Clinical Study Protocol
4WARD

Looking **4WARD**: The role of dipeptidyl peptidase 4 (DPP-**4**) in inflammatory bo**w**el disease (IBD) as a novel biomarker for predicting disease **a**ctivity and monitoring **r**esponse to therapy in IB**D** patients

Study code: DPP-4WARD

Study type: Observational, non-interventional, clinical study

Protocol date: 27/04/2021

Protocol version: 3.4

Study Sponsor: Grupo de Estudo da Doença Inflamatória Intestinal (GEDII)

Contact: gedi@med.up.pt

Sponsor-Investigator: Fernando Magro (MD, PhD)

Contact: fm@med.up.pt

Financial support: This study was supported by an IIS grant provided by Janssen-Cilag Farmacêutica, Lda.

Sponsor-Investigator Signature Page

Study title: Looking 4WARD: The role of dipeptidyl peptidase 4 (DPP-4) in inflammatory bowel disease (IBD) as a novel biomarker for predicting disease activity and monitoring response to therapy in IBD patients.

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Sponsor-Investigator:

Name: Fernando Magro

E-mail: fm@med.up.pt

I, the undersigned, am responsible for overseeing and coordinating the conduct of this study on a national level.

I understand and will conduct the study according to the protocol, any approved protocol amendments, and all applicable Health Authority requirements and national laws.

I will not deviate from the protocol without prior written permission from the GEDII, except where necessary to prevent immediate danger to the subject.

_____ ____ / _____ / _____

Signature *Date*

Principal Investigator Signature Page

To be signed by the PI of each participating center

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Center name: _____

Principal Investigator:

Name: _____

E-mail: _____

I, the undersigned, am responsible for the conduct of the study at this site and affirm that I understand and will conduct the study according to the protocol, any approved protocol amendments, and all applicable Health Authority requirements and national laws.

I will not deviate from the protocol without prior written permission from the GEDII, except where necessary to prevent immediate danger to the subject.

_____ ____ / _____ / ____

Signature *Date*

Table of Contents

1. Study Synopsis

Title	**Looking 4WARD: The role of dipeptidyl peptidase 4 (DPP-4) in inflammatory bowel disease (IBD) as a novel biomarker for predicting disease activity and monitoring response to therapy in IBD patients.**
Study code	DPP-4WARD
Study sponsor	Grupo de Estudo da Doença Inflamatória Intestinal (GEDII)
Sponsor-Investigator	Fernando Magro (MD, PhD)
Principal investigator	To be designated in each participating hospital center
Disease/condition	Crohn's disease and Ulcerative colitis
Rational	DPP-4 appears to be associated with the regulation of inflammatory processes in several inflammatory diseases, including IBD. However, the role of DPP-4 in IBD, is not yet fully elucidated and further studies need to be conducted to clarify its potential use as a biomarker of disease activity and treatment response.
Research hypothesis	We hypothesize that DPP-4, in patients with IBD, is associated with levels of inflammation, disease activity, and response to treatment.
Primary objectives	▪ To explore the associations between plasma/fecal DPP-4 levels and clinical activity in IBD patients. ▪ To explore the predictive potential of plasma/fecal DPP-4 values with regards to treatment response (escalation and de-escalation of therapy). ▪ To explore the associations between plasma/fecal DPP-4 levels, and fecal and plasma biomarkers. ▪ To explore the association between plasma/fecal DPP-4 and fecal calprotectin levels with serum infliximab/vedolizumab/ustekinumab levels throughout a follow-up period of 2 years. ▪ To explore the association between plasma/fecal DPP-4 and fecal calprotectin levels with the development of anti-infliximab/anti-vedolizumab/anti-ustekinumab antibodies throughout a follow-up period of 2 years.
Study design	▪ Prospective cohort of IBD patients, with a follow-up period of 2 years.
Inclusion criteria	For inclusion, **all of the following** criteria must be met: ▪ Patients aged 18 years or older; ▪ Patients with active luminal Crohn's Disease (moderate to severe) or active ulcerative colitis (moderate to severe); ▪ Patients who will initiate biologic agents (infliximab, vedolizumab, ustekinumab [reference product or biosimilar]) or tofacitinib at the time of inclusion according to physician's criteria: both anti-TNF-naïve or patients previously treated with monoclonal antibodies; ▪ Patients who have been submitted to colonoscopy at the baseline (4 weeks before or after baseline);

	▪ Patients who have signed the informed consent.
Exclusion criteria	Subjects will be excluded **<u>if at least one</u>** of the following criteria is met: ▪ Failure to meet any of the inclusion criteria; ▪ Antibiotics and probiotics' use in the four weeks prior to inclusion; ▪ Patients under current treatment or treated in the four weeks prior to inclusion with the following pharmacological classes: biguanides (metformin), gliptins (DDP-4 inhibitors), incretin mimetics (GLP-1 analogues), or GLP-2 analogues; ▪ Patients who have any condition precluding treatment with biologics; ▪ Patients under treatment with any investigational agent; ▪ Patients with active cancer; ▪ Subjects who are pregnant or breastfeeding; ▪ Subjects who are not willing to comply with appointments or procedures.
Expected number of patients:	400 patients (Crohn's disease: 240; Ulcerative colitis: 160)
Expected number of sites:	Approximately 10 centers are expected to participate.
Data collection	Data will be collected at baseline (prior to treatment start), day 1 (treatment start), and at each drug infusion (timepoints will vary depending on the treatment scheme), until the end of the follow-up period of two years. Data will also be collected at one year of follow-up (between 48 and 56 weeks) after treatment start. Main data to collect: ▪ Anthropometric measures ▪ IBD location, activity, and disease course ▪ Extra-abdominal manifestations ▪ Concomitant pharmacological therapies (for IBD and other conditions) ▪ Dose of biologic agent ▪ Routine laboratory parameters (hemoglobin, leukocytes, neutrophils, eosinophils, lymphocytes, C-reactive protein, ferritin, iron, transferrin, and albumin) ▪ Stool samples (fecal calprotectin and DPP-4 levels) ▪ Blood samples (drug concentration and anti-drug antibodies levels) ▪ Adverse events ▪ Status: ongoing/discontinuation. (If discontinued, reason)
Study timeline	Study start: second trimester of 2021 Recruitment: one year Follow-up: two years per patient Study closure: last trimester of 2023 Overall duration: three years

2. Introduction

Crohn's disease (CD) and ulcerative colitis (UC) are chronic and idiopathic inflammatory diseases that share clinical and pathological features.[1] In Europe, incidence rates range from 0.7 to 9.8 cases per 100,000 person-years in CD and 1.5-20.3 cases per 100,000 person-years in UC. In addition, a north-south gradient is evident, with incidence rates 40-80% higher in the Nordic countries.[2]

Inflammatory bowel disease (IBD) results from an imbalance between anti-and pro-inflammatory mediators. Although its cause is not determined, IBD is thought to stem from a complex set of genetic and environmental factors, culminating in intestinal inflammation.[3,4]

CD can potentially affect the entire digestive tract, although in 30% of cases it primarily involves the small intestine (particularly the terminal ileum), in about 20% of cases it involves only the colon and in 50% of cases both sites are involved. There is predominant involvement of the mouth or gastroduodenal area in only 5-15% of cases. Gastrointestinal symptoms depend on the location, extent, and severity of involvement of the affected region.[1]

UC is characterized by recurrent episodes of inflammation limited to the mucous layer of the colon. It often involves the rectum and can extend proximally and continuously, affecting other parts of the colon and in some cases with global reach, being called ulcerative pancolitis.[5]

The current therapeutic arsenal for the treatment of IBD includes aminosalicylates, corticosteroids, immunosuppressants and biological therapies. The advent of monoclonal antibody therapies has revolutionized inflammatory bowel disease (IBD) treatment and delivered great benefits to patients. Currently, seven agents are approved: four are anti-TNF-α agents (infliximab, adalimumab, golimumab and certolizumab); two are anti-integrin (natalizumab and vedolizumab) while ustekinumab targets interleukin-12/23 pathways.[6] Tofacitinib, an oral small-molecule that targets Janus kinase (JAK) 1, 2, and 3, was approved in 2018 for the treatment of moderate-to-severe active UC with inadequate response, loss of response or intolerance to corticosteroids, immunosuppressive agents and/or biological therapies. JAK inhibitors present several advantages in comparison with monoclonal antibodies, among which: oral administration, predictable pharmacokinetics with a reduced plasma half-life, rapid onset of action and quick clearance (beneficial in cases of severe infections and need for surgery and the lack of immunogenicity[7].

However, these agents are not completely effective in the long-term, which is evidenced by recurrence rates of up to 50%.[8] In addition, these therapeutic strategies are often associated with undesirable side effects. There is, therefore, a pressing need to develop new therapeutic strategies that are not only effective and able

to reduce disease exacerbations, but also able to repair the often extensive damage to the intestinal mucosa, ideally with minimal adverse side-effects.[9]

2.1. Dipeptidyl peptidase 4

Dipeptidyl peptidase 4 (DPP-4) is a glycoprotein bound to the cell membrane, acting as a receptor, but also fulfilling proteolytic functions. It is expressed on the surface of various cell types including enterocytes and related cell lines, such as Caco-2 cells.[10]

Due to its broad biochemical function, the effects mediated by DPP-4 reach multiple physiological systems. For example, due to its regulatory role in enteroendocrine hormone activity, DPP-4 has effects on the permeability of the intestinal mucosa and the regulation of appetite; it also leads to decreases in the bioavailability of the anorectic hormone GLP-1 (glucagon-like peptide-1), intestinotrophic enterohormone GLP-2 (glucagon-like peptide-2) and incretin GIP (gastric inhibitory peptide). DPP-4, via degradation of GLP-2, which acts as a growth factor and anti-inflammatory factor in the intestinal tissue, have therefore the potential to amplify intestinal inflammation.[10,11]

DPP-4 activity also appears to be altered in several inflammatory diseases such as systemic lupus erythematosus, rheumatoid arthritis, multiple sclerosis, among others.[10]

The few studies published in this area have demonstrated that individuals with active CD have elevated levels of GLP-2 and reduced levels of DPP-4. It is postulated that this alteration appears as an innate compensatory mechanism for the intestinal lesion associated with the disease and as a mechanism for the preservation of biologically active GLP-2.

2.2. Rationale

DPP-4 appears to be associated with the regulation of inflammatory processes in several inflammatory diseases, including IBD. However, its role is not yet fully elucidated and further studies need to be conducted to clarify the role of DPP-4 in IBD, as well as evaluate its potential use as a biomarker of disease activity and treatment response.

2.3. Research hypothesis

We hypothesize that DPP-4, in patients with IBD, is associated with levels of inflammation, disease activity, and response to treatment.

3. Objectives

3.1. Primary objectives

- To explore the associations between plasma/fecal DPP-4 levels and clinical activity in IBD patients.
- To explore the predictive potential of plasma/fecal DPP-4 values with regards to treatment response (escalation and de-escalation of therapy).
- To explore the associations between plasma/fecal DPP-4 levels, and fecal and plasma biomarkers.
- To explore the association between plasma/fecal DPP-4 and fecal calprotectin levels with serum infliximab/vedolizumab/ustekinumab levels throughout a follow-up period of 2 years.
- To explore the association between plasma/fecal DPP-4 and fecal calprotectin levels with the development of anti-infliximab/anti-vedolizumab/anti-ustekinumab antibodies throughout a follow-up period of 2 years.

3.2. Secondary objectives

- To explore the association between serum infliximab/vedolizumab/ustekinumab levels and development of anti-drug antibodies.
- To explore the association between serum infliximab/vedolizumab/ustekinumab levels and clinical activity (CD – Harvey-Bradshaw index and UC – partial Mayo score).
- To explore the association between serum infliximab/vedolizumab/ustekinumab levels and physician reported clinical outcomes.

4. Study design

The design will consist of a prospective, multicenter, observational study of IBD patients. **Patients enrolled in this study will receive treatment according to routine clinical practice**. There is no imposed experimental intervention, and prescribing decisions from the study physicians will be independent of patient's participation in this study.

The enrolment period of the prospective component of the study will start on day of the study visit of the first included patient and will run, within the defined period, until the expected number of patients is attained. The total observation time for each patient will be approximately 2 years. During this period, patients will be evaluated at different timepoints, following the schedule of their routine clinical appointments, according to clinical decision.

Clinical data, peripheral blood samples (to be collected before monoclonal antibody administration), and stool samples will be collected at each visit, as per routine practice. Blood samples will be used to measure levels of biomarkers of interest (DPP-4, CRP), drug levels, and anti-drug antibody levels. Stool samples will be used to measure levels of biomarkers of interest (DPP-4, FC). A detailed schedule of assessments is provided in Appendix 1 (15.1.)

5. Study timeline

This study is expected to start during the second trimester of 2021 and will last until the observation period of two years is completed for all patients. The overall expected duration is three years: one-year recruitment period (may be extended if the target number of participants is not achieved within the defined timeframe); two-year follow-up period per patient, including data analysis and manuscript drafting. (Appendix 2, 15.2.)

Patients will be observed and data will be collected at baseline (prior to treatment start), day 1 (treatment start), and at each drug infusion (timepoints will vary depending on the treatment scheme).

6. Study population

This study will enroll IBD patients recruited from an expected number of 10 national hospital centers. Overall, 400 IBD patients are expected to be included in this study:

- 240 patients with Crohn's disease
- 160 patients with Ulcerative colitis

6.1. Inclusion criteria

For inclusion, **all of the following** criteria must be met:

- Patients aged 18 years or older;
- Patients with active luminal Crohn's Disease (moderate to severe) or active ulcerative colitis (moderate to severe);
- Patients who will initiate biologic agents (infliximab, vedolizumab, ustekinumab [reference product or biosimilar]) or tofacitinib at the time of inclusion according to physician's criteria: both anti-TNF-naïve or patients previously treated with monoclonal antibodies;
- Patients who have been submitted to colonoscopy at the baseline (4 weeks before or after baseline);
- Patients who have signed the informed consent.

6.2. Exclusion criteria

Subjects will be excluded **if at least one** of the following criteria is met:

- Failure to meet any of the inclusion criteria;
- Antibiotics and probiotics' use in the four weeks prior to inclusion;
- Patients under current treatment or treated in the four weeks prior to inclusion with the following pharmacological classes: biguanides (metformin), gliptins (DDP-4 inhibitors), incretin mimetics (GLP-1 analogues), or GLP-2 analogues;
- Patients who have any condition precluding treatment with biologics;
- Patients under treatment with any investigational agent;
- Patients with active cancer;
- Subjects who are pregnant or breastfeeding;
- Subjects who are not willing to comply with appointments or procedures.

6.3. Discontinuation from observation period

IBD patients receiving monoclonal antibodies will be followed for 24 months. However, observation may be prematurely stopped for different reasons including (but not limited to):

- Failure to meet any of the inclusion criteria;
- Protocol violation;
- Failure to submit blood and stool samples as indicated at each phase of the study;
- Need for use of antibiotics and/or probiotics during the study;
- Need for use of biguanides (metformin), gliptins (DDP-4 inhibitors), incretin mimetics (GLP-1 analogues), or GLP-2 analogues;
- Cancer diagnosis;
- Death;
- Pregnancy;
- Patient withdrawal of consent or loss to follow up.

In case of drop-out, the date of study discontinuation and the associated reason will be recorded in an electronic Case Report Form (eCRF).

7. Data collection

All data will be obtained from medical charts, laboratory reports and, when relevant, complemented with subject's interview or other medical sources. The investigator will be responsible for ensuring that all data is accurately recorded in the case report form (CRF) and that the subject's confidentiality is always maintained.

7.1. IBD patients

7.1.1. Baseline characteristics

The following data will be collected for all eligible IBD patients, before starting monoclonal antibody therapy:

- Date of birth (MM/YYYY)
- Sex at birth
- Residence (district)
- Anthropometric parameters
- Smoking status:
  - Non-smoker: never smoked before or smoked very occasionally
  - Former smoker: patients who stopped smoking more than 6 months before inclusion
  - Current smoker: more than 7 cigarettes per week in the last 6 months (≤ 10 cigarettes/day, 11 to 20 cigarettes/day or > 20 cigarettes/day)
- Family history of IBD
- Date (MM/YYYY) of IBD diagnosis (based on clinical, endoscopic, histologic, and radiographic criteria)
- Disease presentation (abdominal, constitutional, abdominal + constitutional, anal disease, acute abdomen, fever, anemia, extra-intestinal manifestations, abdominal mass)
- Clinical characteristics
  - IBD location/behavior, according to the Montreal Classification
    - CD patients: regarding location as ileal (L1), colonic (L2), ileocolonic (L3) ± upper gastrointestinal tract (L4); regarding behavior as non-stricturing and non-penetrating (B1), structuring (B2) and penetrating (B3), ± perianal disease (p)
    - UC patients: regarding extent as ulcerative proctitis (E1), left-sided UC (E2) or pancolitis (E3); regarding severity as moderate UC (S2) or severe UC (S3)

  - Disease activity:
    - CD patients: Harvey Bradshaw Score (5 items: general well-being, abdominal pain, number of liquid or soft stools per day, abdominal mass, and complications)
    - UC patients: Partial Mayo Score (3 items: stool frequency, rectal bleeding, physician's global assessment)

- Endoscopy findings:
  - as part of routine practice, patients undergo endoscopic studies in the 4 weeks prior to starting treatment with monoclonal antibodies; data pertaining to endoscopic findings from eligible patients will be collected; disease activity will be classified using the Simple Endoscopic Score (for CD patients) and the Mayo Score (for UC patients); routinely, 4 biopsies are expected to be performed in the lesioned colonic segments, with 4 additional biopsies from adjacent non-lesioned colonic segments, for a total of 8 biopsies in IBD patients.

    This procedure does not pose additional risks to the patients, is not interventional in nature, and does not deviate from standard clinical care[12,13].
- Extra-abdominal manifestations
- Comorbidities (including heart disease, hypertension, bile acid malabsorption, celiac disease, rheumatoid arthritis, diabetes mellitus type I, Sjögren syndrome, psoriasis, thyroiditis, hyperthyroidism or hypothyroidism or other auto-immune disorders)
- History of bowel surgery for IBD
- Previous pharmacological therapies for IBD
- Current pharmacological therapies for IBD, including: 5-aminosalicylate, corticosteroids, thiopurines (azathioprine or 6-mercaptopurine), methotrexate, cyclosporine, tacrolimus, mycophenolate mofetil and biologic agents
- Current medical treatments for other conditions, including NSAIDs, PPIs, SSRIs, or statins
- Use of antibiotics in the last year
- Name of the biologic agent (brand or biosimilar), dosage, and date of start

7.1.2. Data collected on each evaluation

The following data will be collected at each clinical assessment, during induction and maintenance phases:

- Anthropometric parameters
- Disease activity (as described above)
- Disease course: relapse of disease activity and need for therapy adjustment; changes in dose and frequency of administration of monoclonal antibody; changes in other IBD therapies
- Concomitant non-IBD pharmacological therapies, if changed since previous data collection point
- Extra-abdominal manifestations
- Routine laboratory parameters (hemoglobin, leukocytes, neutrophils, eosinophils, lymphocytes, C-reactive protein [CRP], iron, transferrin, ferritin, and albumin)
- Central Laboratory Parameters:
  - Stool samples: assessment of fecal calprotectin and DPP-4 levels
  - Blood samples: assessment of DPP-4, drug, and anti-drug antibodies levels
- Use of health resources (surgeries, consultations, admission to emergency rooms, hospitalizations; related or not with IBD)
- Adverse events (AE):
  - Corresponding to any harmful manifestation in a patient (that does not necessarily have a causal relationship with the treatment): information on drug, therapy duration, event description (characteristics and duration), action taken, outcome, causality assessment and severity. The following cases will be considered serious AE: event associated to life-threatening risk or leading to death; event that prolongs hospitalization, causes persistent or significant disability; if there is suspicion of transmission of an infectious agent through medication; another situation considered severe by the investigator

    All adverse events must be reported to the responsible pharmacovigilance units
- Status: ongoing/discontinuation; reason for discontinuation

Week 52 (±4): Data regarding the endoscopic studies performed between week 48 and 56, according to routine practice, will be recorded and classified using the Mayo score (for UC) or the Simple endoscopic score (for CD).

8. Sample collection and processing

Samples will be used for the study analysis. After study completion, all patient data and samples will be irreversibly anonymized, and archived in a secure, validated biobank.

8.1. Blood samples for central laboratory parameters

IBD patients' blood samples will be collected before administration with new dosage of monoclonal antibody.

For evaluation of DPP-4, drug and anti-drug antibodies' levels, blood will be collected prior to administration of drug into gel tubes for coagulation of the sample. At least one hour after collection, the sample will be centrifuged, and the serum transferred to tubes and stored at -20° C.

8.2. Stool samples for central laboratory parameters

Stool collection devices will be provided by GEDII. Samples must be collected according to instructions provided alongside the stool collection kits. Samples should be stored at room temperature immediately after collection and delivered to the clinician within 24-48h. At the hospital, stool samples must be kept at room temperature for the maximum of 48h until shipment to the central laboratory (GEDII, Centro de Investigação Médica, Faculdade de Medicina da Universidade do Porto). The transport must be done within 24h, at room temperature. Exceptionally, stool samples may be delivered to the participating center in the 48h following the infusion/follow-up visit.

8.3. Assessment of serum and fecal DPP-4

Quantification of plasma and fecal DPP-4 will be conducted using commercial DPP-4 ELISA Kits, according to manufacturer's instructions.

8.4. Assessment of fecal calprotectin (FC)

Quantification of fecal calprotectin from extracted fecal samples will be conducted using an automated clinical chemistry analyzer, a turbidimetric immunoassay, fCAL turbo.

8.5. Assessment of drug and anti-drug antibodies' levels

Quantification of infliximab drug levels will be performed using Quantum Blue® (Buhlmann®), and infliximab anti-drug antibodies will be performed using an *in-house* ELISA assay[14]. Vedolizumab and ustekinumab drug levels and anti-drug antibodies will be quantified using commercially available ELISA kits, according to manufacturer's instructions.

9. Statistical analysis

9.1. General considerations

The association between two quantitative variables will be tested through the Pearson correlation coefficient, or the Spearman correlation coefficient if the normality assumption is not verified. The association of two categorical variables will be tested through the Chi-Square test or Fisher Exact test (if applicable).

The comparison of two independent samples for quantitative variables will be performed through t-test for independent samples or the Mann-Whitney non-parametric test, according to the assumption validations of the statistical test (if applicable).

The association between quantitative variables and a categorical binary variable will be analyzed by Area Under the ROC Curve.

The concordance kappa will be used for binary variables, if applicable.

For each subset, the proportion of patients with active disease, clinical response, clinical remission (physician reported Harvey-Bradshaw Index or partial Mayo score), presence of anti-drug antibodies at each data collection time points will be summarized using 95% confidence intervals.

In all hypothesis testing a significance level of 5% will be considered. The analysis will be performed using IBM SPSS Statistics, Version 23. (IBM Corp, Armonk, NY, USA).

9.2. Sample size

The sample size is not based on formal statistical assumptions; 400 patients (Crohn's disease: 240; Ulcerative colitis: 160) are expected to be recruited. This sample will allow a descriptive analysis of clinical characteristics among pre-defined subsets. Furthermore, this sample will allow the exploratory analysis of correlations between independent variables and outcomes of interest within subsets during the follow-up period.

10. Pharmacovigilance

10.1. Notification of adverse events, special situations and/or quality complaints

The participating investigators shall be responsible for the compliance with the Pharmacovigilance obligations defined in the applicable law and regulations. This includes, registration and notification of safety issues, within the time frames required by law, to the Competent Health Authorities, Ethics Committee(s) and notification to the Sponsor-Investigator. Safety issues include adverse events, quality complaints and special situations, including pregnancy, related to any medication involved in the study.

The Sponsor-Investigator will share the following safety information with Janssen's Pharmacovigilance Unit:

- All serious and non-serious adverse events (regardless of causality with the suspected medication), special situations including exposures during pregnancy, adverse events of special interest, and complaints of product quality since the first use of a Janssen drug, and respective follow-up information, by fax or secure email, in English, within 3 calendar days of becoming aware of them.
- A list of all serious and non-serious adverse events occurring with a Janssen drug at least annually and in final report form within six (6) months after the completion of the study.
- For reasons of reconciliation and every 3 months, the Janssen Pharmacovigilance Unit shall send to the Sponsor-Investigator a listing of all serious adverse events received up to that time with a Janssen drug. The Sponsor-Investigator shall confirm this information and, in case of detection of an unreported reaction, inform the Pharmacovigilance unit as soon as possible.

11. Ethical and legal aspects

11.1. Ethics

The study will be conducted according to the ethics principles originated from the Declaration of Helsinki and the Portuguese Clinical Research law (Law #21/2014, 16th April 2014).

A copy of the protocol, the proposed informed consent forms and other written subject information will be submitted to the competent Ethics Committee for written approval. A copy of the written approval of the protocol and of the informed consent forms must be received, by the Investigator, before the recruitment of subjects and data collection are initiated.

The investigator will submit and, where necessary, obtain approval from the competent Ethics Committee for all subsequent protocol amendments and changes to the informed consent documents.

11.2. Informed consent

Before any protocol specific procedures are performed, the investigator is responsible for obtaining written informed consent from the subject (or authorized representative) and after an adequate and clear explanation of the aims, methods, anticipated benefits, and potential hazards of the study.

The informed consent process should be documented in the subject's medical charts, and the informed consent form should be signed and personally dated by the subject (or authorized representative) and by the person who conducted the informed consent discussion (not necessarily an investigator). The original signed informed consent form should be retained in accordance with institutional policy, and a copy of the signed consent form should be provided to the subject (or authorized representative).

12. Quality control

The study will involve a GEDII monitor who will be responsible for ensuring that the study is conducted according to the protocol and to the local regulatory/ethical requirements.

Before the start of activities, a study monitor will conduct Initiation Visits at the sites in order to train the investigational team on the protocol and other protocol-related procedures.

During the study, the monitor will also be responsible for conducting periodic monitoring visits at the sites to ensure that the protocol is being followed and the data recorded is accurate and collected according to the defined procedures.

The sites may be subject to review by the Independent Ethics Committee and/or to quality assurance audits performed by a GEDII designated representative, and/or to inspection by appropriate regulatory authorities. The Investigator(s) and their relevant staff should be available during the monitoring visits and possible audits or inspections. The investigator and institution will allow the appropriate regulatory authorities direct access to source documents to perform this verification.

Prior to study start, the investigator is required to sign a protocol signature page confirming his/her agreement to conduct the study in accordance with this protocol and to give access to all relevant data and records to monitors, auditors, Ethics Committees, and regulatory authorities, as required.

All investigators and study staff will receive training on the protocol and other protocol-related procedures prior to start of activities. The training will be provided by GEDII or its representative.

13. Data handling

13.1. Confidentiality

The investigator is responsible for ensuring that the subject's confidentiality is maintained, according to the Regulation (EU) 2016/679 of the European Parliament and the Counsil of 27 April 2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data, and the Law #58/2019, 8th August 2019. Questionnaires, database profiles and other documents generated in this study will be identified by a unique subject identification number.

13.2. Study archive

The investigator will keep an adequate archive of all study documentation with access restricted to study team. The study archive will be kept at each site for at least 5 years from the study close out.

13.3. Publication policy

All documents and results generated from this clinical study are exclusive property of the Sponsor-Investigator and GEDII.

The results of the study may be presented by the Sponsor-Investigator or associates in national and international meetings and will be published in international papers.

The study results can only be published after the clinical study is terminated; data analysis is completed only upon the agreement of the study's scientific board. The publication should include the results from all the centers that participated in the clinical investigation. The publication of results should be agreed by all participating investigators and in strict adherence to the principles originated from the Helsinki Declaration and according to Portuguese Clinical Research law (Law #21/2014, 16th April 2014).

Any investigator involved in the study who intends to develop any sub-analysis using the collected data may submit a proposal to the study sponsor (GEDII). Any related publications must be previously approved in written by the Sponsor-Investigator and GEDII.

13.4. Authorship criteria

For all publications related to this study, the GEDII will comply with recognized ethical standards concerning publications and authorship established by the International Committee of Medical Journal Editors (*Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals – Updated December 2013*).

For all publications, related with this clinical study, the order of the authors is as follows:

- The first author position will correspond to the scientific coordinator;
- The subsequent authors' positions will correspond to the principal investigators from each center, who will be ranked decreasingly according to the number of patients included in the study, as far as the number of co-authors allowed by the journal is not exceeded;
- All the participating investigator not figuring in the authorship (due to journal's limitations in the number of co-authors) will be cited in the acknowledgement section of the publication;

14. References

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15. Appendix

15.1. Appendix 1 – IBD group data collection timepoints

Information to collect	Baseline	Induction phase	Maintenance phase	One-year visit
400 patients: 240 with Crohn's disease 160 with Ulcerative colitis	Prior to treatment start	IFX/VEDO: day 1, weeks 2 and 6 UST/TOFA: day 1, week 8	IFX-iv/VEDO: every 4/4, 6/6, or 8/8 weeks UST: 4/4 or 8/8 weeks TOFA/IFX-sc 8/8 weeks	Between 48 and 56 weeks
Date of birth	x			
Sex at birth	x			
Residence (district)	x			
Anthropometric parameters[1]	x	x	x	x
Smoking status	x			
Date of IBD diagnosis	x			
Symptoms at disease onset[2]	x			
IBD location/behavior [3]	x			x
IBD clinical score/disease activity[4]		x	x	x
History of bowel surgery	x			
Familial history of IBDs	x			
Extra-abdominal manifestations	x	x	x	x
Past medical history	x			
Previous or current pharmacological therapies	x	x	x	x
Endoscopic findings	x			x
Dose of biologic agent, date of start		x	x	x
Routine laboratory parameters[5]		x	x	x
Levels of biological drug and anti-drug antibodies		x	x	x
Fecal calprotectin levels		x	x	x
Serum/fecal DPP-4 levels		x	x	x
Use of health resources[6]		x	x	x
Adverse events		x	x	x

[1]Height, weight, body mass index (BMI), waist circumference
[2]Abdominal, constitutional, abdominal + constitutional, anal disease, acute abdomen, fever, anaemia, extra-intestinal manifestations, abdominal mass.
[3]IBD location/behavior, according to the Montreal Classification
[4]Disease activity according to the Harvey Bradshaw Score (for CD) and Partial Mayo Score (for UC).
[5]Haemoglobin, leukocytes, neutrophils, eosinophils, lymphocytes, CRP, iron, transferrin, ferritin, and albumin.
[6]Surgeries, consultations, admission to emergency rooms, hospitalizations; related or not with IBD
All dates must be registered in the format MM/YYYY
Abbreviations: Crohn's disease (CD), infliximab (IFX), infliximab intravenous (IFX-iv), infliximab subcutaneous (IFX-sc), tofacitinib (TOFA), ulcerative colitis (UC), ustekinumab (UST), vedolizumab (VEDO)

15.2. Appendix 2 – Study timeline

Tasks	2021				2022				2023			
	Jan-Mar	Apr-Jun	Jul-Sep	Oct-Dec	Jan-Mar	Apr-Jun	Jul-Sep	Oct-Dec	Jan-Mar	Apr-Jun	Jul-Sep	Oct-Dec
Patients recruitment												
Observation period												
Data analysis, manuscripts drafting												