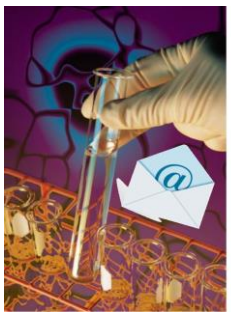
CLINICAL STUDY PROTOCOL

Grupo de Estudo da Doença Inflamatória Intestinal (GEDII)



Title: Impact of AZAthioprine in maintaining clinical, BIOmarkers and endoscopic remission among new Pediatric patients with Crohn's disease treated with exclusive polymeric diet for induction of remission: a 2-year longitudinal analysis from the GEDII Registry – P-BIOAZA

Study code:P- **BIOAZA**

Type of study: Observational

Date of protocol: *5 March 2015*

Version no.: 1

Study Sponsor:	Grupo de Estudo da Doença Inflamatória Intestinal (GEDII)
Name of Scientific Coordinator	Dra. Eunice Trindade
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This document contains confidential information.
This document must not be disclosed to anyone other than the study staff and members of the independent ethics committee or regulatory agencies.

PRINCIPAL INVESTIGATOR SIGNATURE PAGE *(to be signed by the PI from each participating center)*

Study Title: Impact of AZAthioprine in maintaining clinical, BIOmarkers and endoscopic remission among new pediatric patients with Crohn's Disease treated with exclusive polymeric diet for induction of remission: a 2-year longitudinal analysis from the GEDII Registry – P-BIOAZA

Study Code: P- BIOAZA

Protocol Version/Date: version 5 Mar 2015

Center Name: _____

Principal Investigator:

Name:

Academic degree:

Address:

Phone:

Email:

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 of Signature

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1. Sinopse

Title:	Impact of AZAthioprine in maintaining clinical, BIOmarkers and endoscopic remission among new pediatric patients with Crohn's disease treated with exclusive polymeric diet for induction of remission: a 2-year longitudinal analysis from the GEDII Registry – P-BIOAZA
	P-BIOAZA
Scientific Coordinator:	Dra. Eunice Trindade
Disease/Condition	Crohn's disease
Rational	Thus far, there is limited data on the impact of azathioprine in maintaining fecal calprotectin remission in CD patients. Taking advantage of the valuable tool such as the GEDII Registry, the GEDII promoted a research to investigate the clinical, biomarker and endoscopic outcomes among patients with Crohn's disease and managed with azathioprine in the real-world practice. This study will follow a cohort of pediatric patients with Crohn's disease registered in the GEDII Registry who have induced remission with exclusive polymeric diet and who did initiate azathioprine at diagnosis based on physician's criteria. The cohort will be followed for a period of 2 years. The primary aim of this study is to explore the potential value of azathioprine in maintaining fecal calprotectin remission in pediatric patients with CD.
Research hypothesis:	We hypothesize that pediatric Crohn's disease patients treated with exclusive polymeric diet and with azathioprine at the diagnosis will achieve and maintain fecal calprotectin remission.
Primary Objectives:	Among patients with Crohn's disease at study inclusion, registered in the GEDII Registry: •To evaluate the impact of azathioprine in inducing calprotectin remission (calprotectin levels < 200 ug/g) at week 12. •To evaluate the impact of azathioprine in maintaining calprotectin remission (calprotectin levels < 200 ug/g) at week 48 and 96 •To evaluate the impact of azathioprine in maintaining calprotectin remission throughout a follow up period of 96 weeks.
Secondary Objective(s):	•To explore the association of the fecal calprotectin assessment regarding clinical and endoscopic outcomes. •To explore the association of fecal calprotectin levels with clinical outcome throughout a follow up period of 96 weeks. •To explore the association of fecal calprotectin levels with endoscopic activity throughout a follow up period of 96 weeks. •To evaluate the impact of azathioprine in inducing clinical remission at week 12. •Among patients who achieve clinical remission with azathioprine, to evaluate the maintenance of clinical remission up to week 96. •To evaluate the impact of azathioprine in inducing endoscopic remission at week 96. •To evaluate the impact of azathioprine in maintaining endoscopic remission throughout a follow up period of 96 weeks. •To evaluate the rate of azathioprine persistence throughout a follow up period of 96 weeks

	•To evaluate the time until loss clinical remission
Study Design:	Multicenter, prospective, observational study designed to gather and analyze data on a consecutive cohort of subjects with CD who initiated therapy with azathioprine, according to physician's clinical decision. There is no imposed experimental intervention and prescribing decisions from the study physicians will be independent of patient's participation in this study. The maximum overall duration of observation for each patient is 96 weeks since the start of observation period (Day 1).
Inclusion Criteria:	Study patients must fulfill the following criteria: 1.Male or female patients, <18 years; 2.Patients who are registered in the GEDII Registry; 3.Patients who have been treated with exclusive polymeric diet and start azathioprine at diagnosis **AND** who meet one of the following: o Moderate/Severe disease (PCDAI>31) at start of study with fecal calprotectin levels > 100 ug/g o Mild disease (PCDAI>11<30) with fecal calprotectin levels > 100 ug/g and/or Endoscopic activity (Simple endoscopic score for Crohn's disease [SES-CD] ≥ 3) 4. Patients who gave their informed consent.
Exclusion Criteria:	Patients will be excluded if at least one of the following criteria is met: 1. Patients on methotrexate or under biologics. 2. Any contraindications regarding the use of azathioprine; 3. Patients who are being treated with any investigational agent; 4. Patients who are not willing to comply with routine clinical appointments.
Expected number of patients:	80 patients
Expected number of sites:	Approximately 11 centers are expected to participate.
Subject selection:	The study will analyze a consecutive sample of 80 patients who are registered in the GEDII Registry and who fulfill the protocol's eligibility criteria. The recruitment period is 12 months but may be extended if the target number of participants is not achieved within the defined timeframe.
Exposure of interest:	Azathioprine is the exposure of interest. This medication may be tailored or optimized according to the physician's clinical criteria and taking in to account the label of the product.
Main data collected:	Socio-demographic, clinical and endoscopic data and patterns of use of azathioprine and other therapies for CD will be collected during the observation period, as well as PCDAI. The data collection time points in this study will reflect the routine schedule for CD patients receiving polymeric diet and azathioprine. The stool samples for assessment of fecal calprotectin will be collected by the patient at home, at the data collection time points.
Endpoints	**Primary endpoints:** •Proportion of patients who achieve fecal calprotectin remission (< 200 ug/g) at week 12. •Proportion of patients who maintain fecal calprotectin remission (< 200 ug/g) at week 48 and 96. •Proportion of patients who maintain fecal calprotectin remission (< 200 ug/g) at each data collection time point up to week 96. **Secondary endpoints:** •Fecal calprotectin levels assessed at each data collection time point. •Fecal calprotectin levels and clinical activity (PCDAI) at each data collection time

	point. •Fecal calprotectin levels and endoscopic activity (Simple endoscopic score for Crohn's disease [SES-CD]) at week 96. •Proportion of patients with clinical remission (PCDAI ≤ 10) at each data collection time up to week 96. •Proportion of patients who achieve endoscopic remission (SES-CD ≤ 2) at week 96. **Among patients with moderate/severe CD (at inclusion) and who maintain clinical remission with azathioprine:** •Median time to loss of clinical remission (PCDAI>11) with azathioprine **Among patients with mild Crohn's disease at study inclusion and who maintain clinical remission with azathioprine:** •Median time to loss of clinical remission (PCDAI>11) with azathioprine •Physician reported clinical outcome (PCDAI) at each data collection time points.
Statistical methods	The association between two quantitative variables will be performed through Pearson correlation coefficient or Spearman correlation coefficient, in case 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 in respect to 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 proportion of patients with clinical (PCDAI), and endoscopic remission (SES-CD) and biomarker remission (fecal calprotectin < 200 ug/g) at each data collection time points will be summarized using 95% confidence intervals, for the overall population and for the subset of patients with active and non-active CD at study inclusion. Results for hemoglobin, leukocytes, neutrophils, eosinophiles, basophiles, monocytes, lymphocytes, erythrocyte sedimentation rate, CRP, ferritin, iron, transferrin and fecal calprotectin, will be summarized by total number of observations (n), mean, median, standard deviation, minimum and maximum. The correlation between fecal calprotectin levels with clinical (PCDAI score) and endoscopic (SES-CD) activity of disease will be analyzed through the Pearson or Spearman (in case the normality assumption is not verified) correlation coefficient at each data collection time point: ○ Fecal calprotectin levels will be correlated with PCDAI score – Day 1, week 8, and every 12 weeks up to week 96. ○ Fecal calprotectin levels will be correlated with Simple endoscopic score for Crohn's disease (SES-CD) – Day 1 and Week 96. Generalized Estimated Equations will be used to investigate clinical and endoscopic remission (homogeneity) throughout data collection time points (time-effect) for each of the two subsets (patients with moderate/severe CD; patients with mild CD at Day 1). It is expected that: patients will achieve clinical and endoscopic remission and will maintain the remission up to week 96.
Overall Study Duration:	The overall duration of the study is approximately three years (1 year of recruitment + 2-year observation period).
Study timelines:	The study is expected to start during the 2nd Quarter of 2016. Study closure is expected to occur 3th Quarter of 2019.

2 INTRODUCTION

2.1 CROHN'S DISEASE

Inflammatory bowel disease (IBD) is a chronic and disabling condition with an increasing incidence in southern Europe. The etiology of IBD remains unknown, but the characteristic disproportionate inflammatory response in the gut may develop through various mechanisms at the cellular and subcellular level.[1] Ulcerative colitis (UC) and Crohn's disease (CD) represent the two main types of IBD.

In Portugal, the prevalence of IBDs increased from 86 to 146 persons per 100 000 inhabitants between 2003 and 2007. Within the same timeframe, the prevalence of UC and CD increased from 42 to 71 persons per 100 000 inhabitants and from 43 to 73 persons per 100 000 inhabitants, respectively. IBDs are more prevalent among women.[2] The prevalence of Portuguese pediatric IBD is unknown. In the last years the incidence of pediatric Crohn´s disease seems to be increasing for uncertain reasons in different parts of the world.[3]

Crohn's disease is a relapsing, transmural inflammatory disease of the gastrointestinal mucosa that can affect the entire gastrointestinal tract from the mouth to the anus. Typical presentations include the discontinuous involvement of various portions of the gastrointestinal tract and the development of complications including strictures, abscesses, or fistulas.[4]

Given the autoimmune nature of CD, many of the therapies used for this disorder are targeted to reduce the inflammatory response. There are several pharmacologic options available to patients with CD, and therapy is often best tailored to the level of disease severity. Therapies for mild disease are aimed at alleviating symptoms while also minimizing medication-related side effects. The two main classes of medications most often used for mild disease are 5-aminosalicylate (5-ASA) and budesonide. Both of these agents are designed to work at the mucosal level, with reduced systemic absorption and effects.[5]

The initial therapy available for the acute management of CD has traditionally been systemic corticosteroids. Several early studies demonstrated the efficacy of corticosteroids in inducing remission in CD, with remission rates in up to 92% of patients.[6,7] Despite the ability to induce remission, corticosteroids have not demonstrated equivalent efficacy at maintaining remission and are also known for relevant long-term side effects.[6] Exclusive polymeric diet has a similar rate of induction of remission as corticosteroids and has no side effects. For that reason exclusive polymeric diet is considered the first line therapy to induce remission in children with active luminal disease usually in association with azathioprine for maintenance of remission. Thiopurines are recommended as one option for maintenance of steroid free remission in children at risk of poor disease outcome. Thiopurines alone are not recommend as induction therapy. When exclusive polymeric diet is not an option corticosteroids may be necessary.[8] There are two main classes of corticosteroid-sparing agents and in moderate to severe CD: the immunomodulators, which include azathioprine (AZA), 6-mercaptopurine (6MP), and methotrexate (MTX), and the newer "biologics," monoclonal antibodies against TNF-α, which include infliximab, adalimumab, and certolizumab pegol. In addition, there has been recent evidence suggesting that combination therapy with an immunomodulator and biologic may be the most efficacious and may potentially alter the underlying prognosis of CD.[5,9]

The thiopurines, which consist of AZA and 6MP, exert their effect via inhibition of purine synthesis. AZA is a prodrug, converted to 6MP within the host. AZA and 6MP dosing must be carefully titrated due to potential marrow-suppressive and hepatotoxic effects. The goal dose of AZA is typically

between 2 to 3 mg/kg/day, whereas the dose for 6MP is half this, at 1 to 1.5 mg/kg/d. As specific metabolites can lead to undesirable side effects, it is advisable to measure TPMT genotype or enzymatic activity prior to initiation of therapy. A rare TPMT genetic variant, for which ~0.3% of individuals are homozygous, can result in decreased TPMT function and elevated levels of 6-TG resulting in life-threatening marrow suppression.[10,11] Approximately 11% of individuals are heterozygotes for this codominant mutation, requiring reduced dosing.

Two recent Cochrane analyses have synthesized the existing data regarding the efficacy of AZA and 6MP in the induction and maintenance of remission in CD.[12,13] Prefontaine et al. (2010) assessed eight RCTs of AZA/6MP for induction of remission in 425 patients with active CD.[12] Overall response in the pooled treatment group was 54 versus 33% in the placebo arm (odds ratio [OR], 2.43; 95% CI, 1.62-3.64). In addition to clinical response, five studies reported data on corticosteroid reduction while on these agents, with 76 of 117 (65%) thiopurine-treated patients exhibiting reduced steroid use, compared with 39 of 109 (39%) in the placebo group (OR, 3.69; 95% CI, 2.12-6.42). This meta-analysis was updated in 2013, showing that AZA and 6MP offer no advantage over placebo for induction of remission or clinical improvement in active Crohn's disease. However, the combination of AZA and infliximab was superior to infliximab alone for induction of steroid-free remission.[14]
With respect to maintenance of remission, a recent systematic review assessing seven trials with AZA and one with 6MP demonstrated, among 550 adult patients, an OR of 2.32 (95% CI, 1.55-3.49) for AZA and 3.32 (95% CI, 1.40-7.87) for 6MP.[13] A significant number of patients receiving AZA for maintenance of remission had to stop taking the medication due to intolerance, compared with placebo, with an OR of 3.74 (95% CI, 1.48-9.45).
Markowitz et al shown, in 55 children submitted to induction of remission with corticosteroids and randomized to receive 6MP or placebo at week 8, a 9% relapse in the group treated with 6 MP compared with 47% of the controls during a follow up period of 18 months. However subsequent observational studies have not been able to reproduce these low relapse rates, as relapse rates of 40 to 60% were found with azathioprine.[15,16,17,18]

Thiopurines such as azathioprine has also been associated with mucosal healing in Crohn's disease.[19] In children the effects of azathioprine on mucosal healing and histologic remission has been insufficiently studied until present. Studies in adults have shown different rates of mucosal healing; Mantarazis et al. found mucosal healing in 58% of patients while in SONIC study only 16,5% of patients on monotherapy with azathioprine achieved mucosal healing.[9,20] Sandborn et al. (1995)[21] evaluated the effect of intravenous loading doses of azathioprine. Six patients with inflammatory CD with both clinically and endoscopically active disease were included at study entry. After 16 weeks of treatment, of the four who achieved clinical remission all had endoscopic improvement and three had complete endoscopic healing. In the postoperative setting, D'Haens et al. (1997)[22] showed that among patients with severe postoperative recurrence of steroid-refractory ileal disease, treatment with azathioprine for at least 6 months resulted in healing of severe lesions. Of the patients who achieved clinical remission 6 of 15 (40%) achieved complete mucosal healing of their ileum. Subtotal mucosal healing was achieved in 5 (33%) and partial healing was achieved in 3 (20%).

In children, as in adult patients, endoscopy is mandatory for the confirmation of the diagnosis of inflammatory bowel disease. Recent literature address the importance of repeat endoscopy for the management of pediatric inflammatory bowel disease. Thakkar et al. have shown an overall rate of management change after endoscopy evaluation in children with inflammatory bowel disease in approximately 42%, with addition of a new medication been the most common intervention**.**[23]

The performance of endoscopic re-evaluation in the adequate time can contribute to the improvement of disease control allowing a best individualized tailored treatment.

Thus far, there is limited data on the impact of azathioprine in maintaining fecal calprotectin remission in CD patients.

2.2 FAECAL CALPROTECTIN

Given the invasive nature of endoscopy, the implementation of an easy, non-invasive method to support the pre-diagnostic screening and monitoring of disease activity is essential especially in children.

Fecal calprotectin has been shown to be useful in the diagnosis of IBD, correlates with mucosal disease activity and can help to predict response to treatment or relapse. In IBD, the presence of active gut inflammation is associated with migration of leucocytes, including neutrophils, to the gut mucosa.[24] As a result the faecal stream contains increased levels of these inflammatory proteins including calprotectin. Faecal calprotectin has been shown to differentiate quiescent from active disease in both patients with CD and UC.[25,26]

A meta-analysis including 30 prospective studies showed that the sensitivity and specificity of fecal calprotectin in diagnosing IBDs could reach up to 95% and 91%, respectively.[27] Fecal calprotectin also showed to be a reliable surrogate marker of treatment response.[28] Calprotectin levels decrease significantly after infliximab treatment for 12 weeks, and it correlates with endoscopic index of severity (CDEIS).[29] Røseth et al. (2004) showed that fecal calprotectin levels correlated with endoscopic mucosal healing.[30] A meta-analysis focusing on fecal calprotectin in IBD relapse showed that the sensitivity and specificity when predicting the relapse are 78% and 73%, separately.[31]

Currently , there is limited data on the impact of fecal calprotectin as a marker of treatment response in children. A meta-analysis including 8 pediatric studies (six prospective and two retrospective) showed that pooled sensitivity and specifity for the diagnosis utility of fecal calprotectin during the investigation of suspected pediatric IBD were 0,978 (95%CI,0.947-0.996) and 0,682 (95%CI, 0.502-0.863)[32]. Hamalain et al show in 36 children that fecal calprotectin levels rapidly decreases by week 2 in one third of patients treated with infliximab[33]. In a preliminary study Kolho et al measured fecal calprotectin level during the induction and maintenance treatment with infliximab in 76 pediatric patients and concluded that the long- term prognosis was related to the response to induction therapy and reflected in low fecal calprotectin values between weeks 2 and 6 and clinical remission [34].

2.3 THE GEDII REGISTRY

Patient registries are a powerful tool to observe the course of disease, understand variations in treatment and outcomes, to assess effectiveness, to monitor safety and harm and to examine factors that influence prognosis and quality of life. It also allows to describe care patterns and to measure quality of care.

From a clinician's perspective, registries can collect data about disease presentation and outcomes on large numbers of patients rapidly, thereby producing a real-world picture of disease, current treatment practices, and outcomes. A registry might also provide data that can be used to assess the degree to which clinicians are managing a disease in accordance with evidence based guidelines, focus attention on specific aspects of a particular disease that might otherwise be overlooked, or provide data for

clinicians to compare themselves with their peers. Overall, the use of patient registries appears to be active and growing.[35]
The "Grupo de Estudo da Doença Inflamatória Intestinal" (GEDII) Registry was created on 2005 and allows the regular and systematic capture of socio-demographic and clinical characteristics of patients diagnosed with IBDs (CD, UC and indeterminate UC). The Registry also captures the clinical and safety outcomes, treatments and the use of health resources.
So far, the Registry covers 20 gastroenterology departments of public hospitals. As of April 2014, the Registry comprised a total of 2500 patients.

2.4 RATIONALE

Thus far, there is limited data on the impact of azathioprine in maintaining fecal calprotectin remission in CD patients.
Taking advantage of the valuable tool such as the GEDII Registry, the GEDII promoted a research to investigate the clinical, biomarker and endoscopic outcomes among patients with Crohn's disease and managed with azathioprine in the real-world practice. This study will follow a cohort of pediatric patients with Crohn's disease registered in the GEDII Registry and who are initiating azathioprine, based on physician's criteria. The cohort will be followed for a period of approximately 2 years.
The primary aim of this study is to explore the potential value of azathioprine in maintaining fecal calprotectin remission in pediatric patients with CD.

2.5 RESEARCH HYPOTHESIS

We hypothesize that pediatric Crohn's disease patients who have induced remission with exclusive polymeric diet, and treated with azathioprine at the diagnosis will maintain fecal calprotectin remission.

3 OBJECTIVES

3.1 PRIMARY OBJECTIVES

Among patients with Crohn's disease at study inclusion, registered in the GEDII Registry:

- To evaluate the impact of **azathioprine** in inducing **calprotectin remission** (calprotectin levels < 200 ug/g) at week 12.
- To evaluate the impact of **azathioprine** in maintaining **calprotectin remission** (calprotectin levels < 200 ug/g) at week 48 and 96
- To evaluate the impact of **azathioprine** in maintaining **calprotectin remission** throughout a follow up period of 96 weeks

3.2 SECONDARY OBJECTIVES

- To explore the association of the **fecal calprotectin assessment** regarding clinical and endoscopic outcomes.

- To explore the association of **fecal calprotectin** levels with **clinical outcome** throughout a follow up period of 96 weeks.
- To explore the association of **fecal calprotectin** levels with **endoscopic activity** throughout a follow up period of 96 weeks.
- To evaluate the impact of **azathioprine** in inducing clinical remission at week 12.
- To evaluate the impact of **azathioprine** in maintenance of clinical remission up to week 96.
- To evaluate the impact of **azathioprine** in maintaining endoscopic remission at week 96.
- To evaluate the impact of **azathioprine** in maintaining endoscopic remission throughout a follow up period of 96 weeks.
- To evaluate the rate of **azathioprine** persistence throughout a follow up period of 96 weeks
- To evaluate the time until loss clinical remission

4 STUDY DESIGN

This is a multicenter, prospective, observational study designed to gather and analyze data on a consecutive cohort of subjects with CD who initiated therapy with azathioprine, according to physician's clinical decision. There is no imposed experimental intervention and prescribing decisions from the study physicians will be independent of patient's participation in this study.
A prospective, observational study is considered an appropriate tool to evaluate the impact of exposures of interest in real-world outcomes and an opportunity to explore biomarkers that can potentially predict clinical response in these settings.
The study will analyze a consecutive sample of 80 patients who are registered in the GEDII Registry and who fulfill the protocol's eligibility criteria. The recruitment period is 12 months but may be extended if the target number of participants is not achieved within the defined timeframe.
The maximum overall duration of observation for each patient is 96 weeks since the start of observation period (Day 1) – see Chronogram in Appendix.
Socio-demographic, clinical and endoscopic data and patterns of use of azathioprine and other therapies for CD will be collected during the observation period, as well as patient reported outcomes (PCDAI). The data collection time points in this study will reflect the routine schedule for CD patients receiving polymeric diet and azathioprine – see Chronogram in Appendix.
The stool samples for assessment of fecal calprotectin will be collected by the patient at home, at the data collection time points.
A total of 11 centers are expected to participate.

5 STUDY TIMELINES

The study is expected to start during the 2nd Quarter of 2016.

The overall duration of the study is approximately three years (1 year of recruitment + 2-year observation period).

6 STUDY POPULATION

6.1 INCLUSION CRITERIA

Study patients must fulfill the following criteria:

1. Male or female patients, <18 years old
2. Patients who are registered in the GEDII Registry;
3. Patients who induce remission with polymeric diet and start azathioprine at diagnosis

   AND who meet one of the following:

   o Moderate/Severe disease (PCDAI>30) at start of study
   o Mild disease (PCDAI<30) with fecal calprotectin levels > 100 ug/g and/or Endoscopic activity (Simple endoscopic score for Crohn's disease [SES-CD] ≥ 3
4. Patients who gave their informed consent.

6.2 EXCLUSION CRITERIA:

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

1. Patients on methotrexate or under biologics.
2. Any contraindications regarding the use of azathioprine;
3. Patients who are being treated with any investigational agent;
4. Patients who are not willing to comply with routine clinical appointments.

6.3 DISCONTINUATION FROM OBSERVATION PERIOD

In this study, patients will be followed up to a maximum period of 96 weeks. However, observation may be stopped prior to the 96 weeks for different reasons including, but not limited to:

- Patient discontinues the exposure of interest (azathioprine). The introduction of new treatments to CD during the follow up period will not lead to study discontinuation as long as treatment with azathioprine is not discontinued.
- Protocol violation
- Lost to follow up
- Patient withdrawal of consent
- Pregnancy
- Death

In the case observation period is stopped prior to the 96 weeks, the date of study discontinuation, the date of last intake of azathioprine and dose, and the reason for discontinuation should be recorded in the electronic CRF.

7 INFORMATION TO BE COLLECTED

7.1 VARIABLES CAPTURED BY THE GEDII REGISTRY

The GEDII Registry allows the collection of socio-demographic, clinical characteristics and outcomes of patients diagnosed with Crohn's disease.

The following variables will be obtained from the Registry:

Basal characteristics

- Date of birth
- Sex
- Height
- Weight
- BMI
- Smoking status
- Diagnosis of CD and date

- Date of start of symptoms
- Familial history of IBDs
- Disease presentation (abdominal, constitutional, abdominal + constitutional, anal disease, acute abdomen, fever, anemia, extra-intestinal manifestation, abdominal mass, similar presentation to UC, growth failure)
- Clinical course
- PCDAI includes (CD activity): Subjective reporting of the degree of abdominal pain; Presence of extraintestinal manifestations, such fever, arthritis, rash, and uveitis; Physical examinations findings; Weight and height; Hematocrit, erythrocyte sedimentation rate, and serum albumin.[36]
  The scale can be assessed at: http://www.gedii.pt/_scores_online
- Routine laboratory parameters (hemoglobin, leukocytes, neutrophils, eosinophiles, basophiles, monocytes, lymphocytes, erythrocyte sedimentation rate, CRP, ferritin, iron and transferrin)
- First episode - Paris classification for CD: age at diagnosis, location, behavior, growth. [37]
  The scale can be assessed at: http://www.gedii.pt/_scores_online
- Anal lesion (strictures, abscesses, or fistulas)
- Extra-abdominal manifestations
- Colonoscopy - Simple endoscopic score for Crohn's disease (SES-CD)[38]
  The scale can be assessed at: http://www.gedii.pt/_scores_online
- Dose of azathioprine, date of start (Day 1)
- Concomitant therapies (from Day 1)
- Stool sampling - assessment of fecal calprotectin levels:
  - The stools will be sent to a central lab (Departamento de Farmacologia Faculdade Medicina do Porto)
  - Calprotectin testing – sample sent to central laboratory

Information to be collected at each data collection time points of follow up (every 12 weeks up to week 96) – see Chronogram.

- Weight/ Height/BMI
- Dose of azathioprine, if changed since previous data collection time point.
- Concomitant therapies (including CD therapies), if changed since previous data collection time point.
- Clinical activity (PCDAI)
- Routine laboratory parameters (hemoglobin, leukocytes, neutrophils, eosinophiles, basophiles, monocytes, lymphocytes, erythrocyte sedimentation rate, CRP, ferritin, iron and transferrin)
- Stool sampling - assessment of fecal calprotectin levels:
  - Calprotectin testing – performed by the Central Laboratory from the samples collected by the patient at home.
- Status: ongoing/discontinuation. If discontinued, reason.

At week 96:

- Colonoscopy - Simple endoscopic score for Crohn's disease (SES-CD).

7.2 ASSESSMENT OF BIOLOGICAL MARKER - FECAL CALPROTECTIN

Faecal calprotectin is not routinely assessed in the medical practice and therefore is not collected in the GEDII Registry.

In this study, faecal calprotectin will be analyzed at Central Laboratory - Departamento de Farmacologia Faculdade Medicina do Porto.

Collection of stool sample

The stool samples will be collected by the patient according to scheduled time points.

Stool samples will be collected in the morning first stools, using the sample collector device.

Sample collector device: stool samples are collected using the sample collector device and send to the laboratory at room temperature. Detailed description can be found in Figure 1. Calprotectin in stools is stable for 7 days at room temperature.

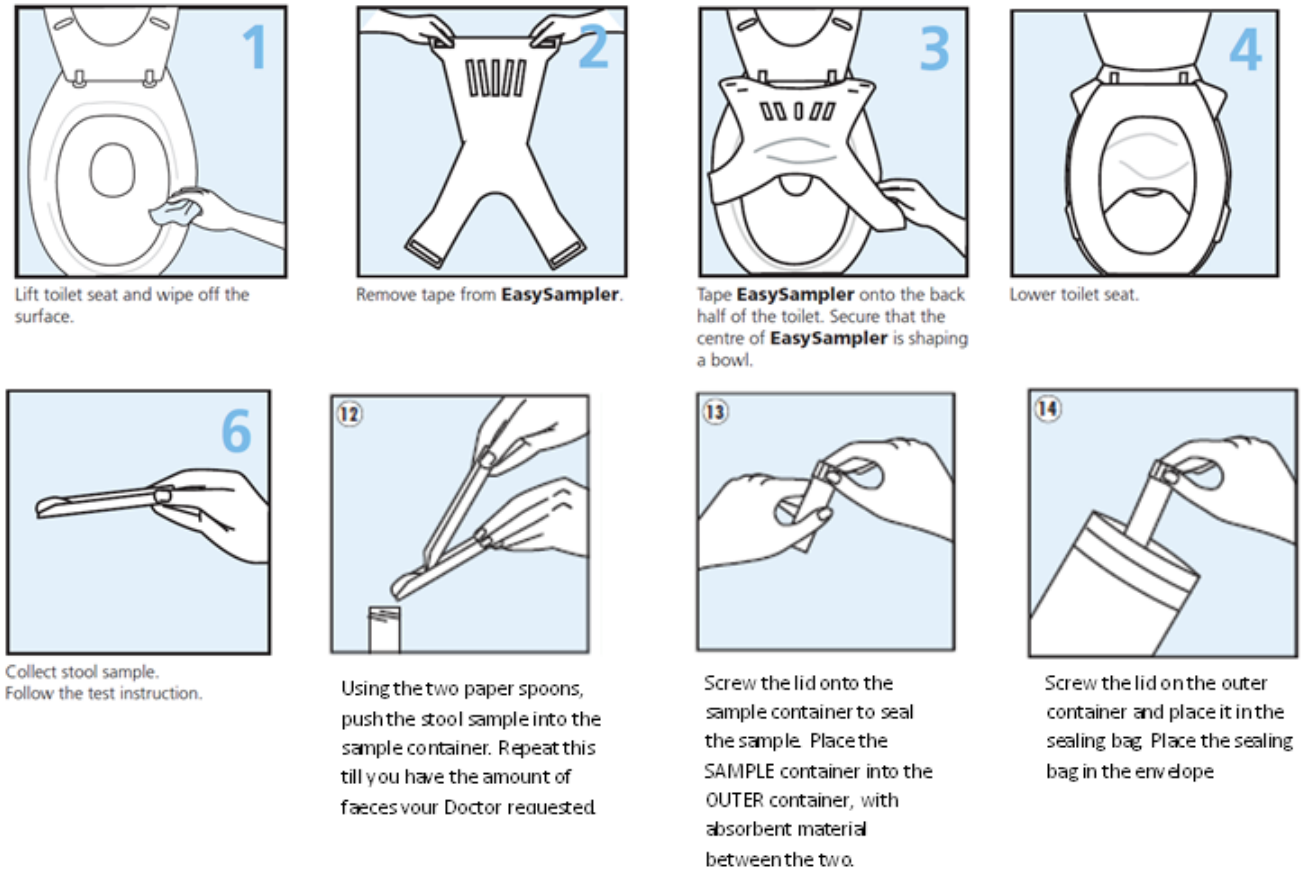

Figure 1 - Collection of stool sample (sample collector device)

Method of calprotectin assessment

Laboratory testing:

The sample (Sample collector device) will be prepared by the patient at home in the morning before attending to the scheduled appointment. The sample will be returned to the study team who will be responsible for sending the sample for laboratory analysis (Departamento de Farmacologia Faculdade Medicina do Porto).

8 EXPOSURE OF INTEREST

Azathioprine is the exposure of interest. This medication may be tailored or optimized according to the physician's clinical criteria **and** taking in to account the label of the product.

9 ENDPOINTS

9.1 PRIMARY ENDPOINT

- Proportion of patients who achieve fecal calprotectin remission (< 200 ug/g) at week 12.
- Proportion of patients who maintain fecal calprotectin remission (< 200 ug/g) at week 48 and 96.
- Proportion of patients who maintain fecal calprotectin remission (< 200 ug/g) at each data collection time point up to week 96.

9.2 SECONDARY ENDPOINTS

- **Fecal calprotectin** levels assessed at each data collection time point.
- **Fecal calprotectin** levels and **clinical activity (PCDAI)** at each data collection time point.
- **Fecal calprotectin** levels and **endoscopic activity (Simple endoscopic score for Crohn's disease [SES-CD])** at week 96.
- Proportion of patients with **clinical remission** (PCDAI<10) at each data collection time up to week 96.
- Proportion of patients who achieve **endoscopic remission** (SES-CD ≤ 2) at week 96.
- Proportion of patients on treatment with **azathioprine** at each data collection time point up to week 96.

Among patients with moderate/severe CD (at inclusion) and who achieved clinical remission with azathioprine:

- Median time to loss of clinical remission (PCDAI>11) with azathioprine

Among patients with mild Crohn's disease at study inclusion (PCDAI>11<30) with fecal calprotectin levels > 100 ug/g and/or Endoscopic activity (Simple endoscopic score for Crohn's disease [SES-CD] ≥ 3

- Median time to loss of clinical remission (PCDAI >10) with azathioprine

- **Physician reported clinical outcome (PCDAI)** at each data collection time points.

9.3 DEFINITIONS OF INTEREST

Clinical activity

- Clinical remission among patients with CD: PCDAI<10 points during therapy with azathioprine.[36]

- Clinically active disease among patients with CD: PCDAI points during therapy with azathioprine:
  - Mildly active disease: PCDAI >11<30
  - Moderately/severely active disease: >31

Endoscopic activity

- Endoscopic remission: SES-CD ≤ 2.[38]

- Endoscopic activity: SES-CD > 2 (3–6 as mildly active disease, 7–15 as moderately active disease and ≥16 as severely active disease).[39]

Observation period

- Start of observation period – corresponds to the date of the first appointment of the study

- End observation period – 96 weeks after the basal assessment

PCDAI (assessment of Crohn's Disease activity)

The PCDAI includes:

a) Subjective reporting of the degree of abdominal pain
b) Presence of extraintestinal manifestations, such fever,arthritis, rash, and uveitis
c) Physical examinations findings
d) Weight and height
e) Hematocrit, erythrocyte sedimentation rate, and serum albumin

The scale The scale can be assessed at: http://www.gedii.pt/_scores_online

Paris classification (first episode)

- CD: age at diagnosis, location, behavior, growth

The scale can be assessed at: http://www.gedii.pt/_scores_online

Simple endoscopic score for Crohn's disease (SES-CD)
The SES-CD includes four variables: ulcer size, the extent of ulcerated surface, extent of affected surface, and stenosis, from 0 to 3 in five segments of the bowel. The scale can be assessed at: http://www.gedii.pt/_scores_online

10 STATISTICAL ANALYSIS

10.1 GENERAL CONSIDERATIONS

All quantitative variables will be summarized through descriptive statistics namely mean, median, standard deviation and range (minimum and maximum) and qualitative variables though absolute (n) and relative frequencies (%) and 95% confidence intervals (if applicable). The statistical analysis will be performed through frequency tables for qualitative variables and tables with descriptive statistics for quantitative variables.

The association between two quantitative variables will be performed through Pearson correlation coefficient or Spearman correlation coefficient, in case 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 in respect to 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 proportion of patients with clinical (PCDAI), endoscopic (SES-CD) and biomarker remission (fecal calprotectin < 200 ug/g) at each data collection time points will be summarized using 95% confidence intervals, for the overall population and for the subset of patients with active and non-active CD at study inclusion.
Results for hemoglobin, leukocytes, neutrophils, eosinophiles, basophiles, monocytes, lymphocytes, erythrocyte sedimentation rate, CRP, ferritin, iron, transferrin and fecal calprotectin (Quantum Blue), will be summarized by total number of observations (n), mean, median, standard deviation, minimum and maximum.

The correlation between fecal calprotectin levels with clinical (PCDAI) and endoscopic (SES-CD) activity of disease will be analyzed through the Pearson or Spearman (in case the normality assumption is not verified) correlation coefficient at each data collection time point:

- Fecal calprotectin levels will be correlated with PCDAI score – Day 1, week 8 and every 12 weeks up to week 96.
- Fecal calprotectin levels will be correlated with Simple endoscopic score for Crohn's disease (SES-CD) – Day 1, and Week 96.

Generalized Estimated Equations will be used to investigate clinical and endoscopic remission (homogeneity) throughout data collection time points (time-effect) for each of the two subsets (patients with severe/moderate CD; patients with mild CD at Day 1).
It is expected that: patients will achieve clinical and endoscopic remission and will maintain the remission up to week 96.

10.2 SAMPLE SIZE

It is expected to analyze a total of 80 new patients. This sample size will allow the evaluation of the potential of fecal calprotectin in predicting the induction and maintenance of clinical and endoscopic remission in this population. Regarding the correlation analysis, with this sample size there is a power probability greater than 95% that the lower limit of a one-sided 95% confidence interval is higher than 0.50, if the observed correlation coefficient is at least 0.75.

11 PHARMACOVIGILANCE

New safety findings that can potentially affect the risk/benefit profile of a medicinal product identified during the conduct of epidemiological studies will be reported promptly to the Health Authorities, according to local pharmacovigilance regulations.

12 ETHICAL AND LEGAL ASPECTS

12.1 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).[40]
A copy of the protocol, proposed informed consent form 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 informed consent form must be received by the Investigator before recruitment of subjects and data collection.
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 document.

12.2 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, if applicable) 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.

12.3 STUDY DISCONTINUATION CRITERIA

Premature termination of this study may occur because of a regulatory authority decision, change in opinion of the ethics committee, or at the discretion of the GEDII. If GEDII decides to terminate prematurely the study, GEDII or designee will promptly notify the appropriate Ethics Committee and regulatory authority (if applicable).

13 QUALITY CONTROL

The study will involve a GEDII monitor who will be responsible to ensure 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 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 the GEDII or its representative.

14 DATA HANDLING

14.1 CONFIDENTIALITY

The investigator is responsible for ensuring that the subject's confidentiality is maintained.
Questionnaires, database and other documents generated in this study will be identified by a unique subject identification number only. This 2-digit number will be assigned sequentially by each investigator, based on subject's recruitment schedule (e.g.: first subject will be assigned No. 01, the second subject will be No. 02 and so on). Each center will also be assigned a predefined two-digit number.
The GEDII Registry received the authorization of Comissão Nacional para Protecção de Dados for the purpose of data processing under the scope of "Lei de Protecção de Dados de Carácter Pessoal Dec. 67/98 de 26 de Outubro".

14.2 DATA COLLECTION

All study data will be obtained from the GEDII Registry, laboratory reports and, when relevant, will be complemented by subject's interview or other medical sources (as appropriate).
The investigator will be responsible for ensuring that all findings and data are accurately and reliably recorded in the case report form.

All eligible subjects who are not enrolled in the study will be recorded in a specific form. No personal data will be collected in this form, only the date of assessment of eligibility criteria and reason for non-enrollment. This form will be kept exclusively at each site.

14.3 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 15 years from the study close out.

14.4 PUBLICATION POLICY

All documents and results generated from this clinical study are exclusive property of Coordinating Investigator and GEDII. Any related publications must be previously approved in written by the Coordinating Investigator and GEDII.
The results of the study will be presented by the Coordinating Investigator in national and international meetings and will published in international papers.
The study results can only be published after the clinical study is terminated, the data analysis is completed and **only** upon the agreement of the study's scientific board. The publication should include the results from all the centers which have 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).[40]

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 Coordinating Investigator;
- The subsequent author's positions will correspond to the principal investigator 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 investigators not figuring in the authorship (due to journal's limitations in the number of co-authors) will be cited in the acknowledgment section of the publication.
- The last author position will correspond to a GEDII member.

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APPENDIX – CHRONOGRAM

		Data collection time points (96-week follow up)								
Information to be collected	**Day 1**	**W8**	**W12**	**W24**	**W36**	**W48**	**W60**	**W72**	**W84**	**W96 or discont.[3]**
Date of birth	X									
Sex	X									
Height	X	X	X	X	X	X	X	X	X	X
Weight	X	X	X	X	X	X	X	X	X	X
BMI	X	X	X	X	X	X	X	X	X	X
Smoking status	X									
Medical history	X									
Comorbidities	X	X	X	X	X	X	X	X	X	X
CD presentation	X									
Diagnosis of CD - location, prognostic classification, extra-abdominal manifestations, anal lesion)	X									
Clinical activity (PCDAI)	X	X	X	X	X	X	X	X	X	X
Colonoscopy (SES-CD)	X									X
Dose of azathioprine administered	X	X	X	X	X	X	X	X	X	X
Other therapies for CD	X	X	X	X	X	X	X	X	X	X
Concomitant therapies	X	X	X	X	X	X	X	X	X	X
Routine laboratory parameters[1]	X	X	X	X	X	X	X	X	X	X
Stool sampling[2]	X	X	X	X	X	X	X	X	X	X

[1] Hemoglobin, leukocytes, neutrophils, eosinophiles, basophiles, monocytes, lymphocytes, erythrocyte sedimentation rate, CRP, ferritin, iron and transferrin.

[2] The stool samples will be collected in the morning first stools by the patient at home.

[3] If patient discontinues the observation period prior to week 96, the date of study discontinuation, the date of last intake of azathioprine and dose, and the reason for discontinuation should be recorded in the electronic CRF

W = week. PCDAI = Pediatric Crohn´s Disease Activity Index. SES-CD = Simple endoscopic score for Crohn's disease.