Medinova AG

CLINICAL STUDY PROTOCOL

Comparative study of the efficacy and safety of vaginally applied Dequalinium Chloride (10 mg) and orally applied Metronidazole (2 x 500 mg) in the treatment of bacterial vaginosis

Study number: 380119

EudraCT Number: 2020-002489-15

Observational Product Name:	Fluomizin® vaginal tablets (Medinova AG)
Sponsor:	Medinova AG Eggbühlstrasse 28 CH-8050 Zurich / Switzerland
Coordinating Investigator	n/a
Protocol Version:	2.0
Protocol Date:	13 APR-2022

Amendments

Nr.	Description	Date
1.	Changes in the statistical analysis of the primary endpoint (see section 9.4): • Primary analysis set has changed from mITT (exclusion of Nugent score ≤ 7) to ITT (all randomized patients), see section 9.3 • Additional subgroup regarding the Nugent score at baseline. The amendment also affects the sample size (see section 9.1), because of a drop our rate adjustment. Minor changes in the statistical methods section (see 9.4), in the Nugent score category definition (see 7.2) and minor typing error	13-APR-2022
2.		
3.		

APPROVAL SIGNATURES

This study protocol has been reviewed and approved by the undersigned persons. It is confirmed that the information and guidance given in this protocol complies with scientific principles, the guidance of Good Clinical Practice, the Declaration of Helsinki in the latest relevant version and the applicable legal and regulatory requirements.

Sponsor Protocol Signatures

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Investigator Protocol Signature

By my signature, I agree to conduct this study according to this protocol and to make no additions or changes without the consent of the Sponsor. In addition, we agree that the study will be carried out in accordance with Good Clinical Practice (GCP), with the Declaration of Helsinki and with the laws and regulations of the country in which the study takes place.

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ABBREVIATIONS

Abbreviations	Description of Abbreviations
AE	Adverse Event
BfArM	Bundesministerium für Arzneimittel und Medizinprodukte in Germany
BV	Bacterial Vaginosis
C1	Control 1 (first control examination)
C2	Control 2 (second control examination)
CDMS	Clinical Data Management System
CRO	Clinical Research Organization
CTA	Clinical Trial Authorisation
Cu	Unscheduled Control
DCF	Data Clarification Form
DMP	Data Management Plan
DVP	Data Validation Plan
E	Entry of the study
eCRF	Electronic Case Report Form
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIV	Human Immunodeficiency Virus
HPV	Human papillomavirus
HSV	Herpes simplex virus
ICH	International Conference of Harmonisation
IEC	Independent Ethics Committee
IRB	Institutional Review Board
ITT	Intention To Treat
IUD	Intrauterine Device
LBG	Lactobacillary Grade
MedDRA	Medical Dictionary for Regulatory Activities
MI	Maturation index
MIC	Minimum inhibition concentration
PCR	Polymerase Chain Reaction
PP	Per Protocol Analysis Set
ePRO	Electronic patient reported outcome/ diary
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SES	Safety Evaluation Set
SOP	Standard Operating Procedure
STD	Sexually Transmitted Diseases
TSS	Total Symptoms Score

1 SYNOPSIS AND FLOWCHART

Title of study:	Comparative study of the efficacy and safety of vaginally applied Dequalinium Chloride (10 mg) and orally applied Metronidazole (2 x 500 mg) in the treatment of bacterial vaginosis
Name of sponsor:	Medinova AG Eggbühlstrasse 28 CH-8050 Zurich / Switzerland Tel.: +41 1 306 13 22 Fax: +41 1 306 2126
Investigational products:	10 mg Dequalinium Chloride (Fluomizin)
Study phase:	Phase IV
Indication:	Bacterial vaginosis
Number of study centres:	15
Study duration:	up to 30 days with treatment period of 7 days
Study Design:	Multicentre, active-controlled, randomised, double-dummy study with parallel-group design
Study Objectives:	
Primary	To evaluate whether vaginal tablets containing 10 mg dequalinium chloride (Fluomizin) are comparable in clinical efficacy to metronidazole 500 mg oral tablets in women suffering from bacterial vaginosis.
Secondary	· To assess the clinical, bacteriological, and therapeutic cure rate on short-term and/or long-term follow-up · To evaluate subjective assessment of efficacy and tolerability · To further explore the safety profile of Fluomizin in this patient population
Study Population:	236
Eligibility criteria for enrolment:	1. Premenopausal woman ≥18 years 2. Diagnosis of bacterial vaginosis (BV): (all 4 Amsel criteria positive, i.e. 1) greyish white thin discharge, 2) KOH test or 'fishy' smell, 3) microscopic presence of > 20% clue cells, 4) vaginal pH > 4.5) 3. Signed Written Informed Consent to participate in this study.
Efficacy Outcome Variables:	Primary: Clinical cure rate at visit C1 (at 8 to 10 study days) based on three Amsel criteria (excluding vaginal pH > 4.5) Secondary: - Clinical cure rate at follow-up (at C2 = at 24 to 30 study days) - Bacteriological cure rate at C1 and C2 - Therapeutic cure rate at C1 and C2 - Evaluation of individual Amsel criteria at E, C1 and C2 - The development of the Nugent Score within 3 categories, i.e. (1) cured, (2) intermediate, and (3) BV positive at E, C1 and C2 - Subjective assessment of efficacy
Safety Parameters:	1. Adverse events (AEs) and serious adverse events (SAEs) 2. Subjective assessment of tolerability

3. Vital signs

Other Parameters: Treatment compliance

- Treatment compliance shall be assessed based on the patient diary with regard to number doses taken and treatment duration.

Resolution of symptoms

- Based on patient diary the time until resolution of symptoms shall be analysed.

Statistical methods: The differences in clinical cure rate between Fluomizin and Metronidazole will be analysed in a confirmatory manner using a one-sided Farrington-Manning test on a 2.5% significance level with following hypothesis:

$$H_0: \pi_F - \pi_M \leq -\delta$$
$$H_1: \pi_F - \pi_M > -\delta$$

It is evaluated whether the observed difference is greater than the non-inferiority margin - δ. The test statistic is based on the asymptotic normality of the score statistic.

Efficacy will be analysed based on the ITT and repeated as sensitivity analysis for the PP population. The Farrington-Manning test will provide a p-value for the outcome variable, on which the decision will be based.

If non-inferiority can be shown, data can furthermore be tested for superiority of Fluomizin over the leading therapy option Metronidazole. The Farrington-Manning test is also providing a confidence interval for this purpose, which will be specified with 0.95 (95%). The hypotheses tested for the evaluation of possible superiority are the following:

$$H_0: \pi_F - \pi_M \leq \delta$$
$$H_1: \pi_F - \pi_M > \delta$$

If Fluomizin exceeds Metronidazole, and the null hypothesis can be rejected, then Fluomizin is considered superior to Metronidazole with respect to efficacy.

There is no multiplicity problem to be considered since this analysis corresponds to a closed hierarchical test procedure.

For primary and secondary endpoints, at least summary statistics will be provided in the following way:

- Quantitative Parameters will be presented in terms of mean, standard deviation, median, Q1, Q3, extreme values, number of patients and missing data.
- Qualitative parameters will be presented in terms of number and percentage of each modality as well as number of patients and missing data.

A Statistical Analysis Plan (SAP) will be written by the study statistician, containing a more detailed explanation of methods used, as well as a list of the output that will be generated when the data is provided. The SAP will be finalized and approved prior to the interim analysis.

Sample size: The minimum number n, of evaluable patients necessary for each treatment groups is determined by the following equation:

$$n = \frac{2\left(z_{1-\alpha} + z_{1-\beta}\right)^2 \pi_M (1 - \pi_M)}{\delta^2}$$

Assuming incidences of treatment successes for both groups of 0.8 (80%), a clinical relevant difference of 0.15 (15%), a power of 0.8 (80%) and a one-sided

	type one error rate of 0.025 (2.5%), the minimum number of evaluable patients per treatment group is 112. Assuming a drop-out rate of 5 %, a total of 118 patients per treatment group is planned.

2 SCIENTIFIC BACKGROUND AND RATIONALE

2.1 Scientific Background

2.1.1 Bacterial Vaginosis

Bacterial vaginosis (BV) is a synergistic polymicrobial syndrome characterized by depletion of Lactobacillus spp., especially those that produce hydrogen peroxide and lactic acid, and an intense increase in the quantity of commensally vaginal anaerobic bacteria to 100- to 1000-fold above normal levels [Marrazzo, 2006]. BV is the most common vaginal disorder in reproductive-age women [Kenyon *et al.*, 2013], causing about 20-40% of all vaginal infections [Sobel, 1997]. BV increases the risk of acquisition and transmission of sexually transmitted infections and is associated with pregnancy complications [Nasioudis *et al.*, 2017].

BV is not called vaginitis because of the absence of inflammation - there are no toxic leucocytes, pain, itching, dyspareunia, redness of the vulva or vagina - only microbial shift to anaerobic pathogens [Donders, 2010]. It is still unknown whether the loss of lactobacilli precedes or follows the upheaval of flora [Srinivasan and Fredricks, 2008]. Although the composition of the bacteria in BV varies between individuals, Gardnerella, Atopobium, Mycoplasma, Prevotella, Bifidobacterium, Megasphaera, Leptotrichia, Sneathia, Dialister, and Clostridium species are found most frequently [Srinivasan *et al.*, 2012]. The development of bacterial vaginosis results in the aforementioned quantitative and qualitative shift from normally occurring lactobacilli species: L. crispatus, L. gasseri, L. jensenii, and L. iners to a mixed flora dominated by anaerobic bacteria [Larsson and Forsum, 2005; Ravel *et al.*, 2011].

Diagnostic criteria of BV established by Amsel have been proved remarkably simple and useful in clinical practice: a) a vaginal pH higher than 4.5; b) the presence of clue (vaginal epithelial) cells in the vaginal fluid; c) a thin, grey or white homogenous discharge; d) a positive KOH "whiff" test (the release of an amine [fishy] odour upon the addition of 10% potassium hydroxide to the vaginal fluid) [Amsel *et al.*, 1983]. Typically, bacterial vaginosis is diagnosed if 3 of the 4 Amsel criteria are present. The presence of clue cells is the single most reliable predictor of BV [Eschenbach *et al.*, 1988]. The four Amsel criteria are usually regarded as the gold standard for diagnosis and clinical cure of BV [Sweet, 2000].

Bacterial vaginosis has been consistently associated with numerous adverse sequelae related to the upper genital tract, including pelvic inflammatory disease and post-surgical infection in the setting of invasive gynaecologic procedures. Pregnant women with bacterial vaginosis experience a high rate of preterm delivery and low-birth-weight infants [Marrazzo, 2006], seven studies (two case-control and five cohort studies) reporting this [Oleen-Burkey and Hillier, 1995].

Concerning the BV therapy, anti-infectives with activity against anaerobes are indicated, but routine treatment of sexual partners is usually not recommended [Mehta, 2012]. The mainstay recommended treatments are oral metronidazole or topical clindamycin, both with comparable 80-90% efficacy [Livengood, 2009] However, three months after the end of therapy the recurrence rate is usually 30% or higher [Bradshaw, Tabrizi, *et al.*, 2006]. There are several disadvantages associated with these two therapies [Mendling *et al.*, 2016]: A significant proportion of patients do not achieve an adequate response, recurrence of BV is frequent, drug resistance may develop, and there is a risk of post-treatment candidosis.

The Bacterial Vaginosis Working Group of the Centre for Disease Control and Prevention (CDC) recommends regimens for the treatment of bacterial vaginosis: metronidazole 500 mg orally twice a day x 7 days, or metronidazole gel 0,75%, one full applicator (5 g) intravaginally once a day x 5 days, or Clindamycin cream 2% one full applicator (5 g) intravaginally at bedtime x 7 days (MMWR, 2002). The effectiveness of CDC recommended therapies at 3–4 weeks is about 80%, the recurrence rate within 1 month after therapy being as high as 20% [Joesoef *et al.*, 1999; Koumans and Kendrick, 2001].

Successful treatment of Bacterial vaginosis with both standard drugs metronidazole and clindamycin poses risks for developing vulvovaginal candidiasis. In one study, 8.5% of the patients treated with clindamycin vaginal cream developed a secondary candidiasis [Fischbach *et al.*, 1993]. In another clinical study, 14.8% of the patients developed candidiasis after clindamycin vaginal cream and 12.5% after oral metronidazole [Ferris *et al.*, 1995].

Increasing resistance against metronidazole and clindamycin has been reported by several investigators [EJ *et al.*, 1993; Goldstein *et al.*, 2002; Austin *et al.*, 2005; Nagaraja, 2008]. So far, true acquired antimicrobial resistance occurring in vaginal infections has not been observed as a major problem in clinical practice [Sobel, 2000, 2001]. Nevertheless, misuse of the over-the-counter (OTC) antimycotics, as well as widespread use of systemic oral azoles, could result in the spread of azole-resistant Candida albicans, and even more likely it could lead to an increase in non-albicans Candida species with intrinsic azole resistance [Sobel, 2001; Ferris *et al.*, 2002].

Thus, despite the current knowledge of BV many questions regarding treatment remain unanswered - all current therapies have disadvantages and gaps [Donders *et al.*, 2014]. The most important challenges are the need for new drugs with better cure rates, possibility to prevent recurrence and to avoid pregnancy complications. For this reason, other promising treatment approaches need to be evaluated. The purpose of the current study is to compare the efficacy and tolerability of such a treatment option, Fluomizin, with the current standard therapy.

Although antibiotics are selective and effective antimicrobials, it is nevertheless still necessary and desirable to broaden the therapeutic possibilities [Mendling *et al.*, 2016]. As alternative therapy, broad-spectrum antimicrobial agents, such as dequalinium chloride (DQC), are used to treat vaginal infections. Typical representatives are phenyl derivates (e.g. chlorhexidine, hexetidine), acids (e.g. salicylic acid), halogens (e.g. povidone-iodine), and quaternary ammonium compounds (e.g. dequalinium chloride). Less specific antimicrobial agents have the advantage that (1) the resistance of pathogenic microorganisms is not expected due to their multiple mechanisms of action, (2) they can also be used for mixed vaginal infections because of their broad antimicrobial spectrum, as well as (3) for pre- and post-operative prophylaxis [Mendling *et al.*, 2016].

2.1.2 Fluomizin (dequalinium chloride) Vaginal Tablets

Fluomizin is available as vaginal tablets, containing 10 mg dequalinium chloride [1,1'-(decane-1,10-diyl)bis(4-amino-2-methylquinolinium) dichloride], an antiseptic and anti-infective agent belonging to the class of quaternary ammonium compounds [D'Auria *et al.*, 1989].

Fluomizin is approved for the treatment of bacterial vaginosis in Switzerland and 23 countries of the European Union, including Czech Republic, Hungary, Slovak Republic and Romania. Fluomizin in its current formulation has been marketed since 1993.

Dequalinium chloride, the drug substance of Fluomizin, has a wide range of antimicrobial activity encompassing Gram positive, Gram negative bacteria, protozoa and fungi. Its mechanism of action is directed toward the cytoplasmatic membrane where dequalinium chloride causes damage and consequently releases cellular components [D'Auria *et al.*, 1989].

As for other surface-active compounds, the primary mode of action of dequalinium chloride is the enhancement of cell permeability and the subsequent loss of enzymatic activity, causing cell death [Mendling *et al.*, 2016]. The bactericidal and fungicidal effect of dequalinium chloride has been demonstrated to occur within 30 to 60 minutes [D'Auria *et al.*, 1989]. Della Casa and colleagues demonstrated the in vitro antimicrobial activity of dequalinium chloride against different pathogens that are relevant for vaginal infections, including anaerobic bacteria (Gardnerella vaginalis, Bacteroides spp., Peptostreptococcus spp., etc.), aerobic bacteria (staphylococci, streptococci, Escherichia coli, etc.), Candida species (C. albicans, C. glabrata, etc.) and Trichomonas vaginalis [Della Casa *et al.*, 2002]. The antimicrobial activity

of dequalinium chloride against Candida spp. is comparable with clotrimazole and ciclopiroxol amine [Della Casa *et al.*, 2002]. A recent in vitro study has also demonstrated a high susceptibility of Atopobium vaginae to dequalinium chloride and confirmed that dequalinium chloride has bactericidal activity [Lopes dos Santos Santiago *et al.*, 2012]. No development of resistance of micro-organisms to dequalinium chloride has been reported in laboratory studies or clinical trials. Resistance of pathogens is unlikely due to the multiple modes of action of dequalinium chloride.

In fact, multidrug resistance to single quaternary cationic anti-infectives is associated rather with hypersensitivity to compounds containing two quaternary cations, including dequalinium chloride [Turner *et al.*, 1997].

In a recently published multicentre, controlled study, Weissenbacher and colleagues compared the efficacy of dequalinium chloride 10 mg vaginal tablets (Fluomizin) once daily for 6 days to clindamycin vaginal cream 2% for 7 days in the treatment of BV [Weissenbacher *et al.*, 2012]. The treatment of BV with a 6-day course of 10 mg dequalinium chloride vaginal tablets had equal efficacy as a 7-day course of clindamycin vaginal cream. At week 4, the overall clinical cure rates observed were 79.5% with 10 mg dequalinium chloride and 77.6% with clindamycin, demonstrating that the efficacy of dequalinium chloride was as good as clindamycin (statistically significant), also after a long-term follow-up. The number of women with positive cultures of Candida spp. ('colonisation') at week 4 was slightly higher in the clindamycin group (14.6%) than in the dequalinium chloride group (9.3%); however, due to small figures the data was statistically not significant. Consistent with these results, symptomatic vulvovaginal candidosis at second follow-up was half as common with dequalinium chloride (2.7%) than with clindamycin (5.8%). No serious adverse drug reactions (ADRs) were observed.

Dequalinium chloride 10 mg vaginal tablet (Fluomizin) has been developed as a directly compressed vaginal tablet to ensure fast disintegration of the tablet and rapid dissolution of the active substance [Mendling *et al.*, 2016]. As soon as the vaginal tablet comes into contact with the vaginal secretion, it begins to disintegrate and dequalinium chloride is released. After dissolution of the dequalinium chloride 10 mg tablet in an estimated 2.5 – 5 ml of vaginal fluid, the dequalinium chloride concentration is estimated at 2000 – 4000 µg/ml, assuming negligible absorption. This concentration is 4 to 8-fold higher than the minimal inhibitory concentration (MIC) of the least susceptible isolate (MIC = 512 µg/ml) [Hugo and Frier, 1969]. An in vivo anti-infective effect is generally obtained if the concentration of the active substance at the site of action is 2 to 4 times higher than the MIC for 20 minutes up to 2 hours [Della Casa *et al.*, 2002].

The broad-spectrum antimicrobial activity covering all relevant pathogens for vaginal infections and the negligible systemic absorption are the key factors of dequalinium chloride that make it suitable for the treatment of most vaginal infections [Mendling *et al.*, 2016]. Within clinical studies, different vaginal dequalinium chloride preparations have been used to treat more than 3000 women with various vaginal infections [Mendling *et al.*, 2016]. The overall share of ADRs was with 2.4 % very low, showing excellent local tolerance of vaginally administered dequalinium chloride.

A total of 181 pregnant women have been intentionally treated with vaginal dequalinium chloride in four clinical studies, and no adverse effects on pregnancy or on the health of the foetus/new-born child (based on pH, Apgar scale, and follow up until 1 year) have been observed [Mendling *et al.*, 2016].

In conclusion, the current formulation dequalinium chloride 10 mg vaginal tablets (Fluomizin) as 6-day therapy with its broad antimicrobial spectrum and excellent tolerability offers a safe and effective option for empiric therapy of different vaginal infections in daily practice.

2.1.3 Metronidazole

Metronidazole [1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole] is a nitroimidazole antiinfective that has been used in clinical practice for over 25 years [Freeman *et al.*, 1997]. Its original indication was the treatment of Trichomonas vaginalis-infections, but over the years it has been discovered to be useful in treatment of anaerobic infections, especially (BV).

Metronidazole is available for oral or vaginal treatment. The best-known brand is Flagyl, but many generics are marketed as well. Metronidazole could be used orally 500 mg twice daily for 5 or 7 days or as a 2g in a single dose ("one shot"); applied intravaginally as 0.75% vaginal gel once to twice daily for 5 days; or as a vaginal ovula 500 mg once daily for 10 days.

Metronidazole (5-nitroimidazole derivative) is an antiprotozoal and an antibacterial agent with both antibacterial and antitrichomonal activity.

If absorbed systemically (oral use) metronidazole is metabolized in the liver. The half-life of metronidazole is 6-11 hours. Major metabolites of metronidazole are excreted in urine.

Main indications in gynaecology are: (1) (BV) due to anaerobic bacteria and Gardnerella vaginalis, and (2) Trichomonas vaginitis due to Trichomonas vaginalis.

In head to head trials, metronidazole and clindamycin (both so-called "gold standard" treatments recommended by CDC and other guidelines) have equal efficacy with cure rates of 60-90% [Larsson and Forsum, 2005; Bradshaw, Morton*, et al.*, 2006], as shown after 1 week and after 1 month. Furthermore, no difference in treatment failures was seen after 1 week or 1 month when oral versus local applications were compared. Resistance has been described.

Metronidazole is generally well tolerated in general, however after oral use, a bitter, metallic taste in the mouth and gastrointestinal adverse effects (abdominal cramps, nausea) are observed in up to 10% of the patients [Donders, 2010]. In combination with alcohol it is known to induce vomiting, heartburn, stomach pains, i.e. its typical (disulfiram) effect. It is not known whether such general side effects also ensue when used vaginally. According to some studies, metronidazole is claimed not to be teratogenic in humans, even when used in the first trimester of pregnancy. Due to the difference in taste it creates in milk, its use is not advised during lactation.

During systemic application many adverse effects have been reported [Donders, 2010] (gastrointestinal, candidosis haematological, immunological, psychiatric, neuropathies, gastrointestinal, liver, kidney, eye watering). Patients with chronic diseases and thus already using other drugs (Alcohol, Warfarin, Disulfiram, Phenobarbital, Cimetidine, Lithium, Ciclosporin, 5-FU, Busulfan) has limited use of metronidazole due to interactions. Vaginal application is not compatible with contraceptive latex products.

Metronidazole is contraindicated in a first trimester (pregnancy category B) and breastfeeding is not possible since metronidazole appears in milk.

In general, metronidazole has a good efficacy profile against all relevant BV associated pathogens [Freeman et al., 1997].

2.2 Rationale and Objectives

2.2.1 Rationale for Performing the Trial

According to the US Food and Drug Administration (FDA) guidelines, clinical cure is recommended as primary efficacy endpoint as follows: resolution of the abnormal discharge,

a negative KOH "whiff" test, and the presence of the clue cells at less than 20 percent of the total epithelial cells on microscopic examination of the saline wet mount. (Guidance for industry: Bacterial vaginosis; Developing Antimicrobial Drugs for treatment, 2019, www.fda.gov).

Since the effectiveness of the current recommended therapies at 3-4 weeks is about 60 -80% depending on product and study design and the BV recurrence rate within 1 month after therapy varies between 9-57% [Arredondo *et al.*, 1992; Koumans and Kendrick, 2001; Larsson and Forsum, 2005], new approaches to BV therapy are required. Moreover, successful therapy of bacterial vaginosis with both standard drugs metronidazole and clindamycin poses risks for developing vulvovaginal candidosis.

Metronidazole is the leading therapy option used in treatment of BV, and the oral method is more common than the vaginal. The comparison against metronidazole and demonstration of cure rates based on Nugent score should be demonstrated.

Vaginal dequalinium chloride has been shown to have equal clinical efficacy as clindamycin in the treatment of BV and to be well-tolerated with negligible systemic effects [Weissenbacher *et al.*, 2012]. It has the major advantage that its broad antimicrobial activity makes it appropriate for the treatment of mixed vaginal infections and in case of an uncertain diagnosis. Moreover, resistance of pathogens is unlikely due to its multiple modes of action, and vaginal dequalinium chloride also provides a reduced risk for post-treatment vaginal infections of different aetiology that is quite frequent after specific antibacterial or antimycotic treatment.

The test drug is a broad-acting anti-infective agent which also exhibits good efficacy against different Candida species with no known resistances so far. Thus, the current study investigates the efficacy and tolerability of Fluomizin, compared to the current standard therapy, and evaluates whether patients benefit from the test drug and have reduced rate of secondary candidosis in comparison to metronidazole oral therapy.

2.2.2 Trial Objectives

The general aim of this study is to evaluate whether vaginal tablets containing 10 mg dequalinium chloride (Fluomizin) are comparable in clinical efficacy to orally 2x500mg/day for 7 days metronidazole in patients suffering from bacterial vaginosis.

2.2.3 Risk Benefit Assessment

The efficacy and safety of treatment with Fluomizin has already been substantiated in clinical studies, and its safety has been proven in these clinical studies as well as from more following 10 years of post-marketing experience. The mode of action of dequalinium chloride bears a clinical advantage of therapeutic activity because the development of resistance to dequalinium chloride has not been reported. The safety profile of Fluomizin is very favourable; mainly local adverse drug reactions with a low incidence have been observed.

Metronidazole 500mg tablets is one of the internationally acknowledged standard therapies for bacterial vaginosis and is approved in all participating countries for the treatment of bacterial vaginosis.

Metronidazole 500mg once daily orally for 7 days represents a standardised regimen in the therapy of bacterial vaginosis which is indicated in the approved Summary of Product Characteristics in the participating countries. Since only severe cases of bacterial vaginosis (based on Amsel criteria) will be enrolled in the study, the treatment period of 7 days has been selected over the shorter one ensuring the best clinical efficacy, and thus is justified.

Current treatment of BV with both standard drugs metronidazole and clindamycin has the following disadvantages: In clinical studies, recurrence rates – 1 month after therapy – have been observed in the range of 10% to 50%; subsequent development of vulvovaginal candidiasis has been observed in 10% to 25% of the treated patients.

These observations demonstrate the need for efficient alternatives. Fluomizin with its broad antimicrobial activity could reduce the risk of development of vulvovaginal candidosis, probably some recurrences, and especially drug resistances.

If the results of this study are as good as expected and efficient, as demonstrated in previous clinical comparative study with Fluomizin and clindamycin [Weissenbacher *et al.*, 2012], the alternative therapy will be strongly supported, from which future patients will benefit, as the risk for development of vulvovaginal candidosis and resistances is reduced.

3 STUDY OBJECTIVES AND ENDPOINTS

The general aim of this study is to evaluate whether vaginal tablets containing 10 mg dequalinium chloride (Fluomizin) are comparable in clinical efficacy to orally 2x500mg/day for 7 days metronidazole in patients suffering from bacterial vaginosis.

3.1 Primary Objective

To evaluate whether vaginal tablets containing 10 mg dequalinium chloride (Fluomizin) are comparable in clinical efficacy to metronidazole 500 mg oral tablets in women suffering from bacterial vaginosis.

3.2 Secondary Objectives

- To assess the clinical, bacteriological, and therapeutic cure rate on short-term and/or long-term follow-up
- To evaluate subjective assessment of efficacy and tolerability
- To further explore the safety profile of Fluomizin in this patient population

3.3 Endpoints

The primary efficacy variable is the clinical cure rate at C1 (8 to 10 days after randomization) defined as all Amsel criteria (greyish white thin discharge; KOH test or 'fishy' smell; presence of > 20% clue cells; vaginal pH > 4.5) are negative excluding the pH.

The secondary endpoints are:

1. Clinical cure rate at follow-up (at C2 = at 24 to 30 days after randomization)
2. Bacteriological cure rate at C1 and C2
3. Therapeutic cure rate at C1 and C2
4. Individual Amsel criteria at C1 and C2
5. The development of the Nugent Score within 3 categories, i.e. (1) cured, (2) intermediate, and (3) BV positive
6. Subjective assessment of efficacy
7. Incidence of adverse events
8. Subjective assessment of tolerability

4 INVESTIGATIONAL PLAN

4.1 Clinical Trial methodology

The effectiveness of the current recommended therapies at 3–4 weeks is about 60 - 80%, and the recurrence rate of BV within 1 month after therapy varies between 9 - 57% with both numbers depend mainly on study design [Arredondo *et al.*, 1992; Koumans and Kendrick, 2001; Larsson and Forsum, 2005; Bohbot *et al.*, 2010]. The present study is a phase IV study in which the efficacy and safety of Fluomizin in bacterial vaginosis (BV) will be assessed in comparison to one of. recommended standard therapies.

4.1.1 Investigational plan description

This is an international, multi-centre, active-controlled, randomized, double-dummy, phase IV study with parallel group design in outpatients suffering from bacterial vaginosis.
Study participants will be recruited from the group of patients consulting a gynaecologist due to the study-specific indication.
The patients will first be asked by the investigators for a general interest to participate in a clinical trial. If they agree, the Informed Consent procedures described in section 11.4 will be followed and afterwards, the screening visit procedures will be performed.

Screening visit (E)

1. Obtaining patient written Informed Consent before any study specific procedures are performed
2. Diagnosis of BV according to 4 Amsel criteria
3. Sampling of vaginal secretion for bacteriological status for Nugent score
4. Assessing clinical signs & symptoms
5. Urine pregnancy test
6. Reviewing exclusion criteria
7. Documenting demographic data
8. Recording medical history
9. Measuring vital signs
10. Documenting concomitant diseases and concomitant medication (anamnesis)
11. Creating new subject entry in electronic case report form (eCRF) and randomization of subject.
12. Dispensing study medication according to kit number provided, and instructing the patient on how to use it
13. Instructing the patient to keep the packaging of the used/unused study medication and to return it at the next visit
14. Providing link to the online diary/questionnaire and instructing the patient on the use of it
15. Scheduling the next visit within 8 to 10 days after screening/entry visit

C1 - First Control Examination (8 to 10 days after Entry Visit):
Patients will visit the investigator site and then following procedures will be done:

1. Checking the results of the laboratory tests for Nugent score
2. Evaluating the BV clinical status based on Amsel criteria
3. Sampling of vaginal secretion for bacteriological status for Nugent score
4. Assessing clinical signs & symptoms
5. Recording (any new) concomitant medication
6. Checking patient diary & questionnaire (ViedocME) for compliance

7. Recording adverse events
8. Assessing subjective efficacy by investigator
9. Assessing subjective tolerability by investigator
10. Scheduling control visit 2 (C2) 24 to 30 days after entry visit

C2 - Second Control Visit (24 to 30 days after Entry Visit):

1. Evaluating the BV clinical status based on Amsel criteria
2. Sampling of vaginal secretion for bacteriological status for Nugent score
3. Assessing clinical signs & symptoms
4. Recording concomitant medication
5. Urine pregnancy test
6. Checking patient diary (ePRO) for compliance
7. Recording adverse events
8. Assessing subjective efficacy by investigator
9. Assessing subjective tolerability by investigator

Unscheduled Visits (C_u)

Unscheduled visits may take place for a number of reasons, including and not limited to the following:

- Patient contacts the investigator with complaints of a worsening of the symptoms
- AE occurred
- Patient may desire to withdraw

In case the patient drops-out early from the study, the following procedures will be performed:

1. Evaluating the BV clinical status based on Amsel criteria
2. Sampling of vaginal secretion for bacteriological status for Nugent score
3. Assessing clinical signs & symptoms
4. Recording concomitant medication
5. Checking patient diary (ePRO) for compliance
6. Recording adverse events
7. Assessing subjective efficacy by investigator
8. Assessing subjective tolerability by investigator

4.2 Methodological bias management

4.2.1 Randomization

At the entry visit all eligible patients will receive either Fluomizin® (investigational product) with placebo or comparator with placebo based on double-dummy design.
The medication is numbered and used consecutively. If a patient discontinues the study, the subject number will not be reused, and the patient will not be allowed to re-enter the study. The study medication will be dispensed at entry by the physician according to the allocated study medication after randomization.

4.2.2 Blinding

As this is a double-blind double dummy study each subject will receive identical medication kit containing both vaginal and peroral medication from which one will be placebo and the second active substance. There are no laboratory or clinical assessments or specific AEs that could unblind the subject.

4.2.3 Site selection

Study will be run at approximately 15 sites in 3 countries. Only sites equipped for and skilled in microscopic examination of native vaginal smear will be included in the study.

4.2.4 Patient recruitment

There will be a competitive patient recruitment.

4.3 Periods and duration of the trial

The duration of the study for participating subjects is up to 30 days with a treatment period of 7 days.

4.4 Source data

Source data are all the information in original records and certified copies of original records of clinical findings, observations, or other activities in the study, which are necessary for the reconstruction and evaluation of the study. The investigator will permit trial-related monitoring, audit(s), IEC review(s) and regulatory inspection(s), providing direct access to source data/records.

4.5 Implemented measures due to COVID-19 pandemic

Due to the ongoing pandemic of COVID-19, measures will be implemented to ensure the safety and well-being of the subjects. These will be chosen and implemented in accordance with the local regulations and in alignment with the most current version of EMA's Guidance on the management of clinical trials during the COVID-19 pandemic, and recommendations by national competent authorities. Sponsor will monitor the situation closely and continuously and adjust these measures if necessary and communicate these changes to the respective investigators to make sure they will follow these measures and precautions.

Bare minimum of precautions implemented at study sites should be as follows:

- Providing hand-sanitizers at the entrance

- Inquiry regarding subjects' health status and potential COVID-19 symptoms
- Maintaining safe distance from others
- Both subjects and site staff should always wear face masks at the study site when possible
- Number of subjects and patients present at the study site should be maintained with respect to the safe distance between individuals and subjects should be warned not to bring family or friends to the appointment

This is not an exhaustive list and study sites may have different practical solutions for implementation of these precautions. Nevertheless, study sites should adhere to the local regulations and the current knowledge to prevent or minimize risk of SARS-COV-2 transmission.

5 STUDY POPULATION

5.1 Number of participants

236 subjects will be enrolled in approximately 15 sites.

5.2 Inclusion Criteria

To be eligible for the study, subjects must fulfil the following criteria:

1. Premenopausal woman ≥18 years
2. Diagnosis of bacterial vaginosis (all 4 Amsel criteria positive, i.e. 1) greyish white thin discharge, 2) KOH test or 'fishy' smell, 3) microscopic presence of > 20% clue cells, 4) vaginal pH > 4.5)
3. Signed Written Informed Consent to participate in this study

5.3 Exclusion Criteria

The following excludes the patient from the study:

1. Pregnancy and/or lactation based on urine Pregnancy test (women with childbearing potential should use any contraception excluding vaginal methods like vaginal ring, etc.)
2. Uterine bleeding (including menstruation) or vaginal bleeding of unknown origin
3. Ulcerations/erosions of vaginal mucosa or cervix uteri
4. Patients with clinical symptoms and findings of Candida vulvovaginitis and/or Aerobic vaginitis
5. Clinically manifest or suspicion of STIs (Neisseria gonorrhoeae, Chlamydia trachomatis, or Trichomonas vaginalis) based on signs, symptoms, and anamnesis
6. Use of any antimicrobial treatment (local or systemic) 14 days before entry the study
7. Use of any vaginal medication or vaginal douching 7 days before entry the study
8. Unwillingness to refrain from alcohol consumption during treatment, and 48 hours after treatment
9. Severe systemic diseases (HIV, cancer, tuberculosis, autoimmune diseases, diabetes mellitus, severe psychiatric conditions, etc.), including diseases treated with immunosuppressive therapies, systemic corticosteroids, or warfarin
10. Known or suspected hypersensitivity to one of the study medications, inclusive of their excipients
11. Inability to follow the procedures of the study, e.g. due to language problems, psychological disorders, dementia, etc. of the participant (including inability to fill-in electronic patient diary)
12. Participation of patient in another investigational drug study concomitantly or within 30 days prior to entry in the study
13. Patient is relative of, or staff directly reporting to, the investigator

5.4 Other Eligibility Criteria Considerations

NA

5.5 Early Patient Withdrawal

In accordance with the current revision of the Declaration of Helsinki and other applicable regulations, a patient has the right to withdraw from the study at any time and for any reason without prejudice to his or her future medical care from the physician or the institution.

Reasons for removal of the patient from investigational product or observation might include:

- Serious Adverse Events (SAEs)
- Intolerable side effects (Adverse Drug Reactions)
- The patient does not adhere to study rules and procedures
- Worsening of the initial findings
- At the request of the patient (withdrawal of consent)
- In the investigator's opinion it is in the patient's best interest to terminate participation
- Lost to follow-up

When a patient decides to withdraw from the study, all efforts will be made to complete and report the observations as thoroughly as possible. If possible, an examination scheduled for early dropouts at the time of the patient's withdrawal will be performed. The reason for discontinuation of therapy will be recorded in any case in the eCRF.

5.5.1 Voluntary discontinuation by a subject

Subjects are free to discontinue their participation in the study at any time without giving any reason, and without prejudice to further treatment. Subjects who discontinue their participation in the study should always be asked about the reason(s) for their discontinuation and about the presence of any adverse events. If possible, they should be seen and assessed by the investigator(s). Adverse events should be followed up and any diary cards and study medication should be returned.

5.5.2 Incorrectly enrolled or randomized subjects

Incorrectly enrolled or randomized subjects will continue to receive study treatment and assessments if, in the opinion of the investigator and/or study team, this is not considered to involve any risk or discomfort for the subject. Otherwise, those subjects will be discontinued from further study treatment and assessments.

5.5.3 Procedures for discontinuation

The reason for discontinuation and the date of discontinuation from the study must be documented on the eCRF provided. The effect of discontinuation will be considered in the analysis to minimize potential bias. For all discontinuations, the assessments that are specified either at the end of the study period or for the early dropouts should be carried out wherever possible.

5.5.4 Dropouts and withdrawals

Patients dropping out after inclusion into the clinical study will not be replaced. The subjects are not allowed to re-enter the study.

5.6 Early Termination of the Study

The end of this study is defined as the date of the last visit of the last patient undergoing this study.
The sponsor reserves the right to discontinue this study at a single site at any time for different reasons, such as:

- Successful completion of the study at the centre
- The required number of subjects for the study has been reached
- Failure of the investigator to comply with the protocol, the sponsor's procedures or GCP guidelines
- Safety concerns
- Inadequate recruitment of subjects by the investigator.

The sponsor reserves the right to discontinue the whole study at any time. Reasons may include:

- The discovery of any unexpected, serious, or unacceptable risk to the subjects enrolled in the study.
- Successful completion of the whole trial.

6 INVESTIGATIONAL PRODUCT

6.1 Description of Investigational Product(s)

Information about the dosage form, manufacturer and batch number for the investigational product is given in Table 1.

Fluomizin and matching placebo: each 6 vaginal tablets
Metronidazole and matching placebo: each 14 oral tablets

Table 1: Identity of Investigational Product

	Investigational product	Comparator
Name	Dequalinium chloride vaginal tablets (Fluomizin)	Metronidazole tablets
Dosage form and strength	Blister containing 6 vaginal tablets (10 mg)	Blister containing 14 oral tablets (500mg)
Manufacturer / Supplier	Medinova AG, Switzerland / IL-CSM	IL-CSM
Manufacturer of the clinical study medication	Rottendorf Pharma	IL-CSM

6.2 Dose Justification

The dose and mode of administration of Fluomizin® - 1 vaginal tablet 10 mg dequalinium chloride daily, before going to sleep, for 6 days is based on the approved Summary of Product Characteristics in the participating countries. Additionally, placebo oral tablets should be applied by patient twice daily for 7 days based on double-dummy design (Group 1).
Comparator 500mg Metronidazole oral tablets should be taken twice daily for 7 days. Additionally, placebo vaginal tablets should be applied by the patient for 6 days based on double-dummy design (Group 2).
The total treatment duration in both groups is 7 days.

6.3 Comparator Justification

Metronidazole is one of two leading and recommended therapy options used in treatment of BV, and the oral method for metronidazole is more common than vaginal. The comparability of Fluomizin against clindamycin the second recommended therapy has already demonstrated non-inferiority. Thus, the comparison to oral metronidazole is justified.

6.4 Randomization

At the entry visit all eligible patients will receive either Fluomizin® (investigational product) with placebo or comparator with placebo based on double-dummy design.
The medication is numbered and used consecutively. If the subject dropouts from the study, the subject number will not be reused, and the patient will not be allowed to re-enter the study. The study medication will be dispensed at entry by the physician according to the allocated study medication after randomization.

6.5 Administration

One tablet of the investigational product, i.e. Fluomizin vaginal tablets (10 mg Dequalinium chloride) will be administered intravaginally daily, before going to sleep, for 6 days.
The comparator Metronidazole 500mg tablets will be administrated orally twice daily for 7 days.
Treatment has not to be initiated during menstruation and in 7 days before the expected period of menstrual bleeding. If unexpected significant vaginal bleeding occurs, the vaginal treatment must be interrupted and resumed afterwards, while the oral treatment continues without interruption. Patients must return all partially used and unused medication, as well as empty containers to the investigator at the end of therapy. **T**reatment compliance will be assessed by the investigator by asking the patients about administrating the study medication as prescribed and recording the drug accountability, by reviewing diary entries and by calculating compliance of the investigational product.

6.6 Product Labelling

The study medication will be supplied in wallets: containing 6 vaginal tablets for patients allocated to Fluomizin and 14 oral tablets Metronidazole Placebo and vice versa. Schematics are depicted in Figure 1, actual photos then in Figure 2. Placebo vaginal and oral tablets will be provided based on double-dummy design requirements.

The labels will contain at least the following information: route of administration, number of doses per day, study code, subject number, batch no., expiry date, as well as the following instructions for use: for clinical study use only, keep out of the reach of children, store at room temperature and not above 30˚C, return at the next visit all partially used / unused study medication. The labelling can be adapted according to national regulations.
Dosage adjustments during the study are not planned.

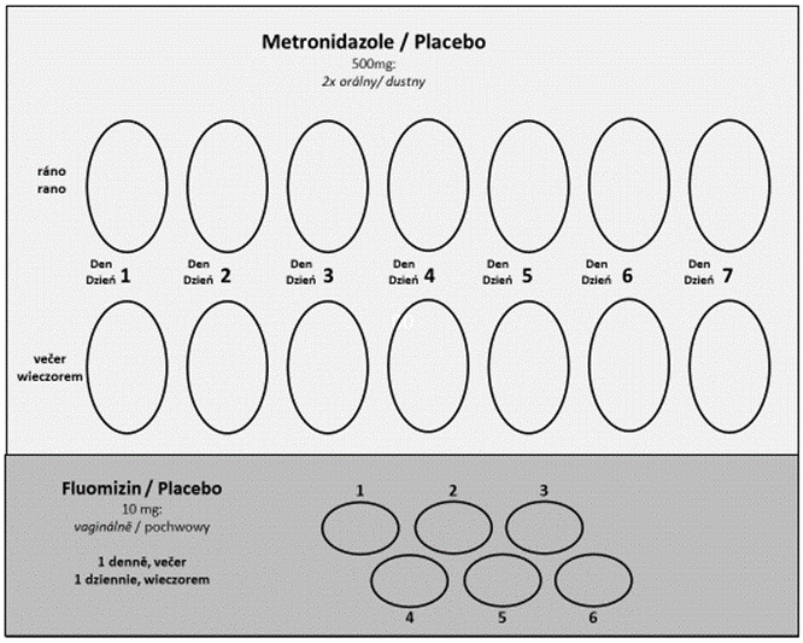

Figure 1: Schematics of the booklet with treatments

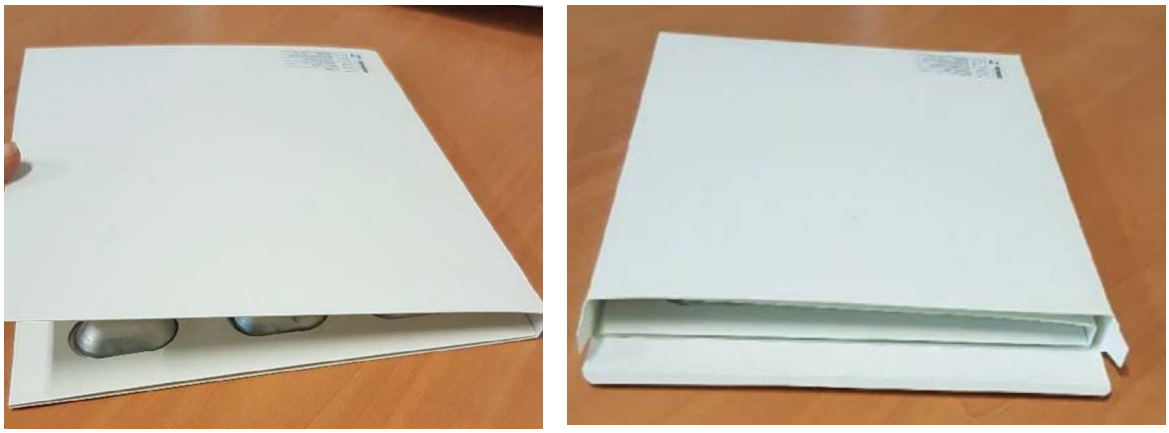

Figure 2: Actual booklet

6.7 Unblinding

Breaking of the blinding of individual subjects is only permitted in case of a serious or unexpected adverse event or other medical event, when knowledge of the type of investigational drug given is required for therapeutic decisions for this event.

6.8 Handling and Storage of Study Drugs

Investigational products and placebo tablets must be kept in a secure place under appropriate storage conditions. All study drugs will be stored at room temperature and not above 30˚C in their original containers, until dispensed to subjects. Study medication must not be frozen.
A drug dispensing log will be kept current by the investigator, detailing the dates and quantities of investigational medicinal product dispensed to each subject. The inventory will be available to the monitor to verify drug accountability during the study. Any unused reusable

investigational medicinal product, either not dispensed or returned by the subject, including empty containers will be accounted for and returned to the sponsor.

When required/allowed by local policies, unused supplies may be destroyed at the study centre. The investigator will ensure that such disposition does not expose subjects to risks from the investigational medicinal product. The investigator will maintain records of any such alternative disposition to permit an accurate drug accountability. Destruction of any unused medication must only be performed after the final drug accountability by the monitor has been done and the concerned medication has been marked for destruction. Documentation of destruction (which IMP, batch numbers, date of destruction etc) must be provided and filed.

7 STUDY ASSESSMENTS AND PROCEDURES

7.1 Study Schedule

7.1.1 Screening

Screening is based on the diagnostic criteria of BV: a) a vaginal pH higher than 4.5; b) the presence of clue (vaginal epithelial) cells in the vaginal fluid; c) a thin, grey or white homogenous discharge, d) a positive KOH "whiff" test (the release of an amine [fishy] odour upon the addition of 10% potassium hydroxide to the vaginal fluid).

7.1.2 Treatment

The treatment duration for both groups is 7 days.
One tablet of the investigational product, i.e. Fluomizin vaginal tablets (10 mg Dequalinium chloride) or matching placebo will be administrated intravaginally daily, before going to sleep, for 6 days. The comparator Metronidazole 500mg tablets or matching placebo will be administrated orally twice daily for 7 days.

7.1.3 Follow-up / End of Study

After the patients have left the study, there will be no special treatment which differs from the normal treatment of the study indication. The investigator must decide if a continuation of medical treatment is necessary and how it will look like. For the follow-up of adverse events after patients have left the study see Section 8.

7.2 Efficacy Assessment

Variables are assessed either clinically by the evaluation of symptoms or using vaginal secretion for microscopy and Nugent score.
There will be a total of 3 vaginal samples:

1. Vaginal sampling, and assessment of pH and Amine test
2. Vaginal sampling for microscopy (native smear with 0.9% NaCl)
3. Vaginal sampling for laboratory examination: Nugent score

The sample of the vaginal secretion will be taken from the posterior vaginal vault with a plastic or wooden Ayre's spatula (no cotton swabs) and spread onto glass slides.

Sample 1: KOH 10% solution will be later added to one of the slides for "whiff" (Amine) test.
Sample 2: One drop of 0.9% NaCl solution will be placed on the second slide for the immediate microscopic (phase contrast 400-fold magnification) evaluation (number of clue cells, presence of Candida, exclusion of *Trichomonas vaginalis*).
Sample 3: This sample of the vaginal secretion is used for Gram staining in the central laboratory for Nugent scoring.

7.2.1 Patient Characteristics

The following variables will be collected in the eCRF and the patient's record:

- Month and year of birth, race, date of last menstruation, number of births and/or pregnancies, body height

- Vital signs: weight, heart rate, systolic and diastolic blood pressure will be measured. Resting blood pressure and heart rate should be measured after 5 minutes sitting
- Number of vaginal infections occurred during the last 12 months; number of bacterial vaginosis occurred during the last 2 years
- Concomitant medication, concomitant diseases

7.2.2 Primary Parameter

The primary efficacy variable is the clinical cure rate at visit C1 based on the Amsel criteria (vaginal pH > 4.5, presence of clue cells, KOH test, greyish white, malodorous discharge, as seen in Table 2), where clinical cure is defined as: 3 criteria negative, excluding pH (Table 3).

Table 2: Amsel Criteria for diagnosis

Greyish white, thin, malodorous discharge	yes ☐ no ☐
Presence of clue cells: ≥ 20% of the total epithelial cells on microscopic examination of the saline wet mount	yes ☐ no ☐
A fishy odour of the vaginal discharge with a drop of 10% KOH	yes ☐ no ☐
Vaginal pH > 4.5	yes ☐ no ☐

Table 3: Cure rate definition

Greyish white, thin, malodorous discharge	**No**
Presence of clue cells: ≥ 20% of the total epithelial cells on microscopic examination of the saline wet mount	**No**
A fishy odour of the vaginal discharge with a drop of 10% KOH	**No**

7.2.3 Secondary Parameters

The secondary efficacy parameters are:

1. Clinical cure rate at follow-up (at C2 = at 24 to 30 study days)
2. Bacteriological cure rate at C1 and C2
3. Therapeutic cure rate at C1 and C2
4. Evaluation of individual Amsel criteria at C1 and C2
5. The development of the Nugent Score within 3 categories, i.e. (1) cured, (2) intermediate, and (3) BV positive
6. Subjective assessment of efficacy

7.2.3.1 Clinical cure rate at follow-up (Amsel criteria)

The clinical cure rate based on the Amsel criteria (vaginal pH > 4.5, presence of clue cells, KOH test, greyish white, malodorous discharge), where clinical cure is defined as: 3 criteria negative, excluding pH. The cure rate will be assessed as secondary efficacy variable at visit C2.

7.2.3.2 Bacteriological cure rate at C1 and C2

The bacteriological status will be determined by the Nugent score. The Nugent score includes the evaluation of:

- Gram positive bacteria (Lactobacillus), with classes from zero to four, where zero stands for more than 30 bacteria/field of view and four for no bacteria.
- Gram negative or gram-labile bacteria (e.g. Gardnerella, Prevotella and Bacteroides), with classes from zero to four, where numbers increase with increasing bacteria numbers.
- and gram-labile bacteria (Mobiluncus), with classes from zero to two, where numbers increase with increasing bacteria numbers.

These single scores are then summed to the overall Nugent score, that ranges between zero and ten, where a score of

- 0-3 is a normal flora and indicates no bacterial vaginosis,
- 4-6 is intermediate flora and indicates that a bacterial vaginosis should be considered with regard to the clinical symptoms,
- and from 7-10 is considered bacterial vaginosis

Bacteriological cure is defined as Nugent score ≤ 3.

7.2.3.3 Therapeutic cure at C1 and C2

The therapeutic cure rate is defined as a combination of clinical and bacteriological cures, i.e. patient has to meet the criteria of both.

7.2.3.4 Evaluation of individual Amsel criteria

a) Clue cells

The number of clue cells will be determined by phase contrast microscopy using a 400× magnification. The proportion of clue cells as compared to the total number of epithelial cells without adherent bacteria is reported semi-quantitatively. The presence of clue cells is considered positive if ≥ 20% of the total epithelial cells of the wet mount are clue cells.

b) KOH test for volatile amines

A positive KOH test for volatile amines involves the presence of a fishy amine odour on mixture of secretions with 10% KOH (due to the volatilisation of the amine products of anaerobic bacterial metabolism).

c) Vaginal pH

The pH of the vaginal secretion will be measured using pH strips Merck, colour scale 4.0-7.0 with a measurement interval of about 0.3. The pH should not be measured at the cervix, but at the lateral vaginal wall in the upper third of the vagina.

d) Abnormal vaginal discharge

The BV-specific discharge and the specific type of smell will be evaluated as present or absent.

The development of the individual Amsel criteria will be analysed over time.

7.2.3.5 The development of the Nugent Score

The development of the 3 Nugent Score categories (1) Normal (score 0 - 3), (2) intermediate (score 4 - 6), and BV positive (score 7 - 10) will be analysed over time.

7.2.3.6 Subjective assessment of efficacy

The subjective assessment of therapy efficacy will be subjectively assessed as the overall satisfaction with the treatment by both the investigators and the patients. At each control, the subjective assessment of efficacy will be rated using 4 categories:

* very good
* good
* moderate
* poor

7.3 Safety Assessments

Safety assessments will consist of recording and monitoring of:

1. Adverse events (AEs) and serious adverse events (SAEs)
2. Subjective assessment of tolerability
3. Vital signs

7.3.1 Adverse events

An adverse event (AE) is any untoward medical occurrence in a patient or clinical investigation subject administered a medicinal product, and which does not necessarily have a causal relationship with this treatment.
AEs will be elicited by a standard non-leading question like "How did you feel since last visit?" All AEs encountered during the study will be recorded in the detail indicated in the CRF, regardless of their relationship to the investigational medicinal product as assessed by the investigator.
See Chapter 8 Safety reporting for more details.

7.3.2 Subjective assessment of tolerability

The subjective assessment of tolerability shall be performed by both the investigator and the patients.
The subjective assessment of tolerability will be rated using 4 categories:

* very good
* good
* moderate
* poor

7.3.3 Vital Signs

The following vital signs will be measured: Weight, heart rate, systolic and diastolic blood pressure. Resting blood pressure and heart rate should be measured after 5 minutes sitting.

7.4 Additional Aspects

7.4.1 Assessment of treatment compliance through patients' diary

Treatment compliance shall be assessed based on the patient diary with regard to number doses taken and treatment duration.

7.4.2 Time to Resolution of symptoms

Based on patient diary the time until resolution of symptoms shall be analysed.

7.5 Study Restrictions

The use of the following medications is not allowed:

- Use of any antimicrobial treatment (local or systemic) during the study
- Use of any vaginal medication or vaginal douching during the study
- Immunosuppressive therapy during the study
- Alcohol consumption during treatment, and 48 hours after the treatment

7.5.1 Confinement

NA

7.5.2 Concomitant Medication

Subjects should receive medical treatment for their condition as per standard of care. Medication history and concomitant medications will be taken at the randomization and recorded on the eCRF.

8 SAFETY REPORTING

8.1 Definitions and categories of Adverse Events

8.1.1 Adverse Events (AEs)

An adverse event (AE) is any untoward medical occurrence (i.e. any unfavourable and unintended sign (including abnormal laboratory findings), symptom or disease) in a patient or clinical investigation subject after providing written informed consent for participation in the study. Therefore, an AE may or may not be temporally or causally associated with the use of a medicinal (investigational) product.

A surgical procedure that was planned prior to the start of the study by any physician treating the subject should not be recorded as AE (however, the condition for which the surgery is required should be documented in the medical history section or may even be reported as an AE).

In the following differentiation between medical history and AEs, the term “condition” may include abnormal e.g. physical examination findings, symptoms, diseases, or laboratory test results.

- Conditions that started before signing of informed consent and for which no symptoms or treatment are present until signing of informed consent are recorded as medical history (e.g. seasonal allergy without acute complaints).

- Conditions that started before signing of informed consent and for which symptoms or treatment are present after signing of informed consent, at unchanged intensity, are recorded as medical history (e.g. allergic pollinosis).

- Conditions that started or deteriorated after signing of informed consent will be documented as adverse events.

Overdose and its subsequent symptoms shall be documented as AE.

The following events are not considered to be adverse events:

- Medical or surgical procedures (e.g., surgery, endoscopy, tooth extraction, transfusion). However, the disorders, which led to the procedures, are AEs, if they did not exist before or if they worsened during or after administration of the investigational medicinal product.

- Pre-existing diseases or conditions, which do not worsen during or after administration of the investigational medicinal product.

- The disease under study or signs and symptoms associated with the disease, unless more severe than expected for the subject’s condition according to the opinion of the investigator.

8.1.2 Adverse Drug Reactions (ADR)

All noxious and unintended responses to a medicinal product related to any dose should be considered adverse drug reactions. The phrase "responses to medicinal products" means that a causal relationship between a medicinal product and an adverse event is at least a reasonable possibility, i.e., the relationship cannot be ruled out.

8.1.3 Unexpected Adverse Drug Reactions (UADR)

An adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g., Investigator's Brochure for an unapproved investigational medicinal product).

8.1.4 Serious Adverse Event (SAE) / Serious Adverse Reaction (SAR)

Serious adverse event (SAE) or serious adverse drug reaction (SADR) is classified as any untoward medical occurrence that at any dose (including overdose) meets at least one the following criteria:

- results in death
- is life-threatening
- requires inpatient hospitalization or prolongation of existing hospitalization
- results in persistent or significant disability or incapacity
- is a congenital anomaly or birth defect
- is an important medical event (e.g. requires medical treatment to avoid one of the above-mentioned conditions)

In this trial SAE reporting period will start with randomization and last until end of study.

Medical and scientific judgment should be exercised in deciding whether a medically important event or an adverse event is serious in other situations. Important adverse events that are not immediately life-threatening or do not result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse. Laboratory abnormalities may only be classified as SAE if deemed clinically significant by the investigator. Suspected Unexpected Serious Adverse Reaction (SUSAR).

8.1.5 Adverse Events of Special Interest (AESI)

Adverse events of special interest (AESI) are events that have by character a special meaning or importance for the particular drug. An AESI might have a serious or non-serious character. In this study the following is determined as an AESI:

- Vulvovaginal candidosis

8.1.6 Adverse Event Intensity/Severity

Severity of AEs will be assessed according to the following definitions:

- **Mild**: sign or symptom of the AE is apparent but is easily tolerated by the subject.
- **Moderate**: the AE interferes somewhat with the subject's usual activities (disturbing).
- **Severe**: the AE prevents the subject from working or performing his/her usual activities (unacceptable).

Note: Severity is not seriousness. An AE may be severe but not serious, as in a severe headache, while an SAE may be mild, as in a mild myocardial infarct.

8.1.7 Assessment of association with the investigational products

Careful medical judgment should be exercised to determine if there is a causal relationship between an AE and the investigational product. The following guidance is provided:

- **Certain:** A clinical event, including laboratory test abnormality, occurs in a plausible time relationship to drug administration, and which cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug (de-challenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, using a satisfactory re-challenge procedure if necessary.
- **Probable:** A clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the drug, unlikely to be attributed to concurrent disease or other drugs or chemicals, and which follows a clinically reasonable response on withdrawal (de-challenge). Re-challenge information is not required to fulfil this definition.
- **Possible:** A clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the drug, but which could also be explained by concurrent disease or other drugs or chemicals. Information on drug withdrawal may be lacking or unclear.
- **Unlikely:** A clinical event, including laboratory test abnormality, with a temporal relationship to drug administration which makes a causal relationship improbable, and in which other drugs, chemicals or underlying disease provide plausible explanations.
- **Unrelated:** A clinical event, including laboratory test abnormalities, for which a causal association between the study drug and the event can be excluded.

8.2 Documentation of Adverse Events and Serious Adverse Events

Adverse events and all information regarding SAEs, whether reported by the subject or observed by the investigator/study personnel, must be documented in the subject's medical record, and recorded on the Adverse Event/Serious Adverse Event Forms in the eCRF.

One of the aims of the study is to assess the safety and tolerability of the study drug. The investigator is responsible for recording and reporting AEs observed from randomization until end of study.

Medical events occurring during the screening period (i.e. after obtaining the subject's written consent, but before randomization) will not be recorded as AE but on the Medical History page in the eCRF, except those resulting from a protocol-mandated procedure, which should be reported on the Adverse Event/Serious Adverse Event Forms.

All AEs and SAEs will be followed until resolution, until the condition stabilizes if irreversible, until the event is otherwise explained, or until the patient is lost to follow-up. Once resolved, the appropriate AE/SAE eCRF sections will be updated. The investigator will ensure that follow-up includes any supplemental investigations as may be indicated to elucidate the nature and/or causality of the AE or SAE. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.

At any time after the end of study, if an investigator learns of an AE or SAE that can be reasonably related to study drug, he should promptly notify the sponsor.

8.3 Reporting of SAEs, SUSARs and AESIS

All SAEs must be reported immediately to the pharmacovigilance department of the CRO GCP-Service International Ltd. & Co. KG as soon as they become known to the investigator and not later than within 24 h of their knowledge of the occurrence of an SAE.

The SAE Report Form is included in the eCRF system and must be completed and submitted according to the instructions provided on the form. In case the CRF cannot be accessed and hence the SAE Report Form cannot be filled in within the eCRF system, a paper SAE Report Form is filed as back-up in the Investigator Site Folder (ISF) which should be used and sent via fax using the contact details below. The information on the form must be transferred to the electronic CRF when it is available again.

CRO: GCP-Service International Ltd. & Co. KG

Email: pv@gcp-service.com

Phone +49-(0)421-8967 6636

Fax +49-(0)421-1669 7547

Expedited Reporting

GCP-Service International Ltd. & Co. KG will report all AEs that are serious, unexpected, and with a reasonable possible causality to the IMP (Suspected Unexpected Serious Adverse Events, SUSARs) as judged by either the investigator or sponsor within the stipulated timelines.

SAEs will be considered reportable, regardless of whether or not the IMP was used in accordance with the provisions in the protocol, Investigator's Brochure, and labelling.

In accordance with national regulations and GCP guidelines the sponsor or his representative should promptly notify all concerned investigators, institutions and the regulatory authorities of SUSARs and all findings that could affect adversely the safety of subjects, impact the conduct of the study or alter IEC/authority approval to continue the study.

All available information relevant for the evaluation of the SAE must be reported by the investigator. Adverse events that are serious but expected (related and/or unrelated to the IMP) will be reported in accordance with local requirements.

Timelines:

Fatal or life-threatening SUSARs must be reported to local competent authorities of countries where the trial is conducted as soon as possible, but not later than 7 calendar days after becoming aware of the event, with a possible follow-up report within 8 additional calendar days.

All other SUSARs must be submitted as soon as possible, but no later than 15 calendar days after becoming aware of the case.

8.4 Pregnancy

To prevent pregnancies during the course of the study, patients are to be informed about highly effective contraception methods. Local barrier methods should be excluded.

Patients will be instructed to notify the investigator in the case of pregnancy during the study. If a pregnancy is suspected during the study, the study medication must be immediately withheld until the result of a pregnancy test is available. If the pregnancy is confirmed, the use of study medication must be stopped by the patient and the patient must be withdrawn from the trial. The Pregnancy Form must be completed and the sponsor (representative of sponsor) must be informed by fax within 24 h after first knowledge of the pregnancy.

A newly diagnosed pregnancy itself will not be considered as an AE but reported as such. Adverse Events related to pregnancy must be reported like any other AE. The mother and the fetus will be followed up at least until the final outcome (e.g. birth, induced or spontaneous

abortion etc.). In general, the follow-up will include the course, duration and the outcome of the pregnancy and the infant. In case of normal outcome, the infant will be followed up for 4-6 weeks after the birth.

If the outcome of the pregnancy meets the criteria for immediate classification as a SAE (e.g. spontaneous abortion [any congenital anomaly detected in an aborted fetus is to be documented], stillbirth, neonatal death or congenital abnormally), the investigator will report the event by completing a SAE form within 24 hours after becoming aware of the event.

8.5 Data Safety Monitoring Board

NA

8.6 Development Safety Update Report

Once a year or on demand, the sponsor will supply a report on the safety of trial subjects in accordance with the guidance ENTR/CT 3 with all relevant information during the reference period to the competent federal authority and the responsible ethics committee.

The reference period for the annual report on the safety of trial subjects begins with the date that the trial is approved by the competent federal authority. This date is the reference date for the start of the year of the annual Development Safety Update Report (DSUR). The sponsor will supply the report once a year within 60 days after the annual reference date (data-lock point).

9 STATISTICAL METHODS

This section describes the main statistical and analytical aspects foreseen at the time of study planning. The main analysis of the primary endpoint will be performed according to the statistical methods described in this study protocol. A Statistical Analysis Plan (SAP) will additionally be written by the study statistician, containing more technical and detailed explanation of methods used, as well as additional information on the analysis of secondary criteria and a list of the output that will be generated. This SAP will be finalized and approved before the first analysis of study data for the interim analysis.

Changes made to the envisaged analysis after the SAP is finalized and prior to the analysis as well as their reason(s) should be described in the SAP or, in case of important changes (e.g. changes affecting the main analysis of the primary endpoint) in amendments to the study protocol. If the actually performed analysis deviates from the SAP, changes, reasons and impact will be described in the clinical study report.

The statistical analysis will be done using SAS® Version 9.4 or higher.

9.1 Determination of Sample Size

The sample size calculation for the trial is based on the proportions of clinical cure rate (greater than or equal to 80%, based on the Amsel criteria) 8 to 10 days after start of treatment as the primary endpoint.

This clinical trial is designed to evaluate if Fluomizin is at least as efficacious as Metronidazole in terms of the clinical cure rate as a non-inferiority comparison.

In this study, the alternative hypothesis H_1 is that the clinical cure rate of Fluomizin, π_F, differs from the clinical cure rate of Metronidazole, π_M, by less than the clinically significant difference, δ (non-inferiority margin: 15%), or Fluomizin has higher cure rate compared to Metronidazole. The null hypothesis, H_0, therefore is that Fluomizin is equal or worse than Metronidazole by the clinically significant difference, δ.

Thus,

H_0: $\pi_F - \pi_M \leq -\delta$

H_1: $\pi_F - \pi_M > -\delta$

The minimum number n, of evaluable patients necessary for each treatment groups is determined by the following equation:

$$n = \frac{2\left(z_{1-\alpha} + z_{1-\beta}\right)^2 \pi_M (1 - \pi_M)}{\delta^2}$$

Assuming

- $z_{1-\alpha}$: The 1- α quantile of the normal distribution, with α=2.5%, the type 1 error
- $z_{1-\beta}$: The 1- β quantile of the normal distribution, with 1-β=80% the power
- an incidence of successful treatment with Fluomizin according to previous studies [Weissenbacher *et al.*, 2012]:
  π_F : 80% (0.80),

- an incidence of successful treatment with Metronidazole according to previous studies that found cure rates between 60-90% [Larsson and Forsum, 2005; Bradshaw, Morton, *et al.*, 2006]: π_M: 80% (0.80)
- smallest clinically relevant differences in the responder rate in treatment of Fluomizin and Metronidazole (δ) of maximum 15% (0.15) are defined as clinically relevant to show non-inferiority of Fluomizin compared to Metronidazole,

the minimum number of evaluable patients per treatment group is 112. Assuming a drop-out rate of 5 %, a total of 118 patients per treatment group is planned. Since in this case the sample size is larger than 50 and the proportion is 0.8, this power calculation gives similar results as if binomial enumeration would be used.

Screening failures and patients withdrawing prior to application of treatments will be replaced.

The sample size was calculated by using nQuery from Statsols, Version 8.5.2.0. For more details regarding efficacy assumptions see sections 2.1.2 for Fluomizin and 2.1.3 for Metronidazole.

9.2 Subject Disposition

The numbers and percentages of subjects will be summarized separately for the following subsets, overall and per site:

- Screened
- Screening failures
- Randomized
- Randomized and treated
- ITT
- PP
- SES
- Nugent score subgroup at baseline (score 0 – 3, score 4 – 6, score 7 - 10)
- Subjects completing the study
- Subjects terminating the study early

Reasons for screening failures and early termination will additionally be tabulated.

Demographics and baseline characteristics will be summarized by randomized groups for the ITT and PP populations and by the actual treatment group for the safety population.

9.3 Analysis Populations

9.3.1 Intention To Treat (ITT)

In the intention to treat set all subjects having been randomized and treated will be included. This set will be used for the primary analysis

For the analysis with the ITT population, subjects will be allocated to the treatment group they were randomized to, following the intention-to-treat principle.

9.3.2 Per protocol population (PP)

The Per Protocol set includes all subjects that have not experienced major protocol violations. This set of subjects is used for additional and supportive sensitivity analyses.

Major protocol deviations are events that have direct impact on the primary endpoint. Possible examples of major protocol violations are:

- Violations of inclusion or exclusion criteria
- Use of any antimicrobial treatment (local or systemic) during the study
- Use of any vaginal medication or vaginal douching during the study
- Immunosuppressive therapy during the study
- Alcohol consumption during treatment, and 48 hours after the treatment
- Major time window violations

9.3.3 Safety Evaluation Set (SES)

The SES includes all study subjects who have received Fluomizin or Metronidazole. For the analysis with the SES population, subjects will be allocated to the treatment group they actually received, rather than to that the one they were randomized to. This set will be used for the safety analysis.

9.3.4 Subgroups

The Nugent score represents an important factor for the severity of the disease. Therefore, subgroups based on the Nugent score at baseline will be defined:

- Normal: subjects with score 0 - 3 at baseline
- Intermediate: subjects with score 4 – 6 at baseline
- BV positive: subjects with score 7 – 10 at baseline

9.4 Statistical Analysis

9.4.1 Descriptive statistics

Summary statistics will be provided at least in the following way:

- Quantitative Parameters will be presented in terms of mean, standard deviation, median, Q1, Q3, extreme values, number of patients and missing data.
- Qualitative parameters will be presented in terms of number and percentage of each modality as well as number of patients and missing data.

If not specified otherwise, percentages will be based on the number of subjects with data available in the group of interest within the analysis set. Percentages will not include missing data. If applicable, the number of missing items for the analysis will be given in a “missing” category.

9.4.1 Demographics and baseline

Additionally to the at baseline taken variables for primary, secondary and safety analysis (see sections 0, 9.4.4 and 9.4.5), only the parameter age will be assessed. It will be presented in terms of mean, standard deviation, median, Q1, Q3, extreme values, number of patients and missing data.

9.4.2 Medical history

Absolute and relative frequency of medical history and concomitant diseases as well as past and current medication will be tabulated based on coded terms (MedDRA or WHO-CC, as applicable). Data will be analysed by system organ class (SOC) and preferred term (PT). Further details will be explained in the SAP. Primary Efficacy Analysis

9.4.3 Main analysis

The main criterion of this trial is to investigate if Fluomizin has an equal or better clinical cure rate in treatment of bacterial vaginosis compared to Metronidazole after 8-10 days. The clinical cure rate is a binary response-variable, defined by the evaluation of the Amsel criteria. Further confirmatory analyses are not foreseen for this trial.

In addition to the standard summary statistics (as described in section 9.4.1), the differences in clinical cure rate between Fluomizin and Metronidazole will be analysed in a confirmatory manner using a one-sided Farrington-Manning test [1990] on a 2.5% significance level with following hypothesis:

H_0: $\pi_F - \pi_M \leq -\delta$

H_1: $\pi_F - \pi_M > -\delta$

where

H_0 = null hypothesis for the non-inferiority test of the primary endpoint
H_1 = alternative hypothesis for the non-inferiority test of the primary endpoint
π_M = clinical cure rate of Metronidazole
π_F = clinical cure rate of Fluomizin
δ = smallest clinically significant difference between the two groups (non-inferiority margin: 15% or 0.15)

With the Farrington and Manning [1990] Likelihood Score test, differences in proportions between two groups can be tested as following:

$$z_{FMD} = \frac{\hat{p}_F - \hat{p}_M - \delta}{\sqrt{\frac{\tilde{p}_M * (1 - \tilde{p}_M)}{n_M} + \frac{\tilde{p}_F * (1 - \tilde{p}_F)}{n_F}}}$$

where

z_{FMD} = the Farrington-Manning test statistic
δ = smallest clinically significant difference between the two groups (non-inferiority margin: 15% or 0.15)
n_M = number of subjects in Metronidazole group
n_F = number of subjects in Fluomizin group
$\hat{p}_M$ = proportion of success rate in Metronidazole group
$\hat{p}_F$ = proportion of success rate in Fluomizin group
$\tilde{p}_M$ = maximum likelihood estimation of the probability of success in Metronidazole group
$\tilde{p}_F$ = e maximum likelihood estimation of the probability of success in Fluomizin group

It is evaluated whether the observed difference is greater than the non-inferiority margin -δ. The test statistic is based on the asymptotic normality of the score statistic.

Efficacy will be analysed based on the ITT population and repeated as sensitivity analysis for PP population. The ITT is selected as primary analysis set to conduct the analysis in a most realistic scenario. The treatment effect could be interpreted as the mean difference in the clinical cure rate of patients diagnosed by using the Amsel criteria. This reflects the practice most realistically, where the treatment decision is also based on the Amsel criteria. The

Farrington-Manning test will provide a p-value for the outcome variable, on which the decision will be based. Furthermore, the analysis will be made by Nugent score subgroups (see section 9.3.4).

If non-inferiority can be shown, data can furthermore be tested for superiority of Fluomizin over the leading therapy option Metronidazole. The Farrington-Manning test is also providing a confidence interval for this purpose, which will be specified with 0.95 (95%). The hypotheses tested for the evaluation of possible superiority are the following:

H_0: $\pi_F - \pi_M \leq \delta$

H_1: $\pi_F - \pi_M > \delta$

where denotations are the same as explained for the non-inferiority hypothesis above.

If the cure rate of Fluomizin exceeds Metronidazole by more than 15% (δ), and the null hypothesis can be rejected, then Fluomizin is considered superior to Metronidazole with respect to efficacy.

There is no multiplicity problem to be considered since this analysis corresponds to a closed hierarchical test procedure.

9.4.3.1 Additional sensitivity analysis

1. *Logistic regression model to consider the parameter time*:

   Apart from the above-mentioned sensitivity analysis, additionally a logistic regression will be calculated for the ITT set to consider the large variance in time given by broader frames of visit schedules. The factor time will be included in the model by a time variable that includes the day on which the visit was performed (8-10). It will be furthermore evaluated, which other considerable variables have an influence on the observed events. Candidates for explanatory parameters included could be age, weight, treatment, and the continuous Variable of the Nugent score:

   Logit (Amsel criteria=1 | $X_i=x_i$) = Intercept + x_{i1}*Time + x_{i2}*Age + x_{i3}*Weight + x_{i4}*Treatment + x_{i5}*Nugent Score

   Odds ratios for the specified variables will be given, and a p-value provided.

2. *Logistic regression to measure effect of Nugent Score*:

   To furthermore evaluate the effect of the Nugent score criteria on the outcome variable, a logistic regression will be calculated including the explaining parameters ‘Nugent score’ and ‘treatment’ only. Odds ratios for the specified variables will be given, and a p-value provided.

3. *Tipping point analysis*:

   Another sensitivity analysis addressing the missing values will be a tipping point analysis, which allows to evaluate the effect of missing data on the outcome. It can determine, for which success probability for the missing subjects in the Fluomizin group the conclusions regarding the primary variable remain stable. For this analysis it is assumed that missing values are missing not at random, and that there is a tipping point that reverses the study outcome.

   This analysis is conducted by the following steps:

   1. Missing values are multiply imputed into the dataset a defined number of times.

2. The imputed values for missing observations in the Fluomizin group are then adjusted by a shift parameter (success probability), to consider that the distribution of missing responses have a lower expected value than those of the observed ones.
3. After a logistic regression will be performed for each imputed dataset, the proportion of tests that would reject the null hypothesis will be calculated.
4. Step 1 to 3 will be repeated until tipping point, meaning the according shift parameter, is found, where the p-value changes to be > 0.05.

This approach is like a stress-test, to assess how severe deviations of success rates within the missing values have to be, to overturn the conclusions from the primary analysis.

9.4.4 Secondary Efficacy Analysis

The following described analyses are of exploratory nature and are used as supportive analyses for the primary endpoint. When possible p-values will be provided to evaluate differences between treatment arms. The analysis will be based at least on the ITT and repeated for the subgroups in section 9.3.4.Further details will be provided in the statistical analysis plan.

9.4.4.1 Clinical cure rate at C2

The clinical cure rate at C2 (after 24 to 30 days) will be analysed as a secondary parameter to test the long-term efficacy based on the Amsel-criteria. Analysis will be conducted as described for the primary endpoint (see section 9.4.3) in an exploratory manner based on the ITT.
A logistic regression model including the factor time will be conducted as a sensitivity analysis according to section 9.4.3.1, point 1.

9.4.4.2 Bacteriological and therapeutic cure rate

The bacteriological cure rate (Nugent score ≤ 3) will be summarized and presented descriptively and in an exploratory nature for Visits C1 (after 8 to 10 days) and C2 (after 24 to 30 days).
The therapeutic cure rate (clinical and bacteriological cures) will be evaluated descriptively for Visits C1 (after 8 to 10 days) and C2 (after 24 to 30 days). If both aspects, the Nugent-score, and the Amsel-criteria have improved as defined (Nugent-score ≤ 3, Amsel criteria all negative except pH), the patient is assumed to be cured on a clinical and bacteriological level.

In both cases groups will be compared with a z-test for two proportions and an exploratory evaluation made, based on the given p-value.

9.4.4.3 Evaluation of Nugent Score

The development of the 3 Nugent Score categories (1) Normal (score 0 - 3), (2) intermediate (score 4 - 6), and BV positive (score 7 - 10) will be analysed over time in an exploratory manner; p-values will be provided comparing the two treatment arms.

9.4.4.4 Evaluation of individual Amsel criteria

The number of clue cells, the KOH test, the vaginal pH and the absence/presence of abnormal vaginal discharge will be analyzed descriptively, and an exploratory evaluation made, based on the given p-value.

9.4.4.5 Subjective assessment of efficacy

A subjective assessment of efficacy of the two treatments will be made by evaluating the patients' and investigators' assessments on an ordinal scale. The number and percentage of patients as well as the number and percentage of investigators reporting each category of the subjective assessment of efficacy (very good, good, moderate, poor) will be reported for each control visit.

The interrater reliability (in this case patients and investigator) will be evaluated by the Cohen`s Kappa statistic.

9.4.5 Safety Analysis

Details regarding the below envisaged descriptive statistics of the safety criteria will be provided in the SAP. At least summary statistics by visit and treatment arm will be provided based on the SES.

9.4.5.1 Adverse Events and Adverse Drug Reactions

The safety profile of Fluomizin will be assessed by descriptive analysis of adverse events, treatment emergent adverse events and adverse drug reactions.

Adverse events, treatment emergent adverse events and adverse drug reactions will be coded prior to the database lock using the MedDRA dictionary in affect at the time of database closure. They will be classified by System Organ Class and Preferred Term.

An adverse event will be considered as treatment-emergent adverse event (TEAE) if:

- It was not present prior to the first treatment with either Fluomizin or Metronidazole
- It was present prior to the first treatment with either Fluomizin or Metronidazole and worsened after start of treatment (increase of intensity/ change to SAE)

TEAEs will be summarised by System Organ Class and Preferred Term. At least the following tables will be provided:

- Number and percentages of patients with TEAE
- Number and percentages of patients with serious TEAE
- Number and percentages of patients with TEAE according to the most severe intensity
- Number and percentages of patients with TEAE according to the relationship to the study drug

The number and percent of subjects that experienced TEAEs will be provided overall and on the System Organ Class/Preferred Term level. Additionally, the number of events will be provided for the first two tables.

A summary table of other serious adverse events, adverse events leading to death, and adverse events leading to discontinuation will be provided. In case of low incidence, only a listing will be provided instead.

9.4.5.2 Subjective assessment of tolerability

The subjective assessment of tolerability is analyzed descriptively. The number and percentage of patients as well as the number and percentage of investigators reporting each

category of the subjective assessment of tolerability (very good, good, moderate, poor) will be reported for each control visit.

The interrater reliability (in this case patients and investigator) will be evaluated by the Cohen`s Kappa statistic.

9.4.5.3 Vital signs

Vital signs, namely: weight, heart rate, systolic and diastolic blood pressure, will be reported descriptively according to section 9.4.1 for both visits and per treatment arms.

Further details for the analysis of the above-mentioned exploratory analysis will be provided in the SAP.

9.4.1 Additional Analysis

9.4.1.1 Treatment compliance

Treatment compliance will be assessed by 'number of medications taken divided by number of medications provided'. Number and percentages of treatment compliance will be provided for both groups at C1 per treatment arm.

9.4.1.2 Clinical symptoms

Clinical symptoms and changes of the physiological status of the
- vaginal epithelium and microflora (including the vaginal pH),
- laboratory values.

These parameters will be summarized and presented descriptively for Visits C1 (after 8 to 10 days) and C2 (after 24 to 30 days) per treatment arm.

9.5 Statistical and Analytical Issues

9.5.1 Adjustment for Covariates

9.5.2 Handling of dropouts and missing data

9.5.2.1 Dropout rate

Considering possible deviations from the assumptions a dropout rate of 5% is assumed.
In case of dropouts or withdrawals of patients they will be documented and described by means of descriptive statistics and frequency tables for each study site and in total.

9.5.2.2 Missing data

Data is used as given. No imputation is foreseen. The impact of missing data will be evaluated in sensitivity analysis (see 9.4.3.1).

9.5.3 Interim Analysis

An interim analysis is planned after 50% of subjects have completed the study. The interim analysis will be performed pseudo-blinded, without revealing the treatment allocation. The following aspects will be reported:

- descriptive summary statistics of baseline values,
- evaluation of drop-out rate,
- assessment of safety parameters,
- percental evaluation of compliance,
- evaluation of responder rates,
- Decision on sample size increase (if applicable to the new sample size)

Within the frame of the interim analysis no confirmatory analysis regarding the primary endpoint will be conducted. Therefore, no control of the alpha error has to be considered.

The conditional power, the probability of correctly rejecting the null hypothesis at the end of the trial, will be calculated based on the unblinded data, given at the time of interim analysis. This opens the possibility to reassess the sample size. The following formula will be used to calculate the conditional power:

$$P(\theta) = \Phi\left(\frac{Z_k\sqrt{I_k} - z_{1-\alpha}\sqrt{I_K} + \theta(I_K - I_k)}{\sqrt{I_K - I_k}}\right)$$

with

θ: the expected difference under the alternative hypothesis: P_2-P_1 with P_1 and P_2 being the population proportions in groups 1 and 2,

I_k: the information level at interim stage: $I_k = \frac{1}{\sigma^2}\left(\frac{1}{n_{1k}} + \frac{1}{n_{2k}}\right)^{-1}$,

I_K: the information level at final stage: $I_K = \frac{1}{\sigma^2}\left(\frac{1}{n_1} + \frac{1}{n_2}\right)^{-1}$,

Z_k: the test statistic calculated from the observed data up to interim analysis,

$z_{1-\alpha}$: standard normal value for the test with α as type one error.

According to Chen et al. [2004] a conditional power of more than 50% allows an increase in sample size without an inflation of the type one error.

The interim analysis regarding the sample size will be conducted by an independent external statistician. Details for the implementation of the interim analysis will be described in the SAP.

9.5.4 Multicenter Studies

Approximately fifteen study sites are included in this trial. Since patients were randomized with site considered as a stratification factor into the treatment groups, and due to the features of the randomization scheme (see Randomization Plan), the possibility of ending up with unbalanced groups within one site was minimized.

10 DATA MANAGEMENT AND QUALITY CONTROL

10.1 Data Collection and Storage

Subject data recorded on CRFs during the study will be documented in a coded fashion. The subject will only be identified by the subject number, and by their year of birth if also required. Confidentiality of subject records must be maintained to ensure adherence to applicable local privacy regulations.

The Investigator must retain essential documents according to the applicable local regulatory requirements after the completion of the study, unless otherwise notified by the Sponsor.

Essential documents include but are not limited to the following:

- study protocol/amendments
- Protocol Signature Page signed and dated by the Principal Investigator
- EC approved blank as well as copies of all signed subject ICFs
- all EC approvals, correspondence, and reports (e.g., SAE reports, protocol deviations, and safety updates)
- curriculum vitae and medical licenses for the PI and all sub-investigators (if applicable)
- regulatory documents (e.g., financial disclosure and DOA forms)
- source documents
- archive of CRFs
- Device Investigator Agreement
- investigational product accountability records
- relevant correspondence from and to the Sponsor
- any other documents relevant to the conduct of the study

In the event that the Investigator withdraws from the study (e.g., retirement, relocation), study records will be transferred to a mutually agreed upon designee (e.g., another Investigator, site EC). The Investigator will provide notice of such transfer in writing to Sponsor.

10.2 Data Management

All data management procedures will be detailed in a separate document known as the Data Management Plan (DMP). The DMP will also describe the Clinical Data Management System (CDMS) that will be used to collect data with an eCRF in detail. The CDMS will be validated prior to data entry based on standard operating procedures (SOP). In compliance with FDA guidance 21 CFR part 11 each user of the CDMS will be assigned a unique user name and will be allocated to a special user role. This special user role controls the user's permissions in the CDMS depending on the function within the study. All staff involved in the study in each site has to be instructed how to maintain the eCRF and therefore be able to edit eCRF entries according to their role permissions. The investigator must sign a declaration on the eCRF attesting to his/her responsibility for the quality of all data entered and that the data represents a complete and accurate record of each subject's participation in the study.

The CDMS is integrated into a general IT infrastructure and safety concept with a firewall and backup system. A regularly backup of all trial data will be ensured.

Trial sites will enter data online via Internet. For each enrolled study participant an eCRF will be maintained. CRFs must be kept current to reflect subject status at each phase during the course of study. Data on subjects collected on eCRFs during the study will be documented in an anonymous fashion and contains an electronic audit trail. Plausibility checks are run during data entry, thereby detecting discrepancies immediately. These computerized edit checks will be performed according to a Data Validation Plan (DVP). In addition, the data management will conduct further checks for completeness and plausibility. Inconsistencies in the data will be queried to the investigators via the electronic data capture system. Manual queries can also be raised during medical or safety review. Answers to queries or changes to the data will be documented in this electronic data capture system directly by an authorized member of the trial site without unreasonable delay. The audit trail in the eCRF documents all changes. These processes will be performed on an ongoing basis as outlined in the DMP until all queries are resolved.

After all data are entered, all queries are solved and quality control procedures have been completed respectively each subject's evaluability is determined, the database will be authorized for lock. In case of any changes to the data after database close, these changes will be documented according to respective SOP. Electronic case report form data per site will be provided to the responsible investigator at the end of the study and will need to be retained by the investigator. The complete data will be provided to the sponsor at the close of the study for archiving.

10.3 Data Quality Assurance

10.3.1 Documentation

Source data are all the information in original records and certified copies of original records of clinical findings, observations, or other activities in the study, which are necessary for the reconstruction and evaluation of the study. The investigator will permit trial-related monitoring, audit(s), IEC review(s) and regulatory inspection(s), providing direct access to source data/records.

For each subject enrolled, the investigator will indicate in the source record(s) that the subject participates in this study. The investigator will maintain adequate case histories for each subject enrolled. Source records should be preserved for the maximum period of time permitted by local regulations.

The investigator will record the following data in the source records in this study:

- Results from diagnostic procedures for BV
- AE and AR
- Concomitant medication
- Subjective assessment of efficacy and tolerability
- Summaries from patient diaries

10.3.2 Monitoring

The monitoring of this study will be done in accordance with the principles of Good Clinical Practice (GCP) as laid out in the International Conference of Harmonization (ICH) guideline.

10.3.3 Audit/Inspection

The investigator will make all trial-related source data and records available to a medically qualified quality assurance auditor mandated by the sponsor, or to regulatory inspectors, after reasonable notice. The main purposes of an audit or inspection are to confirm that the rights and welfare of the subjects have been adequately protected, and that all data relevant for the assessment of safety and effectiveness of the investigational medicinal product have been reported to the sponsor. A quality assurance audit/inspection of this study may be conducted by the sponsor/regulatory authorities/IEC, respectively. The quality assurance auditor/inspector will have access to all medical records, the investigator's study related files and correspondence, and the Informed Consent documentation that is relevant to this clinical study.

10.3.4 Investigator Responsibilities

The Investigator shall ensure that all work and services described herein, or incidental to those described herein, shall be conducted in accordance with the highest standards of medical and clinical research practice. The Investigator will provide current copies of the study protocol to all Sub-Investigators or other site personnel responsible for study conduct.

The Investigator will provide Medinova AG or designee with copies of all EC actions regarding the study.

11 ETHICAL PRINCIPALS

11.1 Basic Principals and Ethical Considerations

The study will be conducted in compliance with the protocol, Good Clinical Practice (GCP) guidelines, including International Conference of Harmonization (ICH) guidelines, and in accordance with the ethical principles in the last version of the Declaration of Helsinki. In addition, all applicable national laws, and regulatory requirements relevant to the use of medicinal products will be adhered to.

11.2 Ethical Review Committee and Regulatory Authority

The final study protocol and the final version of the Written Informed Consent have to be approved by the responsible Independent Ethics Committee (IEC) / Institutional Review Board (IRB) and the national competent authority (if required by national regulations) as appropriate. If national regulations require the investigator to obtain the ethics committee's opinion, the investigator must submit the written IEC/IRB approval to Medinova before he/she may enrol any patient into the study. If national regulations require that the sponsor obtains the consideration from the ethics committee and the authorization by the competent authority, the investigator may enrol patients into the study only after Medinova has provided the approval documents to him/her.
The clinical trial start will only commence after an IEC/IRB approval and the authorization from the competent authority have been obtained.
Approval for any amendment to the protocol must be obtained by the IEC/IRB and the competent authority in accordance with national requirements. In addition, the IEC/IRB must approve all advertising if used to recruit patients for the study.
Progress reports and notifications of serious adverse events (SAEs) will be provided to the IEC/IRB and competent authorities according to national regulations and guidelines.
Under no circumstances will the investigation be extended beyond the limitations defined in this protocol or any subsequent amendments.

11.3 Protocol Modifications

All amendments must be documented, dated and signed by the investigator and sponsor. Before implementation, the amendment must be notified to or approved by the relevant IEC and competent authority, if required, National requirements must be followed.

11.4 Subject Information and Informed Consent

The investigator must explain to each patient in a comprehensive way the nature of the study, its purpose, the procedures involved, the expected duration and the potential risks and benefits of the study. Each patient must be informed that participation in the study is voluntary and that she may withdraw from the study at any time without giving any reasons and that withdrawal of consent will not affect her subsequent medical treatment. The patient must be given the opportunity to ask questions and allowed time to consider the information provided. The patients will be informed that their participation in the study as well as their identity will be treated confidentially. The patients will also be informed that authorised representatives of local / foreign drug regulatory authorities, IEC/IRB and the study sponsor need direct access to their medical records at the investigational site to compare data available there to data reported in the study, and that this may be done after approval from the investigator responsible for their medical records and provided secrecy is maintained. The subject should read and consider the statement before signing and dating it and should be given a copy of

the signed document. By signing the Written Informed Consent Form, the patient agrees to her participation in the study.
The signed and dated Written Informed Consent must be obtained from each patient before conducting any procedures related to the study. The principal investigator must store the original, signed Written Informed Consent Form. A copy of the Written Informed Consent Form must be given to the patient. The consent form must also be signed and dated by the investigator (or his designee) and it will be retained as part of the study records.

11.5 Participant Confidentiality

Individual subject medical information obtained as a result of this study is considered confidential and disclosure to third parties is prohibited. Subject confidentiality will be further ensured by utilising subject identification code numbers, corresponding to treatment data in the computer files and eCRFs.
All personal patient data collected and processed for the purposes of this study should be managed by the investigator and his/her staff with adequate precaution to ensure the confidentiality of this data in accordance with applicable national laws and regulations on personal data protection.
Monitors, auditors, regulatory authorities and in some countries the ethics committees approving this trial will be granted to direct access to the study patients' original medical records for verification of clinical study procedures and/or data, without violating the confidentiality of the patients, to the extent permitted by the law and regulations. Within all presentations of the results of this study at meetings or in publications, the patient's identity will remain confidential.

11.6 Investigator Qualification

The investigator will be informed of the methods for rating relevant study outcomes and for completing CRFs to reduce discrepancies between participating investigators and study centers. The investigator will be kept informed of important data which relate to the safe use of the investigational medicinal product as the study proceeds.

12 DATA HANDLING AND RECORD KEEPING

The Investigator is responsible for ensuring that the source data are accurate, legible, contemporaneous, original and attributable, whether the data are hand-written on paper or entered electronically. If source data are created (first entered), modified, maintained, archived, retrieved, or transmitted electronically via computerized systems (and/or any other kind of electronic devices) as part of regulated clinical trial activities, such systems must be compliant with all applicable laws and regulations governing use of electronic records and/or electronic signatures. When paper records from such systems are used in place to perform regulated activities, such paper records should be certified copies. A certified copy consists of a copy of original information that has been verified, as indicated by a dated signature, as an exact copy having all of the same attributes and information as the original.

All medical information obtained at each study visit must be recorded in the subject's record (source documentation) in real time as it is collected and then transcribed onto the eCRF by site personnel. Source documentation consists of original subject documents, data and records with all information relevant to the subject and his/her participation in the clinical trial.

Examples of acceptable source documents include for example: hospital records, laboratory notes, evaluation checklists. Source data also includes information initially recorded in an electronic format.

Source documentation worksheets may be provided by the Sponsor to record pertinent information. The completed worksheets can then be incorporated into the subject's medical chart. If it is preferred to not use the worksheets in the subject's permanent record, then the worksheets should be used as a reference to determine the type of study data to record in the subject's permanent record.

12.1 Study Monitoring and Source Data Verification

The monitor will contact and visit the investigator periodically to review all trial-related source data/records, verify the adherence to the protocol and the completeness, correctness and accuracy of all CRF entries compared to source data. The investigator will cooperate with the monitor to ensure that any discrepancies identified are resolved.

Additionally, this clinical trial may be reviewed by representatives of sponsor's or CRO's Quality Assurance department and/or regulatory authorities. This implies that auditors/inspectors have the right to inspect the sponsor, CRO and the trial sites at any time during or after completion of the clinical trial and will have access to source documents, including subject's medical records. By participating in this clinical trial, investigators agree to this requirement.

Detailed information about monitoring arrangements including access to source data and the extent of source data verification will be described in the Monitoring Plan, separately from the Clinical Trial Protocol.

12.1.1 Used Subject and Medication Logs

It is the responsibility of the investigators to ensure that a current disposition record of investigational product (inventoried and dispensed) and application of radiotherapy is maintained at the trial site. Records or logs must comply with applicable regulations and guidelines and should include:

- amount received and placed in storage area
- amount currently in storage area
- label identification number or batch number
- amount dispensed to and returned by each subject, including unique subject identifiers
- amount transferred to another area/site for dispensing or storage
- non-study disposition (e.g. lost, wasted)
- amount destroyed at study site, if applicable
- amount returned to sponsor
- dates and initials of person responsible for Investigational Product dispensing/accountability, as per the Delegation of Authority Form.

The sponsor will provide forms to facilitate inventory control if the trial site does not have an established system that meets these requirements.

12.1.2 Protocol Compliance

The clinical trial shall be conducted as described in this approved protocol. All revisions to the protocol must be discussed with and be prepared by the sponsor. The investigator should not implement any deviation or change to the protocol without prior review and documented approval/favorable opinion from the IRB/IEC of an amendment, except where necessary to eliminate an immediate hazard(s) to trial subjects.

If a deviation or change to a protocol is implemented to eliminate an immediate hazard(s) prior to obtaining IRB/IEC approval/favorable opinion, as soon as possible the deviation or change will be submitted to:

- IRB/IEC for review and approval/favorable opinion
- The sponsor
- Regulatory Authority

Documentation of approval signed by the chairperson or designee of the IRB(s)/IEC(s) must be sent to the sponsor.
If an amendment substantially alters the study design or increases the potential risk to the subject: (1) the informed consent form must be revised and submitted to the IRB(s)/IEC(s) for review and approval/favorable opinion; (2) the revised form must be used to obtain consent from subjects currently enrolled in the study if they are affected by the amendment; and (3) the new form must be used to obtain consent from new subjects prior to enrollment. If the revision is done via an administrative letter, investigators must inform their IRB(s)/IEC(s).

12.2 Data Management

12.2.1 Case Report Form

Subject data required by this protocol are to be recorded on a CRF. The investigators and their staff will be responsible for completing the CRF. Data that are derived from source documents and reported on the CRF must be consistent with the source documents or the discrepancies must be explained. Additional clinical information may be collected and analyzed in an effort to enhance understanding of product safety. CRFs will be requested for AEs and/or laboratory abnormalities that are reported or identified during the course of the clinical trial.

For each enrolled trial participant, a CRF will be maintained. CRFs must be kept current to reflect subject status at each phase during the course of the clinical trial. Data on subjects collected on CRFs during the clinical trial will be documented in an anonymous fashion and contains an audit trail.

The confidentiality of records that could identify subjects must be protected, respecting the privacy and confidentiality rules in accordance with the applicable regulatory requirement(s). The encoded subject ID does not identify the patient directly without the use of a confidential encoder or lookup list.

The investigators will maintain a signature sheet to document signatures and initials of all persons authorized to make entries and/or corrections on CRFs.

The completed CRF must be promptly reviewed, signed, and dated by the investigator or qualified physician who is a sub-investigator and who is delegated this task on the Delegation of Authority Form. For electronic CRFs, review and approval/signature are completed electronically through the sponsor's electronic data capture tool.

Each individual electronically signing electronic CRFs must meet the sponsors training requirements and must only access the sponsors electronic data capture tool using the unique user account provided by the sponsor. User accounts are not to be shared or reassigned to other individuals.

The monitor will be responsible for reviewing and verifying the data recorded on the CRF, utilizing the original source documentation and will query discrepant findings. In addition, computerized edit checks will be performed according to a Data Management Plan (DMP). Inconsistencies in the data will be queried to the investigators. Answers to queries or changes to the data will also be documented directly by an authorized member of the investigator’s staff. The audit trail in the CRF documents all changes. Edit checks fire automatic queries during data entry when a field is not populated to specifications defined in the DMP. Queries can be raised during medical or safety review and data management review. The investigator and staff will be responsible for answering all queries.

The CRFs will be retained by the CRO, who must ensure that it is stored in a secure place. All CRFs will be reviewed for completeness, and evidence recording errors will be rectified by contact with the clinical site.

12.3 Record Keeping

The investigator must retain essential documents indefinitely after the completion of the clinical trial, unless otherwise notified by the sponsor. The investigator agrees to adhere to the document retention procedures when signing the protocol signature page.

The documents should be stored for at least 15 years. The investigator must contact the sponsor prior to destroying any records associated with the clinical trial. The sponsor will notify the investigator when the study records are no longer needed.

Essential documents include but are not limited to the following:

- IRB/IEC approvals for the clinical trial protocol, all amendments, informed consent form(s), and advertisements
- IRB/IEC correspondence and reports
- Regulatory documents (e.g. financial disclosure and delegation of authority forms)
- All source documents
- CRFs
- Subject's signed ICFs
- Any other documents relevant to the conduct of the clinical trial

13 FINANCE AND INSURANCE

The trial is financially supported by Medinova AG.

13.1 Compensation to Subjects

No remuneration and reimbursement are intended for subjects participating in the trial as trial visits schedule is in compliance with local standard practice.

13.2 Insurance and Indemnity

Medinova AG confirms that it carries liability insurance, in compliance with national laws governing the clinical study insurance.
The sponsor will be responsible for any damage caused to third parties by the study medication used during this clinical study.
The sponsor confirms that he carries a liability insurance covering the participants in this study and that the present study has been notified to its insurance company.

The sponsor warrants that the insurance policy is valid during the complete period of this clinical study.

13.3 Investigators' Fee

The investigators will be remunerated for the clinical trial performance and maintenance of proper documentation.

14 PUBLICATION POLICY

14.1 Reporting and Publication

14.1.1 Reporting

A draft study report will be prepared by the sponsor after the completion of the study. The coordinating principal investigator will sign the final study report intended to be submitted to regulatory authorities.

14.1.2 Publication

Medinova shall retain ownership of the data collected during the study and of the reports, which result from this study.

All information obtained as a result of the study will be regarded as confidential, until appropriate analysis and review by the investigators and Medinova are completed. The results of the study shall be published or presented by the investigators after the study has been completed.

Authorship will be determined by mutual agreement. The coordinating investigator will be first name author of the resulting publication.

Medinova must receive copies of any intended communication in advance of publication (at least 15 working days for an abstract or oral presentation and 30 working days for a journal submission) for review of proprietary information and to allow the company an opportunity to comment. If additional time is required in order for Medinova to ensure protection of its intellectual property Medinova will notify the publication panel who will delay submission of the abstract or manuscript.

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16 APPENDICES

16.1 Study schedule

Activities and assessments	Screening Visit	Control Examinations		Early drop-out
	E Day 1	C1 Day 8-10	C2 Day 24-30	
Inclusion criteria				
• Reproductive age ≥18 years	x			
• Diagnosis of BV				
1) Amsel criteria all positive (4+)	x			
• Written Informed Consent	x			
Exclusion criteria	x			
Pregnancy test	x		x	
Medical history				
• Demographic characteristics	x			
• Anamnesis & concomitant diseases	x			
• Concomitant medication	x	x	x	x
Assessment of efficacy parameters				
• Sampling / Cure rates				
1) Clinical cure (Amsel criteria)	x	x	x	x
2) Bacteriological cure (Nugent score)	x	x	x	x
3) Therapeutic cure (Clin. & Bact. cure)		x	x	x
• Subjective assessment of efficacy[2]		x	x	x
• Reason for discontinuation		x	x	x
Assessment of safety parameters				
• Treatment compliance (from diary)		x	x	x
• Adverse events and adverse reactions		x	x	x
• Subjective assessment of tolerability[2]		x	x	x
• Vital signs	x	x	x	x
Study management				
• Randomization & schedule next visit	x	x		
• Dispense / collect study medication	x		x	
• Hand out of online link to patient's diary & questionnaire	x			
• Check patient questionnaire compliance		x	x	x

[1]Swab for Nugent Score will be taken; result will be available 2-3 days later

[2]By both the patient and the investigator

STATISTICAL ANALYSIS PLAN

MNFM380119

Comparative study of the efficacy and safety of vaginally applied Dequalinium Chloride (10 mg) and orally applied Metronidazole (2 x 500 mg) in the treatment of bacterial vaginosis

Sponsor: Medinova AG
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2.0	15-JUN-2022	Simona Botta	Finalization after Sponsor review

Signatures

I confirm that this Statistical Analysis Plan accurately describes the planned statistical analyses to the best of my knowledge and was finalized before breaking the blind/database close.

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Table of Contents

List of Abbreviations and Key Terms

AE	Adverse Event
ADR	Adverse Drug Reaction
ATC	Anatomical Therapeutic Classification System
BL	Baseline
C1	Control Examination 1
C2	Control Examination 2
DRM	Data Review Meeting
E	Examination at Screening Visit
ITT	Intention To Treat
KOH Test	Potassium hydroxide Test
MedDRA	Medical Dictionary for Regulatory Activities
PP	Per Protocol
PT	Preferred Term
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SES	Safety Evaluation Set
SOC	System Organ Class
TEAE	Treatment Emergent Adverse Event
WHO-CC	WHO Collaborating Centre for Drug Statistics Methodology

1. Introduction

This statistical analysis plan (SAP) contains a more technical and detailed elaboration of the principal features of the statistical analyses as described in the Study Protocol *MNFM380119 v2.0*, dated 13th April 2022. The purpose of the SAP is to serve as a guideline for the statistical analysis and the creation of the analysis tables, figures, and listings for the clinical study report. The SAP is finalised and signed prior to hard lock of the database.

2. Study Design and Objectives

2.1 Study Design

This is an international, multi-centre, active-controlled, randomized, double-dummy, phase IV study with parallel group design. It will be assessed whether vaginal tablets containing 10 mg dequalinium chloride (Fluomizin) for 6 days are comparable in clinical efficacy to orally 2x500mg/day for 7 days metronidazole in patients suffering from bacterial vaginosis. The clinical cure rate 8-10 days after start of treatment will be assessed. Patients will be followed up to 30 days after start of treatment. For this trial 236 patients will be recruited in 15 sites within Europe.

2.2 Treatments

Patients will be randomized into one of the two following treatment groups:

- Treatment 1: Fluomizin vaginal tablets (10 mg Dequalinium chloride) administrated intravaginally daily, before going to sleep, for 6 days and comparator (Metronidazole) matching placebo administrated orally twice daily for 7 days

- Treatment 2: Fluomizin matching placebo administrated intravaginally daily, before going to sleep, for 6 days and Metronidazole 500mg tablets administrated orally twice daily for 7 days

2.3 Trial Schedule

Outpatients suffering from bacterial vaginosis will be informed about the study at initial visit. After signing the informed consent, baseline characteristics will be recorded, and randomization applied. Patients will start treatment on screening day. They will have two more control examinations after start of treatment, until latest 90 days after the initial visit. A more detailed overview of the visit schedule and the assessed parameters can be taken from Table 1.

Table 1: Visit schedule

Activities and assessments	Screening Visit E Day 1	Control Examinations C1 Day 8-10	Control Examinations C2 Day 24-30	Early drop-out
Inclusion criteria				
• Reproductive age ≥18 years	x			
• Diagnosis of BV				
1) Amsel criteria all positive 4+	x			
• Written Informed Consent	x			
Exclusion criteria (incl. Pregnancy test)	x			
Pregnancy test	x		x	
Medical history				
• Demographic characteristics	x			
• Anamnesis & concomitant diseases	x			
• Concomitant medication	x	x	x	x
Assessment of efficacy parameters				
• Sampling / Cure rates				
1) Clinical cure (Amsel criteria)	x	x	x	x
2) Bacteriological cure (Nugent score[1])	x	x	x	x
3) Therapeutic cure (Clin. & Bact. cure)		x	x	x
• Subjective assessment of efficacy[2]		x	x	x
• Reason for discontinuation		x	x	x
Assessment of safety parameters				
• Treatment compliance (from diary)		x	x	x
• Treatment compliance (from returned medications)			x	x
• Adverse events and adverse reactions		x	x	x
• Subjective assessment of tolerability[2]		x	x	x
• Vital signs	x	x	x	x
Study management				
• Randomization & schedule next visit	x	x		
• Dispense / collect study medication	x		x	
• Hand out of online link to patient´s diary and questionnaire	x			
• Check patient questionnaire compliance		x	x	x

[1]: Swab for Nugent Score will be taken; result will be available 2-3 days later

[2]: by both, patient and investigator

2.4 Study Objectives

2.4.1 Primary Objective

The main objective is to evaluate whether vaginal tablets containing 10 mg dequalinium chloride (Fluomizin) for 6 days are comparable in clinical efficacy to metronidazole orally 2x500mg/day for 7 days oral tablets in women suffering from bacterial vaginosis.

2.4.2 Secondary Objectives

As secondary objectives the following aspects will be evaluated:

- the clinical, bacteriological, and therapeutic cure rate on short-term and/or long-term follow-up
- the physiological status of the vaginal ecosystem during the respective treatments
- the subjective assessment of efficacy and tolerability
- the safety profile of Fluomizin in this patient population

2.5 Study Hypothesis

The main hypothesis of this study is that Fluomizin is not inferior to Metronidazole in the clinical cure rate of patients with acute bacterial vaginosis. The null hypothesis to reject therefore is that Fluomizin (π_F) is equal or worse than Metronidazole (π_M) by the clinically relevant difference (δ=15%). The alternative hypothesis is that the clinical cure rate of Fluomizin differs from the clinical cure rate of Metronidazole by less than the clinically significant difference, or Fluomizin has higher cure rate compared to Metronidazole.

H_0: $\pi_F - \pi_M \leq -\delta$

H_1: $\pi_F - \pi_M > -\delta$

In case the null hypothesis can be rejected, the primary endpoint will hierarchically be tested for superiority of Fluomizin over the leading therapy option Metronidazole. The hypotheses tested for the evaluation of possible superiority are the following:

H_0: $\pi_F - \pi_M \leq \delta$

H_1: $\pi_F - \pi_M > \delta$

where denotations are the same as explained for the non-inferiority hypothesis above.

If the cure rate of Fluomizin exceeds Metronidazole by more than 15% (δ), and the null hypothesis can be rejected, then Fluomizin is considered superior to Metronidazole with respect to efficacy.

2.6 Handling of Screening Failures and Drop-outs

Patients dropping out after inclusion into the clinical study will be replaced, but the subjects will not be allowed to re-enter the study.

2.7 Randomization and Stratification

For the allocation of subjects to treatment arms, a centre-stratified block randomization with variable block size will be used. The randomisation ratio between treatments will be 1:1. The randomization list will be prepared by a statistician and an interactive randomization tool will be used.

2.8 Blinding

As this is a double-blind double dummy study each subject will receive an identical medication kit containing both vaginal and peroral medication from which one will be placebo and the second active substance. There are no laboratory or clinical assessments or specific AEs that could unblind the subject.

2.9 Sample Size Calculation

The sample size calculation for the trial is based on the proportions of clinical cure rate (greater than or equal to 80%, based on the Amsel criteria) 8 to 10 days after start of treatment as the primary endpoint.

This clinical trial is designed to evaluate if Fluomizin is at least as efficacious as Metronidazole in terms of the clinical cure rate as a non-inferiority comparison.

In this study, the alternative hypothesis H_1 is that the clinical cure rate of Fluomizin, π_F, differs from the clinical cure rate of Metronidazole, π_M, by less than the clinically significant difference, δ (non-inferiority margin: 15%), or Fluomizin has higher cure rate compared to Metronidazole. The null hypothesis, H_0, therefore is that Fluomizin is equal or worse than Metronidazole by the clinically significant difference, δ.

Thus,

H_0: $\pi_F - \pi_M \leq -\delta$

H_1: $\pi_F - \pi_M > -\delta$

The minimum number n, of evaluable patients necessary for each treatment groups is determined by the following equation:

$$n = \frac{2\left(z_{1-\alpha} + z_{1-\beta}\right)^2 \pi_M(1-\pi_M)}{\delta^2}$$

Assuming

- $z_{1-\alpha}$: The 1- α quantile of the normal distribution, with α=2.5%, the type 1 error
- $z_{1-\beta}$: The 1- β quantile of the normal distribution, with 1-β=80%, the power
- an incidence of successful treatment with Fluomizin according to previous studies [Weissenbacher *et al.*, 2012]: π_F : 80% (0.80),
- an incidence of successful treatment with Metronidazole according to previous studies that found cure rates between 60-90% [Larsson and Forsum, 2005; Bradshaw, Morton, *et al.*, 2006]: π_M : 80% (0.80),

- smallest clinically relevant differences in the responder rate in treatment of Fluomizin and Metronidazole (δ) of maximum 15% (0.15) are defined as clinically relevant to show non-inferiority of Fluomizin compared to Metronidazole.

the minimum number of evaluable patients per treatment group is 112. Assuming a drop-out rate of 5%, a total of 118 patients per treatment group is planned. Since in this case the sample size is larger than 50 and the proportion is 0.8, this power calculation gives similar results as if binomial enumeration would be used.

Screening failures and patients withdrawing prior to application of treatments will be replaced.

The sample size was calculated by using nQuery from Statsols, Version 8.5.2.0.

2.10 Planned Interim or Sequential Analysis

An interim analysis is planned after approximately 50% of subjects have completed the study. The exploratory interim analysis is performed pseudo-blinded, without revealing the treatment allocation. Sample size will be recalculated by an independent statistician, such that blinding is preserved. Details are given in section 7.9.3.

2.11 Handling of Changes to Study Protocol

Any change which is not only editorial but indicates a change in the statistical analyses planned in the CIP will be justified and documented at least in the SAP text part if it was decided before database close. Any change made to the statistical analysis after database close might be described in a new version of the SAP but has to be justified and documented at least in the final study report. It has to be specified that these changes occurred after database close and whether they were data-driven or not.

3. Technical Aspects and Coding Conventions

All programs will be written using SAS® version 9.4 or higher. There will be an individual SAS® program written for each table, figure, and listing. Each analysis program will be validated by a second qualified SAS® programmer to ensure a correct output and a correct presentation of the data. The validation process is documented in the validation sheet (GCPS_DMF_033 A-C), which also prespecifies criteria for risk categorization of programs and the corresponding validation actions.

All relevant outputs of the SAS® programs will be transferred into RTF documents with DIN A4 format and saved as write-protected PDF documents with a table of content preceding the content of the file. There will be one RTF/PDF document for tables, one for figures and one for listings. Courier New is used as font. The font size should be consistent within each document and reasonable, i.e. there should be as much required information as possible on one page, but the text should be still easy to read.

In headings, titles, and listings only the first word will be capitalised. Whenever data are derived, and the derivation method is not obvious from the information given in the header, a footnote should be added to clarify the derivation method. A footnote should also be considered if additional information facilitates the interpretation of the data.

Patient listings will be sorted by site and subject number unless specified otherwise. Furthermore, derived data will be marked by "#" in patient listings, while missing data will be represented as blank field. If data from a subject are presented by visit, only attended visits will be listed.

3.1 Date Coding and Day Numbering

The format for presentation of date variables will be DDMMMYYYY. The format for presentation of time variables will be hh.mm.

Missing or incomplete dates will not be completed. For additional considerations towards the time allocation of diseases and medication, refer to sections 3.2.2 and 3.2.5, respectively.

3.2 Coding Systems and Conventions

3.2.1 Coding of Adverse Events, Medical History, Concomitant Pathology and Prior and Concomitant Procedures

All medical terms reported as adverse events (AE), as medical history/concomitant pathology or as concomitant procedures are coded according to the Medical Dictionary for Regulatory Activities (MedDRA) version in effect at the time the database is closed for the final analysis. At least the primary System Organ class (SOC) as well as the Preferred Term (PT) should be available for the statistical analysis.

3.2.2 Separation of Medical History from Concomitant Pathology

Separation of past and current medical conditions will be done by comparison of the stop date of the medical condition with the date of start of the treatment. Each medical condition will be allocated unambiguously either to medical history or concomitant pathology.

- **Medical history**: If the stop date is before start of the treatment, the medical condition will be allocated to medical history. If the stop date is partially given and unambiguously before starting with the treatment, the medical condition will also be allocated to medical history.

- **Concomitant diseases**: If the stop date is at or after the start of the treatment or the medical condition is active at start of study participation, the medical condition will be allocated to concomitant diseases.

3.2.3 Separation of Prior from Concomitant Procedures

Separation of past and concomitant procedures will be done by comparison of the stop date of the medical procedure with the date of treatment start. Each medical procedure will be allocated unambiguously either to past or concomitant procedures.

- **Prior procedures**: If the stop date is before the start of the treatment, the medical procedure will be allocated to past procedures. If the stop date is partially given and unambiguously before start of the treatment, the medical procedure will also be allocated to past procedures. Furthermore, if the stop date is missing and the medical procedure is not known to be active at start of study participation, findings will also be allocated to past procedures.

- **Concomitant procedures**: If the stop date is at or after start of the treatment or the medical procedure is concomitantly performed at start of study participation, the medical condition will be allocated to concomitant procedures.

3.2.4 Coding of Medications

Concomitant medications will be coded using the Anatomical Therapeutic Chemical classification system provided by the WHO Collaborating Centre for Drug Statistics Methodology (WHO-CC). At least the ATC level 2 and 3 should be available for the statistical analysis.

3.2.5 Separation of Prior from Concomitant Medication

Separation of prior and concomitant medication will be done by comparison of the stop date of the medication with the date of treatment start. Each medication will be allocated unambiguously either to previous or concomitant medication.

- **Prior medication**: If the stop date is before the start of the treatment, the medication will be allocated to prior medication. If the stop date is partially given and unambiguously before treatment start, the medication will also be allocated to prior medication.

- **Concomitant medication**: If the medication is ongoing or the stop date is at or after treatment start, the medication will be allocated to concomitant medication. Furthermore, if the stop date is partially missing and ambiguous or completely missing, and the medication is not known to be active at the start of study participation, it will also be allocated to concomitant medication.

4. Analysis Populations and Subgroups

4.1 Analysis Populations

In the following the analysis sets for the statistical analysis of this clinical study will be defined.

4.1.1 Intention To Treat (ITT)

In the intention to treat set all subjects having been randomized and treated will be included. This set will be used for the primary analysis. For the analysis with the ITT population, subjects will be allocated to the treatment group they were randomized to, following the intention-to-treat principle.

A subject is considered treated if at least one medication is taken. This will be checked using the drug accountability done by the site at C2 (i.e., the number of medications given to the subject and the number of medications returned).

4.1.2 Per protocol population (PP)

The Per Protocol set includes all subjects that have not experienced major protocol violations. Patients whose menstruation starts within the 7 days of treatment will also be excluded from this analysis set, since they will have to pause the vaginally applied treatment during menstruation. For additional details on protocol deviations and examples of them see section 5.4. All patients will be evaluated based on the treatment they actually received. This set of subjects is used for primary supportive analysis, as well as for sensitivity analyses.

4.1.3 Safety Evaluation Set (SES)

The SES includes all study subjects who have received Fluomizin or Metronidazole. For the analysis with the SES population, subjects will be allocated to the treatment group they actually received, rather than to the one they were randomized to. This set will be used for the safety analysis.

4.2 Subgroups

To evaluate the effect of the Nugent score, the patients will be divided into three subgroups based on the Nugent score at baseline:

- Normal: subjects with score 0 – 3 at baseline
- Intermediate: subjects with score 4 – 6 at baseline
- BV positive: subjects with score 7 – 10 at baseline

4.3 Analyses of the subgroups are described in respective sections 7.4 - 7.9Stratification

Since for the randomization ‘study sites’ have been considered as a stratification factor, it will furthermore be used for stratified analysis. See section 5.4 to find more details.

5. Data Handling

5.1 Handling of Missing Data and Outliers

Missing data will only be considered in a sensitivity analysis (see section 7.5.2). No imputation is foreseen for the main analysis, in which data is used as given.

5.2 Handling of Withdrawals and Drop-outs

A dropout rate of 5% is assumed in this study. In case of dropouts or withdrawals of patients they will be documented and described in the disposition (see section 7.4.1).

5.3 Handling of Multiple Comparisons and Multiple Primary Variables

Since the analysis of non-inferiority and superiority hypotheses follows a closed hierarchical testing procedure in this trial, the problem of multiplicity does not apply here, therefore no adjustment of the type I error has to be considered. Secondary endpoints and subgroup analysis will be done in an exploratory manner.

5.4 Data Review

A data review meeting (DRM) will take place after database (soft)lock prior to the - final analysis, in order to discuss remaining issues (outstanding queries, unresolved errors) and to confirm and approve relevant protocol deviations on an individual base. The DRM is also responsible for the exclusion of outliers and exclusion of subjects from the analysis populations. It will be also investigated the study sites effect on the primary endpoint variable, and if a clinically significant sites effect is found the site variable will be considered in the analysis being included in the sensitivity analysis.

After the final DRM has taken place and the database is considered cleaned, the database will be hard locked for the final statistical analysis.

Protocol deviations will be identified in a joined effort by the Sponsor, project management, data management, clinical monitors, and statisticians prior to the DRM. A complete list of all identified protocol deviations will be prepared by the Statistician and provided to the DRM members for classification. It is important to note that protocol deviations may be rated as major for the clinical data monitoring during the conduct of the trial although they do not affect data integrity (e.g., delayed SAE reporting, incorrect informed consent process) and should not be rated as major for the determination of analysis sets for the statistical analysis.

Possible examples of major protocol violations are:

- Violations of inclusion or exclusion criteria
- Use of any antimicrobial treatment (local or systemic) during the study
- Use of any vaginal medication or vaginal douching during the study
- Immunosuppressive therapy during the study
- Alcohol consumption during treatment, and 48 hours after the treatment
- Major time window violations of C1

6. Variables for Analysis

6.1 Disposition of Subjects

Disposition of subjects will be presented by means of:

- Screened subjects defined as subjects with a date for the screening visit
- Eligible subjects defined as all screened subjects fulfilling the eligibility criteria of this study
- Subjects eligible and randomized
- Subjects treated - -
- Subjects completing the study
- Subjects in the different analysis sets (ITT, PP and SES) as assigned in the data review meeting
- Subjects in the different subgroups (see section 4.2, normal, intermediate and BV positive)
- Screening failure (incl. reason for screening failure)
- Discontinuation (completion status, reason for discontinuation)

6.2 Demographics and Baseline Characteristics

The following demographics and baseline variables are planned to be evaluated:

- Age (in years)
- Gender (male/female)
- Ethnicity (Hispanic or Latino/ not Hispanic or Latino)
- Race (American Indian or Alaska native/ Asian/ Black or African American/ Native Hawaiian or other Pacific islander/ White/ Other)
- Height (in cm)
- Childbearing potential by subject (yes/no)
- Use of highly effective contraceptive method by subject (yes/no)
- Number of pregnancies by subject
- Number of births by subject
- Number of vaginal infections during the last 12 months by subject
- Number of bacterial vaginosis during the last 2 years by subject
- Subjects with at least one finding in relevant medical history and concomitant diseases (see section 3.2.1) as well as SOC and PT
- Subjects with at least one finding in prior and concomitant medications (see section 3.2.4) as well as ATC level 2 and 3

6.3 Primary Variable

The primary efficacy variable is the clinical cure rate at C1 (8 to 10 days after start of treatment), defined as having the following three Amsel criteria negative:

- greyish white thin discharge

- KOH test or 'fishy' smell
- presence of > 20% clue cells

6.4 Secondary Variables

The variables for the secondary endpoints are:

1. Clinical cure rate at follow-up (at C2 = at 24 to 30 study days, derivation in section 6.3)
2. Bacteriological cure rate at C1 and C2 (for derivation see section 7.6.2)
3. Therapeutic cure rate at C1 and C2 (derivation see section 7.6.3)
4. Nugent Score
    a. as continuous variable at C1 and C2
    b. within 3 categories, i.e. (1) normal, (2) intermediate, and (3) BV positive at C1 and C2
5. Individual Amsel criteria at E, C1 and C2
6. Subjective assessment of efficacy (very good, good, moderate, poor)
    a. By the investigator at C1 and C2
    b. By the patients at day 8-10 and 10 – 30

6.5 Safety Variables

Variables for evaluating safety rate:

1. Number of adverse events
    a. Occurrences of treatment-emergent adverse events by SOC and PT (see section 7.7.1)
    b. Occurrence of treatment-emergent adverse events by severity
    c. Occurrence of treatment-emergent adverse events by relatedness to study drug
    d. Occurrences of adverse events by SOC and PT (see section 3.2.1)
2. Subjective assessment of tolerability (very good, good, moderate, poor)
    a. By the investigator at C1 and C2
    b. By the patients at day 8 – 10 and 10 – 30
3. Vital signs: Weight (in kg), heart rate (in bpm), systolic and diastolic blood pressure (in mmHg) at visits E, C1, C2

6.6 Other Variables

Additional aspects will be assessed, based on the following variables:

1. Treatment compliance based on number of medications distributed/returned (derivation defined in section 7.8.1)
2. Time to resolution of symptoms based on patient diary (derivation defined in section **Error! Reference source not found.**)
3. Patients' satisfaction with treatment
4. Incidence of bacterial vaginosis reoccurrences rate by the patients at day 10 - 30
5. Clinical improvement rate (see section 7.8.5)

7. Statistical Analysis Methods

7.1 Descriptive Statistics

The default summary statistics for quantitative variables will be the number of non-missing observations (n) and the number of missing observations (miss) as well as arithmetic mean, standard deviation (SD), lower quartile (Q1), upper quartile (Q3), minimum (min), median, and maximum (max).

For categorical variables, the number (n) and percentage (%) of subjects per category will be the default summary presentation, and, if applicable, the number of missing values is provided in a “miss” category. For the number of missing values, all subjects in the respective study population will be counted. Percentages will be calculated using a denominator of all subjects in a specified population with non-missing data. If necessary, the denominator will be specified in a footnote to the tables for clarification.

7.2 Rounding Rules

7.2.1 Estimates of the Mean and Standard Deviation

When using actual data, the mean and standard deviation will both be calculated to at least 2 extra places than the actual data and the result will be rounded to one more decimal place than the original data.

When using derived means/standard deviations, i.e. means/standard deviations already derived from actual data, results are calculated to at least one more decimal place than these derived means/standard deviations and subsequently rounded to the same number of decimal places as the used values.

7.2.2 Other data

Quartiles, confidence intervals (CIs) and median will be presented with the same number of decimal places as the mean. Minimum and maximum will be presented with the same number of decimal places as the data used. For estimates of proportions, the result will be rounded to 3 decimal places. If proportions are displayed as percentage, 1 decimal place will be displayed. For example, a proportion of 0.655 will be presented in percentage as 65.5%.

7.3 Data derivation

7.3.1 Categorization of subjects

Subjects will be categorized for frequency calculations associated with several variables defined in section 6.4 into responder and non-responder (for details, see section 7.6). The definition for categorization will be highlighted in a footnote to each of the respective tables.

7.3.2 Change from baseline

Whenever the change from baseline is analysed, the value at baseline is subtracted from the post-baseline value:

Change from baseline = Post-baseline value – Baseline value

7.3.3 Time to onset and duration of adverse events

Time to onset and duration of adverse events will be calculated as follows:

- Time to onset = start date of AE - date of start of treatment
- Duration of adverse event = stop date – onset/worsening date + 1

7.3.4 Time to resolution of clinical symptoms

Time to resolution of symptoms will be based on the information provided in the patient diary, being calculated using the date of resolution reported by the patients at day 8 – 10, and it is defined as follow for the subjects that experienced the event:

- Time to symptom's resolution = date reported of symptoms disappeared – randomization date

If at day 8 – 10 symptoms are declared to be still present, the data will be presented as censored in the analysis with the date of entry of the data. If the patient does not enter the ePRO or has no entry for the resolution of clinical symptoms, data will be excluded from the analysis.

7.4 Evaluation of Demographics and Baseline Characteristics

7.4.1 Disposition of Patients

The disposition of subjects will be presented according to section 6.1.

Primary reasons for early termination will additionally be tabulated (withdrawn consent, inability to continue, death, discontinued by investigator, lost to follow up). Early termination will include frequency and percentage, where percentage is based on the number of subjects with early discontinuation. A table detailing the number of patients per visit will further be provided.

A flow-chart detailing the number of subjects at the different stages will be prepared.

7.4.2 Demographics and Baseline Characteristics

Demographics and baseline characteristics (see section 6.2) will be summarized by randomized groups for the ITT, PP and SES according to the analysis described in section 7.1.

7.4.3 Medical History, Concomitant Diseases, Previous and Concomitant Medication

Absolute and relative frequencies (n, %) of medical history, concomitant pathology as well as past and concomitant procedures will be described based on MedDRA system organ class (SOC) and preferred term (PT) levels for the -ITT on subject level by treatment arm.

Absolute and relative frequencies (n, %) of past and concomitant medication will be provided based on Anatomical Therapeutic Chemical (ATC) Classification code levels 2 and 3 for the ITT on subject level by treatment arm.

7.5 Evaluation of Primary Variable

7.5.1 Main analysis

The main criterion of this trial is to investigate whether Fluomizin has a non-inferior clinical cure rate in treatment of bacterial vaginosis (with regard to the 15% non-inferiority margin) compared to Metronidazole after 8-10 days. The clinical cure rate is a binary response-variable, defined by the evaluation of three individual Amsel criteria (see section 6.3 for more details). Further confirmatory analyses are not foreseen for this trial.

In addition to the standard summary statistics (as described in section 7.1**Error! Reference source not found.**), the differences in clinical cure rate between Fluomizin and Metronidazole will be analysed in a confirmatory manner using a one-sided Farrington-Manning test on a 2.5% significance level with following hypothesis:

H_0: $\pi_F - \pi_M \leq -\delta$

H_1: $\pi_F - \pi_M > -\delta$

where

H_0 = null hypothesis for the non-inferiority test of the primary endpoint

H_1 = alternative hypothesis for the non-inferiority test of the primary endpoint

π_M = clinical cure rate of Metronidazole

π_F = clinical cure rate of Fluomizin

δ = smallest clinically significant difference between the two groups

(non-inferiority margin: 15% or 0.15)

With the Farrington and Manning [1990] Likelihood Score test, differences in proportions between two groups can be tested using the following procedure in SAS version 9.4:

```
PROC freq data=<data> order=data;
        tables group*response/ riskdiff(noninf margin=.15 method=fm);
        format response response.;
RUN;
```

Efficacy will be analysed based on the ITT set. The Farrington-Manning test will provide a p-value for the outcome variable, on which the decision will be based. If the test produces a p-value lower than 0.025, non-inferiority with regard to the non-inferiority margin can be declared. The same analysis will be repeated as a sensitivity analysis on the PP set.

If non-inferiority can be shown, data can furthermore be tested for superiority of Fluomizin over the leading therapy option Metronidazole. The Farrington-Manning test is also providing a confidence interval for this purpose, which will be specified with a significant level of 5% (i.e., 95% confidence interval). The hypotheses tested for the evaluation of possible superiority are the following:

1. H_{0a}: $\pi_F - \pi_M \leq 0$

   H_{1a}: $\pi_F - \pi_M > 0$

where denotations are the same as explained for the non-inferiority hypothesis above.

If the clinical cure rate of Fluomizin is superior to Metronidazole, the null hypothesis can be rejected.

If statistical significance on the above-described superiority test is given, it can furthermore be tested, if it is also clinically relevant using the smallest clinically significant difference between the two groups (15%):

2. H_{0b}: $\pi_F - \pi_M \leq \delta$

   H_{1b}: $\pi_F - \pi_M > \delta$

where denotations again are the same as explained for the non-inferiority hypothesis above.

If the null hypothesis can be rejected to the clinically relevant margin of 15% (δ), Fluomizin is considered superior to Metronidazole with respect to clinical efficacy.

There is no multiplicity problem to be considered since this analysis corresponds to a closed hierarchical test procedure.

7.5.2 Additional sensitivity analysis

1. *Logistic regression model to consider the parameter time*:

   A logistic regression will be calculated using the ITT, as well as the PP set to consider the variance in time given by broader frames of visit schedules. The factor time will be included in the model by a time variable that includes the day on which the visit was performed (8-10). The effect of the Nugent score criteria on the outcome variable will be considered as a continuous variable in the model. Further considerable variables will be included as explanatory parameters into the model, such as treatment as well as age or weight. Which variables are included, is finally decided based on a statistical criterion for model selection.

Logit (Amsel criteria=1 | $X_i=x_i$) =

$$\text{Intercept} + \beta_1*\text{Time}_{[i]} + \beta_2*\text{Age}_{[i]} + \beta_3*\text{Weight}_{[i]} + \beta_4*\text{Treatment}_{[i]} + \beta_5*\text{Nugent Score}_{[i]}$$

Odds ratios with its 95%CI for the specified variables as well as corresponding p-values will be given.

2. *Logistic regression to measure the effect of Nugent Score*:
   To furthermore evaluate the effect of the Nugent score criteria at baseline on the outcome variable, a logistic regression will be calculated including the explaining parameters 'Nugent score' and 'treatment' only. Odds ratios with its 95%CI for the specified variables will be given, and a p-value provided. If meaningful, an interaction between treatment and the Nugent score at baseline will be added to the model and investigated.

3. *Subgroup analysis*

   To investigate the impact of the Nugent score, the primary analysis (as described in section 7.5.1) will be repeated for the subgroups listed in section 4.2.

4. *Stratified analysis*

   In case it's found a clinically significant effect site on the primary endpoint (see section 5.4), the primary analysis as described in section 7.5.1 will be repeated for each site.

5. *Tipping point analysis*

   Another sensitivity analysis addressing the missing values will be a tipping point analysis, which allows to evaluate the effect of missing data on the outcome. It can determine, for which success probability for the missing subjects in the Fluomizin group the conclusions regarding the primary variable remain stable. For this analysis it is assumed that missing values are missing not at random, and that there is a tipping point that reverses the study outcome. It will be conducted on the ITT set and only if the non-inferiority null hypothesis can be rejected and if the missing values rate is at least of 10%.

   This analysis is conducted by the following steps:

   1. Missing values are imputed into the dataset one thousand times-.
   2. The imputed values for missing observations in the Fluomizin group are then adjusted by a shift parameter (success probability), to consider that the distribution of missing responses have a lower expected value than those of the observed ones.
   3. After a logistic regression will be performed for each imputed dataset, the proportion of tests that would reject the null hypothesis will be calculated.
   4. Step 1 to 3 will be repeated until tipping point, meaning the according shift parameter, is found, where the p-value changes to be > 0.05.

   This approach is like a stress-test, to assess how severe deviations of success rates within the missing values have to be, to overturn the conclusions from the primary analysis.

7.6 Evaluation of Secondary Variables

The following described analyses are of exploratory nature and are used as supportive analyses for the primary endpoint. Descriptive statistics will be conducted as explained in section 7.1. When possible, p-values will be provided to evaluate differences between treatment arms. Furthermore, if converge, it will be conducted a logistic regression including, in addition to the treatment group, the factor time and the subgroups will be included as covariates. If necessary and due to the exploratory character of the analysis, the model will be extended or reduced to improve the model fit by the study. For the evaluation of the secondary endpoints the ITT set is used. In case the ITT and the PP sets differ at least by 10% of subjects in both arms, the analysis will be repeated also in the PP set.

7.6.1 Clinical cure rate at C2

The clinical cure rate at C2 (after 24 to 30 days) will be analysed as a secondary parameter to test the long-term efficacy based on the Amsel-criteria. Analysis will be conducted as described for the primary endpoint (see section 7.5.1) by subgroups (defined in section 4.2), in an exploratory manner, based on the ITT, and eventually in the PP set, as described above in section 7.6. A logistic regression model including the factor time will be conducted additionally as a sensitivity analysis, as described in section 7.6. An exploratory evaluation will be made, based on the given p-value.

7.6.2 Bacteriological cure rate

The bacteriological cure rate is defined as a Nugent score ≤ 3. It will be presented and summarized descriptively in an exploratory manner for Visits C1 (after 8 to 10 days) and C2 (after 24 to 30 days). Treatment groups will be compared as described for the primary endpoint (see section 7.5.1) in an exploratory manner based on the -ITT, and eventually on the PP set. A logistic regression model including the factor time will be conducted additionally as a sensitivity analysis according to section 7.6. An exploratory evaluation will be made, based on the given p-value.

7.6.3 Therapeutic cure rate

If both aspects, the Nugent-score, and the Amsel-criteria have improved as defined (Nugent-score ≤ 3, Amsel criteria all negative except pH), the patient is assumed to be cured on a clinical and bacteriological level. Therefore, a new binary variable will be created, that rates the patient as cured if both criteria are fulfilled: clinical cure (see section 6.3) and bacteriological cure (see section 7.6.2), and as not cured if at least one of the criteria are rated as not cured. The therapeutic cure rate (clinical and bacteriological cures) will be evaluated descriptively by subgroups (defined in section 4.2) for Visits C1 (after 7 to 14 days) and C2 (after 24 to 30 days).

Treatment groups will be compared as described for the primary endpoint (see section 7.5.1) in an exploratory manner based on the ITT. A logistic regression model including the factor time will be conducted

additionally as a sensitivity analysis according to section 7.6. An exploratory evaluation will be made, based on the given p-value.

7.6.4 Nugent score development over time and in categories

The Nugent score will be reported in two approaches. Firstly, mean and standard deviation of the raw numeric values will be presented in descriptive statistics and graphically over time and per treatment arm for the ITT. Secondly the development of the single categories of the Nugent score ((1) normal, (2) intermediate, and (3) BV positive) will be presented in descriptive statistics, providing the frequencies per category and distinguishing between the two treatment groups. An exploratory Wilcoxon-Mann-Whitney-test will provide p-values comparing the two treatment arms at each visit. To illustrate the development, the mean Nugent score with its 95% CIs will be plotted for each visit and separately by treatment group. In addition, a shift table will be provided to see the development of the Nugent score categories during the study visits, including a figure that will show graphically the development by categories.

7.6.5 Individual Amsel criteria

The presence of clue cells, the KOH test, the vaginal pH, and the absence/presence of abnormal vaginal discharge will be evaluated descriptively according to section 7.1 for each visit.

-. An exploratory Chi square test will provide p-values comparing the two treatment arms for each criterion.

7.6.6 Subjective assessment of treatment efficacy

An assessment of the efficacy of the two treatments will be made by evaluating the patients' and investigators' subjective evaluation on an ordinal scale. The number and percentage of patients as well as the number and percentage of investigators evaluating each category of the global assessment of tolerability (very good, good, moderate, poor), will be reported for each control visit and separately by treatment group. An exploratory evaluation will be made, based on the - p-value given by the Wilcoxon-Mann-Whitney-test.

The interrater reliability (in this case patients and investigator) will be evaluated by the weighted Cohen`s Kappa statistic by treatment group.

7.7 Evaluation of Safety Variables

Safety variables will be envisaged descriptively. At least summary statistics by visit and treatment arm will be provided based on the SES, according to the description in section 7.1.

7.7.1 Adverse Events

The safety profile of Fluomizin will be assessed by descriptive analysis of adverse events, treatment emergent adverse events and adverse drug reactions.

Adverse events will be coded prior to the database lock using the MedDRA dictionary in affect at the time of database closure. They will be classified by System Organ Class (SOC) and Preferred Term (PT).

An adverse event will be considered as treatment-emergent adverse event (TEAE) if:

- It was not present prior to the first treatment with either Fluomizin or Metronidazole and has emerged prior to study day 24 (17 days after start of treatment)
- It was present prior to the first treatment with either Fluomizin or Metronidazole and worsened after start of treatment (increase of intensity/ change to SAE)

For the classification in TEAES and non-TEAES the date of onset / worsening will be compared with the date of the screening visit.

TEAEs will be summarised by System Organ Class and Preferred Term. At least the following tables will be provided:

- Number and percentages of patients with TEAE
- Number and percentages of patients with serious TEAE (if more than 10 serious TEAEs are observed, otherwise they will be only listed)
- Number and percentages of patients with TEAE according to the most severe intensity
- Number and percentages of patients with TEAE according to the relationship to the study drug

Incidences (number and percentage) of treatment-emergent adverse events (TEAEs) will be calculated on the system organ class (SOC) level and on the preferred term (PT) level, on the subject level for the SES. Percentages will only be calculated for the number of subjects and not the number of events. The denominator used will be the number of subjects in the SES.

Incidences of TEAEs will further be summarized by intensity and relationship to the study drug, where each subject will be counted only once towards the worst intensity or highest relationship to the study drug within a SOC/PT. For the analysis by relationship to the study drug, all TEAEs will be categorized into either related (all TEAEs at least possibly related to the study device) or unrelated.

If meaningful (more than 10 drug related TEAEs), Kaplan-Meier plots will be presented for the time to first drug-related TEAE, where subjects discontinuing the study for any reason will be included as censored observations unless they previously experienced a drug-related TEAE.

Listings and, if applicable, tables displaying incidences for TEAEs leading to discontinuation, serious AEs, drug-related serious AEs and deaths will also be provided. In case at least 10 serious AEs were observed, summary statistics will be provided on the SOC and PT level.

7.7.2 Subjective assessment of tolerability

The subjective assessment of tolerability will be analyzed descriptively. The number and percentage of patients as well as the number and percentage of investigators evaluating each category of the global assessment of tolerability (very good, good, moderate, poor) will be reported for each control visit. An exploratory evaluation will be made, based on the p-value given by the Wilcoxon-Mann-Whitney-test.

The interrater reliability (in this case patients and investigator) will be evaluated by the weighted Cohen`s Kappa statistic by treatment group.

7.7.3 Vital signs

Vital signs, namely weight, heart rate, systolic and diastolic blood pressure, will be reported descriptively according to section 7.1 for both visits (C1, C2) and per treatment arm.

7.8 Evaluation of Other Variables

The in the following described analyses are of exploratory nature. Descriptive statistics will be conducted in the ITT set as explained in section 7.1. When possible, p-values will be provided to evaluate differences between treatment arms.

7.8.1 Treatment compliance from medication returned

Treatment compliance will be assessed by 'number of medications taken divided by number of medications provided'. The number of medications taken will be calculated from the difference between the number of medications provided (visit E) and the number of the medications returned (visit C2 or study termination). Number and percentages of treatment compliance will be provided for both groups at C2 per treatment arm.

7.8.2 Time to resolution of clinical symptoms

Descriptive statistics of the time to resolution will be provided according to section 7.1 for both treatments.

If meaningful, time to resolution of clinical symptoms will additionally be prepared as Kaplan-Meier plot, where patients discontinuing the study will be included as censored observations unless they previously experience clinical cure based on the patient diary.

7.8.3 Patients' satisfaction with treatment

The satisfaction of the patient with the treatment will be assessed descriptively. The number and percentage of patients evaluating each category of the satisfaction with the treatment (very satisfied, satisfied, moderate, not satisfied), will be reported for each control visit. An exploratory evaluation will be made, based on the p-value given by the Wilcoxon-Mann-Whitney-test.

7.8.4 Incidence of bacterial vaginosis reoccurrences

Confirmed healed cases at visit C1, that are diagnosed with bacterial vaginosis again at visit C2, will be flagged as recurrent disease subjects. The number and percentage of these subjects will be reported for both treatment arms.

7.8.5 Clinical improvement rate

The clinical improvement rate will be presented as numbers and percentages of subjects that have at least two Amsel criteria negative at both visits, C1 and C2, per treatment arm.

7.9 Special Analytical Issues

7.9.1 Unscheduled visits

Unscheduled visits may be performed, as necessary, to ensure the safety and well-being of subjects. Data from these visits will only be included in the patient listings but not in the statistical analysis, tables, and figures, with the following exceptions:

- AEs
- Concomitant diseases
- Concomitant medications

7.9.2 Adjustment for Covariates

Since with a range of 8 to 10 days a higher variance is assumed for the primary endpoint, data will be adjusted for the factor time in the sensitivity analysis, where the number of days after randomization will be considered by including them into the model. Therewith possible influences of the factor time can be evaluated exploratory. Further considerable variables will be included in the model, as specified in section 7.5.2.

7.9.3 Interim Analysis

An interim analysis is planned after approximately 50% of subjects have completed the study. The interim analysis will be performed pseudo-blinded, without revealing the treatment allocation. The aim of the interim analysis is to check the assumptions made for the sample size calculation by using a conditional power approach. The idea behind is to consider the possibility that the assumption made in the planning phase of the study can differ from the real situation due to lack of knowledge in the planning phase. Therefore, it is checked the possibility of having the necessity to increase the sample size. Further details can be obtained in Chen et al. 2004.

The data export for the interim analysis will be placed direct after approval of the amendment of the CIP version 2.0. In any case, only the subjects that attended visit C1 by that time will be included in the analysis. The following aspects will be reported descriptively at interim time point:

- summary statistics of baseline values, considering the variables described in section 6.2 and the methodology of section 7.1;
- evaluation of drop-out rate;
- assessment of safety parameters
    - List of adverse events overall, without considering the treatment allocation,
    - Table of the incidence of the adverse events overall,

  - Table of vital signs listed in section **Error! Reference source not found.**, following always the rules of section 7.1;

- percental evaluation of compliance, calculated as described in section 7.8.1, only for the patients who completed C2 already;
- evaluation of responder rates by pseudo-blinded treatment group (A vs. B) calculated following the derivation's rules described in section **Error! Reference source not found.**; decision on how to continue the study (with or without sample size recalculation).

Since the number of medications returned is recorded at visit C2, only for the interim analysis patients will be considered treated, and thus will be in the ITT set, if the patient received at least one medication according to the patient diary report.

Within the frame of the interim analysis no confirmatory analysis regarding the primary endpoint will be conducted. Therefore, no control of the alpha error has to be considered.

The conditional power, the probability of correctly rejecting the null hypothesis at the end of the trial, will be calculated based on the unblinded data, given at the time of interim analysis by an external statistician. This opens the possibility to reassess the sample size. The following formula will be used to calculate the conditional power:

$$P(\theta) = \Phi\left(\frac{Z_k\sqrt{I_k} - z_{1-\alpha}\sqrt{I_K} + \theta(I_K - I_k)}{\sqrt{I_K - I_k}}\right)$$

with

θ: the expected difference under the alternative hypothesis: P_2-P_1 with P_1 and P_2 being the population proportions in groups 1 and 2,

I_k: the information level at interim stage: $I_k = \frac{1}{\sigma^2}\left(\frac{1}{n_{1k}} + \frac{1}{n_{2k}}\right)^{-1}$,

I_K: the information level at final stage: $I_K = \frac{1}{\sigma^2}\left(\frac{1}{n_1} + \frac{1}{n_2}\right)^{-1}$,

Z_k: the test statistic calculated from the observed data up to interim analysis,

$z_{1-\alpha}$: standard normal value for the test with α as type one error.

According to Chen et al. [2004] a conditional power of more than 50% would allow an increase in sample size without an inflation of the type one error. Sample size would then be recalculated, based on the process given in section 2.9, by an external statistician to preserve blinding for the statistician conducting the final analysis, and if the result is in the promising region, meaning a conditional power between 50% and 80%, otherwise no action will be made.

A meeting will take place to discuss the interim analysis results, providing in advance a Power Point presentation with the contents that will be discussed, joined to the previous list of the tables with the results.

8. Changes in the Planned Analysis

This SAP include all the changes encompassed in the new protocol after the amendment, with some additional changes to clarify definitions and analysis descriptions. They are summarized in the following

1. Primary analysis set has changed from mITT (exclusion of Nugent score ≤ 7) to ITT (all randomized and treated patients), as described in section 4.1
2. Addition of subgroups regarding the Nugent score at baseline, as described in section 4.2
3. Adjustment of sample size because of a drop out decreasing as a consequence of 1.
4. Change of the compliance definition to make possible a check from the sites (section 7.8.1)
5. Specifications on the site effects analysis (sections 5.4 and 7.5.2)
6. Extension of the PDs description (section 5.4)
7. Rewording of variables definitions to make them clearer and more specific (section 6)
8. Clarifications of the analysis with more details in the descriptions (sections 7.3 - 7.9)

The aim of the main change, the new definition of the analysis sets, is to be more in line with the population that effectively take the study drug, the one diagnosed ill based on the Amsel criteria, and investigate further the relationship with the Nugent score value additionally.

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10. Literature

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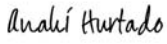
Anahí Hurtado hurtado.anahi@medinova.ch Security Level: Email, Account Authentication (Required)	 Signature Adoption: Pre-selected Style Signed by link sent to hurtado.anahi@medinova.ch Signature ID: A70B50B6-A5A3-4203-B2D9-BA1244A53DE9 Using IP Address: 31.10.245.122 With Signing Authentication via DocuSign password With Signing Reasons (on each tab): I approve this document	Sent: 15-Jun-2022 | 17:38 Viewed: 15-Jun-2022 | 17:42 Signed: 16-Jun-2022 | 09:55
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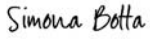
Simona Botta sbotta@gcp-service.com Security Level: Email, Account Authentication (Required)	 Signature Adoption: Pre-selected Style Signed by link sent to sbotta@gcp-service.com Signature ID: 93EC3AD1-5FD5-4F8D-B795-8F510B17839B Using IP Address: 31.17.253.159 With Signing Authentication via DocuSign password With Signing Reasons (on each tab): I am the author of this document	Sent: 15-Jun-2022 | 17:38 Viewed: 15-Jun-2022 | 17:39 Signed: 15-Jun-2022 | 17:40
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