Beyond eosinophils: proteomics to identify potential biomarkers of organ damage and response to MEPOLIZUMAB in EGPA

Protocol Number: 14918

Compound Number or Name: **BEOMICS study**

Brief Title: **Beyond EOsinophils: proteoMICS to identify potential biomarker of organ damage and response to MEPOLIZUMAB in EGPA**

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SCIENTIFIC RATIONALE

Eosinophilic granulomatosis with polyangiitis (EGPA), former Churg Strauss Syndrome, is a systemic disease characterized by small vessel vasculitis, extravascular granulomas and peripheral blood eosinophilia, which typically occurs in the context of late-onset asthma and polypoid rhinosinusitis [1]. Although effective immunosuppressive treatment significantly improved the overall survival of EGPA patients, relapses are still common during follow-up [2], especially isolated asthma/rhinosinus exacerbations [3]. Recent evidence of pathogenetic mechanism shared among EGPA, severe asthma and other eosinophilic disorders, has supported the effective role of mepolizumab, an anti-IL-5 humanised monoclonal antibody, especially in the prevention of EGPA-related ENT and/or asthmatic relapses, with a significant GC- sparing effect [4–5]. There are still scant data on biomarkers that may predict of organ damage in EGPA patients and the heterogeneous spectrum of clinical symptoms makes more difficult the identification of a single biomarker.

A better understanding of potential biomarkers of response to immunosoppressive drugs, as well as the impact of novel anti-IL-5 agents on these clinical domains is essential to guide treatment choice and to improve long-term management of the disease.

In the OMICS era, an approach based on proteomics may highlight new insights of several molecular pathways all over different diseases. It was previously applied this methods in diseases such as primary immunodeficiency, non-histaminergic angioedema and mastocytosis[6]. In EGPA there is by now no data about proteomics, and, similarly, in severe asthma there is still limited available data of proteomics profiling albeit asthma is the most common chronic respiratory disease and has been declared a global public health problem by the World Health Organization [7].

RESEARCH QUESTION

Only a few studies investigated potential biomarkers in EGPA patients and their relation and evolution during treatment.

Aim of the study is to identify potential biomarkers, through a proteomic approach, which could be used to evaluate organ damage and predict the response to mepolizumab in a cohort of patients affected by EGPA. Proteomic analyses will be performed using a proteomic platform, based on a nano-HPLC- couplet to an high resolution ESI-MS device, on three types of biological matrices: blood, saliva and sputum samples in both EGPA and severe asthmatic patients (as controls) at baseline and at different time points after starting treatment with mepolizumab, an anti-IL-5 drug, in order to cluster patients and to analyze the effect of the therapy during treatment, assessing the disease progression on three key aspects: lung function and symptoms control, vasculitis and neuropathy. Plasma analysis will provide an overview of quantitative/qualitative proteomic variations at systemic level after drug administration; however, a less invasive procedure is often sufficient and would improve trial recruitment. On this regard, saliva is a biological fluid well suitable to be used in proteomic investigations for suggestion of potential disease biomarkers and includes various potential advantages compared with blood sample collection such as lower overall cost, lower infection risk, increased patient convenience, acceptability, compliance and uptake. Moreover, the protein composition of the human saliva includes both specific proteins of the oral cavity and proteins common to other tissues and bodily fluids, so saliva prognostic and diagnostic role is particularly interesting [8-9-10-]. Consequently, it was planned to compare the proteomic results of the non- invasive saliva testing to that of blood examination.

These data may be a further step to untangle the mechanisms of the disease and to characterize treatment's response, in the contest of a phenotype/endotype asthma management.

PRIMARY OBJECTIVES

the plan is to collect blood, salivary and sputum samples in both EGPA and severe asthmatic patients (as controls) at baseline and at different timepoints after starting treatment with mepolizumab, an anti-IL-5 drug, in order to cluster patients and to analyse the effect of the therapy during treatment and assess the disease progression on three key aspects of the disease: lung function and symptoms control, vasculitis and neuropathy.

SECONDARY OBJECTIVES

to untangle the mechanisms of the disease and to characterize response to treatment, in the context of a phenotype/endotype asthma and EGPA management.

STUDY POPULATION

According to the patients' groups evaluated in other publications with a robust statistical method and sample size calculation performed by the team, a sample of 90 patients can be considered sufficient to allow the realization of the study.

The plan is to enroll 90 patients followed in Allergy and Clinical Immunology Centers divided into three groups:

- ● 30 patients with EGPA with predominant ENT/asthmatic phenotype (longitudinal sampling before and after Mepolizumab treatment)
- ● 30 patients with EGPA with vasculitic phenotype before and after immunosuppressive drugs (CYC, AZA, MTX) and/or Mepolizumab.
- ● 30 patients with a diagnosis of severe eosinophilic asthma (longitudinal sampling before and after Mepolizumab treatment)

Inclusion criteria

A patient will be eligible for inclusion in this study if all the following criteria apply:

1. Informed Consent: Prior to start any study related activities, participants must be able and willing to provide written informed consent;
2. Participants must have a current clinical diagnosis of asthma or EGPA;
3. Physician decision to initiate treatment with mepolizumab.
4. Patient has to be in treatment with medium-high dose ICS plus an additional controller in the least 6 months before screening and, if on OCS therapy, a stable dosage of prednisone (or equivalent dose of other steroids) in the 4 weeks before screening will be allowed. The above-indicated treatment has to be maintained by the patient all along the study.
5. Adults aged 18 years or over.

Exclusion Criteria

A participant will not be eligible for inclusion in this study if any of the following criteria

apply:
1. asthmatic patients receiving other biological treatment
2. Participation in an interventional clinical trial in which the treatment regimen

and/or monitoring is dictated by a protocol during the previous 12 months. 3. pregnant and breastfeeding woman

STUDY DESIGN AND METHODS

During months 1-2, the Allergy and Clinical Immunology Centers will prepare the documentation to obtain Ethics Committee approval; an electronic database will also be created from this group to collect the data obtained during the study. At the end of this time, it is expected to have obtained approval of the Ethics Committee for samples and data collection.

Primary END POINTS

- Obtain a proteomic profile of EGPA patients and severe asthmatic patients from blood, saliva and sputum
- Analyse the samples after starting anti IL – 5 treatment (mepolizumab) and evaluate the possible disease modifying effect of the mepoliumab on vasculitis and lung function.

Secondary END POINTS
• to untangle the mechanisms of the disease and to characterize response to

treatment, in the context of a phenotype/endotype asthma and EGPA management.

Patients will be screened at visit 1 (T0) and if eligible will be enrolled. After obtaining informed consent, will collect the samples, respiratory lung function tests, and the clinical questionnaire.
Mepolizumab administration will be done every 4 weeks (±7days), as per the drug administration plan (i.e. 100mg SC Q4W). Other drugs will be administrated according to their administration plan and investigated in medical history. At each visit will be collected the clinical questionnaire and assessed the respiratory lung function.
Patients will receive mepolizumab at visit 2 (after 4 weeks ±7days). **At visit 3 (a time interval of 8 weeks ±7days from visit 1), mepolizumab administration will happen,** plus clinical assessment (questionnaire and respiratory lung function test). **At visit 4 (also identified as T1, 12 weeks ±7days after visit 1), the same assessments and analysis as at T0 will be performed,** as well as mepolizumab administration. Follow-up visit will be performed at **visit 5 (after 24 weeks ±7days from visit 1)** and patients will undergo somatosensory evoked potentials together with the collection of clinical questionnaire.

Then, at visit 6, which corresponds to T2 (after 52 weeks _± 7 days from visit 1), the same assessments and analysis as at T0 and T1 will be performed, as well as mepolizumab administration.
Study's total duration is 52 weeks, with follow-up after 4 weeks.

Safety profiles (AEs, SAEs and AEs of special interest) will be recorded throughout the study and at follow-up visit. The plan is to use our local template for adverse events. At the end of the study , GSK will be informed about the reported adverse events. Also, for pregnancies, they would be registered and strictly follow up on the pregnancy during the delivery and the breastfeeding period. If an enrolled patient will be found on pregnancy, she will exit the study.

The same timeline will be used for those patients with vasculitis phenotype and those affected by severe eosinophilic asthma, collecting biological samples before and after immunosuppressive and mepolizumab treatment.
Samples obtained from the patients at T0, T1 and T2, treated as reported in "Laboratory assessment and Samples preparation" section, will be sent to the proteomics unit for analysis.

Demographics and medical history

At study visits, patients' demographics and medical history (asthma disease and therapy history, immunosuppressive drugs) will be documented. Comorbidities in association with severe eosinophilic asthma, in particular chronic rhinosinusitis (CRS), with or without polyps (NP), will be recorded as analysis will be performed taking into account those three subgroups (i.e. according to the presence of CRS and/or NP).

Clinical Assessment and Questionnaires

Patients will be clinically evaluated. Clinical assessment will include physical examination, spirometry, patient's reports outcome [Asthma control test (ACT), Asthma Control Questionnaire 5 (ACQ-5), Sino- nasal outcome test 22 (if patient with CRSwNP), BIVAS, and VDI [10] [11]. Spirometry measurements will be obtained using spirometry equipment that meets or exceeds the minimal performance recommendations of the ATS [Miller, 2005]. All subjects will have spirometry performed at Screening and scheduled clinic visits during the treatment period as indicated in the Time and event table.
For FEV1 and FVC determinations, at least three acceptable spirometry efforts (with no more than 8) should be obtained. Acceptable spirometry efforts should have a satisfactory start of test and end of test (i.e. a plateau in the volume-time curve) and be free from artifacts due to cough, early termination, poor effort, obstructed mouthpiece, equipment malfunction, or other reasons [Miller, 2005]. The largest FEV1 and FVC from the three acceptable efforts should be recorded, even if they do not come from the same effort.

Spirometry must be performed as follows: After withholding salbutamol for 4 hours and ICS/LABA for 12 h. FeNO assessment will be performed after spirometry and even after four puffs of salbutamol.

In order to assess the adequacy of asthma control and the modification of rhinosinusitis, symptoms ACT, ACQ-5 and SNOT-22, respectively, will be submitted to patients at every visit. ACT and ACQ scores at T1 and T2 will be compared to baseline. The current dose of OCS and/or the withdrawal of OCS will be recorded at T0, T1, and T2.

The number of asthma exacerbations will be recorded and evaluated during the12 months.
Asthma exacerbations, defined as any of the following events, would be assessed: asthma attack needing an increase in oral prednisone dose, asthma-related emergency department admission, and/or use of acute oral glucocorticoids, antibiotics, or short-acting beta agonists.
For vasculitic patients, similarly to the previous published MIRRA trial a complete response to treatment was defined as no disease activity (BVAS = 0) and a prednisolone or prednisone dose (or equivalent) of ≤4.0 mg/day.
Blood samples, salivary samples and sputum samples are collected in fasting condition before respiratory measurements.

LABORATORY ASSESSMENT AND SAMPLES PREPARATION

Plasma, saliva and sputum proteome will be analyzed on a proteomic platform based on a nano-HPLC-high resolution ESI-MS device (LTQ Orbitrap Elite, Thermo Fisher Scientific) available at the "proteomic laboratory" of the University Service Center for Research of Cagliari (CeSAR) and by a Surveyor HPLC system connected to a low resolution LTQ XL mass spectrometer (Thermo Fisher Scientific San Jose, CA, USA), available at the proteomic laboratory of the Dept. of life and Environmental Sciences.

Peripheral blood samples of each patient will be collected into BD Vacutainer EDTA. To obtain cell-free plasma samples, the blood samples will be centrifuged at 1600 g for 10 minutes at +4°C. The plasma fraction of each patient will be stabilized by protease inhibitor, divided into aliquots, transferred to clean tubes and stored at –80°C for subsequent studies.

Unstimulated whole saliva will be collected during the morning and in fasting conditions with a plastic Pasteur pipette and will be added with an equal amount of PBS containing protease inhibitor and stored at -80°C (whole saliva for bottom-up proteomics).

Sputum samples will be collected after 15–30 min inhalation of hypertonic saline in concentrations of 3%, treated as salivary samples and stored at -80°C. the procedure for analyzing the proteomic profile in the sputum is the same as saliva samples. The traditional use of sputum induction is not the goal of our research, indeed the eosinophils count in the sputum is not one of our purposes.

PROTEOMIC ANALYSIS

Proteomic analysis based on mass spectrometry (MS) analysis will be performed to evaluate qualitative and quantitative alterations in the saliva, sputum and plasma proteomes associated with:

• EGPA with predominant ENT/asthmatic phenotype (before and after Mepolizumab treatment)

• EGPA with vasculitic phenotype before and after immunosuppressive drugs (CYC, AZA, MTX) and/or Mepolizumab.

• Severe eosinophilic asthma (already with established Mepolizumab treatment)

1. saliva and sputum: the comparison of the protein profiles among the three groups of samples will allow us to highlight quali/quantitative changes associated with EGPA before and after Mepolizumab treatment, immunosuppressive drugs and eosinophilic asthma. The approach will be suitable to identify peptides/proteins that are potentially useful as biomarkers for EGPA diagnosis. Salivary and sputum samples (n=30 from each patient group at T0 T1 and T2 of medical treatment, total 300 samples) will be submitted to protein technique processing in bottom-up proteomics by filter aided sample preparation (FASP), including tryptic digestion, after reduction/alkylation of Cys residues, recovery of tryptic fragments and their analysis by HPLC-ESI-MS for quantification and identification.
2. Plasma: plasma is a very complex protein matrix where the MS signals of high- abundance proteins hide those from other proteins, so plasma samples (n=30 from each patient group at T0 T1 and T2 of medical treatment, total 180 samples) will undergo to depletion of albumin and the 20 most abundant plasma proteins with a commercial kit to improve the detectability of low abundance proteins. Plasma samples will be used to specifically analyze the peptides and/or proteins

individuated as potential biomarkers in EGPA samples. Thus, to detect them, depending on their molecular weight (MW), suitability for top-down proteomics, and abundance, there will applied one of these different approaches: Bottom-up proteomics based on FASP processing of plasma proteins and HPLC-ESI-MS analysis to detect proteins not suitable for a top-down proteomic analysis or with medium/high abundance; ultrafiltration with 30 KDa cut-off membranes to obtain two fractions with a simpler composition that facilitates the detection of low-abundance proteins. A protein fraction <30 kDa is useful for low-MW protein and peptide characterization by a top- down approach, and a protein fraction >30 kDa is useful for proteins with high MW, which will be analyzed by HPLC-ESI-MS analysis with a bottom-up approach. Before any MS analysis, the total protein concentration will be measured in each sample to normalize quantitative data. Proteomic analysis will be performed by both high-resolution (HR), 120,000 at m/z 200 in full MS mode, and low-resolution (LR) mass spectrometry, 60,000 at m/z 200 in full MS mode. For HR-MS it will be used the nano- HPLC-ESI-LTQ Orbitrap Elite (Thermo Fisher Scientific San Jose, CA, USA) available by payment at the University Service Center for Research of Cagliari (CeSAR); for the LR-MS, it will be used the HPLC- ESI-LTQ XL-MS instrument (Thermo Scientific) available in the Proteomic Lab of the Department of Life and Environmental Sciences, Biomedical section.

-The HR-MS platform will be used for the identification of peptides/proteins, either in top-down or in bottom-up proteomics modalities, through amino acid sequencing based on data-dependent MS/MS experiments. In addition, post-translational modifications will be characterized. All MS/MS data will be elaborated by the ProteomeDiscoverer software v.2.4 (Thermo Scientific). Spectra will be recorded in the 300–2000 m/z scanning range in the positive ion mode and ions fragmented with higher energy collisional dissociation (HCD) or collision induced dissociation (CID). Protein identification will be performed using the reviewed Swiss-Prot human database with a precision tolerance of 10 ppm for peptide masses and 0.02 Da for fragment ion masses. Proteins will be identified with at least two unique peptides and a significance threshold FDR = 0.01. In plasma samples, beyond full protein profiling, targeted peptides/proteins will be selectively searched and identified by consecutive reaction monitoring.

- The LR-MS platform will be used for explorative experiments and to quantify target proteins/peptides, due to the high sensitivity of the instruments, which are able to detect up to femtomolar concentrations.

Moreover, target components detectable either as intact proteins or peptides in top- down mode or through their unique tryptic fragments in bottom-up mode, can also be quantified by the LR-MS platform by measuring the AUC (area under the curve) of either multiple components with the extracted ion current technique, or few components with the selected ion monitoring technique.

- Quantification of peptides and proteins can be performed in label-free way (Levin, Y.; Schwarz, E.; Wang, L.; Leweke, F. M.; Bahn, S. Label-free LC-MS/MS quantitative proteomics for large-scale biomarker discovery in complex samples. J. Sep. Sci. 2007, 30 (14), 2198–203.) or via selected reaction monitoring (SRM) by spiking unlabeled samples with known concentrations of isotopically-labeled synthetic peptides [Allergy. 2020 Dec;75(12):3171-3183.doi: 10.1111/all.14406.].

d) Quantitative MS data will be compared by statistical analyses with GraphPad Prism 5.0 and Perseus software, to highlight significant differences between the protein profiles of:

• EGPA with predominant ENT/asthmatic phenotype (before and after Mepolizumab treatment)

•EGPA with vasculitic phenotype before and after immunosuppressive drugs (CYC, AZA, MTX) and/or Mepolizumab.

•severe eosinophilic asthma
(already with established Mepolizumab treatment).

-MS results will undergo technical verification by immune-detection methods (western-blot or dot-blot) in the same samples for target proteins and peptides individuated as candidate biomarkers.

SAFETY MONITORING AND REPORTING
Definition of adverse events and reporting requirements Adverse events (AEs)

An AE is any untoward medical occurrence (e.g. any unfavorable and unintended sign [including abnormal laboratory findings], symptom or disease) in a subject or clinical investigation subject after providing written informed consent for participation in the

study. Therefore, an AE may or may not be temporally or causally associated with the use of a medicinal product. The investigator has the responsibility for managing the safety of individual subject and identifying AEs. The occurrence of AEs must be sought by non-directive questioning of the subject at each visit or, for self-administering subjects at each scheduled safety phone call, during the study. AEs also may be detected when they are volunteered by the subject during or between visits or telephone calls or through physical examination findings, laboratory test findings, or other assessments. AEs must be recorded under the signs, symptoms or diagnosis associated with them, accompanied by the following information (as far as possible) (if the event is serious refer to Section 1.2): the Common Toxicity Criteria (CTC) AE grade (version 5 or higher);

- its relationship to the investigational study treatment and other study treatment.

-its duration (start and end dates), or if the event is ongoing, an outcome of not recovered/not resolved, must be reported;

- whether it constitutes a SAE (see Section 1.2 for definition of SAE) and which seriousness criteria have been met.

- action taken regarding with study treatment
- its outcome i.e., its recovery status or whether it was fatal.
All AEs must be treated appropriately. Treatment may include 1 or more of the following: Dose not changed - Dose Reduced/increased - Drug interrupted/permanently discontinued;

Relevant conditions that were already present at the time of informed consent should be recorded in medical history of the subject. AEs (including lab abnormalities that constitute AEs) should be described using a diagnosis whenever possible, rather than individual underlying signs and symptoms. Once an AE is detected, it must be followed until its resolution or until it is judged to be permanent (e.g., continuing at the end of the study), and assessment must be made at each visit (or more frequently, if necessary) of any changes in severity, the suspected relationship to the interventions required to treat it, and the outcome.

Serious adverse events (SAEs)

An SAE is defined as any AE, i.e. appearance of (or worsening of any pre-existing) undesirable sign(s), symptom(s), or medical conditions(s) which meets any one of the following criteria: fatal OR life-threatening.

Life-threatening in the context of a SAE refers to a reaction in which the subject was at risk of death at the time of the reaction; it does not refer to a reaction that hypothetically might have caused death if it were more severe (please refer to the ICH- E2D Guidelines) results in persistent or significant disability/incapacity constitutes a congenital anomaly/birth defect requires inpatient hospitalization or prolongation of existing hospitalization, unless hospitalization is for: routine treatment or monitoring of the studied indication, not associated with any deterioration in condition elective or pre- planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since signing the informed consent social reasons and respite care in the absence of any deterioration in the subject's general condition treatment on an emergency outpatient basis for an event not fulfilling any of the definitions of a SAE given above and not resulting in hospital admission is medically significant, e.g. defined as an event that jeopardizes the subject or may require medical or surgical intervention to prevent one of the outcomes listed above. Medical and scientific judgment should be exercised in deciding whether other situations should be considered serious reactions, such as important medical events that might not be immediately life threatening or result in death or hospitalization but might jeopardize the subject or might require intervention to prevent one of the other outcomes listed above. Such events should be considered as "medically significant." Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias, or convulsions that do not result in hospitalization or development of dependency or abuse (please refer to the ICH-E2D Guidelines).

All malignant neoplasms will be assessed as serious under "medically significant" if other seriousness criteria are not met. Any suspected transmission via a medicinal product of an infectious agent is also considered a serious adverse reaction.

All reports of intentional misuse and abuse of the product are also considered SAE irrespective of whether a clinical event has occurred or not.

STATISTICAL PLAN

Data will be analyzed by SPSS for MS Windows, version 18.0 (SPSS, Inc, Chicago, IL, USA).

The primary analysis is to determine the difference of proteomic profile at T1 and T2 versus T0 (baseline) and to evaluate this percentage in relation to subject condition (CRS, NP). This will be addressed using parametric and non-parametric tests and clustering techniques on flow cytometry data (cytograms and sample composition). Results will be expressed as mean values SEM. Mann-Whitney U test will be used for two-group comparisons. Wilcoxon signed-rank test will be used for paired data. When more than two groups will need to be analyzed, ANOVA and Dunnett's multiple comparison test will be used. Correlation will be evaluated by the Spearman rank test.

Differences will be considered significant if P values were <.05.

LIMITATIONS

The study could be limited from the relatively small size group of vasculitis patients.

Another limit could be due to the collection of anamnestic data regarding the disease onset from patients already in mepolizumab treatment or under immunosuppressive drug(s).

Funding

The study is supported by GSK.

Ethics

The study will be performed after collecting a valid informed consent in accordance with the Good Clinical Practice and with the Declaration of Helsinki.

Data policy and informed consent

At visit 1 the study is presented to potential eligible patients. They will be suggested to read with attention the information sheet and the informed consent. Only those patients who read and sign the informed consent can be enrolled in the study.

REFERENCES

1. Jennette JC, Falk RJ, Bacon PA, Basu N, Cid MC, Ferrario F, et al. 2012 revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides. Arthritis Rheum. 2013;65:1–11.
2. Samson M, Puéchal X, Devilliers H, Ribi C, Cohen P, Stern M, et al. Long-term outcomes of 118 patients with eosinophilic granulomatosis with polyangiitis (Churg-Strauss syndrome) enrolled in two prospective trials. J Autoimmun. 2013;43:60–9.

3. Puéchal X, Pagnoux C, Baron G, Lifermann F, Geffray L, Quémeneur T, et al. Non-severe eosinophilic granulomatosis with polyangiitis: long-term outcomes after remission- induction trial. Rheumatol Oxf Engl. 2019;58:2107–16.
4. Kahn J-E, Grandpeix-Guyodo C, Marroun I, Catherinot E, Mellot F, Roufosse F, et al. Sustained response to mepolizumab in refractory Churg-Strauss syndrome. J Allergy Clin Immunol. 2010;125:267–70.

5. Wechsler ME, Akuthota P, Jayne D, Khoury P, Klion A, Langford CA, et al. Mepolizumab or Placebo for Eosinophilic Granulomatosis with Polyangiitis. N Engl J Med. 2017;376:1921–32.

6. Serrao S, Firinu D, Olianas A, Deidda M, Contini C, Iavarone F, Sanna MT, Boroumand M, Amado F, Castagnola M, Messana I, Del Giacco S, Manconi B, Cabras T. Top-Down Proteomics of Human Saliva Discloses Significant Variations of the Protein Profile in Patients with Mastocytosis. J Proteome Res. 2020 Aug 7;19(8):3238-3253. doi: 10.1021/acs.jproteome.0c00207. Epub 2020 Jul 6. PMID: 32575983; PMCID: PMC8008451.

7. Yanting Lan, Xiaoyin Zeng, Jing Xiao, Longbo Hu, Long Tan, Mengdi Liang, Xufei Wang, Shaohua Lu, Fei Long & Tao Peng (2021) New advances in quantitative proteomics research and current applications in asthma, Expert Review of Proteomics, 18:12, 1045- 1057, DOI: 10.1080/14789450.2021.2017777

8. Tabak, L. A. A revolution in biomedical assessment: the development of salivary diagnostics. J. Dent. Educ. 2001, 65, 1335-1339.
9. Cabras, T.; Iavarone, F.; Manconi, B.; Olianas, A.; Sanna, M. T.; Castagnola, M.; Messana,I. Top-down analytical platforms for the characterization of the human salivary proteome.Bioanalysis 2014, 6, 563, 581.

10. Bandhakavi, S.; Stone, M. D.; Onsongo, G.; Van Riper, S. K.; Griffin, T.J. A dynamic range compression and three-dimensional peptide fractionation analysis platform expands proteome coverage and the diagnostic potential of whole saliva. J. Proteome Res. 2009, 8, 5590-5600.

11. Steinfeld, J., Bradford, E. S., Brown, J., Mallett, S., Yancey, S. W., Akuthota, P., Cid, M. C., Gleich, G. J., Jayne, D., Khoury, P., Langford, C. A., Merkel, P. A., Moosig, F., Specks, U., Weller, P. F., & Wechsler, M. E. (2019). Evaluation of clinical benefit from treatment with mepolizumab for patients with eosinophilic granulomatosis with polyangiitis. The Journal of allergy and clinical immunology, 143(6), 2170–2177. https://doi.org/10.1016/j.jaci.2018.11.041

12. Doubelt, I., Cuthbertson, D., Carette, S., Chung, S. A., Forbess, L. J., Khalidi, N. A., Koening, C. L., Langford, C., McAlear, C. A., Moreland, L. W., Monach, P. A., Seo, P., Specks, U., Spiera, R. F., Springer, J. M., Sreih, A. G., Warrington, K. J., Merkel, P. A., Pagnoux, C., & Vasculitis Clinical Research Consortium (2021). Clinical Manifestations and Long-Term Outcomes of Eosinophilic Granulomatosis With Polyangiitis in North America. ACR open rheumatology, 3(6), 404–412. https://doi.org/10.1002/acr2.11263

EPOCH	Screening/Enrolled	Study			Follow-up		EOT
VISIT NUMBER	**Visit 1 (T0)**	Visit 2	Visit 3	**Visit 4 (T1)**	Visit 5	**Visit 6 (T2)**	Visit 7
Days	**1**	**29**	**57**	**85**	**169**	**365**	**393**
Weeks	**0**	**4 ±7days**	**8 ±7days**	**12 ±7days**	**24 ±7days**	**52 ±7days**	**56 ±7days**
Informed consent/assent	X						
Inclusion/exclusion criteria	X						
Medical history	X1	X1	X1	X1	X1	X1	X1
AE/SAE			X	X	X	X	X
Blood count	X			X		X	
Clinical Assessment and Questionnaires							
Physical examination	X	X	X	X	X	X	X
Current dose of OCS	X	X	X	X	X	X	X
Number of asthma exacerbations	X	X	X	X	X	X	X
ACT	X		X	X	X	X	X
ACQ5	X		X	X	X	X	X
SNOTT22	X		X	X	X	X	X
BIVAS	X2			X2		X2	X2
VDI	X2			X2		X2	X2
Lung function tests							
Spirometry	X		X	X		X	X
FeNO	X		X	X		X	X
Drug administration							
Mepolizumab 100mg		X3	X3	X3	X3	X3	X3
Proteomics sample							
Saliva	X			X		X	
Sputum	X			X		X	
Plasma	X			X		X	
Diagnostic							
Somatosensory evoked potentials (SSEPs)					X		

X1: all the therapy taken by the patient, like asthma therapy or immunosoppressive drug, and their specific dose.
X2: not applicable in patients without a diagnosis of EGPA
X3: if the patient has EGPA with vasculitic phenotype and takes immunosuppressive drugs (CYC, AZA, MTX) without taking Mepolizumab, don't administrate it.

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