Intestinal mucosal concentrations from three mesalazine pharmaceutical formulations and correlation with efficacy in patients with mild/moderate ulcerative colitis

CLINICAL INVESTIGATION REPORT

Version 1 - February 10nd, 2020

STUDY CODE: 5-ASA/12/2015

EudraCT Number: 2016-000516-15

STUDY PHASE: POST-MARKETING (NO-PROFIT)

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Protocol Study No.:	5-ASA/12/2015
Study Type:	Clinical trial
Product Name:	Mesalazine
Date of Issue:	*November 19th,2019*
Study Title:	Intestinal mucosal concentrations from three mesalazine pharmaceutical formulations and correlation with efficacy in patients with mild/moderate ulcerative colitis.
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Principal Investigator:	Prof. Gaetano Federico Inserra Azienda Ospedaliero-Universitaria Policlinico - Vittorio Emanuele Via S. Sofia 78 95123 Catania (Italy) Tel: +39 095 3782907-2903 Fax: +39 095 3782376 Email: g.inserra@unict.it
Study Site:	Azienda Ospedaliera-Universitaria - Policlinico Vittorio Emanuele - Università degli Studi di Catania - Via S. Sofia 78 - 95123 Catania (Italy) Inflammatory bowel diseases and gastroenterology Service of Internal Medicine U.O., P.O. Gaspare Rodolico, directed by Pietro Castellino. In collaboration with the Inflammatory bowel diseases Service of Gastroenterology U.O.C., P.O. Vittorio Emanuele, directed by Dott. Giacomo Bonanno.

If any ethically relevant changes are subject to Study Protocol Amendment, the Ethics Committee must review the Study Protocol Amendment and approve it. Ethically relevant changes are e.g. amount of blood taken, dosing alternatives and/or number of subjects in the study. Before the Ethics Committee gives its written approval, the study must neither begin nor be continued.

SIGNATURE
I, here undersigned, approve the content of the Clinical Investigational Report

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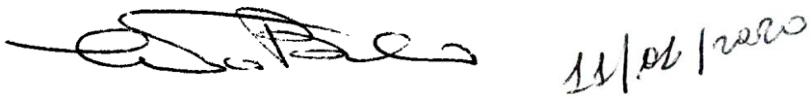

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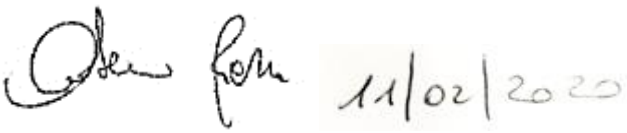

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SUMMARY

TITLE	Intestinal mucosal concentrations from three mesalazine pharmaceutical formulations and correlation with efficacy in patients with mild/moderate ulcerative colitis.
STUDY CODES	Protocol no.: 5-ASA/07/2015 EudraCT. no.: 2016-000516-15
STUDY PHASE	Post-marketing (no-profit)
STUDY PERIOD	12 months (planned for patients'enrollment). Each patient enrolled will be followed for 12 weeks
AIM OF THE STUDY	Evaluation of tissue concentrations in endoscopic intestinal biops ies of different mesalazine formulations and correlation with efficacy.
STUDY OBJECTIVES	PRIMARY OBJECTIVE: • Evaluation of tissue concentrations in endoscopic intestinal biopsies of different mesalazine formulations. SECONDARY OBJECTIVE • Efficacy of the three formulations and correlation with tissue concentration in endoscopic intestinal biopsies. • Safety profile of the three formulations.
STUDY DESIGN	Single centre, open label, randomized study evaluating different mesalazine formulations in patients with mildly/moderately active ulcerative colitis. Eligible patients will be randomly assigned in a 1:1:1 ratio to receive one of the three formulations of mesalazine.
SUBJECT SELECTION CRITERIA	*Inclusion criteria* • Adults male and female aged 18 to 65 years. • Patients with mildly/moderately active ulcerative colitis (Mayo scoregreater than or equal to 3 but less than or equal to 10 and with an endoscopy score of at least 1). • If patient is treated with immunosuppressants a maintenance stable dosage has to be used within 8 weeks prior to randomization. • If patient is treated with infliximab or other biologics a maintenance stable dosage has to be used within 8 weeks prior to randomization. • Willingness and ability to give informed consent. *Esclusion criteria* • Patients with severe ulcerative colitis (Mayo score greater than 10). • Ulcerative colitis only affecting rectum (proctitis). • Patients with active rectal bleeding at the time of screening. • Treatment with topical aminosalicylate within 1 week prior to randomization. • Treatment with 5-aminosalicylic acid (5-ASA) at a dose of >2.0g/day within 1 week prior to randomisation. • Failed treatment with a mesalazine dose of > 2.0 g/day. • Treatment with systemic or rectal steroids within 4 weeks prior to randomization. • Treatment with anti-diarrheals within 7 days prior to randomization. • History of colectomy or partial colectomy. • Gastrointestinal infection. • Immediate or significant risk of toxic megacolon. • Known or suspected colonic perforation • Hypersensitivity to salicylates, aspirin, sulfasalazine or 5-ASA. • Patients with peptic ulcer. • Renal and/or hepatic failure. • Females of childbearing potential (those who are not surgically sterilized or postmenopausal for at least 1 year) will be excluded from participation in the study if they meet any one of the following conditions: ○ are currently pregnant or, ○ have a positive result on the urine pregnancy test at the Baseline Visit

	or, ○ intend to become pregnant during the study treatment period or, ○ are breast-feeding or, ○ not willing to use highly effective birth control measures, such as: hormonal contraceptives – oral, implanted, transdermal, or injected - and/or mechanical barrier methods – spermicide in conjunction with a barrier such as a condom or diaphragm or intrauterine device (IUD) during the entire course of the study treatment period. • Enrollment in another study protocol within 30 days prior to randomization. • History of alcohol or drug abuse. • Any other significant disorders which, in the opinion of the investigator, may influence the participation in the study or affect study result.
SUBJECT WITHDRAWAL CRITERIA	• Worsening to a severe ulcerative form despite treatment. • Development of serious adverse events. • Severe protocol violations, such as an incorrect treatment administration, or a concomitant use of not permitted medications. • Subject request. • Subject becomes pregnant • Treating physician decision in the subject's best interest. • Onset of conditions requiring additional not allowed treatments or surgery.
NUMBER OF SUBJECTS	36
MEASUREMENTS	Mesalazine (5-ASA) will be determined by a validated LC-MS-MS method, in mucosal biopsy specimen homogenates.
PRIMARY ENDPOINT	Tissue concentrations in endoscopic intestinal biopsies of different mesalazine formulations.
SECONDARY ENDPOINT – EFFICACY PARAMETERS	- Clinical response defined as a decrease from baseline in Mayo score of at least 3 points and at least 30 percent, with an accompanying decrease in the subscore for rectal bleeding of at least 1 point or an absolute subscore for rectal bleeding of 0 or 1. - Clinical remission defined as a total Mayo score of 2 points or lower, with no individual subscore exceeding 1 point. - Mucosal healing defined as an absolute subscore for endoscopy of 0 or 1. Clinical response, clinical remission, and mucosal healing will be assessed at weeks 8. - Time to remission. - Remission rate. - Maintainance of remission (up to the follow-up date)
STATISTICS	The study will be analyzed using an Intent-to-Treat (ITT) approach. All randomized patients will be included in both efficacy and safety analyses. Descriptive statistics of all relevant variables will be performed. The significance level of statistical tests will be set at 0.05. Missing data will not be replaced unless specified otherwise. The main objective of the study is to evaluate the intestinal mucosa concentration of mesalazine. The comparison among the three treatment groups will be performed using ANOVA, with Tukey's HSD for pairwise comparisons. T-test will be also used to compare two treatments at a time. The rates of clinical response, clinical remission and mucosal healing assessed at week 8 and the maintainance of remission up to the follow-up date will be compared among treatment groups using logistic regression. Correlation between these efficacy endpoints and the intestinal mucosa concentration of mesalazine will be assessed using logistic regression.

	Time to remission will be analyzed using Kaplan-Meier curves with log-rank test and Cox propotiornal harzard regression. Correlation between this efficacy endpoint and the intestinal mucosa concentration of mesalazine will be assessed using Cox propotiornal harzard regression. Adverse events will be coded using the last updated version of the MedDRA dictionary to give a preferred term (PT) and a system/organ class term (SOC) for each event. The number of patients who experienced at least one AE, study product-related AE, serious AE, and the number of patients withdrawn due to AE will be summarized. No interim analysis is planned.
INVASIVE TECHNIQUES	Colonoscopy will be performed at baseline and after 8 weeks of treatment in order to evaluate changes in Mayo score. Mucosal biopsies will be performed during the second endoscopic procedure (after 8 week of treatment) in order to evaluate mesalazine tissue concentrations.
INVESTIGATIONAL MEDICINAL PRODUCTS	Pentasa® (mesalazine prolonged-release tablet): 500 mg tablet x5/die for at least 8 weeks. Pentasa® modified-release tablets consist of microgranules of mesalazine coated with ethylcellulose; following the administration and the disintegration of the tablets, the active substance is released continuously by the individual micro-granules for the entire gastro-intestinal tract at different enteral pH values; micro-granules reach duodenum within one hour after the administration, irrespective of concomitant meal. The average speed of transit in the small intestine is approximately 3-4 hours in healthy volunteers. Pentacol® (mesalazine delayed-release tablet): 800 mg tablet x3/die for at least 8 weeks. The gastro-resistant tablets release the active substance in a pH-dependent manner, in particular in the distal ileum and colon, in which ph value is above 6.5 Mesalazine Sandoz® (mesalazine generic): 500 mg tablet x5/die for at least 8 weeks. Coated tablets disintegrate coating at pH greater than 6; this feature allows the tablet to pass through the stomach intact and to make available the active ingredient in correspondence of the terminal ileum and colon. A total dose > 2000 mg of mesalazine will be administered to each subject according to the randomization list. Consider adding oral prednisolone if there is no improvement within 4 weeks of starting aminosalicylate therapy or if symptoms worsen despite treatment.
ROUTE OF ADMINISTRATION	Oral, with 200 mL of water.
TISSUE COLLECTIONS	Samples for tissue concentration of mesalazine will be collected 8 weeks after starting treatment. The total number of bioptic samples will be 16 for each subject (two adjacent biopsy specimens will be taken from eight different sites in the colon, approximately one site every 10 cm).
SPECIAL SAMPLING AND STORAGE	The specimens will be weighed immediately, frozen and stored at -20 C. The interval between taking and freezing the biopsy specimens will be less than 30 minutes.
DRUG ASSAY	Mesalazine concentrations in tissue samples will be determined by a validated LC-MS-MS method in accordance with Principles of GLP.
REPORTING	Clinical Study Report will be prepared in accordance with the ICH E3 guideline
CLINICAL INVESTIGATION INITIATION DATE:	March 03th, 2017 (first visit of first patient).

CLINICAL INVESTIGATION COMPLETION DATE:	June 13th, 2017 (last visit of last patient).
RESULTS OF THE CLINICAL INVESTIGATION	During all period of screening only one patient have responded to all inclusion/exclusion criteria planned in the protocol of study. The patient has been enrolled after the confirmation of his willingness to participate in a clinical trial by signed and dated informed consent form. During the vistit 1(enrolment, March 03th, 2017) the patient have a partial Mayo score (without endoscopic assessment) of 5 and a total Mayo score (with endoscopic assessment) of 7. At follow up visit the patient have a partial Mayo score (without endoscopic assessment) of 0, that have determinated the remission of phatology. Despite, the encouraging result, the few number of patient treted (only one) exclude the possibility to obtain results statistically significant.
CONCLUSIONS	In conclusions, despite the protocol study could have provide rilevant information about the efficacy of intestinal mucosal concentrations from three mesalazine pharmaceutical formulations in patients with mild/moderate ulcerative colitis, probably the stringent inclusions/exclusion parameters have prevent to enrol the total numer of patients planned in the simple size preventing, in this way, the obtainment of specific results.

Flow chart

Procedure	Visit 1 (Enrolment-randomization week 0)	Telephone contact 1[a]	Telephone contact 2[b]	Telephone contact 3[c]	Visit 2[d]	Visit 3[e]
Informed Consent	√					
Inclusion/exclusion criteria evaluation	√					
Medical history	√	√	√	√	√	√
Physical examination	√				√	√
Previous and concomitant medication	√	√	√	√	√	√
Blood tests	√				√	
Endoscopic procedure (with or without sample biopsy collection)	√*				√**	
Clinical Mayo Score	√	√	√	√	√	√
Total Mayo Score	√				√	
Study drugs delivery	√				√	
Diary delivery	√					
Study drugs/diary collection						√
Check Compliance (number of daily administrations)		√	√	√	√	√
Adverse Events		√	√	√	√	√

[a] 2 weeks after treatment start. [b]4 weeks after treatment start. [c]6 weeks after treatment start. [d]8 weeks after treatment start.[e]12 weeks after treatment start.[*] without sample biopsy collection (unless considered appropriate by the investigator beyond the study). [**]with sample biopsy collection

TERMS AND ABBREVIATIONS

5-ASA = 5-aminosalicylic acid
AE = Adverse Event
ALP = alkaline phosphatase
ALT = alanine aminotransferase
AST = aspartate aminotransferase
CA = Competent Authorities
CRF = Case Report Forms
CRO = Contract Research Organization
EC = Ethics Committee
EMA = European Medicines Agency
GCP = Good Clinical Practice
GLP = Good Laboratory Practice
GI = Gastro-Intestinal
IB = Investigation Brochure
IBD = Inflammatory Bowel Disease
ICF = Informed Consent Form
ICH = International Conference on Harmonization
IF = Investigator File
IRB/IEC = Institutional Review Board / Independent Ethics Committee
IS = Investigational Site
LC-MS = Liquid-Chromatography Mass Spectrometry
LLOQ = Lower Limit Of Quantification
MedDRA = Medical Dictionary of Regulatory Activities
NA = Not Applicable
OTC = Over the counter
PI = Principal Investigator(s)
QC = Quality control
RA = Regulatory Authorities
RSD = Relative Standard Deviation
SAE = Serious Adverse Events
SAP = Statistical Analysis Plan
SOP = Standard Operating Procedure
UC = Ulcerative Colitis

TABLE OF CONTENTS

1 GENERAL INFORMATION

1.1 Study Title

Intestinal mucosal concentrations from three mesalazine pharmaceutical formulations and correlation with efficacy in patients with mild/moderate ulcerative colitis.

1.2 Study Code

This study is coded: 5-ASA/12/2015 Eudra CT no. 2016-000516-15

1.3 Sponsor

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1.4 Investigator and Facility

1.4.1 Principal Investigator and Clinical Research Facility

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Inflammatory bowel diseases and gastroenterology Service of Internal Medicine U.O., P.O. Gaspare Rodolico, directed by Pietro Castellino.

In collaboration with the Inflammatory bowel diseases Service of Gastroenterology U.O.C., P.O. Vittorio Emanuele, directed by Dott. Giacomo Bonanno.

1.4.2 Clinical Laboratory and Analytical Facility

All samples will be analyzed by a specialized company:

Azienda Ospedaliero – Universitaria
Policlinico – Vittorio Emanuele
Laboratorio Centralizzato di Analisi
Via Santa Sofia, 78
95123 Catania
Tel: +39 095 3781337
Responsible person: Dr. Ferdinando Di Vincenzo

UNIFARM Research Centre
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Tel: +39 095 7167514
Fax: +39 095 7167514
Email: lab.hplc@unifarm.org
Responsible person: Mr. Cateno Piazza

1.4.3 Statistics

All data collected will be analyzed by a specialised CRO (Latis srl), who will produce a statistical report.

1.4.4 Quality Assurance Unit

The Study Protocol review, inspection of the clinical facilities and methods used as well as the audit on the data records and CRFs for accuracy and compliance will be performed by the UNIFARM Research Centre Quality Assurance personnel.

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2 PRODUCT BACKGROUND INFORMATION

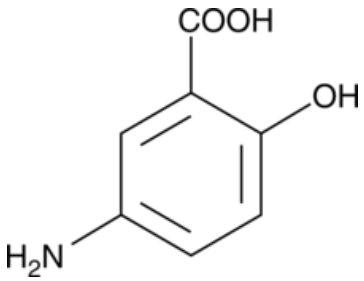
2.1 Active Ingredient

Mesalazine

2.1.1 Chemical name

5-amino-2-hydroxybenzoic acid

2.1.2 Structural formula



2.1.3 Molecular formula

$C_7H_7NO_3$

2.1.4 Molecular weight

153.135 g/mol

2.1.5 CAS

89-57-6

2.1.6 Physical form

Mesalazine occurs as a white or light grey or light pink powder or crystals and is very slightly soluble in water, practically insoluble in alcohol. It dissolves in dilute solutions of alkali hydroxides and in dilute hydrochloric acid.

3.2 Properties

Mesalazine (5-aminosalicylic acid, 5-ASA) has been identified as the therapeutic moiety of sulfasalazine in inflammatory bowel disease (IBD) [1-2-3]. Sulfasalazine is a conjugate of 5-aminosalicylic acid (mesalazine) and sulfapyridine. Following oral administration, it reaches the colon, where it is metabolized by colonic bacteria into sulfapyridine and 5-aminosalicylic acid (mesalazine) [4-5].
The precise mechanism of action of mesalazine is still unknown, but it appears to act topically rather than systemically, and it may be related to the modulation of chemical mediators of the inflammatory response [6-7-8]. Arachidonic acid metabolites, through the cyclooxygenase

pathway (prostanoids) and the lipoxygenase pathway (leukotrienes), and hydroxyeicosatetrasnoic acids appear to be influenced by mesalazine. Increased levels of prostaglandin E2 in the rectal mucosa of patients suffering from ulcerative colitis have suggested that prostaglandin E2 may be the inflammatory response mediator in ulcerative colitis [9]. If given without any release modifications, mesalazine is absorbed rapidly from the proximal gastrointestinal tract [10], with little potential therapeutic benefit. In fact, when administered orally as an unformulated 1-g aqueous suspension, mesalazine is approximately 80% absorbed [11], therefore this formulation is not used in clinics due to the relative low concentrations of the active principle in the distal gastrointestinal tract.

Upon oral administration, 5-ASA exhibits rapid and nearly complete absorption from the upper intestine, resulting not only in systemic side effects but also in a lower concentration reaching the colon, with the subsequent decreased probability of therapeutic success [12].

In order to achieve an effective oral 5-ASA treatment with minimal side effects and an acceptable patient compliance, the delivery system has to overcome these issues. An "ideal" 5-ASA containing preparation would give a low plasma concentration and an high faecal concentration [13].

The first generation of release-modified oral mesalazine formulations include the following: Eudragit S-coated tablets, which dissolve at pH 7 and release mesalazine in the terminal ileum and right colon (delayed-release mesalazine); and ethylcellulose-coated microgranules of mesalazine, which releases mesalazine throughout the gastrointestinal tract (prolonged or controlled-release mesalazine) [11].

The effective use of most of the current 5-ASA formulations requires multiple daily dosing with up to 12 tablets or capsules. Reduced patient compliance and disease control are the results of this inconvenience of frequent daily dosing and the number of tablets or capsules required per day [14]. Accordingly, in order to overcome these problems, in formulating 5-ASA in a successful delivery system, it is critical to minimize the release of 5-ASA in the upper gastrointestinal (GI) tract and to localize it in the colon in a sustained-release manner. Single-unit dosage formulations (tablets and capsules) for modified release colonic delivery suffer from problems like an unpredictable gastric emptying, GI transit variations resulting from inter-subject variability in transit patterns, and an incomplete drug delivery in GI tract due to the risk of not dissolving the polymer coat of the large, low surface area coated tablets. On the other hand, the multiparticulate drug delivery system for colonic delivery presents several advantages compared to single-unit dosage formulations. Being of smaller size, it is expected to provide less inter- and intra-individual variability, more rapid and uniform gastric emptying, more uniform dispersion and reproducible transit through GI tract [15-16]. However, in the microparticulate delivery systems, it is challenging to develop a colon-targeted sustained-release dosage formulation. It suffers from the risk of early dissolution and release of the drug before reaching the colon due to its large surface area [17]. This is more difficult in the case of 5-ASA because of its physico-chemical properties. 5-ASA exhibits amphoteric properties, and its solubility is increased at acidic pH values (pH<2) in the stomach and at more alkaline values (pH>5.5) in the lower portions of the small intestine [18].

The enteric-coated targeted-release formulations have shown to release mesalazine in the terminal ileum and the large bowel [19]. However, it has been designed as a preparation consisting of a myriad of prolonged-release ethylcellulose-coated mesalazine microgranules [20]. Upon administration, the tablet disintegrates in the stomach to provide individual microgranules that act

as discrete prolonged-release units of mesalazine. Pharmacoscintigraphic studies in healthy volunteers have evaluated the location of mesalazine release from ethylcellulose-coated microgranules in the gastrointestinal tract under fasted and fed conditions [21]. Two studies confirmed that mesalazine release from ethylcellulose-coated microgranules occur throughout the gastrointestinal tract, and that food effects on the in vivo administration of the preparation were minimal. Higher dosages of mesalazine are now being utilized to treat inflammatory bowel disease, as clinical trials have demonstrated greater efficacy at higher dosages in both ulcerative colitis (2±4 g/day) and Crohn's disease (2±4 g/day) [22-23]. As a consequence of the increase in recommended daily doses of mesalazine (up to 4 g), the sachet formulation has been developed as an alternative to mesalazine tablets, in order to improve patient compliance and acceptability [24-25]. When 1 g of a single dose of controlled-release mesalazine capsules was administered to 24 healthy volunteers under fasting condition, the mean plasma 5-ASA and acetyl 5-ASA concentrations peaked at 0.53 μg*ml−1 and 1.33 μg*ml−1 from 3 to 4 hours following administration, respectively. The half-lives of both compounds could not be determined as the absorption of 5-ASA continued throughout the gastrointestinal tract. An average of 29.4% of the dose was excreted in the urine primarily as acetyl 5-ASA. Up to 91.1% of the dose was released from the capsules. 40% of the dose was eliminated in the feces, with 8.9% of the dose remained as bounded 5-ASA, indicating that controlled-release capsules continue to release drug throughout the GI tract. 5-ASA contributed to 46.7% of the salicylates eliminated in the feces and acetyl 5-ASA accounted for the balance.
Controlled-release capsules produced three times more total salicylates and 10 times more total and free 5-ASA in the feces than 5-ASA suspension. Thus, while lower systemic levels of salicylates were absorbed, greater therapeutic quantities of 5-ASA were available in the bowel [26].
Compliance is one of the most critical aspect of a successful therapy and, in the case of a long-term treatment, every approach that reduces or limits patient's discomfort, like reducing the number of administrations per day, results in a better adherence to therapy and, subsequently, in a better clinical outcome. The administration of a relatively higher dose of the drug, having the characteristics of the controlled-release capsules described above, could result in a reduction of the total number of administration per day, thus enhancing confort and compliance for the single patient.

2.3 Justification of the Study

Although randomized clinical trials have demonstrated that various formulations of 5-ASA are effective in either treating symptoms or inducing remission in ulcerative colitis (UC), it has been difficult to elucidate how each formulation or delivery system releases 5-ASA in the GI tract. While data exist pertaining to the predicted release mechanism of the various 5-ASA formulations, these data are not precise, which is in part due to limitations in the techniques available to perform these measures. As 5-ASA acts topically in the treatment of UC, systemic exposure to 5-ASA is not necessarily related to therapeutic efficacy. Moreover, because only low levels of 5-ASA are released into the plasma via the colonic mucosa, it is difficult to relate mucosal concentrations of 5-ASA to plasma concentration or to define how 5-ASA is distributed along the length of the colon [27]. Faecal concentrations could represent an alternative method to compare different 5-ASA formulations, but it provide only an indirect estimate of the drug available in the tissues. No two 5-ASA formulations and specifically no two formulations delivering the drug by different

technologies are currently considered to be bioequivalent. The European regulatory authorities have stated that for "locally acting products", bioequivalence generally is not a suitable way to show therapeutic equivalence, since plasma levels are not relevant for local efficacy, although they may play a role for safety [28]. A concept paper on the development of a guideline on this topic is under EMA (European Medicines Agency) evaluation [29].

The concentration of 5-ASA in mucosal tissues assessed from biopsy samples [27] could be usefull to compare different 5-ASA formulations and delivery systems. Because of its invasive nature (requiring endoscopy with mucosal biopsy), direct assessment of mucosal concentration is not always feasible in randomized studies. Studies that have been performed using mucosal biopsy have shown that high mucosal 5-ASA concentrations are associated with improved mucosal healing in patients with UC [30, 31, 32]. In a study of 21 patients with UC taking oral 5-ASA therapy, mucosal concentrations of 5-ASA were found to be significantly higher in patients with no or only mild mucosal damage on endoscopy compared with those having moderate or severe mucosal damage. The association between high mucosal 5-ASA levels and low disease activity was also shown when assessing 5-ASA concentrations from rectal biopsies in a study of 29 patients taking oral sulfasalazine, or delayed-release 5-ASA, with or without rectally administered 5-ASA. The link between mucosal 5-ASA levels and disease activity was strengthened further by the results from a study of 18 patients with UC deemed at high risk of relapse, despite existing 5-ASA therapy. Differences in tablet delivery systems, as well as in transit time, may lead to differences in drug availability at colonic mucosa level, as assessed by tissue biopsy. Lower tissue 5-ASA concentrations during maintenance therapy may predispose patients to relapse. Moreover, there is growing evidence that mucosal healing, evaluated endoscopically or histologically, reflects long term clinical outcome better than remission/response based on classical clinical indices [33].

Purpose of the present study is to assess mucosal concentration of three formulations of mesalazine in patient with mild/moderate UC and correlate it with efficacy, analyzing the relationship between tissue concentrations and disease activity.

2.3.1 Benefit/risk Assessment

5-ASA-containing formulations represent the first-line therapy for the treatment of mild/moderate UC and for the prevention of recurrence [34-36]. To date, a clinical advantage of one mesalazine preparation over another has not been demostrated.
A meta-analysis suggested that doses of at least 2 g per day are more effective in inducing remission and preventing relapse, but that exceeding 2.5 g per day might not have any additional benefit [37]. After remission, long-term maintenance therapy is encouraged, but compliance rate is low. Patients who do not continue with maintenance therapy experience high rates of relapse. Systemic steroids are indicated when disease fails to quickly respond to aminosalicylates (if there is no improvement within 4 weeks of starting aminosalicylate therapy or if symptoms worsen despite treatment). Consider adding other treatment (immunosuppressive agents) to induce remission in people with mild to moderate ulcerative colitis if there is an inadequate response to systemic steroids after 2–4 weeks.
Overall, aminosalicylates are well tolerated and safe. Therefore, the potential benefits of these drugs outweigh the potential risks but it is important to be aware of the possible side effects before

starting treatment [38].
Side effects, such as nausea, heartburn, headache, anaemia, skin rashes, reversible abnormalities of sperm number and morphology, and, rarely, hepatitis and nephritis, occur primarily due to an high plasma sulphapyridine concentrations [39]. Rare side effects affect less than 1 percent of people, but are potentially serious. Interstitial nephritis can usually be detected with routine blood tests performed at six weeks and at six months after starting a 5-ASA medication, and yearly thereafter. The newer aminosalicylates offer targeted delivery of mesalazine to the bowel, with fewer side effects [40].
The aim of this study is to demonstrate if these three formulations achieve different mucosal concentrations, that could be related to a better benefit/risk profile.

In order to obtain these data, mucosal biopsies from endoscopic procedure will be performed after 8 weeks of treatment with one of the three mesalazine formulations.
Unless contraindicated because of severe colitis or possible toxic megacolon, a full colonoscopy should be performed during the initial evaluation of patients with a clinical presentation suggestive of IBD [41].
Moreover, endoscopy is usually used to assess the disease extent and activity, to evaluate response to therapy, to perform a preoperative assessment and detect of postoperative recurrence, and to monitor the occurrence of malignancies.
The procedure is safe and effective in the visual inspection of the entire large bowel from the distal rectum to the cecum and allows the execution of multiple biopsies is order to carry out differential diagnosis. It is performed by inserting a device, called colonoscope, into the anus and advancing through the entire colon. The procedure usually lasts from 45 minutes to one hour. With sedation/analgesia provided during the colonoscopy, many people sleep during the test, while others are very relaxed, comfortable, and generally not aware.

Complications are rare but can occur:

- Bleeding can occur from biopsies or the removal of polyps, but it is usually minimal and can be controlled.
- The colonoscope can cause a serious complication such as tear or hole in the colon, but it is uncommon.
- Some complications can arise not directly related to endoscopy, such as electrolyte imbalances and alterations of circulating volume resulting from the preparation or side effects from sedation.

Relative contraindications to the procedure include known or suspected colonic perforation, toxic megacolon, and fulminant colitis or severe inflammatory bowel disease with ulceration, because these conditions increase the risk of perforation [42].

In this study a colonscopy will be performed at basaline in order to define the stage of the disease. A second endoscopic procedure will be performed after 8 weeks of treatement with one of the three formulation of mesalazine, in order to evaluate patient's response and drug concentration in mucosal biopsies. Overall, the procedure performed by staff with experience and in an hospital setting is safe. In order to guarantee subjects' safety, patients with contraindication to the procedure will be excluded from the study.

2.3.2 Study Population

The study population will include 36 male and female patients with mild/moderate UC, between 18 and 65 years of age inclusive.

3 STUDY OBJECTIVES AND PURPOSE

3.1 Primary objective

Purpose of the present study is the evaluation of tissue concentrations in endoscopic intestinal biopsies of different mesalazine formulations in patients with mild/moderate UC.
Mucosal biopsies from endoscopic procedure will be performed after 8 weeks of treatment with one of the three mesalazine formulations.

3.2 Secondary objective

One of the secondary objective is to assess efficacy of different mesalazine formulations, taking into account their tissue concentrations.
Patients will be evaluated (during a visit or with a phone contact) at weeks 0, 2, 4, 6, 8, and 12. Mayo score will be use to assess ulcerative colitis activity, with higher scores indicating more severe disease. Mayo score (range 0-12 points) is made up of four sub-scores (stool frequency, bleeding, physicians assessment of illness severity and endoscopic findings), each carrying equal numerical weight (0-3 points). Remission is defined as a score of 2 or less.
Total Mayo score will be determined at weeks 0 and 8. Clinical Mayo score (Mayo score without endoscopy) will be determined at all visits.

Safety profile of the three formulations will be evaluated, reporting all the suspected advese drug reactions (ADRs) occurring during the study period. See section 10 (Safety assessment) for further details.

4 STUDY DESIGN

4.1 Type of Study

This study will be a multicentre, open-label, randomized study.

4.2 Study Scheme

Procedure	Visit 1 (Enrolment-randomization-basal week 0)	Telephone contact 1[a]	Telephone contact 2[b]	Telephone contact 3[c]	Visit 2[d]	Visit 3[e]
Informed Consent	√					
Inclusion/exclusion criteria evaluation	√					
Medical history	√	√	√	√	√	√
Physical examination	√				√	√
Previous and concomitant medication	√	√	√	√	√	√
Blood tests	√				√	
Endoscopic procedure (with or without sample biopsy collection)	√*				√**	
Clinical Mayo Score	√	√	√	√	√	√
Total Mayo Score	√				√	
Study drugs delivery	√				√	
Diary delivery	√					
Study drugs/diary collection						√
Check Compliance (number of daily administrations)		√	√	√	√	√
Adverse Events		√	√	√	√	√

[a] 2 weeks after treatment start. [b]4 weeks after treatment start. [c]6 weeks after treatment start. [d]8 weeks after treatment start.[e]12 weeks after treatment start.[*] without sample biopsy collection (unless considered appropriate by the investigator beyond the study). [**]with sample biopsy collection

4.3 Randomization
Eligible patients will be randomly assigned in a 1:1:1 ratio to receive one of the three formulations of mesalazine. Randomization list will be generated by Latis, using the module RALLOC [43] of STATA/IC 13.1 for Windows (StataCorp LP, College Station, TX, USA).

4.4 Study Drugs Shipping
It is responsibility of the Sponsor to provide individual 36 boxes containing an adequate supply of the medications to be administered during the study to each patient.

The Sponsor will deliver the product to:
Dr. Francesca Lo Monaco
Azienda Ospedaliero-Universitaria
Policlinico di Catania Vittorio Emanuele
Università degli Studi di Catania
Servizio Farmacologia Clinica e Farmacia
Via Santa Sofia, 78 - 95123 Catania (Italy)
Tel: 095 3782740
Fax: 095 3782322
Email: f.lomonaco@ao-ve.it

After receiving the drug supply, Pharmacist will provide confirmation through a written standard form with date and signature.

4.5 Study Drugs Accountability
Upon receiption of the study drug the Principal Investigator (PI), or a delegate, will maintain an inventory record of the drug received.
The trial medication will be stored below 25°C in a dry, locked and secure storage facility, sheltered from light. The storage place will be accessible only to those individuals authorised by the Investigator/Pharmacist.

At the conclusion of the study, a final study drug accountability will be performed. If any supplies are missing, this will be indicated together with an explanation for the discrepancy.

5 ELECTION AND WITHDRAWAL OF SUBJECTS

5.1 Source of Subjects
Patients with an established diagnosis of mild/moderate UC afferent to the study sites of Azienda Ospedaliero-Universitaria Policlinico di Catania Vittorio Emanuele (Catania), that meet inclusion/exclusion criteria and provide informed consent to study participation.
Each subject will be interviewed by the Investigator who will record medical history, previous/concomitant pharmacological treatments, history of alcohol or drug abuse, and conduct a routine medical examination.
Venous blood samples will be obtained from each subject for the following lab tests, in order to confirm the absence of significant laboratory abnormalities:

• Haematology: full blood count, haemoglobin, haematocrit, platelet count, and leukocyte differential count.
• Clinical chemistry: ALT (SGPT), AST (SGOT), GMT (gamma-GT), alkaline phosphatase (ALP), total bilirubin, azotaemia, creatinine.

The same laboratory tests will be performed after 4 weeks of treatment in order to assess and confirm that the subject's state of health was not affected by/during the study.

5.2 Subject inclusion criteria

- Adults male and female aged 18 to 65 years .
- Patients with mildly/moderately active ulcerative colitis (Mayo score greater than or equal to 3 but less than or equal to 10 and with an endoscopy score of at least 1).
- If patient is treated with immunosuppressants a maintenance stable dosage has to be used within 8 weeks prior to randomization.
- If patient is treated with infliximab or other biologics a maintenance stable dosage has to be used within 8 weeks prior to randomization.
- Willingness and ability to give informed consent.

5.3 Subject esclusion criteria

- Patients with severe ulcerative colitis (Mayo score greater than 10).
- Ulcerative colitis only affecting rectum (proctitis).
- Patients with active rectal bleeding at the time of screening.
- Treatment with topical aminosalicylate within 1 week prior to randomisation.
- Treatment with 5-ASA at a dose of >2.0g/day within 1 week prior to randomisation.
- Failed treatment with a mesalazine dose of > 2.0 g/day.
- Treatment with systemic or rectal steroids within 4 weeks prior to randomization.
- Treatment with anti-diarrheals within 7 days prior to randomization.
- History of colectomy or partial colectomy.
- Gastrointestinal infection evident from stool culture and testing for clostridium difficile toxin (in the opinion of the investigator).
- Immediate or significant risk of toxic megacolon.
- Known or suspected colonic perforation
- Hypersensitivity to salicylates, aspirin, sulfasalazine or 5-ASA.
- Patients with peptic ulcer.
- Renal and/or hepatic failure.
- Females of childbearing potential (those who are not surgically sterilized or postmenopausal for at least 1 year) will be excluded from participation in the study if they meet any one of the following conditions:
    - are currently pregnant or,
    - have a positive result on the urine pregnancy test at the Baseline Visit or,
    - intend to become pregnant during the study treatment period or,
    - are breast-feeding or,
    - not willing to use highly effective birth control measures, such as: hormonal

contraceptives – oral, implanted, transdermal, or injected - and/or mechanical barrier methods – spermicide in conjunction with a barrier such as a condom or diaphragm or intrauterine device (IUD) during the entire course of the study period [44].

- Enrollment in another study protocol within 30 days prior to randomization.
- History of alcohol or drug abuse.
- Any other significant disorders which, in the opinion of the investigator, may influence the participation in the study or affect study result.

5.4 Subject withdrawal criteria

A subject should be withdrawn from the trial if, in the opinion of the Investigator, it is medically necessary or if it is the wish of the subject.

The study treatment will be discontinued at any time if any of the following events occur:

- Worsening to a severe ulcerative form despite treatment.
- Development of AE or unacceptable toxicity, precluding further therapy with the IDs.
- Severe protocol violations, such as an incorrect treatment administration, or a concomitant use of not permitted medications.
- Subject request.
- Subject becomes pregnant
- Treating physician decision in the subject's best interest.
- Onset of conditions requiring additional treatments or surgery.

Any treatment discontinuation must be recorded on the case report form (CRF) by the Investigator, who will indicate date and reason(s) for treatment withdrawal.

6 TREATMENT OF SUBJECTS

6.1 Study Medication

	Pentasa® 500 mg tablets (5 tablets)	**Pentacol® 800 mg tablets (3 tablets)**	**Mesalazine Sandoz® (generic) 500 mg tablets (5 tablets)**
Active ingredients:	***Mesalazine***	***Mesalazine***	***Mesalazine***
Dosage form:	Tablets	Tablets	Tablets
Strength:	Pentasa® 500mg controlled-release tablets (5 tablet)	Pentacol® 800mg delayed-release tablets (3 tablet)	Mesalazine Sandoz ® 500 mg controlled-release tablets (5 tablet)
Storage conditions:	Below 25°C	Below 25°C	Below 25°C
Packaging:	Blister	Blister	Blister
Manufacturer:	Ferring GmbH Fabrikstraße 7, 24103 Kiel, Germania	SOFAR S.p.A. Via Firenze 40 20060 - Trezzano Rosa – MILANO (prodotto finito) BIOLAB S.p.A. VIMODRONE, Via Bruno Buozzi n° 2 (analisi microbiologiche)	Sandoz S.p.A. Largo U. Boccioni, 1 21040 Origgio (VA)
Marketing Authorisation Holder (MAH):	Ferring S.p.A. Via Senigallia 18/2 20161 – MILANO	SOFAR S.p.A. Via Firenze 40 20060 - Trezzano Rosa - MILANO	Sandoz S.p.A. Largo U. Boccioni, 1 21040 Origgio (VA)

Pentasa® (mesalazine prolonged-release tablet): 500 mg tablet x5/die for at least 8 weeks. Pentasa® modified-release tablets consist of microgranules of mesalazine coated with ethylcellulose; following the administration and the disintegration of the tablets, the active substance is released continuously by the individual micro-granules for the entire gastro-intestinal tract at different enteral pH values; micro-granules reach duodenum within one hour after the administration, irrespective of concomitant meal. The average speed of transit in the small intestine is approximately 3-4 hours in healthy volunteers.

Pentacol® (mesalazine delayed-release tablet): 800 mg tablet x3/die for at least 8 weeks. The gastro-resistant tablets release the active substance in a pH-dependent manner, in particular in the distal ileum and colon, in which ph value is above 6.5

Mesalazine Sandoz® (generic): 500 mg tablet x5/die for at least 8 weeks. Coated tablets disintegrate coating at pH greater than 6; this feature allows the tablet to pass through the stomach

intact and to make available the active ingredient in correspondence of the terminal ileum and colon.

A total dose > 2000 mg of mesalazine will be administered to each subject according to the randomization list.

Consider adding oral prednisolone if there is no improvement within 4 weeks of starting aminosalicylate therapy or if symptoms worsen despite treatment.

6.2 Study Procedures

Each subject enrolled will be treated for at least 8 week and will be evaluated during 3 programmed visits e 3 telephone contacts: at baseline (Visit 1, week 0), at week 2, 4, 6 (telephone contacts 1, 2 and 3 rispectively), 8 and 12 (Visit 2 and 3) after treatment start. Endoscopic procedure will be performed at Visit 1 (week 0), in order to evaluate total Mayo score at baseline, and at Visit 2 (week 8), when tissue samples will be collected in order to evaluate mesalazine concentration.

6.3 Concomitant medications

All therapies, topic or systemic, that might interfere with the evaluation of the treatment under investigation are forbidden, excluding drugs foreseen in the protocol (eg. immunosuppressants at maintenance stable dosage has to be used within 8 weeks prior to randomization.

- If patient is treated with infliximab or other biologics a maintenance stable dosage has to be used within 8 weeks prior to randomization.

systemic corticosteroid if there is no improvement within 4 weeks from starting treatment with mesalazine).

If any medication, including OTC medicine, is administered or taken during the course of the study, the medication(s) will be recorded in the appropriate Case Report Form reporting name, date(s) and time(s) of intake, reason(s) for use, and dosage information. If it is necessary for medical reasons that the subject has to take drugs, it will be the PI's decision whether the subject can further participate in the study.

This decision obviously depends on the type of illness and the kind of drug to be used.

Patients that must be treated with forbidden therapies will be excluded from the prosecution of the study, but their data until that moment will be used in the statistical analysis.

7 DRUG ASSAY

Mesalazine will be determined by a validated LC-MS-MS method, in mucosal biopsy specimen homogenates in accordance with Principles of GLP (Good Laboratory Practice).

7.1 Mesalazine Drug Assay method and Validation

Mesalazine concentrations will be determined at Unifarm by an LC-MS/MS method, validated according to Guideline on bioanalytical method validation [45].

All analytical data obtained during bioassay of study samples including re-analyzed samples data, calibration standards and QC samples as well as Summary of QC Standard in all Sequences, Regression Parameters of Calibration Standard in all Sequences and copies of chromatograms of the study samples, including all associated calibration and QC chromatograms for 20% of all subjects, chosen at random, will be reported. The validation report including the method sensivity (LLOQ), linearity, calibration range, selectivity inter- and intra-assay accuracy and reproducibility (%RSD) as well as recovery, incurred sample re-analysis, and stability data, will be provided togheter with the Analitical Report.
All analytical procedures applied for this study will be carried out in accordance with the applicable principle of GLP and applicable guidelines [46]

7.2 Batch Acceptance Criteria

Each anlytical batch will consist of the following samples:

Calibration curve standards	Blank, blank spiked with internal standard and six spiked calibration standard
Quality control (QC) samples	In duplicates three concentration (low, medium, and high) interspesed evenly among study study
Study samples	Mesalazine samples except the batch for the repeat assay

The calibration curve will be determined through linear regression of the drug to internal standard responce ratios versus drug concentration. Individual responce ratios of calibration standards, QCs and study samples will be then substituted on the regression curves to determine values of concentration found.

The batch will be considered acceptable if the following criteria are met:

- correlation coefficient of the calibration curve is 0.990 or better.
- the lowest calibration standard lies within ±20% of the nominalvalue and the remaining calibration standards are within ±15% of their nominal values, 75%, should fall within the limits, the values falling outside these limits can be discarded.
- at least four out of six QC samples are within ±15% of their nominal values (QCs found outside the limit not both at the same concentration).

7.3 Re-analysis of Study Samples

The study samples will be re-analyzed in the following circumstances:

- the complete batch will be re-analyzed when the batch acceptance criteria are not met
- the samples were lost due to wrong processing or poor chromatography
- study samples with results unexpected from the pharmacological point of view may be re-assayed (in duplicate, subject to availability of sample/time) upon request of pharmacokineticist; they will be evaluated and reported according to the rules of UNIFARM.

The reason for the sample re-assay will be provided.

8 DATA COLLECTION

8.1 Case report form

Specific CRFs for the collection of data pertaining to this study will be provided to the Investigator(s), who is responsible for ensuring that the data required are carefully reported. CRFs will be designed in order to act as a reminder both as far as the timing and the nature of the data to be collected are concerned.
CRFs must be filled in with permanent ink and corrections have to be made with a single line through, so that one can see what has been crossed out. Each correction or new entry must be initialized and dated by the personnel who made that correction. The reason why the correction was made will be also provided, if necessary. Each completed CRF must be reviewed for accuracy and will be signed by the PI. The PI assumes responsibility for ensuring the completeness and accuracy of the forms.

A copy of the CRF is retained by the Investigator who must ensure that it is stored in a secure place with the other trial documents.

The Investigator must keep source documents for each subject in the study. All information on CRFs must be traceable to theses source documents, which are generally stored in the subject's medical file. The source documents should contain all demographic and medical information, , as well as the original signed informed consent form which should indicate the trial number and title of the trial.

8.2 Source Documents

Source documents are defined as original documents, data and records. This may include hospital records, clinical and office charts, laboratory data/information, pharmacy dispensing and other records. Data collected during this study must be recorded on the appropriate source documents.

9 MONITORING

9.1 Study monitoring

UNIFARM will designate qualified person(s) to maintain a close liaison with the Investigators and study staff to ensure the study is being conducted with respect to GCP and according to the approved protocol. This liaison will consist of document e monitoring visits, and/or telephone call or e-mail communication prior to study initiation and at regular intervals during the study to enable periodic reviews of the progress of the study with the Investigators as well as the opportunity to clarify any questions that may appear during the study.
Study monitor will have access to all records (including source documents when applicable) necessary to ensure the integrity of the recorded data. During monitoring visits, the Investigators and authorized staff will assist the monitor to check the CRFs and other documents pertinent to the project.

10 SAFETY ASSESSMENT

Safety profile of each treatment will be assessed through the recording, reporting and analyzing of baseline medical conditions, adverse events, physical examination findings including vital signs and laboratory tests.
Incidence, type, and severity of the adverse events will be summarized by presentation of the number of patients with any adverse event (AE). AEs will be coded by using the Medical Dictionary for Regulatory Activities (MedDRA).
AEs frequently observed with mesalamine are diarrhea, nausea, vomiting, abdominal pain, headache, rash.
Occasionally patient treated with mesalazine may experience hypersensitivity reactions, fever, anorexia and dizziness.

10.1 Safety monitoring

The physician will monitor each patient for serious adverse events on a routine basis. The physician will assess and record any AE in detail including the date of onset, description, severity, time course, duration and outcome (when known), relationship of the adverse event to drug, an event diagnosis, if known, and any action(s) taken. Information about any concomitant medications taken at the time of the AE will be collected. For all AEs the physician must pursue and obtain all the above mentioned information in order to determine the outcome and to assess whether it meets the criteria for classification as a SAE as well as to determine a causal relationship.

10.1.1 Definitions

An adverse event (AE) is defined as any untoward medical occurrence in a patient, which does not necessarily have a causal relationship with treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not the event is considered causally related to the use of the product. Any worsening of a pre-existing condition or illness is considered an adverse event.

- **Adverse Drug Reaction (ADR)** is any noxious and unintended responses to a medicinal (investigational) product related to any dose. Responses to a medicinal product means that a casual relationship between a medicinal producy and an adverse event is at least a reasonable possibility, i.e. the relationship cannot be ruled out.
- **Serious Adverse Event (SAE)** is considered any untoward medical occurrence at any dose that result in death, is life-threatening, result in persistent or significant disability/incapacity, or requies inpatient hospitalization or prolongation of existing hospitalization. Congenital anomaly/birth defects are serious events. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Example of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

• **Unexpected Adverse Drug Reaction** is an adverse drug reaction whose nature or severity are not consistent with the applicable product information.

10.1.2 Frequency (onset data and time, frequency and duration)

The Investigator will indicate the adverse event onset date and time and the frequency as:

- *single (1);*
- *recurrent (2).*

The Investigator will report the duration of the adverse event: if the frequency is recurrent be should indicate the entire period in which the episodes occurred (expressed in days, hours, minutes). Moreover he will report only the grade of intensity associated with the most severe adverse event.

10.1.3 Correlation

The physician will use the following definitions to assess the relationship of the adverse event to the use of study treatment:

- *definite:* an adverse event, including abnormal laboratory values, that follow a reasonable temporal sequence from the administration of a drug, that is unrelated to concomitant disease/s, other drug/s or chemical substance/s, that is confirmed by a positive responce upon suspension of the suspected drug (dechallenge), and the reappearance of the reaction on repeated exposure (rechallenge) (1);
- *probable:* an adverse event, including abnormal laboratory values, that follows a reasonable temporal sequence from the administration of a drug, that is unrelated to concomitant disease/s, other drug/s or chemical substance/s, that is confirmed by a positive response upon suspension of the suspected drug (dechallenge) (2);
- *possible:* an adverse event, including abnormal laboratory values, that follows a reasonable temporal sequence from the administration of a drug, but that could have been produced by the concomitant administration of other drug/s or chemical substance/s, when data available on the suspension of the suspected drug are not available or are insufficient (3);
- *unlikely:* an adverse event, including abnormal laboratory values, that does not follw a reasonable temporal sequence from the administration of a drug and that could have been produced by concomitant disease/s, therapy/s or chemical substance/s (4);
- *unrelated:* an adverse event, not corresponding to the above mentioned criteria (5).

10.1.4 Corrective action taken

- none (1);
- pharmacological (2);
- not pharmacological (3).

10.1.5 Outcome

- recovered/resolved (1);
- recovering/resolving (2);
- recovered with sequelae (3);
- not recovered/not resolved (4);

- fatal (5);
- unknown (6).

If the outcome is indiacted as "recovering/resolving" or "nor recovered/nor resolved", the Investigator will follow up the AE until resolution and, at the time of its resolution, must fill in the "Follow-up form" of the Case Report Form (following the Initial form) and a follow up SAE form if applicable (for serious adverse events). If the outcome is indicate as "unknown" a reason should be provided (e.g. lost to follow up, etc.).

10.1.6 Registration procedures of AE/SAE

The Investigator is responsible for recording all adverse events that have occurred during the study, regardless of their relationship to the study medication, in the CRF.
The occurrence of any SAE will be reported by Investigator to the Sponsor and to the Study Director within 24 hours after he becomes aware of the occurrence of such an event. The SAE report should contain a detailed description of the observed symptoms and the concomitant therapy. The Investigator has to judge the possible casual relationship between the event and the study drug, and should arrange additional axaminations at his own discretion to clarify if the event is connected with the study medication. He should consult a specialist if necessary.
The occurrence of any serious unexpected ADR will be reported by the Sponsor to the competent Authorities within 15 days (7 days in case of death or life threatening event)-Promoter and Sponsor by the Clinical Study Report.
Contact person in Sponsor's pharmacovigilance department, who will be announced about SAE:
Dr. Valentiva Drago
EU QP Responsible for Pharmacovigilance
SCF Società di Consulenza Farmacologica srl
Via del Bosco 222
95125 Catania (Italy)
Tel: +39 095 338246
Fax: +39 095 338246
Cell: +39 347 1889202
Email: valentinadrago@essecieffe.it

10.2 Safety data evaluation

All safety data from medical examinations will be documented in the CRFs, summarized by descriptive statistics and the results will be included in the Clinical Study Report.

11 STATISTICS

11.1 Sample size

On the basis of the literature review [13], the concentration of mesalazine (5-Asa) in the intestinal mucosa can widely vary according to the oral preparation tested and the intestinal section where specimens can be taken, then a formal sample size estimate for the primary endpoint is not imaginable. Twenty subjects per group are considered enough to perform the explorative analyses planned in the protocol. As an example, with 12 patients per group, it is possible to assess a

significant difference in intestinal mucosa concentrations of 50% with a power cery close to 90%, when two groups are compared, with a coefficient of variation of 0.3 and a significance level of 0.05.

11.2 Definition of Study Populations for Analysis

The study will be analyzed using an Intent-to-Treat (ITT) approach. All randomized patients will be included in both efficacy and safety analyses. Eventual exclusions will be carefully checked and motivated. Despite three treatment groups will be analyzed, no correction for multiple comparison is considered necessary, as the primary endpoints is not efficacy.

11.3 Statistical Analysis

Descriptive statistics of all relevant variables will be performed. Continuous variables will be summarized by the number of patients (N), mean, standard deviation, median, minimum, maximum. Where appropriate, 95% confidence intervals for the target variables will be estimated. Categorical variables will be summarized by the number (N) and the proportion of patients (%).
The significance level of statistical tests will be set at 0.05. Details of statistical analysis are provided in the following paragraphs.
The statistical analysis will be performed using SAS 9.4 for Windows (SAS Intitute Inc., Cary, NC, USA).

11.4 Missing Data

Missing data will not be replaced unless specified otherwise.

11.5 Multiplicity

A list of endpoints has been provided for this study, anyway no correction for multiplicity will be needed as the aim of the study is exploratory and not confirmatory. In case of clinically and statistically significant results, the exact p-value will be reported to support the strength of the findings.

11.6 Covariates, Interactions and Subgroups

For both continuous and discrete variable, multivariate analyses can be performed (e.g., ANOVA, ANCOVA, and logistic regression). Subgroup analysis are not planned, due to the limited sample size; if any subgroup analysis will be performed, its purpose will be exploratory only.

11.7 Analysis of Demographics and Baseline Variables

Demographic (gender, age) and baseline characteristics will be summarized using mean, median, standard deviation, minimum and maximum for continuous variables and frequencies and percentages for categorical variables. Chi-square or ANOVA will be used to compare discrete and continuous variable, respectively, among the three treatment groups.

11.8 Primary Endpoint Analysis

The main objective of the study is to evaluate the intestinal mucosa concentration of mesalazine. The comparison among the three treatment groups will be performed using ANOVA, with Tukey HSD for pairwise comparisons. T-test will be also used to compare two treatments at a time.

11.9 Secondary Endpoint Analysis - Efficacy Endpoints

The rates of clinical response, clinical remission and mucosal healing assessed at week 8 and the maintainance of remission up to the follow-up date will be compared among treatment groups using logistic regression.
Correlation between these efficacy endpoints and the intestinal mucosa concentration of mesalazine will be assessed using logistic regression.
Time to remission will be analyzed using Kaplan-Meier curves with log-rank test and Cox propotiornal harzard regression.
Correlation between this efficacy endpoint and the intestinal mucosa concentration of mesalazine will be assessed using Cox propotiornal harzard regression.

11.10 Safety Analysis

Adverse events will be coded using the last updated version of the Medical Dictionary for Regulatory Activities (MedDRA) dictionary to give a preferred term (PT) and a system/organ class term (SOC) for each event. The number of patients who experienced at least one AE, study product-related AE, serious AE, and the number of patients withdrawn due to AE will be summarized.
For each SOC and preferred term, summaries will be made with respect to the proportion of patients having at least one occurrence of that event during the trial and the total number of events. The incidence of AEs will be presented overall, by SOC and preferred term, and additional grouping by severity and relationship to the trial treatment will be performed.

11.11 Planned Interim Analysis(es)

No interim analysis is planned.

12 ETHICS

12.1 Ethics Committee

This study will be conducted in accordance with GCP, Declaration of Helsinki, local laws and regulations relevant to the use of new and approved therapeutic agents in human subjects.
It's responsibility of the Sponsor to submit the study protocol, the information sheet and informed consent to the approval of the Ethical Committee (EC) of the clinical centre. It's responsibility of the Sponsor to request the Competent Authority (CA) of the permission to conduct the study by after the approvation of the EC.

12.2 Amendments and EC/CA Additional Requirements

Any amendment to this study protocol will be sent to the EC/CA (if required), any amendments that may lead to increased risks and/or inconvenience to the subject must first be approved by the EC and CA, if appropriate.
Copies of all documents must be stored also by the Investigator/s. Any revision of the study protocol shall be reported on the CRF. Any additional EC/CA requirement will be followed as appriopate.

12.3 Informed Consent

Before being admitted to the observational study, the subjects must have expressed their consent to the participation, explanations after the nature, scope and possible consequences of the study will be given by the Investigator in a form understandable to them. Information will be given in both oral and written form. The information sheet and informed consent form must be approved by the EC and regulatory authorities (RA). It will include all the elements required by law according to the ICH-GCP recommendations.
In addition to the standard requirements that physicians are currently obliged to observe when providing information, the following points must also be covered:

- a description of the aims of the study and how it will be organized
- the type of treatment
- any potential negative effects attributable to the study treatment
- the freedom to ask for further information at any time
- the subjects ‟ right to withdraw from the clinical study at any time without giving reasons and without compromising their further course of medical treatment
- the existence of subject insurance cover and obligations following from this cover.

Adequate time and opportunity to satisfy questions will be given to the subjects.
The investigator will be supplied with an adequate number of blank informed consent forms to be used. The forms will be signed and dated by both the investigator and the subjects.
A copy of the signed form will be given to the patient.
To ensure medical confidentiality and data protection, the signed informed consent forms will be stored in the investigator's study file according to the regulatory requirements.

The investigator will allow inspection/audit of the forms by authorized representatives of the Sponsor, EC members and RA. He will confirm, by signing and dating the forms, that informed consent has been obtained. In case any new information regarding the IDs will be available during the study the subject will be contacted by the Investigator and if the case a new informed consent will be prepared, submitted to the EC/CA and after approval proposed to the subject.

13 STUDY TERMINATION

- Regular Termination of the Study

The study completion date is the date that the final subject is examined or receives an intervention for the purposes of final collection of data, whether the clinical trial for this subject is concluded according to the protocol or is terminated.

- Premature Termination of the Study

The Sponsor and PI reserve the right to close the study at any time if any of the following reasons occur:

- the safety parameters used for subject's assessments clearly show that it would be unethical to continue the study;
- incidence of serious adverse events is significantly higher than expected.

As far as possible, this should occur after mutual consultation. Reasons for discontinuation must be documented appropriately.

14 INSURANCE POLICY

An insurance cover has been issued in favor of the subjects participating in this clinical study. The insurance is in compliance with the local regulation and with the requirements of the Health Authorities.

14.1 Liability Statement

On behalf of the Sponsor, the investigational sites will take out reasonable third-party liability insurance cover in accordance with all local legal requirements.

The civil liability of the investigators, the persons instructed by them and the hospital, practice or institute in which they are employed and the liability of the Sponsor in respect of financial loss due to personal injury and other damage which may arise as a result of the carrying out of this study are governed by the applicable local laws.

As a precautionary measure, the investigators, the persons instructed by them and the hospital, practice or institute are included in such cover in respect of work done by them in carrying out this study to the extent that the claims are not covered by their own professional indemnity insurance.

15 RESULTS OF THE CLINICAL INVESTIGATION

During all period of screening only one patient have responded to all inclusion/exclusion criteria planned in the protocol of study. The patient has been enrolled after the confirmation of his willingness to participate in a clinical trial by signed and dated informed consent form.
During the vistit 1(enrolment, March 03th, 2017) the patient have a partial Mayo score (without endoscopic assessment) of 5 and a total Mayo score (with endoscopic assessment) of 7.
At follow up visit the patient have a partial Mayo score (without endoscopic assessment) of 0, that have determinated the remission of phatology.
Despite, the encouraging result, the few number of patient treted (only one) exclude the possibility to obtain results statistically significant.

16 CONCLUSIONS

In conclusions, despite the protocol study could have provide rilevant information about the efficacy of intestinal mucosal concentrations from three mesalazine pharmaceutical formulations in patients with mild/moderate ulcerative colitis, the stringent inclusions/exclusion parameters

have prevent to enrol the total numer of patients planned in the simple size preventing, in this way, the obtainment of specific results.

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