



Dipartimento di Medicina Interna

U.O.C. Gastroenterologia ed Endoscopia - Direttore: Prof. Maurizio Vecchi

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Efficacy of Lactobacillus Rhamnosus GG add-on therapy in mild-to-moderate Ulcerative Colitis

LaRhGG-UC

Versione 1.0 del 26/07/2021

Promotore: Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico,
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Centro coordinatore: U.O. Gastroenterologia ed Endoscopia
Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico,

Sperimentatore Principale: Prof. Maurizio Vecchi

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Flowchart

	Screening	Baseline/randomization	Induction	Maintenance			Follow-up
Study activity	Visit 1 (week - 2)	Visit 2 (week 0)	Visit 3 (week 4)	Visit 4* (Week 8)	Visit 5 * (Week 12)	Visit 6 (Week 16)	Visit 7 (Week 24)
Informed consent	X						
Inclusion/exclusion criteria	X						
Demographic and medical history	X						
Prior/concomitant medication	X						
Randomization		X	X				
Physical exam	X	X	X			X	X
Blood pressure and pulse	X	X	X			X	X
Weight	X	X	X			X	X
Height	X	X	X			X	X
Temperature	X	X	X			X	X
Partial Mayo Score	X	X	X	X	X	X	X
Stool sample for fecal calprotectin	X		X			X	X
Blood exams	X		X			X	X
Quality of life questionnaire		X	X			X	X



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(IBD-Q, SF-36)							
FACIT-F questionnaire		X	X			X	X
IBS-SSS questionnaire		X	X			X	X
Collection of blood and fecal sample	X		X			X	X
Dispensation of study treatment		X	X				

Abbreviation list

IBD: Inflammatory Bowel Disease

UC: Ulcerative colitis

CD: Crohn's disease

RCT: Randomized clinical trial

IL-10: Interleukin-10

APC: Antigen presenting cell

LGG: Lactobacillus Rhamnosus GG

WMS: Whole metagenome shotgun



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Responsibility (promoter and investigator role)

*Principal investigator: Prof. Maurizio Vecchi: - Head of Gastroenterology and Endoscopy Unit -
Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico.*

Internal collaborations

<i>Unit</i>	<i>Name</i>	<i>role</i>
<i>Gastroenterology and Endoscopy Unit</i>	<i>Prof. Flavio Caprioli</i>	<i>investigator</i>
<i>Gastroenterology and Endoscopy Unit</i>	<i>Dott. Francesco Conforti</i>	<i>investigator</i>
<i>Gastroenterology and endoscopy unit</i>	<i>Dr.ssa Nicole Piazza O Sed</i>	<i>investigator</i>

External collaboration

<i>Center</i>	<i>Name</i>	<i>Role</i>
<i>Istituto Europeo di Oncologia</i>	<i>dott.ssa Federica Facciotti</i>	<i>Investigator</i>



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Background

In the last years, the role of microbiome in the pathogenesis and in the course of Inflammatory Bowel Diseases (IBD) – namely Ulcerative Colitis (UC) and Crohn's disease (CD) - was highlighted by



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several observations in animal models and human subjects.(1) Many changes in microbiome was seen in IBD patients compared to healthy individuals,(2) often leading to a condition of dysbiosis, which is characterized by loss of beneficial bacteria, overgrowth of potentially pathogenic bacteria and loss of bacterial diversity. (3)

Thus, this condition could contribute to pathogenesis of IBD by increasing host pro-inflammatory immune responses, decreasing of energy source in epithelial cell growth and differentiation, alteration of regulatory T cell differentiation, degradation of mucus and changes in mucosal permeability. (4)

As a consequence, modulation of the gut microbial ecologies in IBD patients has been proposed as an efficient tool to restore an health-promoting microbiota, thus reducing the inflammatory trigger for the intestinal immune system.

Several therapeutic strategies have been proposed to modulate gut microbiota in IBD. Antibiotics, probiotics and fecal microbiota transplantation are currently the most considered treatment approaches.(1,5)

Probiotics are defined as living microorganism that exert beneficial effects on the host.(6) The efficacy in IBD has been evaluated for many probiotics. It has been observed that some bacteria species, such as *Lactobacillus*, *Bifidobacterium* and *Enterococcus* have a clinical effect on gastrointestinal inflammation.(7) VSL#3 - a highly concentrated probiotic supplement (450 billion bacteria/sachet) containing eight different probiotic strains - has shown efficacy in obtaining clinical remission in active UC patients compared to placebo,(8-9) and this findings was confirmed by a recent meta-analysis.(10) VSL#3 was found to be superior to placebo in maintaining remission in patients with acute pouchitis treated with antibiotics and in the primary prevention of acute pouchitis.(11-12) RCTs have shown an efficacy comparable to salicylate of *E.Coli* Nissle 1917, *S.Boulardii*, *B.Breve* and *B.Bifidum* in maintaining remission of UC.(13) Data on the efficacy of probiotics in induction and maintenance of the remission in CD are limited, nevertheless in this setting probiotics failed to show a significant better efficacy compared to placebo.(14)

Fecal microbiota transplantation is a therapeutic procedure of transplanting normal fecal microbiota obtained from healthy donors in order to treat patients' dysbiosis. Some studies have shown a promising role in UC, even if to date evidence are scarce and findings are often conflicting. (15)

Although there is a lack of large well-designed clinical trial and of strong evidence of probiotics' efficacy in IBD, these findings are promising and suggest an important role of modulating-microbiome treatment approaches, especially in UC.

Furthermore, up to 30% of patients with UC may suffer from IBS-like symptoms, such as bloating, constipation, recurrent diarrhoea.(16) These symptoms often do not reflect inflammatory activity but they are associated with low quality of life. Considering the efficacy of probiotics in treatment



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of IBS and IBS-like symptoms, probiotics could be a possible therapeutic option for the management of these patients.

Lactobacillus Rhamnosus GG is a bacterial strain belonging to the genus Lactobacillus. It is able to survive in acid pH and in medium containing bile and to adhere to enterocytes.

The health-promoting role of Lactobacilli, including Lactobacillus GG, has been largely demonstrated, leading to the hypothesis of administering it also to IBD patients to counterbalance the observed microbial dysbiosis. A recent study has demonstrated that Lactobacillus promotes the release of anti-inflammatory IL10 by APC and T cells, which is pivotal for the resolution of intestinal inflammation by reducing the proliferative capacity of proinflammatory T cell in murine models of ulcerative colitis (18-19). Upon transfer of a lactobacilli-enriched microbiota, different mucosal immune populations produce IL10 in colitic animals, concurring to the resolution of inflammation. Moreover, the exposure of lamina propria cells isolated from active ulcerative colitis patients to lactobacilli enriched- faecal water promoted the secretion of IL10 (20), suggesting a direct role of metabolic products from lactobacilli in the functional shaping of pro-inflammatory immune cells. Altogether these data support the hypothesis that a lactobacillus-enriched microenvironment favors a better control of colitis severity and inflammation by directly acting on the functional status of mucosal immune cells.

However, it is presently not known which and how Lactobacilli and its metabolites can influence the phenotype and function of pro-inflammatory mucosal immune cells , and how stable is this interaction over time upon supplementation.

Indeed, to date only few studies have been performed to evaluate efficacy of LGG in IBD.

An open label trial was performed by Zocco et al. to assess efficacy of LGG in maintenance of remission in UC. (21) 187 patients were randomized to receive LGG, mesalazine 2.4g/d and LGG + mesalazine for 12 months, overall analysis showed no significant differences among the three treatment groups. However the treatment with LGG appeared to be more effective than standard treatment in prolonging the relapse-free time.

A double-blind trial was performed by Prantera et al. in CD patients to assess efficacy of LGG compared to placebo in preventing post-operative recurrence.(22) No benefit compared to placebo was shown neither in clinical recurrence rate nor endoscopic recurrence rate. These findings could be explained by the ileal location of the disease in most patients. In fact, LGG is able to colonize colonic mucosa while there are inconsistent data on the adhesion to ileal mucosa. (23)

Considering the potential beneficial effect on colonic inflammation and IBS-like symptoms we aim to explore the efficacy of Lactobacillus Rhamnosus GG in UC patients with mild-to-moderate symptoms, in addition to treatment with mesalamine compounds. By integrating gut microbiome,



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immunological and clinical data we aim at identifying lactobacillus GG-associated metabolic signatures capable to shape the function of pro-inflammatory mucosal immune cells.

Study design

Single-centre, randomized, double-blind, placebo-controlled, two phases-integrated trial

Objectives and outcomes

Primary objective

The primary objective of the study is to evaluate the efficacy of Lactobacillus Rhamnosus GG on health-related quality of life, in addition to standard therapy, in UC patients with mild-to-moderate symptoms.

Secondary objectives

Secondary objectives are to evaluate clinical efficacy of LGG, variation in fecal calprotectin and IBS-like symptoms in patients with mild-to-moderate ulcerative colitis. We aim also to evaluate the effects of Lactobacillus GG-derived metabolites on the mucosal immune system modulation in UC patients.

Induction phase:

The primary outcome will be:

- ☐ the mean change of IBD-related quality of life (IBDQ) from Week 0 to Week 4

The secondary outcomes:

- ☐ Clinical remission rate (defined as partial Mayo Score ≤ 2) at Week 4
- ☐ Clinical response rate (defined as a reduction > 2 point of partial Mayo Score and decrease of ≥ 1 point in rectal bleeding subscore or absolute rectal bleeding subscore ≤ 1) at Week 4
- ☐ the mean change of IBS-SSS from week 0 to week 4
- ☐ the mean change of SF-36 from week 0 to week 4
- ☐ the mean change of FACIT-F from week 0 to week 4
- ☐ the mean change of Fecal Calprotectin from week 0 to week 4
- ☐ Change in gut microbiome composition, microbial community structure, inflammatory status and fecal metabolome from week 0 to week 4

Maintenance phase:

The primary outcome of the study will be:

- ☐ the mean change of IBD-related quality of life (IBDQ) from Week 4 to Week 16 and Week 24



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The secondary outcomes:

- ☐ Clinical remission rate (defined as partial Mayo Score ≤ 2) at Week 16 and week 24
- ☒ Clinical response rate (defined as a reduction > 2 point of partial Mayo Score and decrease of ≥ 1 point in rectal bleeding subscore or absolute rectal bleeding subscore ≤ 1) at Week 16 and week 24
- ☐ Clinical relapse rate (defined as partial Mayo Score > 5) at Week 16 and Week 24
- ☐ the mean change of clinical activity index (partial Mayo Score) from Week 4 to Week 16 and Week 24
- ☐ the mean change of IBS-SSS from week 4 to week 16 and 24
- ☐ the mean change of FACIT-F from week 4 to week 16 and 24
- ☐ the mean change of SF-36 from week 4 to week 16 and 24
- ☐ the mean change of Fecal Calprotectin from week 4 to week 16 and 24
- ☐ Change in gut microbiome composition, microbial community structure, inflammatory status and fecal metabolome from week 4 to week 16 and 24

Inclusion and exclusion criteria

Inclusion criteria

Subjects older than 18 years of age at the inclusion

Able to understand the study procedures and sign the informed consent form

Established diagnosis of ulcerative colitis at least 3 months before enrolment

Mildly-to-moderately, clinically active UC defined as a baseline Partial Mayo Score of 3 to 7 and rectal bleeding subscore ≥ 1 at baseline

Exclusion criteria

Severely clinically active UC defined as a Partial Mayo score > 7

Use of probiotic and/or systemic antibiotic therapy in the last 4 weeks.

Pregnancy and lactation

History of severe renal, hepatic, cardiac or metabolic disease

Unable to conform to study protocol

Concomitant therapy allowed (in accordance to RCP)

Oral Mesalamine

Mesalamine enema

Concomitant therapy not allowed



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- ☐ Oral, parenteral or rectal corticosteroids, including budesonide and beclomethasone dipropionate.
- ☐ Immunosuppressants such as 6-MP, AZA or MTX
- ☐ Tofacitinib
- ☐ Anti-TNF agents (Infliximab, Adalimumab, Golimumab)
- ☐ Vedolizumab
- ☐ Systemic antibiotic therapy
- ☒ Probiotics

Study procedures

At the screening visit (Week -2) patients will sign informed consent and will be evaluated according to inclusion and exclusion criteria. Blood exams and assay of fecal calprotectin will be performed.

The eligible patients after enrollment will be randomized with 1:1 ratio to receive LGG or placebo, one sachet twice daily for the 4-weeks induction treatment period (from week 0 to week 4), in addition to conventional therapy with mesalamine compounds prescribed according to current guidelines and to usual clinical practice.

The patients who achieve clinical response at week 4 (defined as a reduction ≥ 2 point of partial Mayo Score and decrease of ≥ 1 point in rectal bleeding subscore or absolute rectal bleeding subscore ≤ 1) will be included in the maintenance phase and will be re-randomized with 1:1 ratio to receive LGG or placebo, one sachet once daily for the 12-weeks maintenance treatment period (from week 4 to week 16), in addition to conventional therapy with mesalamine compounds prescribed according to current guidelines and to usual clinical practice.

Stool and blood sampling will be performed at week -2, week 4, week 16 and week 24 to evaluate the microbial community structure, metabolic shifts and inflammatory status before and after modulation of gut microbiota composition after lactobacillus supplementation.

Faecal samples will be analysed by using the cost-effective shallow whole metagenome shotgun (WMS) to obtain species-resolved metagenomics data and microbiome functional profiles. The faecal metabolome will be analysed as well to decipher how the functional output of combined host and UC microbiota interactions promotes inflammation. Serum levels of pro-inflammatory and anti-inflammatory cytokines will be analysed by using ELISA assays.

Metabolites-containing faecal waters obtained from the longitudinal stool samples from lactobacillus GG treated or untreated UC patients will be used in *in vitro* experiments to modulate the functional profile of ex-vivo isolated LPMC. At the end of the experiment, cells will be analysed



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with flow cytometry (to evaluate Th1-Th2-Th17-Treg frequency and phenotype) and supernatants will be collected for ELISA assays to evaluate IFN-gamma, IL13, IL17, IL4, IL10 concentrations.

Stool and blood samples will be stored at the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico of Milan Biobank, under the responsibility of the Biobank director.

Fecal calprotectin will be measured at week -2, 4, 16 and 24.

Clinical assessment of UC symptoms during the study will also be performed.

Ulcerative colitis clinical activity will be evaluated according to Partial Mayo Score at week -2, 0, 4, 8, 12, 16 and 24. Visit at week 8 and at week 12 could be performed by phone call.

The questionnaire IBDQ and SF-36 will be administered at week 0, 4, 16 and 24 to evaluate health-related quality of life. The IBS-SSS will be administered at week 0, 4, 16 and 24 to evaluate IBS-like severity symptoms. The questionnaire FACIT-F will be administered at week 0, 4, 16 and 24 to evaluate fatigue.

Randomization and blindness

The patients will be randomized with a 1:1 ratio, with a block randomization method in order to balance the number of patients among treatment groups. A random number generator will be used to assign any patient to a treatment. Investigator, sub-investigators and patients will be blinded to the treatment assigned. We will appoint unblind research staff members, who will deliver to the patients the treatment assigned according to the randomization.

Study duration

Study enrollment will be completed in 12 months. Study duration will be completed in approximately 21 months.

For each patient the duration of the study will be of 26 weeks, articulated as follows:

- ☐ 2 weeks run-in
- ☐ 4 weeks induction treatment period
- ☐ 12 weeks maintenance treatment period
- ☐ 8 weeks follow up post treatment

Statistical analysis and sample size calculation



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A descriptive analysis will be performed on the collected data. The categorical variables will be described as absolute frequency and percentage. The continuous variables with normal distribution will be described as mean and standard deviation (SD), whereas the continuous variables without normal distribution will be given as median and range. The D'Agostino-Pearson omnibus normality test will be used to check for normality of continuous variables. Comparisons between treatment groups will be performed by Mann-Whitney test or Student's t-test for continuous variables, and by Chi-square or Fisher's exact tests for categorical variables. P-values less than 0.05 will be considered to be statistically significant.

According to a recent meta-analysis (24), we assumed the mean difference in IBD-Q score in the placebo arm were 17.5 ($\pm$ 5) for the induction phase and 27.5 ($\pm$ 9) for the maintenance phase. Therefore, we estimate that to find a difference of 30% between two groups with statistically significant difference ($p < 0.05$), a sample size of 40 patients (20 for each arm) will be necessary to obtain a study power of 80%.

Adverse events

The project does not involve the administration of drugs or other substances or invasive clinical practices. No adverse events are therefore expected.

Ethical considerations

The study will be performed after the approval by the local Ethic Committee. The Ethical Committee must also approve any modification to the protocol and the advertising used to recruit subjects for the study, according to local regulations.

The study will be conducted in accordance with the Standards of Good Clinical Practice and with the ethical principles deriving from the Helsinki Declaration.

Each patient will be informed about the aims and procedures of the study by the investigator or sub-investigators and will sign an informed consent. Each patient will sign an informed consent to the collection and use of biological samples for the current study and for future studies.

Duties of the Investigator

In accordance with applicable local regulations, the investigator must send periodic reports regarding the progress of the study in his center to the EC and notify the EC of the closure of the study. Periodic reporting and termination notification are part of the investigator's responsibilities.

Risk/benefit considerations



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No significant adverse events are expected with the use of *Lactobacillus Rhamnosus GG* supplementation. Any adverse events will occur during the study will be recorded and will be graded according to CTCAE classification which is widely used in clinical trials. We will be consider as serious adverse events (SAE) any adverse event leading to death, life-threatening condition or hospitalization. In case of occurrence of a SAE patients will be discontinued from the study. We hypotize beneficial effect for patients in the treatment arm on disease related quality of life and symptoms, however the patients in placebo arm will be treated with standard therapy for their disease.

Data management and study quality assurance

Data entry and data check will be performed by investigator, sub-investigator or appointee data manager. All data will be recorded in an electronic CRF.

In this study an independent data monitoring committee is not provided, such as this trial is of short duration, known risks are minimal and patients with severe disease or life-threatening conditions will not be included.

As promoter of the study, Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico may perform, at any moment and at its discretion, a quality control of the study. In this case investigator must consent access to any documentation and data of the study.

Outcome measures

Partial Mayo score will be used to evaluate clinical activity. The Partial Mayo Score (PMS) considers the three clinical parameters (stool frequency, rectal bleeding and Physician's global assessment) of the full Mayo score, with a score for each of these parameters ranging between 0 and 3, according to the clinical evaluation. Partial Mayo score demonstrated a good correlation with Global Mayo score in defining disease activity. (25)

The Inflammatory Bowel Disease Questionnaire (IBDQ) is a tool to evaluate disease-specific quality of life, widely used in randomized clinical trials for Inflammatory bowel disease. The IBDQ considers the patient's experience of IBD on 4 domains of functioning and well-being: bowel and systemic symptoms; emotional and social function.(26)

Irritable bowel syndrome - severity symptoms score (IBS-SSS) is the most frequently used severity measure for evaluating IBS symptoms severity. Items relate to pain, bowel dysfunction and overall well being. (27)

The FACIT-fatigue (FACIT-F) is a subscale of the general questionnaire, the FACIT-G. It is a 13-item instrument to assess fatigue and its impact on daily life. The FACIT-F scale has been validated in the general population as well as in IBD patients.(28)



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Fecal calprotectin is a non-invasive biomarker of intestinal inflammation. The high correlation of FC with clinical disease activity, endoscopic, and histological indices has been described in patients with ulcerative colitis. (29)

Archiving of documents

In accordance with applicable national regulations, the investigator must keep a copy of all documentation and keep it in a dry and safe place after the closure of the study

Disclosure of information regarding the study

Confidentiality

The investigator and research staff involved in the study will hold all information relating to the study (including the protocol, data obtained and all documentation produced during the study) in strict confidence. These information, data or reports will not be used for purposes other than those described in the protocol. These restrictions do not apply if:

- Information becomes publicly available not due to investigator's negligence
- information requires confidential disclosure to ethical committee in order to evaluate the study
- information must be disclosed in order to offer adequate medical care for a study subject

Data ownership and publications policy

Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, as study promoter, is the only owner of the data. Only investigator and research staff will have access to the data.

At the end of the study investigator will draft a manuscript with the results of the study that will be submitted for publication to a major gastroenterological journal, regardless of the results of the study.

Indemnity and compensation in the event of damages

In the event of undesirable events or any damage that may derive from participation in the study, the Insurance Policy of our institute is also extended to subjects participating in research projects.

Financial disclosure

This study will be performed with unconditional financial support by DicoPharm S.p.A. The funder renounces any claim to ownership of study data.



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