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~~**CONFIDENTIAL**~~

CLINICAL STUDY REPORT

Product: Rifaximin

Pharmaceutical form: Rifaximin 550 mg tablets

Indication: Non-Constipation Irritable Bowel Syndrome

Protocol No.: RIBS-MIC/002/2010, version 1.0, dated 20/12/2010 (EudraCT number: 2010-024177-39) and Amendment 01, dated 23/09/2011

Phase of development: IIb

PROSPECTIVE MICROBIOLOGICAL STUDY ON PATIENTS WITH NON-CONSTIPATION IBS TREATED WITH RIFAXIMIN 550 MG TABLETS

Study start date (First patient enrolled): 9th May 2011

Study completion date (Last patient completed): 14th June 2012

Investigator and Centre: [redacted] Internal Medicine and Gastroenterology Division, Catholic University of Rome, Largo A. Gemelli 8, 00168, Italy

Sponsor's Clinical Study Manager and contact for any question:
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This study was performed in compliance with the current Good Clinical Practice (GCP), including the archiving of essential documents

Date of version Final 1: 16th September 2013

~~2.~~ SYNOPSIS

Name of Sponsor/Company: Alfa Wassermann SpA, Via Ragazzi del '99, 5; 40133 Bologna, Italy

Name of Active Ingredient: Rifaximin

Name of Finished Product: Rifaximin 550 mg

Title of the study: Prospective microbiological study on patients with non-constipation IBS treated with Rifaximin 550 mg tablets.

Investigators: [redacted] MD

Study centres: Internal Medicine and Gastroenterology Division – Catholic University of Rome, Largo Agostino Gemelli 8, 00168 Roma, Italy

Publication (reference): None

Study period: First subject enrolled: 9 May 2011; Last subject completed: 14 Jun 2012	**Phase of development:** IIb

Objective: To determine the effect of Rifaximin 550 mg treatment on the faecal microflora of patients with non-constipation Irritable Bowel Syndrome (non-C IBS).

Study Design:

This is a prospective, phase 2b study, planned to determine the effect of Rifaximin 550 mg tablet treatment, administered thrice a day (TID) (1650 mg daily dose) for 14 days, on the faecal microflora of patients with non-constipation Irritable Bowel Syndrome (non-C IBS). Microbiological and molecular analyses were performed at different timepoints during the study to detect treatment-induced changes in faecal microflora.

Both healthy volunteers and patients with non-C IBS were enrolled in the study. Only patients with non-C IBS received treatment with Rifaximin 550 mg tablets TID for 14 days and were followed for 12 weeks after treatment.

The study consisted of the following phases :

- Screening phase – Prospective subjects underwent a screening evaluation within 14 days prior to randomization. The diagnosis of SIBO was performed by the lactulose H2/CH4/CO2 breath test (LBT). A faecal sample was provided during the screening period (Baseline sample).
- Treatment phase – Starting on Day 0 (Visit 2 - Enrolment Visit), eligible patients with non-C IBS received Rifaximin 550 mg TID for 14 days. Healthy volunteers did not receive any treatment At the end of each week of treatment patients were administered a weekly questionnaire for the evaluation of the effect of rifaximin on IBS symptom relief. At the end of treatment (EOT) (Visit 3 - Day 14) non-C IBS patients were required collect a faecal sample. Patients with non-C IBS and SIBO at baseline (Group B) performed the LBT.
- Follow-up phase – At the end of each week during follow-up and up to day 56 patients were administered the weekly IBS symptoms questionnaire. At Day 56 non-C IBS patients were required to collect a faecal sample (FU sample) and to perform a LBT (Visit 4). At the end of a 86-day follow-up period, patients provided an additional faecal sample (End of Study Visit - EOS Visit 5 – Day 96 ± 2).

Number of patients:

Planned: The study was planned to include 3 groups of subjects:

- Group A: 10 healthy volunteers;
- Group B: 20 patients with non-C IBS with a diagnosis of SIBO;
- Group C: 20 patients with non-C IBS and SIBO-negative.

An interim analysis was planned after the enrolment and collection of stool samples from 5 healthy volunteers, 10 patients with non-C IBS and SIBO and 5 patients with non-C IBS and a negative diagnosis of SIBO.

Analysed: The interim analysis was performed on 6 healthy volunteers, 12 patients with non-C IBS and SIBO and 3 patients with non-C IBS and a negative diagnosis of SIBO.

Diagnosis and main criteria for inclusion:

Main inclusion criteria for Healthy Volunteers

Female or male subjects aged ≥ 18 and < 75 , without ongoing or significant gastrointestinal symptomatology and clinical laboratory tests at screening showing no clinically significant abnormalities and no relevant concomitant diseases in the opinion of the Investigator.

Subjects with signed and dated written informed consent prior to admission to the study and able to understand and comply with protocol requirements, instructions and protocol-stated restrictions.

Main inclusion criteria for non-C IBS patients

Female or male subjects aged ≥ 18 and < 75 at the time of the screening visit and being diagnosed with non-constipation IBS, according to Rome II diagnostic criteria.

Subjects must have signed and dated written informed consent prior to admission to the study and be able to understand and comply with protocol requirements, instructions and protocol-stated restrictions.

Female subjects of childbearing potential was allowed to enter and participate in this study if she was not lactating and had a negative pregnancy test both at screening (Visit 1) and baseline (Visit 2) prior to investigational product administration.

Exclusion criteria specific for Healthy Volunteers

- Subjects with a positive LBT;
- Subject with symptoms related to IBS;
- Subject with significant history of gastrointestinal, renal, hepatic, pulmonary, oncologic, endocrine or cardiovascular disease; or history of epilepsy, asthma, psychosis, glaucoma, severe head injury, immunological, haematological or neoplastic disease.

Exclusion criteria specific for non-C IBS patients

- Subject with the following symptoms of constipation IBS: less than 3 bowel movements a week; hard or lumpy stools, and straining during a bowel movement;
- Subject is a candidate for GI surgery or has a history of GI surgery (exceptions: benign polypectomy and inguinal hernia);
- Subjects with known hypersensitivity to Rifaximin or rifampin or excipients;
- Subjects with severe hepatic insufficiency, renal insufficiency (creatinine >2.2 mg/dl), severe cardiac insufficiency (NYHA - New York Heart Association classes 3 – 4);
- Subject with a history of human immunodeficiency virus (HIV) or hepatitis (B or C);
- Subjects with immunological, haematological or neoplastic disease.

Main exclusion criteria for both Healthy Volunteers and non-C IBS patients

- Subject with current evidence of duodenal ulcer, gastric ulcer, diverticulitis, or infectious gastroenteritis;
- Subject with a history of celiac disease, inflammatory bowel disease (eg, Crohn's disease, ulcerative colitis, celiac disease), GI malignancy, GI obstruction, gastroparesis, carcinoid syndrome, pancreatitis, amyloidosis, ileus or cholelithiasis;
- Subject with hyperthyroidism and/or diabetes (Type 1 or Type 2);
- Subject with lactose intolerance not controlled by lactose free diet;
- Subject with a positive stool culture for pathogenic bacteria, yeast, parasites and viruses;
- Patients who have used any investigational drug within the 3 months prior to screening;
- Subjects taking one or more of the following prohibited medications within 4 weeks prior to and during the study: rifaximin and other antibiotics, probiotics, antipsychotic drugs, antispasmodics, bismuth subsalicylate or kaopectate, IBS drugs (e.g., Alosetron), laxatives, lubiprostone, proton-pump inhibitors, narcotics, prokinetic drugs, chronic nonsteroidal anti-inflammatory drugs usage.

Test product, dose and mode of administration, batch no:

Rifaximin 550 mg film coated tablets, to be orally administered TID for 14 days.

Batch No.: 7956

Expiry Date: December 2012

Duration of treatment: 14 days.

Reference therapy, dose and mode of administration, batch no: Not applicable.

Criteria for evaluation:

Exploratory End-points:

- To evaluate any difference in faecal microbiota between healthy volunteers and non-C IBS patients at baseline, at the end of the 14 day treatment and after a six-week follow-up period;
- To analyse the effect of rifaximin treatment on the composition of faecal microbiota samples in non-C IBS patients, with particular interest to any qualitative/quantitative change in *Firmicutes*, *Bacteroidetes*, *Enterobacteriaceae*, *Bifidobacteria*.
- To evaluate whether at the end of the treatment period bacterial strains resistant to rifaximin are selected and whether they are still present in faecal sample after a six- and a twelve-week follow-up period.

Efficacy End-points:
Subject achieving adequate relief of global IBS symptoms, evaluated through a weekly (every 7 days) binary questionnaire. Clinical responders were defined as those subjects achieving adequate relief of global IBS symptoms, for at least 2 of the 4 weeks during the evaluation period (i.e., Weeks 2 through 5).

Other efficacy endpoints were the adequate relief of symptom of bloating and abdominal pain/discomfort.

Safety:
Safety variables were adverse events and withdrawals due to adverse events; vital signs (heart rate, blood pressure, body temperature and body weight); routine laboratory parameters (haematology and chemistry).

Methods:

Clinical Efficacy:
The relief of global IBS symptoms, symptom of bloating and abdominal pain/discomfort was evaluated to asses clinical efficacy. Adequate relief of IBS symptoms, symptom of bloating and abdominal pain/discomfort was defined as a response of "yes" to a weekly (every 7 days) binary questionnaire.

LBT was performed by all subjects at baseline. Non-C IBS patients with a positive diagnosis of SIBO repeated the LBT at the end of treatment (Day 14) and at Day 56, while patients without SIBO at baseline underwent an additional LBT at Day 56.

Microbiological and molecular analyses on faecal microbiota:
To assess the effect of Rifaximin treatment on the faecal microbiota, faecal samples were collected from healthy volunteers at the enrolment into the study and from patients with non-C IBS during the screening period (baseline sample), at the end of the 14-day treatment with Rifaximin (End of Treatment - EOT sample), at Day 56 (Follow-up, FU sample) and at Day 98 (End of Study – EOS sample).

Qualitative/quantitative changes in faecal microbiota were measured by PCR-DGGE and RT-PCR. DGGE results were analysed by means of similarity matrices among profiles belonging to different subjects and using Dice' and Pearson's correlation coefficient.

Statistical evaluations of Real-Time PCR results were performed using mean, median, standard deviation of the values, obtained in duplicate, among different collection moments and considering selected bacterial groups, as established after DGGE analyses results. RealPlex Instrument and Software were used for Real-Time amplification and data statistics.

The capability for rifaximin to select the growth of resistant bacteria was investigated in five genera: *Bifidobacterium spp., Lactobacillus spp. , Enterococcus spp, Enterobacteriaceae,* and *Clostridium spp*. Intestinal rifaximin-resistant strains were isolated on selective agar plates, supplemented with increasing concentrations of rifaximin. A representative number of the grown colonies were randomly selected, to obtain an identification of the species harbouring some potential resistance.

Study population:

The study population included 6 patients (2 males and 4 females, with a mean age of 27.7 years; range 22-44) and 15 patients with non-C IBS (5 males and 10 females, with a mean age of 34.5 years; range 19-62).

Extent of exposure and compliance:

All patients took the study treatment according to the dose regimen planned by protocol. The compliance was higher than 90% for all patients.

RESULTS:

Clinical efficacy results:
Twelve of the 15 patients (80%) achieved an adequate relief of global IBS symptoms for at least 2 of 4 weeks during the evaluation period, i.e. clinical responders. Among these, 9 patients had a sustained response up to Day 56 (Visit 4).

Normalization of LBT at the end of treatment (Day 14) was achieved by 11 of 12 patients with a diagnosis of SIBO at baseline.

Microbiological and molecular results:
The faecal samples collected from all enrolled non-C IBS (i.e. 15) patients and 5 of the 6 enrolled healthy volunteers were processed according to study protocol.

Assessment of bacterial resistance to rifaximin:

Rifaximin showed the capability to select the growth of resistant bacteria, among all the investigated genera, with the exception of *Lactobacillus spp*. This ability greatly differed among genera.

Bifidobacteria showed to be extremely prone to select resistant populations in presence of antibiotic pressure and to maintain resistance during the 12-week follow up period (15 of 15 had rifaximin resistant strains in faecal sample collected at Day 14, 56 and 98). All resistant *Bifidobacteria* detected belonged to *Bifidobacterium Longum* subsp. *longum/infantis*.

Enterococcal resistant strains appeared overall in 13 of 15 patients at Day 14 or Day 56 and most frequently detected resistant strains were *Enterococcus Faecium* and *Durans*. Resistance to rifaximin in this genera had the tendency to revert, disappearing at T98 in all except two cases.

Resistant strains of *Enterobacteriaceae* were detected in 11 of 15 patients and were mainly identified as *Escherichia coli*. Resistance in this genera did not completely disappear at 12 week after treatment and was maintained in 4 patients.

Clostridium spp. appeared more affected by rifaximin activity, with a significant reduction of the counts and an extremely poor development of resistant features.

Analysis of qualitative/quantitative change in faecal microbiota:

Although the high inter-individual variability limits the interpretation of the results, real-Time PCR data showed that some bacterial groups (*Bifidobacteria* and *Enterobacteriaceae*) were poorly influenced by rifaximin administration, probably in agreement with the selection of resistant clones, replacing the most susceptible species within the families.

The quantification of *Firmicutes* and *Bacteroidetes* showed individual-specific fluctuations between different collection timepoints. However, the *Firmicutes* to *Bacteroidetes* ratio, that was used as exploratory index in signaling the human gut microbiota status, as suggested by recent scientific publications, showed no significant changes over time (Day 0 mean ± SD = 1.15±0.06 at Day 0, mean ± SD = 1.09±0.04 at Day 14, mean ± SD = 1.11±0.06 at Day 56), suggesting that rifaximin intake did not have a significant impact on the overall microbiota.

Based on the comparison of this ratio between healthy subjects and non-C IBS patients, it was not possible to define a trend for “sick” and “healthy” microbiota, since similar values were observed in faecal samples from healthy subjects (mean ± SD = 1.16±0.05).

DGGE analyses confirmed the finding that rifaximin did not affect the overall composition of the core microbiota, evidencing a substantial uniformity of bands abundance and richness in the 15 subjects and in the 3 samplings for each of them. Samples at Day 0 differed from those at Day 14 showing a higher probability to revert at the initial condition at Day 56 for about 50% of the subjects, whereas for the remaining subjects bacterial variations appeared more prominent at Day 56. It could be hypothesised that changes caused by rifaximin administration were probably recovered during the follow up period, and at Day 56 sample an intermediate condition was captured.

Band sequencing revealed that some specific bacterial populations underwent modifications, such as an increase in *Actinobacteria* presence and fluctuations in clostridial representatives, further confirming the action of the antibiotic toward this group. Moreover, bands belonging to *Lachnospiraceae* were frequently isolated, besides a good presence of *Faecalibacterium prausnitzii*, for some subjects specifically after rifaximin administration.

However, because of the huge variability among subjects it was not possible to determine a common trend in the analysis of the profiles in the sampling points. Based on the results of those analyses, the enrolment of further subjects and collection/analysis of further faecal samples was stopped.

Safety results:

Adverse events:

No adverse events were registered during the study.

Laboratory parameters;

None of the registered changes in laboratory parameters (haematology, blood chemistry and urinalysis) from the screening to the last visit was considered clinically relevant.

Vital signs:

There were no substantial changes in any of the measured vital sign parameters (blood pressure, heart rate and body temperature) from the screening to the last visit.

Conclusions:

Despite the limited sample size, Rifaximin was able to induce adequate relief of global IBS symptoms and normalization of LBT at the end of the treatment in 80% and 92% of patients, respectively.

At the end of the 14-day treatment most of the evaluated strains developed resistance to rifaximin. However, there was a tendency for resistance to revert, except for *Bifidobacterium spp* and in few cases for *E.coli*, where bacterial strains resistant to rifaximin persisted up to Day 98. Nevertheless, the emergence of rifaximin resistant strains seemed not to hamper clinical efficacy.

All the subjects had relatively stable microbiota genomic profiles over time, suggesting that treatment with rifaximin did not induce significant change on the composition of the core microbiota and that observable changes were probably limited to fluctuations of specific bacterial strains. In this regard, rifaximin treatment induced an increase *Actinobacteria* and a good presence of *Faecalibacterium Prausnitzii* was observed in samples collected after the baseline.

However, due to high inter-individual variability it was not possible to provide an indication of the presence of a common feature in the composition of faecal microbiota of healthy subjects and IBS patients and a clear evaluation of changes of the faecal microbiota following treatment with rifaximin. For this reason the enrolment of further subjects and collection/analysis of further faecal samples was stopped.