation of treatment with mepolizumab according to physicians decision, and a history of at least 2 asthma exacerbations in the previous year despite optimal treatment with high doses of inhaled corticosteroids and β2 agonists. The only exclusion criterion was the patients’ refusal to participate in the study. The study was approved from all local ethic committees and all patients provided oral and written informed consent. The study was registered in clinical trials.gov: ClinicalTrials.gov Identifier: NCT04084613.

**Study design**

During screening, the physician recorded the patients’ demographics, medical history, usual medication (including OCS), exacerbation history, asthma control Test (ACT) score, pulmonary function (simple spirometry) and blood eosinophils in the most recent Complete Blood Count (CBC) as % and absolute count. All aforementioned information was also recorded 4, 8 and 12 months after mepolizumab treatment initiation. Mepolizumab was administered at the dosage of 100 mg every 4 weeks for at least a year.

**Lung function tests**

Forced Expiratory Volume in 1st sec (FEV1) and Forced Vital Capacity (FVC), were measured using different spirometers. All values were post bronchodilation.

***Atopic Status***

A positive skin prick test (mean wheal diameter of 3mm or greater) to any of twenty common aeroallergens (including mites, grasses, trees, fungus, domestic animals) guided by clinical symptoms was used to confirm atopy. Data was retrieved from patients’ medical records.

**Statistical analysis**

All patients enrolled with the objective to be treated with mepolizumab for at least a year. The baseline is defined as the measured period preceding the starting date of add-on treatment with mepolizumab. For the purpose of this interim analysis, description and analysis of clinical benefit were primarily conducted on patients who have continued mepolizumab treatment for 12 months (per protocol analysis). Analysis was performed using available data. No data imputation was performed. The missing data were data that were not recorded in the database. Adverse Event (AE) shall mean any untoward medical occurrence to have been identified. In order to evaluate the target sample size, a power analysis was performed using the PS Power and Sample Calculation Program [[1](#_ENREF_1)], based on data from a previous study of mepolizumab an effect size of 0.35, based on an exacerbation reduction of 55% of asthma exacerbations after one year of treatment [[2](#_ENREF_2)] estimated that a sample size of 137 patients was required to achieve a power of 0.99 using an alpha significance level of 0.05 (two-sided).

All statistical analyses, including the generation of tables, listings and figures were performed using either R or Python frameworks. Categorical variables were summarised as the number of patients and percentages (%) of patients in each category. Percentages were calculated over the number of patients with available (non-missing) data. Continuous variables were summarised using the mean, medians, standard deviation (SD) and the number of missing observations. Computed metrics were presented up to two decimal places. Normality tests were applied before choosing between parametric (e.g. Student’s t-test) or non-parametric (e.g Wilcoxon’s signed rank test) statistic tests. p-values of less than 0.05 were considered statistically significant.

**Results**

87 patients were initially screened and followed up. Out of the baseline 87 patients, 70 were included in the interim analysis. Seventeen patients were not included due to: missing data (7pts), lost to follow up (6 pts) or discontinuation due to AE (muscle pain-1 patient) or lack of efficacy (3 pts). The flow chart of the study participants is shown in Figure E1. Demographic characteristics are provided in table E1.

The annual rate of exacerbations was even greater in patients with greater numbers of blood eosinophils. Compared to the 1 year preceding the beginning of treatment , after 1 full year of anti-IL-5 therapy the number of asthma exacerbations dropped from 3.41 ± 2.71 to 1.58 ± 1.56 (54% reduction, p < 0.0001) [150, 300) blood eosinophils cells/μl, from 4.6 ± 2.17 to 1.35 ± 1.92 (71% reduction, p < 0.0001) for ≥300 cells/μl and from 4.42 ± 2.27 to 1.24 ± 1.55 (72% reduction, p < 0.0001) for ≥400 cells/μl (Figure E2).

42 patients received maintenance dose of OCS at baseline with stable dose for at least one year before enrolment. From these patients 8 (20%) achieved total discontinuation at 4 months of follow up, 15 (30%) at 8 months of follow up and at the end of one year of therapy with mepolizumab totally 17 patients (40%) had achieved OCS discontinuation (Figure E3).

With respect to Adverse Events of treatment with mepolizumab, 19 patients (27%) were recorded to have at least one such occurrence during their 1-year treatment. Table E2 shows the kind of AE recorded, as well as their frequency. The most frequent adverse events were rhinitis-nasal congestion and upper respiratory tract infection each of which occurred in 5 (26.3%) patients. No severe adverse events were reported in our group of patients. One patient stopped the treatment due to muscle pain.

**References**

E1. Dupont WD, Plummer WD, Jr. Power and sample size calculations. A review and computer program. Control Clin Trials. 1990 Apr;11(2):116-28.

E2. Ortega HG, Liu MC, Pavord ID, Brusselle GG, FitzGerald JM, Chetta A, et al. Mepolizumab treatment in patients with severe eosinophilic asthma. N Engl J Med. 2014 Sep 25;371(13):1198-207.

**Figure legends**

**Figure E1:** Flow chart of the study participants.

**Figure E2**: Exacerbation evolution based on blood eosinophils at baseline. For data see text.

**Figure E3:** Patients who discontinued OCS during the one year of treatment with mepolizumab. For data see text.