the Italian Group for the study of
Inflammatory Bowel Disease

Study Title	**Comparative Effectiveness of First-Line Biologic Therapies in Ulcerative Colitis**
Study Acronym	**FIRST-UC**
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Sponsor	Italian Group for the Study of Inflammatory Bowel Disease (IG-IBD)
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Study indication	Ulcerative Colitis
Study population	We will include patients with a confirmed diagnosis of ulcerative colitis (UC) who initiated treatment with a biologic agent—specifically anti-TNFα agents (infliximab, adalimumab, golimumab), vedolizumab, ustekinumab, or mirikizumab—as first-line targeted therapy. Only biologic-naïve patients, receiving one of these agents as their initial biologic treatment, will be eligible for inclusion.

Background and rationale	For about a decade, anti-Tumour Necrosis Factor (TNF)-α agents have represented the only available targeted therapies for the treatment of ulcerative colitis (UC). In more recent years, however, several new biologics with distinct mechanisms of action have been introduced, including vedolizumab (anti-α4β7), ustekinumab (anti-IL-12/23), and mirikizumab (anti-IL-23). While the expanding therapeutic landscape offers a broader range of treatment options, the optimal positioning of these agents—particularly in the first-line setting—remains unclear. It is widely recognized that all these biologics tend to be more effective when used as first-line therapies in biologic-naïve patients, compared to when administered after prior exposure to other biologics. Despite this, no head-to-head studies have been conducted to directly compare the real-world effectiveness and safety of anti-TNF agents, vedolizumab, ustekinumab, and mirikizumab when used as first-line treatment in patients with mild-to-moderate UC. This study aims to fill this critical gap by retrospectively assessing and comparing the real-world effectiveness and safety of these biologics in a multicentre cohort of biologic-naïve ulcerative colitis (UC) patients, all of whom initiated therapy in the first-line setting. According to current Italian regulatory indications, anti-TNFα agents, vedolizumab, ustekinumab, and mirikizumab can be prescribed as first-line biologic therapies in UC, whereas JAK inhibitors are restricted to second-line use and were therefore excluded from this analysis.

Study Objectives	In this study, we aim to address the existing knowledge gap regarding the optimal first-line positioning of anti-TNFα agents, vedolizumab, ustekinumab, and mirikizumab in the treatment of ulcerative colitis (UC). We will retrospectively compare the real-world effectiveness and safety of these biologics in a multicentre cohort of biologic-naïve patients with mild-to-moderate UC, all of whom received one of these agents as their first-line biologic therapy.

Study Endpoints/Outcomes	• The **primary objective** will be to compare the clinical effectiveness of the three treatments in terms of clinical remission. • **Secondary objectives** • The **Secondary objectives** will include: comparative endoscopic and biochemical effectiveness, comparative persistence on therapy, comparative safety. **Endpoints** **Primary endpoint** Steroid-free clinical remission (SFCR) at 6 and 12 months **Secondary endpoints** • Biochemical remission at 6 and 12 months Defined as normalization of inflammatory biomarkers, including a fecal calprotectin level <250 µg/g and a C-reactive protein (CRP) level within the normal range, at 6 and 12 months. • Endoscopic improvement during follow-up Defined as a Mayo endoscopic subscore of 1 or less during any follow-up endoscopic assessment. This indicates a reduction in mucosal inflammation but not complete healing. • Endoscopic remission during follow-up Defined as a Mayo endoscopic subscore of 0 during follow-up endoscopy, indicating complete mucosal healing. • Deep remission during follow-up Defined as the simultaneous achievement of clinical remission (no rectal bleeding and normal stool frequency), biochemical remission (normal CRP and fecal calprotectin <250 µg/g), and endoscopic remission (Mayo endoscopic subscore = 0) during follow-up. • Treatment persistence during the first year of treatment • Occurrence of AEIs throughout the entire follow-up

Study design	This is a real-life, multicenter, retrospective cohort study. We will include patients with ulcerative colitis (UC) who initiated biologic therapy with anti-TNFα agents, vedolizumab, ustekinumab, or mirikizumab as first-line treatment, with no prior exposure to any other biologic or small molecule. Only biologic-naïve patients with mild-to-moderate UC at the time of treatment initiation will be considered eligible. Data will be retrospectively extracted from medical records; the date of first prescription of the selected biologic will be defined as the baseline for the study.
Eligibility Criteria	**Inclusion criteria**: ✓ Established diagnosis of UC according to the current European Crohn's and Colitis Organization (ECCO) guidelines[7]; ✓ Age ≥ 18 years-old; ✓ Capability of expressing informed consent; ✓ Clinically active ulcerative colitis (cf. 'operative clinical measures', below) at baseline; ✓ Start of anti-TNFα, vedolizumab, ustekinumab or Mirikizumab as first-line target therapy at baseline; ✓ At least 1 follow-up visit after baseline **Exclusion criteria**: ✓ Diagnosis of Crohn's colitis, IBD-U or other gastrointestinal inflammatory conditions; ✓ Incapability of expressing informed consent; ✓ Previous exposure to biologic therapies and/or JAK inhibitors;

Study Procedures	Investigators will screen their databases to identify eligible patients in each participating center. All consecutive patients who meet the inclusion criteria will be offered to participate and informed consent will be obtained. Demographic data (including gender, age, smoking status, BMI), comorbidities (assessed with the Charlson comorbidity index[11]) and disease-related characteristics, such as disease duration and extension (according to the Montreal Classification for UC[12]), previous and concurrent non-target therapies for UC (*i.e.*, aminosalicylates, steroids, immunosuppressants) will be extrapolated from patients' clinical records. At baseline, clinical disease activity – assessed by Stool Frequency (SF) and Rectal Bleeding (RB) scores and by the presence of urgency (that is, 'yes' or 'no') –, concomitant oral therapies (aminosalicylates, steroids and immunosuppressants), C-reactive protein (CRP) serum levels, fecal calprotectin (FCP) levels, and endoscopic activity (according to Mayo endoscopic score[13]) – if an endoscopy performed within 3 months before enrollment is available – will be recorded. In regard to urgency, the Urgency Numerical Rating Scale (NRS) is a 11-item tool that has been recently developed for patients with UC[14]. Indeed, urgency is a common and disabling symptom in UC that is often overlooked in clinical trials and real-life research; the retrospective design of our study prevents us from adopting the NRS, hence we decided to include urgency assessment as a dichotomous outcome (*i.e.*, 'yes' or 'no') Data on follow-up assessments will be collected for each visit performed during the 12 months following induction or until drug discontinuation (if it occurs before the 12-month study period); the minimum required number of follow-up visits is going to be one. For each follow-up visit, investigators will collect data on: - Clinical disease activity (assessed by SF and RB scores, and by the presence of urgency) - Study drug dosing regimen - Concomitant oral therapies (aminosalicylates, steroids and immunosuppressants), - CRP and FCP levels - Endoscopic activity (according to Mayo endoscopic score) – if available Adverse events of interest (AEIs - *cf*. 'operative clinical definition', below) and reasons for treatment discontinuation will also be collected

Number of patients (planned), sample size	The sample size was calculated based on the primary objective of demonstrating non-inferiority in clinical remission rates between treatment groups at 12 months. Assuming a clinical remission rate of 65% in both groups, a non-inferiority margin (Δ) of 12%, a two-sided alpha level of 0.05, and 80% statistical power, 294 patients in the anti TNF group (test group) and 147 in the three control groups would be required under a 2:1 allocation ratio.
Investigational Sites (planned)	Multi-centre study

Sample size and statistical consideration	Categorical variables will be described with absolute numbers and percentage. Continuous variables will be expressed either with mean and standard deviation (SD) or with median and interquartile range (IQR), according to their distribution, which will be previously tested with the Shapiro-Wilk test. Inter-group comparisons between variables will be performed with chi-squared and Mann-Whitney U tests. Propensity score with inverse probability of treatment weighting (IPTW) will be used for the analysis of primary and secondary outcomes. Potential confounding variables considered for propensity score calculation are listed above. Propensity scores will be calculated by multinomial logistic regression including all confounding variables, with treatment as the dependent variable, for each cohort, using the vedolizumab cohort as the reference. Extreme weights will be dealt with by adopting weight trimming and stabilization. Propensity scores will be cut at the 1st and 99th percentile to exclude extreme outliners. For each patient, stabilized weight will be calculated as r_u/p_u for ustekinumab-treated patients, r_t/p_t for JAK inhibitors-treated patients, and as r_v/p_v for vedolizumab treated patients, where r is the probability of receiving a specific treatment and p is the propensity score. IPTW will be independently calculated and applied for the entire cohort of analysed patients (*i.e.*, all-patient cohort) to evaluate clinical outcomes (clinical response, SFCR, biochemical remission and treatment persistence) and for the sub-cohort of patients who have baseline and follow-up endoscopic evaluations to investigate endoscopic outcomes. Statistical differences between treatment groups for primary and secondary dichotomous outcomes will be calculated with Pearson's chi-squared test. Odds ratio (OR) and 95% confidence intervals (CI) will be estimated by logistic regression models weighted by stabilized and truncated weights, with treatment as the single independent variable. Kaplan-Meier survival analysis will be performed to estimate persistence in therapy and maintenance of remission. Cox regression will be used to estimate the potential association of baseline clinical predictors with the primary outcome. Variables with a p-value<0.1 at univariate Cox-regression will be included in a multivariable logistic regression analysis. For each test, statistical significance level will be set at $p<0.05$. Statistical analysis will be performed with IBM SPSS Statistics 26.

Study timetable	Planned study duration (from FPI to LPO): 6 months
GCP Statement:	This study will be conducted in compliance with the protocol, the current version of the Declaration of Helsinki and applicable guidelines as well as all national legal and regulatory requirements.