ClinicalTrials.gov Protocol Registration and Results System (PRS) Receipt
Release Date: July 7, 2021

ClinicalTrials.gov ID: [Not yet assigned]

Study Identification

Unique Protocol ID:	EoAG
Brief Title:	Flow Cytometry Analysis of Eosinophils in Severe Asthma Patients.
Official Title:	Flow Cytometry Analysis of Eosinophils Activity and Membrane Receptors Expression in Severe Asthma Patients: Basal Characterization and Evaluation of Changes Induces by Biological Drugs.
Secondary IDs:	

Study Status

Record Verification:	April 2021
Overall Status:	Recruiting
Study Start:	March 18, 2021 [Actual]
Primary Completion:	March 2022 [Anticipated]
Study Completion:	March 2023 [Anticipated]

Sponsor/Collaborators

Sponsor:	Scarlata, Simone, M.D.
Responsible Party:	Sponsor
Collaborators:	IRCCS San Raffaele

Oversight

U.S. FDA-regulated Drug:	No
U.S. FDA-regulated Device:	No
U.S. FDA IND/IDE:	No
Human Subjects Review:	Board Status: Approved Approval Number: 94/20 OSS ComEt CBM Board Name: Ethical Commitee of Campus Bio Medico University Board Affiliation: Campus Bio Medico University and Teaching Hospital Phone: +39 06-22541 Email: comitato.etico@unicampus.it Address: Via Álvaro del Portillo 5, 00128 Roma Italy

Data Monitoring:	No
FDA Regulated Intervention:	No

Study Description

Brief Summary: Asthma is a heterogeneous disease, characterized by reversible airflow obstruction, airway hyperresponsiveness, and airway inflammation, in which 40% of patients exhibit eosinophil-driven pathobiology.The main treatment of asthma is the use of corticosteroid, whose use induces a reduction in eosinophils that is considered a strong predictor of response to treatment. Corticosteroids have remained the mainstay treatment of asthma and reduction in eosinophils has remained the unequivocal predictor of steroid response. The prevalence of asthma, which is expected to increase, it is about 300 million people worldwide. About 5-10% of asthma patients have severe disease, which is defined as asthma that requires high-dose inhaled corticosteroids (ICSs) plus a second controller to prevent it from becoming “uncontrolled” or which remains “uncontrolled” despite this therapy. Patients with severe disease have worse quality of life, and disproportionately high morbidity, mortality, and use of health care resources when compared with their peers with well-controlled disease.The pathophysiology of asthma is complex and heterogeneous between patients, as the disease itself; however, on the basis of immune system involvement, it is possible to define 2 subtypes – or endotypes- of asthma. These endotypes are named T2 (for type 2 cells) high or low, and are defined by the levels of expression of the T2 cytokines, IL-4, IL-5, and IL-13 produced by T helper 2 lymphocytes, and innate lymphoid cell-2.T2 high endotype patients display an increase in the number of blood and sputum eosinophils, and have a better response to the current available biological therapies , such as the administration of mepolizumab (anti IL-5 antibody). Eosinophilic asthma is associated to a more severe clinical phenotype,but patients with a T2 endotype respond better to biological therapies. Our hypothesis is that the activation status of these cells, analyzed by the expression of activation markers, can be used to define a new, different, endotype, in which eosinophils, although quantitatively low or normal, are qualitatively more active and aggressive, and could therefore act as an indicator of the progression toward a T2 high endotype.Moreover, we will verify whether a different expression of these molecules on eosinophil’s surface might be associated with different clinical response to biologic medications.

Detailed Description: Subject satisfying eligibility criteria will be asked to participate the study; all will receive a detailed explanation of the nature of the study, of the aims and objectives. After having signed informed consent, patients will be divided into two groups, based on their clinical status: controlled asthma group (group A) and severe asthma group (group B). Visits will be planned at recruitment, after a run-in period (3-months), meant to optimize first and second lines medical treatment and confirm diagnosis of severe asthma. After the run-in period, patients will be re-evaluated and appropriate medical therapy will be tailored (T0). To group B, therapy with Mepolizumab or Omalizumab will be given, according to standard guidelines. At T0 we will also withdrawn a blood sample (3ml) to evaluate eosinophils phenotype by flow cytometry (see below). After 6 months (T1/2) and12 months (T1) patients will be visited again and a blood sample will be taken for flow cytometry analysis.All samples will be properly stored and shipped for analysis, according to best of practice and state of art criteria, within 6 hours from sample’s collection. Data will be collected and stored according to the GDPR (General Data Protection Regulation, EU 2016/679). Biological material collected will be used only for the indicated assay and excess material will be discharged.

- Baseline evaluation (T'0) In case of acceptance, and after signing an informed consent, participants will be asked to collect a complete medical history, including allergies and Asthma related comorbidities; a comprehensive physical examination and respiratory function tests consisting of: flow-volume spirometry plus residual volume and DLCO determination. In case of a first diagnosis of asthma a bronchodilator response or a methacholine challenge tests will be obtained. In case of a preexistent diagnosis of asthma, previous examination confirming and proving the diagnosis will be collected.
- Evaluation Tool The following data will be collected at T'0, T'1/2 (six months) and T'1 (twelve months): Clinical assessment: ACT, on demand or rescue therapy, acute exacerbations, medical visits, working absences, hospital admissions. Functional assessment: Flow volume curve, Residual volume, Ig E and eosinophils' count, FeNO, 6 minutes walk test.
- Flow cytometry We will evaluate, by multicolor flow cytometry, the expression of molecules on eosinophils'membrane, using a whole-blood staining protocol, so that blood samples will be minimally manipulated, thus reducing the possibility of artifacts. Briefly, we will treat peripheral blood samples within 2 hours from withdrawal. Samples will be stored at room temperature until processing. We will perform staining on 200 ul of blood samples, using fluorochrome conjugates antibodies directed towards eosinophils'surface molecules. In particular, we will use antibodies against CD45, CD66, CD15, CD16 and Siglec-8, which will be used to identify eosinophils between other leukocytes; CD63, CD294 (CRTH2, prostaglandin D2 receptor), CD125 (IL-5 receptor), CD193 (receptor for several chemokines, as for example RANTES, Eotaxin, MCP-3, MIP1α), and HLA-DR: these molecules are upregulated with eosinophils activation, so they can have an altered expression in severe asthma patients. After 30 minutes incubation at 4°C, we will add FACS lysing solution (BD Bioscience) to remove erythrocytes, according to manufacturer's instruction. Samples will be resuspended in phosphate buffered saline (PBS) and acquired on a LSRFortessa X-20 (BD Bioscience) flow cytometer, equipped with four lasers. We will perform analysis using FlowJo software.

Conditions

Conditions:	Severe Asthma
Keywords:	severe asthma, eosinophils phenotype, anti-IL5R antibody

Study Design

Study Type:	Interventional
Primary Purpose:	Diagnostic
Study Phase:	Phase 4
Interventional Study Model:	Parallel Assignment
Number of Arms:	2
Masking:	None (Open Label)
Allocation:	Randomized
Enrollment:	80 [Anticipated]

Arms and Interventions

Arms	Assigned Interventions
Experimental: Group A Subject with severe asthma, treated with anti IL5R antibodies	Biological/Vaccine: anti IL5 receptor antibodies Subjects with severe uncontrolled asthma will be assigned to treatment with Omalizumab (anti Ig E antibodies) or Mepolizumab (anti IL-5 antibodies) according to medical advice and standardized protocols for asthma treatment.
No Intervention: Group B Subject with severe asthma, treated with conventional therapy	

Outcome Measures

Primary Outcome Measure:

1. Differences in the expression of molecules CD193, CD63, CD294, CD125 and HLA-DR on eosinophils membrane exists and associate them to different asthma endotypes.
   - CD193 (also known as CCR3), is a receptor for several chemokines, and it is upregulated in response to an inflammatory status; its expression drives eosinophils towards inflamed tissues.
   - CD63: this molecule (LAMP-3) is associated to intracellular vescicles, and migrates on cell surface as granules contents is released in the extracellular space, so its upregulation is associated with secretory activity of eosinophils.
   - CD294 (CRTH2) is the prostaglandin D2 receptor, its expression is indicative of aT2 response.
   - CD125 is the receptor for IL-5, which is the major cytokine for eosinophils development and proliferation; high amounts of IL-5 induce a downregulation of CD125
   - HLA-DR is one of the class II MHC molecules; it is involved in antigen presentation to CD4+ T cells and it is upregulated in activated eosinophils.

   [Time Frame: 12 months]

Secondary Outcome Measure:

2. (i) Association between activated eosinophils phenotype and disease progression; (ii) effect of biological therapies on eosinophils phenotype.
   Statistically significant difference surface molecular expression between controlled and uncontrolled asthma, responders and not responders to regular and biologic treatment subgroups by stratifying eosinophils receptor expression according to main clinical subgroups

   [Time Frame: 12 months]

Eligibility

Minimum Age:	18 Years
Maximum Age:	
Sex:	All
Gender Based:	No
Accepts Healthy Volunteers:	No
Criteria:	Inclusion Criteria:

- proved diagnosis of severe, refractory eosinophilic asthma, according to GINA recommendations and International ERS/ATS guidelines;
- agreeing to participate this study and signing an informed consent.

Exclusion Criteria:

- current smoking habit (both tobacco and e-cigarettes),
- concomitant diseases requiring chronic administration of immunosuppressors, biologic medications or systemic corticosteroids for any disease other than asthma
- History of previous or concomitant acute or chronic disease known to directly or indirectly affect eosinophil count, both in a quantitative and qualitative manner (eosinophilic lung and gastrointestinal diseases, systemic vasculitis, allergic bronchopulmonary aspergillosis, parasitic infections, etc)
- COPD/Asthma overlap.
- Inability or denial to sign the informed consent.

Contacts/Locations

Central Contact Person: Simone Scarlata, M.D.
Telephone: +393939259912
Email: s.scarlata@unicampus.it

Central Contact Backup:

Study Officials:

Locations: **Italy**
Campus Bio Medico University and Teaching Hospital
[Recruiting]
Roma, Italy, 00136
Contact: Simone Scarlata, MD +393939259912 s.scarlata@unicampus.it

IPDSharing

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References

Citations:

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