Study report

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The ANANKE study

ChAracterisation of ItaliaN severe uncontrolled Asthmatic patieNts Key features and long-term outcomes when receiving Benralizumab in a real life setting: an observational rEtrospective study

Sponsor:	AstraZeneca
Author:	[redacted]

CONTENTS

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviation or special term	Explanation
ADR	Adverse Drug Reaction
AE	Adverse Event
AIFA	Agenzia Italiana del Farmaco [Italian Medicines Agency]
AQLQ	Asthma Quality of Life Questionnaire
ATS	American Thoracic Society
AZ	AstraZeneca
BMI	Body Mass Index
CDM	Clinical Data Manager
CI	Confidence Interval
COPD	Chronic Obstructive Pulmonary Disease
CRO	Clinical Research Organization
EC	Ethics Committee
eCRF	electronic Case Report Form
EMA	European Medicine Agency
ER	Emergency Room
ERS	European Respiratory Society
EU	European Union
FeNO	Fractional exhaled nitric oxide
FEV1	Forced Expiratory Volume in 1 second
FVC	Forced Vital Capacity
GAMP5	Compliant GxP Computerized Systems
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
GINA	Global Initiative for Asthma
GP	General Practitioner
GPP	Good Pharmacoepidemiology Practice
HR-QoL	Health-Related Quality of Life
IC	Informed Consent
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ICS	Inhaled Corticosteroids
IEC	Independent Ethics Committee
IgE	Immunoglobulin E
IRB	Institutional Review Board
IT	Information Technology
LABA	long-acting β-agonist

Abbreviation or special term	Explanation
MC	Marketing Company
MDT	Multi-disciplinary team
mOCS	maintenance OCS treatment
MCID	Minimum Clinically Important Difference
MID	Minimum Important Difference
N/A	Not assessed
OCS	Oral corticosteroids
PRO	Patient Reported Outcomes
RCT	Randomized Controlled Trial
SABA	Short-acting beta agonists
SAE	Serious Adverse Event
SANI	Severe Asthma Network in Italy
SAP	Statistical Analysis Plan
SAS	Statistical Analysis System
SD	Standard deviation
SEC	Self Evident Correction
SmPC	Summary of Product Characteristics
SOP	Standard Operating Procedure
SUA	Severe/uncontrolled asthma
WHO	World Health Organization

RESPONSIBLE PARTIES

Name	Professional title	Role in study	Affiliation	Email address
[redacted]				

STUDY REPORT SYNOPSIS

Authoring instructions

- *The synopsis should be written as a standalone document, such that it can be understood without reference to the main study report. The synopsis should be prepared, reviewed and approved in parallel with the observational study report.*
- *The body of the synopsis should preferably not exceed five pages in length. All abbreviations used in the synopsis should be spelled out on first use.*
- *If a new edition of an observational study report is created and the changes have an effect on the synopsis as well (e.g. changes in safety/efficacy results or conclusions), the synopsis should be updated with the relevant changes, including the edition number and date. The edition number of the synopsis should always correspond with the edition number of the observational study report.*

<<INSERT STUDY NAME>>

<<INSERT STUDY DESCRIPTION>>

Milestones:

Milestone	Planned date
First subject/patient in	03 December 2019
Interim analysis 1 (results on 52 patients)	04 April 2020
Last subject/patient in	15 July 2020
Last subject/patient last visit	15 July 2020
Database lock 1	28 October 2020
Clinical study report 1	27 November 2020
Protocol amendment	17 March 2021
Ethics submission of extension study	29 March 2021
Interim analysis 2 (48 weeks results)	31 October 2021
Final database lock	31 June 2022
Clinical study report 2 (96 weeks results)	25 October 2022

Phase of development:	IV
Sponsor:	AstraZeneca
Author:	[redacted]

This study was performed in compliance with Good Clinical Practice (GCP) and Good Pharmacoepidemiology Practice (GPP), including the archiving of essential documents.

Background/rationale: Severe asthma is of remarkable interest for the scientific community, as documented by the work of some research groups in the United States [Moore WC et al, 2007; Chipps BE et al, 2012], in Europe [ENFUMOSA Study Group, 2003; Heaney LG et al, 2010; Schleich F et al, 2014], and in Italy as well [Maio S et al 2018; Heffler E et al, 2018]. Severe asthma has a high social and economic burden, accounting for 50% of global costs of the disease due to high healthcare utilization, drugs used, hospital admissions and days lost from work [Accordini S et al, 2008; Heaney LG et al, 2013; Ojeda P et al, 2013; Zeiger RS et al, 2016]. Severe eosinophilic asthma is a well-recognised but still imprecisely characterised sub-classification of severe asthma, driven by a distinct pathophysiological process involving the abnormal production of type 2 cytokines from T-helper 2 and innate lymphoid cells. In order to optimize the management of patients with severe asthma, during the last years there have been included monoclonal antibodies in the therapeutic armamentarium as omalizumab (monoclonal antibody anti immunoglobulin E, which was shown to be effective in the treatment of severe allergic asthma) [Humbert M et al, 2005], as well as mepolizumab [Ortega HG et al, 2014] and reslizumab [Castro M et al, 2015] effective in patients with severe eosinophilic asthma. The latest monoclonal antibody approved was benralizumab, a humanized monoclonal antibody which binds specifically to the alpha subunit of the receptor of the human interleukin-5 (IL-5) present in eosinophils and basophils that suppresses these cells through apoptosis mediated by natural killer cells [Kolbeck R et al, 2010]. Benralizumab has been reimbursed in Italy from February 2019, however data about eosinophilic asthma in real world populations are lacking in Italy. There is a current need to gain information on the clinical profile of patients eligible for treatment with benralizumab and of its effectiveness in a real-world setting in Italy. The present observational study aims at filling in this knowledge gap: the ANANKE study allowed to describe the features of patients with severe eosinophilic asthma at start of benralizumab treatment in a real-world setting, in terms of clinical characteristics, asthma exacerbations in the previous year, and other previous asthma treatments. However, long-term data utilisation and clinical outcomes in real-world setting are also important to demonstrate the impact of benralizumab on patients asthma outcomes. This extension of the ANANKE study generated long-term insights into patients' treatment experience and associated outcomes in patients treated in a real world setting with benralizumab during the sampling program and after the reimbursement. The study also explored treatment persistence and adherence levels over the observation period, which will be increased to 96 weeks maintaining a retrospective data collection from patients's medical chart setting.

Objectives: The primary objective is to describe the clinical characteristics, asthma exacerbations in the previous year, and other previous asthma treatments of patients with severe eosinophilic asthma when starting benralizumab treatment in a real-world setting. The secondary objectives are the following: (1) To describe the observed severe, moderate/severe, moderate and any exacerbation occurred during the benralizumab treatment including 48 and 96 weeks; (2) to describe the reduction in inhaled corticosteroids (ICS) and oral corticosteroids (OCS) use and reasons for ICS reduction during benralizumab treatment including 48 and 96 weeks; (3) to describe IgE and eosinophil count during benralizumab treatment including 48 and 96 weeks and changes over time with respect to benralizumab treatment start; (4) to describe lung function parameters during benralizumab treatment including 48 and 96 weeks, and changes over time with respect to benralizumab treatment start; (5) to describe the patients' asthma control level and quality of life at benralizumab treatment start and during the observation period including 48 and 96 weeks, and changes over time with respect to benralizumab treatment start; (6) to describe the patient's adherence to benralizumab treatment, benralizumab discontinuation, subsequent biologic treatments for asthma and correlation of adherence to age and age at diagnosis, at 48 and 96 weeks; (7) to describe the healthcare resource utilization in terms of GP/specialist visits for asthma during the benralizumab treatment including 48 and 96 weeks, and ER admissions and hospitalizations for asthma during benralizumab treatment including 24, 48 and 96 weeks. [redacted] Since this is a study with a descriptive aim, analytical objectives were not defined, and no formal hypotheses were pre-specified.

Study design: This is an observational, Italian multi-center, retrospective cohort study with enrollment visit on patients suffering from severe eosinophilic asthma who started benralizumab in the Sampling Program or as per normal clinical practice in Italy. The current amendment to the previous version 1.0 of the Study Protocol enlarged the observational period and it will allow to retrospectively observe the already enrolled patients up to96 weeks after the benralizumab treatment start, from now on defined as end of observation. The retrospective data collection will be performed after the signature of an additional and amended study informed consent and privacy forms by the eligible patients already enrolled, asking them for the authorization to collect the data up to the updated end of observation as previously defined.

Data source: In agreement with the observational nature of the Study Protocol, the medical records available as per routine clinical practice at the Investigational Site for each patient were the primary data source for data collection (see further details in section 3.1.1). After patient's signature of study informed consent and privacy form, patient clinical data were collected from the hospital medical charts of participant sites according to their ordinary clinical practice.

Study population: The ANANKE study enrolled adult patients with severe eosinophilic asthma, requiring a stable treatment of high doses of inhaled corticosteroids and a long acting β2 agonist ± additional asthma controller, who started benralizumab at least 3 months prior to enrollment, either within the sampling program or as per routine clinical practice; patients who interrupted temporarily or permanently benralizumab treatment before enrollment were included. Patients received benralizumab either within the sampling program or as per routine clinical practice were enrolled as well. Participating sites enrolled patients in a consecutive manner when patients came for their regular visit, in order to minimize the risk of selection bias.

Inclusion criteria: Patients meeting all the following characteristics were included into the study:

1. Adult patients (age ≥18 years) at the start of benralizumab treatment within the sampling program or per clinical practice ("index date").

2. Patients with severe eosinophilic asthma requiring a stable treatment of high doses of inhaled corticosteroids and a long acting β2 agonist ± additional asthma controller (according to clinician's judgment).
3. Patients who started benralizumab and received at least one injection at least 3 months before enrollment, either within the sampling program or as per routine clinical practice.
4. Patients who signed the informed consent and privacy form at enrollment visit.
5. Patients with available hospital medical chart since the start of benralizumab treatment within the sampling program or per clinical practice ("index date").

Exclusion criteria: Patients meeting any of the following characteristics were not included in this study:

1. Patients who, during the observation period, received benralizumab in a clinical experimental trial.
2. Patients who, during the observation period, participated in studies imposing a specific patient's management strategy which does not correspond to the site's normal clinical practice.

Statistical methods: The statistical analyses were performed considering the set of evaluable patients, defined as eligible subjects with available information regarding at least one of the defined primary .

For patients excluded from the statistical analyses, descriptives of the reasons for non-evaluability were provided.

Evaluable patients with missing data for certain variables were not excluded from the study and their data were not replaced (unless differently specified), but were not considered for the analyses for which that variables are taken into account. Frequency of missing data was given for all analyzed variables.

Descriptive analyses were performed, since all objectives are descriptive in nature. No formal hypotheses were pre-specified (this is not an analytical study), and inferential statistical significance testing was not foreseen.

The analyses were performed using SAS software (SAS Institute, Cary, NC, USA).

Results: Among 218 patients enrolled in the ANANKE study, 167 (76.6%) patients were considered eligible for the 96-week report.

Among evaluable patients, the median (25th-75th percentile) duration of observation accounted for 22.3 (21.0 – 23.7) months; all patients had at least three months of observation, as required by Study Protocol selection criteria.

Demographics and life habits at the start of benralizumab treatment

- The mean (SD) age was 56.0 (12.7) years out of 162 evaluable patients;
- Females were 99 (61.1%);

[redacted]

- Non-smokers were 106 out of 155 patients with available information about smoking status (68.4%).

Clinical characteristics at the start of benralizumab treatment

[redacted]

- Patients positive to perennial allergens were 63 (38.9%); in particular: 45 (27.8%) to Dust mite (D. Pteronyssinus), 15 (9.3%) to Cat hair, 13 (8.0%) to Dog hair and 12 (7.4%) to Aspergillus.

Key features at Benralizumab treatment start - Primary objective

- Overall, 141 (87.0%) patients had relevant comorbidities;
- Patients with current or past nasal polyposis were 86 (53.4% out of 161 patients with available data) (see Table 10);
- Current asthma-related conditions were observed in 91 (56.2%) patients. The most frequent ones were Chronic rhinosinusitis (n=43, 26.5%), Gastroesophageal reflux (GERD; n=39, 24.1%) and Allergic rhinitis (n=38, 23.5%);
- Patients with any current OCS-related conditions were 64 (39.5%). In particular: Hypertension (n=38, 23.5%) and Osteoporosis (n=17, 10.5%).
- The blood eosinophil count showed a median (25th-75th percentile) of 600.0 (430.0–890.0) cells/mm3;
- The observed mean (SD) of lung function assessment were the following:
    - Pre-bronchodilator FEV1: 2.0 (0.8) L (n=121);
    - Pre-bronchodilator FEV1 predicted: 70.2 (21.1) % (n=125);
    - Pre-bronchodilator FVC: 3.0 (1.0) L (n=116);
    - Pre-bronchodilator FEV1/FVC ratio: 0.7 (0.2) (n=116).
- Post-bronchodilator parameters showed a similar distribution, even if parameters were available for a lower number of patients (results shown in Table 9).
- Before starting benralizumab, 144 (93.5%; 95% CI limits: [89.6% - 97.4%]) out of 154 patients experienced at least one asthma exacerbation of any kind (mild, moderate or severe). In particular (see Table 11):
    - 125 (81.2%; [75.0% - 87.3%]) had moderate/severe exacerbations, with annual exacerbation rate accounting for 2.82 cases / person-year;
    - 57 (37.0%; [29.4% - 44.6%]) had severe exacerbations, with annual exacerbation rate accounting for 0.98 cases / person-year.
- Thirty-eight (23.5%) patients were previously treated with biologics: 21 patients (13.0%) received omalizumab, 13 patients (8.0%) received Mepolizumab; 4 patients (2.5%) received both treatments (Omalizumab as first treatment, followed by Mepolizumab);
- The median (25th-75th percentile) duration of exposure to biological drugs before starting benralizumab was 21.0 (10.6 – 52.9) months, out of 29 patients with available evaluation;
- The median (25th-75th percentile) time gap between prior biologic end and benralizumab treatment start was 2.3 (1.3 – 4.9) months, out of 29 patients.
- Consistently with inclusion criteria, 160 (98.8%) patients were treated with ICS/LABA and 2 with ICS+LABA;
- Moreover, the following therapies were administered to patients in addition to ICS/LABA: 83 (51.2%) patients were treated with LAMA, 41 (25.3%) with OCS, 12 (7.4%) with ICS, 1 (0.6%) with LABA, 76 (46.9%) with OTHER asthma treatments;
- The maintenance ICS dose according to GINA 2019 Guidelines was reported to be high in 143 (88.3%) out of 162 evaluable patients;

- The median (25th-75th percentile) OCS daily (prednisone-equivalent) dose at index date was 10.0 (5.0 – 25.0) mg, considering 39 patients with available dose at the index date.

Secondary objective #1: Exacerbations during benralizumab treatment including 48 and 96 weeks

A total of 30 (20.7%) patients, out of the 145 evaluable for secondary analysis at 48 weeks, experienced exacerbations of any kind during benralizumab treatment. In particular, 18 (12.4%) patients experienced moderate exacerbations, 24 (16.6%) moderate/severe exacerbations and 9 (6.2%) patients experienced severe exacerbations. For these patients the Annualized severe exacerbation rate during benralizumab treatment at 48 weeks was 0.07 cases / person-year and the annualized moderate/severe exacerbation rate at 48 weeks was 0.25 cases / person-year;

- A total of 26 (23.0%) patients, out of the 113 evaluable for secondary analysis at 96 weeks, experienced exacerbations of any kind during benralizumab treatment. In particular, 19 (16.8%) patients experienced moderate exacerbations, 22 (19.5%) moderate/severe exacerbations and 6 (5.3%) patients experienced severe exacerbations. For these patients the Annualized severe exacerbation rate during benralizumab treatment at 96 weeks was 0.03 cases / person-year and the annualized moderate/severe exacerbation rate at 96 weeks was 0.16 cases / person-year.

Secondary objective #2: ICS and OCS use and reasons for ICS reduction during benralizumab treatment including 48 and 96 weeks

- Eight patients (5.5% of evaluable patients for secondary analyses at 48 weeks) reduced/interrupted ICS within 48 weeks after the index date, 2 of them because of Improvement of patient symptoms, 2 due to lack of clinical efficacy, 1 because of lack of compliance, 1 because of patient decision;
- Nine patients (8.0% of evaluable patients for secondary analyses at 96 weeks) reduced/interrupted ICS within 96 weeks after the index date, 3 of them due to lack of clinical efficacy, 2 because of Improvement of patient symptoms, 1 because of lack of compliance, 1 because of patient decision;
- As agreed with Sponsor, analyses within the subgroups of patients experiencing/not experiencing ICS dose reduction during benralizumab treatment were not included in this statistical report due to the low frequency of patients experiencing ICS dose reduction.
- The number of patients achieving at 48 weeks after index date a dose OCS reduction of any extent compared to the start of benralizumab was 21 (61.8%), out of the 34 patients evaluable for secondary analyses at 48 weeks (with OCS treatment ongoing at the start of benralizumab and available dose reduction). In particular, 18 of them (52.9%) interrupted OCS;
- At 96 weeks after index date 20 patients (66.7%) achieved a dose OCS reduction of any extent compared to the start of benralizumab, considering 30 evaluable patients with OCS treatment ongoing at the start of benralizumab and available dose reduction at 96 weeks. In particular, 18 patients (60.0%) interrupted OCS.

Secondary objective #3: IgE and eosinophil count during benralizumab treatment including 48 and 96 weeks and changes over time compared to benralizumab treatment start

- The available observations of IgE count (IU/mL) at the prespecified time points after the index date (16, 24, 48 weeks) were <=8. Due to the limited number of patients, it is not possible to evaluate with sufficient precision the extent of variation over time compared to baseline values.
- Considering the evaluable patients for secondary analyses at 96 weeks, blood eosinophil count was available for:

- 113 patients at the index date: at least 75% of the sample had ≤850 cells/mm3;
- 37 patients at 16 weeks: at least 75% of the sample had 0 cells/mm3;
- 31 patients at 24 weeks: at least 75% of the sample had 0 cells/mm3;
- 37 patients at 48 weeks: at least 75% of the sample had 0 cells/mm3;
- 38 patients at 96 weeks: at least 75% of the sample had 0 cell/mm3

- In all cases, the median change of blood eosinophil count indicated a numerical decrease of 500 cells/mm3 or more from baseline values.

Secondary objective #4: Lung function parameters during benralizumab treatment including 48 and 96 weeks and changes over time compared to benralizumab treatment start

Pre- and Post-bronchodilator FEV1 / FEV1 predicted considering evaluable patients for secondary analyses at 96 weeks

- The time points with the highest number of observations were recorded at 16, 48 and 96 weeks after starting benralizumab; for this reason, descriptive statistics at these time points vs index date are reported.
  - Observations of Pre-bronchodilator FEV1 after 16 weeks were made available for 43 patients, with a mean (SD) of 2.3 (0.9) L, after 48 weeks were made available for 45 patients, with a mean (SD) of 2.1 (0.8) L and after 96 weeks were made available for 47 patients, with a mean (SD) of 2.3 (0.8) L;
  - The median (25th-75th percentile) intra-patient changes at 16, 48 and 96 weeks vs index date of Pre-bronchodilator FEV1 were 0.2 (0.0; 0.4) L (n=39), 0.3 (- 0.0; 0.6) L (n=35) and 0.2 (- 0.1; 0.7) L (n=36), respectively;
  - Observations of Post-bronchodilator FEV1 after 16 weeks were made available for 28 patients, with a mean (SD) of 2.4 (0.8) L, after 48 weeks were made available for 21 patients, with a mean (SD) of 2.5 (0.8) L and after 96 weeks were made available for 29 patients, with a mean (SD) of 2.6 (1.0) L;
  - The median (25th-75th percentile) intra-patient changes at 16, 48 and 96 weeks vs index date of Post-bronchodilator FEV1 were 0.1 (- 0.2; 0.5) L (n=18), 0.2 (- 0.1; 0.7) L (n=16) and 0.3 (0.1; 0.7) L (n=22), respectively;
  - Observations of Pre-bronchodilator FEV1 predicted after 16 weeks were made available for 44 patients, with a mean (SD) of 83.3 (32.8) %, after 48 weeks were made available for 47 patients, with a mean (SD) of 84.0 (28.5) % and after 96 weeks were made available for 51 patients, with a mean (SD) of 83.4 (23.0) L;
  - The median (25th-75th percentile) intra-patient changes at 16, 48 and 96 weeks vs index date of Pre-bronchodilator FEV1 predicted were 6.5 (-2.5; 15.0) (n=40) %, 9.0 (3.0; 29.0) (n=37) % and 11.0 (-3.0; 27.0) % (n=37), respectively;
  - Observations of Post-bronchodilator FEV1 predicted after 16 weeks were made available for 28 patients, with a mean (SD) of 83.2 (21.0) %, after 48 weeks were made available for 20 patients, with a mean (SD) of 86.8 (27.3) % and after 96 weeks were made available for 29 patients, with a mean (SD) of 89.0 (23.1) %;
  - The median (25th-75th percentile) intra-patient changes at 16, 48 and 96 weeks vs index date of Post-bronchodilator FEV1 predicted were: 0.0 (- 9.0; 18.0) % (n=18), 8.0 (- 9.0; 21.0) % (n=15) and 16.0 (0.0; 29.0) % (n=22), respectively.

Pre- and Post-bronchodilator FVC considering evaluable patients for secondary analyses at 96 weeks

- The time points with the highest number of observations were recorded at 16, 48 and 96 weeks after starting benralizumab; for this reason, descriptive statistics at these time points vs index date are reported.
    - Observations of Pre-bronchodilator FVC (L) after 16 weeks were made available for 43 patients, with a mean (SD) of 3.1 (1.1) L, after 48 weeks were made available for 45 patients, with a mean (SD) of 2.9 (1.0) L and after 96 weeks were made available for 47 patients, with a mean (SD) of 3.2 (1.1) L;
    - The median (25th-75th percentile) intra-patient changes at 16, 48 and at 96 weeks vs index date of Pre-bronchodilator FVC (L) were 0.2 (- 0.1; 0.4) L (n=39), 0.2 (0.0; 0.7) L (n=34) and 0.1 (- 0.1; 0.7) L (n=35), respectively;
    - Observations of Post-bronchodilator FVC (L) after 16 weeks were made available for 26 patients, with a mean (SD) of 3.3 (1.0) L, after 48 weeks were made available for 22 patients, with a mean (SD) of 3.6 (1.1) L, and after 96 weeks were made available for 28 patients, with a mean (SD) of 3.5 (1.3) L;
    - The median (25th-75th percentile) intra-patient changes at 16, 48and 96 weeks vs index date of Post-bronchodilator FVC (L) were 0.0 (- 0.5; 0.5) L (n=16), 0.1 (- 0.3; 0.5) L (n=16) and 0.1 (- 0.2; 0.5) L (n=19), respectively.

<u>Secondary objective #5: Patients' asthma control level and quality of life at benralizumab treatment start, during the observation period including 48 and 96 weeks and changes over time compared to benralizumab treatment start</u>

Considering evaluable patients for secondary analyses at 96 weeks after index date

- Asthma Control Test (ACT) total score was available (see Table 38):
    - at the start of benralizumab for 96 patients, with a mean (SD) of 14.9 (4.6);
    - after 4 weeks for 26 patients, with a mean (SD) of 20.2 (4.2);
    - after 16 weeks for 77 patients, with a mean (SD) of 20.0 (4.2);
    - after 24 weeks for 70 patients, with a mean (SD) of 20.2 (3.8);
    - after 48 weeks for 75 patients, with a mean (SD) of 20.7 (4.7);
    - after 96 weeks for 75 patients, with a mean (SD) of 21.5 (3.8);
- Well-controlled asthma (i.e. ACT score >=20) was reported in 16 (16.7%), 16 (61.5%), 48 (62.3%), 47 (67.1%), 58 (77.3%) and 59 (78.7%) patients at benralizumab start, after 4, 16, 24, 48 and 96 weeks after the index date, respectively;
- At every evaluation during benralizumab treatment (at 16, 24, 48, 96 weeks after index date), at least 72% of the patients with filled questionnaires achieved the minimum importance difference.

Considering evaluable patients for secondary analyses at 48 weeks after index date

- The available observations of Asthma Quality of Life Questionnaire (AQLQ) total score during benralizumab treatment for patients were 28 at the start of benralizumab and less than 25 at the other

time points. Due to the limited number of patients with an available questionnaire, it is not possible to provide precise estimates of the distribution of AQLQ scores in the sample (results are available in Table 40) and of patients achieving MID in the AQLQ total score.

Secondary objective #6: Patient's adherence, benralizumab discontinuation and subsequent biologic treatment and correlation of adherence at 48 and 96 weeks to age and age at diagnosis

Considering evaluable patients for secondary analyses at 48 weeks after index date

- Out of the 150 eligible patients with consistent data at 48 weeks, the level of patient's adherence to benralizumab treatment at 48 weeks, measured as the ratio between the number of actual injections and the number of expected injections at 48 weeks (in percentage), showed a mean (SD) of 98.9 (6.8) %;
- Between index date and week 48 the mean (SD) number of benralizumab injections was 7.7 (0.6) and 122 (81.3%) patients performed >90% of the expected injections;
- The permanent discontinuation of benralizumab was observed in six patients (4.0% out of 150) for lack of clinical efficacy (N=3, 50.0% of patients who discontinued), patient decision (N=2, 33.3%), and presence of allergic rhinitis (N=1, 16.7%);
- Three patients, out of six eligible patients with consistent data who discontinued benralizumab within 48 weeks, had subsequent biologic treatments for asthma (Omalizumab) due to exacerbations, recurrence of symptoms and other reasons;
- No statistically significant correlation was observed between the level of patient's adherence to benralizumab treatment at 48 weeks and age at start of benralizumab treatment or age at diagnosis of asthma.

Considering evaluable patients for secondary analyses at 96 weeks after index date

- Out of the 115 eligible patients with consistent data at 96 weeks, the level of patient's adherence to benralizumab treatment at 96 weeks, measured as the ratio between the number of actual injections and the number of expected injections at 96 weeks (in percentage), showed a mean (SD) of 95.1 (10.4) %;
- Between index date and week 96 the mean (SD) number of benralizumab injections was 13.0 (1.6) and 96 (83.5%) patients performed >90% of the expected injections;
- The permanent discontinuation of benralizumab was observed in twelve patients (10.4% out of 115) for lack of clinical efficacy (N=6, 50.0% of patients who discontinued with consistent data), lack of efficacy-adverse events (N=2, 16.7%), patient decision (N=2, 16.7%), and presence of allergic rhinitis (N=1, 8.3%);
- Seven patients, out of twelve eligible patients with consistent data who discontinued benralizumab, had subsequent biologic treatments for asthma (Omalizumab or Dupilumab) due to inadequate clinical response, exacerbations, recurrence of symptoms and other reasons;
- No statistically significant correlation was observed between the level of patient's adherence to benralizumab treatment at 96 weeks and age at start of benralizumab treatment or age at diagnosis of asthma.

Secondary objective #7: Healthcare resource utilization during benralizumab treatment up to 48 and 96 weeks

- The number of primary care physician/GP office or specialist visits, for asthma, per patient up to 48 or 96 weeks after the index date was neglectable. The same goes for the number of ER admissions and hospitalizations up to 48 and 96 weeks after the index date.

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

Conclusion: The population of severe eosinophilic asthmatic patients evaluated in the ANANKE study had a remarkable and durable response to benralizumab. The long-term improvements in all clinical outcomes, in combination with patients' clinical characteristics at index date, underline a specific "eosinophilic-driven" asthma profile (as recently defined by Couillard et al.) [48] that features the ANANKE severe eosinophilic asthmatic population. Of note, the specific design of the ANANKE study, its retrospective nature (enrolment at least 3 months after benralizumab initiation) and the possibility to follow up patients until 96 weeks of treatment were pivotal to allow a thorough investigation of the patients'key features as well as the achievement of such profound results. Once again, these data highlight that when benralizumab is administered to the right patient, with an authentic "eosinophilic-driven" asthma phenotype, it ensures a great, long-term efficacy thanks to the continuous and nearly complete depletion of eosinophils.

Publications:

- Menzella F, Bargagli E, Aliani M, Bracciale P, Brussino L, Caiaffa MF, et al. ChAracterization of ItaliaN severe uncontrolled Asthmatic patieNts Key features when receiving Benralizumab in a real-life setting: the observational rEtrospective ANANKE study. Respir Res. England; 2022;23:36.
- D'Amato M, Menzella F, Altieri E, Bargagli E, Bracciale P, Brussino L, et al. Benralizumab in Patients With Severe Eosinophilic Asthma With and Without Chronic Rhinosinusitis With Nasal Polyps: An ANANKE Study post-hoc Analysis. Front Allergy. Switzerland; 2022;3:881218.
- Caruso C, Cameli P, Altieri E, Aliani M, Bracciale P, Brussino L, et al. Switching from one biologic to benralizumab in patients with severe eosinophilic asthma: An ANANKE study post hoc analysis. Front Med (Lausanne). Switzerland; 2022;9:950883.
- Senna G, Aliani M, Altieri E, Bracciale P, Brussino L, Caiaffa MF, et al. Clinical Features and Efficacy of Benralizumab in Patients with Blood Eosinophil Count Between 300 and 450 Cells/mm(3): A Post Hoc Analysis from the ANANKE Study. J Asthma Allergy. New Zealand; 2022;15:1593–604.

AMENDMENT HISTORY

Not Applicable.

MILESTONES

Milestone	Planned date
First subject/patient in	03 December 2019
Interim analysis 1 (results on 52 patients)	04 April 2020
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Ethics submission of extension study	29 March 2021
Interim analysis 2 (48 weeks results)	31 October 2021
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Clinical study report 2 (96 weeks results)	25 October 2022

1. BACKGROUND AND RATIONALE

1.1 Background

Asthma is a chronic disease with significant impact at the personal, social and economic level [Martinez-Moragon E et al, 2009] and the increasing prevalence of asthma is associated with important implications at the level of health care resources use.

In the whole of Europe, about 30 million children and adults under 45 years of age have asthma. In most European countries, the prevalence and incidence of asthma increased substantially at some time between 1950 and 2000 but, at least in Western Europe, the increase has levelled off in the past decade. The prevalence of asthma in Italy among adults aged between 18 and 44 years is currently estimated to be <3% [European Respiratory Society. European Lung White Book 2019]; the incidence of disease tends to be higher in northern and western countries where the prevalence may be higher than 10%. Adult asthma tends to be more common in females [European Respiratory Society. European Lung White Book 2019].

The majority of patients has mild to moderate disease and can be controlled with relative ease. However, over an estimated 340 million people worldwide with asthma, between 5 to 10% of patients develop severe asthma and require long-term treatment with high doses of inhaled corticosteroids (ICS) combined with β2-adrenergic agonists with or without oral treatment to reach the target of asthma control [Olaguibel JM et al, 2012; Chung KF et al, 2014; Global Burden of Disease Study Collaborators, 2015].

Severe asthma is of remarkable interest for the scientific community, as documented by the work of some research groups in the United States [Moore WC et al, 2007; Chipps BE et al, 2012] and in Europe [ENFUMOSA Study Group, 2003; Heaney LG et al, 2010; Schleich F et al, 2014]. In Italy two registries exist specifically focused on severe asthma, namely RItA, the Italian severe/uncontrolled asthma (SUA) web-based registry encompassing demographic, clinical, functional, and inflammatory data [Maio S et al 2018] and the Severe Asthma Network in Italy [SANI] [Heffler E et al, 2018] aimed to analyze epidemiological, clinical, inflammatory, functional and treatment characteristics of patients affected by severe asthma.

The American Thoracic Society (ATS) and European Respiratory Society (ERS) define severe asthma as asthma which requires treatment with guidelines suggested medications for Global Initiative for Asthma (GINA) stages 4-5 asthma (i.e. ICS and and a second controller) for the previous year or systemic corticosteroids for ≥50% of the previous year to prevent it from becoming “uncontrolled” or which remains “uncontrolled” despite this therapy [Chung KF et al, 2014].

Severe asthma is a currently attracting a lot of interest since quite a few unmet needs are still to be answered. In fact, patients affected by severe eosinophilic asthma suffer from frequent

exacerbations, require Emergency Room visits and hospitalizations, and receive oral steroids on a regular basis [Chung KF et al, 2014, Vennera Mdel C, 2014]. Moreover, patients with SUA are commonly affected by comorbidities such as allergic rhinitis, gastroesophageal reflux, sinusitis, nasal polyposis, allergic conjunctivitis and bronchiectasis [Maio S et al, 2018; Heffler E et al, 2018]. For all these reasons, severe asthma has a high social and economic burden, accounting for 50% of global costs of the disease due to high healthcare utilization, drugs used, hospital admissions and days lost from work [Accordini S et al, 2008; Heaney LG et al, 2013; Ojeda P et al, 2013; Zeiger RS et al, 2016]. Severe eosinophilic asthma also determines much greater deterioration in health-related quality of life (HR-QoL) as compared to stable asthma [Papi A et al 2018].

As a consequence of advances in the development of precision medicines for patients with severe asthma, the need to identify asthma subtypes by phenotype based on clinical, functional or inflammatory parameters is becoming a mandatory part of patient's management [Chung KF et al 2014; de Groot JC et al 2015; Castro M et al 2016; Fajt ML et al, 2015]. Different phenotypes have been described basing on the clinical features, pulmonary function and histological parameters [Haldar P et al, 2008]. According to the inflammatory cells present in sputum or bronchial biopsy, there are different types of them regarding the predominance of eosinophils, neutrophils, both cell types or none of them (the so-called "paucicellular") [Ray A et al, 2016].

Severe eosinophilic asthma is a well-recognised but still imprecisely characterised sub-classification of severe asthma, driven by a distinct pathophysiological process involving the abnormal production of type 2 cytokines from T-helper 2 and innate lymphoid cells. Sputum eosinophilia is found in slightly under half of all patients with asthma and both blood and sputum eosinophilia are associated with more severe disease, worse control, and worse prognosis [Schleich FN et al, 2014].

Introduction of new biological drugs is a valid support in controlling severe asthma. In order to optimize the management of patients with severe asthma, during the last years there have been included monoclonal antibodies in the therapeutic armamentarium as omalizumab (monoclonal antibody anti immunoglobulin E, which was shown to be effective in the treatment of severe allergic asthma) [Humbert M et al, 2005], as well as mepolizumab [Ortega HG et al, 2014] and reslizumab [Castro M et al, 2015] effective in patients with severe eosinophilic asthma.

The latest monoclonal antibody approved was benralizumab, a humanized monoclonal antibody which binds specifically to the alpha subunit of the receptor of the human interleukin-5 (IL-5) present in eosinophils and basophils that suppresses these cells through apoptosis mediated by natural killer cells [Kolbeck R et al, 2010]. Benralizumab is indicated as an add-on maintenance treatment in adult patients with severe eosinophilic asthma inadequately controlled despite high-dose inhaled corticosteroids plus long-acting β-agonists. Its efficacy and safety in large populations of severe asthmatics was evaluated in particular through the

WINDWARD program consisting of 6 Phase III studies, including SIROCCO, CALIMA, ZONDA, BORA, BISE, and GREGALE [Pelaia C et al 2018]. Benralizumab has already demonstrated efficacy [Bleecker ER et al, 2016; FitzGerald JM et al, 2016] in reducing severe exacerbations by up to 51%, increasing FEV1 vs placebo, improving control, regardless of the atopic profile [Chipps BE et al, 2018] and reducing or even eliminating oral corticosteroids [Nair P et al, 2017]. Compared with placebo, benralizumab treatment demonstrated also significant improvements in HR-QoL [Liu T et al 2018].

Benralizumab is intended for long-term asthma treatment. Patients enrolled in Phase III studies were followed up to 24 months in the BORA study [Busse et al 2019] and up to 60 months in the MELTEMI study [NCT02808819]. On January 18, benralizumab was approved for use in Italy. Before reimbursement, in response to requests from healthcare professionals, AstraZeneca implemented an Italian Sampling Program in order to make benralizumab available to patients according to the approved indication. Currently, in some Italian regions, benralizumab is already available for use in clinical practice also outside the Sampling Program.

1.2 Rationale

Benralizumab has been reimbursed in Italy from February 2019. As of December 2020, over 2780 patients were receiving benralizumab for treatment of their severe eosinophilic asthma.

Its efficacy and safety in large populations of severe asthmatics was evaluated in the WINDWARD program [Pelaia C et al 2018]. Recent national registries (the RitA and the SANI) have been prompted and set up in Italy in order to study severe asthmatic patients in a real-world setting, to raise knowledge and awareness of this special population [Maio S et al, 2018; Heffler E et al, 2018].

However data about eosinophilic asthma in real world populations are lacking in Italy. There is still a current need to gain information on the clinical profile of patients eligible for treatment with benralizumab and of its effectiveness in a real-world setting in Italy. The present observational study aims at filling in this knowledge gap.

The ANANKE study enrolled 218 Italian patients affected by severe eosinophilic asthma and described their features at start of benralizumab treatment in a real-world setting, in terms of clinical characteristics, asthma exacerbations in the previous year, and other previous asthma treatments.
The ANANKE study provides valuable data for early benralizumab utilisation and associated clinical outcomes. However, long-term data utilisation and clinical outcomes in real-world setting are also important to demonstrate the impact of benralizumab on patients asthma outcomes.

This extension of the ANANKE study generated long-term insights into patients'treatment experience and associated outcomes in patients treated in a real world setting with benralizumab during the sampling program and after the reimbursement. The study explored treatment persistence and adherence levels over the observation period, which was increased to 96 weeks maintaining a retrospective data collection from patients' medical chart setting.

2. OBJECTIVES AND HYPOTHESES

The primary, secondary, and exploratory objectives of this observational study are listed in the following paragraphs. Since this is a study with a descriptive aim, analytical objectives were not defined, and no formal hypotheses were pre-specified.

2.1 Primary objectives and hypotheses

To describe the clinical characteristics, asthma exacerbations in the previous year, and other previous asthma treatments of patients with severe eosinophilic asthma when starting benralizumab treatment in a real-world setting.

2.2 Secondary objectives and hypotheses

(1) To describe the observed severe, moderate/severe, moderate and any exacerbation occurred during benralizumab treatment including 48 and 96 weeks.
(2) To describe the reduction in inhaled corticosteroids (ICS) and oral corticosteroids (OCS) use and reasons for ICS reduction, during benralizumab treatment including 48 and 96 weeks.
(3) To describe IgE and eosinophil count during benralizumab treatment including 48 and 96 weeks and changes over time with respect to benralizumab treatment start..
(4) To describe lung function parameters during benralizumab treatment including 48 and 96 weeks, and changes over time with respect to benralizumab treatment start.
(5) To describe the patients' asthma control level and quality of life at benralizumab treatment start and during the observation period including 48 and 96 weeks and changes over time with respect to benralizumab treatment start.
(6) To describe the patient's adherence to benralizumab treatment, benralizumab discontinuation, subsequent biologic treatments for asthma and correlation of adherence to age and age at diagnosis, at 48 and 96 weeks.
(7) To describe the healthcare resource utilization in terms of GP/specialist visits for asthma during benralizumab treatment including 48 and 96 weeks, and ER admissions and hospitalizations for asthma during benralizumab treatment including 24, 48 and 96 weeks .

2.3 Exploratory objectives and hypotheses

[redacted]

3. METHODOLOGY

3.1 Study design – general aspects

This is an observational, Italian multi-center, retrospective cohort study with enrollment visit on patients suffering from severe eosinophilic asthma who started benralizumab in the Sampling Program or as per normal clinical practice in Italy.

The study is classified as 'non-interventional' per Directive 2001/20/EC with secondary data collection with an enrollment visit, and will follow the guidelines for Good Pharmacoepidemiology Practice (GPP) (Epstein, 2016).

The current amendment to the previous version 1.0 of the Study Protocol enlarged the observational period and it allowed to retrospectively observe the already enrolled patients up to 96 weeks after the benralizumab treatment start, from now on defined as end of observation. The retrospective data collection was performed after the signature of an additional and amended study informed consent and privacy forms by the eligible patients already enrolled, asking them for the authorization to collect the data up to the updated end of observation as previously defined. The data collection kept its retrospective design as it took place once the patients will have reached the previously defined timepoints, defining the end of observation.

Among patients selected using the outlined inclusion and exclusion criteria (see paragraphs 3.3 and 3.4), the date of benralizumab treatment initiation (started within the Italian Sampling Program or per clinical practice) defined the study "index date". The index date corresponded to the start of exposure period for each patient, while the end of observation window enlarged up to 96 weeks, as authorized by additional and amended study informed consent and privacy forms. Patients were not required to be still on treatment with benralizumab at enrollment visit in the study in order to be considered for inclusion in the study.
Retrospective data were mostly referred to the period comprised between index date and the updated end of observation, but also selected information such as details about asthma diagnosis, relevant medical history, and any prior biologic treatments for asthma (which may be even prior to the 12 months before index date) were collected as well to better characterize the enrolled patient sample (see **Table 1** in Chapter 4). A retrospective cohort study design

involving secondary data collection (during the foreseen retrospective observation period) was chosen to appropriately address the primary objective in a relatively short period of time after enrollment phase initiation.
Moreover, besides retrospective data collection, in order to reach the secondary objectives, clinical variables and Patient-Reported Outcomes (PROs), i.e. ACT and AQLQ scores, were collected also at the predefined timepoints (as described in **Table 1**; see Chapter 4).

This is an observational study and for this reason no treatments will be administered per protocol requirement, but instead according to normal clinical practice. Since benralizumab first administration occurred before inclusion in the study (as per chosen study design), the decision to include the patient in the study was clearly separated from the prescription of benralizumab, in accordance with the observational nature of the study.

In order to be eligible for the study, for each patient, the index date had to be at least 3 months prior to enrollment. As a result, the data collected from the patients' medical charts covered a total period accounting for a minimum of 15months (i.e. minimum 3 months between index date and enrollment, plus the 12 months prior to index date to collect a restricted set of clinical details.

3.1.1 Data source

Data recorded in the electronic Case Report Form (eCRF) derived from source documents consistent with the data recorded on the source documents. After patient's signature of study informed consent and privacy form, patient clinical data were collected from the hospital medical charts of participant sites according to their ordinary clinical practice.

In agreement with the observational nature of the Study Protocol, the medical records available as per routine clinical practice at the Investigational Site for each patient were the primary data source for data collection. The eligibility of the patient and the majority of data required for the analysis of the primary objective were documented in patients' medical records in order to allow the source data verification of the data entered into the eCRF.

Retrospective clinical data were retrieved from hospital medical charts or from other documents. Data and information on the diagnostic process might be not always reported in medical chart, because they are part of ordinary clinical practice and set-up at the site.

Data sources that were used were deemed to be appropriate for the purposes of the study. It was foreseen that, in some cases, retrospective information may not be complete for all enrolled patient. However, most of the primary variables assessed at benralizumab treatment start were expected to be available in the patient's medical chart; in fact, such information should be taken into account when prescribing benralizumab.

All data required for the study were collected and entered into the eCRF at the Investigator's site; the eCRF were filled in by the investigator and/or his/her representative designee. The principal investigator at each site was responsible for ensuring that the required retrospective data from the charts are correctly extracted, collected and entered into the eCRF.

Furthermore, the investigators and/or delegated members retained full responsibility for the accuracy and authenticity of all data that they entered into the eCRF. The site was also provided with data entry instructions.

3.2 Study population

The ANANKE study enrolled 218 patients with severe eosinophilic asthma treated with benralizumab by 21 sites, according to Study Protocol version 1.0 of 10 July 2019. Patients received benralizumab either within the sampling program or as per routine clinical practice were enrolled as well.

Participating sites enrolled patients in a consecutive manner when patients came for their regular visit, in order to minimize the risk of selection bias.

3.3 Inclusion criteria

Patients meeting all the following characteristics were included into the study:

(1) Adult patients (age ≥18 years) at the start of benralizumab treatment within the sampling program or per clinical practice ("index date").
(2) Patients with severe eosinophilic asthma requiring a stable treatment of high doses of inhaled corticosteroids and a long acting β2 agonist ± additional asthma controller (according to clinician's judgment).
(3) Patients who started benralizumab and received at least one injection at least 3 months before enrollment, either within the sampling program or as per routine clinical practice.
(4) Patients who signed the informed consent and privacy form at enrollment visit.
(5) Patients with available hospital medical chart since the start of benralizumab treatment within the sampling program or per clinical practice ("index date").

3.4 Exclusion criteria

Patients meeting any of the following characteristics were not included in this study:

(1) Patients who, during the observation period, received benralizumab in a clinical experimental trial.
(2) Patients who, during the observation period, participated in studies imposing a specific patient's management strategy which does not correspond to the site's normal clinical practice.

Eligible patients enrolled in Study protocol version of 10 July 2019 were included in amended protocol version 1.1 of 17 March 2021 after the signature of the study informed consent and privacy forms for the updated end of observation, which will be up to 96 weeks from their own index date.

4. VARIABLES AND EPIDEMIOLOGICAL MEASUREMENTS

Table 1 *Variables collected during the ANANKE study.*

VARIABLES	**Index date** (benralizumab start)	**16 weeks (+/-4 weeks)** after benralizumab start	**24 weeks (+/-6 weeks)** after benralizumab start	**48 weeks (+/-8 weeks)** after benralizumab start	**96 weeks (+/-8 weeks)** after benralizumab start (**end of observation**)	**Concluded enrollment**
Eligibility criteria Inclusion/exclusion criteria,						X
Informed consent and privacy form signature date.				X***	X***	X
Demographics and life habits Age, gender, smoking habits	X*					
Physical examination Height, weight, Body Mass Index (BMI)	X*					
Comorbidities and relevant medical history Details on allergies (skin prick test results, if available). Rhinitis, nasal polyposis, gastroesophageal reflux, COPD, atopic dermatitis and other eosinophilic conditions, conditions related to OCS chronic use (osteoporosis, cataract, etc), other relevant conditions.	X*					
Asthma history Time from diagnosis of asthma, of severe eosinophilic asthma. Number and severity of exacerbations in the prior 12 months, and details on exacerbations that required only OCS treatment, that required ER access, and that required hospitalization. Number of GP/specialist visits, hospitalization, ER admissions for asthma in the prior 12 months.	X					
Prior asthma medications and maintenance treatments Inhaled treatments, systemic corticosteroids, other respiratory treatments received by the patient in the 12 months prior to benralizumab, and ongoing at benralizumab start. Any prior biologic treatments for asthma (if applicable) since diagnosis, time on treatment,	X					

VARIABLES	Index date (benralizumab start)	16 weeks (+/-4 weeks) after benralizumab start	24 weeks (+/-6 weeks) after benralizumab start	48 weeks (+/-8 weeks) after benralizumab start	96 weeks (+/-8 weeks) after benralizumab start (end of observation)	Concluded enrollment
and reason for switching to benralizumab. Maintenance treatments (inhaled, OCS, others) and rescue medications (SABA, ICS/formoterol) in the 12 months prior to benralizumab, and ongoing at benralizumab start.						
Lung function assessments FEV1, FEV1% predicted, FVC, FEV1/FVC, FeNO (both pre-bronchodilator and post-bronchodilator, if available)	X*	X §, †	X **, †	X **, †	X **, †, &	
Clinical assessments Eosinophil count in peripheral blood, total IgE	X*	X †	X **, †	X **, †	X **, †, &	
Patient-Reported Outcomes as per ordinary clinical practice followed by each site ACT questionnaire score, AQLQ questionnaire score	X *, **	X§, ^	X **, ^	X **, ^	X **, ^	
GP and specialist visits for asthma Total number of visits per patient performed during the observational period					X (up to benralizumab permanent discontinuation)	
Adherence to benralizumab Actual injections of benralizumab received by the patient					X (up to benralizumab permanent discontinuation)	
Benralizumab treatment Start date, dose, frequency of administration and changes. Temporary and permanent discontinuation dates and reasons	Data collected in continuum					
Asthma treatments received since index date (including post-benralizumab treatments) Drug name and type (i.e. inhaled treatments, systemic corticoids, other respiratory treatments, biologics; including maintenance treatments), dose, frequency and route of administration, start and end dates and changes.	Data collected in continuum					
Other relevant concomitant medications Drug, start and end dates, indication (e.g. to treat comorbidities, to treat adverse events, etc.).	Data collected in continuum					
Exacerbations	Data collected in continuum (up to benralizumab permanent discontinuation)					

VARIABLES	**Index date** (benralizumab start)	**16 weeks (+/-4 weeks)** after benralizumab start	**24 weeks (+/-6 weeks)** after benralizumab start	**48 weeks (+/-8 weeks)** after benralizumab start	**96 weeks (+/-8 weeks)** after benralizumab start (**end of observation**)	**Concluded enrollment**
Date of onset, severity, action taken, outcome, resolution date; details on exacerbations that required only OCS treatment, that required ER access, and that required hospitalization.						
Hospitalizations and ER admissions Dates of inpatient hospitalization and ER admissions, reasons (adverse events, exacerbations).	Data collected in continuum (up to benralizumab permanent discontinuation)					

* if no information referred to index date is available, latest available data before the first injection of benralizumab.
** it is expected that such data might not be available for all patients: in fact, patients must have started benralizumab at least 3 months before enrollment, therefore it is expected that not all patients perform a visit at 24, 48 and 96 weeks after index date.
*** Informed consent and privacy form for the data collection at 48 and 96 weeks will be collected at the first convenient visit.
§ the results of the lung function assessments and ACT scores performed at 4 weeks after first benralizumab injection will also be collected, if available.
^ scores will also be collected also for patients not anymore in treatment with benralizumab. Moreover, if also the ACT/AQLQ score referred to the time of benralizumab discontinuation (if occurred) are available, they will be collected too. AQLQ wil not be collected at 96 weeks.
† in case of benralizumab permanent discontinuation, data should be referred to the time of benralizumab discontinuation; whereas if the patient has not discontinued benralizumab, data will be collected at the specified time point, as per routine practice. & Total Ige and FeNO will not be collected at 96 weeks.

4.1 Exposure

The exposure in common to all enrolled patients suffering from severe eosinophilic asthma was benralizumab treatment started at least 3 months before enrollment (either within the sampling program or as per routine clinical practice), requiring also a stable treatment of high doses of inhaled corticosteroids and a long acting β2 agonist ± additional asthma controller. Patients who interrupted temporarily or permanently benralizumab treatment before enrollment were included, if they met the above-mentioned inclusion criteria and did not meet any exclusion criteria.

4.2 Outcomes

The study outcomes are listed below.

- *Primary objective (key features at benralizumab treatment start)*
  - **Total IgE** and **eosinophil count** in peripheral blood as measured at index date (*).
  - **Lung function assessments** (FEV1, FEV1% predicted, FVC, FEV1/FVC, FeNO, both pre-bronchodilator and post-bronchodilator; if available) as measured at index date (*).

- Presence of **comorbidities** such as rhinitis (allergic and not), gastroesophageal reflux, COPD, sinusitis, nasal polyposis, atopic dermatitis, chronic rhinosinusitis, other eosinophilic conditions, conditions related to OCS chronic use (osteoporosis, cataract, etc) and other relevant conditions at index date (*).
- **Previous any exacerbations** in the 12 months before index date.
- **Previous mild exacerbations** in the 12 months before index date
- **Previous moderate exacerbations** in the 12 months before index date
- **Previous moderate/severe exacerbations** in the 12 months before index date
- **Previous severe exacerbations** in the 12 months before index date.
- **Previous treatments with biologics** for asthma before index date.
- **Maintenance asthma treatment(s) ongoing at index date** (inhaled treatments, systemic corticoids, biologics, other respiratory treatments).
- **GP/specialist visits**, **ER admissions**, and **hospitalizations** for asthma in the 12 months prior to index date.

(*) or latest available data before index date.

- *Secondary objective #1 (the observed severe, moderate/severe, moderate and any exacerbation occurred during benralizumab treatment including 48 and 96 weeks.)*

  The following exacerbations will be observed during benralizumab treatment including 48 and 96 weeks:
  - Any **severe** exacerbation.
  - Any **moderate/severe** exacerbation.
  - Any **moderate** exacerbation.
  - **Any** exacerbation.

  In case of benralizumab permanent discontinuation prior to 96 weeks, this outcome will be evaluated as long as patients are under benralizumab treatment.

- *Secondary objective #2 (the reduction in inhaled corticosteroids (ICS) and oral corticosteroids (OCS) use and reasons for ICS reduction during benralizumab treatment including 48 and 96 weeks)*

  - **ICS dose reduction** of any extent during benralizumab treatment including 48 and 96 weeks (final dose* with respect to index date).
  - **Reasons** for ICS reduction.

  - Within patients experiencing / not experiencing ICS dose reduction during benralizumab treatment including 48 and 96 weeks (final dose* with respect to index date):
    variation of exacerbations, **lung function variation** and **asthma control variation**

- **OCS dose reduction** during benralizumab treatment including 48 and 96 weeks (final dose*with respect to index date; if applicable) of the following extents:
  - any extent;
  - a 100% reduction
  - a ≥90% reduction;
  - a ≥75% reduction;
  - a ≥50% reduction;
  - a ≥25% reduction;
  - a reduction to ≤5 mg/day (if OCS dose at index date >5 mg/day);
  - no reduction.

*as evaluated at end of observation or at benralizumab permanent discontinuation (whichever occurs earlier).
In case of benralizumab permanent discontinuation prior to enrollment visit, these outcomes will be evaluated as long as patients are under benralizumab treatment.

- *Secondary objective #3 (IgE and eosinophil count during benralizumab treatment including 48 and 96 weeks, and changes over time with respect to benralizumab treatment start.)*

  - **Total IgE** in peripheral blood as measured at the following time points:
    - at 16 weeks (±4 weeks) after index date;
    - at 24 weeks (±6 weeks) after index date, if available;
    - at 48 weeks (±8 weeks) after index date, if available;
    - at enrollment

  - **Eosinophil count** in peripheral blood as measured at the following time points:
    - at 16 weeks (±4 weeks) after index date;
    - at 24 weeks (±6 weeks) after index date, if available;
    - at 48 weeks (±8 weeks) after index date, if available;
    - at 96 weeks (±8 weeks) after index date, if available;
    - at enrollment

  - **Total IgE and eosinophil count changes over time** with respect to index date (according to data availability). Total IgE count will not be collected at 96 weeks.

  These outcomes will be evaluated as long as patients are under benralizumab treatment. In case of benralizumab permanent discontinuation, it will be evaluated the opportunity to consider also the assessment at end of benralizumab treatment, according to data availability.

- *Secondary objective #4 (lung function parameters during benralizumab treatment including 48 and 96 weeks, and changes over time with respect to benralizumab treatment start)*

- **Lung function parameters** (FEV1, FEV1 % predicted, FVC, FEV1/FVC, FeNO*) as measured at the following time points:
  - at 4 weeks after index date, if available;
  - at 16 weeks (±4 weeks) after index date;
  - at 24 weeks (±6 weeks) after index date, if available;
  - at 48 weeks (±8 weeks) after index date, if available;
  - at 96 weeks (±8 weeks) after index date, if available;
  - at enrollment.

- **Lung function parameters changes over time** with respect to index date (according to data availability).

*FeNO will not be collected at 96 weeks.

These outcomes will be evaluated as long as patients are under benralizumab treatment. In case of benralizumab permanent discontinuation, it will be evaluated the opportunity to consider also the assessment at end of benralizumab treatment, according to data availability.

- *Secondary objective #5 (patients' asthma control level and quality of life at benralizumab treatment start and during the observation period including 48 and 96 weeks, and changes over time with respect to benralizumab treatment start.)*

  - **Asthma Control Test (ACT) total score** as measured at the following time points:
    - at index date (if available*);
    - at 16 weeks (±4 weeks) after index date;
    - at 24 weeks (±6 weeks) after index date, if available;
    - at 48 weeks (±8 weeks) after index date, if available;
    - at 96 weeks (±8 weeks) after index date, if available;
    - at enrollment.

  - **ACT total score changes over time** with respect to index date (according to data availability).

  - **Asthma Quality of Life Questionnaire (AQLQ) total score** as measured at the following time points:
    - at index date (if available*);
    - at 16 weeks (±4 weeks) after index date;
    - at 24 weeks (±6 weeks) after index date, if available;
    - at 48 weeks (±8 weeks) after index date, if available;
    - at enrollment.
  - **AQLQ total score changes over time** with respect to index date (according to data availability).

(*) or latest available data before index date.

In case of benralizumab permanent discontinuation, it will be evaluated the opportunity to consider also the assessment at end of benralizumab treatment, according to data availability.

- *Secondary objective #6 (patient's adherence to benralizumab treatment, benralizumab discontinuation, subsequent biologic treatments for asthma and correlation of adherence to age and age at diagnosis, at 48 and 96 weeks)*

    - **Patient's adherence to benralizumab treatment** during the observation period (based on actual injections received) at 48 and 96 weeks.

    - **Benralizumab permanent discontinuation** during the observation period at 48 and 96 weeks

    - **Reasons of benralizumab permanent discontinuation** during the observation period at 48 and 96 weeks.

    - **Subsequent biologic treatments** for asthma and reason for switch at 48 and 96 weeks.

- *Secondary objective #7 (healthcare resource utilization in terms of GP/specialist visits for asthma during benralizumab treatment 48 and 96 weeks, and ER admissions and hospitalizations for asthma during benralizumab treatment including 24, 48 and 96 weeks)*

    - **GP/specialist visits** for asthma during benralizumab treatment
        - at 48 weeks (±8 weeks) after index date, if available;
        - at 96 weeks (±8 weeks) after index date, if available;

    - **ER admissions** for asthma during benralizumab treatment.
        - at 24 weeks (±6 weeks) after index date, if available;
        - at 48 weeks (±8 weeks) after index date, if available;
        - at 96 weeks (±8 weeks) after index date, if available;

    - **Hospitalizations** for asthma during benralizumab treatment.
        - at 24 weeks (±6 weeks) after index date, if available;
        - at 48 weeks (±8 weeks) after index date, if available;
        - at 96 weeks (±8 weeks) after index date, if available;

  In case of benralizumab permanent discontinuation prior to enrollment visit, these outcomes will be evaluated as long as patients are under benralizumab treatment.

[redacted]

[redacted]

[redacted]

[redacted]

4.3 Other variables and covariates

Prior biologic treatments received before benralizumab was considered as stratification factor for exploratory analyses on selected secondary endpoints (see paragraph 4.2 for details), as exposure to prior biologics might potentially influence the investigated outcomes.
Patients with missing data for covariates were not excluded from the study and their data were not replaced, but were not considered for the analyses for which those covariates are taken into account.

5. STATISTICAL ANALYSIS

5.1 Statistical methods – general aspects

A general description of the planned statistical methods to be used to analyze the data collected in this study is presented in the following subsections. Specific details are provided in a Statistical Analysis Plan (SAP).
The statistical analyses were performed considering the set of evaluable patients, defined as eligible subjects (i.e. those meeting all inclusion criteria and not meeting any of the exclusion criteria listed at paragraphs 3.3 and 3.4, respectively) with available information regarding at least one of the defined primary outcomes (listed in paragraph 4.2).

For patients excluded from the statistical analyses, descriptives of the reasons for non-evaluability was provided.

Evaluable patients with missing data for certain variables were not excluded from the study and their data were not replaced (unless differently specified), but were not considered for the analyses for which that variables are taken into account. Frequency of missing data was given for all analyzed variables.

Descriptive analyses were performed, since all objectives are descriptive in nature. No formal hypotheses were pre-specified (this is not an analytical study), and inferential statistical significance testing was not foreseen.

General descriptive statistics for continuous numerical variables included: the number of observations, the mean, the standard deviation (SD), the median and quartiles, the minimum, and the maximum values. For categorical variables, the proportion (with count) of subjects with a certain event/characteristic was presented. Where relevant, two-sided 95% confidence interval (95% CI) limits of the mean for numerical variables and 95% CI limits for proportions were computed.

The analyses were performed using SAS software (SAS Institute, Cary, NC, USA).

5.1.1 Primary objectives – calculation of epidemiological measures of interest (e.g. descriptive statistics, hazard ratios, incidences, test/retest reliability)

The analyses performed for the primary objective of the study (namely, the description of key features of patients with severe eosinophilic asthma when starting benralizumab) required the computation of the following indicators:

- descriptive statistics of available information on **total IgE** and **eosinophil count** in peripheral blood as measured at index date (*);
- descriptive statistics of available **lung function parameters** (i.e. FEV1, FEV1 % predicted, FVC, FEV1/FVC, FeNO) as measured at index date (*);
- proportion of patients with ≥1 relevant comorbidity at index date (*), and prevalence of each **comorbidity** at index date (*);
- descriptive statistics of number of **previous annual any**, **mild, moderate , moderate/severe, severe exacerbations** per patient at index date;
- **annual severe exacerbation rate** in the year before index date, calculated as the number of severe exacerbations occurred during the 12 months prior to index date divided by the persons at risk throughout the 12 months prior to index date;
- **annual any exacerbation rate** in the year before index date, calculated as the number of severe exacerbations occurred during the 12 months prior to index date divided by the persons at risk throughout the 12 months prior to index date;

- proportion of patients with ≥1 **maintenance asthma treatment** ongoing at index date (including oral corticosteroids), and prevalence of each type of asthma maintenance treatment ongoing at index date;
- proportion of patients previously treated with **biologics** to treat their asthma before index date, and prevalence of each type of biologic treatment before index date (pathways of treatment). Moreover, descriptive statistics of duration of treatment with prior biologics, and time gap between prior biologic end and benralizumab treatment start, will be computed as well;
- descriptive statistics of number (per patient) of **GP/specialist** visits, **ER admissions**, and **hospitalizations** for asthma in the 12 months prior to index date.

(*) or latest available data before index date.

The 95% CI limits of above-listed primary endpoints estimates was computed, where relevant.

5.1.2 Secondary objectives – calculation of epidemiological measures of interest (e.g. hazard ratios, incidence rates, test/retest reliability)

The analyses performed for the secondary objectives of the study required the computation of the following indicators.

Secondary objective #1 (the observed severe, moderate/severe, moderate and any exacerbation occurred during benralizumab treatment including 48 and 96 weeks)

- Proportion of patients experiencing **≥1 severe, moderate/severe, moderate** and **any exacerbation** during benralizumab treatment including 48 and 96 weeks.
- **Annualized severe, moderate/severe, moderate** and **any exacerbation rate**, calculated as the ratio between the total number of severe, moderate/severe, moderate and any exacerbations occurred in the sample and the total number of person-years (i.e. the actual time-at-risk that all evaluable patients contributed to the study while they were in treatment with benralizumab).

Secondary objective #2 (reduction in inhaled corticosteroids (ICS) and oral corticosteroids (OCS) use and reasons for ICS reduction, during benralizumab treatment including 48 and 96 weeks)

- Proportion of patients with any **ICS dose reduction** of any extent (final dose* with respect to index date).
- The **reasons for ICS reduction** (per medical judgement or per patient decision) was provided by descriptive statistics.
- Within the subgroups of patients experiencing / not experiencing ICS dose reduction (final dose*with respect to index date), descriptive statistics during benralizumab treatment including 48 and 96 weeks from index date, was calculated for:
  - **variation of exacerbations**
  - **lung function variation**
  - **asthma control variation**

- Proportion of patients with **maintenance OCS treatment (mOCS)** at index date achieving a **reduction** in OCS final dose* with respect to index date as specified in chapter 4.2:
- Proportion of patients with maintenance OCS treatment at index date not achieving reduction in OCS final dose* with respect to index date.

*as evaluated at end of observation or at benralizumab permanent discontinuation (whichever occurs earlier).

Secondary objective #3 (IgE and eosinophil count during benralizumab treatment including 48 and 96 weeks and changes over time with respect to benralizumab treatment start)

- Descriptive statistics of **total IgE** and **eosinophil count** in peripheral blood as measured at the time points specified in chapter 4.2.
- Descriptive statistics of intra-patient **changes over time** in total IgE and eosinophil count in peripheral blood, at each time points specified in chapter 4.2 with respect to index date (according to data availability).

These indicators were calculated considering the patients during benralizumab treatment, including 48 and 96 weeks (total IgE count will not be collected at 96 weeks).

Secondary objective #4 (lung function parameters during benralizumab treatment including 48 and 96 weeks, and changes over time with respect to benralizumab treatment start)

- Descriptive statistics of **lung function parameters** as measured at the time points specified in chapter 4.2.
- Descriptive statistics of intra-patient **changes over time** in lung function parameters, at each time points specified in chapter 4.2 with respect to index date (according to data availability).

These indicators werecalculated considering patients as long as they are under benralizumab treatment.

Secondary objective #5 (patients' asthma control level and quality of life at benralizumab treatment start and during the observation period including 48 and 96 weeks, and changes over time with respect to benralizumab treatment start.)

- Descriptive statistics of **Asthma Control Test (ACT)** total score at index date (if available*).
- Descriptive statistics of ACT total score at the time points specified in chapter 4.2 (according to data availability), along **with proportion of patients with well-controlled asthma** (i.e. ACT score ≥20).
- **Proportion of patients achieving the Minimal Important Difference** (MCID, i.e. a change accounting for ≥3 points with respect to index date) **in ACT total score** at the time points specified in chapter 4.2 (according to data availability).
- Descriptive statistics of intra-patient **changes in ACT total score**, at each time points specified in chapter 4.2 with respect to index date (according to data availability).
- Descriptive statistics of **Asthma Quality of Life Questionnaire (AQLQ)** total score at index date (if available*).
- Descriptive statistics of AQLQ total score at the time points specified in chapter 4.2 (according to data availability).

- Descriptive statistics of intra-patient **changes in AQLQ total score**, at each time point specified in chapter 4.2 with respect to index date (according to data availability).
- **Proportion of patients achieving the Minimal Important Difference** (MID, i.e. a change accounting for ≥0.5 points with respect to index date) **in AQLQ total score** at the time points specified in chapter 4.2 (according to data availability).

(*) or latest available data before index date.

Secondary objective #6 (patient's adherence to benralizumab treatment, benralizumab discontinuation, subsequent biologic treatments for asthma and correlation of adherence to age and age at diagnosis, at 48 and 96 weeks)

- Descriptive statistics of **level of patient's adherence to benralizumab treatment** at 48 and 96 weeks, computed as the ratio (in percentage) between the number of actual injections received during the observation period over the number of expected injections (which will be estimated considering the injection schedulation specified in the SmPC). In particular, the proportion of patients with 100%, 75% ,50% and <50% injections as scheduled (+/- 35 days injection window) at 48 and 96 weeks.
- Proportion of patients with **benralizumab permanent discontinuation** at 48 and 96 weeks, and description of **reasons**.
- Proportion of patients with **subsequent biologic treatments for asthma**, description of **type of treatment** and **reason for switch** at 48 and 96 weeks**.**
- Spearman correlation coefficient (or analogous according to data distribution) of adherence to age and age at diagnosis will be calculated.

Secondary objective #7 (healthcare resource utilization in terms of GP/specialist visits for asthma during benralizumab treatment including 48 and 96 weeks, and ER admissions and hospitalizations for asthma during benralizumab treatment including 24, 48 and 96 weeks)

- Descriptive statistics of total number (per patient) of the following **healthcare resource utilization** occurred during benralizumab treatment including 48 and 96 weeks: GP/specialist visits for asthma.
- Mean of the following **healthcare resource utilization** occurred during benralizumab treatment including 24, 48 and 96 weeks: ER admissions for asthma, and hospitalizations for asthma.

In case of benralizumab permanent discontinuation prior to enrollment visit, these indicators will be calculated considering patients as long as they are under benralizumab treatment.

5.1.3 Exploratory objectives – calculation of epidemiological measures of interest (e.g. hazard ratios, incidences, test/retest reliability)

The analyses to be performed for the exploratory objective of the study will require the computation of the following indicators, that will be computed considering the set of eligible patient with available information regarding prior use of biologic treatments before benralizumab.

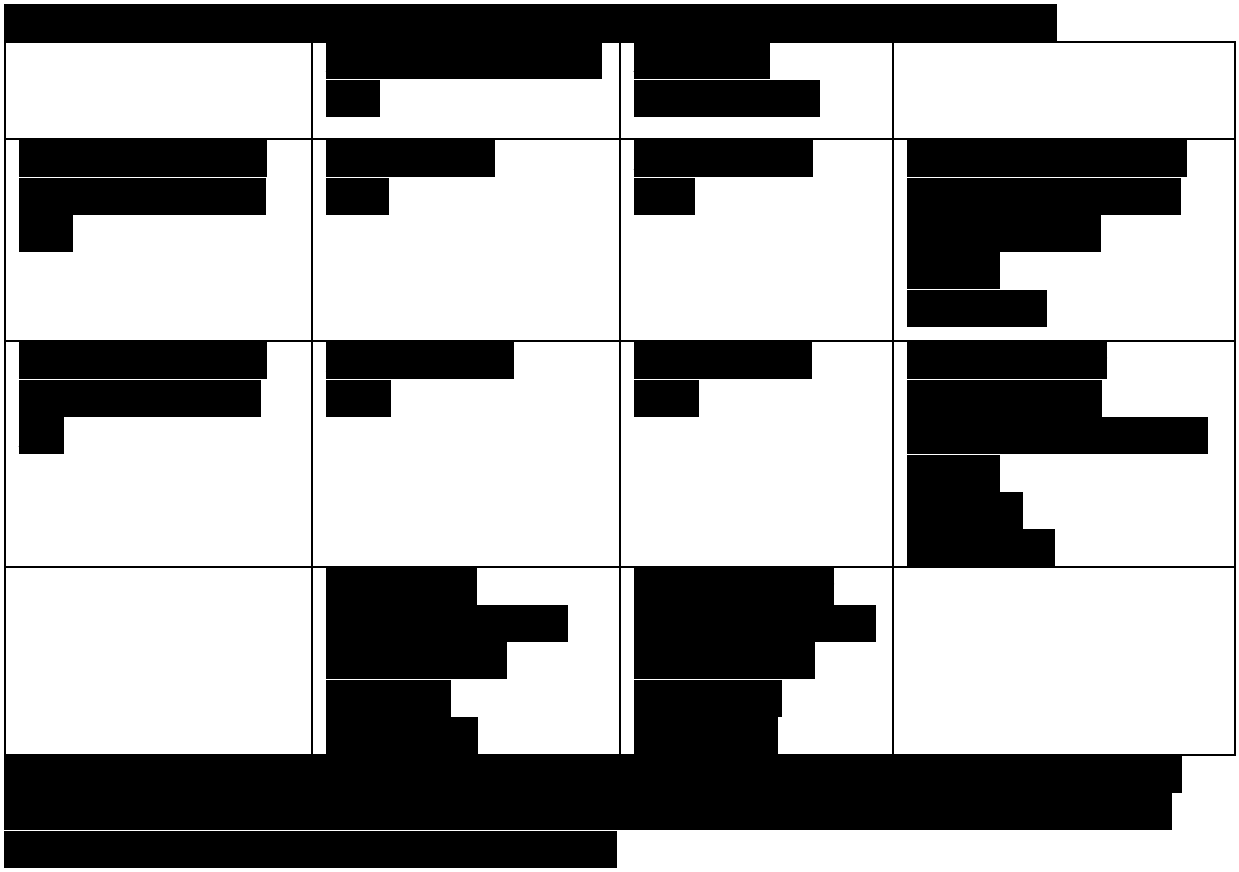
[redacted]

[redacted]

[redacted]

[redacted]

[redacted]



[redacted]

5.2 Bias

5.2.1 Methods to minimize bias

This is an observational (non-interventional) study with a retrospective data collection Information, selection and recall bias have to be taken into account, as in all observational studies.

Information and recall bias might affect the quality of collected retrospective data; however, the most relevant retrospective information such as total IgE, eosinophils count, lung function parameters, number of previous exacerbations and asthma treatment(s) were not affected by

the recall bias, since one of the inclusion criteria is to have medical chart data available as per clinical practice.

By design, patients who started benralizumab treatment less than 3 months before enrollment were not included in the study. The systematic exclusion of these patients introduces a selection of the target population. However it is of interest in the study to have a period of observation: 3 months were considered clinically acceptable to describe also the clinical outcomes. Rather, in order to minimize patient selection during recruitment, sampling is based on consecutive enrollment.

5.3 Sample size and power calculations

[redacted]

5.4 Data quality

The Clinical Data Manager (CDM) of the Clinical Research Organization (CRO) working on behalf of AstraZeneca performed the cleaning session. In particular, the data entered into the eCRFs by investigational staff were checked for completeness and accuracy. After data entry, online checks were run on primary variables, in order to verify implausible and out-of-range data, other than missing data and inconsistencies among variables. For missing, incoherent, implausible data, an online query was raised, which had to be solved by the investigator. The CDM performed the cleaning session using the SAS software running post-entry checks by means of validation programs and data listings specific for the study.

Self Evident Corrections (SECs) could be made to the database in case of obvious mistakes in order to substantially decrease the number of queries to be handled by investigators without affecting data quality nor original data.

Once the database was declared complete and accurate, it was locked and used for statistical analysis.

6. RESULTS

Among 218 patients enrolled in the ANANKE study, 167 (76.6%) patients were considered eligible for the 96-week report.

Among evaluable patients, the median (25th-75th percentile) duration of observation accounted for 22.3 (21.0 – 23.7) months; all patients had at least three months of observation, as required by Study Protocol selection criteria.

Demographics and life habits at the start of benralizumab treatment

- The mean (SD) age was 56.0 (12.7) years out of 162 evaluable patients;
- Females were 99 (61.1%);
- Non-smokers were 106 out of 155 patients with available information about smoking status (68.4%).

Clinical characteristics at the start of benralizumab treatment

- Patients positive to perennial allergens were 63 (38.9%); in particular: 45 (27.8%) to Dust mite (D. Pteronyssinus), 15 (9.3%) to Cat hair, 13 (8.0%) to Dog hair and 12 (7.4%) to Aspergillus.

Key features at Benralizumab treatment start - Primary objective

- Overall, 141 (87.0%) patients had relevant comorbidities;
- Patients with current or past nasal polyposis were 86 (53.4% out of 161 patients with available data) (see Table 10);
- Current asthma-related conditions were observed in 91 (56.2%) patients. The most frequent ones were Chronic rhinosinusitis (n=43, 26.5%), Gastroesophageal reflux (GERD; n=39, 24.1%) and Allergic rhinitis (n=38, 23.5%);
- Patients with any current OCS-related conditions were 64 (39.5%). In particular: Hypertension (n=38, 23.5%) and Osteoporosis (n=17, 10.5%).

- The blood eosinophil count showed a median (25th-75th percentile) of 600.0 (430.0–890.0) cells/mm3;
- The observed mean (SD) of lung function assessment were the following:
    - Pre-bronchodilator FEV1: 2.0 (0.8) L (n=121);
    - Pre-bronchodilator FEV1 predicted: 70.2 (21.1) % (n=125);
    - Pre-bronchodilator FVC: 3.0 (1.0) L (n=116);
    - Pre-bronchodilator FEV1/FVC ratio: 0.7 (0.2) (n=116).
- Post-bronchodilator parameters showed a similar distribution, even if parameters were available for a lower number of patients (results shown in Table 9).
- Before starting benralizumab, 144 (93.5%; 95% CI limits: [89.6% - 97.4%]) out of 154 patients experienced at least one asthma exacerbation of any kind (mild, moderate or severe). In particular (see Table 11):
    - 125 (81.2%; [75.0% - 87.3%]) had moderate/severe exacerbations, with annual exacerbation rate accounting for 2.82 cases / person-year;
    - 57 (37.0%; [29.4% - 44.6%]) had severe exacerbations, with annual exacerbation rate accounting for 0.98 cases / person-year.
- Thirty-eight (23.5%) patients were previously treated with biologics: 21 patients (13.0%) received omalizumab, 13 patients (8.0%) received Mepolizumab; 4 patients (2.5%) received both treatments (Omalizumab as first treatment, followed by Mepolizumab);
- The median (25th-75th percentile) duration of exposure to biological drugs before starting benralizumab was 21.0 (10.6 – 52.9) months, out of 29 patients with available evaluation;
- The median (25th-75th percentile) time gap between prior biologic end and benralizumab treatment start was 2.3 (1.3 – 4.9) months, out of 29 patients.
- Consistently with inclusion criteria, 160 (98.8%) patients were treated with ICS/LABA and 2 with ICS+LABA;
- Moreover, the following therapies were administered to patients in addition to ICS/LABA: 83 (51.2%) patients were treated with LAMA, 41 (25.3%) with OCS, 12 (7.4%) with ICS, 1 (0.6%) with LABA, 76 (46.9%) with OTHER asthma treatments;
- The maintenance ICS dose according to GINA 2019 Guidelines was reported to be high in 143 (88.3%) out of 162 evaluable patients;
- The median (25th-75th percentile) OCS daily (prednisone-equivalent) dose at index date was 10.0 (5.0 – 25.0) mg, considering 39 patients with available dose at the index date.

Secondary objective #1: Exacerbations during benralizumab treatment including 48 and 96 weeks

A total of 30 (20.7%) patients, out of the 145 evaluable for secondary analysis at 48 weeks, experienced exacerbations of any kind during benralizumab treatment. In particular, 18 (12.4%) patients experienced moderate exacerbations, 24 (16.6%) moderate/severe exacerbations and 9 (6.2%) patients experienced severe exacerbations. For these patients the Annualized severe exacerbation rate during benralizumab treatment at 48 weeks was 0.07 cases / person-year and the annualized moderate/severe exacerbation rate at 48 weeks was 0.25 cases / person-year;

- A total of 26 (23.0%) patients, out of the 113 evaluable for secondary analysis at 96 weeks, experienced exacerbations of any kind during benralizumab treatment. In particular, 19 (16.8%) patients experienced moderate exacerbations, 22 (19.5%) moderate/severe exacerbations and 6 (5.3%) patients experienced severe exacerbations. For these patients the Annualized severe exacerbation rate during benralizumab treatment at 96 weeks was 0.03 cases / person-year and the annualized moderate/severe exacerbation rate at 96 weeks was 0.16 cases / person-year.

Secondary objective #2: ICS and OCS use and reasons for ICS reduction during benralizumab treatment including 48 and 96 weeks

- Eight patients (5.5% of evaluable patients for secondary analyses at 48 weeks) reduced/interrupted ICS within 48 weeks after the index date, 2 of them because of Improvement of patient symptoms, 2 due to lack of clinical efficacy, 1 because of lack of compliance, 1 because of patient decision;
- Nine patients (8.0% of evaluable patients for secondary analyses at 96 weeks) reduced/interrupted ICS within 96 weeks after the index date, 3 of them due to lack of clinical efficacy, 2 because of Improvement of patient symptoms, 1 because of lack of compliance, 1 because of patient decision;
- As agreed with Sponsor, analyses within the subgroups of patients experiencing/not experiencing ICS dose reduction during benralizumab treatment were not included in this statistical report due to the low frequency of patients experiencing ICS dose reduction.
- The number of patients achieving at 48 weeks after index date a dose OCS reduction of any extent compared to the start of benralizumab was 21 (61.8%), out of the 34 patients evaluable for secondary analyses at 48 weeks (with OCS treatment ongoing at the start of benralizumab and available dose reduction). In particular, 18 of them (52.9%) interrupted OCS;
- At 96 weeks after index date 20 patients (66.7%) achieved a dose OCS reduction of any extent compared to the start of benralizumab, considering 30 evaluable patients

with OCS treatment ongoing at the start of benralizumab and available dose reduction at 96 weeks. In particular, 18 patients (60.0%) interrupted OCS.

Secondary objective #3: IgE and eosinophil count during benralizumab treatment including 48 and 96 weeks and changes over time compared to benralizumab treatment start

- The available observations of IgE count (IU/mL) at the prespecified time points after the index date (16, 24, 48 weeks) were <=8. Due to the limited number of patients, it is not possible to evaluate with sufficient precision the extent of variation over time compared to baseline values.
- Considering the evaluable patients for secondary analyses at 96 weeks, blood eosinophil count was available for:
    - 113 patients at the index date: at least 75% of the sample had ≤850 cells/mm3;
    - 37 patients at 16 weeks: at least 75% of the sample had 0 cells/mm3;
    - 31 patients at 24 weeks: at least 75% of the sample had 0 cells/mm3;
    - 37 patients at 48 weeks: at least 75% of the sample had 0 cells/mm3;
    - 38 patients at 96 weeks: at least 75% of the sample had 0 cell/mm3
- In all cases, the median change of blood eosinophil count indicated a numerical decrease of 500 cells/mm3 or more from baseline values.

Secondary objective #4: Lung function parameters during benralizumab treatment including 48 and 96 weeks and changes over time compared to benralizumab treatment start

Pre- and Post-bronchodilator FEV1 / FEV1 predicted considering evaluable patients for secondary analyses at 96 weeks

- The time points with the highest number of observations were recorded at 16, 48 and 96 weeks after starting benralizumab; for this reason, descriptive statistics at these time points vs index date are reported.
    - Observations of Pre-bronchodilator FEV1 after 16 weeks were made available for 43 patients, with a mean (SD) of 2.3 (0.9) L, after 48 weeks were made available for 45 patients, with a mean (SD) of 2.1 (0.8) L and after 96 weeks were made available for 47 patients, with a mean (SD) of 2.3 (0.8) L;
    - The median (25th-75th percentile) intra-patient changes at 16, 48 and 96 weeks vs index date of Pre-bronchodilator FEV1 were 0.2 (0.0; 0.4) L (n=39), 0.3 (- 0.0; 0.6) L (n=35) and 0.2 (- 0.1; 0.7) L (n=36), respectively;
    - Observations of Post-bronchodilator FEV1 after 16 weeks were made available for 28 patients, with a mean (SD) of 2.4 (0.8) L, after 48 weeks were

made available for 21 patients, with a mean (SD) of 2.5 (0.8) L and after 96 weeks were made available for 29 patients, with a mean (SD) of 2.6 (1.0) L;

- The median (25th-75th percentile) intra-patient changes at 16, 48 and 96 weeks vs index date of Post-bronchodilator FEV1 were 0.1 (- 0.2; 0.5) L (n=18), 0.2 (- 0.1; 0.7) L (n=16) and 0.3 (0.1; 0.7) L (n=22), respectively;

- Observations of Pre-bronchodilator FEV1 predicted after 16 weeks were made available for 44 patients, with a mean (SD) of 83.3 (32.8) %, after 48 weeks were made available for 47 patients, with a mean (SD) of 84.0 (28.5) % and after 96 weeks were made available for 51 patients, with a mean (SD) of 83.4 (23.0) L;
- The median (25th-75th percentile) intra-patient changes at 16, 48 and 96 weeks vs index date of Pre-bronchodilator FEV1 predicted were 6.5 (-2.5; 15.0) (n=40) %, 9.0 (3.0; 29.0) (n=37) % and 11.0 (-3.0; 27.0) % (n=37), respectively;
- Observations of Post-bronchodilator FEV1 predicted after 16 weeks were made available for 28 patients, with a mean (SD) of 83.2 (21.0) %, after 48 weeks were made available for 20 patients, with a mean (SD) of 86.8 (27.3) % and after 96 weeks were made available for 29 patients, with a mean (SD) of 89.0 (23.1) %;
- The median (25th-75th percentile) intra-patient changes at 16, 48 and 96 weeks vs index date of Post-bronchodilator FEV1 predicted were: 0.0 (- 9.0; 18.0) % (n=18), 8.0 (- 9.0; 21.0) % (n=15) and 16.0 (0.0; 29.0) % (n=22), respectively.

Pre- and Post-bronchodilator FVC considering evaluable patients for secondary analyses at 96 weeks

- The time points with the highest number of observations were recorded at 16, 48 and 96 weeks after starting benralizumab; for this reason, descriptive statistics at these time points vs index date are reported.
    - Observations of Pre-bronchodilator FVC (L) after 16 weeks were made available for 43 patients, with a mean (SD) of 3.1 (1.1) L, after 48 weeks were made available for 45 patients, with a mean (SD) of 2.9 (1.0) L and after 96 weeks were made available for 47 patients, with a mean (SD) of 3.2 (1.1) L;
    - The median (25th-75th percentile) intra-patient changes at 16, 48 and at 96 weeks vs index date of Pre-bronchodilator FVC (L) were 0.2 (- 0.1; 0.4) L (n=39), 0.2 (0.0; 0.7) L (n=34) and 0.1 (- 0.1; 0.7) L (n=35), respectively;

- Observations of Post-bronchodilator FVC (L) after 16 weeks were made available for 26 patients, with a mean (SD) of 3.3 (1.0) L, after 48 weeks were made available for 22 patients, with a mean (SD) of 3.6 (1.1) L, and after 96 weeks were made available for 28 patients, with a mean (SD) of 3.5 (1.3) L;
- The median (25th-75th percentile) intra-patient changes at 16, 48and 96 weeks vs index date of Post-bronchodilator FVC (L) were 0.0 (-0.5; 0.5) L (n=16), 0.1 (- 0.3; 0.5) L (n=16) and 0.1 (- 0.2; 0.5) L (n=19), respectively.

Secondary objective #5: Patients' asthma control level and quality of life at benralizumab treatment start, during the observation period including 48 and 96 weeks and changes over time compared to benralizumab treatment start

Considering evaluable patients for secondary analyses at 96 weeks after index date

- Asthma Control Test (ACT) total score was available (see Table 38):
  - at the start of benralizumab for 96 patients, with a mean (SD) of 14.9 (4.6);
  - after 4 weeks for 26 patients, with a mean (SD) of 20.2 (4.2);
  - after 16 weeks for 77 patients, with a mean (SD) of 20.0 (4.2);
  - after 24 weeks for 70 patients, with a mean (SD) of 20.2 (3.8);
  - after 48 weeks for 75 patients, with a mean (SD) of 20.7 (4.7);
  - after 96 weeks for 75 patients, with a mean (SD) of 21.5 (3.8);
- Well-controlled asthma (i.e. ACT score >=20) was reported in 16 (16.7%), 16 (61.5%), 48 (62.3%), 47 (67.1%), 58 (77.3%) and 59 (78.7%) patients at benralizumab start, after 4, 16, 24, 48 and 96 weeks after the index date, respectively;
- At every evaluation during benralizumab treatment (at 16, 24, 48, 96 weeks after index date), at least 72% of the patients with filled questionnaires achieved the minimum importance difference.

Considering evaluable patients for secondary analyses at 48 weeks after index date

- The available observations of Asthma Quality of Life Questionnaire (AQLQ) total score during benralizumab treatment for patients were 28 at the start of benralizumab and less than 25 at the other time points. Due to the limited number of patients with an available questionnaire, it is not possible to provide precise estimates of the distribution of AQLQ scores in the sample (results are available in Table 40) and of patients achieving MID in the AQLQ total score.

Secondary objective #6: Patient's adherence, benralizumab discontinuation and subsequent biologic treatment and correlation of adherence at 48 and 96 weeks to age and age at diagnosis

Considering evaluable patients for secondary analyses at 48 weeks after index date

- Out of the 150 eligible patients with consistent data at 48 weeks, the level of patient's adherence to benralizumab treatment at 48 weeks, measured as the ratio between the number of actual injections and the number of expected injections at 48 weeks (in percentage), showed a mean (SD) of 98.9 (6.8) %;
- Between index date and week 48 the mean (SD) number of benralizumab injections was 7.7 (0.6) and 122 (81.3%) patients performed >90% of the expected injections;
- The permanent discontinuation of benralizumab was observed in six patients (4.0% out of 150) for lack of clinical efficacy (N=3, 50.0% of patients who discontinued), patient decision (N=2, 33.3%), and presence of allergic rhinitis (N=1, 16.7%);
- Three patients, out of six eligible patients with consistent data who discontinued benralizumab within 48 weeks, had subsequent biologic treatments for asthma (Omalizumab) due to exacerbations, recurrence of symptoms and other reasons;
- No statistically significant correlation was observed between the level of patient's adherence to benralizumab treatment at 48 weeks and age at start of benralizumab treatment or age at diagnosis of asthma.

Considering evaluable patients for secondary analyses at 96 weeks after index date

- Out of the 115 eligible patients with consistent data at 96 weeks, the level of patient's adherence to benralizumab treatment at 96 weeks, measured as the ratio between the number of actual injections and the number of expected injections at 96 weeks (in percentage), showed a mean (SD) of 95.1 (10.4) %;
- Between index date and week 96 the mean (SD) number of benralizumab injections was 13.0 (1.6) and 96 (83.5%) patients performed >90% of the expected injections;
- The permanent discontinuation of benralizumab was observed in twelve patients (10.4% out of 115) for lack of clinical efficacy (N=6, 50.0% of patients who discontinued with consistent data), lack of efficacy-adverse events (N=2, 16.7%), patient decision (N=2, 16.7%), and presence of allergic rhinitis (N=1, 8.3%);
- Seven patients, out of twelve eligible patients with consistent data who discontinued benralizumab, had subsequent biologic treatments for asthma (Omalizumab or Dupilumab) due to inadequate clinical response, exacerbations, recurrence of symptoms and other reasons;
- No statistically significant correlation was observed between the level of patient's adherence to benralizumab treatment at 96 weeks and age at start of benralizumab treatment or age at diagnosis of asthma.

Secondary objective #7: Healthcare resource utilization during benralizumab treatment up to 48 and 96 weeks

- The number of primary care physician/GP office or specialist visits, for asthma, per patient up to 48 or 96 weeks after the index date was neglectable. The same goes for the number of ER admissions and hospitalizations up to 48 and 96 weeks after the index date.

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[redacted]

[redacted]

[redacted]

[redacted]

7. SAFETY EVALUATION

Not applicable

8. DISCUSSION AND CONCLUSION

8.1 Discussion

ANANKE (NCT04272463) is one of the largest RWE studies conducted to examine the key features of SEA patients and the clinical outcomes achieved during benralizumab treatment. The data reported here complement previously published results from the ANANKE study, related to a shorter treatment period (median treatment duration: 9.8 months).

The clinical characteristics of the SEA population treated in the ANANKE study (diagnosis of asthma during adulthood, presence of circulating blood eosinophils, moderate airflow obstruction, more than half of the patients suffering from nasal polyposis and other asthma-related comorbidities, frequent exacerbations and a considerable proportion of OCS users) are consistent with the adult-onset, eosinophilic asthma phenotype. As already well-established benralizumab is highly effective in this subset of patients regardless of baseline BEC levels and its effectiveness has been further corroborated in this study with novel long term data.

The frequency of asthma exacerbations represents the leading endpoint to evaluate how effective are therapies in alleviating the burden of severe asthma. The well-known efficacy of benralizumab in reducing exacerbations is strengthened in this study and its effectiveness is sustained over a prolonged period; at 96 weeks, AER of any severity and severe AER were decreased by 94.9% and 96.9% respectively. Notably, 77% patients free from any exacerbations and 94.9% patients remained without severe exacerbations for the whole duration of the study. These results are consistent with data obtained from the Phase III MELTEMI study, where exacerbations decreased progressively over the 5-year treatment period; a similar drop of AER (from 4.1 to 0.33) has been found in the RWE study published by Sposato et al, in which 95 patients were treated for a mean period of 19.7 months. In addition, a recent real-life study by Vitale and colleagues showed that benralizumab eliminated exacerbations in 85% patients (all OCS-dependent) after 2 years of treatment.

Exacerbation frequency is known to be associated with greater BEC; in light of benralizumab exclusive MoA and the results presented here and elsewhere, the strong effect of benralizumab in reducing exacerbations could be considered a hint to unlock clinical remission in SEA.

Severe asthma patients typically experience a progressive deterioration in lung function over time. Even if spirometric data were limited and not all patients were evaluated at all time points, our results suggest that overall, benralizumab treatment enhances FEV1 from the very first administration (4 weeks) and preserves lung function over time as demonstrated by spirometry parameters collected at various time points. While post-BD levels of FEV1 and FVC were increased at 48 weeks and remained stable up until 96 weeks, a sharp

improvement in pre-BD FEV1 (change in median volume: +400 mL) was evident only after 96 weeks of treatment.

Albeit additional measurements at later time points would be needed to confirm this result, we speculated this effect to be a direct consequence of benralizumab-induced sustained depletion of eosinophils within the lung tissue. As a matter of fact, our data showed a nearly complete depletion of BEC maintained over time, yet it is known that benralizumab has a similar effect in depleting eosinophils in sputum. Moreover, exacerbation frequency is known to be associated with a more pronounced decline in lung function; consistently, we found exacerbations to be almost completely eradicated with a parallel amelioration in pre-BD parameters. Of note, FEV1 increased more than 300 mL in the 1-year long phase III SIROCCO and CALIMA studies and such level was maintained, but did not further improve, during the 1-year extension study BORA. In general, the majority of RWE studies highlighted the rapid action of benralizumab in improving lung function, with only few studies that investigated benralizumab long-term effects on multiple respiratory parameters. One of these studies reported a progressive improvement in FEV1, FVC and FEV1/FVC levels after 26 and 52 weeks of treatment in a small cohort of 18 SEA patients, with FEV1 increasing more than 1000 mL in patients with nasal polyposis and BEC> 500 cells/mm3. Three other studies assessed FEV1 over a period greater than 1 year. The data published by Sposato and colleagues, showing an improvement of +300 mL in FEV1 are comparable with our results, yet the measurements were taken only at baseline and at the end of the study, hence it is difficult to determine if the improvement was continuous or limited to the first period of treatment. The multiple respiratory assessments performed at various time points in the study by Vitale et al. demonstrated a significant and stable increase in all respiratory parameters, with the greatest improvement detected within the first 6 months of treatment with benralizumab.

Asthma control was also enhanced, as assessed by median ACT score and proportion of patients achieving total control of asthma and MCID; notably, all these variables sharply increased at 4 weeks and continued to rise. ACT score has been extensively shown to be improved in the SEA population, nevertheless the various time points assessed in our study reinforce both the rapidity and the durability of action of benralizumab in ameliorating patients' asthma control.

To manage asthma-related symptoms and avoid exacerbations, SEA patients are often treated with OCS in a chronic manner. Here we describe a median OCS reduction of 100% achieved at 48 weeks and maintained at 96 weeks, accompanied by a parallel increase in the proportion of patients who discontinued OCS treatment. In this context, it is important to reiterate the impact of benralizumab on exacerbation rate, asthma control, and lung function in parallel with the concomitant elimination or reduction of OCS therapy.

SEA is associated with a substantial need of healthcare services and high healthcare-related costs; this is particularly true when patients experience frequent exacerbations. Given the

improvement in the asthma-related outcomes described above, it is not surprising to find that the average rate of emergency department admissions and hospitalization was eliminated after 96 weeks of benralizumab treatment.

[redacted]

[redacted]

8.2 Conclusion

The population of severe eosinophilic asthmatic patients evaluated in the ANANKE study had a remarkable and durable response to benralizumab. The long-term improvements in all clinical outcomes, in combination with patients' clinical characteristics at index date, underline a specific "eosinophilic-driven" asthma profile (as recently defined by Couillard et

al.) that features the ANANKE severe eosinophilic asthmatic population. Of note, the specific design of the ANANKE study, its retrospective nature (enrolment at least 3 months after benralizumab initiation) and the possibility to follow up patients until 96 weeks of treatment were pivotal to allow a thorough investigation of the patients'key features as well as the achievement of such profound results. Once again, these data highlight that when benralizumab is administered to the right patient, with an authentic "eosinophilic-driven" asthma phenotype, it ensures a great, long-term efficacy thanks to the continuous and nearly complete depletion of eosinophils.

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10. ASTRAZENECA SIGNATURE

The ANANKE study

ChAracterisation of ItaliaN severe uncontrolled Asthmatic patieNts Key features and long-term outcomes when receiving Benralizumab in a real life setting: an observational rEtrospective study

I have read this Study Report and I confirm that it describes the procedure and the results of the study.

AstraZeneca representatives

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