~~1~~ Study Synopsis

Name of Sponsor/Company: Alfa Wassermann S.p.A., Italy	Individual Study Table Referring to Part of the Dossier	*(For National Authority Use only)*
Name of Investigational Product: Rifaximin vaginal tablet	Volume:	
Name of Active Ingredient: Rifaximin	Page:	

Title of Study: A phase II, multicentre, double-blind, randomised, placebo-controlled study on efficacy and tolerability of rifaximin vaginal tablets in the treatment of bacterial vaginosis.

Co-ordinating Investigator:
[redacted]

Study Centre(s):
There were 13 active centres: 6 centres in Belgium, 4 centres in Germany, and 3 centres in Italy.

Publication (Reference): None	**Phase of Development:** II

Studied Period:
Date of first patient, first visit: 14 Aug 2009
Date of last patient, last visit: 19 Oct 2010

Objectives: To evaluate the efficacy and tolerability of two doses (100 mg and 25 mg) of rifaximin vaginal tablets vs. placebo in the treatment of bacterial vaginosis, administered either as 100 mg x 5 days or 25 mg x 5 days or 100 mg x 2 days vs. placebo to 112 female patients affected by bacterial vaginosis.
The diagnosis of bacterial vaginosis was assessed on the basis of at least 3 of 4 fulfilled Amsel's criteria and a Gram stain Nugent score ≥ 4.

Methodology: This was a multicentre, double-blind, randomised, placebo-controlled study comparing efficacy and tolerability of rifaximin vaginal tablets vs. placebo for the treatment of bacterial vaginosis. The study consisted of a screening phase of up to 7 days, a treatment period of 5 days, and a follow-up phase of 35 days from the last study drug administration. Patients were evaluated for study eligibility at the screening visit (V 1). At the randomisation visit (V 2), eligible subjects were randomised in a 1:1:1:1 ratio to 1 of the 4 treatment groups. Study medication was administered intra-vaginally at bedtime (in the evening) of the randomisation visit (Day 0) and then once daily at the same time for a total of 5 days. Patients attended the first follow-up visit (V 3) 7 to 10 days after the end of therapy. At this visit, patients who did not show remission according to Amsel's criteria and Gram stain Nugent score were withdrawn from the study as treatment failures. Patients showing remission attended the second follow-up visit/final visit (V 4) 28 to 35 days after the end of treatment. Efficacy and safety assessments were performed at each follow-up visit, or at the early discontinuation visit (EDV), if applicable.

Number of Patients (Planned and Analysed):
Planned: 112
Randomised: 114
Analysed datasets (number of patients):

	Rifaximin 100 mg /5 d	Rifaximin 25 mg /5 d	Rifaximin 100 mg /2 d	Placebo	Total
All patients randomised	28	29	29	28	114
Randomised, but not treated	0	3	3	2	8
Safety evaluation set (SES)	28	26	26	26	106
Full analysis set (FAS)	27	25	25	26	103
Per protocol set (PPS)	17	19	17	14	67

d = days.

Diagnosis and Main Criteria for Inclusion:

- Caucasian race.
- Post-menarchal, pre-menopausal female patient.
- Non-pregnant (negative urine pregnancy test at screening and randomisation) nor breast-feeding patient.
- Patient aged between 18 - 50 years, inclusively.
- Patient who was willing to be asked questions about personal medical health and sexual history.
- Patient capable of and willing to conform to the study protocol.
- Patient who had been thoroughly informed of the aim of the study and the study procedures and who provided signed and dated written informed consent form.
- Patient who agreed to abstain from intercourse during 5-day treatment period.
- Patient who agreed also to abstain from intercourse 3 days before the scheduled visits of follow-up.
- Patient who agreed to abstain from the use of any other intravaginal product (i.e. douching, feminine deodorants sprays, tampons, spermicides, gels, foams, and diaphragms) during the entire study period.
- Patient who agreed to use an adequate method of birth control for the duration of the study to avoid pregnancy. Acceptable methods include a history of bilateral tubaric ligature, male partner with a vasectomy, a steroidal contraceptive (oral, patch, injectable or implantable), IUD or abstinence.
- Clinical diagnosis of bacterial vaginosis with Amsel's criteria (at least 3 of 4 fulfilled criteria).
- Diagnosis of bacterial vaginosis confirmed by Gram stain Nugent score (score ≥ 4).

Test Product: Rifaximin vaginal tablets.

Dose: 100 mg vaginal tablet once daily for 5 days or 25 mg vaginal tablet once daily for 5 days or 100 mg vaginal tablet once daily for 2 days + placebo vaginal tablet once daily for the remaining 3 days.
Mode of Administration: 1 tablet administered intra-vaginally at bedtime for a total of 5 days.
Batch Numbers: F869/05.2 (100 mg), F869/06.1 (25 mg), F869/05.4 (100 mg plus placebo).

Duration of Treatment:
The study duration for each patient was up to 47 days. The study had a 5-day treatment period and a follow-up period of up to 35 days.

Reference Therapy, Dose and Mode of Administration, Batch Number:
Placebo vaginal tablets.
Mode of Administration: 1 tablet administered intra-vaginally at bedtime for a total of 5 days.
Batch Number: F869/03.3.

Criteria for Evaluation:
Efficacy:
Primary Endpoint:

- Remission of bacterial vaginosis assessed via Amsel's criteria and Gram stain Nugent score 7-10 days after the end of therapy at the first follow-up visit, V 3 (or EDV).

Secondary Endpoints:

- Remission evaluated by Amsel's criteria at the first follow-up visit (V 3 or EDV).
- Remission evaluated by Gram stain Nugent score at the first follow-up visit (V 3 or EDV).
- Maintenance of remission evaluated by Amsel's criteria and Gram stain Nugent score at the second follow-up visit / final visit (V 4).
- Evaluation of composition of the vaginal microbiota by polymerase chain reaction denaturating gradient gel electrophoresis (PCR-DGGE) and real-time PCR (see separate report in Appendix 16.4).

Safety:

- AEs
- Local objective and subjective tolerability (from patient diary).
- Vital signs (including blood pressure, pulse, temperature and body weight).
- Routine laboratory parameters (haematology, clinical chemistry, urinalysis.
- Physical examination findings.
- Gynaecological findings.

Statistical Methods:
The remission of bacterial vaginosis assessed via Amsel's criteria and Gram stain Nugent score 7-10 days after the end of therapy at the first follow-up visit, V 3 (or EDV) served as the primary endpoint.
Specifically:

- Amsel's criteria 7-10 days after the end of therapy (remission according to Amsel): resolution of the clinical findings assessed at baseline with 2 or less signs

and

- Gram stain Nugent score 7-10 days after the end of therapy (remission according to Nugent): score from 0 to 3.

The analysis of efficacy was performed primarily on the full analysis set (FAS). The analysis of the primary endpoint and the maintenance of remission were also performed on the per protocol set (PPS) for supportive and sensitivity purposes.
Descriptive 95% Clopper-Pearson 2-sided confidence intervals of remission rates were calculated for each treatment group. Comparisons of the remission rates between

individual active treatment group as well as pooled group of subjects receiving any dose of rifaximin vs. placebo group were performed using a 2-sided Fisher's exact test. Bonferroni-Holm procedure was implemented to assess multiplicity. Secondary efficacy endpoints were analysed in a similar manner to the analysis of the primary endpoint.
Safety variables, i.e. AEs, local objective and subjective tolerability, laboratory evaluations, vital signs, physical examination findings, and gynaecological signs and symptoms were analysed using descriptive statistics.

Determination of sample size

The sample size estimation was based on the planned confirmative comparisons of the rifaximin treatment groups vs. the placebo group with respect to the remission rates according to Amsel's criteria and Gram stain Nugent score. The power estimation was based on a 2-sided Fisher's exact test. The level of significance, accounting for the multiplicity of the planned comparisons, was fixed at a 1.25%.
The study aimed to detect an effective dosage of rifaximin, able to produce a 60% remission rate according to Amsel's criteria and Gram stain Nugent score for this study. Assuming a remission rate of 10% in the placebo group, a sample size of 22 patients per group would provide at least 80% power to each single comparison, which resulted in a required overall sample size of 88 patients.
Assuming a 20% rate of screening failures and a 20% drop-out rate, 140 patients were planned to be screened to attain 112 randomised patients (28 patients per group).

Summary – Conclusions:

Efficacy Results:

Primary Efficacy Analysis

Analysis of the primary endpoint on the FAS showed that the rate of remission at V 3 (or EDV) was highest in the rifaximin 25 mg / 5 days group (48.0%), followed by the rifaximin 100 mg / 2 days group (36.0%) and the rifaximin 100 mg / 5 days group (25.9%). In the placebo group, the rate of remission was 19.2%.
Superiority of the individual and pooled rifaximin regimens vs. placebo was tested using a 2-sided Fisher's exact test. Multiplicity was assessed via the implementation of a Bonferroni-Holm procedure. According to this procedure, a statistically significant test result is shown if the smallest p-value is less than 0.0125. As the p-value for the test of rifaximin 25 mg / 5 days vs. placebo was 0.0399, the null hypothesis (H_o) could not be rejected, and thus none of the rifaximin treatment regimens was superior to placebo in terms of remission rate. These results were supported by the analysis on the PPS.
The subgroup analysis of the primary endpoint stratified by history of bacterial vaginosis on the FAS showed higher remission rates for all rifaximin groups in patients with 'recurrence of bacterial vaginosis' compared to patients with 'first episode of bacterial vaginosis'. Moreover, the remission rate was higher in patients treated with rifaximin 25 mg / 5 days compared to the other groups, particularly in patients with 'first episode'. Similar results were obtained for the PPS.

Secondary Efficacy Analysis

Evaluated by Amsel's criteria alone, remission rates in the rifaximin groups were 80.0% in the rifaximin 25 mg / 5 days group, 72.0% in the rifaximin 100 mg / 2 days group, and 66.7% in the rifaximin 100 mg / 5 days group. The rate of remission in the placebo group was clearly lower (42.3%). The comparisons of the remission rates in the rifaximin 25 mg / 5 days, rifaximin 100 mg / 2 days and the pooled rifaximin group to placebo showed p-values of 0.0095, 0.0483, and 0.0082, respectively.
The rates of remission evaluated by Nugent score alone were noticeably lower than

those evaluated by Amsel's criteria in all treatment groups, and were identical to the results obtained for the primary efficacy endpoint. i.e., for both evaluation methods assessed together (48.0% in the rifaximin 25 mg / 5 days, 36.0% in the rifaximin 100 mg / 2 days, 25.9% in the rifaximin 100 mg / 5 days, and 19.2% in the placebo group). Nevertheless, the rate of remission in the rifaximin 25 mg / 5 days group was clearly higher than in the placebo group ($p = 0.0399$).

In accordance with the protocol, patients who did not show remission at V 3 were to be withdrawn from the study as treatment failures. A total of 33 patients showed remission at V 3. Two of these patients had no V 4 data as they were lost to follow-up. A further 10 patients should have been withdrawn as treatment failures at V 3 but were not. Consequently, V 4 data were available for 41 of the 103 patients included in the FAS, 31 of whom showed remission at V 3. The percentage of patients maintaining remission at V 4 (or EDV) was highest in the rifaximin 25 mg / 5 days group (28.0%) followed by the rifaximin 100 mg / 5 days group (14.8%). The rates of remission in the rifaximin 100 mg / 2 days group and the placebo group were similar (4.0% and 7.7%, respectively). The results for the supportive analysis on the PPS were similar to those for the FAS.

Safety Results: A total of 106 patients received at least 1 dose of the study medication and were included in the SES. The median treatment duration was 5.0 days in all 4 treatment groups.

During the study, a total of 146 TEAEs were reported for 60 of the 106 patients (56.6%) included in the SES. Of these, 80 TEAEs were assessed as treatment-related.

The overall rate of patients reporting TEAEs during treatment was 23.1% in the rifaximin 100 mg / 2 days group, 39.3 % in the rifaximin 100 mg / 5 days group, 42.3% in the rifaximin 25 mg / 5 days group and 50.0% in the placebo group.

TEAEs classed as "Reproductive system and breast disorders" were most frequent in all 4 treatment groups. At the preferred term level, vulvovaginal pruritus was the most frequent TEAE in the rifaximin 100 mg / 5 days group (25.0%) and, together with vulvovaginal burning sensation, in the rifaximin 25 mg / 5 days group (19.2% each). In the placebo group, the most frequent TEAE was vulvovaginal burning sensation (30.8%), followed by vulvovaginal pruritus (19.2%). Vulvovaginal burning sensation was the only TEAE to be reported for more than 1 patient in the rifaximin 100 mg / 2 days group (7.7%). Very few of the other TEAEs were reported for more than 1 patient per treatment group. No TEAEs associated with vulvovaginal candidiasis were reported during treatment.

The overall rates of TEAEs reported post-treatment were lower than those reported during treatment in all treatment groups except for the rifaximin 100 mg / 2 days group, which showed a higher rate post-treatment than during treatment. This was mainly due to the higher rate of TEAEs classed as "Reproductive system and breast disorders" reported for this group post-treatment. At the preferred term level, very few post-treatment TEAEs were reported for more than 1 patient per treatment group. Events concerning vulvovaginal candidiasis were reported by 4 patients in the rifaximin 25 mg / 5 days group (15.4%), 2 patients each in the 100 mg / 5 days and 100 mg / 2 days groups (7.1% and 7.7%, respectively) and for 1 patient in the placebo group (3.8%).

The vast majority of TEAEs reported for patients in each treatment group were of mild or moderate intensity. Severe TEAEs were reported for 4 patients during treatment (vulvovaginal pruritus in 1 patient of the rifaximin 100 mg / 5 days group, diarrhoea in

2 patients of the rifaximin 25 mg / 5 days group, and nausea, vomiting, abdominal pain, and headache in 1 patient each of the rifaximin 25 mg /5 days group) and for 1 patient post-treatment (vulvovaginal candidiasis in a patient in the rifaximin 25 mg / 5 days group). During treatment, the overall rate of treatment-related TEAEs was highest in the placebo group (46.2%) and lowest in the rifaximin 100 mg / 2 days treatment group (7.7%). Local symptoms such as vulvovaginal burning sensation, vulvovaginal pain, and vulvovaginal pruritus were the most frequent related TEAEs in all treatment groups. During the follow-up post-treatment, related TEAEs were reported by 3 patients: vaginal inflammation in 1 patient treated with rifaximin 100 mg / 5 days, vulvovaginal burning sensation and vulvovaginal discomfort in 1 patient of the rifaximin 25 mg / 5 days group, and vulvovaginal candidiasis in 1 patient in the placebo group**.**

None of the patients experienced serious TEAEs, TEAEs leading to withdrawal or TEAEs leading to dose changes. Evaluation of objective and subjective local symptoms showed that all 4 treatments were well tolerated. The objective local symptom score (sum of the scores for erythema, oedema, petechial haemorrhage and ulcer) was 0 for almost all patients. For the evaluation of subjective local symptoms, the majority of patients graded the maximum intensity of pain as none. A similar trend was observed for itching and burning, although higher percentages of patients graded these symptoms as mild or moderate.

Analyses of haematology, clinical chemistry and urinalysis parameters did not reveal any noteworthy changes over the course of the study in any treatment group. Clinical laboratory values remained within normal range in the vast majority of patients. No TEAEs associated with laboratory parameters were reported.

There were no noteworthy findings regarding vital signs, physical examination, and gynaecological data.

Conclusion: When adjusted for multiple comparisons, superiority of rifaximin compared to placebo could not be demonstrated for the primary efficacy endpoint, remission of bacterial vaginosis assessed via Amsel's criteria and Gram stain Nugent score 7-10 days after the end of therapy. There was, however, a consistent trend for the rifaximin 25 mg / 5 days group to show better efficacy compared to both placebo and the other rifaximin groups with respect to the rate of remission and to the maintenance of remission. All 3 rifaximin treatment regimens were safe and well tolerated.

Date of Report: 01 Mar 2011