*Journal of Antimicrobial Chemotherapy* (2002) **50**, 231–240
DOI: 10.1093/jac/dkf101

JAC

# Efficacy, tolerability and development of resistance in HIV-positive patients treated with fluconazole for secondary prevention of oropharyngeal candidiasis: a randomized, double-blind, placebo-controlled trial

**Jean-Luc Pagani[1]*, Jean-Philippe Chave[1], Chantal Casjka[2], Michel-Pierre Glauser[1] and Jacques Bille[1]**

*Divisions of [1]Infectious Diseases and [2]Clinical Pharmacology, Department of Internal Medicine, Centre Hospitalier Universitaire Vaudois, 1011 Lausanne, Switzerland*

*Received 11 June 2001; returned 28 December 2001; revised 4 February 2002; accepted 26 April 2002*

**Over 37 months, we conducted a prospective double-blind, randomized study in a cohort of 138 HIV-infected patients to compare the effect of two different strategies on the prevention and treatment of oropharyngeal candidiasis relapses and on the development of clinical and microbiological resistance to fluconazole. Each episode was treated with a 7 day course of fluconazole 200 mg/day, followed by secondary prophylaxis with fluconazole 150 mg once weekly matched to placebo. The duration of the double-blind phase of the study, from the day of randomization to the first primary end-point, was 347 ± 186 days for the fluconazole group and 196 ± 128 days for the placebo group ($P < 0.001$). A total of 33 patients remained relapse-free during the course of the study. Clinical failure was observed in a total of five patients (four in the fluconazole group, one in the placebo group; $P = 0.15$). Microbiological resistance was recorded in 12 patients (eight in the fluconazole group, four in the placebo group; $P = 0.20$). There were no significant treatment group differences in microbiological resistance whether comparisons were made for all cases or for cases up to 1 month post-study. In the few patients who developed clinical and/or microbiological resistance, the cumulative dose of fluconazole before entry into the study was a mean value of 8.6 g (compared with 2.9 g in patients without clinical and/or microbiological resistance). In summary, patients treated with secondary prophylaxis suffered fewer relapses of oropharyngeal candidiasis. Development of resistant candidiasis (clinical and/or microbiological) was rarely seen in either group and its incidence was not significantly different.**

## Introduction

Before the introduction of highly active antiretroviral therapy (HAART), oropharyngeal candidiasis occurred in >90% of patients with symptomatic HIV infection.[1] Today, oropharyngeal candidiasis still represents the most frequent opportunistic infection in these patients. Taking a global perspective, only a minority of HIV-infected patients receive HAART. Various topical treatments, as well as ketoconazole, are associated with sub-optimal efficacy and/or poor tolerability.[2,3] In contrast, the efficacy and safety of fluconazole in the treatment of oral thrush in AIDS patients has been well demonstrated.[4–6]

Relapse of oropharyngeal candidiasis is frequent in patients with untreated HIV infection,[7,8] and should be considered a warning sign of possible HIV progression and prompt a re-evalution of the patient's HAART with determination of viral load and $CD4^+$ cell counts.[7] Mean disease-free intervals have been reported to vary from 18 to 42 days,[4,9] and the frequency of relapses seems to be related to the degree of immunosuppression.[7,10,11] Secondary prevention with fluconazole has been employed by several centres, with well-established efficacy and safety at dosages varying from 50 mg daily to 400 mg weekly.[12–17]

Punctual treatment of relapse is an acceptable alternative, since oropharyngeal candidiasis usually responds rapidly to

*Corresponding author. Tel: +41-21-314-17-67; Fax: +41-21-314-16-34; E-mail: jean-luc.pagani@chuv.hospvd.ch

treatment and is associated with no mortality and low morbidity. This strategy (150 mg in single-dose therapy), adopted in our institution from 1987 to 1994,[18] presents certain advantages with respect to cost, compliance, drug interactions and lack of potential for the development of resistance, particularly in severely immunosuppressed patients taking several other drugs.

Oropharyngeal candidiasis resistant to fluconazole has been described previously. In these reports, the infections were caused by *Candida* species intrinsically resistant to fluconazole, such as *Candida krusei*,[19] or may have been due to the secondary development of resistance in *Candida albicans*[20,21] or *Candida glabrata*[22] isolates during fluconazole treatment. An increase in clinical resistance to fluconazole has been observed in our centre since 1990,[23] and other authors, likewise, have also observed an increase in resistance to fluconazole during prophylactic therapy.[24–27]

The primary objective of the present study was to compare the effect of two strategies for the therapy of oropharyngeal candidiasis relapses on the development of clinical and/or microbiological resistance to fluconazole: (i) secondary prevention with weekly administration of fluconazole; (ii) iterative fluconazole treatment of relapse(s).

The secondary objectives were the prospective evaluation of the clinical significance of *C. albicans* resistance to fluconazole and the confirmation of the efficacy and tolerability of fluconazole for secondary prevention of oropharyngeal candidiasis in HIV-positive patients over the long-term.

## Materials and methods

### *Study population*

The trial subjects were HIV-positive patients, most of whom were recruited from the Swiss HIV Cohort Study. Patients with documented oropharyngeal candidiasis who responded to a 7 day course of treatment with oral fluconazole 200 mg daily, and who provided their written informed consent, were considered eligible.

The following exclusion criteria were applied: age younger than 16 years; known hypersensitivity to azole compounds; a documented *Candida* isolate intrinsically resistant to fluconazole from baseline swab culture (especially *C. glabrata* or *C. krusei*); ongoing systemic or topical secondary prevention for oropharyngeal candidiasis; ongoing fluconazole therapy for any other reason; previous systemic (but not topical) antifungal drug within 15 days of planned study entry; creatinine >150 μmol/L; alanine aminotransferase (ALAT) or alkaline phosphatase more than five times the upper normal value.

### *Study design*

This was a prospective, randomized, double-blind, placebo-controlled trial. The study protocol was approved by the Ethics Committee of the Centre Hospitalier Universitaire Vaudois (CHUV). Eligible patients were randomized to fluconazole 150 mg weekly or to matching placebo capsules, and remained in the double-blind phase of the study until one of the primary end-points was reached (see below). All subsequent oropharyngeal candidiasis episodes were treated with a 7 day course of fluconazole, 200 mg/day.

At baseline (day 0, before randomization) a complete physical examination was performed and medical history, including historical data on number, treatment and clinical outcome of previous oropharyngeal candidiasis episodes, was obtained. Oropharyngeal swabs and physiological analysis including complete blood count (CBC) with differential leucocyte count, creatinine, ALAT and alkaline phosphatase were obtained. CD4 lymphocyte counts were also determined if this had not been done within the preceding 3 months. The patients were initiated on a 7 day treatment regimen with fluconazole 200 mg/day, and those who responded clinically (disappearance of all oropharyngeal candidiasis symptoms and signs) were randomized to a secondary prevention regimen (weekly dose of 150 mg of fluconazole or matching placebo).

Before randomization, the patients were stratified according to CD4 lymphocytes (≤50 cells/mm$^3$ versus >50 cells/mm$^3$) and number of previous oropharyngeal candidiasis episodes at randomization (<2 versus ≥2).

At the occurrence of a relapse (clinically and microbiologically documented oropharyngeal candidiasis), patients were given a 7 day course of fluconazole 200 mg/day. Before initiation of treatment, changes in concomitant medication were recorded, an oropharyngeal swab was taken (both hard and soft palate) and physiological analysis was performed. Oropharyngeal candidiasis was considered to be clinically documented when examination showed raised confluent white patches on a hyperaemic base, or erythema alone. In this latter case it was required that the oropharyngeal candidiasis be confirmed by direct microscopic examination of the lesions. Typical symptoms included burning, pain and inflammation, and these were graded as absent, mild, moderate or severe.

In the absence of oropharyngeal candidiasis, follow-up visits took place at least every 3 months. On these occasions, as upon relapse, swabs were taken and physiological analysis was performed.

### *Primary end-points*

Patients remained in the double-blind phase of the study until one of the following primary end-points was reached.

(i) Third relapse of oropharyngeal candidiasis.

(ii) Occurrence of an adverse event requiring drug discontinuation. In particular, a patient was to be discontinued if a liver function test increased by a factor of 3 (relative to normal baseline levels) or 2 (relative to above-normal baseline levels).

(iii) Development of microbiological resistance to fluconazole, in association with clinical resistance defined as follows:

(a) Acquisition of *in vitro* resistance in comparison with previous determinations performed on the 'same' strain. Identity of the strains was established by phenotypic methods (biocodes), and when necessary, molecular methods (C1a probe) were used to assess genotypic relatedness between clinical isolates.

(b) Appearance of an isolate belonging to a species of *Candida* intrinsically resistant to fluconazole in a mixed fungal population (comprising different species or different clones belonging to the same species).

(c) Re-infection with a strain intrinsically resistant to fluconazole after eradication of the susceptible strain(s).

(iv) A total duration of 18 months in the study.

Patients were followed up clinically and microbiologically after the randomized phase of the study until the end of the trial.

### *Definitions*

*Clinical response*. Complete clinical response to treatment was defined as disappearance of all clinical signs and symptoms of oropharyngeal candidiasis. Partial response was defined as disappearance of 50% of clinical signs and symptoms according to the scores used.

*Fluconazole resistance.* Clinical resistance (i.e. lack of clinical response) to treatment was defined as the persistence or a decrease of <50% in clinical signs and symptoms following a 7 day treatment course with 200 mg of fluconazole daily. Microbiological resistance was defined as a decrease in fluconazole susceptibility as measured with an agar disc diffusion method, and corresponding to an MIC ≥ 64 mg/L when measured with a broth dilution method according to the reference method.[23,28–30]

### *Microbiology*

Oropharyngeal swabs were transferred to the bacteriology laboratory of the CHUV, where the following examinations were performed. (i) Direct examination (Gram's stain). (ii) Primary cultures on chocolate blood agar, blood agar, Sabouraud dextrose agar and Albicans ID agar plates; in addition, a Sabouraud broth medium was inoculated; incubation at 37°C for 72 h, with daily examination. (iii) Yeast colonies were identified at the species level by classic procedures [biochemical identification system: API-Candida (bio-Merieux, Marcy l'Étoile, France) and morphology on rice agar Tween medium]; all pre- and post-randomization isolates were saved and stored at –80°C, in BHI broth with 10% glycerol. Strains were differentiated according to their phenotypes (API-Candida). For consecutive isolates belonging to the same species, when some of them demonstrated acquisition of resistance, genotyping was carried out using the C1a probe method.[31] (iv) Determination of susceptibility to fluconazole using a disc diffusion agar test developed in our laboratory.[23]

### *Blood tests*

Blood samples were obtained for CBC with differential leucocyte count, creatinine, ALAT, and alkaline phosphatase, as appropriate. Biological analyses were performed at the central laboratory of the CHUV.

### *Statistical analysis*

Statistical analysis was performed using Statistica (Release 4.5). Differences in proportions were assessed by $\chi^2$. The Mann–Witney *U* test, the Kruskal–Wallis test and the Wilcoxon matched pairs test were used when between-group variances were unequal for unpaired and paired samples analysis, respectively. ANOVA/MANOVA was used to test between differences for normally distributed variables. Kaplan–Meier analysis (with LogRank test) was performed to estimate differences between fluconazole and placebo in time to occurrence of oropharyngeal candidiasis episodes. Multiple regression analysis was used to explore factors associated with the appearance of microbiological and clinical resistance to fluconazole.

### *Multiple regression analysis*

In order to identify a factor that might be associated with clinical or microbiological failure, multiple regression analyses were performed. Factors taken into consideration were: gender, age, infection source, disease stage, number of previous oropharyngeal candidiasis episodes, time to the first occurrence of oropharyngeal candidiasis, cumulative fluconazole dose before study entry, total fluconazole dose during study, monthly adjusted fluconazole dose during study, concomitant medication. Pertinent data on the individual patients developing clinical and/or microbiological resistance are presented in Table 1.

## Results

### *Patients: baseline characteristics*

For 37 months, 143 HIV-positive patients were randomized to fluconazole or placebo. Five patients (four fluconazole and one placebo) had no follow-up visits: three of them refused further participation and two were lost to follow-up. There remained 138 patients who completed the study and were evaluable.

**Table 1.** Detailed data on the individual patients who developed clinical and/or microbiological resistance

| | Gender/ age | Stage | CD4 (cells/mm$^3$) | Risk[b] | Previous OPC[c] | Fluco cumul (g)[d] | Study drug/ placebo F/P | No. of relapse(s)[e] | Clinical failure (OPC episode)[f] | Microbiological resistance[a] | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | During study (OPC episode) | After study (week) |
| 1 | M/37 | C3 | 2 | ivdu | 20 | 16 | P | 3 | – | + (1st) | |
| 2 | M/25 | C3 | 27 | homo | 24 | 24.3 | F | 3 | – | + (3rd) | – |
| 3 | M/36 | C3 | 5 | homo | 9 | 15.8 | F | 3 | +(3rd) | + (3rd) | – |
| 4 | M/39 | C3 | 9 | ivdu | 2 | 0.6 | F | 1 | – | + (1st) | – |
| 5 | F/31 | C3 | 18 | hetero | 13 | 3.9 | F | 2 | + (2nd) | + (2nd) | – |
| 6 | M/36 | C3 | 11 | homo | 2 | 2.8 | F | 0 | – | + (no OPC) | – |
| 7 | M/38 | C3 | 13 | ivdu | 20 | 17.9 | P | 3 | +(3rd) | + (2nd) | – |
| 8 | M/40 | B3 | 36 | ivdu | 25 | 3.3 | F | 3 | +(3rd) | – | + (1) |
| 9 | M/28 | C3 | 44 | hetero | 10 | 10.1 | P | 3 | – | + (2nd) | – |
| 10 | M/33 | C2 | 312 | ivdu | 10 | 2.6 | F | 3 | +(3rd) | – | + (4) |
| 11 | M/34 | C3 | 57 | homo | 4 | 4.6 | P | 3 | – | – | + (3) |
| 12 | M/36 | C3 | 132 | ivdu | 7 | 1.7 | F | 3 | – | + (1st) | – |

[a] *C. albicans* strains with fluconazole diameter of inhibition corresponding to an MIC correlate of ≥64 mg/L.
[b] Risk: ivdu, iv drug user; homo, homosexual patient; hetero, heterosexual patient.
[c] Before study entry; OPC, oropharyngeal candidiasis.
[d] Total dose of fluconazole before study entry.
[e] During the study.
[f] Persistence of confluent white patches and/or erythema.

**Table 2.** Evaluation of relapses among 138 patients randomized to receive or not weekly fluconazole prophylaxis for oropharyngeal candidiasis

| | *n* (%) | | | |
|---|---|---|---|---|
| | all | fluconazole group | placebo group | *P* |
| Total no. of patients | 138 | 67 | 71 | ns |
| No relapse | 33 (24) | 26 (39) | 7 (10) | |
| Any relapse(s) | 105 (76) | 41 (61) | 64 (90) | $10^{-5}$ |
| one relapse | 29 | 17 | 12 | |
| two relapses | 21 | 8 | 13 | |
| three relapses | 55 | 16 | 39 | <0.03 |
| Median time interval to relapse (in days) | | | | |
| 1st relapse | | 175 | 35 | $10^{-5}$ |
| 2nd relapse | | 68 | 43 | 0.027 |
| 3rd relapse | | 41 | 41 | ns |
| Median time interval free of OPC after randomization (in days)[a] | | 312 | 38 | $10^{-5}$ |

[a]All patients included.
ns, not significant.

There were no significant differences between the fluconazole and placebo groups for the following parameters: patients randomized and evaluable, gender, infection source, CD4 count, stage of HIV infection, anti-HIV therapy, primary or secondary prevention of opportunistic infections, number or frequency of previous oropharyngeal candidiasis episodes, or cumulative fluconazole dose. Patients who were to receive fluconazole as secondary prevention were slightly older (38 ± 8 years) than those in the placebo group (35 ± 7 years) (unpaired *t*-test, $P < 0.05$) but this was not considered likely to interfere with the interpretation of the results. Although randomization was stratified according to baseline CD4 count and number of previous oropharyngeal candidiasis episodes, there was a trend (not statistically significant: $\chi^2$ test, $P > 0.05$) towards an imbalance between the number of fluconazole ($n = 24$) and placebo ($n = 40$) patients at stage C3. Furthermore, more placebo than fluconazole patients ($n = 45$ versus 31, Pearson's $\chi^2$ test, $P < 0.05$) were taking concomitant medication at study entry.

## *Efficacy*

*Duration in study—time to first primary end-point*. The patients receiving fluconazole for secondary prevention remained in the trial longer than did those on placebo. The duration of the double-blind phase of the study, from the day of randomization to the first primary end-point, was 347 ± 186 days for the fluconazole group and 197 ± 128 days for the placebo group (mean ± S.D., Mann–Whitney *U*-test, $P < 0.001$).

Patients who did not relapse remained in the study for a mean duration of 326 ± 197 days (fluconazole group, $n = 26$) and 321 ± 173 days (placebo group $n = 7$). For patients who relapsed at some point during the course of the study, mean duration was 362 ± 180 days (fluconazole group, $n = 41$) and 184 ± 116 days (placebo group, $n = 64$). Application of the Mann–Whitney *U*-test gave $P < 0.001$.

## *Relapses*

Relapses occurred in a total of 105 of 138 evaluable patients. As detailed in Table 2, 61% of the patients in the fluconazole group experienced at least one relapse, compared with 90% of those in the placebo group (Pearson $\chi^2$, $P < 0.001$).

A total of 33 patients (fluconazole group $n$ = 26, 39%; placebo group $n$ = 7, 10%) remained relapse-free during the course of the study. Twenty-nine patients experienced one relapse (fluconazole group $n$ = 17; placebo group $n$ = 12), 21 patients two relapses (fluconazole group $n$ = 8; placebo group $n$ = 13) and 55 patients three relapses (fluconazole group $n = 16$; placebo group $n = 39$).

## *Time interval to relapse*

Table 2 shows the median time intervals to relapse in the fluconazole- and placebo-treated patients. The median time interval from study entry to first relapse was 175 days in the fluconazole-treated patients, and 35 days in those treated with placebo (Mann–Whitney *U*-test, $P < 0.001$). Similarly, the

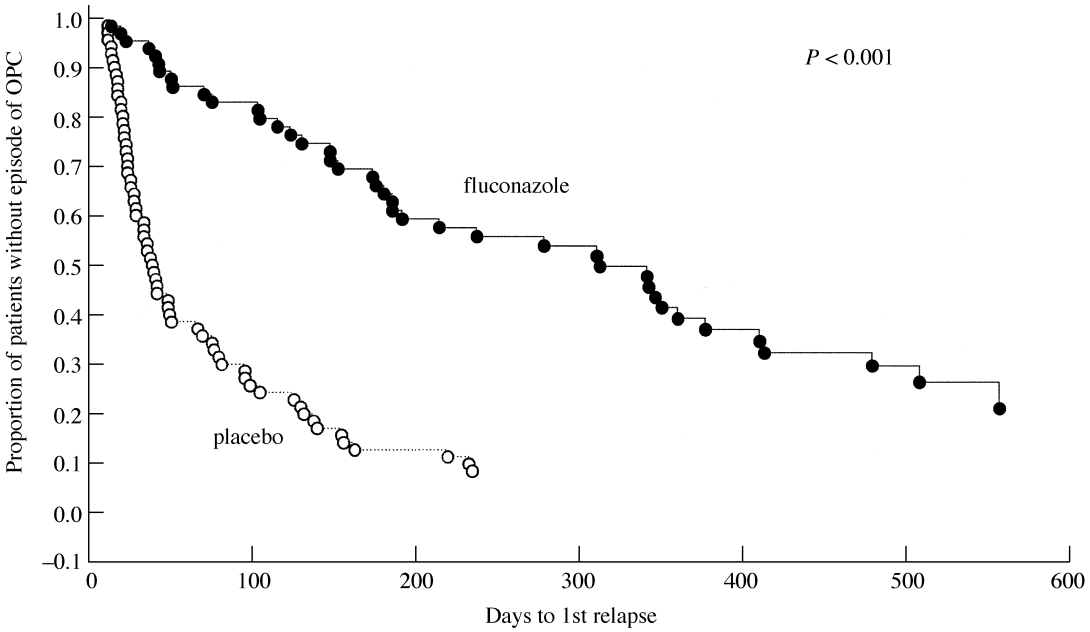


**Figure 1.** Kaplan–Meier curve of time to first relapse of oropharyngeal candidiasis for prevention (filled circles, fluconazole) and intermittent (open circles, placebo) therapy groups.

median time interval from first to second relapse was prolonged by fluconazole (fluconazole group: 68 days; placebo group: 43 days; Mann–Whitney *U*-test, $P < 0.05$). In contrast, there was no difference between the two groups in the median time interval from second to third relapse (fluconazole and placebo group: 41 days).

### *OPC-free intervals*

The median time duration to first relapse following randomization was 312 days in fluconazole-treated patients, compared with 38 days for those receiving placebo. The difference was highly significant ($P < 0.001$). Figure 1 (Kaplan–Meier regression analysis) illustrates this highly significant difference between placebo- and fluconazole-treated patients. Likewise, there was a marked and highly significant difference between the fluconazole- and placebo-treated patients regarding intervals free of multiple relapses. Figure 2 illustrates that while 50% of the placebo patients had experienced three oropharyngeal candidiasis relapses by day 196 post-randomization, only 25% of those who received fluconazole had suffered a third relapse by day 382 ($P < 0.001$).

### *Tolerability*

Fluconazole, whether administered as prevention or as punctual treatment, was generally well tolerated throughout the course of the study. There were no allergic reactions, nor did any patient drop out of the study because of a fluconazole-related adverse event. A total of 108 of the 138 evaluable patients (fluconazole group $n = 50$; placebo group $n = 58$) experienced one or more adverse events unrelated to the study drug (study-emergent illness, worsening of pre-existing disease, hospitalization, death). No serious adverse events considered to be related to the study drug occurred.

### *Resistance to fluconazole*

The detailed data on the individual patients who developed clinical and/or microbiological resistance are presented in Table 1. Clinical failure or resistance to fluconazole, as defined by the persistence of confluent white patches and/or erythema, was observed in a total of five patients, four having been randomized to fluconazole and one to placebo. Those patients had a cumulative dose of fluconazole before study entry of a mean value of 8.7 g (range 2.6–17.9 g) compared with 2.9 g (range 0–28.1 g) in patients without clinical failure.

Clinical resistance observed in five patients was associated with the isolation of a *C. albicans* strain resistant to fluconazole (microbiological resistance), either during the study (two patients in the fluconazole group; one patient in the placebo group), or within 1 month of study end (two patients in the fluconazole group). One case occurred during the second episode of oropharyngeal candidiasis and four cases during the third episode.

Microbiological resistance of *C. albicans* isolates to fluconazole was documented in 12 patients. During the course of the study, microbiological resistance occurred in six patients of the fluconazole group, and in three patients in the placebo group. Within 4 weeks of study completion, microbiological resistance was documented in two additional cases in the fluconazole group and in one case in the placebo group. Among

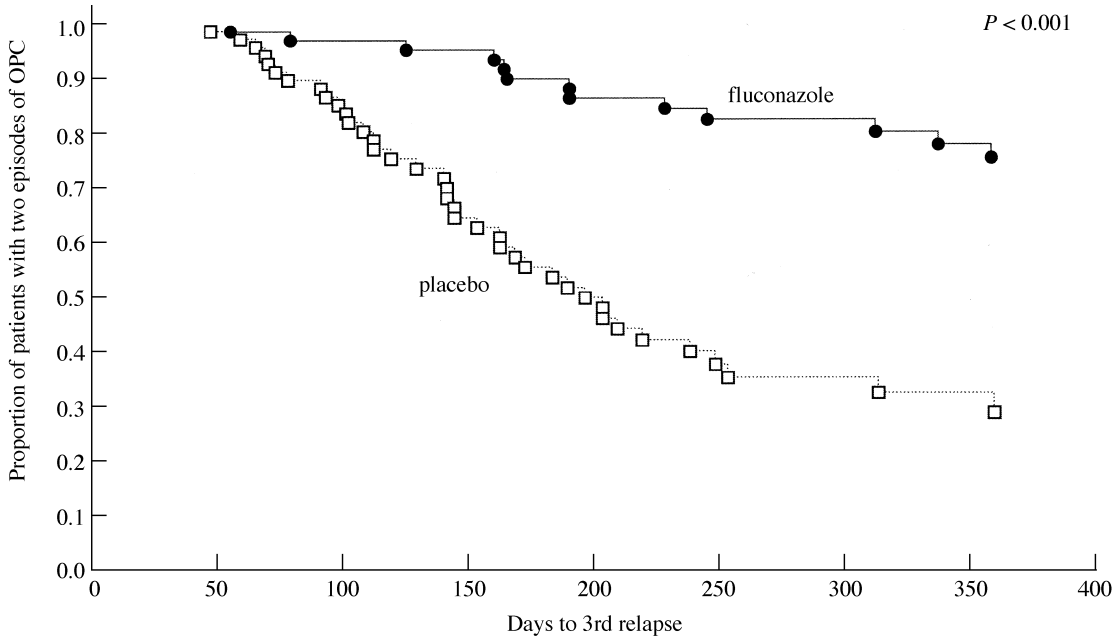


**Figure 2.** Kaplan–Meier curve of time to third relapse of oropharyngeal candidiasis for prevention (filled circles, fluconazole) and intermittent (open squares, placebo) therapy groups.

these 12 cases, five were recorded in patients who also manifested clinical resistance, while seven were not associated with clinical resistance.

In the period following the 30 days post-study, we detected three other patients from whom fluconazole-resistant *C. albicans* was isolated, one patient in the fluconazole group and two patients in the placebo group. This microbiological resistance occurred 3–6 months after the end of the study. For clinical resistance, there was no statistically significant difference between the two treatment groups ($\chi^2$ test, $P = 0.15$). Likewise, there were no significant treatment group differences in microbiological resistance whether comparisons were made for all cases, for cases up to 1 month post-study or for cases later than 1 month post-study.

These results demonstrate that secondary prevention with fluconazole did not lead to a significant increase in clinical or microbiological resistance to the drug, either during or following the study.

### *Multiple regression analysis*

Microbiological resistance was positively and significantly associated with the cumulative fluconazole dose before study entry.[32] In addition, newly emergent resistance was associated with the monthly adjusted fluconazole dose during the study. For clinical failure, a significant association with the mean quarterly rate of oropharyngeal candidiasis episodes before study entry was found, as well as with the monthly adjusted fluconazole dose during the trial. Multiple regression analysis did not reveal any further associations.

## Discussion

The present study confirmed the efficacy and tolerability of weekly fluconazole for the secondary prevention of oropharyngeal candidiasis in HIV-infected patients; it has also expanded previous data to include long-term prevention. In addition, the present study demonstrated that secondary prevention with weekly administration of fluconazole 150 mg is not associated with a significant increase in clinical or microbiological resistance to the drug.

As described in previous studies,[12–17,33,34] a secondary prevention fluconazole regimen was effective. Doses and administration schedules varied from 50 mg daily to 400 mg weekly. In a recent study,[17] a once-weekly dose of fluconazole 400 mg was relatively less successful than 200 mg daily in preventing thrush.

Two recent prospective clinical trials investigating prophylaxis for mucosal candidiasis in HIV-infected patients had not been published when our study started. The first one, conducted by Schuman *et al.*,[33] was a randomized, double-blind, placebo-controlled trial, with a collective of 323 HIV-infected women, and a median follow-up of 29 months. The conclusion was that weekly fluconazole (200 mg) administration appeared to be safe and effective in preventing oropharyngeal and vaginal candidiasis. The occurrence of resistance in *C. albicans* was documented in <5% of patients in each group. In this study only vaginal specimens were routinely cultured for surveillance of resistance. Oropharyngeal specimens were collected only in documented candidiasis.

The second study, conducted by Revankar *et al.*,[34] evaluated the effects of continuous or intermittent therapy with flu-

conazole on the recurrence of candidiasis and development of fluconazole resistance. Sixty-two patients were enrolled with a mean follow-up of 11 months (18 patients with a follow-up of <3 months). Patients were randomized at enrolment in a ratio of 2:1 to either intermittent or continuous therapy with open-labelled fluconazole. Forty-four patients were evaluable. There was no significant difference in the development of resistance, either microbiologically or clinically in the two evaluated groups, but in patients with frequent recurrences, continuous suppressive therapy significantly reduced relapses and colonization.

Between 1994 and 1996, Fichtenbaum *et al.*[35] conducted a multi-centre prospective study to determine the incidence, risk factors and natural history as well as the outcome of fluconazole-refractory mucosal candidiasis in 832 patients with advanced HIV infection (median CD4, 14 cells/mm$^3$). This study was conducted before the use of HAART. In their population, they observed an incidence of refractory candidiasis of 4.2 events per 100 person-years. Those data are comparable with a previous study.[33]

Our data clearly demonstrate that fluconazole is effective in the secondary prevention of oropharyngeal candidiasis over periods longer than those investigated previously. In observations extending up to 18 months, the reduction in the number of clinical oropharyngeal candidiasis episodes was highly significant when compared with placebo. Within the study, the average duration of inclusion to the third relapse was increased from 196 days in the placebo group to 347 days in the fluconazole group.

Likewise, the present study confirmed the excellent tolerability of fluconazole over the long-term. This tolerability has been demonstrated previously in shorter-term trials.

In the present study, secondary prevention with fluconazole did not lead to a significant increase in clinical resistance. The proportion of patients developing clinical resistance remained low in both groups. These findings are consistent with previous reports from short-term observations.[33–39]

In conclusion, the present study has demonstrated the efficacy and tolerability of fluconazole in the secondary prevention of oropharyngeal candidiasis in HIV patients, for up to 18 months. Within this time period, no significant increase in clinical or microbiological resistance to the drug occurred.

Among the few patients who developed clinical and/or microbiological resistance, the cumulative dose of fluconazole taken before the start of the study was a mean value of 8.6 g. These data confirm previous retrospective studies showing correlation between the cumulative dose of fluconazole and the emergence of resistance.[32]

This study ended at the time when clinical trials including anti-proteases had just started. With HAART, the course of HIV infection was profoundly modified. In common with others we observed a drastic reduction in the occurrence of opportunistic infections including oropharyngeal candidiasis.[39–41] The beneficial effect of protease inhibitors on the reduction of opportunistic infections and mortality has also been observed in the most severely immunosuppressed patients. Episodes of oropharyngeal candidiasis resistant to fluconazole in severely immunosuppressed patients (CD4 lymphocytes <10 cells/mm$^3$) with a history of long-term use of fluconazole, have been cured after initiation of HAART.[42,43] The treatment of underlying HIV infection with HAART is indeed very important for the prevention and management of many opportunistic infections.[44] But our observation remains valid, considering the restricted access to effective antiretroviral therapy worldwide as well as instances of failure or intolerance to it.

In conclusion, patients who received secondary prevention with weekly fluconazole had fewer relapses of oropharyngeal candidiasis. Development of clinically and/or microbiologically resistant candidiasis was rarely observed in either the treated or placebo group, without significant differences between the two. However, due to the increasing risk of developing resistance after long-term prevention therapy, our expert recommendation is that such treatment should be considered only for exceptional situations.[41,42,45,46]

## Acknowledgements

We are indebted to C. Durussel for identification and maintaining the *C. albicans* strain collection, and to E. Guex and C. Lauzon for their invaluable help.

## References

**1.** Darouiche, R. O. (1998). Oropharyngeal and esophageal candidiasis in immunocompromised patients: treatment issues. *Clinical Infectious Diseases* **26**, 259–74.

**2.** Tavitian, A., Raufman, J. P., Rosenthal, L. E., Weber, J., Webber, C. A. & Dincsoy, H. P. (1986). Ketoconazole-resistant *Candida* esophagitis in patients with the acquired immunodefiency syndrome. *Gastroenterology* **90**, 443–5.

**3.** Lake-Bakaar, G., Tom, W., Lake-Bakaar, D., Gupta, N., Beidas, S., Elsakr, M. *et al.* (1988). Gastropathy and ketoconazole malabsorption in the acquired immunodeficiency syndrome. *Annals of Internal Medicine* **109**, 471–3.

**4.** De Wit, S., Weerts, D., Goessens, H. & Clumeck, N. (1989). Comparison of fluconazole and ketoconazole for oropharyngeal candidiasis in AIDS. *Lancet* **1**, 746–7.

**5.** Hay, R. J. (1990). Overview of studies of fluconazole in oropharyngeal candidiasis. *Reviews of Infectious Diseases* **12**, *Suppl. 3*, 334–7.

**6.** Just-Nubling, G., Gentschew, G., Dohle, M., Bottinger, C., Helm, E. B. & Stille, W. (1990). Fluconazole in the treatment of oropharyngeal candidosis in HIV-positive patients. *Mycoses* **33**, 435–40.

**7.** Powderly, W. G., Gallant, J. E., Ghannoum, M. A., Mayer, K. H., Navarro, E. E. & Perfect, J. R. (1999). Oropharyngeal candidiasis in patients with HIV: Suggested guidelines for therapy. *Aids Research and Human Retroviruses* **15**, 1619–23.

**8.** Quereda, C., Polanco, A. M., Giner, C., Sanchez-Sousa, A., Pereira, E., Navas, E. *et al.* (1996). Correlation between *in vitro* resistance to fluconazole and clinical outcome of oropharyngeal candidiasis in HIV-infected patients. *European Journal of Clinical Microbiology and Infectious Diseases* **15**, 30–7.

**9.** Stevens, D. A., Greene, S. I. & Lang, O. S. (1991). Thrush can be prevented in patients with aquired immunodeficiency syndrome and the acquired immunodeficiency syndrome-related complex. Randomized, double-blind, placebo-controlled study of 100-mg oral fluconazole daily. *Archives of Internal Medicine* **151**, 2458–64.

**10.** Powderly, W. G., Mayer, K. H. & Perfect, J. R. (1999). Diagnosis and treatment of oropharyngeal candidiasis in patients infected with HIV: a critical reassessment. *Aids Research and Human Retroviruses* **15**, 1405–12.

**11.** Fichtenbaum, C. & Powderly, W. G. (1998). Refractory mucosal candidiasis in patients with human immunodeficiency virus infection. *Clinical Infectious Diseases* **26**, 556–65.

**12.** Esposito, R., Castagna, A. & Uberti Foppa, C. (1990). Maintenance therapy of oropharyngeal candidiasis in HIV-infected patients with fluconazole. *AIDS* **4**, 1033–5.

**13.** Just-Nübling, G., Gentschew, G., Meissner, K., Odewald, J., Staszewski, S., Helm, E. B. *et al.* (1991). Fluconazole prophylaxis of recurrent oral candidiasis in HIV-positive patients. *European Journal of Clinical Microbiology and Infectious Diseases* **10**, 917–21.

**14.** Leen, C. L., Dunbar, E. M., Ellis, M. E. & Mandal, B. K. (1990). Once-weekly fluconazole to prevent recurrences of oropharyngeal candidiasis in patients with AIDS or AIDS-related complex: a double-blind study. *Journal of Infection* **21**, 55–60.

**15.** Marriott, D. J., Jones, P. D., Hoy, J. F., Speed, B. R. & Harkness, J. L. (1993). Fluconazole once a week as secondary prophylaxis against oropharyngeal candidiasis in HIV-infected patients. A double-blind placebo-controlled study. *Medical Journal of Australia* **158**, 312–6.

**16.** Powderly, W. G., Finkelstein, D. M., Feinberg, J., Frame, P., He, W., Van der Horst, C. *et al.* (1995). A randomized trial comparing fluconazole with clotrimazole troches for the prevention of fungal infections in patients with advanced human immunodeficiency virus infection. *New England Journal of Medicine* **332**, 700–5.

**17.** Havlir, D. V., Dubé, M. P., McCutchan, J. A., Forthal, D. N., Kemper, C. A., Dunne, M. *et al.* (1998). Prophylaxis with weekly versus daily fluconazole for fungal infections in patients with AIDS. *Clinical Infectious Diseases* **27**, 1369–75.

**18.** Chave, J. P., Cajot, A., Bille, J. & Glauser, M. P. (1989). Single-dose therapy for oral candidiasis with fluconazole in HIV-infected adults: a pilot study. *Journal of Infectious Diseases* **159**, 806–7.

**19.** Akova, M., Akalin, H. E., Uzun, O. & Gur, D. (1991). Emergence of *Candida krusei* infections after therapy of oropharyngeal candidiasis with fluconazole. *European Journal of Clinical Microbiology and Infectious Diseases* **10**, 598–9.

**20.** Dupouy-Camet, J., Paugam, A., Di Donato, C., Viguie, C., Vicens, I., Volle, P. J. *et al.* (1991). Résistance au fluconazole en milieu hospitalier: concordance entre la resistance de *Candida albicans in vitro* et l'échec thérapeutique. *Presse Médicale* **20**, 1341.

**21.** Willocks, L., Leen, C. L. S., Brettle, R. P., Urquhart, D., Russel, T. B. & Milne, L. J. R. (1991). Fluconazole resistance in AIDS patients. *Journal of Antimicrobial Chemotherapy* **28**, 937–9.

**22.** Warnock, D. W., Burke, J., Cope, N. J., Johnson, E. M., von Fraunhofer, N. A. & Williams, E. W. (1988). Fluconazole resistance in *Candida glabrata*. *Lancet* **2**, 1310.

**23.** Troillet, N., Durussel, C., Bille, J., Glauser, M. P. & Chave, J. P. (1993). Correlation between *in vitro* susceptibility of *Candida albicans* and fluconazole resistant oropharyngeal candidiasis in HIV-infected patients. *European Journal of Clinical Microbiology and Infectious Diseases* **12**, 911–5.

**24.** Sanguineti, A., Carmichael, K. & Campbell, K. (1993). Fluconazole-resistant *Candida albicans* after long-term suppressive therapy. *Archives of Internal Medicine* **153**, 1122–4.

**25.** Maenza, J. R., Keruly, J. C., Moore, R. D., Chaisson, R. E., Merz, W. G. & Gallant, J. E. (1996). Risk factors for fluconazole-resistant candidiasis in human immunodeficiency virus-infected patients. *Journal of Infectious Diseases* **173**, 219–25.

**26.** Maenza, J. R., Merz, W. G., Romagnoli, M. J., Keruly, J. C., Moore, R. D. & Gallant, J. E. (1997). Infection due to fluconazole-resistant *Candida* in patients with AIDS: Prevalence and microbiology. *Clinical Infectious Diseases* **24**, 28–34.

**27.** Newman, S. L., Flanigan, T. P., Fisher, A., Rinaldi, M. G., Stein, M. & Vigilante, K. (1994). Clinically significant mucosal candidiasis resistant to fluconazole treatment in patients with AIDS. *Clinical Infectious Diseases* **19**, 684–6.

**28.** Bille, J. (1996). Correlation between *in vitro* and *in vivo* activity of antifungal agents against yeasts. *Journal de Mycologie Médicale* **6**, 118–22.

**29.** Bille, J., Glauser, M. P. & the Fluconazole Global Susceptibility Study Group. (1997). Evaluation of the susceptibility of pathogenic *Candida* species to fluconazole. *European Journal of Clinical Microbiology and Infectious Diseases* **16**, 924–8.

**30.** National Committee for Clinical Laboratory Standards. (1996). *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts. Approved Standard M27-A*. NCCLS, Villanova, PA.

**31.** Sanglard, D., Kuchler, K., Ischer, F., Pagani, J. L., Monod, M. & Bille, J. (1995). Mechanisms of resistance to azole antifungal agents in *Candida albicans* isolates from AIDS patients involve specific multidrug transporters. *Antimicrobial Agents and Chemotherapy* **39**, 2378–86.

**32.** Vuffray, A., Durussel, C., Boerlin, P., Boerlin-Petzold, F., Bille, J., Glauser, M. P. *et al.* (1994). Oropharyngeal candidiasis resistant to single-dose therapy with fluconazole in HIV infected patients. *AIDS* **8**, 708–9.

**33.** Schuman, P., Capps, L., Peng, G., Vazquez, J., el-Sadr, W., Goldman, A. I. *et al.* (1997). Weekly fluconazole for the prevention of mucosal candidiasis in women with HIV infection. A randomized, double-blind, placebo-controlled trial. *Annals of Internal Medicine* **126**, 689–96.

**34.** Revankar, S. G., Kirkpatrick, W. R., McAtee, R. K., Dib, O. P., Fothergill, A. W., Redding, S. W. *et al.* (1998). A randomized trial of continuous or intermittent therapy with fluconazole for oropharyngeal candidiasis in HIV-infected patients: clinical outcomes and

development of fluconazole resistance. *American Journal of Internal Medicine* **105**, 7–11.

**35.** Fichtenbaum, C. J., Koletar, S., Yiannoutsos, C., Holland, F., Pottage, J., Cohn, S. E. *et al.* (2000). Refractory mucosal candidiasis in advanced human immunodeficiency virus infection. *Clinical Infectious Diseases* **30**, 749–56.

**36.** Ruhnke, M., Eigler, A., Tennagen, I., Geiseler, B., Engelmann, E. & Trautmann, M. (1994). Emergence of fluconazole-resistant strains of *Candida albicans* in patients with recurrent oropharyngeal candidiasis and human immunodeficiency virus infection. *Journal of Clinical Microbiology* **32**, 2092–8.

**37.** Redding, S., Smith, J., Farinacci, G., Rinaldi, M., Fothergill, A., Rhine-Chalberg, J. *et al.* (1994). Resistance of *Candida albicans* to fluconazole during treatment of oropharyngeal candidiasis in a patient with AIDS: documentation by *in vitro* susceptibility testing and DNA subtype analysis. *Clinical Infectious Diseases* **18**, 240–2.

**38.** Baily, G. G., Perry, F. M., Denning, D. W. & Mandal, B. K. (1994). Fluconazole-resistant candidiasis in an HIV cohort. *AIDS* **8**, 787–92.

**39.** Ledergerber, B., Egger, M., Erard, V., Weber, R., Hirschel, B., Furrer, H. *et al.* (1999). AIDS-related opportunistic illnesses occurring after initiation of potent antiretroviral therapy: the Swiss HIV Cohort Study. *Journal of the American Medical Association* **282**, 2220–6.

**40.** Michelet, C., Arvieux, C., François, C., Besnier, J. M., Rogez, J. P., Breux, J. P. *et al.* (1998). Opportunistic infections occurring during highly active antiretroviral treatment. *AIDS* **12**, 1815–22.

**41.** Kovacs, J. A. & Masur, H. (2000). Prophylaxis against opportunistic infections in patients with human immunodeficiency virus infection. *New England Journal of Medicine* **342**, 1416–29.

**42.** Ruhnke, M., Adler, A. & Muller, F. M. (2000). Clearance of a fluconazole-resistant *Candida albicans* strain after switching antifungal therapy and initiation of triple therapy for HIV infection. *Clinical Microbiology and Infection* **6**, 220–3.

**43.** Zingman, B. S. (1996). Resolution of refractory AIDS-related mucosal candidiasis after initiation of didanosine plus saquinavir. *New England Journal of Medicine* **334**, 1674–5.

**44.** Martin, M. V. (1999). The use of fluconazole and itraconazole in the treatment of *Candida albicans* infections: a review. *Journal of Antimicrobial Chemotherapy* **44**, 429–37.

**45.** Rex, J. H., Walsh, T. J., Sobel, J. D., Filler, S. G., Pappas, P. G., Dismukes, W. E. *et al.* (2000). Practice guidelines for the treatment of candidiasis. *Clinical Infectious Diseases* **30**, 662–78.

**46.** USPHS/IDSA Prevention of Opportunistic Infections Working Group. (1999). USPHS/IDSA guidelines for the prevention of opportunistic infections in persons infected with human immunodeficiency virus. *Annals of Internal Medicine* **11**, 873–908.