

# Discontinuation of Primary and Secondary *Toxoplasma gondii* Prophylaxis Is Safe in HIV-Infected Patients after Immunological Restoration with Highly Active Antiretroviral Therapy: Results of an Open, Randomized, Multicenter Clinical Trial

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**Background.** To our knowledge, no randomized trials have evaluated whether prophylaxis against toxoplasmic encephalitis can be safely discontinued after the CD4<sup>+</sup> T cell count increases in response to highly active antiretroviral therapy.

**Methods.** We conducted a randomized, nonblinded, multicenter clinical trial of the discontinuation of primary or secondary prophylaxis against toxoplasmic encephalitis in human immunodeficiency virus (HIV)–infected patients with a sustained response to antiretroviral therapy (defined as a CD4<sup>+</sup> T cell count of  $\geq 200$  cells/mm<sup>3</sup> and a plasma HIV type 1 [HIV-1] RNA level of  $< 5000$  copies/mL for at least 3 months). Prophylaxis was restarted if the CD4<sup>+</sup> T cell count decreased to  $< 200$  cells/mm<sup>3</sup>.

**Results.** The 381 patients receiving primary prophylaxis had a median CD4<sup>+</sup> T cell count on study entry of 343 cells/mm<sup>3</sup>, and 318 (83%) of 381 patients had undetectable HIV-1 RNA in plasma. After a median follow-up period of 25 months (409 person-years), there were no episodes of toxoplasmic encephalitis among the 196 patients who discontinued prophylaxis (at 1 year, the upper limit of the 95% confidence interval for relapse rate was 2.40%). For the 57 patients receiving secondary prophylaxis, the median CD4<sup>+</sup> T cell count on entry was 407 cells/mm<sup>3</sup>, and 49 (86%) of 57 patients had undetectable HIV-1 RNA in plasma. After a median follow-up period of 30.5 months (69 person-years), there were no episodes of toxoplasmic encephalitis among the 28 patients who discontinued prophylaxis (at 1 year, the upper limit of the 95% confidence interval for relapse rate was 16%).

**Conclusions.** In HIV-infected adult patients receiving effective highly active antiretroviral therapy, primary and secondary prophylaxis against toxoplasmic encephalitis can be safely discontinued after the CD4<sup>+</sup> T cell count has increased to  $\geq 200$  cells/mm<sup>3</sup> for  $> 3$  months.

The advent of HAART has completely changed the course of HIV infection, with a striking reduction in morbidity and mortality in western countries [1, 2]. Several articles report a dramatic decrease in the in-

cidence of most opportunistic infections, including toxoplasmic encephalitis (TE), after institution of HAART [3]. During the last few years, several studies have demonstrated that primary or secondary prophylaxis can be safely discontinued for several opportunistic infections, such as *Pneumocystis jirovecii* pneumonia, *My-*

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*cobacterium avium* complex disseminated infection, or cytomegalovirus retinitis, in patients with immune reconstitution induced by HAART [4–7]. As far as TE is concerned, several observational studies [8–10] and 1 clinical trial [11] have suggested that primary prophylaxis can be safely discontinued in patients with a CD4<sup>+</sup> T cell count >200 cells/mm<sup>3</sup> for at least 3 months. However, these studies were not primarily aimed at stopping TE prophylaxis; they were substudies of *P. jirovecii* pneumonia prophylaxis discontinuation studies. On the other hand, discontinuation of secondary TE prophylaxis has been examined only in observational studies with a small number of patients [12–14] or in studies that analyze several small cohort studies together as 1 large cohort study [15] and show that maintenance therapy may be safely discontinued in patients who have a CD4<sup>+</sup> T cell count >200 cells/mm<sup>3</sup> for at least 3–6 months. On the basis of these data, the 2004 Centers for Disease Control and Prevention (CDC), National Institutes of Health, and HIV Medicine Association–Infectious Diseases Society of America (IDSA) Task Force panel has rated discontinuation of primary TE prophylaxis as “AI,” but it only rated TE maintenance therapy as “CIII,” because there are no randomized clinical trials that have studied it and because of “the number of such patients who have been evaluated remain limited” [16, p. 16].

In this study, we present the final results of the first randomized, multicenter, nationwide trial to examine the discontinuation of TE prophylaxis with a very extended follow-up period. Our data strongly support the recommendation that primary and secondary TE prophylaxis can be safely discontinued in patients with immune reconstitution induced by HAART.

## METHODS

**Patients.** Patients were eligible for the Grupo de Estudio de Sida (GESIDA) 04/98-B study if they fulfilled the following criteria: having serologic analysis results positive for *Toxoplasma gondii*, a previous CD4<sup>+</sup> T cell count of <200 cells/mm<sup>3</sup>, a previous episode of TE (only for the secondary prophylaxis discontinuation trial), receipt of treatment with any of the regimens accepted as being prophylaxis against TE, a sustained response to HAART (defined by a CD4<sup>+</sup> T cell count ≥200 cells/mm<sup>3</sup> and a plasma HIV type 1 [HIV-1] RNA level <5000 copies/mL for >3 months), and a Karnofski score >80. Patients were excluded if they were <18 years old, pregnant, or had adherence to antiretroviral treatment that was considered to be poor.

**Study design.** The study was a randomized, nonblinded, multicenter trial that evaluated whether primary and secondary prophylaxis against TE could be safely discontinued in HIV-infected patients. Patients were recruited from 22 Spanish public hospitals with broad experience in the care of HIV-infected

patients. These patients were not included in other Spanish studies exploring discontinuation of *P. jirovecii* pneumonia prophylaxis. The randomization, based on permuted blocks, was stratified according to center and for primary prophylaxis according to the nadir level of CD4<sup>+</sup> T cells (more or less than 100 cells/mm<sup>3</sup>). The trial was approved by the institutional review board of each participating hospital, and all patients gave written informed consent. Patients were randomly assigned to continue or to discontinue prophylaxis against TE. Accepted regimens of prophylaxis were those recommended in the 1997 guidelines of the US Public Health Service and the IDSA [17]. HAART was considered when at least 3 antiretroviral drugs, 1 of which was a protease inhibitor, or a nonnucleoside reverse-transcriptase inhibitor (NNRTI) were included. When the CD4<sup>+</sup> T cell counts of patients assigned to discontinue prophylaxis decreased to <200 cells/mm<sup>3</sup>, prophylaxis was immediately restarted if this figure was confirmed by a second cell count determination, although such patients were kept in the study. An increase in the HIV-1 RNA level was not a criterion for restarting prophylaxis.

Chronic *T. gondii* infection was diagnosed if patients had positive results (IgG antibodies) of serologic analysis using ELISA or immunofluorescence assay. All patients had 2 tests with positive results, the first of which occurred at screening and the second of which occurred at study entry. Acute TE was diagnosed on the basis of a clinical and radiological response to specific treatment for 4–8 weeks in patients with clinical and neuroradiological evidence (obtained by either CT scanning or MRI) of ≥1 space-occupying encephalic lesion and the presence of IgG antibodies against *T. gondii* [18]. None of the cases was diagnosed on the basis of brain biopsy results.

Patients were evaluated at least every 3 months with a clinical assessment and laboratory monitoring that included measurements of CD4<sup>+</sup> T cell counts and HIV-1 RNA levels, which were performed at each site. Lymphocyte subpopulations were measured at all centers by 3-color flow cytometry. HIV-1 RNA levels were determined by either a PCR assay (Amplicor HIV-1 Monitor Assay; Roche Molecular Systems) or a branched-chain DNA assay (Chiron). When the study was designed, most of the hospitals used techniques with a detection limit of 400 copies/mL for the PCR assay or 500 copies/mL for the branched DNA assay. For this reason, we kept 500 copies/mL as the limit of detection for the HIV-1 RNA level throughout the study. Study patients were instructed to contact the medical center if they had any signs or symptoms suggesting TE or another “C” event (as defined by the CDC). Patients with previous TE who discontinued secondary prophylaxis were scheduled to have a cranial CT scan or MRI during the first 6 months of follow-up.

**End points and follow-up.** The primary end point in the assessment of safety was the occurrence of TE. The secondary end points were the development of an AIDS-defining event

other than TE (a "C" event), the occurrence of drug-related adverse effects, the development of a non-AIDS-defining bacterial infection, changes in CD4<sup>+</sup> T cell counts and HIV-1 RNA levels, and death. Patients were removed from the study during the follow-up period whenever 1 of the following events occurred: an AIDS-defining event (including TE), hypersensitivity to the prophylactic agents, discontinuation of HAART, or voluntary withdrawal from the study. A decrease in CD4<sup>+</sup> T cell counts to <200 cells/mL alone was not a criterion for removal from the study.

**Statistical analysis.** We assumed that TE would develop in 3% of patients receiving primary prophylaxis during the first 12 months of follow-up and in at least 13% of patients who discontinued prophylaxis [19, 20]. We estimated that at least 173 at-risk patients would be needed in each group for the study to be able to detect a 10% difference with 90% power and a significance level of .05. Ten percent of patients were expected to be lost to follow-up.

We assumed that TE would develop in 10% of patients receiving secondary prophylaxis during the first 12 months of follow-up and in at least 65% of patients who discontinued prophylaxis [21, 22]. We estimated that at least 20 at-risk patients would be needed in each group to enable us to detect a 55% difference with 90% power and a significance level of .05. Ten percent of patients were expected to be lost to follow-up.

An intention-to-treat analysis was performed. Median and interquartile ranges (25th–75th percentiles) were used as measures of central tendency and dispersion. CIs for both groups were calculated using Poisson distribution tables. For baseline variables, comparisons between groups were made using the  $\chi^2$  test for categorical variables and the Mann-Whitney nonparametric test for quantitative variables. A random coefficient model was used to compare CD4<sup>+</sup> T cell counts at enrollment and at the first, second, third, and fourth follow-up visits. A polynomial contrast was used to model the within-group sum of squares and a difference contrast to model the between-group sum of squares. All reported *P* values are 2-sided.

## RESULTS

**Primary prophylaxis.** A total of 381 patients with no history of TE were enrolled in the study between 1 September 1997 and 30 June 2000. Of these, 196 patients were randomly assigned to discontinue prophylaxis, and 185 were assigned to continue. The groups were well balanced with respect to demographic, clinical, immunological, and virological characteristics and type of HAART regimen (table 1). One hundred eighty-nine patients (96 in the group discontinuing prophylaxis and 93 in the group continuing prophylaxis) had a nadir CD4<sup>+</sup> T cell count of no more than 100 cells/mm<sup>3</sup>. Prophylaxis with trimethoprim-sulfamethoxazole, dapsone plus pyrimethamine,

or other drugs was given to 91%, 6%, and 3% of patients, respectively, for a median of 33 months (range, 3–108 months).

The median duration of follow-up was 23.7 months (range, 3–42 months). The CD4<sup>+</sup> T cell count evolution and the proportion of patients with an HIV-1 RNA level of <500 copies/mL during the follow-up period were similar in the 2 groups (figure 1). In 17 patients (5 in the group discontinuing prophylaxis and 12 in the group continuing prophylaxis), CD4<sup>+</sup> T cell counts decreased to <200 cells/mm<sup>3</sup>, and prophylaxis had to be reintroduced to patients in whom it had been discontinued. Thirty-two patients in the group discontinuing prophylaxis and 27 in the group continuing prophylaxis had HIV-1 RNA levels of >500 copies/mL for 128.1 person-years of follow-up (the median HIV-1 RNA level for the group discontinuing prophylaxis was 1434 copies/mL [range, 504–3650 copies/mL], and the median HIV-1 RNA level for the group continuing prophylaxis was 1820 copies/mL [range, 561–4848 copies/mL]). During the follow-up period, the protease inhibitor was replaced with an NNRTI for 87 patients (47 in the group discontinuing prophylaxis and 40 in the group continuing prophylaxis).

There were no TE episodes in either group during follow-up. The 95% CI for relapse rate at 6 and 12 months and the incidence of TE per 100 person-years in both groups are shown in table 1. There were no statistically significant differences between both groups. At 1 year, the upper limit of the 95% CI for relapse rate for the group discontinuing prophylaxis was 2.40%.

One patient in the group discontinuing prophylaxis had a "C" event. Other events are shown in table 1. Four patients died (3 in the group discontinuing prophylaxis and 1 in the group continuing prophylaxis) because of non-HIV-related causes (table 1).

One patient who discontinued prophylaxis developed Hodgkin disease. Drug-related adverse effects occurred in 48 patients (31 in the group discontinuing prophylaxis and 17 in the group continuing prophylaxis); these events were mostly attributed to the use of protease inhibitors, and only in 1 case was the event attributed to the use of prophylactic agents. Finally, antiretroviral treatment had to be modified in 76 patients (42 in the group discontinuing prophylaxis and 34 in the group continuing prophylaxis; *P* is not significant), either because of adverse effects of the antiretroviral drugs or because of virologic evidence of treatment failure.

**Secondary prophylaxis.** Between 1 July 1998 and 30 June 2000, a total of 57 patients with a previous episode of TE were enrolled in the study (tables 2 and 3). Twenty-eight patients were randomly assigned to discontinue secondary prophylaxis, and 29 patients were randomly assigned to continue. Their clinical and neuroradiological characteristics and the specific acute and maintenance therapies are shown in table 2. The

**Table 1. Main characteristics of patients continuing or discontinuing primary *Toxoplasma gondii* prophylaxis.**

Characteristic	Continuing PPG	Discontinuing PPG
<b>At baseline</b>		
No. of patients	185	196
Age, median years (IQR)	37 (33–42.5)	37 (33–42)
Male sex	133 (74)	151 (79)
Mode of acquisition of infection		
Injection drug use	93 (50)	96 (49)
Homosexual activity	37 (20)	45 (23)
Heterosexual activity	46 (25)	47 (24)
Other	9 (5)	8 (4)
Time from diagnosis of HIV infection to study entry, median years (IQR)	6 (3–9)	6 (3–8)
CDC group		
A-III	90 (49)	78 (40)
B-III	26 (14)	34 (17)
C-III	69 (37)	84 (43)
CD4 <sup>+</sup> T cell count, median cells/mm <sup>3</sup> (IQR)		
Nadir	98 (44.5–154)	104 (48.25–154)
At baseline	330 (272.5–431)	361 (286.5–477)
HIV-1 RNA level		
<500 copies/mL	154 (83)	164 (84)
Level among patients with >500 copies/mL, median copies/mL (range)	1820 (561–4848)	1434 (504–3650)
Time with a CD4 <sup>+</sup> T cell count >200 cells/mm <sup>3</sup> and an HIV-1 RNA level <500 copies/mL, median months (IQR)	8 (5–13)	9 (6–14)
Time receiving prophylaxis, median months (IQR)	33 (20.3–49.6)	34 (17.28–49.13)
Time receiving HAART, median months (IQR)	17 (11.4–23.3)	16 (10.3–22.1)
Treatment received		
Lamivudine	145	157
Stavudine	126	132
Indinavir	115	120
Zidovudine	61	56
Saquinavir	38	38
Ritonavir	26	27
Nelfinavir	25	24
Didanosine	30	24
Nevirapine	13	13
Dideoxycytidine	2	5
Receipt of protease inhibitor-sparing regimen	0	3
<b>At follow-up</b>		
No. of episodes of toxoplasmic encephalitis	0	0
Patients with relapse at 6 months, % (95% CI) <sup>a</sup>	0 (0–2.50)	0 (0–2.34)
Patients with relapse at 12 months, % (95% CI) <sup>b</sup>	0 (0–2.75)	0 (0–2.40)
Duration of follow-up after randomization		
Overall, median months (IQR)	24.9 (20.8–29.6)	24.9 (19.5–29.1)
Total person-years	378.7	400.0
No. of episodes per 100 person-years, 95% CI	0–0.86	0–0.80
No. of episodes per 100 person-years, 99% CI	0–1.26	0–1.16
Patients with a nadir CD4 <sup>+</sup> T cell count <100 cells/mm <sup>3</sup> , person-years	184.4	182.5
No. of episodes per 100 person-years, 95% CI	0–1.78	0–1.8
No. of episodes per 100 person-years, 99% CI	0–2.59	0–2.61
Patients with a nadir CD4 <sup>+</sup> T cell count of 100–199 cells/mm <sup>3</sup> , person-years	194.4	225.3
No. of episodes per 100 person-years, 95% CI	0–1.69	0–1.46
No. of episodes per 100 person-years, 99% CI	0–2.15	0–2.12

(continued)

**Table 1. (Continued.)**

Characteristic	Continuing PPG	Discontinuing PPG
Duration of follow-up with a CD4 <sup>+</sup> T cell count $\geq$ 200 cells/mm <sup>3</sup>		
Median months (IQR)	24.2 (19.6–28.9)	24.9 (21–29.5)
Person-years	359.3	404.2
No. of episodes per 100 person-years, 95% CI	0–0.91	0–0.81
Duration of follow-up with a CD4 <sup>+</sup> T cell count <200 cells/mm <sup>3</sup>		
Median months (IQR)	19.3 (13.6–26.9)	25.8 (11.3–25.8)
Person-years	19.4	5.8
No. of episodes per 100 person-years, 95% CI	0–16.9	0–56.6
Patients who dropped out of the study	12	11
Lost to follow-up	10	8
No. of protocol violations <sup>c</sup>	2	3
No. of “C” events	0	1 <sup>d</sup>
No. of other events	8 <sup>e</sup>	9 <sup>f</sup>
No. of deaths <sup>g</sup>	1	3

**NOTE.** Data are no. (%) of patients, unless otherwise indicated. Category A includes patients who have had no HIV-related diseases; category B includes patients who have had HIV-related diseases that are not in category C; category C includes patients who have had HIV-related diseases that are considered to be AIDS-defining (reference). CDC, Centers for Disease Control and Prevention; IQR, interquartile range; PPG, primary prophylaxis group.

<sup>a</sup> *N* = 181 for the continuing primary prophylaxis group, and *N* = 194 for the discontinuing primary prophylaxis group.

<sup>b</sup> *N* = 164 for the continuing primary prophylaxis group, and *N* = 186 for the discontinuing primary prophylaxis group.

<sup>c</sup> One patient assigned to continue prophylaxis discontinued after enrollment, and 2 patients in the group discontinuing prophylaxis decided to resume because they were concerned about the risk of TE. One patient in each group discontinued HAART.

<sup>d</sup> Cryptosporidiasis.

<sup>e</sup> Six cases of community-acquired bacterial pneumonia and 2 cases of herpes-zoster virus infection.

<sup>f</sup> Seven cases of community-acquired bacterial pneumonia, 2 cases of herpes-zoster virus infection, and 1 case of Hodgkin disease. In all cases of community-acquired pneumonia, *Pneumocystis jirovecii* was ruled out as the cause of infection by microbiological methods. No patient received empirical anti-*P. jirovecii* treatment in therapeutic doses during the follow-up period.

<sup>g</sup> The causes of death were acute respiratory failure due to *Legionella pneumophila* pneumonia, acute brain hemorrhage secondary to the rupture of a cerebral aneurysm, sudden death in a patient who previously received a diagnosis of progressive multifocal leucoencephalopathy, and complications related to end-stage liver disease, in 1 case each.

main epidemiological, clinical, immunological, and virological characteristics at study entry and during follow-up are shown in table 3. During follow-up, the protease inhibitor was replaced by an NNRTI for 10 patients (5 in the group discontinuing prophylaxis and 5 in the group continuing prophylaxis).

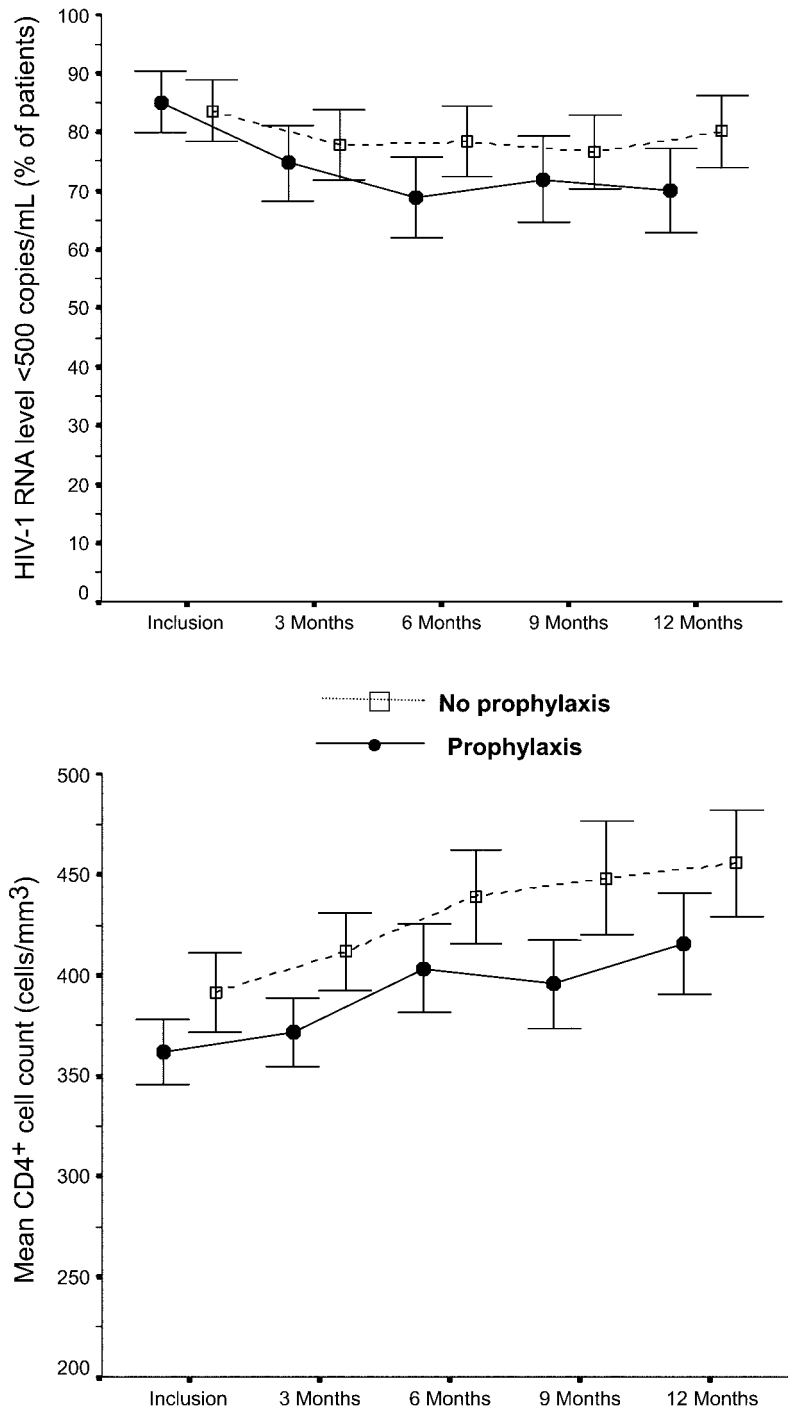
There were no relapses of TE in either group during follow-up. The 95% CI for relapse rate at 6 and 12 months and the incidence of TE per 100 person-years in both groups are shown in table 3. There were no statistically significant differences between both groups. At 1 year, the upper limit of the 95% CI for relapse rate for the group discontinuing prophylaxis was 16%. All patients who discontinued prophylaxis had a control cranial CT scan or MRI (at a median of 6 months [interquartile range, 5–6 months]). None of them had radiological signs of TE reactivation. None of the patients had a “C” event or died. Other events are shown in table 3. Secondary prophylaxis adverse effects occurred in 2 patients. HAART was modified in 7 patients (4 in the group discontinuing prophylaxis and 3 in the group continuing prophylaxis; *P* is not significant), because of virologic evidence of treatment failure or because of adverse effects.

After analyzing these results, the GESIDA 04/98 Steering Committee decided to stop secondary prophylaxis in the con-

tinuing prophylaxis group on 31 October 2000. Patients from both groups were followed up during this open-label phase until 30 May 2002. The median duration of follow-up after study entry was 29 months (range, 7–42 months). There were no patients lost to follow-up or discontinuations of HAART. There were no TE relapses in either group during the long-term follow-up period (the upper limit of the 95% CI for TE incidence for the group discontinuing prophylaxis was 4.78 episodes per 100 person-years). None of the patients had a “C” event. One patient randomized to discontinue prophylaxis died.

## DISCUSSION

After starting HAART, there is an immediate improvement in immunological parameters [23], although it has been suggested that a complete immune reconstitution could only be achieved after several years of HAART [24]. The decrease in the incidence of opportunistic infections and death among HIV-infected patients supports the clinical relevance of HAART-associated immunological reconstitution [1, 2]. There are few studies on the discontinuation of primary TE prophylaxis in patients with immunological recovery after receipt of HAART. In fact, all were subanalyses of *P. jirovecii* prophylaxis discontinuation



**Figure 1.** Mean CD4<sup>+</sup> T cell counts and proportions of patients who had undetectable HIV-1 RNA levels at baseline (month 0) and during follow-up, according to whether they were assigned to discontinue or continue primary prophylaxis against toxoplasmic encephalitis. The bars represent 95% CIs. Only data for the first 12 months of follow-up are included. The curves have been offset for ease of viewing; all measurements were made at 3-month intervals. The mixed-effects model fitted a model with a different intercept ( $P < .05$ ) for each arm (difference in CD4<sup>+</sup> T cell count between both groups, 33 cells/mm<sup>3</sup>) and the same slope. The estimate for interaction between time since entry and group variable was not statistically significant.

**Table 2. Clinical and neuroradiological characteristics of 57 episodes of toxoplasmic encephalitis (TE) in patients randomized to discontinue or continue secondary *Toxoplasma gondii* prophylaxis.**

Characteristic	Continuing SPG	Discontinuing SPG
No. of patients	29	28
Duration of symptoms, median months (IQR)	13.5 (3–30)	14 (5–30)
Clinical manifestations		
Headache	17 (59)	11 (39)
Seizures	6 (21)	7 (25)
Focal signs	19 (65)	18 (64)
Abnormal mental status	7 (24)	7 (25)
Fever	17 (59)	11 (40)
CT and/or MRI characteristics of acute episode		
No. of lesions, median (range)	2 (1–4)	2 (1–3)
Contrast enhancement	25 (86)	22 (79)
Edema/mass effect	20 (70)/13 (45)	22 (79)/16 (57)
Received therapy of acute episode		
Sulfadiazine plus pyrimethamine plus folinic acid	26 (90)	25 (80)
Clindamycin plus pyrimethamine plus folinic acid	1 (3)	1 (4)
Other antitoxoplasma therapies	2 (7)	2 (8)
Corticosteroids	10 (35)	6 (21)
Duration of acute therapy, median weeks (range)	6 (2–8)	6 (5–8)
Clinical sequelae after the acute episode		
No sequelae	23 (79)	25 (89)
Any sequelae	6 (21)	3 (11)
Type of secondary prophylaxis		
Sulfadiazine plus pyrimethamine plus folinic acid	20 (69)	21 (75)
Clindamycin plus pyrimethamine plus folinic acid	6 (21)	4 (14)
Other antitoxoplasma therapies	3 (10) <sup>a</sup>	3 (11) <sup>b</sup>
TE relapse before HAART	0 (0)	3 (11)

**NOTE.** Data are no. (%) of patients, unless otherwise indicated. IQR, interquartile range; SPG, secondary prophylaxis group.

<sup>a</sup> Two patients (7%) took trimethoprim-sulfamethoxazole, and 1 patient (3%) took atovaquone.

<sup>b</sup> Two patients (7%) took trimethoprim-sulfamethoxazole, and 1 patient (4%) took atovaquone.

studies, and patients were included with a CD4<sup>+</sup> T lymphocyte count of <200 cells/mm<sup>3</sup>. More than 300 patients were evaluated [8–11], and only 1 had an episode of TE. In our trial, patients were stratified according to the level of CD4<sup>+</sup> T cells (equal to or more or fewer than 100 cells/mm<sup>3</sup>), because those at highest risk of TE reactivation are those with a cell count of <100 cells/mm<sup>3</sup>. None of the patients had an episode of TE during the follow-up period. At 1 year, the upper limit of the 95% CI for relapse rate for the group discontinuing prophylaxis was only 2.40%. This relapse rate is below the TE risk rate of patients who received primary prophylaxis in the pre-HAART era [20, 25]. These data support the CDC recommendation to discontinue primary prophylaxis in patients with a sustained increase in the CD4<sup>+</sup> T lymphocyte count of >200 cells/mm<sup>3</sup> for at least 3 months. Although there are no guidelines for the reintroduction of primary TE prophylaxis in these patients, it seems reasonable to resume prophylaxis in accordance with the same criteria used for primary prophylaxis (i.e., when the CD4<sup>+</sup>

T cell count decreases to <100 cells/mm<sup>3</sup>). However, in clinical practice, prophylaxis is initiated when the CD4<sup>+</sup> T cell count decreases to <200 cells/mm<sup>3</sup>, because this is the threshold for initiating *P. jirovecii* pneumonia prophylaxis.

Fifty-seven of our patients had had a previous episode of TE and were receiving secondary prophylaxis. It is well known that the risk of relapse after a TE episode is high among patients not receiving secondary prophylaxis, with an incidence of 50%–80% among patients surviving more than 6–12 months [21, 26]. There are few data regarding stopping prophylaxis in these patients, and most data are from observational studies [12–15] with few patients. If we take all the cases studied as a whole, only 1 patient experienced relapse of 126 patients who stopped maintenance therapy after a median follow-up period of 18 months. However, observational cohort studies have limitations and biases, and the safety of interrupting secondary prophylaxis ideally should be addressed in randomized controlled trials [15]. To our knowledge, our study represents the only clinical

**Table 3. Main characteristics of patients discontinuing or continuing secondary *Toxoplasma gondii* prophylaxis.**

Characteristic	Continuing SPG	Discontinuing SPG
<b>At baseline</b>		
No. of patients	29	28
Age, median years (IQR)	36 (33–43.5)	36.5 (33–41)
Male sex	26 (89)	21 (75)
Mode of acquisition of infection		
Injection drug use	10 (34.5)	16 (57)
Homosexual activity	10 (34.5)	5 (18)
Heterosexual activity	8 (29)	7 (25)
Other	1 (4)	...
Time from diagnosis of HIV infection to study entry, median years (IQR)	6 (2–9)	7 (3–11.7)
CD4 <sup>+</sup> T cell count, median cells/mm <sup>3</sup> (IQR)		
Nadir	24 (10–54)	35 (14.2–59)
At baseline	364 (255–473)	416 (317.7–521)
HIV-1 RNA level		
<500 copies/mL	25 (86)	24 (86)
Level among patients with >500 copies/mL, median copies/mL (range)	3150 (690–4060)	3700 (3000–4690)
Time with a CD4 <sup>+</sup> T cell count >200 cells/mm <sup>3</sup> and an HIV-1 RNA level <500 copies/mL, median months (IQR)	9 (6–16.7)	12 (6–19.7)
Time receiving prophylaxis, median months (IQR)	33 (22.2–60.8)	29 (22.3–42.8)
Time receiving HAART, median months (IQR)	28 (19.7–30.8)	25 (21.2–28.6)
Treatment received		
Lamivudine	23	27
Stavudine	6	1
Indinavir	20	14
Zidovudine	6	9
Saquinavir	2	3
Ritonavir	2	3
Nelfinavir	6	6
Didanosine	6	1
Nevirapine	0	2
Dideoxycytidine	0	0
Receipt of protease inhibitor-sparing regimen	0	2
<b>At follow-up</b>		
No. of episodes of toxoplasmic encephalitis	0	0
Patients with relapse at 6 months, % of patients (95% CI) <sup>a</sup>	0 (0–12)	0 (0–12)
Patients with relapse at 12 months, % of patients (95% CI) <sup>b</sup>	0 (0–23)	0 (0–16)
Duration of follow-up after randomization and open-label phase		
Median months (IQR)	16.2 (9.8–19.3)	18.4 (11.2–22.3)
No. of person-years	36.9	42.3
No. of episodes per 100 person-years, 95% CI	0–8.9	0–7.8
Duration of follow-up after randomization and at end of study		
Median months (IQR)	25.1 (21.6–31.5)	30.5 (22.7–34.1)
Person-years	63.4	68.7
No. of episodes per 100 person-years, 95% CI	0–5.19	0–4.78
No. of episodes per 100 person-years, 99% CI	0–7.53	0–6.94
CD4 <sup>+</sup> T 'cell count during follow-up, cells/mm <sup>3</sup>		
Month 3, median (IQR)	403 (305–466)	435 (328–544.2)
Month 6, median (IQR)	396 (343–489.5)	444 (353–599)
Month 9, median (IQR)	408 (292.5–566)	435 (347.5–584.5)
Month 12, median (IQR)	455 (322–616)	472 (357.5–575.7)
Month 24, median (IQR)	452 (299.8–676.3)	517 (396.5–645.5)

*(continued)*

**Table 3. (Continued.)**

Characteristic	Continuing SPG	Discontinuing SPG
Patients who dropped out of the study	0	0
No. of "C" events	0	0
No. of other events	1 <sup>c</sup>	1 <sup>d</sup>
No. of deaths	0	1 <sup>e</sup>

**NOTE.** Data are no. (%) of patients, unless otherwise indicated. IQR, interquartile range; SPG, secondary prophylaxis group.

<sup>a</sup> *N* = 27 for the continuing secondary prophylaxis group, and *N* = 27 for the discontinuing secondary prophylaxis group.

<sup>b</sup> *N* = 14 for the continuing secondary prophylaxis group, and *N* = 21 for the discontinuing secondary prophylaxis group.

<sup>c</sup> One case of relapse of visceral leishmaniasis.

<sup>d</sup> One case of herpes-zoster virus infection.

<sup>e</sup> The patient died of an acute brain hemorrhage secondary to the rupture of a mycotic aneurysm.

trial to randomize patients receiving secondary prophylaxis to discontinue prophylaxis or not. We have enrolled a relatively large sample of patients who have been followed up over a long period. Furthermore, in our study, patients in the discontinuation arm were systematically evaluated for disease recurrence by MRI or CT scanning. Therefore, it is unlikely that any subclinical relapse would have been missed. None of our patients developed a TE relapse during follow-up. At 1 year, the upper limit of the 95% CI for relapse rate for the group discontinuing prophylaxis was 16%. This figure is similar to that for patients who received maintenance therapy in the pre-HAART era [22, 27, 28]. Taken together, these results suggest that secondary prophylaxis can be safely discontinued in patients with a previous TE episode. However, this group is at maximum risk for developing a new TE episode, and discontinuation of prophylaxis should be made under continuous medical supervision.

Most patients included in prophylaxis discontinuation studies were receiving a protease inhibitor-containing regimen. In our study, only 5 of 438 patients were receiving a protease inhibitor-sparing regimen at the time of enrollment, because at the end of the 1990s, protease inhibitor-based regimens represented the gold standard of HAART. However, the magnitude of the CD4<sup>+</sup> T cell count increase observed in patients taking a protease inhibitor-sparing regimen is very similar to that of patients receiving a protease inhibitor-containing regimen [23], even that of very advanced patients [29, 30]. For this reason, we can extrapolate this recommendation to patients receiving protease inhibitor-sparing regimens.

It is difficult to establish precisely the optimal time point to discontinue TE prophylaxis after beginning HAART. Several reports of opportunistic infections during the first months of HAART give cause for concern, especially in patients with a CD4<sup>+</sup> T cell count of <50 cells/mm<sup>3</sup> [31]. Several studies have demonstrated that antigen-specific T cell responses may require several months to be regenerated in patients receiving HAART [23, 32]. With regard to *T. gondii*, Fournier et al. [33] have demonstrated in a cross-sectional study that the in vitro *T. gondii*-specific T cell responses can be restored with HAART

in severely immunosuppressed, HIV-1-infected patients. However, there are few studies of the timing of reconstitution of the *T. gondii*-specific T cell immune responses in patients with AIDS who developed acute TE after starting HAART. Our group performed a prospective, multicenter, longitudinal study that enrolled 20 patients with acute TE patients who started HAART [34]. T cell responses to *T. gondii* antigens were restored in most patients after at least 1 year of HAART. These studies showed a positive correlation between the *T. gondii* lymphoproliferative responses, IFN- $\gamma$  production, and CD4<sup>+</sup> T cell count, and almost all patients with a CD4<sup>+</sup> T cell count of >200 cells/mm<sup>3</sup> had a positive response. For these reasons, the inclusion criteria for stopping TE prophylaxis should take into consideration that patients should be on HAART for at least 1 year, with an increase in CD4<sup>+</sup> T cell count to >200 cells/mm<sup>3</sup> and with totally or partially suppressed viral replication for at least 3–6 months. This conclusion can be applied to patients living in Europe and the United States, where the majority of *T. gondii* strains can be clustered into 3 main clonal genotypes, of which type II is the most prevalent [35]. However, other geographical regions (e.g., South America) can have different genotypes, and HAART-induced immune recovery of *T. gondii* infection may not be protective against reinfection with these atypical strains, as Ghosn et al. [35] have recently reported.

The importance of the CD4<sup>+</sup> T cell count and HIV-1 RNA level in the development of opportunistic infections is well documented [36, 37]. It has been suggested that patients receiving HAART whose CD4<sup>+</sup> T cell counts increase to >200 cells/mm<sup>3</sup>, but who have partial suppression of viral replication, may not be as well protected as patients who achieve full viral suppression. However, in our study, none of these patients developed TE or any other opportunistic infection. Furthermore, in a study from the Johns Hopkins HIV Clinic [38], no patient receiving combination treatment developed an opportunistic infection after the CD4<sup>+</sup> T cell count increased to >200 cells/mm<sup>3</sup>. Thus, immunological studies performed in patients with partial viral suppression (plasma HIV-1 RNA level, <10,000 copies/mL) have shown that antigen-specific T cell

responses are maintained [39]. In a report from the CDC regarding patients receiving HAART who have CD4<sup>+</sup> T counts >200 cells/mm<sup>3</sup>, plasma HIV-1 RNA level was only predictive for the development of opportunistic infections if it was >150,000 copies/mL [36]. These data suggest that when patients are receiving HAART with a CD4<sup>+</sup> T cell count >200 cells/mm<sup>3</sup>, plasma HIV-1 RNA level is less predictive of AIDS outcome than it is in patients with a CD4<sup>+</sup> T cell count <200 cells/mm<sup>3</sup>. However, because HIV-1 RNA level probably predicts future CD4<sup>+</sup> T cell counts, discontinuation of prophylaxis in these patients should be carefully monitored.

In summary, the results of this randomized, open-label, clinical trial support that primary and secondary TE prophylaxis may be safely discontinued in patients with immunological recovery after receipt of HAART when the CD4<sup>+</sup> T cell count increases to >200 cells/mm<sup>3</sup> for >3 months, even in patients with incomplete suppression of viral replication (<5000 copies/mL). In the absence of further data, it seems prudent to reintroduce prophylaxis whenever the CD4<sup>+</sup> T cell count decreases to <200 cells/mm<sup>3</sup>.

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