«ESSAI RANDOMISÉ, CONTROLÉ, MULTICENTRIQUE, EN DOUBLE-AVEUGLE, ÉVALUANT L'EFFICACITÉ DE L'ERADICATION D'E. COLI ADHERENTE ET INVASIVE DANS LA MALADIE DE CROHN ILÉALE OU ILEOCOLIQUE DROITE DE L'ADULTE»

« MULTICENTER RANDOMIZED DOUBLE-BLIND CLINICAL TRIAL ASSESSING THE BENEFIT OF ADHERENT INVASIVE E.COLI ERADICATION IN ADULT ILEAL AND ILEO-COLONIC CROHN DISEASE»

TEOREM

BIOMEDICAL RESEARCH PROTOCOL RELATING TO A MEDICINAL PRODUCT FOR HUMAN USE

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The research will be carried out in accordance with the protocol, with current good practices and with the legislative and regulatory provisions in force.

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Suivi des modifications du protocole

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V1.2 du 30/10/2015	16/09/2015	06/11/2015	Première version autorisée
V2.0 du 21/07/2016	29/11/2016	30/08/2016	-Critères d'inclusion et de non inclusion : élargissement des critères aux formes modérées de maladie de Crohn Augmentation de la période sans biothérapie avant l'inclusion -Critères d'évaluation : évaluation de l'évolution des lésions par segment -Ajout de deux biopsies rectales -Mise à jour de la liste des centres
V 3.0 du 12/04/2017	17/05/2017	22/05/2017	-Critères d'inclusion : élargissement des critères aux formes cliniquement inactive de maladie de Crohn - Mise à jour de la liste des centres

CONTENTS

1. SUMMARY 7

2. SCIENTIFIC JUSTIFICATION FOR THE RESEARCH 12

2.1 RESEARCH HYPOTHESIS 12
2.2 SUMMARY OF OF KNOWLEDGE CONCERNING THE DISEASE 12
2.3 SUMMARY OF RELEVANT PRE-CLINICAL EXPERIMENTS AND CLINICAL TRIALS 13
2.3.1 Ciprofloxacin (21) 13
2.3.2 Rifaximin (19, 22) 13
2.4 DESCRIPTION OF THE STUDIED POPULATION AND JUSTIFICATION FOR THE CHOICE OF PARTICIPANTS 13
2.5 IDENTIFICATION AND DESCRIPTION OF THE EXPERIMENTAL MEDICATION OR MEDICATIONS 14
2.6 JUSTIFICATION OF THE DOSAGE, ADMINISTRATION METHOD/DESIGN, AND TREATMENT PERIOD 14
2.7 SUMMARY OF THE KNOWN AND FORESEEABLE BENEFITS AND RISKS FOR THE RESEARCH PARTICIPANTS 14

3. OBJECTIVES 14

3.1 PRIMARY OBJECTIVE 14
3.2 SECONDARY OBJECTIVES 14
3.3 OBJECTIVE OF ANY POSSIBLE ANCILLARY RESEARCH* 15

4. RESEARCH PLAN 15

4.1 CONCISE DESCRIPTION OF THE PRIMARY AND SECONDARY ASSESSMENT CRITERIA 15
4.1.1 Primary endpoint 15
4.1.2 Secondary endpoints 16
4.2 EXPERIMENTAL PLAN 16
4.3 NUMBER OF CENTRES PARTICIPATING 16
4.4 IDENTIFICATION OF THE SUBJECTS 16
4.5 RANDOMIZATION 16
4.6 BLINDING METHODS AND PROVISIONS PUT IN PLACE TO MAINTAIN BLINDNESS 16
4.7 PROCEDURES FOR BREAKING THE BLIND 17

5. PROCEDURE FOR THE RESEARCH 17

5.1 PREINCLUSION VISIT 17
5.2 INCLUSION VISIT 18
5.3 FOLLOW-UP VISITS 19
5.3.1 Week 4 visit (±4 days) 19
5.3.2 Week 8 visit (±4 days) 19
5.3.3 Week 12 visit (±4 days) in patients who did not discontinue the treatment 19
5.3.4 Additional visit for early treatment discontinuation (this visit replaces Week 12 visit in case of treatment discontinuation before Week 12) 20
5.3.5 Week 24 visit (±4 days) 20
5.3.6 Week 36 visit (±4 days) 20
5.3.7 End of research visit: Week 48 visit (±4 days) 20
5.4 EXPECTED LENGTH OF PARTICIPATION AND DESCRIPTION OF THE CHRONOLOGY AND DURATION OF THE RESEARCH 20
5.5 RESEARCH SCHEME 22
5.6 TABLE SUMMARISING THE CHRONOLOGY OF THE RESEARCH 23
* ONLY IN CASE OF TREATMENT DISCONTINUATION 23
5.8 DISTINCTION BETWEEN CARE AND RESEARCH 24
5.9 BIOLOGICAL COLLECTION 24
5.10 TERMINATION RULES 25
5.10.1 Criteria and methods for prematurely terminating the research treatment 25
5.10.1.2 Criteria and methods for the premature termination of the research 25
5.10.2 Follow-up of the subjects after the premature termination of treatment 26
5.10.3 Methods for replacing subjects 26
5.10.4 Terminating part or all of the research 26

6. ELIGIBILITY CRITERIA 26

6.1 PREINCLUSION AND INCLUSION CRITERIA 26
6.2 NON-PREINCLUSION AND NON-INCLUSION CRITERIA 27
6.3 RECRUITMENT METHODS 28

7. TREATMENT ADMINISTERED TO RESEARCH PARTICIPANT 28

7.1 DESCRIPTION OF THE EXPERIMENTAL TREATMENT 28
7.1.1 Experimental medication 1 28
7.1.2 Experimental medication 2 28
7.1.3 Experimental medication 3 29
7.1.4 Experimental medication 4 29
7.2 DESCRIPTION OF THE NON-EXPERIMENTAL TREATMENT 29
7.2.1 Non-experimental medication 1 29
7.2.2 Non-experimental medication 2 29
7.3 DESCRIPTION OF THE TRACEABILITY ELEMENTS THAT ACCOMPANY THE EXPERIMENTAL MEDICATIONS 29
THE EXPERIMENTALS PRODUCTS WILL BE PRESCRIBED BY SPECIFIC COMPUTER PRESCRIPTION (CLEANWEB) 29
7.4 AUTHORISED AND PROHIBITED TREATMENTS (MEDICINAL, NON MEDICINAL, SURGICAL), INCLUDING RESCUE MEDICATIONS 29
7.5 METHODS FOR MONITORING COMPLIANCE WITH THE TREATMENT 30

8. SPECIFIC RESEARCH COMMITTEES 30

8.1 STEERING COMMITTEE 30
8.2 ENDPOINT ADJUDICATION COMMITTEE 30

9. SAFETY ASSESSMENT RISKS AND RESTRICTIONS ADDED BY THE RESEARCH 31

9.1 PROCEDURES IN PLACE FOR RECORDING AND REPORTING ADVERSE EVENTS 31
9.1.2 The investigator's roles 32
9.1.3 Specific features of protocol 32
9.1.4 Procedures and deadlines for notifying the sponsor 34
9.1.5 Period for notifying the sponsor 35
9.1.6 The sponsor's roles 35

10. DATA MANAGEMENT 36

10.1 DATA COLLECTION METHODS 36
10.2 IDENTIFICATION OF DATA COLLECTED DIRECTLY IN THE CRFS AND THAT WILL BE CONSIDERED AS SOURCE DATA 36
10.3 RIGHT TO ACCESS SOURCE DATA AND DOCUMENTS 36
10.3.1 Access to data 36
10.3.2 Source documents 37
10.3.3 Data confidentiality 37
10.4 DATA PROCESSING AND STORAGE OF DOCUMENTS AND DATA 37
10.4.1 Identification of the manager and the location(s) for data processing 37
10.4.2 Data entry 37
10.4.3 Data processing (CNIL, the French Data Protection Authority) in France 37
10.4.4 Archival 38
10.5 OWNERSHIP OF THE DATA 38

11. STATISTICAL ASPECTS 38

11.1 DESCRIPTION OF STATISTICAL METHODS TO BE USED INCLUDING THE TIMETABLE FOR THE PLANNED INTERIM ANALYSES 38
11.2 CALCULATION HYPOTHESES FOR THE NUMBER OF SUBJECTS REQUIRED AND THE RESULT 39
11.3 SPECIFY IF SUBJECTS WHO LEAVE THE RESEARCH PREMATURELY WILL BE REPLACED AND IN WHAT PROPORTION 40
11.4 LEVEL OF STATISTICAL SIGNIFICANCE 41
11.5 METHOD FOR TAKING INTO ACCOUNT MISSING DATA 41
11.6 MANAGEMENT OF MODIFICATIONS MADE TO THE ANALYSIS PLAN FOR THE INITIAL STRATEGY 41
11.7 SELECTION OF POPULATIONS 41
11.8 ADDITIONAL REFERENCES 41

12. CONTROL QUALITY AND ASSURANCE 42

12.1 GENERAL ORGANISATION 42

12.2 Strategy for opening the centres 42
12.3 Level of centre monitoring 42
12.4 Quality control 42
12.5 Case Report Form 43
12.6 Management of non-compliances 43
12.7 Audits/inspections 43
12.8 Primary investigator's commitment to assume responsibility 43
12.9 Pharmacist's commitment to assume responsibility 44

13. ETHICAL AND LEGAL CONSIDERATIONS 44

13.1 Methods for obtaining information and consent from research participants 44
13.2 Subject prohibited from participating in another research 44
13.3 Compensation for subjects 44
13.4 Legal obligations 44
13.4.1 The sponsor's role 44
13.5 Request for an opinion from the Comité de Protection des Personnes (CPP, ethical review board) 44
13.6 Request for authorisation to ANSM 45
13.7 Commitment to compliance with the MR 001 "Méthodologie de Reference" 45
13.8 Modifications to the research 45
13.9 Final research report 45

14. FUNDIND AND INSURANCE 45

14.1 Funding source 45
14.2 Insurance 45

15. PUBLICATIONS RULES 46

15.1 Mention of the affiliation of AP-HP for projects sponsored or managed by AP-HP 46
15.2 Mention of the AP-HP manager (DRCD) in the acknowledgements of the text 46
15.3 Mention of the financier in the acknowledgements of the text 46

16. REFERENCES 47

17. LIST OF APPENDIX 50

17.1 Appendix 1: List of Investigators 51
17.2 Appendix 2: Form for reporting Serious Adverse Events 54
17.3 Appendix 3: Form for monitoring a pregnancy that developed during a biomedical research 55
17.4 Appendix 4: Patient card 56
17.5 Appendix 5: Crohn's Disease Activity Index (CDAI) 57
17.6 Appendix 6: Crohn's Disease Endoscopic Index of Severity (CDEIS) 58
17.7 Appendix 7: Inflammatory Bowel Disease Questionnaire (IBDQ) 59
17.8 Appendix 8: Sampling, conservation and transport of biopsies 66
17.9 Appendix 9: SmPC for Ciprofloxacin 67
17.10 Appendix 10: SmPC for Rifaximin 67

1. SUMMARY

Full title	Multicenter randomized double-blind clinical trial assessing the benefit of adherent invasive E.coli eradication in adult ileal and ileo-colonic Crohn disease
Acronym	**TEOREM**
Coordinating Investigator	*Dr Franck Carbonnel* Gastroenterology Department Kremlin Bicêtre Hospital, 78, rue du général Leclerc 94275 Le Kremlin-Bicêtre
Sponsor	Assistance Publique – Hôpitaux de Paris
Scientific justification	Current medical treatment of Crohn disease (CD) consists of immunosuppressive drug therapy (corticosteroids, azathioprine, methotrexate, anti-TNF monoclonal antibodies). These agents control symptoms of CD in most patients but their withdrawal leads to disease relapse. They are also associated with serious side effects (infections, lymphoma, cancer, liver toxicity). Anti-TNF monoclonal antibodies are the most efficient but are cost prohibitive. Moreover, each year, roughly 10% of responders to anti-TNF become refractory to treatment, most often because they become tolerized to these drugs. Future CD drugs are small molecules or biologics that antagonize pro-inflammatory cytokines or gut homing of lymphocytes. Yet, they have similar safety issues as the immunosuppressive agents currently in use. The current therapeutic algorithm for CD does not take into account the site of inflammation or composition of intestinal microbiota. Moreover, dysregulated intestinal immunity and overproduction of pro-inflammatory cytokines are not the only components of CD pathophysiology. Intestinal microbiota is abnormal and plays an important role in the pathogenesis of CD. An *E. coli* specie with adhesive and invasive properties, adherent-invasive *E. coli* (AIEC), has been described in ileal mucosa of patients with CD. AIEC has been found in 36% of patients with ileal CD and 6% of controls The role of AIEC in CD has been thoroughly investigated in several laboratories around the world and has shown that AIEC is pro-inflammatory and could play an important role in intestinal inflammation associated with CD (1). As such, it may be an important therapeutic target. This randomized trial proposes a novel approach to the treatment of CD. It is individualized to patients colonized with AIEC who will be prescribed FDA-approved antibiotics that have been shown to be active against the bacteria *in vitro*. The treatment will be discontinued after 12 weeks. We hope to obtain clinical and endoscopic remission in patients who have been randomized into the experimental arm. If this trial is successful, it will be the first of its kind and will facilitate a new era of clinical research in patients

	with CD and concomitant infection with AIEC.
Primary objective and assessment criterion	The **primary objective** of this trial is to assess whether a 12-week treatment with Ciprofloxacin and Rifaximin is superior to placebo to obtain endoscopic remission in AIEC-colonized patients with ileal CD, with or without involvement of the caecum or the right colon. **The primary endpoint is the overall response** at week 12, using centralized, anonymous and blinded reading of ileocolonoscopies. ***Overall response*** is defined by segmental response in at least one segment in which the CDEIS was ≥8 at baseline, with no more than 20% increase of CDEIS in the other segment and a CDEIS<8 in this segment. ***Segmental response*** in the segment in which the CDEIS was ≥8 at baseline, is defined by: - In the case of isolated aphtoid ileitis and/or colitis: decrease ≥ 50% of lesions. **-** Otherwise, <u>either</u> decrease ≥ 50% of surface of ulcerations (either deep or superficial), <u>or</u> loss of deep ulcerations (if present at inclusion) with no more than 20% increase in superficial ulcerated surface.
Secondary objectives and assessment criteria	The **secondary objectives** are: ▪ To test the correlation between endoscopic remission and AIEC eradication at 12 weeks, ▪ To assess the evolution of endoscopic lesions at week 12, ▪ To assess the rate of clinical remission without steroids, anti-TNF, and surgery at 48 weeks, in patients whose AIEC has, or has not been eradicated, and in patients who have, or have not, reached the primary endpoint, ▪ To assess the rate of biological remission, during the study, ▪ To assess side effects in the experimental and placebo group. The **secondary endpoints** are : • <u>Endoscopic remission at 12 weeks</u> (assessed by centralized reading of ileocolonoscopies), defined by a CDEIS < 6 in all the segments ▪ Mean variation of segmental CDEIS, at 12 weeks (centralized reading of ileocolonoscopies), ▪ Complete endoscopic remission at 12 weeks (centralized reading of ileocolonoscopies) defined by a CDEIS <3, in all the segments ▪ No ulceration at 12 weeks, ▪ Clinical remission as defined by CDAI<150 without steroids, anti-TNF, and resection surgery for CD at 12 and/or 48 week, ▪ Biological remission as defined CRP serum level ≤5 mg/L and fecal calprotectin <250 µg/g at week 4, 8, 12, 24, 36 and 48, ▪ Side effects.

Experimental design	This is a phase II clinical trial. It is a multicentre, prospective, randomized, double-blind, placebo-controlled adaptive clinical trial, with two parallel arms: ▪ **Experimental arm**: oral Ciprofloxacin 500 mg bid and oral Rifaximin 800 mg bid for 12 weeks ▪ **Control arm**: a placebo of Ciprofloxacin bid and a placebo of Rifaximin bid for 12 weeks
Population involved	CD adult patients infected with AIEC.
Preinclusion criteria	▪ Age 18 to 80 years ▪ CD of the ileum, with or without involvement of the caecum or the right colon ▪ Most severe lesions are confined within the ileum and/or the right colon. This is defined by: - segmental CDEIS ≥8 in the ileum and/or right colon; - and segmental CDEIS <8 in each of the other segments (transverse, left colon and rectum). ▪ Informed consent to participate in this study ▪ CD either clinically inactive or active, but not severe (CDAI<450) ▪ For patients with clinically active disease (CDAI>150) : Prescription of steroid treatments : Budesonide, Prednisone (or Prednisolone) independently from entry in study Note: patients with or without previous surgery for CD, with or without CD anal lesions, with or without draining fistulas and setons, can be enrolled.
Inclusion criteria	▪ Patients with clinically active disease (CDAI>150) who respond to budesonide (initial dose 9 mg/d) or prednisone or prednisolone (initial dose 40 mg/d), by a 70 points decrease in CDAI between the pre-inclusion and the inclusion visit, or patients with clinically inactive disease (CDAI<150) ▪ Patients colonized with AIEC on initial ileal biopsies.
Non-Preinclusion and **Non-inclusion criteria**	▪ Ileal stenosis that cannot be crossed by the endoscope, ▪ Infliximab treatment received less than 8 weeks before **pre**inclusion in this study, ▪ Methotrexate and azathioprine started less than three months before **pre**inclusion ▪ Adalimumab treatment received less than 4 weeks before **pre**inclusion in this study, ▪ Vedolizumab treatment received less than 8 weeks before **pre**inclusion, in the study ▪ Hypersensitivity to Ciprofloxacin, to other quinolones, or to any of the excipients (cellulose microcrystalline, crospovidone, maize starch, magnesium stearate, silica colloidal anhydrous, hypromellose titanium dioxide E171, macrogol 4000), ▪ Treatment with Tizanidine, Probenecid,

	Theophylline, Xanthine derivatives, Phenytoin, oral anticoagulants, and Ropinirole, ▪ Hypersensitivity to Rifaximin, or to any excipients (sodium starch glycolate type A, glycerol distearate, colloidal anhydrous silica, talc, microcrystalline cellulose, hypromellose, titanium dioxide, disodium edentate, propylene glycol, red iron oxide E172), ▪ Previous extensive ileal surgery (≥ 1 meter as measured on the pathology and/or surgical report), ▪ Short bowel syndrome, ▪ Need for an intestinal resection for fistula, abscess or intestinal obstruction, ▪ Renal failure (creatinine clearance<30 mL/min/1.73m^2), ▪ Liver failure (V factor<50%), ▪ Past history of epilepsy, ▪ No health insurance, ▪ Pregnant or lactating women, ▪ Refusal to have a double effective contraception, ie Hormonal + barrier method, Intrauterine device + barrier method, double barrier method). Highly effective methods of contraception are: hormonal contraceptives (e.g. combined oral contraceptives, patch, vaginal ring, injectables, and implants); intrauterine device (IUD) or intrauterine system (IUS); vasectomy and tubal ligation. Effective methods are: barrier methods of contraception (e.g. male condom, female condom, cervical cap, diaphragm, contraceptive sponge). ▪ Patients already included in a biomedical research other than an observational study (e.g:registry, cohort).
Treatment being tested	<u>Association of two antibiotics:</u> ▪ Oral Ciprofloxacin, tablets of 500 mg, every 12 hours during 12 weeks ▪ Oral Rifaximin (tablets of 200 mg), 800 mg every 12 hours during 12 weeks
Benchmark treatment	Association of two placebos: A placebo corresponding to each antibiotic will be manufactured (tablet of the same appearance). It will be administrated every 12 hours during 12 weeks.
Other procedures added by the research	▪ Ciprofloxacin and Rifaximin or their placebos ▪ 6 additional biopsies, including 4 biopsies of the ileum or the right colon (2 for the AIEC search and 2 for the biological collection) and 2 biopsies of the rectum (for AIEC search) during the 1st ileocolonoscopy ▪ Ileocolonoscopy at week 12 including 6 additional biopsies ▪ Study of fecal microbiota using PCR 16S ▪ Screening for fecal AIEC lpf+
Risks added by the research	*Risk C*
Practical procedure	252 patients with CD will screened to realize biopsies to check for infection by AIEC bacteria. 62 evaluable patients

	are anticipated. These are patients infected with this bacterium and responding to the eligibility criteria of the study. They will be included in the study and randomized to the experimental arm (Rifaximin+Ciprofloxacin) or control (double placebo) arm. An ileocolonoscopy and biopsies will be made at week 12, to research AIEC infection and thereby assess the eradication of the bacteria after taking the study drugs or placebos. Bacterial infection will also be sought from the stool samples.
Number of subjects chosen	252 screened patients will be needed on average to obtain 62 randomized patients
Number of centres	26
Research period	*Period of inclusion: 36 months* *Period of pre-inclusion: 3 months* *Maximum duration of participation (treatment+follow-up): about 13 months* *Total duration of the study: 52 months*
Number of inclusions expected /centre and month	0.1
Statistical analysis	Data will be analyzed on an **intent-to-treat** basis The trial is based upon an adaptive scheme, allowing the re-estimation of the required number of included patients (« *Sample size re-estimation (SSR) design »*), taking into account the uncertainty of endoscopic remission rate at week 12 in the placebo arm. Thus, one **interim unblind statistical analysis** will be performed once one half of the patients have been successively enrolled. **Primary endpoint** analysis concerns the comparison of the main end point, ie, the percentage of endoscopic remission in each group that will be compared using the chi-square test unless validity violated (or the Fisher exact will be used). **Secondary endpoints** will be analyzed once the whole sample size will be reached.
Funding source	Broad Medical Research Program, Mayoly-Spindler, Alpha Wassermann, and PHRC national.
Data Safety Monitoring Board anticipated	No

2. SCIENTIFIC JUSTIFICATION FOR THE RESEARCH

2.1 Research hypothesis

Crohn's disease (CD) is a chronic inflammatory bowel disease. Intestinal microbiota is composed by several billions of microbes in healthy adults. The dysregulated intestinal immunity and overproduction of proinflammatory cytokines are not the only components of CD pathophysiology. Intestinal microbiota is abnormal and plays an important role in the pathogenesis of CD. An *E. coli* specie with adhesive and invasive properties, adherent-invasive *E. coli* (AIEC), has been described in ileal mucosa of patients with CD. AIEC has been found in 36% of patients with ileal CD and 6% of controls. The role of AIEC in CD has been thoroughly investigated in several laboratories around the world and it has been shown that AIEC is proinflammatory and could play an important role in intestinal inflammation associated with CD (1). As such, it may be an important therapeutic target.
The research hypothesis is that antibiotics targeting AIEC (Ciprofloxacin + Rifaximin) will achieve mucosal healing in the CD patients colonized by AIEC. If the primary hypothesis is validated, this will be the first evidence that an eradication of a particular bacteria species by an antibiotic treatment can lead to remission in CD. If these results are confirmed, patients with CD who are AIEC+ will benefit from an antibiotic treatment in terms of prolonged remission without the use of immunosuppressants. Future trials will determine optimal treatment duration, biomarkers of early response, recolonization rate, and replication of our results at a larger scale.

This randomized trial proposes a novel approach to the treatment of CD. It is individualized to patients colonized with AIEC who will be prescribed FDA-approved antibiotics that have been shown to be active against the bacteria *in vitro*. The treatment will be discontinued after 12 weeks. We hope to obtain clinical and endoscopic remission in patients who have been randomized into the experimental arm.

2.2 Summary of of knowledge concerning the disease

Crohn's disease (CD) is a chronic inflammatory bowel disease affecting approximately one million people in Europe, including 100,000 in France and 700,000 in the United States. Most affected patients are teenagers and young adults. Patients with CD have an altered quality of life and an increased mortality rate of 30% to 50% as compared with age-matched controls. Most CD patients will undergo intestinal resection at least once, generally for a complication such as intestinal obstruction, abscess, fistula or intestinal carcinoma.
Current medical treatment of CD consists of immunosuppressive drug therapy (corticosteroids, azathioprine, methotrexate, anti-TNF monoclonal antibodies). These agents control symptoms of CD in most patients but their withdrawal leads to disease relapse. They are also associated with serious side effects (infections, lymphoma, cancers, liver toxicity). Anti-TNF monoclonal antibodies are the most efficient but are cost prohibitive. Moreover, each year, roughly 10% of responders to anti-TNF become refractory to treatment, most often because they become tolerized to these drugs. Future CD drugs are small molecules or biologics that antagonize pro-inflammatory cytokines or gut homing of lymphocytes. Yet, they have similar safety issues as the immunosuppressive agents currently in use.
The current therapeutic algorithm for CD does not take into account the site of inflammation or composition of intestinal microbiota.

2.3 Summary of relevant pre-clinical experiments and clinical trials

A number of fundamental studies have investigated the biology of AIEC and its role in CD. The role of AIEC in CD has been discovered in the laboratory of Pr Arlette Darfeuille Michaud, who was the scientific coordinator of this project. She died in June 2014. Her successor, Dr Nicolas Barnich, is now the head of the lab and the scientific coordinator of the project. His lab can reliably analyse biopsy specimens for the presence of AIEC in our trial. Results can be obtained in 30 days or less.
Several trials have investigated the efficacy of antibiotics in CD, but none of them was individualized to patients colonized with AIEC. The present trial is the first of its kind. It attempts to eradicate AIEC in the aim to treat CD. It is individualized to patients colonized with AIEC, who will be prescribed antibiotics that are active against the bacteria *in vitro.*
Antibiotics such as Ciprofloxacin and Rifaximin have fewer side effects than the immunosuppressive treatment used as a first-line treatment of CD and may be a safer alternative to immunosuppressive therapy in those patients colonized with AIEC.

2.3.1 Ciprofloxacin (21)

A randomised trial based upon 134 patients with CD has compared the combination of Ciprofloxacin + métronidazole with placebo in patients treated with budesonide. Adverse events were more frequent in patients who received antibiotics but they were not serious: diarrhea, nausea, vertigo, glossitis, dysgueusia and vaginitis (18). In addition, some of them were due to metronidazole.

2.3.2 Rifaximin (19, 22)

A review based upon 10 randomized trials of Rifaximin in travelers' diarrhea has been published (22). These trials have included 1500 patients. Tolerance of Rifaximin was similar with that of placebo and better than that of Ciprofloxacin. Adverse events were mild and non-specific (headache, constipation, flatus, nausea and vomiting).
42 patients with CD have been included in a randomised trial which compared Rifaximin (n=300) to placebo (=102) (19). The rate of adverse events was not different in both groups (12% and 13% respectively). Side effects of Rifaximin were: nausea, headache, respiratory infection, pharyngitis… Combination of Rifaximin and Ciprofloxacin has been studied in an open trial of 8 patients with pouchitis no side effect was reported (20). There is no known interaction between both drugs.

2.4 Description of the studied population and justification for the choice of participants

The role of AIEC in CD has been thoroughly investigated in several laboratories around the world. These researches have shown that AIEC could play an important role in the intestinal inflammation associated with CD. As such, it may be an important therapeutic target. This randomized trial proposes a new and alternative approach to treat CD. It is individualized to patients colonized with AIEC who will be prescribed antibiotics that have been shown to be active against AIEC *in vitro.*

2.5 Identification and description of the experimental medication or medications

Trial treatments are Ciprofloxacin, Rifaximin and placebos. Ciprofloxacin and Rifaximin are antibiotics. Ciprofloxacin has a marketing authorization in France for several infections. Rifaximin has not a marketing authorization in France. It is marketed in several EC countries and in the USA where it has obtained the FDA agreement for the treatment of traveler's diarrhea and hepatic encephalopathy (see chapiter 7).

2.6 Justification of the dosage, administration method/design, and treatment period

Ciprofloxacin will be administered orally at a dose of 500 mg every 12 hours during 12 weeks (20).
Rifaximin will be administered orally at a dose of 800 mg every 12 hours during 12 weeks (19) (see section 7).

2.7 Summary of the known and foreseeable benefits and risks for the research participants

If the primary hypothesis is validated, this will be the first evidence that an eradication of a particular bacteria species by an antibiotic treatment can lead to remission in CD.
Currently CD patients are treated with immunosuppressive treatments, including anti-TNF. These drugs are associated with harmful side effects, such as opportunistic infections and, rarely, lymphoma. In addition, approximately 10% of patients loose response to anti TNF each year. Antibiotics such as Ciprofloxacin and Rifaximin have fewer side effects than the immunosuppressive treatment used as a first-line treatment of CD and may be a safer alternative to immunosuppressive therapy in those patients concomitantly colonized with AIEC.
If antibiotic treatment of CD patients who are colonized with AIEC is efficient, these patients will be more likely to go into prolonged remission without steroids and immunosuppressants, avoid hospitalizations, surgeries and have a better quality of life. Also antibiotic therapy cost much less than some immunosuppressive therapies and will reduce healthcare costs.

3. OBJECTIVES

3.1 Primary objective

The primary objective of this trial is to assess whether a 12-week treatment with Ciprofloxacin and Rifaximin is superior to placebo to obtain endoscopic remission in AIEC-colonized patients with ileal CD, with or without involvement of the caecum or the right colon.
Endoscopic remission has been chosen as a primary endpoint because it is associated with improvement of CD evolution in the midterm.

3.2 Secondary objectives

- To test the correlation between endoscopic remission and AIEC eradication at week 12,
- To assess the evolution of endoscopic lesions at week 12,
- To assess the rate of clinical remission without steroids, anti-TNF, and surgery at 48 weeks in patients whose AIEC has or has not been eradicated and in patients who have or have not reached the primary endpoint,
- To assess the rate of biological remission, during the study,
- To assess side effects in the experimental and placebo group.

3.3 Objective of any possible ancillary research*

Ancillary studies

- Clinical characteristics of patients AIEC$^+$ will be compared with AIEC$^-$ screened patients,
- Effect of antibiotics and placebo upon fecal and mucosal microbiota will be assessed using PCR 16S at inclusion, week 12 and week 48 and will be correlated with primary and secondary endpoints of the study. This will allow to assess the effects of antibiotics upon dysbiosis and lead to a microbiota signature associated with endoscopic remission,
- Search for AIEC lpf+ using PCR within stool specimens will be performed at the screening visit, week 12, and week 48. This will be aimed to correlate mucosal and fecal AIEC as well as fecal AIEC with clinical and endoscopic remission.

4. RESEARCH PLAN

4.1 Concise description of the primary and secondary assessment criteria

4.1.1 Primary endpoint

- **The primary endpoint is the overall response** at week 12, assessed by a panel of GETAID expert endoscopists, using centralized, anonymous and blinded reading of ileocolonoscopies. For this, in each investigator centre, after anonymisation, ileocolonoscopies will be recorded on DVD, USB keys or on a dedicated internet platform.
  Overall response is defined by segmental response in at least one segment, in which the CDEIS was ≥8 at baseline with no more than 20% increase of CDEIS in the other segment and a CDEIS<8 in this segment.
  Segmental response in the segment in which the CDEIS was ≥8 at baseline, is defined by:
  - In the case of isolated aphtoid ileitis and/or colitis: decrease ≥ 50% of lesions.
  - Otherwise, either decrease ≥ 50% of surface of ulcerations (either deep or superficial), or loss of deep ulcerations (if present at inclusion) with no more than 20% increase in superficial ulcerated surface.

- The Crohn's Disease Endoscopic Index of Severity (CDEIS) is a validated score of endoscopic severity in CD (Mary JY, Modigliani R. Gut 1989;30: 983-9). The segmental CDEIS is adapted to the patients included in the trial who have CD limited to the ileum and right colon. The preinclusion criteria (CDEIS>8) and the definition of endoscopic response and remission have been defined with JY Mary. The segmental CDEIS will be measured by an endoscopist during an ileocolonoscopy performed at the preinclusion visit and at week 12 of the treatment. During these two ileocolonoscopies, 4 ileal biopsies will be taken (2 for the AIEC search and 2 for the biological collection), at the edge of ulcerations; 2 rectal biopsies will be taken, for AIEC search.
- The primary endpoint is assessed at week 12 after randomization, that is, at least 18 weeks after the start of the budesonide or prednisone or prednisolone treatment, which lasts 12 weeks. Therefore, at the moment of the primary endpoint, most patients will have stopped budesonide. This will avoid any interaction between steroids (budesonide or prednisone or prednisolone) and antibiotics.

  The same endoscopist will perform the ileocolonoscopy at the preinclusion visit and at week 12. The ileocolonoscopies will be performed within the gastroenterology unit.

4.1.2 Secondary endpoints

The secondary endpoints are:

- Endoscopic remission at 12 weeks (assessed by centralized, anonymous and blinded reading of ileocolonoscopies), is defined by a CDEIS < 6 in all the segments
- Mean variation of segmental CDEIS, at 12 weeks (assessed by centralized, anonymous and blinded reading of ileocolonoscopies),
- Complete endoscopic remission at 12 weeks (assessed by centralized, anonymous and blinded reading of ileocolonoscopies) defined by a CDEIS <3, in all the segments
- No ulceration at 12 weeks,
- Clinical remission as defined by CDAI<150 without steroids, anti-TNF, and resection surgery for CD at 12 and/or 48 week,
- Biological remission as defined CRP serum level ≤5 mg/L and fecal calprotectin <250 µg/g at week 4, 8, 12, 24, 36 and 48,
- Side effects.

4.2 Experimental plan

This study is an adaptive phase II biomedical research with health products. This is a multicenter prospective randomized double-blind, placebo-controlled clinical trial, with two parallel arms (allocated ratio: 1/1):

- **Experimental arm:** oral Ciprofloxacin 500 mg bid and oral Rifaximin 800 mg bid for 12 weeks.
- **Control arm:** a placebo of Ciprofloxacin bid and a placebo of Rifaximin bid for 12 weeks.

4.3 Number of centres participating

This is a national multicenter research involving 26 investigation centres
Patient recruitment will take place within the recruiting hospitals, in gastroenterology units.

4.4 Identification of the subjects

For this research, the subjects will be identified as follows:
Centre No. (3 numerical positions) – Selection order No. of the person in the centre (4 numerical positions) - surname initial - first name initial**. This reference is unique and will be retained for the entire research period for any patient.**

4.5 Randomization

Randomization will be centralized, stratified on two factors: immunosuppressants (yes or no) and steroids prescribed (budesonide or prednisone). Therefore, four pre-specified lists will be established by the SBIM before the start of the study, according to a method based upon permutation block (which size will be kept confidential) to control the disequilibrium in sample sizes across the two arms.
Patients will be randomized during the inclusion visit. Randomization of the two parallel groups will be performed once the inclusion criteria have been verified (compliance with inclusion and exclusion criteria), and centralized on the web (see below 4.6).
A treatment number will be assigned by randomization.

4.6 Blinding methods and provisions put in place to maintain blindness

The concealment of allocation, which is the process for actually assigning a patient to a group without breaking blinding, will be performed by the internet software named Cleanweb/CTMS.
Trial treatments (Ciprofloxacin, Rifaximin and their respective placebo) will be manufactured in accordance with GMPs. Experimental drugs and placebo will have a similar appearance, in order to preserve blinding. Clinical trial department, AGEPS, insures the manufacture, labelling of treatments depending on internal procedures, in accordance with the regulations applicable to clinical trials for experimental drugs (GMP).

4.7 Procedures for breaking the blind

Unblinding will be requested for any reason considered essential by the investigating doctor by calling upon:

- the DRCD **in a situation other than an emergency** during the work day and during working hours, addressed to the DRCD's project referent**:** Pauline Cavelier
  Tel : +33 1 44 84 17 48,
- the poison centre of Fernand Widal Hospital, in the case of an emergency (see emergency situations requiring unblinding), on weekends, bank holidays, when the DRCD is closed and when unblinding cannot be carried out at the DRCD Telephone: +33 1 40 05 48 48,

5. PROCEDURE FOR THE RESEARCH

Before any examination or act related to the research, the investigator collects the free and informed, written consent of the patient undergoing research.

5.1 Preinclusion visit

During this visit, preinclusion and non-preinclusion criteria will be checked and the data below will be collected.

- Patient's information
- Signed consent
- Medical consultation including Crohn's Disease Activity Index (CDAI, see appendix 5) and Inflammatory Bowel Disease Questionnaire (IBDQ, see appendix 7)
- Stool collection before bowel preparation for
  - Fecal calprotectin
  - Search for AIEC lpf+ bacteria using PCR within the stools
  - Study of fecal microbiota using PCR 16S
- Ileocolonoscopy with CDEIS evaluation, ileal biopsies for pathology search for AIEC and biological collection (sent to Clermont-Ferrand laboratory). The results of the search of AIEC will be sent to the investigator by mail no later than 30 days after the reception of the biopsies.
- For patients with clinically active CD (CDAI>150): prescription of budesonide at the initial dose of 9 mg/d or prednisone or prednisolone at the initial dose of 40 mg/d. Patients who relapse at the maximal dose of budesonide will be treated with prednisone or prednisolone. Patients who relapse at the maximal dose of prednisone or prednisolone will not be eligible for the randomized trial.

- Prescription of blood tests for the inclusion visit: CBC, ALT, AST, γ-GT, phosphatases alcalines, creatinine, β-HCG (if applicable) and CRP
- Prescription of the fecal calprotectin for the inclusion visit

The preinclusion will be performed by cleanWeb®. The physician responsible for the patient will be provided the **anonymous and unique** identification number for each patient.

I ___I I ___I I ___I / I ___I I ___I I ___I I ___I / I___I I ___I

1- code centre / 2- N° inclusion in centre / 3- initiales patient

Budesonide will be prescribed during the preinclusion visit, at an initial dose of 9 mg/d during 8 weeks, then 6 mg/d during 2 weeks, and then 3 mg/d during 2 weeks (in accordance with the WMA). Prednisone or prednisolone will be prescribed during the preinclusion visit, at an initial dose of 40 mg/d during 3 weeks, then 30 mg/d during 10 days and then 20 mg/d during 10 days, then 15 mg/d during 10 days, then 10 mg/d during 10 days, then 7.5 mg/d during 10 days, then 5 mg/d during 10 days, then 2.5 mg/d during 10 days (in accordance with the WMA). In case of relapse (defined as a CDAI>150 and increase of 70 points in CDAI in patients who had reached clinical remission (CDAI<150) and increase of 50 points in those who had not reached clinical remission) occurring between preinclusion and inclusion, the dose of budesonide or prednisone or prednisolone will be increased to the previous dose during 14 days. Then, the tapering will be started again, according to the abovementioned scheme. The maximum time relapse between preinclusion and randomization (inclusion visit) will be 12 weeks. Patients eligible within the randomized trial who need an increase in budesonide or prednisone or prednisolone dosage will be included when they will reach response, as defined by a 70 points decrease in baseline CDAI.
Patients who fail to respond to the maximal dose of budesonide will be treated with prednisone or prednisolone at the initial dose of 40 mg/d or 60 mg/d and will be eligible in the randomized trial, if they respond (70 points decrease in baseline CDAI) to prednisone or prednisolone. Patients who have a severe relapse (CDAI>450) or do not respond to the maximal dose of prednisone or prednisolone and/or in whom budesonide or prednisone or prednisolone is poorly tolerated will need to step up to immunosuppressants or biologics (anti TNF or vedolizumab) and will not be eligible to the randomized trial.
Prednisone or prednisolone can be associated with budesonide. Whenever possible, they will be stopped concomitantly with budesonide, in order to avoid any interaction between these drugs and antibiotics.
The primary endpoint will be evaluated 6 weeks after steroids have been stopped (ie, 12 weeks after randomization).

5.2 Inclusion visit

During the inclusion visit, the eligibility criteria will be checked again.

- Medical consultation including CDAI and IBDQ
- Review of blood tests prescribed at the preinclusion visit
- Review of inclusion and non-inclusion criteria
- Randomisation, performed by CleanWeb®
    - the name of the physician and his(her) centre (with fax number)
    - the identification number of the patient (from the preinclusion visit)
    - the past history of immunosuppressive drugs (naive patient or not)
    - the administered corticosteroid for the past 6 weeks : prednisone, prednisolone or budesonide

A randomization e-mail will be sent to the physician, SBIM, clinical trial unit and clinical trial department AGEPS.

- Prescription of the allocated treatment
  - Prescription of the allocated treatment, performed by CleanWeb® (units treatments numbers)
  - Experimental drugs will be sent to the pharmacy of the centre by clinical trial department AGEPS. The patient will be invited to collect the drug at the hospital's pharmacy within 5 days after the randomisation. The dispensed unit will contain the whole treatment (12 weeks)
- A patient card (indicating participation in the trial and treatments) will be issued to each patient (see appendix 4)
- Search for AIEC lpf+ bacteria using PCR within the stools
- Study of fecal microbiota using PCR 16S
- Prescription of blood tests for the week 4 visit: CBC, ALT, AST, γ-GT, alkaline phosphatase, creatinine, β-HCG (if applicable), CRP

5.3 Follow-up Visits

5.3.1 Week 4 visit (± 4 days)

- Medical consultation including CDAI
- Review of symptoms and treatment observance (pill counts and patient's interview)
- Review of blood tests results prescribed at the previous visit
- Prescription of blood tests for the week 8 visit: CBC, ALT, AST, γ-GT, alkaline phosphatase, creatinine, β-HCG (if applicable), CRP

5.3.2 Week 8 visit (± 4 days)

- Medical consultation including CDAI
- Review of symptoms and treatment observance (pill counts and patient's interview)
- Review of blood tests results prescribed at the previous visit
- Prescription of blood tests for the week 12 visit : CBC, ALT, AST, γ-GT, alkaline phosphatase, creatinine, β-HCG (if applicable), CRP
- Prescription of the fecal calprotectin for the week 12 visit

5.3.3 Week 12 visit (± 4 days) in patients who did not discontinue the treatment

- Medical consultation including CDAI and IBDQ
- Review of symptoms and treatment observance (pill counts and patient's interview)
- The investigator will be asked to say in what arm of the trial he thinks the patients have been allocated to.
- Review of blood tests results prescribed at the previous visit
- Prescription of blood tests for the week 24 visit: CBC, ALT, AST, γ-GT, alkaline phosphatase, creatinine, β-HCG (if applicable), CRP

- Stool collection before bowel preparation for
  - Fecal calprotectin
  - Search for AIEC lpf+ bacteria using PCR within the stools
  - Study of fecal microbiota using PCR 16S

- Ileocolonoscopy with CDEIS evaluation, biopsies for pathology search for AIEC and biological collection

5.3.4 Additional visit for early treatment discontinuation (this visit replaces Week 12 visit in case of treatment discontinuation before Week 12)

- Medical consultation including CDAI and IBDQ
- Review of symptoms and treatment observance (pill counts and patient's interview)
- The investigator will be asked to say in what arm of the trial he thinks the patients have been allocated to.
- Review of blood tests results prescribed at the previous visit
- Prescription of blood tests for the week 24 visit: CBC, ALT, AST, γ-GT, alkaline phosphatase, creatinine, β-HCG (if applicable), CRP
- Stool collection before bowel preparation for
    - Fecal calprotectin
    - Search for AIEC lpf+ bacteria using PCR within the stools
    - Study of fecal microbiota using PCR 16S
- Ileocolonoscopy with CDEIS evaluation, biopsies for pathology and search for AIEC

In case of suspected CD relapse see chapter 5.10.1.1

5.3.5 Week 24 visit (± 4 days)

- Medical consultation including CDAI
- Review of blood tests results prescribed at the previous visit
- Prescription of blood tests for the week 36 visit: CBC, ALT, AST, γ-GT, alkaline phosphatase, creatinine, β-HCG (if applicable), CRP.

5.3.6 Week 36 visit (± 4 days)

- Medical consultation including CDAI
- Review of blood tests results prescribed at the previous visit
- Prescription of blood tests for the week 48 visit: CBC, ALT, AST, γ-GT, alkaline phosphatase, creatinine, β-HCG (if applicable), CRP
- Prescription of the fecal calprotectin for the week 48 visit

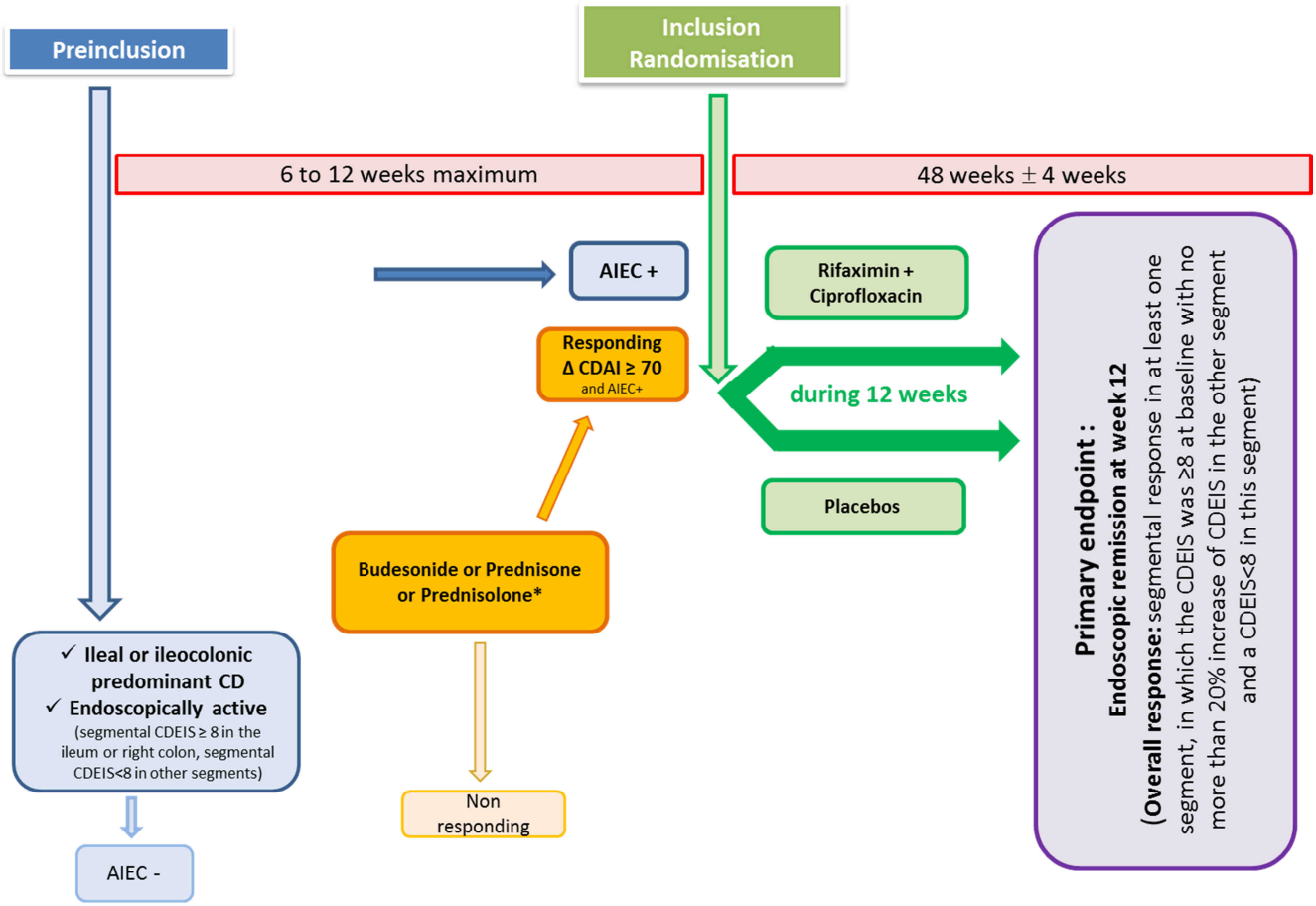
5.3.7 End of research visit: Week 48 visit (± 4 days)

- Medical consultation including CDAI and IBDQ
- Review of blood tests and fecal calprotectin results prescribed at the previous visit
- Stool collection for
    - Fecal calprotectin
    - Search for AIEC lpf+ bacteria using PCR within the stools
    - Study of fecal microbiota using PCR 16S

5.4 Expected length of participation and description of the chronology and duration of the research

Maximum period between preinclusion and inclusion	3 months
Inclusion period	36 months
Maximum length of participation for each included patient	13 months
of which:	
• Treatment period:	3 months (± 12 days)
• Follow-up period:	9 months (± 12 days)
Total research period:	52 months

5.5 Research Scheme



* Budesonide, Prednisone or Prednisolone will be stopped at least 6 weeks before primary endpoint evaluation.

5.6 Table summarising the chronology of the research

	Preinclusion visit	Inclusion						
DATE	Preinclusion	D0	W4 ± 4 days	W8 ± 4 days	W12 ± 4 days or Additionnal visit*	W24 ± 4 days	W36 ± 4 days	W48 ± 4 days (end of study)
Medical visit (with CDAI and IBDQ)	x	x	x	x	x	x	x	x
Informed consent form	x							
Verification of preinclusion and non-preinclusion criteria	x							
Verification of inclusion and non-inclusion criteria		x						
Randomization		x						
Blood samples collection (CBC, ALT, AST, γ-GT, alkaline phosphatases, creatinine, β-HCG, CRP)		x	x	x	x	x	x	x
Stool collection for Fecal calprotectin	**x**	x			x			x
Stool collection for Study of fecal microbiota using PCR 16S	x	**x**			x			x
Stool collection for Screening for fecal AIEC lpf+	x				x			x
Ileocolonoscopy with CDEIS and 4 ileal biopsies (2 for AIEC search : primary endpoint and 2 for the biological collection) and 2 rectal biopsies for AIEC search	x				x			
Prescription of non-experimental drug medication: budesonide or prednisone or prednisolone for clinically active patients	x							
Prescription of experimental drug medication		x						
Administration of experimental drug medication		x	x	x	x			
Review of treatment observance			x	x	x			
Opinion of investigator concerning the patient's arm					x			

* Only in case of treatment discontinuation

5.8 Distinction between care and research

Procedures and treatments carried out as part of the research	**Procedures and treatments associated with <u>care</u>**	**Procedures and treatments added because of <u>the research</u>**
Treatments	Budesonide and/or other steroids	Ciprofloxacin and Rifaximin or their placebos
Consultations	All the study visits	none
Blood samples	All blood samples	none
Endoscopies	Initial ileocolonoscopy	6 additional biopsies, including 4 biopsies of the ileum or the right colon (2 for the AIEC search and 2 for the biological collection) and 2 biopsies of the rectum (for AIEC search) during the 1st ileocolonoscopy (preinclusion visit) Ileocolonoscopy at week 12 including 6 additional biopsies, including 4 biopsies of the ileum or the right colon (2 for the AIEC search and 2 for the biological collection) and 2 biopsies of the rectum (for AIEC search)
Stool samples	Fecal calprotectin dosage	Study of fecal microbiota using PCR 16S Screening for fecal AIEC lpf+

5.9 Biological Collection

Sampling, conservation and transport of biopsies is described in appendix 8

The fecal samples and the biopsies samples taken as part of the research will be included in a biological collection.
The collections will be stored at the laboratory of Pathogénie Bactérienne Intestinale, CBRV, 28 Place Henri Dunant, 63000 Clermont-Ferrand under the supervision of Nicolas Barnich (scientific director of the study). The collection will be used to exhaustion if patients do not express opposition to this.

The samples may be used with the explicit agreement of the subject on the consent form for further analyses not included in the protocol and which could be beneficial for the pathology (Crohn disease), based on evolution in scientific knowledge. The collection will be used to exhaustion if patients do not express opposition to this.
The collections will be declared to the ANSM in the context of biomedical research. At the end of the research, the samples will be preserved. Thus, the collection will be declared to the minister responsible for research (Article L. 1243-3 of the CSP (French Public Health Code).

5.10 Termination rules

5.10.1 Criteria and methods for prematurely terminating the research treatment

5.10.1.1 Different situations

- Temporary termination of treatment (minor side effect, oversight, etc…): the investigator must document the reason for stopping and restarting the treatment in the patient's source file and the case report form (CRF). The treatment can be stopped prematurely (on a decision of investigator) without breaking the blinding. The treatment can be started again, at the initial dosage, if the discontinuation was not due to a serious adverse event.
- Premature and definitive termination of the treatment. In this case, the patient is still followed according to the scheduled visits of the protocol, unless he decides to withdraw his/her participation. The investigator must document the reason for discontinuation, the date of premature termination of treatment and of the end of follow up within the patient's source file and the CRF
- In case of suspected CD relapse, care will be taken to eliminate *Clostridium difficile* infection. In those patients without *Clostridium difficile* infection, an ileocolonoscopy will be performed to assess endoscopic activity, as it is the case in the usual care setting. Faecal calprotectin can be proposed to replace ileocolonoscopy in patients who refuse to undergo ileocolonoscopy. If relapse is confirmed (no improvement in endoscopic lesions and/or faecal calprotectin above 150 µg/g), the experimental treatment will be discontinued and a new treatment of CD can be started (steroids, immunosuppressive drug, anti-TNF (Infliximab, Adalimumab) or Vedolizumab) as it is the case in the usual care setting. The patient will still be followed according to the scheduled visits of the study, until week 48, unless he decides to withdraw his/her participation.

The investigator must:

- Document the reason(s)
- Collect the assessment criteria when participation in the research ends, if the subject agrees

5.10.1.2 Criteria and methods for the premature termination of the research

- Patients included can withdraw their participation in the study, at any time and for any reason.
- The investigator can temporarily or permanently end the participation of a patient to the study, for any reason such as safety (occurrence of an adverse event) or for the patient's sake.
- A patient is considered as lost to follow up if the investigator does not know what has happened to her/him. The investigator should make every effort to contact the patient (and trace her/him to his source file) to, at least, know if the patient is alive or dead.

- If a patient leaves the research prematurely, data related to this patient can be used unless an objection was recorded when the subject signed the consent form.
- If consent is withdrawn, no data about the patient can be used, unless the subject writes that he/she does not object. In practice, the subject is excluded from the research.
- The case report form must list the various reasons for ending participation in the research :

✓ Ineffective
✓ Adverse reaction

- ✓ Other medical problem
- ✓ Subject's personal reasons
- ✓ Explicit withdrawal of consent

5.10.2 Follow-up of the subjects after the premature termination of treatment

- After the premature termination of treatment, patients will still be followed according to the scheduled visits of the study, until week 48, unless he decides to withdraw his/her participation. In this latter case, they will be followed for side effects, 30 days after study withdrawal, according to standard criteria of follow up.

If the treatment is stopped prematurely due to a serious adverse event, a serious adverse event notification form will be sent by fax (**33+1 44 84 17 99**) to the sponsor. The serious adverse event will be monitored until it is resolved.

5.10.3 Methods for replacing subjects

In case of study withdrawal, patients will not be replaced.

5.10.4 Terminating part or all of the research

AP-HP as sponsor or the Competent Authority (ANSM) can prematurely terminate all or part of the research, temporarily or permanently, in the following situations:

- firstly, if unexpected serious adverse reactions (SUSARs) are seen in the experimental arm or if there is a discrepancy in the rate of serious adverse reactions between the 2 arms, the benefit-risk ratio of the research will be reassessed.
- Likewise, unexpected facts, new information about the experimental drugs, which show that the objectives of the research are unlikely to be achieved, can lead AP-HP as sponsor or the Competent Authority (ANSM) to prematurely halt the trial.
- AP-HP as the sponsor has the right to permanently suspend inclusions at any time if it appears that the inclusion objectives are not met.

If the research is terminated prematurely, the decision and justification will be given by the sponsor, AP-HP, to the Competent Authority (ANSM) and to the CPP within 15 days.

6. ELIGIBILITY CRITERIA

6.1 Preinclusion and inclusion criteria

Preinclusion criteria

- Age 18 to 80 years
- CD of the ileum, with or without involvement of the caecum or the right colon
- The most severe lesions are confined within the ileum and/or the right colon. This is defined by:
  - segmental CDEIS ≥8 in the ileum and/or right colon;
  - and segmental CDEIS <8 in each of the other segments (transverse, left colon and rectum).
- Informed consent to participate in this study

- CD clinically inactive (CDAI<150) or clinically active but not severe (CDAI<450)
- For patients with clinically active disease (CDAI>150) : Prescription of steroid treatments : Budesonide, Prednisone (or Prednisolone) independently from entry in study

Note: patients with or without previous surgery for CD, with or without CD anal lesions, with or without draining fistulas and setons, can be enrolled.

Inclusion criteria

- Patients with clinically active disease (CDAI>150) who respond to budesonide (initial dose 9 mg/d) or prednisone or prednisolone (initial dose 40 mg/d), by a 70 points decrease in CDAI between the preinclusion and the inclusion visit, or patient with clinically inactive disease (CDAI<150)
- Patients colonized with AIEC on initial ileal biopsies. Testing for AIEC will be performed by phenotypic analysis of the interaction between bacteria and intestinal epithelial cells as well as macrophages by the UMR Inserm/Université d'Auvergne laboratory of Pr Nicolas Barnich in Clermont Ferrand, France. Biopsies of ileal mucosa taken during ileocolonoscopy will be cultured in a selective medium for Enterobacteriaceae. The identification of bacteria will be performed for confirmation of *E. coli* species. Five colonies will be sampled and analysed for their adhesive and invasive properties of I-407 epithelial cells in culture as well as for their survival and multiplication in THP-1 macrophages. AIEC will be defined as *E. coli* able to adhere and invade epithelial cells and to divide within macrophages. As in our previous studies, results will be presented as: AIEC+ or AIEC- (see appendix 8).

6.2 Non-preinclusion and non-inclusion criteria

- Ileal stenosis that cannot be crossed by the endoscope
- Infliximab treatment received less than 8 weeks before preinclusion, in this study
- Methotrexate and azathioprine started less than three months prior to preinclusion
- Adalimumab treatment received less than 4 weeks before preinclusion in this study
- Vedolizumab treatment received less than 8 weeks before preinclusion in the study
- Hypersensitivity to Ciprofloxacin, to other quinolones, or to any of the excipients (cellulose microcrystalline, crospovidone, maize starch, magnesium stearate, silica colloidal anhydrous, hypromellose titanium dioxide E171, macrogol 4000),
- Treatment with tizanidine, Probenecid, Theophylline, Xanthine derivatives, Phenytoin, oral anticoagulants, and Ropinirole
- Hypersensitivity to Rifaximin, or to any excipients (sodium starch glycolate type A, glycerol distearate, colloidal anhydrous silica, talc, microcrystalline cellulose, hypromellose, titanium dioxide, disodium edentate, propylene glycol, red iron oxide E172)
- Previous extensive ileal surgery (≥ 1 meter as measured on the pathology and/or surgical report)
- Short bowel syndrome
- Need for an intestinal resection for fistula, abscess or intestinal obstruction
- Renal failure (creatinine clearance<30 mL/min/1.73m^2)
- Liver failure (V factor<50%)
- Past history of epilepsy
- No health insurance
- Pregnant or lactating women

- Refusal to have double effective contraception, ie Hormonal + barrier method, Intrauterine device + barrier method, double barrier method). Highly effective methods of contraception are: hormonal contraceptives (e.g. combined oral contraceptives, patch, vaginal ring, injectables, and implants); intrauterine device (IUD) or intrauterine system (IUS); vasectomy and tubal ligation. Effective methods are: barrier methods of contraception (e.g. male condom, female condom, cervical cap, diaphragm, contraceptive sponge
- Patients already included in a biomedical research other than an observational study (e.g: registry, cohort)

6.3 Recruitment methods

Patients will be recruited during outpatient consultation or hospitalization.

Number of subjects	
Total number of subjects chosen	62
Number of centres	26
Inclusion period (months)	36
Number of patients/centre	3
Number of patients/centre/month	0.1

7. TREATMENT ADMINISTERED TO RESEARCH PARTICIPANT

7.1 Description of the experimental treatment

This study assesses an association of two antibiotics: Ciprofloxacin and Rifaximin

- Units (sealed cases) including antibiotics or their respective placebo in the amount necessary to cover the treatment protocol will be prepared, labelled within the clinical trial department of AGEPS in accordance with GMP. AGEPS will send the units to the hospital pharmacy of centres.
- Experimental drugs will be stored at ambient temperature.

7.1.1 Experimental medication 1

Ciprofloxacin, 500 mg, has a marketing authorization in several infections (see appendix 9). Ciprofloxacin will be administered orally (tablets) at a dose of 500 mg every 12 hours during 12 weeks.

The dose of Ciprofloxacin can be decreased to 250 mg bid or 250 mg daily, in patients with non-serious adverse events. The dose adjustment will be decided by the investigator during scheduled study visits or additional study visits, if needed.

7.1.2 Experimental medication 2

Rifaximin, 200 mg, does not have a marketing authorization in France. Rifaximin will be provided by Alfa Wassermann, the pharma that manufactures Rifaximin, for the whole

trial. Rifaximin will be administered orally (tablets) at a dose of 800 mg (4 tablets) every 12 hours during 12 weeks.
The dose of Rifaximin can be decreased to 400 mg bid or 200 mg bid, in patients with non-serious adverse events. The dose adjustment will be decided by the investigator during scheduled study visits or additional study visits, if needed.

7.1.3 Experimental medication 3

Placebo **Ciprofloxacin, 500 mg, is manufactured by AGEPS –EPHP (subcontracting).**
Placebo Ciprofloxacin, 500 mg, will be administered orally (tablets) every 12 hours during 12 weeks.

7.1.4 Experimental medication 4

Placebo **Rifaximin, 200 mg, is manufactured by AGEPS–EPHP (subcontracting).**
Placebo Rifaximin, 200 mg, will be administered orally (tablets) every 12 hours during 12 weeks.

7.2 Description of the non-experimental treatment

7.2.1 Non-experimental medication 1

Budesonide treatment during 12 weeks, at an initial dose of 9 mg/d during 8 weeks, then 6 mg/d during 2 weeks, and then 3 mg/d during 2 weeks, as it is usually done in current clinical practice, in accordance with WMA.
In case of clinical relapse occurring between preinclusion and inclusion, the dose of budesonide will be increased to the previous dose during 14 days. Then, the tapering will be started again, according to the abovementioned scheme.

7.2.2 Non-experimental medication 2

Prednisone or prednisolone during 12 weeks, at an initial dose of 40 mg/d during 3 weeks, then 30 mg/d during 10 days and then 20 mg/d during 10 days, then 15 mg/d during 10 days, then 10 mg/d during 10 days, then 7.5 mg/d during 10 days, then 5 mg/d during 10 days, then 2.5 mg/d during 10 days, as it is usually done in current care in accordance with WMA.
In case of clinical relapse occurring between preinclusion and inclusion, the dose of prednisone or prednisolone will be increased to the previous dose during 14 days. Then, the tapering will be started again, according to the abovementioned scheme.

7.3 Description of the traceability elements that accompany the experimental medications
The experimentals products will be prescribed by specific computer prescription (Cleanweb)

They are manufactured and labelled for the research in accordance with a unit number list and GMP.
The products have a peel off label to ensure dispensation traceability, in the hospital pharmacy.
The empty, only partly used or full boxes and blisters will be returned by the patient at the end of research.

7.4 Authorised and prohibited treatments (medicinal, non medicinal, surgical), including rescue medications

Salicylates are authorized all along study protocol. Methotrexate and azathioprine are allowed, provided that they have been started at least three months prior to preinclusion in the study and that they are maintained at a stable dose during the trial. However, dose reductions of these drugs are allowed for safety purpose. In case of confirmed CD relapse, the experimental treatment will be discontinued and a new treatment of CD can be started (steroids, immunosuppressive drug, anti-TNF (Infliximab, Adalimumab) or Vedolizumab) as it is the case in the usual care setting. (see chapter 5.10.1.2).
Patients who have received Infliximab more than 8 weeks before inclusion and patients who have received Adalimumab more than 4 weeks before inclusion may be included. Moreover, Tizanidine, Probenecid, Theophylline, Xanthine derivatives, Phenytoin, oral anticoagulants, and Ropinirole are not authorized because of their incompatibility with Ciprofloxacin. Adequate double contraception will be necessary in women of childbearing age to reduce likelihood of pregnancy during the study.

7.5 Methods for monitoring compliance with the treatment

Once the patient is randomized, the system Cleanweb/CTMS will assign a treatment number. This treatment number will be printed on the prescription; the pharmacist in each site will provide the amount of experimental drugs for the whole trial to the patient.
At each visit, the patient will bring his/her own treatment and the investigator will count the tablets at each visit. This information will be reported on the CRF.
At week 12, the patient will bring the case containing the blisters, either empty, partly empty or full.
These units will be sent back to the centre pharmacy and conserved until count by the CRA and subsequently destroyed after Sponsor authorization.

8. SPECIFIC RESEARCH COMMITTEES

8.1 Steering committee

- Members of the committee: F. Carbonnel (coordinating investigator), A Buisson, N Barnich, S. Chevret (data manager and statistician), H Agostini (Responsible delegate URC Paris Sud), DRCD-Siège project referent, DRCD-URC project referent, Clinical research associate.
- Missions: To monitor patient inclusion and any acute unexpected complication and suggest corrective measures as required
- Operating methods: one conference call or face-to-face meeting every six months.

8.2 Endpoint Adjudication Committee

- Franck Carbonnel and Anthony Buisson will check the criteria of eligibility and study end points, blindly to the randomization arm.

9. SAFETY ASSESSMENT RISKS AND RESTRICTIONS ADDED BY THE RESEARCH

9.1 Procedures in place for recording and reporting adverse events

According to Article R1123-39 of the French Public Health Code and the guideline on good pharmacovigilance practices (EMA, 2012):

- **Adverse event:** Any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment.

- **Adverse drug reaction**
- Any response to a medicinal product which is noxious and unintended.
- **Serious adverse event**
- Any untoward medical occurrence that at any dose results in death, is life-threatening, requires inpatient hospitalisation or prolongation of existing hospitalisation, results in persistent or significant disability/incapacity, or is a congenital anomaly/birth defect.
- **Unexpected adverse reaction**
- An adverse reaction, the nature, severity or outcome of which is not consistent with the applicable product information: the summary of product characteristics (SmPC) for an authorised product or the investigator's brochure for an unauthorised investigational product.

According to the notice to sponsors of clinical trials for medications (ANSM):

- **New safety issue**

Any new information regarding safety:
- that could significantly alter the assessment of the benefit-risk ratio for the experimental medication, or for the trial
- or which could lead to the possibility of altering the administration of the experimental medication or altering the conduct of the trial

Examples

a) Any clinically significant increase in the frequency of an expected serious adverse reaction occurring

b) Suspected unexpected serious adverse reactions (SUSAR) occurring in patients who have finished the trial and about whom the sponsor is notified by the investigator, who also provides any follow-up reports

c) Any new fact relating to the conduct of the clinical trial or the development of the experimental medication, if the new fact is likely to affect participant safety

Examples:

- A serious adverse event likely to be related to the investigations and to the trial's diagnostic procedures and which could modify the conduct of this trial

- A significant risk for the trial participants such as ineffectiveness of the experimental medication used in the trial in treating a life-threatening illness

- Significant safety results from a recently completed research carried out on animals (such as a carcinogenicity research)

- The premature termination, or temporary interruption, of a trial conducted with the same experimental medication in another country, for safety reasons

- An unexpected serious adverse reaction associated with a non-experimental medication required for carrying out the trial, (e.g., challenge agents, rescue treatment)

d) Any unexpected serious adverse reaction reported to the sponsor by another sponsor of a trial carried out in a different country but relating to the same medication

9.1.2 The investigator's roles

9.1.2.1 Regulatory obligations of the investigator (Art R1123-54 of the French Public Health Code)

The investigator must notify the sponsor, **immediately on the day when the investigator becomes aware**, of all the serious adverse events, except those that are listed in the protocol (see. section 9.1.3.1) or in the investigator's brochure as not requiring immediate notification. These serious adverse events are recorded in the "adverse event" section of the case report form and the investigator must immediately notify the sponsor's Vigilance division (see9.1.4).

9.1.2.2 The investigator's other roles

The investigator must document the serious adverse event as thoroughly as possible and provide the medical diagnosis, if possible.

The investigator assesses the severity of the adverse events according to The NCI Common Terminology Criteria for Adverse Events v4.0, This is a descriptive terminology which can be utilized for Adverse Event (AE) reporting. A grading (severity) scale is provided for each AE term. It can be found on the following website: http://ctep.cancer.gov/forms/CTCAEv4.pdf

Due to the well-known and low risk nature of the medications, adverse events graded as Grade 2 or below need not be reported. All other adverse events should continue to be collected on the CRF pages.

The investigator assesses the causal relationship between the serious adverse events and the experimental medication(s) check the study main end points, the procedures added by the research. This causality can be defined as:

- ✓ Definitely: A causal relationship that can only be the result of the investigational medicinal product and there is no other plausible cause of the AE.
- ✓ Probable: A causal relationship is clinically / biologically highly plausible and there is a plausible time sequence between onset of the AE and administration of the investigational medicinal product and there is a reasonable response on withdrawal.
- ✓ Possible: A causal relationship is clinically / biologically plausible and there is a plausible time sequence between onset of the AE and administration of the investigational medicinal product.
- ✓ Unlikely: A causal relation is improbable and another documented cause of the AE is most plausible.
- ✓ Unrelated: A causal relationship can be definitely excluded and another documented cause of the AE is most plausible.

9.1.3 Specific features of protocol

All serious and non-serious adverse events must be reported in the CRF.

9.1.3.1 Serious adverse events that do not require the investigator to immediately notify the sponsor

These serious adverse events are only recorded in the "adverse event" section of the case report form.

- **Normal and natural evolution of the pathology:**

- Hospitalization for routine treatment or monitoring of the disease, not associated with worsening of the patient's condition

- **Special circumstances**

Hospitalizations that do not require immediate notification by the investigator:
- Hospitalization for pre-existing disease
- Hospitalization for medical or surgical treatment before the planned research admission for social or administrative reasons
- Consultation at the emergency ward for less than 12 hours

- **Adverse events likely associated with the treatments prescribed as part of the usual patients' care during the study**

Adverse events related to budesonide or prednisone (or prednisolone) will not be immediately notified to the sponsor but will be documented in the CRF.

9.1.3.2 Serious adverse events that require the investigator to immediately notify the sponsor

Methotrexate and azathioprine are allowed, provided that they have been started at least three months prior to preinclusion.

The investigator must report all adverse events that meet one of the seriousness criteria below, except for events listed in section 9.1.3.1 as not requiring immediate notification:
1- Death
2- Life threatening situation
3- Requiring hospitalisation or prolonging hospitalisation
4- Persistent or significant disability or incapacity
5- Congenital abnormality or birth defect
6- Or any other adverse event considered "medically significant"

❖ For serious adverse events related to the experimental medication(s) and which are expected:
the SmPC for **«Ciprofloxacin »** and SmPC **for « Rifaximin »** , found in Appendix 9, and 10 should be consulted.

❖ The serious adverse events associated with specific research procedures or exams, and which are expected, are:

Main serious adverse events related to the added Ileocolonoscopy at 3 months and the biopsies are: perforation (1/1000), bleeding (exceptional), aspiration pneumonia (low risk; ref 37) infections, and reactions due to general anesthesia.

9.1.3.3 Other events that require the investigator to immediately notify the sponsor

- **Non serious adverse events**

The investigator must notify the sponsor about these "non serious" adverse events, in accordance with the same procedures and deadlines as serious adverse events (see section 10.3.4). These events can be considered "medically significant".

- ***In utero* exposure**

The sponsor must be notified immediately about any pregnancy during which the foetus (from the pre-embryonic stage up to birth) could have been exposed, at a given time, to an experimental medication, even if the pregnancy is not associated with an adverse event.
Notification is required if the exposure involves the mother.

- **Exposure while breastfeeding**

Exposure while breastfeeding occurs if an infant or child could have been exposed to a medication *via* the breast milk of a mother being treated with an experimental medication. Even if such exposure is not associated with an adverse event, the investigator must always notify the sponsor about exposure while breastfeeding as soon as the investigator is aware.

9.1.4 Procedures and deadlines for notifying the sponsor

Notification of an SAE must initially be provided in a written report using the special form for reporting SAE (see Appendix 2). The report must be signed by the investigator.
Each item in the form must be completed by the investigator so that the sponsor can carry out the appropriate analysis.
This initial notification must be followed by one or more detailed follow-up report(s), in writing and signed, within a maximum of 8 days in the case of a fatal or life-threatening event and within 15 days for all other cases.
Whenever possible, the investigator will provide the sponsor with any documents that may be useful (medical reports, laboratory test results, results of additional exams, etc.). These documents must be made anonymous. In addition, the documents must include the following: research acronym, number and initials of the subject, nature and date of the serious adverse event.
Any adverse event will be monitored until fully resolved (stabilisation at a level considered acceptable by the investigator, or return to the previous state) even if the subject has left the trial.

The initial notification, the SAE follow-up reports and all other documents must be sent to the sponsor via fax only to the Vigilance Division of the DRCD, fax No. **01 44 84 17 99**.

The investigator must comply with all requests from the sponsor for additional information.
For all questions relating to the notification of an adverse event, the Vigilance Division of the DRCD can be contacted via email: vigilance.drcd@drc.aphp.fr

***In utero* exposure**

The investigator completes the "form for monitoring a pregnancy that developed during a biomedical research", found in Appendix 3 and sends it by fax to the Vigilance Division at **01 44 84 17 99**.
The investigator must monitor the pregnant woman throughout her pregnancy or until the pregnancy is terminated, and must notify the sponsor of the outcome of the pregnancy, using this form.
If the outcome of the pregnancy falls within the definition of a serious adverse event (miscarriage, pregnancy termination, foetal death, congenital abnormality, etc.), the investigator must follow the procedure for reporting SAE.

If the exposure involves the father, the investigator must obtain the mother's permission before collecting information about the pregnancy.

The initial pregnancy notification, the SAE follow-up reports and all other documents must be sent to the sponsor via fax only to the Vigilance Division - of the DRCD, fax **01 44 84 17 99**

9.1.5 Period for notifying the sponsor

The investigator must report all SAE that occur in research subjects:

- after the date on which the consent was signed
- throughout the period during which the participant is monitored, as determined by the research
- for up to 30 days after the participant stops treatment using the experimental medication
- with no time limit, if the SAE is likely to be due to the experimental medication or to the research procedures (for example, serious reactions that could appear long after exposure to the medication, such as cancers or congenital abnormalities)

9.1.6 The sponsor's roles

The sponsor, represented by its Vigilance Division, continuously assesses the safety of each experimental medication throughout the research.

9.1.6.1 Analysis and declaration of serious adverse events

The sponsor assesses:

- the seriousness of all adverse events reported
- the causal relationship of these events with each experimental medication and/or specific medical procedures/exams added by the research and with other possible treatments
- the expected or unexpected nature of these adverse reactions

All serious adverse events which the investigator and/or the sponsor believe could reasonably have a causal relationship with the experimental medication are considered as suspected adverse reactions.

All suspected unexpected serious adverse reactions (SUSAR) are declared by the sponsor, within the legal time frame, to the Agence Nationale de Sécurité des Médicaments et des Produits de Santé (ANSM, French Health Products Safety Agency) and to the relevant Comité de Protection des Personnes (CPP, ethical committee).

- The initial declaration must be made no later than 7 calendar days after the date on which the serious adverse event occurs in the case of death or of a life-threatening diagnosis.
- The initial declaration must be made no later than 15 calendar days after the date on which the serious adverse event occurs in the case of other serious situations.
- The follow-up declaration must be made no later than 8 days after the 7- or 15-day deadline (depending on the seriousness).

Any suspected unexpected serious adverse reaction must also be declared electronically in the Eudravigilance European database for adverse events due to medications, established by the European Medicines Agency (EMA). The sponsor must notify all relevant investigators about any data that could adversely affect the safety of the research subjects.

Specific case of double-blind trials

As a general rule, the sponsor declares a suspected unexpected serious adverse reaction to the competent authorities and to the CPP after having broken the blind on the experimental medication.
In exceptional situations, and if the ANSM grants permission when requested by the sponsor in the sponsor's clinical trial authorisation application, the methods for unblinding and for declaring suspected unexpected serious adverse reactions can be modified. These methods will then be defined in detail in the research protocol (see section 4.2.6).

9.1.6.2 Analysis and declaration of other safety data

This relates to any safety data or new fact that could significantly alter the assessment of the benefit-risk ratio for the experimental medication, or for the research, or which could lead to the possibility of altering the administration of the experimental medication or altering the conduct of the research.
New facts must be declared to the competent authorities within 15 calendar days of the sponsor becoming aware. Additional relevant information must be sent within an additional 8 days after the 15 day deadline.

9.1.6.3 Annual safety report

Once a year for the duration of the clinical trial, the sponsor must draw up an annual safety report (Development Safety Update Report - DSUR) which includes, in particular:
- An analysis of the safety of the research subjects
- A description of the patients included in the trial (demographic characteristics, etc.)
- A line listing of suspected serious adverse reactions that occurred during the period covered by the report
- A cumulative summary tabulation of serious adverse events that have occurred since the start of the research

The report must be delivered no later than 60 days after the anniversary of the date on which the ANSM authorised the trial.

10. DATA MANAGEMENT

10.1 Data collection methods

Data will be collected on a paper case report form (CRF)

10.2 Identification of data collected directly in the CRFs and that will be considered as source data

All data collected in the CRFs will come from source data.

10.3 Right to access source data and documents

10.3.1 Access to data

In accordance with GCPs:
- the sponsor is responsible for obtaining the permission of all parties involved in the research to guarantee direct access to all locations where the research will be carried out, to the source data, to the source documents and the reports, with the goal of quality control and audit by the sponsor
- the investigators will make available to those in charge of monitoring, quality control and audit relating to the biomedical research the documents and personal data strictly necessary for these

controls, in accordance with the legislative and regulatory provisions in force (Articles L.1121-3 and R.5121-13 of the French Public Health Code)

10.3.2 Source documents

Source documents are defined as any original document or object that can prove the existence or accuracy of a piece of information or a fact recorded during the research. These documents will be kept for 15 years by the investigator or by the hospital in the case of a hospital medical file.

10.3.3 Data confidentiality

Those responsible for biomedical research quality control (Article L.1121-3 of the French Public Health Code) will take all necessary precautions to ensure the confidentiality of information about the experimental medications, the research, the research subjects and in particular the identity of the subjects and the results obtained.
These individuals, as well as the investigators themselves, are subject to professional secrecy (in accordance with the conditions set out in Articles 226-13 and 226-14 of the Penal Code). During or after the biomedical research, the data collected about the research subjects and sent to the sponsor by the investigators (or any other specialised parties) will be made non-identifying. Under no circumstances should the names and addresses of the subjects involved be shown. **Only the initials of first and last names and own research coded number will be recorded**. The sponsor will ensure that each research subject has given permission in writing for access to personal information about him or her which is strictly necessary for the quality control of the research.

10.4 Data processing and storage of documents and data

10.4.1 Identification of the manager and the location(s) for data processing

Quality control and follow-up of the protocol will be made by Clinical Research Unit of Paris Sud. The data management and statistical analysis will be made by the SBIM at the Saint Louis Hospital (APHP).

10.4.2 Data entry

Data will be entered by the SBIM at the Saint Louis Hospital (APHP) by staff dedicated to this task via independent duplicate data entry in forms for collecting anonymised data, then where data handling and storage will be done.

10.4.3 Data processing (CNIL, the French Data Protection Authority) in France

This research falls under the "Méthodologie de référence" (MR-001) according to the provisions of Article 54, paragraph 5 of modified Law No. 78-17 of 6 January 1978 relating to information technology, data files and privacy. This change was approved in a decision made on 5 January 2006. AP-HP, the research sponsor, has signed a commitment to comply with this " Méthodologie de référence ".

10.4.4 Archival

Specific documents for biomedical research relating to a medication for human use will be archived by the investigator and the sponsor for a period of 15 years after the end of the research.

This indexed archival includes, in particular:

- A sealed envelope containing the original copies of all information sheets and consent forms signed for all individuals at the centre that participated in the research for the investigator
- A copy of all the information notes and consent forms signed for all subjects at the centre that participated in the research for the sponsor
- "Research" binders for the Investigator and the sponsor, including:
    - the successive versions of the protocol (identified by the version no. and date), and the appendices
    - the ANSM authorisations and CPP favourable opinions
    - letters of correspondence
    - the inclusion list or register
    - the appendices specific to the research
    - the final research report
- The data collection documents

10.5 Ownership of the data

AP-HP is the owner of the data, which cannot be used or disclosed to a third party without its prior approval.

11. STATISTICAL ASPECTS

11.1 Description of statistical methods to be used including the timetable for the planned interim analyses

An interim analysis will be made once the first half of the planned number of randomized patients has been recruited consecutively into the study. It will be performed in an Intent-to-treat basis to confirm that the rate of endoscopic remission is similar to that expected in this protocol or re-estimate the required sample size, as described below (section 11.2).

The terminal analysis will deal with all the end points, based on all the population has been randomized and followed-up for 12 weeks.
First, a descriptive analysis will be first conducted on the following parameters:

- The characteristics of patients in control and experimental groups
- The estimation of primary and secondary endpoints

Qualitative data will be described in frequency and percentage and will be represented using histograms or diagrams of distribution. Quantitative data will be described using the calculations

of median and inter-quartile range (IQR). The toxicities rate will be calculated and will be given with their 95% confidence intervals (95CI).

Then, all the primary and secondary end points will be compared across randomized arms, with statistical tests and models adapted to the criterion.

- comparison of continuous end points will be based on the non-parametric Wilcoxon rank sum test
- comparison of binary end points will use the chi-square or the exact Fisher test
- comparison of right-censored end points will use the log-rank test, and semi-parametric proportional hazards models unless competing risks where the Gray test will be used together with the cause-specific Cox model

At last, all secondary analyses will be performed

- To measure the correlation between endoscopic remission and AIEC eradication (no AIEC within ileal biopsies) at 12 weeks, as well as the association between remission without steroids, immunosuppressives, anti TNF and surgery and AIEC eradication at 48 week, logistic models will be used, testing any interaction with the treatment arm

11.2 Calculation hypotheses for the number of subjects required and the result

The main hypothesis is that 60% of patients in the experimental group and 25% of patients in the placebo group will exhibit endoscopic remission at week 12. The difference of efficacy between the 2 groups is high and consistent with the hypothesis which states that the role of AIEC is important in Crohn disease.
A total of 31 patients in each arm will be recruited to demonstrate such a difference using a two-tailed test, to control a type I error rate of 0.05, with a statistical power of 80%. The proportion of 25% with endoscopic remission in the placebo group is based upon data from literature (endoscopic remission obtained with budesonide treatment (7)). The anticipated 60% of patients with endoscopic remission in the experimental group is consistent with the efficacy of this association upon AIEC (96 to 99.6% of eradication *in vitro*) as well as with the hypothesized role of AIEC in intestinal inflammation in the subgroup of patients colonized with AIEC.
We expect that roughly 250 pre-screened patients will be needed to obtain the appropriate number of randomized patients, taking into account the rate of responders to budesonide (75% 8, 9) and the prevalence of AIEC colonization in patients with ileal CD (36%).
The trial is based upon an adaptive scheme, allowing the re-estimation of the required number of included patients (« *Sample size re-estimation (SSR) design* »), taking into account the uncertainty of endoscopic remission rate at week 12 in the placebo arm, a nuisance parameter that takes part in the calculation of included patients. Such a re-estimation is justified by the relative imprecision concerning the proportion of responses within the control arm (double placebo).
This parameter is mandatory for the calculation of the number of patients to include, in order to have a sufficient statistical power to detect a clinically meaningful difference or to decide to stop the trial in case of futility/benefit (23, 24). As the treatment effect is not assessed, these adaptive trials are also referred to *« Designs with internal pilot study » (*25).

Nevertheless, if these schemes are based upon the reevaluation of the probability of response in the control arm, this would suppose unblinding, at least for the statistician in charge of the study, since only the results of the control arm will be analyzed. In order to avoid what is contrary to

good clinical practice of the statistical analysis of a randomized trial (26), designs with internal pilot study allowing blinding of randomization have been proposed (27,28, 29).
They are based upon binary judgment criteria on the re-estimation of the overall response probability (and not only the control arm only) as a nuisance parameter.
Data will be analyzed on an intent-to-treat basis: all randomized patients will be analyzed in the arm to which they have been allocated by randomization.
The trial scheme will be divided into three steps:

- **Initial calculation of a provisional sample size**, $2n_0$, from an initial estimation of the proportion of response within the control arm the number of patients to include is given by the following formula :

$$n_0 = 2\frac{\left(z_{1-\alpha/2} + z_{1-\beta}\right)^2}{\Delta^2}\pi(1-\pi)$$

  where π is the assumed likely overall response rate, and Δ *is* the minimal expected difference (clinically interesting) in terms of endoscopic remission at week 12 between the treated and control arms.
- **Re-estimation of the size of the sample size** to include in each arm, $\widehat{N}$, after observation of the responses of a sample of size $n_1 = n_0/2$ first included patients. This reestimation will be based upon the estimated overall proportion of response, obtained by substituting to π its estimate $p = \frac{x_c + x_t}{n_1}$ where $x_c + x_t$ is the total number of observed responses in these n_1 patients, while other quantities are unchanged (32,35-36). The re-estimated sample size can then be expressed as a function of the initial sample size n_0 :$\widehat{N} = n_0\frac{p(1-p)}{\pi(1-\pi)}$. This formula shows that such a computation is independent from the type I and II error rates (25). This re-estimated sample size to be further included in each arm is given by $n_2 = max(n_1, \widehat{N}) - n_1$(« unrestricted design », 23). Of note, this sample size may be limited due to budget or recruitment constraints (25).

- **Terminal analysis** on the $n = n_1 + n_2$ observations, based on the exact Fisher test, similarly to a fixed design (27). Even if there is a theoretical possibility of inflated type I error rate using this re-estimation process based on the reevaluation of the overall proportion of response, blinded to treatment arm, this approach has been shown asymptotically valid without any substantial inflation of the type I error rate I (27,32, 36). A logistic model will allow adjusting treatment comparison on prognostic factors.Search for interactions will be conducted based on the whole sample, testing heterogeneity in treatment effect according to (i) disease location (ileal vs. ileo-caecal), (ii) thiopurins or not, (iii) in remission or not at the time of randomization. Such search will use the Gail et Simon's interaction test (1985).

This procedure will concern the primary endpoint. Secondary endpoints will be analyzed once the whole sample size will be reached.
Percentages of endoscopic and clinical remission will be compared using chi square test. A logistic model will allow adjustment for a therapeutic comparison upon prognostic factors.
Mean variation of CDEIS at 3 months will be compared between the groups using a Wilcoxon rank sum test.

11.3 Specify if subjects who leave the research prematurely will be replaced and in what proportion

No patients will be replaced, according to the ITT principle.

11.4 Level of statistical significance

All the tests will be two-sided, with $p \leq 0.05$ defining statistical significance.

11.5 Method for taking into account missing data

Multiple imputation (MI), which is a popular approach for handling the pervasive problem of missing data in biostatistics, will be used (Rubin 1987). It is usually performed under a missing at random (MAR) assumption. MI by chained equation is to our knowledge the most flexible approach to handle complex patterns of missing data (including categorical data, quantitative data, and survival data) (Little 2002).

11.6 Management of modifications made to the analysis plan for the initial strategy

All modifications will be submitted for approval to the CPP.

11.7 Selection of populations

The interim analysis will be made once the first half of the planned number of randomized patients has been recruited consecutively into the study.
Every patient included in the study will be taken into account in the terminal intent-to-treat analysis.
Note that some secondary analyses (estimating the rate of clinical and biological remission at 48 weeks) will be restricted to patients whose AIEC has or has not been eradicated and in patients who have or have not reached the primary endpoint

11.8 Additional References

- ✓ Gail M, Simon R. Testing for Qualitative Interactions between Treatment Effects and Patient Subsets. Biometrics 1985;41:361-372
- ✓ Rubin. Multiple imputation for nonresponse in surveys. Wiley Online Library 1987.
- ✓ Little R. Statistical Analysis with Missing Data, 2nd edition. Wiley 2002.

12. CONTROL QUALITY AND ASSURANCE

Each biomedical research project managed by AP-HP is ranked from A to D according to the projected risk incurred by research subjects using the classification of biomedical research sponsored by AP-HP.

12.1 General organisation

The sponsor must be responsible for the safety and respect of those subjects who have agreed to participate in the research. The sponsor must implement a quality assurance system to best monitor the conduct of the research in the investigation centres.
For this purpose, the sponsor shall delegate Clinical Research Associates (CRA) whose primary role is to carry out regular follow-up visits at the research locations, after having carried out initial visits.
The objectives of monitoring the research, as defined in the French Good Clinical Practices (BPC section 5.18.1), are to verify that:

- the rights, safety and protection of the research subjects are met
- the data reported is exact, complete and consistent with the source documents
- the research is carried out in accordance with the protocol in force, with the French GCPs and with the legislative and regulatory provisions in force.

12.2 Strategy for opening the centres

The strategy for opening the centres established for this research is determined using the appropriate monitoring plan.

12.3 Level of centre monitoring

In the case of this research, which is considered as C risk, the appropriate monitoring level has been determined based on the complexity, the impact and the budget for the research. Thus, the sponsor and the coordinating investigator have agreed on the logistic score and impact, resulting in a research monitoring level to be implemented: maximal.

12.4 Quality control

A Clinical Research Associate (CRA) appointed by the sponsor will be responsible for the proper conduct of the research, for collecting and documenting, recording and reporting the data generated in writing, in accordance with the Standard Operating Procedures applied within the DRCD and in accordance with the French Good Clinical Practices as well as with the legislative and regulatory provisions in force.
The investigator and the members of the investigator's team agree to make themselves available during Quality Control visits carried out at regular intervals by the Clinical Research Associate. During these visits, the following elements will be reviewed:

- written consent
- compliance with the research protocol and with the procedures defined therein
- quality of the data collected in the case report form: accuracy, missing data, consistency of the data with the "source" documents (medical files, appointment books, original copies of laboratory results, etc.)
- management of the treatments used

12.5 Case Report Form

All information required under the protocol must be entered in the case report forms and an explanation must be given for all missing data. The data must be collected as and when they are obtained, and must be clearly and legibly transcribed.
Erroneous data found in the case report forms will be stricken and the new data will be provided, next to the stricken data, initialled, with the date and, when applicable, with a justification from the investigator or the authorised individual who made the correction.

12.6 Management of non-compliances

Any events that occur as a result of non-compliance, by the investigator or any other individual involved in conducting the research, with the protocol, with the standard operating procedures, with the good clinical practices or with the legislative and regulatory provisions in force must be noted in a declaration of non-compliance addressed to the sponsor. As a first step, major or critical non-compliances will be reviewed and processed by the DRCD's medical coordinator in order to implement the necessary corrective or preventive actions. Next, the non-compliances will be sent to the Quality - Risk Management Division of the DRCD for verification and analysis. These verifications could result in the investigator in charge of the research location in question being asked for information or could lead to compliance or audit visits.

12.7 Audits/inspections

The investigators agree to accept the quality assurance audits carried out by the sponsor as well as the inspections carried out by the competent authorities. All data, documents and reports may be subject to regulatory audits and inspections. Medical secrecy cannot be invoked in opposition to these audits and inspections.

An audit can be carried out at any time by individuals appointed by the sponsor and who are not associated with the research directors. The objective of the audit is to ensure the quality of the research, the validity of the results and compliance with the legislation and regulations in force.
The individuals who lead and monitor the research agree to comply with the sponsor's requirements and with the competent authority regarding research audits or inspections.
The audit may be applicable to all stages of the research, from the development of the protocol to the publication of the results and the organisation of the data used or produced as part of the research.

12.8 Primary investigator's commitment to assume responsibility

Before starting the research, each investigator will give the sponsor’s representative a copy of his/her personal curriculum vitæ, signed and dated, with his/her number in the RPPS (Répertoire Partagé des Professionnels de Santé, Collective Database of Health Professionals).
Each investigator will undertake to comply with the legislation and to carry out the research according to French GCP, adhering to the Declaration of Helsinki terms in force.
The primary investigator at each participating centre will sign a responsibility commitment (standard DRCD document) which will be sent to the sponsor's representative.
The investigators and their employees will sign a delegation of duties form specifying each person's role.

12.9 Pharmacist's commitment to assume responsibility

This study also involves the participation of a pharmacy unit for each recruiting centre.

13. ETHICAL AND LEGAL CONSIDERATIONS

13.1 Methods for obtaining information and consent from research participants

In accordance with Article L1122-1-1 of the French Public Health Code, no biomedical research can be carried out on a person without free and informed consent, obtained in writing after the person has been given the information specified in Article L.1122-1 of said Code.

The subject will be granted a reflection period of a few hours between the time when the subject receives the information and the time when he or she signs the consent form.
The free and informed consent, in writing, of the subject is obtained by the investigator, or by a doctor representing the investigator, before the preinclusion and the inclusion of the subject in the research
The information sheet and a copy of the consent form signed and dated by the research subject and by the investigator or the doctor representing the investigator, are given to the individual prior to his or her participation in the research.
In addition, the investigator will specify in the research participant's medical file the methods used for obtaining his or her consent as well as the methods used for providing information with the goal of obtaining their consent. The investigator will retain the original signed and dated copy of the subject's consent form. To optimize patient recruitment, poster of the study will be published.

13.2 Subject prohibited from participating in another research

There will be no exclusion period defined in the context of the study. The patient cannot take part in another clinical trial on medicinal products as long as he/she will be participating in this trial. However, the patients will may take part in observational studies

13.3 Compensation for subjects

No compensation is anticipated for the patients/control subjects as compensation for the inconveniences relating to the research.

13.4 Legal obligations

13.4.1 The sponsor's role

Assistance Publique - Hôpitaux de Paris (AP-HP) is the sponsor of this research and by delegation, the Clinical Research and Development Department (DRCD) carries out the research's missions in accordance with Article L.1121-1 of the French Public Health Code. Assistance Publique - Hôpitaux de Paris reserves the right to halt the research at any time for medical or administrative reasons. In this case, notification will be sent to the investigator

13.5 Request for an opinion from the Comité de Protection des Personnes (CPP, ethical review board)

AP-HP, as sponsor, obtains for this biomedical research relating to a medication for human use and prior to starting the research, the favourable opinion of the appropriate CPP, within the scope of its authority and in accordance with the legislative and regulatory provisions in force.

13.6 Request for authorisation to ANSM

AP-HP, as sponsor, obtains for this biomedical research relating to a medication for human use and prior to starting the research, authorisation from the ANSM, within the scope of its authority and in accordance with the legislative and regulatory provisions in force.

13.7 Commitment to compliance with the MR 001 "Méthodologie de Reference"

AP-HP, the research sponsor, has signed a commitment to comply with this "Méthodologie de reference".

13.8 Modifications to the research

Any substantial modification to the protocol by the coordinating investigator must be sent to the sponsor for approval. After approval is given, the sponsor must obtain, prior to starting the research, a favourable opinion from the CPP and authorisation from the ANSM within the scope of their respective authorities.
The information sheet and the consent form can be revised if necessary, in particular if there is a substantial modification to the research or if adverse reactions occur.

13.9 Final research report

The final biomedical research report referred to in Article R1123-60 of the French Public Health Code is drawn up and signed by the sponsor and the investigator. A summary of the report written according to the competent authority's reference plan will need to be sent to the competent authority and ethical review board within one year after the end of the research, meaning the end of the participation of the last research subject.

14. FUNDIND AND INSURANCE

14.1 Funding source

The funding sources of this clinical trial are: PHRC (national funds) and HAO (private funds). Private funds will be given by Broad Foundation, Mayoly-Spindler and Alfa Wassermann.

14.2 Insurance

For the duration of the research, the Sponsor will take out an insurance policy covering the sponsor's own civil liability as well as the civil liability of all the doctors involved in carrying out the research. The sponsor will also provide full compensation for all harmful consequences of the research for the research subjects and their beneficiaries, unless the sponsor can prove that the harm is not the fault of the sponsor or any agent. The act of a third party or the voluntary withdrawal of the person who initially consented to participate in the research cannot be invoked against said compensation.

Assistance Publique- Hôpitaux de Paris (AP-HP) has taken out insurance from HDI-GERLING through BIOMEDIC-INSURE for the full research period, covering its own civil liability and that of any agent (doctor or research staff), in accordance with Article L.1121-10 of the French Public Health Code.

15. PUBLICATIONS RULES

It will be mentioned in any paper related to this research that "The sponsor was Assistance Publique – Hôpitaux de Paris (Département de la Recherche Clinique et du Développement, Clinical Research and Development Department).

15.1 Mention of the affiliation of AP-HP for projects sponsored or managed by AP-HP

The first affiliation of the author will be defined by APHP, Hospital, team, city, CP, France.

15.2 Mention of the AP-HP manager (DRCD) in the acknowledgements of the text

"The sponsor was Assistance Publique – Hôpitaux de Paris (Département de la Recherche Clinique et du Développement, Clinical Research and Development Department)"

15.3 Mention of the financier in the acknowledgements of the text

The research was funded by a grant from Programme Hospitalier de Recherche Clinique - PHRC 2014 (Health minister)" and private funds given by Broad Foundation, Mayoli-Spindler and Alphawassermann.
This research has been registered on the website http://clinicaltrials.gov/ under **number *registration number***.

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17. LIST OF APPENDIX

Appendix 1: List of Investigators
Appendix 2: Form for reporting Serious Adverse Events
Appendix 3: Form for monitoring a pregnancy that developed during a biomedical research
Appendix 4: Patient card
Appendix 5: Crohn's Disease Activity Index (CDAI)
Appendix 6: Crohn's Disease Endoscopic Index of Severity (CDEIS)
Appendix 7: Inflammatory Bowel Disease Questionnaire (IBDQ)
Appendix 8: Sampling, conservation and transport of biopsies
Appendix 9: SmPC for Ciprofloxacin
Appendix 10: SmPC for Rifaximin

17.1 Appendix 1: List of Investigators

Nom, Prénom		Lieu de recherche	Code centre
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Moreau, Jacques	Dr	Service de Gastro-Entérologie et nutrition du Pr. escourrou CHU RANGUEIL 1 avenue du Pr Jean Poulhès TSA 50032 31059 TOULOUSE	018
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Yoram Bouhnik	Pr	Service de gastro-entérologie et assistance nutritive du	021

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Peyrin-Biroulet, Laurent	Pr	Service d'Hépato-gastroentérologie, du Pr Jan-Pierre Bronowicki CHU Nancy-Barbois, Rue du Morvan, 54 511 Vandoeuvre-lès-Nancy	025
Nancey, Stéphane	Pr	Service de Médecine Hépato-Gastro-Entérologie CHU de Lyon HCL - GH Sud 165 Chemin du Grand Revoyet 69495 PIERRE-BENITE CEDEX	026

17.2 Appendix 2: Form for reporting Serious Adverse Events

17.3 Appendix 3: Form for monitoring a pregnancy that developed during a biomedical research

17.4 Appendix 4: Patient card

CARTE PATIENT

Merci de garder cette carte en permanence avec vous

Nom :…… Prénom : ……

Je participe à la recherche *: TEOREM*
dont le promoteur est l'Assistance Publique – Hôpitaux de Paris
Je reçois le traitement suivant : **Rifaximine et Ciprofloxacine ou leurs placebos**

A la dose de : 800 mg, 2 fois par jour pendant 12 semaines (Rifaximine**)**

500 mg, 2 fois par jour pendant 12 semaines (Ciprofloxacine)

Date de début de traitement : __ __ /__ __ /__ __ __ __

Traitements reçu n° : …… (Rifaximine or placebo**)**

……(Ciprofloxacine or placebo)
Je suis suivi(e) par le Dr…….
A l'Hôpital ……..
☎……..

En cas de nécessité de connaître votre traitement en urgence, votre médecin peut contacter le Centre Anti-Poison de l'hôpital Fernand Widal, à Paris
☎ : 01 40 05 48 48

17.5 Appendix 5: Crohn's Disease Activity Index (CDAI)

Groupe d'Etude Thérapeutique des Affections Inflammatoires du tube Digestif

GETAID

Date [_ _ / _ _ / _ _] Initiales patient |___|-|___|___|/|___|___|

Recueil du CDAI
(À remplir par le médecin à partir de la fiche d'auto évaluation remplie par le patient la semaine précédant la visite)

Calcul du CDAI : les données sont recueillies sur une semaine à l'aide de la carte journalière remise au patient.

LA FICHE DE RECUEIL ORIGINAL EST UN DOCUMENT SOURCE A GARDER DANS LE DOSSIER DU PATIENT

1. Nombres de selles liquides ou très molles : L ⌋L ⌋L ⌋ ×2 = L ⌋L ⌋L ⌋

2. Douleurs abdominales : L ⌋L ⌋ ×5 = L ⌋L ⌋L ⌋
(0=aucune ; 1=légères ; 2=moyennes ; 3=intenses)

3. Bien être général : L ⌋L ⌋ ×7 = L ⌋L ⌋L ⌋
(0=bon ; 1=moyen ; 2=médiocre ; 3=mauvais ; 4=très mauvais)

4. Autres éléments liés à la maladie : L ⌋ ×20 = L ⌋L ⌋L ⌋
Compter 1 pour chaque catégorie d'éléments présents et souligner l'élément présent :

- arthrite, arthralgie
- iritis, uvéite
- érythème noueux, pyoderma gangrenosum,
- stomatite aphteuse
- fissure, fistule, abcès anal ou péri rectal
- autre fistule
- fièvre>38°C dans la dernière semaine

5. Prise d'anti-diarrhéiques : L ⌋ × 30 = L ⌋L ⌋L ⌋
(0 = non; 1 = oui)

6. Masse abdominale : L ⌋ × 10 = L ⌋L ⌋L ⌋
(0 = absente; 2 = douteuse; 5 = certaine)

7. Hématocrite : Hématocrite = L ⌋L ⌋ %
Ajouter ou soustraire selon le signe : Hommes : 47 – Hte = L ⌋L ⌋ × 6 = L ⌋L ⌋
Femmes : 42 – Hte = L ⌋L ⌋

8. Poids :

$$\frac{\text{Poids théorique}^* \; | \_\_ | \_\_ | - \text{Poids actuel} \; | \_\_ | \_\_ |}{\text{Poids théorique}^* \; L \; ⌋L \; ⌋L \; ⌋} \times 100 = L \; ⌋L \; ⌋ \times 1 = L \; ⌋L \; ⌋$$

TOTAL **CDAI** = L ⌋L ⌋L ⌋

* Les chiffres avec virgule seront arrondis : – au chiffre supérieur si le chiffre après la virgule est ≥ 5
– au chiffre inférieur si le chiffre après la virgule est < 5

CDEIS

1. Estimer la surface occupée par les lésions et les ulcérations en pourcentage pour chaque segment exploré:

		0%	50%	100%
Iléon	lésions	l_______________________________________l		
	ulcérations	l_ l		
Caecum et	lésions	l_______________________________________l		
côlon droit	ulcérations	l_ l		
Transverse	lésions	l_______________________________________l		
	Ulcérations	l_ l		
Sigmoïde et	lésions	l_______________________________________l		
côlon gauche	ulcérations	l_ l		
Rectum	lésions	l_______________________________________1		
	ulcérations	l_ l		

2. Mesurer à l'aide d'un centimètre chacun des segments de droite, reporter les chiffres au niveau des colonnes 4 et 5 du tableau suivant et remplir les colonnes 1 et 2 pour calculer le CDEIS

	Ulcérations creusantes *Noter 12 si présentes*	Ulcérations superficielles *Noter 6 si présentes*	Surface des ulcérations *(0-10 cm)*	Surface des lésions *(0-10 cm)*	Somme
Iléon					
Côlon droit					
Transverse					
Côlon gauche					
Rectum					

TOTAL (somme de toutes les cases) = [_ _]
TOTAL/nombre de segments explorés = [_ _]
+ 3 si sténose ulcérée = [_ _]
et + 3 si sténose non ulcérée = [_ _]

CDEIS : [_ _]

17.7 Appendix 7: Inflammatory Bowel Disease Questionnaire (IBDQ)

Le but de ce questionnaire est de nous permettre de savoir comment vous vous êtes senti(e) au cours des 2 dernières semaines. On vous demande de répondre à des questions sur les symptômes que vous avez eus du fait de votre maladie de Crohn, sur la manière dont vous vous êtes senti(e) en général, ainsi que sur votre moral.

1. Quelle a été la fréquence de vos selles au cours des deux dernières semaines ?
Indiquez la fréquence de vos selles au cours des deux dernières semaines en choisissant l'une des réponses suivantes :
1. SELLES AUSSI FREQUENTES OU PLUS FREQUENTES QUE JAMAIS
2. EXTREMEMENT FREQUENTES
3. TRES FREQUENTES
4. UNE AUGMENTATION MOYENNE DE LA FREQUENCE DES SELLES
5. UNE LEGERE AUGMENTATION DE LA FREQUENCE DES SELLES
6. UNE TRES LEGERE AUGMENTATION DE LA FREQUENCE DES SELLES
7. NORMALES, PAS D'AUGMENTATION DE LA FREQUENCE DES SELLES

2. Au cours des deux dernières semaines, la sensation de fatigue, d'épuisement ou la sensation d'être fourbu(e) vous a-t-elle posé problème ? Veuillez indiquer si cette sensation de fatigue ou d'épuisement a été un problème pour vous au cours des deux dernières semaines en choisissant l'une des réponses suivantes :
1. TOUT LE TEMPS
2. PRESQUE TOUT LE TEMPS
3. ASSEZ SOUVENT
4. PARFOIS
5. RAREMENT
6. TRES RAREMENT
7. JAMAIS

3. Au cours des deux dernières semaines, vous êtes-vous senti(e) frustré(e), agité(e) ou avez vous manqué de patience? Veuillez choisir l'une des réponses suivantes :
1. TOUT LE TEMPS
2. PRESQUE TOUT LE TEMPS
3. ASSEZ SOUVENT
4. PARFOIS
5. RAREMENT
6. TRES RAREMENT
7. JAMAIS

4. Au cours des deux dernières semaines, vos problèmes intestinaux vous ont-il empêché(e) d'aller sur votre lieu d'études ou de travailler ? Veuillez choisir l'une des réponses suivantes:
1. TOUT LE TEMPS
2. PRESQUE TOUT LE TEMPS
3. ASSEZ SOUVENT
4. PARFOIS
5. RAREMENT
6. TRES RAREMENT
7. JAMAIS

5. Au cours des deux dernières semaines, avez-vous eu des selles liquides ?
Veuillez choisir l'une des réponses suivantes :
1. TOUT LE TEMPS
2. PRESQUE TOUT LE TEMPS
3. ASSEZ SOUVENT
4. PARFOIS

5. RAREMENT
6. TRES RAREMENT
7. JAMAIS

6. Au cours des deux dernières semaines, avez-vous eu de l'énergie ?
Veuillez choisir l'une des réponses suivantes
1. PAS D'ENERGIE DU TOUT
2. PRESQUE PAS D'ENERGIE
3. TRES PEU D'ENERGIE
4. UN PEU D'ENERGIE
5. UNE QUANTITE MOYENNE D'ENERGIE
6. BEAUCOUP D'ENERGIE
7. PLEIN(E) D'ENERGIE

7. Au cours des deux dernières semaines, avez-vous été inquiet(e) à l'idée de devoir vous faire opérer un jour à cause de vos problèmes intestinaux ? Veuillez choisir l'une des réponses suivantes :
1. TOUT LE TEMPS
2. PRESQUE TOUT LE TEMPS
3. ASSEZ SOUVENT
4. PARFOIS
5. RAREMENT
6. TRES RAREMENT
7. JAMAIS

8. Au cours des deux dernières semaines, avez-vous dû retarder ou annuler une sortie avec des amis, de la famille, etc. en raison de vos problèmes intestinaux ? Veuillez choisir l'une des réponses suivantes :
1. TOUT LE TEMPS
2. PRESQUE TOUT LE TEMPS
3. ASSEZ SOUVENT
4. PARFOIS
5. RAREMENT
6. TRES RAREMENT
7. JAMAIS

9. Au cours des deux dernières semaines, avez-vous eu des spasmes intestinaux ?
Veuillez choisir l'une des réponses suivantes :
1. TOUT LE TEMPS
2. PRESQUE TOUT LE TEMPS
3. ASSEZ SOUVENT
4. PARFOIS
5. RAREMENT
6. TRES RAREMENT
7. JAMAIS

10. Au cours des deux dernières semaines, vous est-il arrivé(e) de ne pas vous sentir bien d'une manière générale ?
Veuillez choisir l'une des réponses suivantes :
1. TOUT LE TEMPS
2. PRESQUE TOUT LE TEMPS
3. ASSEZ SOUVENT
4. PARFOIS
5. RAREMENT
6. TRES RAREMENT
7. JAMAIS

11. Au cours des deux dernières semaines, avez-vous été gêné(e) par la crainte de ne pas trouver de toilettes ?
Veuillez choisir l'une des réponses suivantes :
1. TOUT LE TEMPS
2. PRESQUE TOUT LE TEMPS
3. ASSEZ SOUVENT
4. PARFOIS
5. RAREMENT
6. TRES RAREMENT
7. JAMAIS

12. Au cours des deux dernières semaines, avez-vous eu des difficultés à pratiquer les activités sportives ou de loisirs que vous auriez aimé faire, à cause de vos problèmes intestinaux ?
Veuillez choisir l'une des réponses suivantes :
1. D'ENORMES DIFFICULTES ; ACTIVITES DEVENUES IMPOSSIBLES
2. BEAUCOUP DE DIFFICULTES
3. PAS MAL DE DIFFICULTES
4. QUELQUES DIFFICULTES
5. PEU DE DIFFICULTES
6. PRESQUE AUCUNE DIFFICULTE
7. PAS DE DIFFICULTE; MES PROBLEMES INTESTINAUX N'ONT PAS LIMITE MES ACTIVITES SPORTIVES OU DE LOISIRS

13. Au cours des deux dernières semaines, avez-vous eu mal au ventre ? Veuillez choisir l'une des réponses suivantes :
1. TOUT LE TEMPS
2. PRESQUE TOUT LE TEMPS
3. ASSEZ SOUVENT
4. PARFOIS
5. RAREMENT
6. TRES RAREMENT
7. JAMAIS

14. Au cours des deux dernières semaines, avez-vous eu des problèmes pour bien dormir ou vous êtes-vous réveillé la nuit ? Veuillez choisir l'une des réponses suivantes :
1. TOUT LE TEMPS
2. PRESQUE TOUT LE TEMPS
3. ASSEZ SOUVENT
4. PARFOIS
5. RAREMENT
6. TRES RAREMENT
7. JAMAIS

15. Au cours des deux dernières semaines, vous êtes-vous senti(e) déprimé(e) ou découragé(e) ?
Veuillez choisir l'une des réponses suivantes :
1. TOUT LE TEMPS
2. PRESQUE TOUT LE TEMPS
3. ASSEZ SOUVENT
4. PARFOIS
5. RAREMENT
6. TRES RAREMENT
7. JAMAIS

16. Au cours des deux dernières semaines, avez-vous dû éviter de sortir dans des endroits où il n'y a pas de toilettes
à proximité ? Veuillez choisir l'une des réponses suivantes :
1. TOUT LE TEMPS
2. PRESQUE TOUT LE TEMPS
3. ASSEZ SOUVENT
4. PARFOIS
5. RAREMENT
6. TRES RAREMENT
7. JAMAIS

17. Globalement, au cours des deux dernières semaines, le fait d'évacuer beaucoup de gaz intestinaux a-t-il été un problème pour vous ? Veuillez choisir l'une des réponses suivantes :
1. UN PROBLEME ENORME
2. UN GROS PROBLEME
3. UN PROBLEME IMPORTANT
4. UN PROBLEME MOYENNEMENT IMPORTANT
5. UN LEGER PROBLEME
6. PRESQUE PAS UN PROBLEME
7. PAS UN PROBLEME

18. Globalement, au cours des deux dernières semaines, le fait de maintenir ou d'atteindre le poids que vous souhaitez avoir a-t-il été un problème pour vous ? Veuillez choisir l'une des réponses suivantes :
1. UN PROBLEME ENORME
2. UN GROS PROBLEME
3. UN PROBLEME IMPORTANT
4. UN PROBLEME MOYENNEMENT IMPORTANT
5. UN LEGER PROBLEME
6. PRESQUE PAS UN PROBLEME
7. PAS UN PROBLEME

19. Beaucoup de patients ayant des problèmes intestinaux ressentent souvent de l'inquiétude et de l'angoisse à propos de leur maladie. Cette inquiétude peut être la crainte d'avoir un jour un cancer, de ne jamais se sentir mieux ou de faire une rechute. Globalement, au cours des deux dernières semaines, vous êtes-vous senti(e) inquiet (ète) ou anxieux (se) ? Veuillez choisir l'une des réponses suivantes :
1. TOUT LE TEMPS
2. PRESQUE TOUT LE TEMPS
3. ASSEZ SOUVENT
4. PARFOIS
5. RAREMENT
6. TRES RAREMENT
7. JAMAIS

20. Au cours des deux dernières semaines, avez-vous eu des ballonnements intestinaux ?
Veuillez choisir l'une des réponses suivantes :
1. TOUT LE TEMPS
2. PRESQUE TOUT LE TEMPS
3. ASSEZ SOUVENT
4. PARFOIS
5. RAREMENT
6. TRES RAREMENT
7. JAMAIS

21. Au cours des deux dernières semaines, vous êtes-vous senti(e) détendu(e) et décontracté(e) ?
Veuillez choisir l'une des réponses suivantes :
7. TOUT LE TEMPS
6. PRESQUE TOUT LE TEMPS
5. ASSEZ SOUVENT
4. PARFOIS
3. RAREMENT
2. TRES RAREMENT
1. JAMAIS

22. Au cours des deux dernières semaines, avez-vous remarqué qu'il y avait du sang dans vos selles ?
Veuillez choisir l'une des réponses suivantes :
1. TOUT LE TEMPS
2. PRESQUE TOUT LE TEMPS
3. ASSEZ SOUVENT 4. PARFOIS
5. RAREMENT
6. TRES RAREMENT
7. JAMAIS

23. Au cours des deux dernières semaines, vous êtes-vous senti(e) embarrassé(e) à cause de vos problèmes intestinaux ? Veuillez choisir l'une des réponses suivantes :
1. TOUT LE TEMPS
2. PRESQUE TOUT LE TEMPS
3. ASSEZ SOUVENT
4. PARFOIS
5. RAREMENT
6. TRES RAREMENT
7. JAMAIS

24. Au cours des deux dernières semaines, avez-vous été gêné(e) par la sensation d'avoir à aller aux toilettes alors que vos intestins étaient vides ? Veuillez choisir l'une des réponses suivantes :
1. TOUT LE TEMPS
2. PRESQUE TOUT LE TEMPS
3. ASSEZ SOUVENT
4. PARFOIS
5. RAREMENT
6. TRES RAREMENT
7. JAMAIS

25. Au cours des 2 dernières semaines, vous êtes-vous senti(e) perturbé(e) ou sur le point de pleurer ?
Veuillez choisir l'une des réponses suivantes:
1. TOUT LE TEMPS
2. PRESQUE TOUT LE TEMPS
3. ASSEZ SOUVENT
4. PARFOIS
5. RAREMENT
6. TRES RAREMENT
7. JAMAIS

26. Au cours des 2 dernières semaines, avez-vous été gêné(e) parce que vous aviez tâché vos sous-vêtements ?
Veuillez choisir l'une des réponses suivantes:
1. TOUT LE TEMPS

2. PRESQUE TOUT LE TEMPS
3. ASSEZ SOUVENT
4. PARFOIS
5. RAREMENT
6. TRES RAREMENT
7. JAMAIS

27. Au cours des 2 dernières semaines, avez-vous éprouvé du ressentiment à cause de vos problèmes intestinaux ?
Veuillez choisir l'une des réponses suivantes:
1. TOUT LE TEMPS
2. PRESQUE TOUT LE TEMPS
3. ASSEZ SOUVENT
4. PARFOIS
5. RAREMENT
6. TRES RAREMENT
7. JAMAIS

28. Au cours des 2 dernières semaines, vos problèmes intestinaux ont-ils limité votre activité sexuelle ?
Veuillez choisir l'une des réponses suivantes:
1. PAS D'ACTIVITE SEXUELLE EN RAISON DE MES PROBLEMES INTESTINAUX
2. LIMITATION IMPORTANTE EN RAISON DE MES PROBLEMES INTESTINAUX
3. LIMITATION MOYENNE EN RAISON DE MES PROBLEMES INTESTINAUX
4. LIMITATION LEGERE EN RAISON DE MES PROBLEMES INTESTINAUX
5. LIMITATION TRES LEGERE EN RAISON DE MES PROBLEMES INTESTINAUX
6. PRESQUE PAS DE LIMITATION EN RAISON DE MES PROBLEMES INTESTINAUX
7. AUCUNE LIMITATION EN RAISON DE MES PROBLEMES INTESTINAUX
29. Au cours des 2 dernières semaines, avez-vous eu des nausées ou avez-vous eu envie de vomir ?
Veuillez choisir l'une des réponses suivantes :
1. TOUT LE TEMPS
2. PRESQUE TOUT LE TEMPS
3. ASSEZ SOUVENT
4. PARFOIS
5. RAREMENT
6. TRES RAREMENT
7. JAMAIS

30. Au cours des 2 dernières semaines, vous êtes-vous senti(e) irritable ?
Veuillez choisir l'une des réponses suivantes :
1. TOUT LE TEMPS
2. PRESQUE TOUT LE TEMPS
3. ASSEZ SOUVENT
4. PARFOIS
5. RAREMENT
6. TRES RAREMENT
7. JAMAIS

31. Au cours des 2 dernières semaines, avez-vous ressenti un manque de compréhension de la part des autres ?
Veuillez choisir l'une des réponses suivantes :
1. TOUT LE TEMPS
2. PRESQUE TOUT LE TEMPS
3. ASSEZ SOUVENT
4. PARFOIS

5. RAREMENT
6. TRES RAREMENT
7. JAMAIS

32. Au cours des 2 dernières semaines, avez-vous été satisfait(e), heureux(se) ou content(e) de votre vie personnelle ? Veuillez choisir l'une des réponses suivantes:
1. TRES INSATISFAIT(E), MALHEUREUX(SE) LA PLUPART DU TEMPS
2. GENERALEMENT INSATISFAIT(E), MALHEUREUX(SE)
3. UN PEU INSATISFAIT(E), MALHEUREUX(SE)
4. GENERALEMENT SATISFAIT(E), CONTENT(E)
5. SATISFAIT(E) LA PLUPART DU TEMPS, HEUREUX(SE)
6. TRES SATISFAIT(E) LA PLUPART DU TEMPS, HEUREUX(SE)
7. EXTREMEMENT SATISFAIT(E), JE N'AURAIS PAS PU ETRE PLUS HEUREUX(SE) OU CONTENT(E)

17.8 Appendix 8: Sampling, conservation and transport of biopsies

Four ileal biopsies will be performed. Two ileal biopsies will be for the AIEC search and two ileal biopsies will be for the biological collection. AIEC isolation will be performed in a tube n°1, a second biopsy will be placed in tube n°2 to quantitate the number of AIEC within the biopsy using PCR. Two other biopsies will be placed in tube tube n°3 to look for CEACAM6 overexpression.
These biopsies will be transported by the TSE firm to the laboratoire de Pathogénie Bactérienne Intestinale, CBRV, 28 place Henri Dunant, 63000 Clermont-Ferrand.
The results will be sent to the investigator by mail no later than 30 days after the receipt of the biopsies.

Biopsy analyses

- *OVEREXPRESSION OF CEACAM6*

Biopsies will be embedded in paraffin and cut. CEACAM6 overexpression will be performed using immunohistochemistry using murine anti-CEACAM6 monoclonal antibodies (Genovac, clone 9A6). Results will be distributed in 4 groups : negative (-) to strongly positive (+++) : (-) negative staining of epithelial cells, (+) weakly positive, (++) moderately positive and (+++) strongly positive.

- *AIEC COLONIZATION*

AIEC search will be performed using bacteriological phenotypic and molecular analysis of the interaction between bacteria and intestinal epithelial cells and macrophages.
From the PBS sample, a culture on a selective milieu for Enterobactericae (Drigalski) will be performed using the dilution method to quantitate the number of bacteria colonizing the mucosa within the biopsy. A bacterial identification will be realized to confirm E. coli specie. 5 colonies will be analysed to test for adhesion and invasiveness of epithelial cells I-407 in culture, as described previously (Boudeau et al, MolecularMicrobiology, 2001) as well as survival and multiplication within macrophages (Glasser et al, Infection and Immunity). AIEC adhere and invade epithelial cells and multiply within macrophages.
DNA will be extracted from the trizol tube and a quantification of E coli-associated with mucosa will be performed using real-time PCR with probes specific for E. coli and for AIEC using probes specific for AIEC virulence genes (fimH, lpfA, gipA, waaW).

- *CEACAM 6*

From AFA biopsies, an immunohistochemical study will be performed to study the expression of CEACAM 6 (carcinoembryonic antigen-related cell adhesion molecule) which is a glycosylated receptor which binds type 1 pili expressed at the surface of AIEC. Overexpression of CEACAM6 identified using immunohistochemistry within ileal mucosa defines a group at high risk for AIEC

colonization. AIEC+ patients will be those colonized by AIEC, with or without overexpression of CEACAM 6. The results of initial analysis of biopsies, will be send to the investigator within 30 days by e-mail.

17.9 Appendix 9: SmPC for Ciprofloxacin

http://agence-prd.ansm.sante.fr/php/ecodex/frames.php?specid=62450285&typedoc=R&ref=R0252314.htm

17.10 Appendix 10: SmPC for Rifaximin

http://www.fda.gov/downloads/advisorycommittees/committeesmeetingmaterials/drugs/gastrointestinaldrugsadvisorycommittee/ucm279646.pdf