Study title	A phase IIa randomized, active-controlled, double-blind, dose-escalation study in patients with vulvovaginal candidiasis to evaluate dose response relationship of clinical efficacy, safety and tolerability of topically administered ProF-001
Investigational product	ProF-001 (Candiplus®)
Name of sponsor	ProFem GmbH Riglergasse 4/I 1180 Vienna, Austria
Health agency reference number	EudraCT no. 2016-004268-21
Sponsor study no.	ProF-001_Phase IIa
Phase of development	IIa
Study initiation	March 24, 2017 (FPFV: May 9, 2017)
Study completion	October 15, 2018 (LPLV: July 30, 2018)
GCP compliance	The clinical conduct of this study (ProF-001) was performed in compliance with Good Clinical Practice (GCP)
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Background and study rationale

A common problem in women worldwide, vulvovaginal candidiasis (VVC) is a fungal infection of the vaginal mucosa, characterized by vulvar and vaginal pruritus, burning, and irritation, dysuria erythema, edema and vulvar excoriations/fissures. In 85-90% of cases the causative pathogen is Candida albicans. The primary diagnosis by clinical evaluation of symptoms should be confirmed by a wet-mount preparation and microscopy to demonstrate the presence of budding yeasts or fungal (pseudo-)hyphae.

In contrast to sporadic VVC, recurrent VVC (RVVC) is defined as at least four episodes of VVC within one year. This distinct population requires long-term suppressive antifungal therapy to control frequent relapses of VVC, which are associated with substantial impact on work and social life.

Candida spp., most commonly C. albicans, are found as commensals in the majority of healthy females. In the absence of immunosuppression or damaged mucosa, the presence of Candida in the vagina is usually not associated with any signs of disease, whereas VVC is defined as signs and symptoms of inflammation in the presence of infectious Candida spp. in the absence of other infectious etiology. Risk factors for developing VVC are pregnancy, diabetes mellitus, use of oral contraceptives and antibiotic therapy.

Treatment of candidiasis is based on antimycotic drugs, which are administered topically or orally such as nystatin, the imidazoles clotrimazole, miconazole, econazole, fenticonazole, terconazole, butoconazole or tioconazole, and the oral triazoles fluconazole and itraconazole, whereas ketoconazole is not recommended for treatment of VVC.

Successful treatment of uncomplicated VVC is achieved with a single-dose or short-course therapy in over 90% of cases with topical antifungal agents, with none of the agents superior to another.

Recurrent VVC does not respond well to the routine treatment described above (i.e. short-course topical treatment or single oral dose of fluconazole). Prolonged local treatment as well as multiple doses of oral treatment has been proposed. Based on regimens by Sobel and co-workers[1] and Donders and co-workers[2] in RVVC treatment of the acute episode should be followed by maintenance therapy with fluconazole. However, more than 50% of women will recur after maintenance therapy is discontinued with a new VVC episode as fluconazole does rather suppress the growth of Candida than eradicate it.

Clotrimazole-containing creams are commonly used to treat VVC. The lowest clotrimazole dose in approved products using a vaginal applicator is 1%. Efficacy and safety of these products are well-established. In patients with recurrent VVC, clotrimazole is successful in inducing clinical and mycologic remission. In the absence of maintenance suppressive anti-mycotic therapy, however, patients relapse frequently after two months.

In this study, diclofenac sodium was combined with 1% clotrimazole cream at concentrations of 0.2%, 0.3% and 0.4% diclofenac. The rationale for the dose selection was based on safety data from dermal application, safety and pharmacokinetic (PK) data from rectal application of diclofenac preparations and our own safety and PK data after vaginal application of ProF-001 with diclofenac sodium at a dose of 0.25% (pilot PK study). In study ProF-001_Phase IIa, diclofenac was supposed to potentiate the therapeutic effect of clotrimazole. Diclofenac is considered to act as anti-adhesive inhibiting hyphal adhesion, invasion and biofilm formation thereby facilitating access of the antifungal clotrimazole to the pathogen. Moreover, diclofenac's anti-inflammatory (inhibitor of cyclooxygenases I and II) and analgesic effects were expected to provide short-term pain relief and

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reduction of edema.

Study design

Multi-center, randomized, prospective, active-controlled, double-blind, dose-escalation study comparing dose-response of clinical efficacy, safety, local tolerability of three different doses of diclofenac in ProF-001 (= Candiplus®) to 1% clotrimazole vaginal cream.

The study was performed as an active-controlled study, using the most widely used topical product (cream containing 1% clotrimazole) as comparator. Duration of treatment and dosage were adjusted to the requirements of the comparator.

Primary study objective

The primary objective of the study was to determine the dose-response and the clinical efficacy of a cream containing a combination of clotrimazole and diclofenac sodium for the topical treatment of acute episodes of VVC after vaginal administration. Three different doses of diclofenac sodium in combination with clotrimazole were compared with clotrimazole alone as reference.

Secondary study objective

Secondary objectives were the evaluation of clinical safety and tolerability, and the assessments of ProF-001 efficacy with additional clinical and surrogate endpoints.

Endpoints and study outcomes

Primary endpoint (composite endpoint)

Dose-response relation of the clinical efficacy defined as:

1. Symptom relief within the first 60 minutes after application of the investigational medicinal product (IMP) defined as reduction of the subjective symptom score ≥2 in combination with
2. Clinical cure (absence of signs and symptoms of VVC) at the test of cure (TOC) visit at day 7 (±3 days)

Secondary endpoints

- Number of patients with local adverse events and serious adverse events (SAE) with causal relation to study medication
- Symptom relief within the first 60 minutes after application of IMP (reduction of the subjective symptom score ≥2)
- Clinical cure (absence of signs and symptoms of VVC) at the TOC visit
- Mycological outcome: vaginal swab culture negative for growth of Candida albicans and/or Candida species at the TOC visit
- Responder outcome: absence of signs and symptoms plus vaginal swab culture negative for growth of Candida albicans and/or Candida species at the TOC visit
- Time to improvement of symptoms after first intervention
- Time to termination of clinical symptoms
- Clinical relapse of VVC during follow-up period

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Study size

Planned: 84 subjects (21 subjects in each of the three study cohorts receiving ProF-001 with additional 21 subjects as reference arm (clotrimazole alone)

Randomized: 86 subjects

Main inclusion criteria

- Premenopausal female patients ≥18 years old
- Patients suffering from an acute episode of vulvovaginal candidiasis, characterized by:
  - Positive vaginal smear (native or KOH) for budding yeasts and/or fungal (pseudo-)hyphae, normal (G I) or intermediate flora (G II) according to the Nugent criteria
  - Clinical symptoms (itching, burning, irritation, edema, erythema, excoriations), with a subjective symptom score of at least 3 (0=absent, 1=mild, 2=moderate, and 3=severe) with at least one moderate subjective symptom and itching being present and a total sign and symptom score of at least 4
- Readiness for sexual abstinence from start of treatment until TOC visit (end of study)
- Written informed consent prior to enrolment.

Main exclusion criteria

- Known hypersensitivity to clotrimazole, diclofenac or any other ingredient of the investigational medicinal product
- Pregnancy or breast feeding at time of screening
- Menstrual bleeding (spotting is not an exclusion criterion) during the first three days of treatment
- Acute cystitis
- Patients with other clinical gynecological abnormalities, such as infections of the upper urogenital tract (pelvic inflammatory disease, adnexitis) or infectious causes of vulvovaginitis: bacterial vaginosis (GIII), trichomonas vaginalis, herpes simplex genitalis
- Subjects with another vaginal or vulvar condition that would confound the interpretation of clinical response (e.g. Lichen sclerosus, neuropathic pain) or clinically relevant conditions that could compromise the study objectives or the patient's compliance (e.g. known immune deficiency syndrome with clinical relevance at time of screening)
- Treatment with antimycotics (systemic or vaginal) within seven days of randomization
- Chronic use of non-steroidal anti-inflammatory drugs (NSAID).

Test product, dose and mode of administration

The new combination tested in this study consisted of two registered drug substances combined in a cream for topical application in the vulvar region and the vagina. While the first substance, clotrimazole, is used in its typical indication, the second substance, diclofenac sodium is applied in a new indication as an antimicrobial anti-adhesive agent with anti-inflammatory and analgesic properties.

Intravaginal application of 2.5 ml of cream via a vaginal applicator and topical application to the vulvar region of 2 cm of cream twice a day (minimum interval of five hours between IMP dosing) for the first three days was followed by once daily 2.5 ml intravaginal application and 2 cm topical

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application to the vulvar region for the remaining three days at bed time. The first dose of IMP was applied by the investigator. Patients were trained on how to correctly apply the IMP for the remaining doses.

Duration of treatment

The overall trial duration for each patient enrolled including the follow-up period was 65 days:

- Active treatment period: 7 days (from day 1 to day 7 = TOC visit)
- Follow-up period: approx. 21 days (from day 8 to day 28)
- Extended follow-up period: approx. 32 days (from day 29 to day 60).

Note: with the amendment of study protocol 4.0, study duration of cohort 3 was reduced to seven days (plus/minus three days).

Treatment schedule

Patients were randomized to receive a daily dose of either 5 ml (intravaginal) of Candiplus® at three different doses for the first three days and 2.5 ml for the remaining three days or 5 ml (intravaginal) application of 1% clotrimazole cream over the first three days and 2.5 ml for the remaining three days according to the following scheme (during each application 2 cm of cream has been applied to the vulvar region):

Cohort 1: Candiplus® with 0.2% diclofenac sodium versus clotrimazole mono
Cohort 2: Candiplus® with 0.3% diclofenac sodium versus clotrimazole mono
Cohort 3: Candiplus® with 0.4% diclofenac sodium versus clotrimazole mono

Randomization into the cohorts was performed at a ratio 3:1 and starting from the lowest dose to the highest dose, i.e. patients were randomized first in cohort 1 and last in cohort 3.

Variables and safety assessments

Adverse event monitoring included subjective and objective symptom assessments applying an established symptom score as defined in the FDA guidance for treatment of VVC from 2016 and by Sobel and co-workers[3] in 2001:

- Subjective symptoms: itching, burning pain and irritation/soreness classified as mild, moderate and severe,
- Objective symptoms: erythma, edema and excoriation as assessed by the gynecologist (classified as mild, moderate and severe).

Study subjects were asked to self-rate their physical condition and adverse reactions associated with the study medication at each visit. Subjective grading of local reactions has been documented by patients in the diary according to severity based on a visual analogue scale (VAS). The treating physician assessed the symptoms itching, burning pain and irritation/soreness according to the above mentioned symptom score (categorized into mild, moderate and severe) and objectively confirmed by gynecological examination.

Special attention of participating subjects has been drawn to document in the diary and to report the occurrence of local irritations such as erythema, peeling, itching or burning.

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Data analysis and statistical methods

For the statistical analysis, all patients treated with clotrimazole 1% cream were pooled to the dose group "clotrimazole mono" and all patients treated with Candiplus® to the corresponding dose group with diclofenac sodium (referred to as dose groups 0.2%, 0.3% and 0.4% Candiplus®).

Safety was assessed using the safety population (receiving at least one dose of IMP), whereas efficacy was analyzed with the full analysis set (FAS) comprising all randomized subjects who received at least six doses (three days) of IMP with sufficient data to determine the primary study endpoint.

For the summary statistics, all variables including score parameters were analyzed descriptively displaying number and percentage of patients per category. All relevant variables (including demographic data) were summarized by descriptive statistics by dose group or by timepoint according to treatment schedule. Continuous endpoints (such as scores) were analyzed as absolute values and changes from baseline. For the primary endpoint dose-response have been tested using a logistic regression and in addition a quadratic model approach. The final model has been selected based on the AIC (Akaike Information Criterion) criterion. Confidence bands for the estimated dose-response curve have been presented.

All variables of the secondary endpoints including single parameter of the scores have been analyzed descriptively. Binary endpoints have been analyzed by displaying the number and percentage of patients in each dose group. Differences between dose groups have been tested using Fisher's exact test. Continuous endpoints (such as scores) have been analyzed as absolute values and changes from baseline by display of basic statistics (mean, standard deviation, minimum, median, maximum) by timepoint.
Additional tests and post-hoc analyses like Kruskal-Wallis test and ANOVA for comparing dose groups and multivariate statistical models that account for baseline characteristics as well as appropriate tests for repeated measurements have been used. For the parameter „Time to improvement of symptoms after first intervention" and "Time to termination of clinical symptoms" Kaplan-Meier estimates have been displayed. These tests are regarded as exploratory.

The statistical data analysis was performed by subgroups of those enrolled subjects with recurrent VVC (subjects with ≤3 episodes of vulvovaginal candidiasis per year versus >3 episodes per year) and dose group. These tests were considered as exploratory.

Results

The study has been designed as a proof of concept study including a variety of efficacy parameters and clinical outcomes to be tested.

For the combined primary endpoint (i.e. symptom relief after 60 min and clinical cure at day 7), no statistically significant results were obtained in the predefined tests in the FAS, which was also true for the single parameters clinical cure and symptom relief after 60 min. Likewise, the secondary endpoints "mycological outcome" (i.e. vaginal swab culture negative for Candida species) and "responder rate" (i.e. absence of signs and symptoms plus vaginal swab culture negative for Candida species at the TOC visit) the Fisher test was not statistically significant. The overall comparison (comparator and the three ProF-001 dose groups using a Log Rank Mantel Cox test) of the secondary endpoints "time to improvement of symptoms after intervention using VAS" and „Time to termination of symptoms after intervention using VAS" showed a significance level of p=0.001 and p=0.005 indicating a subjective patient reported benefit when administering

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The explorative analysis of the subgroups with recurrent VVC (>3 episodes per year) showed a statistically significant impact (without adjustment for multiple testing) on the combined primary endpoint for Candiplus® 0.3% using a 95% confidence interval favoring Candiplus® 0.3% over the reference clotrimazole alone. Very similar results were obtained regarding the parameters "clinical cure". For "responder rate" a significant difference between Candiplus® 0.3% and clotrimazole has been observed (p=0.034; unadjusted for multiple testing). In analogy, there was a trend for a significant difference of the parameters "Time to improvement of symptoms after intervention using VAS" (p=0.02; unadjusted for multiple testing) in subjects with recurrent VVC using the VAS data from the subject's diaries.

Considering safety, a total of 273 adverse events (AE) was documented during the trial. Among these, 234 events were reproductive system disorders (234 out of 273 AE) with vulvovaginal burning as the dominant adverse reaction (172 events or 63% of all AE) followed by vulvovaginal pruritus (8% of all AE).

The vast majority of AE was classified as mild (73% of all AE) or moderate (23% of all AE). All severe AE – mostly itching and burning – were documented in a single subject, probably experiencing a hypersensitivity reaction against clotrimazole or diclofenac as she was receiving Candiplus® 0.3%. Due to the nature of the study and the repeated topical intravaginal application of the cream twice daily, almost all AE were treatment-related with lower figures for the reference 1% clotrimazole cream.

Symptoms upon exposure to the IMP were readily controlled with a median duration between 5 and 15 min independent of the administered dose of diclofenac sodium and with episodes sporadically exceeding the duration of 60 minutes. There was, however, a trend towards a higher incidence of AE in the treatment groups receiving Candiplus® 0.3% and Candiplus® 0.4%. In summary, there were no safety issues associated with the application of Candiplus® with vulvovaginal burning as the most frequently observed adverse reaction, which was mild and transient in nature.

Conclusions

In conclusion, the addition of 0.3% diclofenac sodium to 1% clotrimazole cream (Candiplus® 0.3%) was associated to beneficial effects in this proof of concept study. Although the composite primary study endpoint combining symptom relief after 60 min and clinical cure at day 7 was not met, a subgroup analysis for patients with recurrent VVC showed encouraging results to be demonstrated in a future clinical trial, which needs to be adequately powered.

References

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