PROTOCOL NAME: REBUC: Role of Epstein-Barr virus in Ulcerative Colitis

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I have read this Protocol Amendment relevant to the study entitled “REBUC: Role of Epstein Barr virus in Ulcerative Colitis” and I agree to conduct the study as detailed herein and in compliance with guidelines for Good Clinical Practice and applicable regulatory requirements. I will provide all study personnel under my supervision with all information provided by the Sponsor and I will inform them about their responsibilities and obligations.

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Glossary of abbreviations:

EBV	Epstein-Barr virus
EBER	EBV-encoded RNAs
eCRF	Electronic Case Report Form
GCP	Good Clinical Practice
HCMV	Human Citomegalovirus
IBD	Inflammatory Bowel Disease
IBS	Irritable Bowel Syndrome
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
ISH	*in situ* hybridization
IU	International Units
qRealTime-PCR	quantitative Real Time Polymerase Chain Reaction
SCCAI	Simple Clinical Colitis Activity Index
UC	Ulcerative Colitis
UCEIS	Ulcerative Colitis Endoscopic Index of Severity
VCA	Viral Capside Antigen
95%CI	95% confidence interval

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1 Summary

Title	**REBUC: Role of Epstein-Barr virus in Ulcerative Colitis**
Investigator sponsor	Italian Group for Inflammatory Bowel Diseases
Study coordinator	Rachele Ciccocioppo
Protocol identifying number	
Protocol version date	**4.0 – 03/10/2022**
Background and rationale	The role and the appropriate management of Epstein-Barr virus (EBV) infection in ulcerative colitis (UC) are the object of a wide debate. Therefore, the possibility to carry out a multicenter study aimed at assessing the true prevalence, the best diagnostic tool, the risk factors and the role of EBV infection in UC is expected to be of meaningful clinical value in the decisional algorithm for the correct management of this condition. Participation of IG-IBD Centers guarantees the recruitment of all cases needed, while the high expertise of the diagnostic tests carried out in both the Molecular Virology Unit in Pavia and Service of Pathology in Brescia guarantee the accuracy of data.
Population and patient selection criteria	A cohort of 100 adults (18-75 years) patients with UC, either naïve or under standard of care (both responders and non-responders to therapies) will be prospectively enrolled. In all cases, the Montreal classification, and the clinical and endoscopic indexes of activity will be carefully assessed. The control group consists of 100 sex- and age-matched subjects, selected in the same Center, undergoing colonoscopy as examination in the diagnostic algorithm for lower abdominal symptoms and that results normal, thus confirming the diagnosis of irritable bowel syndrome, and who were not taking drugs possibly affecting immune response and microbiota composition. A small cohort of patients (n=20) suffering from microscopic colitis will be also enrolled as pathological control group. All enrolled patients and controls will undergo lower endoscopy with mucosal sampling as diagnostic workup for their condition. Patients with active UC undergo colonscopy and laboratory tests according to international

guidelines and depending on physician judgement. More in depth, active UC patients will undergo colonscopy at baseline, while laboratory tests will be performed at baseline, and at 3 and 6 months. As optional follow-up, accordingly with physician evaluation, some patients will undergo medical examination and laboratory tests at 9 and 12 months, and colonscopy at 12 months. The aim of the study is settled at 6 months, but adding the optional follow-up at 9 and 12 months, will aid us to understand if patients with EBV colitis undergo colectomy more frequently than patients whithout EBV colitis. Patients with UC in remission will undergo colonscopy and laboratory tests according to ECCO-ESGAR guidelines [52]. Patients with suspicion of irritable bowel syndrome will undergo colonscopy and laboratory tests if alarm features are present (aged>45-50 years, anemia, weight loss, familiar history of colon cancer, blood mixed in the stool). The exclusion criteria include: significant comorbidities, malignancy, pregnancy, organ failure, immunodeficiencies, active infections, recent abdominal surgery, or invalidating psychiatric-neurological disorders.

The sample size has been calculated based on the primary endpoint, i.e., the prevalence of EBV infection in UC patients. The power to detect a significant difference with the Fisher exact test from an expected proportion in controls of 1% is above 80% for proportions of infection of 20% or greater.

Study design and study duration

This is an investigator-initiated prospective multicentre interventional without drug and medical device study evaluating the prevalence, role, life phase and the most accurate diagnostic method of EBV infection in UC as assessed by means of traditional method (*in situ* hybridization) and quantitative RealTime-PCR assay. A longitudinal study is included to assess whether EBV infection has an impact on patients' outcome. The study duration is three years.

Objectives and end-points

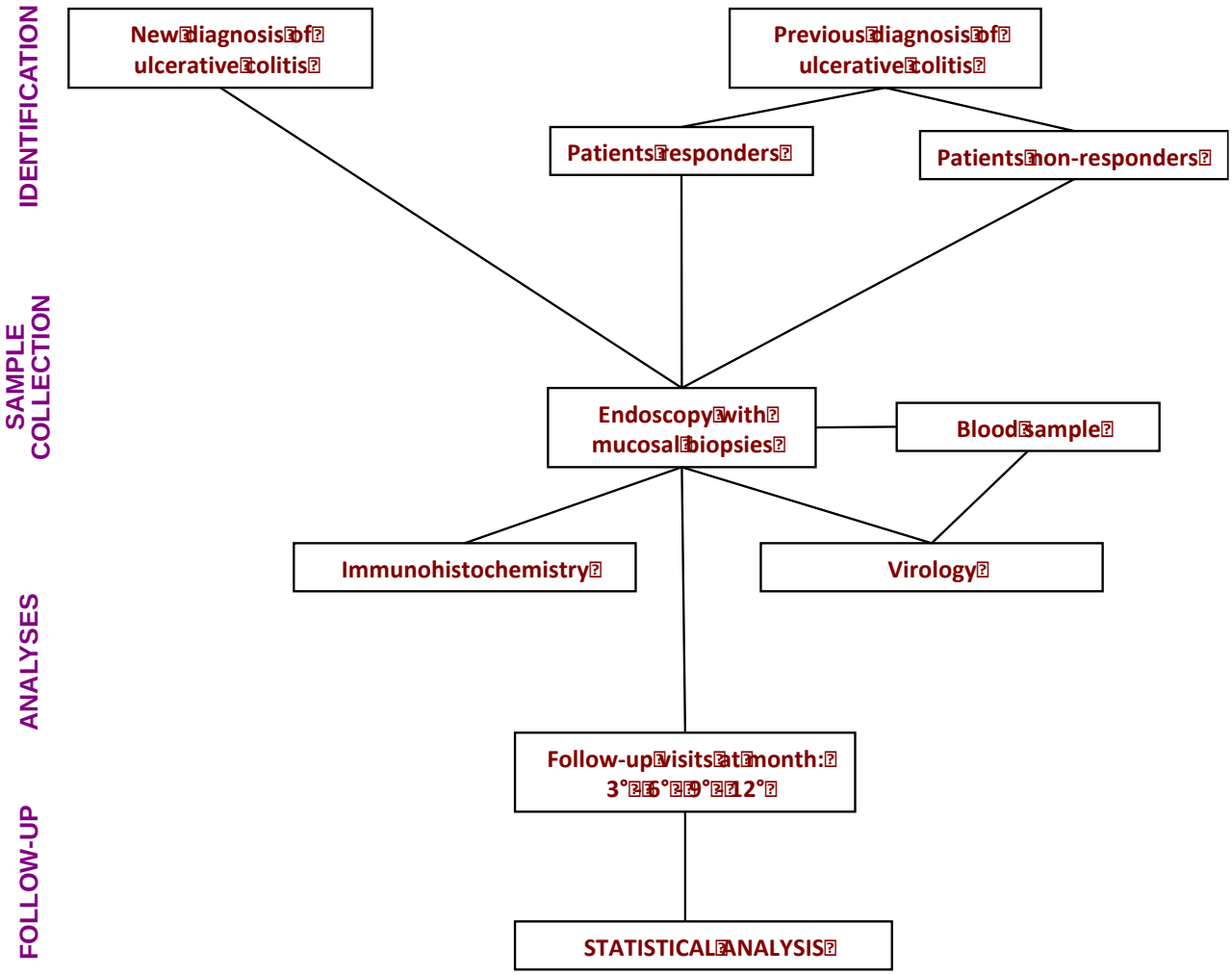
The primary objective consists of the evaluation of the prevalence of EBV infection, defined as isolation of the EBV or detection of viral proteins or nucleic acids in any body fluid or tissue specimens. ~~in~~ in patients suffering from UC. The same analyses will be performed in a comparable cohort of non-IBD patients who undergo lower endoscopy during their work-up for irritable bowel syndrome (IBS) and microscopic colitis. ~~(new diagnosis, responders, non-responders) in order to unravel whether it is a bystander or causative of the onset/relapse of the disease and whether it is responsible for refractoriness to standard of care.~~ The secondary objectives include: a) comparison of the presence of EBV infection in the cohort of patients suffering from UC and divided into the following subgroups: new diagnosis, responders, non-responders, in order to unravel whether it is a bystander or causative of the onset/relapse of the disease and whether it is responsible for refractoriness to standard of care; b) evaluation of the diagnostic ability in detection of EBV infection of ISH performed on mucosal speciemens with respect to qRealTime-PCR; ~~of *in situ* hybridization with respect to quantitative RealTime-PCR;~~ c) evaluation of diagnostic ability in detection of EBV infection between qRealTime-PCR performed on peripheral blood samples with respect to mucosal specimens; ~~association between viral load and clinical and endoscopic indexes of activity~~ c) evaluation of association between therapeutic agents and EBV infection; d) evaluation of association between mucosal/blood viral load and mucosal damage as endoscopically assessed; e) evaluation of association between clinical index of UC activity and EBV infection; f) evaluation of association between steroid therapy (absent, previous or current) and EBV infection; g) evaluation of association between immunosuppressive therapy (absent, previous or current) and EBV infection; h) evaluation of association between biological therapy (absent, previous or current) and EBV infection; i) evaluation of the prognostic role of EBV infection on the 6 and 12 month outcome (colectomy / number of hospitalization / cause of

	hospitalization). The exploratory endpoints include the study of the virus life cycle phase and its correlation with disease activity.
Statistical methods, data analysis	Data will be described with the mean and standard deviation or the median and 25th-75th percentiles if continuous and as counts and percent if categorical. For the primary endpoint we will compute the prevalence as the ratio of the number of patients diagnosed with EBV infection to the number of patients evaluated, together with its 95%CI. We will use the Fisher exact test to compare the prevalence in UC patients to that of control. We will use binomial regression models to adjust for potential confounders. We will compute sensitivity, specificity, area under the ROC curve and predictive values with 95%CIs. We will use the Spearman R and the Fisher exact test to assess the association, for continuous and categorical independent variables, respectively, with dependent variables. We will use Cox regression to assess the association of EBV infection with colectomy and of hospitalization for any cause; we will use Poisson regression to assess the association of EBV infection with the number of hospitalization from any cause. We will use Stata 17 or later versions for computations. A 2-sided p-value <0.05 will be considered statistically significant. We will apply the Bonferroni correction for post-hoc comparisons.
Ethical considerations	The protocol and its annexes are subject to review and approval by the competent Independent Ethics Committee(s). Each patient and control gives written informed consent after approval by the local Ethics Committee is obtained. Both the Principal Investigators and the Study Coordinator will ensure that this study is conducted in agreement with either the Declaration of Helsinki and the Italian laws and regulations.
Study time table	Project starting date: 30.10.2022 Project completion of data collection: 30.05.2025

Project data analysis: 31.07.2025

Project presentation of scientific report: 30.10.2025

2 STUDY FLOW CHART FOR ACTIVE ULCERATIVE COLITIS PATIENTS



3 BACKGROUND AND INTRODUCTION

- Inflammatory bowel disease (IBD), namely Crohn's disease and ulcerative colitis (UC), are emerging chronic and disabling enteropathies triggered and sustained by a dysregulated immune response towards antigens of the gut microbiota in genetically susceptible individuals (1,2). The growing use of immunosuppressive therapies, mainly based on biological agents, to achieve mucosal healing and prevent disease progression (3), has led to an increased risk of opportunistic infections (4,5,6), including that caused by the Epstein-Barr virus (EBV) (7). In this regard, although a considerable number of studies support the notion that colonic involvement through opportunistic viral infection in existing IBD is associated with a worsening of symptoms, increased prevalence of refractoriness and surgical intervention [8,9,10,11], the role played by EBV in UC, whether contributor or innocent bystander, remains a topic of ongoing controversy [12,13]. EBV infection is usually acquired early in the life and is generally asymptomatic in healthy people where it establishes a lifelong latency in target cells (memory B-cells) (14), while it reactivates in case of reduced host immunity (15). EBV, indeed, exists in two possible states of its life cycle, latent and lytic (16,17,18). The latter usually occurs in immunocompromised hosts where it eventually results in systemic or end-organ diseases leading to increased morbidity and mortality (19). Localizing in the gastrointestinal tract (20,21) represents a clinical challenge in IBD since it becomes hard to distinguish between a superimposed viral colitis and a relapse of the underlying disease. In this regard, the ECCO guidelines state that: "*Screening for EBV infection* (through the search of specific anti-virus immunoglobulin) *before initiation of immunomodulator therapy should be considered. In severe primary clinical EBV infection during immunomodulator therapy, antiviral therapy may be considered and immunomodulator therapy discontinued. In the event of EBV-driven lymphoproliferative disease during immunomodulator therapy, the patient should be managed in conjunction with appropriate specialists. Immunomodulators should be stopped*" (22). It appears clear that the main focus is lymphoma-driven (23), with almost no mention of secondary symptomatic infection due to reactivation of the lytic phase of the virus life cycle, that is the most frequent condition in clinical practice. As a consequence, several crucial points still remain unsolved, such as its true prevalence and role in tissue damage, the best diagnostic and therapeutic approach, as well as the identification of risk factors still remains elusive.
- This is why a number of important issues related to EBV infection in the specific clinical setting of IBD, namely its life cycle, its interaction with the host immune system, and virus–

cell interaction has received little or no attention. Interestingly, the clinical features of UC, such as the pick of onset at young age, the association with a life-stressing event, the unpredictable clinical course, and even the evolution towards toxic megacolon, all fit with EBV infection. Moreover, our previous demonstration of the presence of EBV DNA in epithelial cells of those samples harvested from diseased UC mucosa but not from healthy mucosa of non-IBD controls (24) gives an explanation of the contiguity of lesions, that is a hallmark of this condition. Indeed, it is known that EBV uses epithelial cells for its lytic cycle and to spread locally in tissues and, possibly, systemically (17,25). It is conceivable, therefore, that in the event of switch of virus infection from latency to lytic phase, the ability to infect neighboring epithelial cells (26,27) is responsible for the extension of its localization to more proximal tracts. Moreover, the loss of the ability of the host immune system to circumvent this switch due to malnourishment and/or therapies (28) whose main target are T-cells which play a crucial role in controlling EBV latency and reactivation (18), represents a potential trigger for a superimposed viral colitis. Also a key event in the life of a person may largely affect the mutual relationship between the virus and the host, giving rise to the onset the disease and/or of its flare-up (29).

- Therefore, we aim to prospectively evaluate the presence of EBV infection with the analysis of its cycle phase (latent or lytic) in a cohort of UC patients, in order to investigate its contribution to disease onset, activity and relapse, together with the analysis of possible risk factors of its activation. Finally, shell we find the capability of EBV to establish latency (30) even in epithelial cells, the possibility of its contribution in the development of dysplasia/carcinoma sequence in inflamed mucosa becomes a valid hypothesis (31).
- To date, the information available on the frequency, role and risk factors of EBV infection/disease in UC as well as its diagnostic and therapeutic approach is conflicting (7,10,12,20,21). The causes for this discrepancy lie in the differences amongst the patients enrolled, the diagnostic methods applied, and the retrospective design of the majority of studies. Therefore, no firm conclusion may be drawn on the real incidence and role of EBV infection in UC. Surely, an increased risk of lymphoma has been found in IBD patients under immunosuppressive therapy, especially among young males under thiopurines, where a role for primary EBV infection has been proposed (23,32) as in the case of post-transplant lymphoproliferative disease (33). Following the ECCO guidelines for the diagnosis and treatment of opportunistic infections, where only a short paragraph is reserved to EBV (22), the diagnosis of primary infection should be based on the evidence of the detection of class M and G immunoglobulin against the EBV viral capsid antigen (VCA) with negative EBNA1

IgG, whereas no mention of reactivation of latent infection is given. Moreover, it is stated that viral load measurement (without specify whether in the blood or at mucosal level) after establishment of an immunomodulatory therapy has negligible relevance without an EBV-associated disease (without specify whether systemic or an end-organ disease, like colitis). It appears clear that the issues regarding the management of EBV infection in IBD are extrapolated from the haematologic conditions, since no robust experience in this gastroenterologic setting is available. At this point, it should be underpinned that the definitions of EBV infection and colitis have been often used interchangeably, thus creating further confusion. Moreover, no recommendations are given about the therapeutic management of a superimposed EBV colitis in UC and, on the other side, no valid treatments are available for EBV infection itself. Finally, as far as the outcome is concerned, a clear association between EBV infection and a poor prognosis, mainly in terms of an increased incidence of colectomy, appears evident from the literature (34).

- Our previous studies have clearly showed the need for quantitative real time polymerase chain reaction (qRealTime-PCR) analysis performed on mucosal samples not only for its better diagnostic accuracy in comparison to *in situ* hybridization (ISH) and immunohistochemistry, but mostly in order to stratify the patients, since a positive result does not necessarily imply that the patient is suffering from an EBV-related end-organ disease (35). Indeed, we identified a cutoff of 1000 copies/100.000 cells as the value to distinguish a superimposed viral colitis from a pure relapse of the underlying disease (35). Furthermore, since no efficacious therapy is currently available for symptomatic EBV infection, the only possible intervention consists of a quick tapering and then discontinuation of steroids, while improving malnutrition if present. By contrast, the immunosuppressive and biological agents could be continued by virtue of their long-lasting effects. We also suggested a note of caution in prescribing steroids and biologics in those patients carrying a mucosal viral load of between 100 and 1000 copies, who should be closely monitored, while no modification of the standard treatment is needed for those with a mucosal viral load lower than 100 copies (36). In this regard, it should be emphasized that the search for circulating class M specific anti-virus antibodies is rather ineffective in detecting an active disease, since elevated levels can persist for up to two years after infection, while immunocompromised patients may not mount an IgM response (37).
- In addition, our evidence of localization of virus infection in colocytes represents the first step towards understanding the mechanisms of viral dissemination and the role of the lytic phase in tissue damage (24). Indeed, a suggested viral tropism to sites of inflammation, and its

ability to affect cytokine production (20) may account for how it escapes host immune surveillance directly at mucosal level. Here, resting memory B cells serve as the long-term reservoir of latent EBV infection, and their differentiation into plasma cells in response to inflammatory stimuli may trigger the lytic phase and subsequent release of viral particles (38), leading to viral replication (15,16,17). Moreover, T-cell action in controlling viral replication (18) may be hampered by concurrent malnourishment and immunosuppressive drugs, thus resulting in an uncontrolled activation of the lytic phase of the viral life cycle which localizes precisely in the target organ (21).

- Therefore, we aim to carry out a multicenter interventional without drug and medical device study in order to definitely prove whether EBV infection represents a causative factor in tissue damage or a surrogate marker of severe colitis (13), an issue that may greatly contribute to understanding the mutual relationship between the host and the virus and to correctly manage the patients. The parallel analysis of the Human Cytomegalovirus (HCMV) load will assure a better understanding of the role of EBV. The possibility to involve the IG-IBD Centers gives the assurance for the study feasibility. Furthermore, the presence of clinicians with high expertise on histologic examination of IBD samples (Doctor Vincenzo Villanacci) and opportunistic viral infections (Prof. Fausto Baldanti) gives more strength to the proposal. We thus intend to recruit patients suffering from UC, either naïve to therapy or under treatment (both responders and non-responders) and non-IBD controls in order to evaluate the real prevalence, role and risk factors of EBV infection in this peculiar clinical setting. Moreover, by using qRealTime-PCR and ISH in parallel, we want to definitely establish the better diagnostic tool. Finally, the duration of three years seems the right length of the study to recruit a sizable number of patients and evaluate their outcome, as well as to perform the analysis and interpretation of results.

4 RATIONALE OF THE STUDY

The role and the appropriate management of EBV infection in UC are the object of a wide debate. Therefore, the possibility to carry out a multicenter study aimed at assessing the true prevalence, the best diagnostic tool, the risk factors and the role of EBV infection in UC is expected to be of meaningful clinical value in the decisional algorithm for the correct management of this condition.

5 OBJECTIVES OF THE STUDY

5.1 General objectives

The main study objective is to evaluate the true prevalence of EBV infection in a cohort of UC patients in comparison with a cohort of non-IBD patients. In parallel, the identification of the more accurate diagnostic approach, the association with clinical and endoscopic indexes of activity, as well as the risk factors will be evaluated. Finally, the outcome of these patients will be assessed to establish the correct prognosis and relationship with EBV infection.

5.2 End-points

5.2.1 Primary endpoint

Evaluation of the prevalence of EBV infection, defined as isolation of the EBV or detection of viral proteins or nucleic acids in any body fluid or tissue specimens, in patients suffering from UC. The same analyses will be performed in a comparable cohort of non-IBD patients who undergo lower endoscopy during their work-up for irritable bowel syndrome (IBS) and microscopic colitis (as stated above). The parallel assessment of the HCMV load will avoid false interpretation of the role of EBV.

5.2.2 Secondary endpoint

a- Comparison of the presence of EBV infection in the cohort of patients suffering from UC and divided into the following subgroups: a) new diagnosis; b) responders to conventional therapies; c) non-responders to conventional therapies, in order to unravel whether it is a bystander or causative of the onset/relapse of the disease and whether it is responsible for refractoriness to standard of care.

b- Evaluation of the diagnostic ability in detection of EBV infection of ISH performed on mucosal specimens with respect to qRealTime-PCR.

c- Evaluation of the diagnostic ability in detection of EBV infection between qRealTime-PCR performed on peripheral blood samples with respect to mucosal specimens.

d- Evaluation of association between mucosal/blood viral load and mucosal damage as endoscopically assessed (see paragraph 5).

e- Evaluation of association between clinical index of UC activity and EBV infection.

f- Evaluation of association between steroid therapy (absent, previous or current) and EBV infection.

g- Evaluation of association between immunosuppressive therapy (absent, previous or current) and EBV infection.

h- Evaluation of association between biological therapy (absent, previous or current) and EBV infection.

i- Evaluation of the prognostic role of EBV infection on the 6 and 12 months outcome (colectomy / number of hospitalization / cause of hospitalization).

5.2.3 Exploratory endpoints

- Identification of the life cycle phase of EBV infection through qRealTime-PCR and ISH. Specifically, the expression of EBER RNA will be assessed to distinguish between the lytic and latent phase of EBV infection into colonic specimens (31,39). In particular, although the presence of EBER is typically considered latency-associated, its expression was also found to promote cell growth and modulate innate immunity in EBV-associated epithelial cancers (31,40).

6 PATIENT SELECTION CRITERIA

For these purposes, a cohort of **100 patients with UC**, as diagnosed following the worldwide accepted criteria (2,41), either naïve or under standard of care (both responders and non-responders), will be prospectively enrolled. In all cases, the clinical and endoscopic indexes of activity will be carefully assessed. Specifically, to establish the refractoriness to current therapy, the following criteria will be applied: steroid-refractoriness is defined as the persistence of active disease despite an adequate dose and duration of steroid treatment (prednisone, 0.75–1 mg/kg day orally for at least two weeks; methylprednisolone, 1 mg/kg day intravenously for one week) (42); primary and secondary non-response to biological agents (infliximab, adalimumab, golimumab, vedolizumab) is considered as the lack of clinical improvement with induction therapy or recurrence of symptoms of disease activity during maintenance therapy despite an appropriate interval adjustment and dose escalation with exclusion of concomitant condition, respectively (43); resistance to immunosuppressive therapy (azathioprine, cyclosporine) on the basis of lack of clinical improvement after at least 4 weeks of treatment or the development of a flare of disease under appropriate maintenance dosage. For those patients following combination therapies, refractoriness is defined as the persistence of symptoms of active disease despite at least 4 weeks' treatment. In all cases, the Montreal classification is applied to assess the behavior, severity, and extent of the illness (44), while the clinical disease activity index is assessed according to the Simple Clinical Colitis Activity Index (SCCAI – Appendix 1) (45). Remission is defined as a SCCAI score of below 3 points. The endoscopic activity score is evaluated according to the Ulcerative Colitis Endoscopic Index of Severity (UCEIS – Appendix 2 - score ranging from 3 to 11, with a higher score indicating more severe disease) (46,47).

The **control group consists of 100 sex- and age-matched subjects**, selected in the same Center, undergoing colonoscopy with mucosal sampling as diagnostic workup for IBS in order to exclude macroscopic or microscopic disorders, or because alarm features are present: aged > 45-50 years, anemia, family history of colon cancer. That are confirmed to be suffering from IBS (C1), functional constipation/diarrhea (C2, C3) and functional abdominal bloating/distension (C4), following the Rome IV criteria (48), and who were not taking drugs possibly affecting immune response (steroids, immunosuppressant and biological agents) and microbiota composition (antibiotics, probiotics, prebiotics, proton pump inhibitors).

In addition, a small cohort of patients (n=20) suffering from **microscopic colitis** will be also enrolled as pathological control group (49).

All enrolled patients and controls undergo lower endoscopy with mucosal sampling and laboratory tests as diagnostic workup for their condition:

- Patients with active UC undergo colonscopy and laboratory tests according to International guidelines and depending on physician judgement: active UC patients will undergo colonscopy at baseline and laboratory tests at baseline, and at 3 and 6 months. A number of these patients, according to medical judgment, undergo medical examination at 9 and 12 months, and laboratory tests and colonscopy at 12 months as optional follow-up. This is why the aim of the study is calculated at 6 months, but adding the optional follow-up at 9 and 12 months, will aid to understand if patients with superimposed EBV colitis undergo .colectomy more frequently than patients whithout EBV colitis
- Patients with UC in remission undergo colonscopy and laboratory tests as standard follow-up of their pathological condition according ECCO-ESGAR guidelines [52] (ex: to evaluate mucosal healing or screening)
- Patients with IBS undergo colonscopy and laboratory tests only if alarm features are present (age > 45-50 years, anemia, family history of colon cancer, weight loss, blood mixed with stool).

Each enrolled case will undergo clinical and/or laboratory examination as standard follow-up of his/her pathological condition. All the laboratory tests are shown in the Appendix 5.

Each patient and control give written informed consent after approval by the local Bio-Ethics Committee is obtained.

6.1 Inclusion criteria

- Patients of either sex, aged 18-75 years (inclusive), without racial restriction.
- Patients undergoing colonoscopy with mucosal sampling.
- Ability to give informed consent according to International Conference on Harmonisation (ICH) Good Clinical Practice (GCP) and local regulations.
- Have given written informed consent to participate in the study before initiation of any study specific procedure.
- Prior and current standard treatments allowed.
- Diagnosis of UC according to the worldwide accepted criteria (2,41) (any age of onset, any localization, any activity of the disease, any severity of symptoms and signs of the disease).

6.2 Exclusion criteria

- Presence of significant comorbidities, such as uncontrolled hypertension and/or diabetes mellitus, invalidating psychiatric or neurological disorders, organ failure (renal impairment defined by creatinine clearance below 50 ml/min or by serum creatinine ≥2.0 mg/dl; hepatic impairment defined by total bilirubin ≥3.0 mg/dl and AST + ALT ≥ 2.5 × upper normal value; cardiac failure with an output fraction ≤40%; respiratory insufficiency with a pO_2 ≤60 mm Hg in arterial blood), or any other clinically significant condition, as determined by the Principal Investigator.
- Primary immunodeficiencies and human immunodeficiency virus infection.
- Pregnancy.
- Known malignancy.
- Patient who has had abdominal surgery, either open or laparoscopic, within the 3 months prior to screening.
- Patient recruited in another trial.
- Patient with a positive test for active hepatitis B or C disease or active severe infections.
- Known history of alcohol or drug abuse in the 12 months prior to inclusion.

7 STUDY DESIGN

This is an investigator-initiated prospective multicentre interventional without drug and medical device study evaluating the prevalence, role, life phase and the most accurate diagnostic method of EBV infection in UC. A longitudinal study is included to assess whether EBV infection has an impact on patients' outcome (see Appendix 3 illustrating the time points of the study).

7.1 General design

- *Type:* cross-sectional study with a longitudinal component.
- *Description of the exposure of interest:* EBV infection, defined as isolation of the EBV or detection of viral proteins or nucleic acids in any body fluid or tissue specimens.
- *Description of the matching factor(s) and how the matching will be done:* Center, sex- and age (5 year classes)-matching will be performed among the IBD patients and concomitant non-IBD control subjects.
- *Methods used to minimize bias.* The assessment of EBV infection through qRealTime-PCR, histology or ISH as primary endpoint is *per se* unbiased due to standardization and reproducibility of the techniques applied. Also calculation of clinical and endoscopic indexes of activity are routinely applied in IG-IBD centres. Moreover, the measurement of exploratory endpoints will be assessed by blinded technicians, whereas monitoring of patients' outcome will be performed by the local Principal Investigator.
- *Describe the strategy/process by which participants will be recruited, screened, and enrolled in the study.* All cases admitted at each IG-IBD center that agrees to participate in this study will be screened for inclusion. All enrolled cases undergo lower endoscopy with mucosal sampling from both inflamed and non-inflamed mucosa in each IBD patient and from healthy mucosa in each control subject, as part of their diagnostic workup for disease diagnosis, relapse or follow-up. Patients' assessment includes: clinical examination, body mass index calculation, evaluation of smoking habits and stratification according to the Montreal classification (44), routine laboratory tests, and calculation of clinical [SCCAI (45)] and endoscopic [UCEIS (46,47) indexes of activity.
- All enrolled cases also undergo peripheral blood harvest. Quantitation of viral DNA load will be performed on both peripheral blood samples and whole mucosal specimens by means of qRealTime-PCR (50). In addition, mucosal specimens will be also evaluated by ISH.

- Quantitation of EBV and HCMV DNA. For each patient and control, the EBV and HCMV load will be assessed in terms of International Units (IU) on both freshly collected whole peripheral blood samples and endoscopic specimens harvested from colonic mucosa, by qRealTimePCR technique as previously reported (51). Specifically, in the IBD group, two snap-frozen biopsies will be taken from either inflamed and/or non-inflamed mucosa as assessed during the endoscopic examination, that is from the edge of the ulcers and the nearby damaged zones for the former and at least 20 cm away from the affected areas for the latter. Two snap-frozen biopsies from healthy mucosa (possibly transverse colon) will be collected from each non-IBD patient. Viral DNA extraction will be performed by using the EZ1 DNA Tissue kit and EZ1Virus Mini Kit v2.0 (Qiagen, Hilden, Germany), while EBV DNA will be quantified by using the artus EBV QS-RGQ PCR Kit (Qiagen), and HCMV DNA will be quantified by using the artus CMV QS-RGQ kit (Qiagen), according to the manufacturer's instructions. Results will be expressed as IU/ml of blood and IU/10^5 cells. Normalization of viral DNA load in tissue samples will be obtained by quantitative determination of β_2-microglobulin gene. The lower detection limit is 1 DNA input target DNA copies (51). This analysis will be carried out at the the Molecular Virology Unit in Pavia.
- *In situ* chromogen hybridization. Mucosal specimens harvested in parallel from the same areas as those for the qRealTimePCR assay and needed for traditional diagnostic immunohistochemistry, will be fixed in 10% neutral buffered formalin and paraffin-embedded. Sections (4µm) will be transferred to pre-treated glass slides (DAKO, Denmark) and stored at 37°C overnight. The haematoxylin-eosin staining will be performed following standard protocol, while the specific *in situ hybridization* for EBV will be carried out on seriate sections. Briefly, the slides will undergo enzyme pretreatment (Leica AR9551) for 30 minutes at room temperature, and then hybridized with a FITC-labeled peptic nucleic acid probe complementary to EBV-encoded RNAs (EBER-PROBE; Leica) and incubated overnight at 55°C. After washing in restricting conditions for 35 minutes, the hybridized cells will be visualized with an ISH detection kit (Leica DS AR0833 and then 9800) according to the manufacturer's instructions. The sections will be then counterstained with Kernechtrot, dehydrated through graded alcohols, immerged in xylene and mounted with a permanent medium. The present ISH method stain the nuclei of EBV infected cells dark blue, whilst the nuclei of non-infected cells appear red. This analysis will be carried out at the Service of Pathology, ASST Spedali Civili (Brescia).

8 STATISTICAL CONSIDERATIONS

8.1 Sample size

Sample size has been calculated based on the primary endpoint, i.e., the prevalence of EBV infection in UC patients. In UC patients non-responders to standard of care the prevalence of EBV is expected around 50%; in UC patients responders to conventional treatment may be around 10%. We hypothesize that the prevalence of EBV infection in the control population is around 0-1%. Based on the feasibility on enrolment given study design, we plan to enroll about 100 UC patients. ~~The precision achieved with 100 patients is replicated. However, the power achieved when comparing 100 patients and 100 controls is not replicated; according to our computations is much larger than that reported by the Authors (76.9% rather than 27% with a prevalence of 10% and 99.8% rather than 87% with a prevalence of 20%).~~

Prevalence	95%CI	precision	power to detect difference from control
50%	40% - 60%	10%	100%
30%	21% - 40%	9%	100%
20%	13% - 29%	8%	99,8%
10%	5% - 18%	6%	76,9%

The power to detect a significant difference with the Fisher exact test from an expected proportion in controls of 1% is above 80% for proportions of infection of 10% or greater, considering 100 patients in each group and an alpha level of 5%. Calculations are performed using Stata 17 (StataCorp, College Station, TX, USA).

8.2 Analysis

Data will be described with the mean and standard deviation or the median and 25th-75th percentiles if continuous and as counts and percent if categorical. For the primary endpoint we will compute the prevalence as the ratio of the number of patients diagnosed with EBV infection to the number of patients evaluated, together with its 95%CI. We will use the Fisher exact test to compare the prevalence in UC patients to that of control. We will report the mean difference in proportions and its 95%CI. We will use binomial regression models with identity link (command binreg in Stata), to adjust for potential confounders, such as, but not limited to, age, gender, duration of disease and treatment. We will apply the 1:10 rule to adapt the number of confounders evaluated to the sample size. We will compare the prevalence of EBV infection between UC subgroups with the Fisher exact test (EP2a). In these cases, given in particular that the ratio of responders to non-responders is at yet unknown, we will compute *a posteriori* the power for the comparison. We will compute

sensitivity, specificity, area under the ROC curve and predictive values with 95%CIs for EP2b and EP2c. We will use the Spearman R and the Fisher exact test to assess the association, for continuous and categorical independent variables, respectively, with the dependent variables of EP2d to EP2h. All estimates will be presented with their 95%CI.

We will use Cox regression to assess the association of EBV infection with colectomy and of hospitalization for any cause; we will use Poisson regression to assess the association of EBV infection with the number of hospitalization from any cause. For secondary endpoints assessing associations, we will also fit regression models to adjust for possible confounders (as for the EP1) and report adjusted mean differences in proportions and 95%CI. We may also use propensity score inverse probability weights to account for the bias by indication when assessing treatment effect on the outcome.

The exploratory endpoints will be only descriptive.

We will use Stata 17 or later versions for computations. A 2-sided p-value <0.05 will be considered statistically significant. We will apply the Bonferroni correction for post-hoc comparisons.

9 WITHDRAWAL OF SUBJECTS

It is under the responsibility of the Principal Investigator in each IG-IBD Centre who has in charge the patient to withdraw her/him from the study and to advise both the Study Coordinator and Data Manager specifying the reason/s. The impossibility to harvest mucosal biopsies is considered a criterion for subject withdrawal. In this case, the patient's data will be removed from the database, but considered when assessing patient disposition. The development of malignancy or death are primarily considered as evolution of the underling disease. In addition, a patient has the right to withdraw from the study at any time for any reason. Subjects who withdraw their consent are considered withdrawn from the study. The Principal Investigator has the right to terminate participation of any patient if it is deemed in the patient's best interest and if she/he develops severe psychiatric or neurological disorders. The reason and circumstances for premature discontinuation must be reported in the patient's electronic case report form (eCRF). This means that a patient may be withdrawn in every phase of the trial, from the inclusion to any time of the follow-up period. In summary, reasons of withdrawal include, but are not limited, to the following:

- Patient's decision,
- Withdrawal of patient consent to participate in the study,
- Physician's decision based on patient's well-being.

For any subject discontinuing the study the Principal Investigator should before do the following:

- Complete the eCRF, indicating in the final visit, the date of termination and the reason for discontinuing the study as well as the protocol deviation section, with all data not corresponding to a formal visit filled in the Early Termination visit.

In patients who do not come back for the scheduled study visits, documented efforts should be performed to convince she/he to continue attending study visits and if unsuccessful, at least the exact reason(s) should be obtained for their discontinuation and any adverse event associated to it should be recorded.

The patient will be replaced if her/his mucosal specimens are not available for the full analyses.

10 FORMS AND PROCEDURES FOR COLLECTING DATA AND DATA MANAGING

a) Quality and Consistency controls. Electronic case report forms (eCRF) will be filled directly by the Principal Investigators. Prior to obtaining the clean file, checks of consistency of inclusion/exclusion criteria, clinical assessment, visit dates, compliance, concomitant treatment, outcome, and withdrawal information will be performed. Data query forms will be generated to the investigator in order to clarify the data inconsistencies through the source data verification.

b) Data Management Plan. A validation plan is set-up according to the protocol requirements, which describes the validation rules to be applied. Actions that should be taken in case of data abnormalities are detailed. In case of missing values, out of range values, data inconsistencies or values that fail logical checks, correction forms (queries) are edited and transmitted to the investigator for clarification.

c) Database. A database will be created in order to collect all clinical and other data from the present study. The data management will be provided by the Service of Clinical Epidemiology & Biometry of the I.R.C.C.S. Policlinico San Matteo Foundation (Pavia, Italy). The IGIBDReg platform will be used (https://igibdreg.ibismed.cloud/) .

d) Source documents. Evaluation of all clinical parameters, including laboratory tests, body mass index, and symptoms will be performed at the IG-IBD Centre where the patient is in charge and shared with the Study Coordinator. Study data will be collected on source documents. Each Principal Investigator is responsible for assuring that collected data are complete and accurate. Source documentation (the point of initial recording of a piece of data) will support data collected on the eCRF. The eCRF is the primary data collection instrument for the study. Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary

for the evaluation and reconstruction of the clinical study (see below). Completed eCRF data will be entered into the database within a week of the patient visit being completed. According to the ICH guidelines on GCP, the Data Manager must check the eCRF entries against the source documents, except for the pre-identified source data directly recorded in the eCRF.

Source Data

- Clinical Charts,

- Patients medical files,

- Signed Informed Consent Forms,

- On-line test results (especially if only held electronically),

- Samples log.

e) Database Access. Access to the eCRF database will be allowed to the Principal Investigators, data managers, members of the Steering Committee and IGIBD Scientific Committee. Any entry in the database will be traceable through its identification and date. Audit trail of data changes will be assured.

f) Data reported in the eCRFs should be consistent with and substantiated by the subject's medical record and original source documents. The final, completed eCRF Casebook for each subject will be electronically signed and dated by the investigator of the IG-IBD Centre to signify that the Investigator has reviewed the eCRF and certifies it to be complete and accurate within a month.

g) Data Queries. Data query forms will be generated to the Coordinator Investigator in order to clarify the data inconsistencies through the source data verification. Only then and after all detected errors, inconsistencies or doubts cleared, will the database declared a clean file and protected accordingly. IGIBDReg integrates a query management system.

h) All data entry, modification or deletion will be recorded automatically in an electronic audit trail. The investigators will retain all copies of the eCRF in the relevant sections of their Investigator Site File with any required anonymised background information from the medical records as required.

i) Clean File. A clean file will be created and registered, to which further changes will be disallowed. The database lock report will be generated which includes the eligibility of patients.

j) Data Quality Assurance. Periodic monitoring will be made by remote for source data verification, and to check compliance with the protocol, GCP and applicable regulatory requirements by the Data Manager. Quality control steps will be taken for the analytical protocol, results, and report.

k) The Trial Statistician will retain the final eCRF data and audit trail as permanent records of the study. A copy of all completed eCRFs will be provided to the investigators. Study document will be retained for 10 years after the completion of the protocol.

l) The Informed Consent Form will include a statement by which the patient allows the Sponsor's duly authorised personnel, the IEC, and the regulatory authorities to have direct access to source data which supports the data on the eCRF (e.g., patient's medical file, appointment books, original laboratory records, etc.). These personnel, bound by professional secrecy, will not disclose any personal identity or personal medical information.

m) Data collection and study report form monitoring. All data obtained for this study will be entered into a regulation compliant Data Management System. Data will be recorded with an Electronic Data Capture system using eCRFs. Specifically, the Electronic Data Capture system will be based on the IGIBDReg platform. IGIBDReg is a novel workflow methodology and software tool that expedites the electronic collection of research data from a single site or multi-site clinical research study The study database will be resident on OVH server (https://www.ovhcloud.com/) as required by European Certification

n) Procedure to account for missing or spurious data. In case of missing clinical assessment at follow-up visit, the last observation carried forward from the latest earlier visit will apply, while in case of missing clinical, endoscopic and histologic data by month 12, the evaluation of outcome will not be possible. We expect to have 10% patients on drop-out. IGIBDReg provides section to recording missing or N/A information.

o) Procedure to account for protocol deviations. Generally, a protocol deviation is not imperative for patient withdrawal. Before the statistical analysis begins, the protocol deviations must be taken into account for the analysis. The mandatory withdrawal reasons will be considered as major protocol deviations. Other protocol deviation will be classified as minor protocol deviation. Additionally, the lack of compliance with any of the inclusion/exclusion criterion, either identified before or after enrolment into the study, will be considered as major protocol deviation. Any protocol deviation must be recorded in the eCRF (specific section in IGIBDReg). Once the study has begun, any modification of the protocol deviation will be specified in the statistical analysis plan. This will be done before closing the database.

Management of biological samples. The histologic slides will be stored at room temperature in a dedicated rack on long-term at the Pathology Unit of each referral Centre, except for a couple of sections that will be send to the Service of Pathology of the ASST Spedali Civili in Brescia for centralized examination (Doctor Vincenzo Villanacci). In addition, snap-frozen mucosal and whole

peripheral blood samples will be collected and stored in liquid nitrogen tanks of each Centre participating in the study and then sent to the Laboratory of Molecular Virology of the I.R.C.C.S. Policlinico San Matteo Foundation (Pavia) for viral load centralized detection (Prof. Fausto Baldanti). These biological samples will be used for qRealTime-PCR assay and will be destroyed once processed. The Principal Investigator of each Centre who has in charge the patient is responsible for the appropriate storage and has full access to the biological samples that are labelled with the date, the patient's code and the trial number. No further researchers will have access to these samples.

11 ETHICAL CONSIDERATIONS

11.1 Patient protection

Both the Principal Investigators and the Study Coordinator will ensure that this study is conducted in agreement with either the Declaration of Helsinki (Tokyo, Venice, Hong Kong and Somerset West amendments) and the Italian laws and regulations (Appendix 6).

The protocol has been written, and the study will be conducted according to the ICH Guideline for GCP. The protocol and its annexes are subject to review and approval by the competent Independent Ethics Committee(s) (IEC). All correspondence with the IEC will be retained in the Investigator Site File.

11.2 Subject identification – Personal Data protection

All records identifying the subject will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, not be made publicly available. The name of the patient will not be asked for nor recorded at the Data Center. A sequential identification number will be automatically attributed to each patient registered in the study. This number will identify the patient and must be included on all case report forms.

Any and all patient information or documentation pertaining to this study, to the extent permitting, through a "key" kept anywhere, regardless of whether such key is supplied along with the information or documentation or not, must be considered as containing sensitive personal data of the patient, and is therefore subjected to the provisions of applicable data protection ("privacy") regulations. The Study Coordinator and all the Principal Investigators will be aware that a breach of such regulations may result in administrative or even criminal sanctions.

An information sheet prepared according to such regulations and a form to evidence the consent of patients to the processing of such data will accompany the informed consent administered to the patient (see Annex no. 1). Such information must (i) identify the roles of the holder and processor (appointed by the holder) of the patient personal data (also if not directly identifying the patient), as well as the purposes of the personal data collection and processing (scientific research), (ii) adequately describe the flows of communication involving them, particularly if third parties should become involved, and (iii) seek the patient's prior and specific consent to such processing.

In addition, anonymized data may be uploaded, with explicit consent, on a single database protected by password that can be accessed through the platform of the IGIBD database. The members of the IG-IBD Scientific and Steering Committee and the Investigators who coordinate this study will have access to the database; access and consultation of the data will be carried only for scheduled review or on the specific mandate of the IGIBD Scientific Committee for the timely assessment of specific variables.3. ***Data Protection & Patient Confidentiality***

All investigators and study site staff involved in this project comply with the requirements of the "Regolamento Generale sulla Protezione dei Dati" (usually known as GDPR) of 25th May, 2018, referring to the Regulamentation of the United Europe no. 2016/679, with regards to the collection, storage, processing and disclosure of personal information. An electronically generated code will be assigned to each case whose identity will be known only at the clinician who has in charge the patient and her/his staff. Each site is responsible for keeping an updated list of patients with their codes. This is to protect the anonymity of the collected data. The Study Coordinator guarantees that only pseudo-anonymized data will be received and recorded at each site. Moreover, only pseudo-anonymized data will be shared with the study team and other investigators.

All information obtained as a result of this study will be considered confidential until the Steering Committee deems it appropriate. This confidential information will be the property of both the Study Coordinator and the Principal Investigators who has in charge the patient and must not be disclosed to third parties without prior written consent from the patients, and must only be used for the study purposes.

To protect patients' privacy, the Informed Consent Form will include a statement by which the patient allows the authorized personnel, the IEC, and the regulatory authorities to have direct access to source data that supports the data on the eCRF. These personnel, bound by professional secrecy, will not disclose any personal identity or personal medical information.

11.3 Informed consent

All patients will be informed of the aims and possible risks of the study. They will be also informed as to the strict confidentiality of their data, but that their medical records may be reviewed for study purposes by authorized individuals other than their treating physician. The patient informed consent is given as an annex to this protocol and will be prospectively approved by the IEC.

It is emphasized that the participation is voluntary and that the patient is allowed to refuse further participation in the protocol whenever she/he wants. This will not prejudice the patient's subsequent care. Documented informed consent will be obtained for all patients included in the study before they are registered at the Data Center. This will be done in accordance with the Italian regulatory requirements and the procedure will be compliant with the ICH guidelines on GCP. This implies that "the written informed consent form should be signed and personally dated by the patient or by the patient's legally acceptable representative" and by the Principal Investigator. The Study Coordinator will retain the original of each patient's signed informed consent form and a copy is given to the participant. Should a patient require a verbal translation of the study documentation by a locally approved interpreter/translator, it is the responsibility of the local Principal Investigator to use locally official translators. Any change to the proposed informed consent form suggested by the Study Coordinator or Principal Investigators must be agreed to by the Steering Committee before submission to the IEC of the Coordinator Centre, and a copy of the approved version must be provided to each Centre and the Data Manager after IEC approval. Any new information which becomes available and which might affect the patient's willingness to continue participating in the trial will be communicated to the patient as soon as possible verbally over the telephone where an additional visit will be arranged.

12 CONFLICT OF INTEREST

Any investigator and/or research staff member who has a conflict of interest with this study (such as patent ownership, royalties, or financial gain greater than the minimum allowable by their institution) will fully disclose the nature of the conflict of interest.

13 DATA OWNERSHIP

The investigators participating in the study are the owners of the data resulting therefrom. All centers and investigators participating in the study are invited not to disseminate information or data without prior express agreement with the Study Coordinator and Principal Investigators.

14 PUBLICATION POLICY

After completion of the study, the Study Coordinator will prepare a draft manuscript containing final results of the study on the basis of the statistical analysis. The manuscript will be derived to the co-authors for comments and after revision will be sent to the appropriately selected peer-reviewed scientific journal. All information concerning this study has to be treated confidentially. In all publication/s the confidentiality of patients' data will be ensured.

All publications, abstracts, presentations, manuscripts and slides including data from the present study will be submitted to and reviewed by the Study Coordinator for coordination and homogeneity purposes. By signing this study protocol, the investigators accept that the results of this study can be presented to national and international authorities. They also accept that in this context their name, address, qualification and grade of involvement in the trial will be published.

The Study Coordinator will be the Senior Author and the Corresponding Author of the relevant publication/s. The Authors' list will include all the investigators (up to the maximum required by the Journal to whom the article will be submitted) in a decreasing order of patients included into the final analysis for the primary outcome, as well as the study statistician.

The funding body will be acknowledged within the publication/s, but they do not have any review and publication right on the data from the study, including their timelines for doing so.

It is responsibility of the Study Coordinator to notify the Principal Investigators of the outcome of the study by provision of the publication/s.

15 Study time table (Gantt chart)

The whole study duration is 36 months, while the time frame from the enrolment of the first patient is 32 months. The patients' participation consists of a 3 weeks screening period, one week to undergo endoscopy with mucosal sampling and peripheral blood harvest, a 6 months mandatory follow-up period, a 6 further months of facultative follow-up period, and a 4 weeks end of study visit. Thus, the recruitment period lasts 29 months and the minimum expected duration of subject participation is of 7 months. The whole study duration is 36 months, and the subdivision of the activities by trimesters and by involved units is represented here below:

Task	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
1a												
1b												

Legend. *Task 1: patients' screening (a), recruitment (b), and outcome evaluation (c) – Task 2: viral load assessment on both mucosal and blood samples – Task 3: histology assessment of ulcerative colitis activity (a), and in situ hybridization for evaluation of EBV infection (b) – Task 4: evaluation of the expression of latency/lytic-associated genes – Task 5: statistical analysis – Task 6: trial monitoring.*

IBD Units: orange; Virology Unit: green; Pathology Unit: violet; Service of Clinical Epidemiology & Biostatistics: light blue.

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List of Appendixes and Annexes included with the protocol

Appendix 1: Simple Clinical Colitis Activity Index (SCCAI)

Appendix 2: Ulcerative Colitis Endoscopic Index of Severity (UCEIS)

Appendix 3: Schedule Assessments UC

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Annex 3: Budget REBUC

Annex 4: Lista Centri REBUC

Appendix 1

SCCAI: Simple Clinical Colitis Activity Index

Variabile	**Punteggio**				
	0	**1**	**2**	**3**	**4**
Numero di scariche diurne	1 - 3	4 - 6	7 - 9	>9	
Numero di scariche notturne		1 - 3	4 - 6		
Urgenza di defecare		di corsa	immediatamente	incontinenza	
Presenza di sangue nelle feci	assente	tracce	occasionale	sempre	
Valutazione clinica globale	molto bene	lievemente al di sotto della normalità	scadente	pessima	terribile
Manifestazioni extraintestinali (1 punto per ciascuna manifestazione)					
Score totale					

Appendix 2

UCEIS: Ulcerative Colitis Endoscopic Index of Severity

Variabile	**Punteggio**				
	1	**2**	**3**	**4**	**5**
Pattern vascolare	normale		perdita a chiazze		obliterato
Eritema mucosale	assente		lievemente arrossata		molto arrossata
Superficie mucosale	normale		granulare		nodulare
Edema mucosale	assente		probabile		definito
Presenza di muco-pus	assente		poco		tanto
Presenza di sangue	assente	mucosale	luminale lieve	luminale moderato	luminale severo
Fragilità mucosale	assente	lieve	moderata	severa	molto severa
Fragilità mucosale al contatto	assente		probabile		definita
Presenza di erosioni-ulcere	assenti	presenza di erosioni	presenza di ulcere superficiali	presenza di ulcere profonde	
Estensione di erosioni-ulcere	assente	limitata	sostanziale	estesa	
Score totale					

Appendix 3

Schedule assessments in active UC

	Screening	Baseline	Mandatory follow-up		Optional follow-up	
			3 months	6 months	9 months	12 months
Clinical assessment	X		X	X	X	X
Body Mass Index		X	X	X	X	X
Diagnosis confirmation	X					
Clinical index of activity		X	X	X	X	X
Laboratory analyses		X	X	X		X
Blood sampling		X	X	X		X
Lower endoscopy		X				X
Endoscopic index of activity		X				X
Mucosal sampling		X				X
Histology examination		X				X
Immohistochemistry		X				X
ISH		X				X
Viral load assessment		X				X

Appendix 4

Schedule assessments in UC in remission and IBS

	Screening	Baseline
Clinical assessment	X	
Body Mass Index		X
Diagnosis confirmation	X	
Clinical index of activity		X
Laboratory analyses		X
Blood sampling		X
Lower endoscopy		X
Endoscopic index of activity		X
Mucosal sampling		X
Histology examination		X
Immohistochemistry		X
ISH		X
Viral load assessment		X

APPENDIX 5: LABORATORY TESTS

WBC
RBC
Hb
MCV
PLT
NEUTROPHILIS
LYMPHOCYTES
SODIUM
POTASSIUM
PROTEINS
ALBUMIN
C REACTIVE PROTEIN
IgG-A-M
anti-EBV IgM/IgG antibodies
Fecal calprotectin

Appendix 6

World Medical Association (WMA) Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964 and amended by the:

29th WMA General Assembly, Tokyo, Japan, October 1975

35th WMA General Assembly, Venice, Italy, October 1983

41st WMA General Assembly, Hong Kong, September 1989

48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996

52nd WMA General Assembly, Edinburgh, Scotland, October 2000

53rd WMA General Assembly, Washington DC, USA, October 2002 (Note of Clarification added)

55th WMA General Assembly, Tokyo, Japan, October 2004 (Note of Clarification added)

59th WMA General Assembly, Seoul, Republic of Korea, October 2008

64th WMA General Assembly, Fortaleza, Brazil, October 2013

Preamble

- The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data. The Declaration is intended to be read as a whole and each of its constituent paragraphs should be applied with consideration of all other relevant paragraphs.
- Consistent with the mandate of the WMA, the Declaration is addressed primarily to physicians. The WMA encourages others who are involved in medical research involving human subjects to adopt these principles.

General Principles

1. The Declaration of Geneva of the WMA binds the physician with the words, “The health of my patient will be my first consideration,” and the International Code of Medical Ethics declares that, “A physician shall act in the patient’s best interest when providing medical care.”
2. It is the duty of the physician to promote and safeguard the health, well-being and rights of patients, including those who are involved in medical research. The physician’s knowledge and conscience are dedicated to the fulfilment of this duty.
3. Medical progress is based on research that ultimately must include studies involving human subjects.
4. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best proven interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.
5. Medical research is subject to ethical standards that promote and ensure respect for all human subjects and protect their health and rights.
6. While the primary purpose of medical research is to generate new knowledge, this goal can never take precedence over the rights and interests of individual research subjects.
7. It is the duty of physicians who are involved in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects. The responsibility for the protection of research subjects

must always rest with the physician or other health care professionals and never with the research subjects, even though they have given consent.

8. Physicians must consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.
9. Medical research should be conducted in a manner that minimises possible harm to the environment.
10. Medical research involving human subjects must be conducted only by individuals with the appropriate ethics and scientific education, training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional.
11. Groups that are underrepresented in medical research should be provided appropriate access to participation in research.
12. Physicians who combine medical research with medical care should involve their patients in research only to the extent that this is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.
13. Appropriate compensation and treatment for subjects who are harmed as a result of participating in research must be ensured.

Risks, Burdens and Benefits

1. In medical practice and in medical research, most interventions involve risks and burdens. Medical research involving human subjects may only be conducted if the importance of the objective outweighs the risks and burdens to the research subjects.
2. All medical research involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and groups involved in the research in comparison with foreseeable benefits to them and to other individuals or groups affected by the condition under investigation. Measures to minimise the risks must be implemented. The risks must be continuously monitored, assessed and documented by the researcher.
3. Physicians may not be involved in a research study involving human subjects unless they are confident that the risks have been adequately assessed and can be satisfactorily managed. When the risks are found to outweigh the potential benefits or when there is

conclusive proof of definitive outcomes, physicians must assess whether to continue, modify or immediately stop the study.

Vulnerable Groups and Individuals

1. Some groups and individuals are particularly vulnerable and may have an increased likelihood of being wronged or of incurring additional harm. All vulnerable groups and individuals should receive specifically considered protection.
2. Medical research with a vulnerable group is only justified if the research is responsive to the health needs or priorities of this group and the research cannot be carried out in a non-vulnerable group. In addition, this group should stand to benefit from the knowledge, practices or interventions that result from the research.

Scientific Requirements and Research Protocols

1. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.
2. The design and performance of each research study involving human subjects must be clearly described and justified in a research protocol. The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, potential conflicts of interest, incentives for subjects and information regarding provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study. In clinical trials, the protocol must also describe appropriate arrangements for post-trial provisions.

Research Ethics Committees

1. The research protocol must be submitted for consideration, comment, guidance and approval to the concerned research ethics committee before the study begins. This committee must be transparent in its functioning, must be independent of the researcher, the sponsor and any other undue influence and must be duly qualified. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration. The committee must have the right to monitor ongoing studies. The researcher must provide

monitoring information to the committee, especially information about any serious adverse events. No amendment to the protocol may be made without consideration and approval by the committee. After the end of the study, the researchers must submit a final report to the committee containing a summary of the study's findings and conclusions.

Privacy and Confidentiality

1. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information.

Informed Consent

2. Participation by individuals capable of giving informed consent as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no individual capable of giving informed consent may be enrolled in a research study unless he or she freely agrees.
3. In medical research involving human subjects capable of giving informed consent, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, post-study provisions and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information. After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed. All medical research subjects should be given the option of being informed about the general outcome and results of the study.
4. When seeking informed consent for participation in a research study the physician must be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent must be sought by an appropriately qualified individual who is completely independent of this relationship.
5. For a potential research subject who is incapable of giving informed consent, the physician must seek informed consent from the legally authorised representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the group represented by the potential subject, the

research cannot instead be performed with persons capable of providing informed consent, and the research entails only minimal risk and minimal burden.

6. When a potential research subject who is deemed incapable of giving informed consent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorised representative. The potential subject's dissent should be respected.
7. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research group. In such circumstances the physician must seek informed consent from the legally authorised representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research must be obtained as soon as possible from the subject or a legally authorised representative.
8. The physician must fully inform the patient which aspects of their care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never adversely affect the patient-physician relationship.
9. For medical research using identifiable human material or data, such as research on material or data contained in biobanks or similar repositories, physicians must seek informed consent for its collection, storage and/or reuse. There may be exceptional situations where consent would be impossible or impracticable to obtain for such research. In such situations the research may be done only after consideration and approval of a research ethics committee.

Use of Placebo

1. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best proven intervention(s), except in the following circumstances: Where no proven intervention exists, the use of placebo, or no intervention, is acceptable; or Where for compelling and scientifically sound methodological reasons the use of any intervention less effective than the best proven one, the use of placebo, or no intervention is necessary to determine the efficacy or safety of an intervention and the patients who receive any intervention less effective than the best proven one, placebo, or no intervention will not be subject to additional risks of serious or irreversible harm as a result of not receiving the best proven intervention. Extreme care must be taken to avoid abuse of this option.

Post-Trial Provisions

1. In advance of a clinical trial, sponsors, researchers and host country governments should make provisions for post-trial access for all participants who still need an intervention identified as beneficial in the trial. This information must also be disclosed to participants during the informed consent process.

Research Registration and Publication and Dissemination of Results

2. Every research study involving human subjects must be registered in a publicly accessible database before recruitment of the first subject.
3. Researchers, authors, sponsors, editors and publishers all have ethical obligations with regard to the publication and dissemination of the results of research. Researchers have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. All parties should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results must be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest must be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

Unproven Interventions in Clinical Practice

1. In the treatment of an individual patient, where proven interventions do not exist or other known interventions have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorised representative, may use an unproven intervention if in the physician's judgment it offers hope of saving life, re-establishing health or alleviating suffering. This intervention should subsequently be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information must be recorded and, where appropriate, made publicly available.