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AIDS-associated central nervous system cryptococcosis: a Brazilian case study

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In recent years the effect of HAART in patients with AIDS has been great at decreasing the incidence of opportunistic infections [1]. Nonetheless, patients with AIDS living in developing countries still present with severe central nervous system cryptococcosis, with high mortality rates [2]. The study of the clinical–epidemiological–laboratory aspects of the patients treated before the HAART era might be useful in an assessment of the impact of these drugs in the prognosis of cases.

Data from 35 patients who had been hospitalized from 1995 to 1997, at a reference hospital for infectious diseases located in São Paulo city, Brazil, were collected at the start of central nervous system cryptococcosis (CNSC) infection, and during further follow-ups. The characteristics of the patients are given in Table 1. The concomitant existence of neoplasia or opportunistic infection in the central nervous system, a CD4 cell count below 100 cells/μl, and treatment of the initial phase with just one drug were all co-factors in therapeutic failure. Furthermore, intracranial hypertension and cryptococcosis external to the neural site are among the most important factors leading to poor prognosis in cryptococcosis, and have been underdiagnosed, possibly contributing to the high lethality rate (62.8%) seen in this series. The data were grouped according to acute phase (first phase of treatment) and prophylactic phase (second phase of treatment). Therapeutic failure was considered to have occurred when there were no remissions of signs or symptoms or when cephalorachidian spinal fluid (CSF) culture remained positive until the 10th week of treatment, or else when there were CNSC-associated deaths. Clinical cure was defined as total or partial remission of the signs and symptoms, and laboratory cure was defined when negative culture of CSF to *Cryptococcus neoformans* was obtained. Relapse or recurrence was characterized by clinical worsening or rehospitalization, by having to increase the fluconazole dose (from 200 to 400 or 800 mg/day) or by having to use amphotericin B again.

In order to assess the lethality attributable to cryptococcosis the following criteria were accepted: CSF-positive culture and clinical presentation at death. A computerized tomography scan of the brain was performed in five patients. Intracranial pressure was assessed in two cases at the first attendance. The laboratory findings of the initial CSF for the 35 cases are: leukocytes 1–453; protein (27–387 mg/ml); glucose (10–60 mg/ml); positive test of China ink (in 20 patients) 90%; positive polysaccharide antigen (in 23 patients) 100%; title of polysacchride antigen 128–131 072. The aetiological agent was isolated from multiple samples

Table 1. Characteristics of 35 patients with AIDS-associated cryptococcosis.

Variables (no. of patients with available data)	No. of patients
Sex (35)	
Female	10 (28.6%)
Male	25 (71.4%)
Race (35)	
Caucasian	33 (94.4%)
Negro	1 (2.8%)
Yellow	1 (2.8%)
Signs and symptoms (35)	
Headache	34 (97.1%)
Nausea/vomiting	18 (51.4%)
Fever	12 (34.3%)
Visual alteration	7 (20.0%)
Mental confusion	4 (11.4%)
Convulsions	3 (8.6%)
Meningeal signs	2 (5.7%)
Intracranial hypertension	2 (5.7%)
CD4 cell count, mm^3 (30)	
< 50	22 (73.4%)
50–100	7 (23.3%)
≥ 100	1 (3.3%)
Neural site external to cryptococcosis (5)	
Lungs	2 (5.7%)
Bone marrow	1 (2.8%)
Blood	1 (2.8%)
Multiples	1 (2.8%)
Antifungal therapy, acute phase (35)	
Desoxycolate amphotericin B	14 (40.0%)
Liposomal amphotericin B	2 (5.7%)
Amphotericin B + flucitosine	2 (5.7%)
Amphotericin B + fluconazole	9 (25.7%)
Amphotericin B + flucitosine +fluconazole	1 (2.9%)
Flucitosine + fluconazole	7 (20.0%)
Antiretroviral agents	
Zidovudine	2 (5.7%)
Zidovudine + didanosine	14 (40.0%)
Zidovudine + didanosine + protease inhibitor	7 (20.0%)
Not informed	12 (34.3%)
Clinical progression	
Acute phase (35)	
Mortality attributed to CNSC	11 (31.5%)
Clinical cure	20 (57.1%)
Laboratory cure	4 (11.4%)
Prophylaxis (24)	
Mortality attributed to CNSC	10 (41.7%)
Mortality due to other causes	1 (4.2%)
Clinical cure with relapse	5 (20.8%)
Laboratory cure	7 (29.2%)
Not followed-up	1 (4.1%)

CNSC, Central nervous system cryptococcosis.

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($N = 3–11$) of CSF for a total of 168 samples, collected at one to 240-day intervals. All 168 isolates were *C. neoformans* negative.

The importance of CNSC as an indication of opportunistic disease in AIDS was confirmed. *C. neoformans* was found to be the agent of CNSC in 100% of cases, reasserting how scarce the association with var. *gatti* infection and HIV is in Brazil, even though this variety has been repeatedly isolated from environmental sources [3–5].

Only three out of 35 cases (9%) were treated according to the recommendation of Saag *et al.* [6], using a regimen of drug combination (amphotericin B plus flucitosine). In Brazil, flucitosine has not been commercially available for a long time. For prophylaxis, fluconazole (21/24; 87.5%) or amphotericin B (12.5%) was used. Of the 21 patients who received fluconazole, nine died (42.9%); of those receiving amphotericin B, two out of three died (66.6%), totalling 11 obits. The period of follow-up among the survivors observed a total of 22 (62.8%) obits, including 16 (72.7%) culture-confirmed cases.

The analyses of cause of death reports and laboratory results showing positive cultures for *C. neoformans* in the CSF of 22 patients have made it possible to confirm the high mortality rate associated with cryptococcosis in this group. The death rate (31.4%) occurring during the acute phase was high according to international literature, which reported 10–25% mortality in the initial 2 weeks. These data could reflect the impact of cryptococcosis in the early 1990s, before the era of HAART, and the lack of facilities for monitoring intracranial pressure.

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Early virological response of zidovudine/lamivudine/abacavir for patients co-infected with HIV and tuberculosis in Uganda

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Triple nucleoside reverse transcriptase inhibitors are recommended as an alternative regimen for HIV-infected patients undergoing tuberculosis treatment in resource-limited settings. Few data exist on the efficacy of such regimens in tuberculosis patients. In 34 tuberculosis/HIV-co-infected patients treated with zidovudine/lamivudine/abacavir, 76% achieved HIV RNA less than 50 copies/ml at 24 weeks. No cases of hypersensitivity or immune reconstitution syndrome were observed. These data support the continuing evaluation of nucleoside-based antiretroviral regimens as an alternative treatment for this population.

Current World Health Organization guidelines for resource-limited settings recommend the initiation of antiretroviral therapy (ART) for individuals with HIV and active tuberculosis at CD4 cell counts below 350 cells/μl [1]. The use of first-line non-nucleoside reverse transcriptase inhibitor (NNRTI)-based ART regimens during the management of tuberculosis is complicated by significant drug interactions between rifamycins and nevirapine or efavirenz [2], an increased risk of hepatotoxicity with the use of nevirapine at CD4 cell counts above 250 cells/μl [3], and safety concerns with efavirenz in early pregnancy [4].

Nucleoside-based regimens represent alternative ART options for patients co-infected with tuberculosis and HIV in resource-limited settings, and have the advantages of being compatible with rifamycin-based tuberculosis regimens, carrying a lower risk of hepatic toxicity at

higher CD4 cell counts than nevirapine-based regimens, and remaining safe to use in early pregnancy. These regimens remain largely untested in the treatment of tuberculosis patients. Among tuberculosis/HIV co-infected patients in Africa, we evaluated the early virological and CD4 cell response to zidovudine/lamivudine/abacavir, and examined the development of immune reconstitution syndrome (IRS) and abacavir hypersensitivity reaction.

HIV-infected, ART-naive adults with acid-fast bacillus sputum smear or culture-positive pulmonary tuberculosis and CD4 cell counts of 350 cells/μl or greater were recruited from the Mulago Tuberculosis Research Unit in Kampala, Uganda. Consenting individuals were initiated on short-course antituberculous therapy (2 months of isoniazid, rifampicin, pyrazinamide, and ethambutol, followed by 4 months of isoniazid and rifampicin). Clinically stable patients were initiated on oral zidovudine 300 mg/lamivudine 150 mg/abacavir 300 mg (as fixed-dose Trizivir) twice a day 2–4 weeks after starting tuberculosis therapy. Subjects received directly observed ART and antituberculous therapy for 6 months, and were clinically evaluated monthly and at patient-initiated visits.

Adverse events were graded using standard NIAID/ACTG criteria (www.nih.gov). Abacavir hypersensitivity reaction was suspected if a subject reported two or more of fever, rash, gastrointestinal symptoms, non-tuberculosis respiratory symptoms, myalgia, arthralgia, headache, or parasthesia; and was confirmed when other possible etiologies were excluded and there was symptom resolution with abacavir discontinuation. Tuberculosis-associated IRS was defined as more than one week of new persistent fevers after ART initiation without other identifiable etiology, or a marked worsening of pulmonary infiltrates, intrathoracic or cervical lymphadenopathy, or other tuberculosis lesions on serial examination. Complete blood count and liver function tests were performed at baseline, 2, 4, 8, 12, and 24 weeks. Urine β-human chorionic gonadotrophin was performed at baseline and monthly for female subjects of childbearing potential, and female subjects were required to use two forms of contraception. CD4 cell counts and plasma HIV-RNA levels were quantified [Roche Amplicor 1.5, limit of detection (LOD) 400 copies/ml; Roche Diagnostic Systems Inc., Branchburg, New Jersey, USA] at baseline, 12, and 24 weeks. Twenty-four week HIV RNA was also quantified using the Roche Amplicor 1.5 Ultra-sensitive assay, LOD 50 copies/ml.

Among 34 tuberculosis/HIV-co-infected subjects who completed 24 weeks of fixed-dose zidovudine/lamivudine/abacavir, the median age was 28 years (range 20–45), and 56% were men. The median baseline CD4 cell count among subjects was 541 cells/μl (range 356–852 cells/μl) and HIV RNA was 4.57 log copies/ml (range 3.23–5.87 log copies/ml). Of the 34 subjects, 29 (85%) and 31 (91%) achieved virological suppression to less than 400 copies/ml HIV RNA at 12 and 24 weeks, respectively (Table 1). Twenty-six subjects (76%) demonstrated virological suppression at less than 50 copies/ml HIV RNA at 24 weeks. The median CD4 cell count increase at 24 weeks was 81 cells/μl (−303–841 cells/μl). Despite virological suppression, 13 of 31 (42%) subjects had CD4 cell count increases of less than 50 cells/μl at 24 weeks, of whom eight had CD4 cell count declines below baseline. A poor CD4 cell response was not associated with age, sex, baseline CD4 cell count, or baseline HIV RNA.

Table 1. Virological and immunological response to zidovudine/lamivudine/abacavir among patients from Uganda co-infected with tuberculosis and HIV.

Characteristic	
Viral response	$N = 34$ (%)
HIV RNA < 400 copies/ml	
12 weeks	29 (85)
24 weeks	31 (91)
HIV RNA < 50 copies/ml	
24 weeks	26 (76)
Immune response	Cells/μl (range)
Median CD4 cell count at 24 weeks	628 (325–1370)
Median CD4 cell count change at 24 weeks	81 (−303–841)
CD4 cell count response in patients with viral suppression at 24 weeks	$N = 31$ (%)
Increase > 50 cells/μl	18 (58)
Increase < 50 cells/< μl	13 (42)
CD4 cell decline from baseline	8 (26)

NIAID grade 3 or 4 neutropenia was detected in four patients, one of whom also had grade 4 anemia. Hematological toxicities resolved in all four patients with dose reduction of zidovudine. Abacavir hypersensitivity reaction was suspected in three of 34 (9%) subjects, but none met the criteria for confirmed hypersensitivity reaction. Suspected hypersensitivity reaction symptoms were determined to be related to zidovudine ($n = 2$) or tuberculosis medications ($n = 1$), and resolved in all patients without abacavir discontinuation. There were no cases of tuberculosis-associated IRS. Pregnancy was detected in one subject, who continued to tolerate zidovudine/lamivudine/abacavir.

We present the first data on short-term virological and clinical outcomes of zidovudine/lamivudine/abacavir among HIV-infected patients with active tuberculosis in Africa. Published reports of efavirenz or nevirapine-based ART in tuberculosis/HIV-co-infected patients in Thailand and South Africa have shown 24-week on-treatment viral suppression rates ranging from 66 to 88% [5–9]. Subjects in our study achieved comparable early viral suppression (76%) to less than 50 copies/ml HIV-RNA. The heterogeneous CD4 cell response to ART in our study, even among patients with viral suppression, further confirms other African data that suggest that the

immunological response to ART is a poor predictor of virological suppression [10,11].

Zidovudine/lamivudine/abacavir was well tolerated among study subjects, with no confirmed episodes of abacavir hypersensitivity reaction. Very low rates of hypersensitivity reaction were also reported among HIV-infected African individuals receiving zidovudine/lamivudine/abacavir in the DART trial [12]. Our study extends these data to the tuberculosis/HIV co-infection setting, in which interactions with tuberculosis medications and the potential development of IRS are additional concerns that complicate the management of suspected hypersensitivity reaction in a resource-limited country. Although zidovudine dose reduction was required in four patients for neutropenia, dosing changes in the study were dictated by NIAID/ACTG toxicity criteria, which may not be applicable to the African setting where normal immunological reference values can differ considerably from those in the United States [13]. Notably, even in a clinical trial setting one subject became pregnant, underscoring the importance of safe ART options for tuberculosis/HIV-co-infected women of childbearing potential in Africa.

At present, alternatives to NNRTI-based ART regimens are either very limited or non-existent in most resource-constrained countries. Nucleoside-based ART regimens represent a possible option for select populations, such as HIV-infected individuals receiving concurrent tuberculosis treatment, who may be unable to tolerate NNRTI. Concerns remain about the potency of such regimens, given the demonstrated inferior virological response to zidovudine/lamivudine/abacavir in comparison with an efavirenz-based regimen in AACTG 5095 [14]. Although our sample size is limited, our preliminary data on safety and efficacy suggest that the development and continued evaluation of potent nucleoside reverse transcriptase inhibitor-based regimens as alternative ART for tuberculosis/HIV co-infection is warranted.

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Virological fitness of HIV in patients with resistance to enfuvirtide

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Resistance to the HIV fusion inhibitor enfuvirtide is associated with mutations in the first heptad repeat region of gp41, but little is known of their impact on replicative fitness *in vivo*. We followed seven patients undergoing salvage therapy that

included enfuvirtide in order to document the temporal generation of genotypic and phenotypic resistance in parallel with replicative fitness. Resistance to enfuvirtide was not associated with decreased replicative fitness of HIV strains infecting these patients.

Long-term treatment and associated adherence issues with HAART have facilitated the emergence of drug resistance, especially to protease inhibitors and reverse transcriptase inhibitors, but also to enfuvirtide (Fuzeon), the only clinically available drug targeting the HIV fusion process [1]. Resistance to enfuvirtide is associated with mutations in the first heptad repeat (HR1) region of gp41 involving amino acids 36–45 [1]. Combinations of these mutations generally lead to higher levels of phenotypic resistance [2]. Mutations in the second heptad repeat (HR2) may play a compensatory role by restoring or improving the interaction between it and HR1 [3].

We followed seven patients treated with optimized HAART that included enfuvirtide, but in whom subsequent virological control was not achieved. This allowed us to study the temporal generation of mutations in HR1 and HR2 in parallel with the replicative fitness of HIV strains present before and during treatment. The patients studied had previously failed treatment with reverse transcriptase and protease inhibitors and had significant resistance to them. All had virological failure at the start of enfuvirtide treatment and six of the seven had CD4 cell counts less than 250 cells/μl. They were treated for at least 3 years with enfuvirtide on an optimized antiretroviral drug background.

HIV RNA was extracted from plasma samples using a QIAmp viral RNA purification protocol (Qiagen, Hilden, Germany). A region of *env* was amplified in a nested reverse transcriptase–polymerase chain reaction encompassing HR1 and HR2. The mutations in HR1 considered to indicate enfuvirtide resistance were G36D/S/V, I37V, V38A/E/M, Q39R, Q40H, N42T, N43D, L44M and L45M [3,4].

HIV strains in 27 plasma samples from seven patients were evaluated for co-receptor tropism, enfuvirtide susceptibility and replicative capacity using a previously described method (Phenoscript) [5]. The fitness of each enfuvirtide-resistant virus was expressed as a percentage compard with the pretreatment strain from that patient (defined as 100%).

The viral load and CD4 cell count were monitored over the course of the study. Before initiation of enfuvirtide therapy, six of the seven patients had CD4 cell counts less than 250 cells/μl. After approximately 3 years of treatment, three patients (1, 2 and 7) maintained CD4 cell counts higher than at the commencement of therapy, despite phenotypic resistance and detectable viral loads (Fig. 1). This observation has been noted by others, and suggests that an enfuvirtide-containing salvage regimen may have immunological benefits even in the face of poor virological response [1,6].

At the time of initiating enfuvirtide treatment, three patients (1, 3 and 4) were infected with R5 tropic HIV strains and three (2, 6 and 7) were infected with mixed/dual tropic (R5/X4) virus. One patient (5) had a mixed/dual tropic strain before commencing enfuvirtide therapy, but only R5 strains were detected at subsequent timepoints.

Pretherapy IC_{50} susceptibilities to enfuvirtide were generally in the range of 10–75 ng/ml (Fig. 1). Patient 5 was infected with a strain that was particularly sensitive to enfuvirtide (IC_{50} 0.002 ng/ml). Natural variation in enfuvirtide susceptibility has been reported previously [6] and may be linked to the fusion rate [7]. Slow fusion, with a relatively protracted exposure of HR1 to enfuvirtide, may facilitate reduced inhibitory concentrations (relatively low IC_{50}) [7]. Enfuvirtide resistance mutations may reduce the exposure time of HR1 to the drug, resulting in higher concentrations needed for inhibition (increased IC_{50}) compared with the pretreatment strain. Of note is the fact that although the virus from patient 5 had the highest pretherapy level of susceptibility ($IC_{50} < 0.002$ ng/ml), on-treatment strains had increased IC_{50}, which remained lower than resistant virus from the other patients (Fig. 1).

All patients developed enfuvirtide-associated mutations in HR1 (Fig. 1). Mutations developed between 3 weeks and 17 months after the commencement of therapy, and were associated with high-level resistance (40-fold to 35 000-fold). Apparently random changes in HR2 were identified, but we were unable to establish whether they contributed to altered enfuvirtide susceptibility, a finding noted by others [8].

Switching from one resistant genotype to a second resistant genotype during enfuvirtide therapy was observed in patients 3, 4 and 5 (Fig. 1), a phenomenon that has been noted previously [7]. It generally resulted in an increased fold-change in resistance, suggesting an evolutionary requirement by the virus, possibly facilitated by the presence of quasi-species containing discrete resistance mutations, and in response to subtle changes in enfuvirtide concentrations *in vivo*.

Despite the presence of genotypic and phenotypic resistance, little or no impact was observed on HIV replicative fitness in the R5 tropic strains of five of the seven patients studied, with changes in fitness from 0.5 to 1.8-fold compared with baseline (Fig. 1). This result was supported by the persistence of resistance mutations for periods ranging from at least 4 to 14 months after the

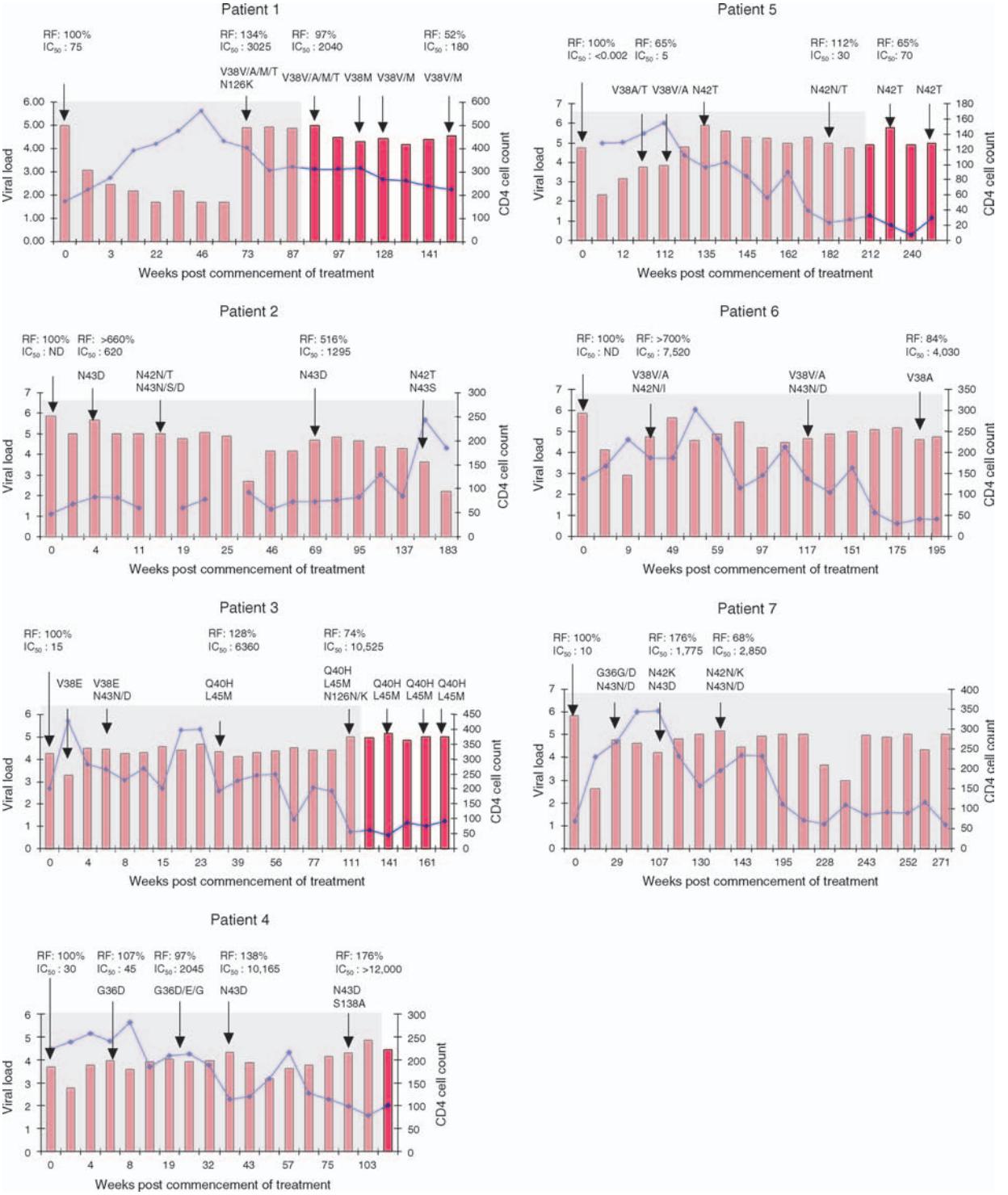

Fig. 1. Plasma HIV-1 viral loads (log_{10} RNA copies/ml) (columns) and CD4 cell counts (line) for the seven patients studied. Relative replicative fitness (RF) compared with the baseline virus (100%), susceptibility to enfuvirtide (IC_{50} in ng/ml) and mutations in the first and second heptad repeat regions (HR1 and HR2) are indicated. Weeks after the commencement of treatment are shown on the horizontal axis with the beginning of enfuvirtide treatment at week 0. The duration of enfuvirtide treatment is shaded. ND, Not determined.

cessation of enfuvirtide treatment in three evaluable patients (1, 3 and 5), an observation recently supported by one study [5] but not by a second [8]. In contrast, the replicative fitness of viruses from patients 2 and 6 increased during enfuvirtide treatment. The increase in fitness was at least sixfold compared with viruses present before enfuvirtide treatment, and involved mixed/dual-tropic variants.

Previous studies involving the site-directed mutagenesis of HR1 have generated virus with impaired fitness [9,10]. The assay we used to determine replication fitness incorporated the entire *env* region from patient-derived virus. The contributions of any compensatory mutations in this region were therefore accounted for in our assay. Despite genotypic and phenotypic resistance to enfuvirtide, no obvious impact on fitness, particularly a negative impact, was observed in our patients. Our findings are in agreement with those of Labrosse and colleagues [5], who also showed improved replication fitness in two enfuvirtide-resistant viruses. Our results suggest that the improvements or stabilization of CD4 cell counts sometimes observed in patients treated with enfuvirtide are not the result of reduced virological fitness of the resistant strains present.

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Combined tipranavir and enfuvirtide use associated with higher plasma tipranavir concentrations but not with increased hepatotoxicity: sub-analysis from RESIST

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In RESIST, enfuvirtide co-administered with ritonavir-boosted tipranavir was associated with higher plasma tipranavir concentrations, which seldom rose above those associated with an increased risk of grade 3/4 transaminase elevations. Transaminase elevation rates (6.5%) and clinical hepatic event rates (5.9 events/100 person exposure years) were lower in the tipranavir/ritonavir with enfuvirtide group than in the tipranavir/ritonavir without enfuvirtide group. Observed increases in plasma tipranavir concentrations thus had no apparent effect on the risk of hepatotoxicity.

Tipranavir, a non-peptidic protease inhibitor (PI) administered with low-dose ritonavir [1] has potent in-vitro activity against the majority of PI-resistant HIV-1 strains isolated from treatment-experienced patients [2]. It was recently reported that the co-administration of tipranavir and ritonavir with the HIV-1 fusion inhibitor enfuvirtide resulted in higher plasma trough concentrations ($C_{min,ss}$) of tipranavir and ritonavir [3].

A case report detailed the development of grade 4 transaminase elevations in a 52-year-old white man with chronic hepatitis B infection who was co-administered tipranavir/ritonavir plus enfuvirtide, but recovered after enfuvirtide was discontinued [4]. This suggested that hepatotoxicity was induced by the use of enfuvirtide, causing higher tipranavir/ritonavir serum levels. The authors recommended close monitoring of tipranavir serum levels and dosage adaptation, including the reduction or discontinuation of ritonavir, to avoid hepatotoxicity [4].

The RESIST studies are ongoing randomized clinical trials, evaluating the efficacy and safety of 500/200 mg tipranavir/ritonavir twice a day compared with a ritonavir-boosted comparator protease inhibitor (CPI) in treatment-experienced patients with advanced HIV-1 infection [2]. Enfuvirtide was co-administered with tipranavir/ritonavir in approximately 22% of patients selected by investigators before random selection as part of an optimized background regimen. We investigated whether the combination of tipranavir/ritonavir plus enfuvirtide was associated with an increase in tipranavir exposure in RESIST trial participants [3], and whether this could affect the rate of hepatotoxicity. Intra-individual geometric mean tipranavir $C_{min,ss}$, derived from individual plasma samples taken at steady state (at week 2 or later) within 10–14 h after the previous tipranavir/ritonavir administration, was used in the analysis. The rates of transaminase elevations and hepatic adverse events were compared in tipranavir/ritonavir-treated patients stratified by enfuvirtide use.

The combined RESIST-1 and 2 study population comprised 746 tipranavir/ritonavir and 737 CPI/ritonavir recipients. Enfuvirtide was used by 22.8% (16.6% for the first time) and 18.3% (13.2% for the first time) of patients in the respective arms. A significantly greater proportion of patients achieved a viral load of less than 400 copies/ml (26.9 versus 10.9%; $P < 0.0001$) and less than 50 copies/ml (20.4 versus 9.1%; $P < 0.0001$) in the tipranavir/ritonavir group than the CPI/ritonavir group at 96 weeks [5]. In patients taking enfuvirtide for the first time, the proportions achieving a viral load less than 400 copies/ml were more than threefold higher in the tipranavir/ritonavir arm (44.4%) than in the CPI/ritonavir arm (14.4%; $P < 0.0001$). Similarly, the proportions of enfuvirtide-naive patients achieving a viral load less than 50 copies/ml were nearly 2.5-fold higher in the tipranavir/ritonavir arm (34.7%) compared with the CPI/ritonavir arm (14.4%; $P = 0.0002$) [5].

Tipranavir/ritonavir patients receiving enfuvirtide had more advanced HIV disease at baseline than tipranavir/ritonavir patients not receiving enfuvirtide. In particular, tipranavir/ritonavir plus enfuvirtide patients had lower median CD4 cell counts (74 versus 179 cells/μl), higher baseline HIV-RNA levels (5.06 versus 4.72 $\log_{10}$ copies/ml) and a higher frequency of Centers for Disease Control and Prevention class C events (65.3 versus 57.8%). A smaller proportion of patients in the tipranavir plus enfuvirtide group had hepatitis co-infection (5.3 versus 11.8%) at baseline, determined by positive hepatitis B surface antigen or hepatitis C virus RNA tests.

Among the 661 patients for whom tipranavir $C_{min,ss}$ data were available, tipranavir/ritonavir plus enfuvirtide recipients had a 31% higher median tipranavir $C_{min,ss}$ than tipranavir/ritonavir without enfuvirtide patients (Table 1). Likewise, higher median lopinavir (+19%) and saquinavir (+39%) $C_{min,ss}$ values were also observed in ritonavir-boosted lopinavir plus enfuvirtide ($n = 60$) and ritonavir-boosted saquinavir plus enfuvirtide recipients ($n = 27$) compared with lopinavir/ritonavir without enfuvirtide ($n = 240$) and saquinavir/ritonavir without enfuvirtide patients ($n = 110$). In previous analyses, tipranavir $C_{min,ss}$ greater than 120 μmol was associated with an increased risk of developing grade 3/4 transaminase elevations compared with lower concentrations [6]. The proportion of tipranavir/ritonavir plus enfuvirtide and tipranavir/ritonavir without enfuvirtide patients in RESIST who had tipranavir $C_{min,ss}$ greater than 120 μmol was small (1.3 versus 1.2%). The average $C_{min,ss}$ for the majority of patients (tipranavir/ritonavir plus enfuvirtide 73.4%; tipranavir/ritonavir without

Table 1. Descriptive statistics of tipranavir and comparator protease inhibitor plasma trough levels by enfuvirtide use.

Treatment	Enfuvirtide use	*N*	Q1	Median	Q3	Geometric mean
Tipranavir (μmol/l)[a]	Total	663[b]	21.56	33.70	50.75	30.29
	Without Enfuvirtide	507	19.83	31.03	46.96	27.53
	With Enfuvirtide	154	31.50	45.17	64.73	41.34
Lopinavir (μg/ml)	Total	300	3.28	5.77	8.22	4.07
	Without Enfuvirtide	240	3.01	5.53	7.93	3.84
	With Enfuvirtide	60	4.29	6.82	8.82	5.12
Saquinavir (μg/ml)	Total	137	0.26	0.42	1.01	0.40
	Without Enfuvirtide	110	0.25	0.38	0.97	0.38
	With Enfuvirtide	27	0.32	0.62	1.27	0.49

Q, Quartile.
[a]1 μmol/l = 0.603 μg/ml of tipranavir.
[b]Enfuvirtide usage data not available for two patients.

enfuvirtide 69.4%) fell between 20 and 80 μmol, and 94.9% of tipranavir/ritonavir without enfuvirtide patients had $C_{min,ss}$ less than 80 μmol.

Grade 3/4 alanine aminotransferase (ALT) elevation rates were lower in the tipranavir/ritonavir plus enfuvirtide arm (6.5%; 11/170) compared with the tipranavir/ritonavir without enfuvirtide arm (12.9%; 73/565) over 96 weeks. Similarly, ALT elevation rates were lower in the CPI/ritonavir plus enfuvirtide arm (0.8%; 1/133) than in the CPI/ritonavir without enfuvirtide arm (2.5%; 15/594) over 96 weeks. When patients with hepatitis co-infection were excluded, ALT elevation rates were marginally lower; 6.3% (10/160) in the tipranavir plus enfuvirtide arm and 12.2% (61/500) in the tipranavir without enfuvirtide arm, and 0.9% (1/116) in the CPI/ritonavir plus enfuvirtide arm and 1.6% (8/499) in the CPI/ritonavir without enfuvirtide arm. Tipranavir/ritonavir plus enfuvirtide recipients had lower rates of clinical hepatic events reported by RESIST trial investigators than tipranavir/ritonavir without enfuvirtide recipients [5.9 events/100 patient exposure years (PEY) versus 9.3 events/100 PEY]. By comparison, in the CPI/ritonavir arm, higher rates of investigator-reported clinical hepatic events were observed in patients who received enfuvirtide compared with those who did not (3.9 events/100 PEY versus 2.9 events/100 PEY).

In summary, this analysis of plasma tipranavir concentrations from the RESIST data is consistent with previously published findings that the co-administration of enfuvirtide with tipranavir/ritonavir is associated with higher tipranavir plasma concentrations [3]. This association is observed as soon as 2 weeks after therapy initiation. An increase in $C_{min,ss}$ was not limited to tipranavir but was also observed with two other ritonavir-boosted PI regimens, lopinavir/ritonavir and saquinavir/ritonavir, when combined with enfuvirtide. The association between enfuvirtide use and higher PI concentrations is thus not unique to tipranavir and may be a general class phenomenon, although the mechanism is unknown. This could also reflect better adherence to the antiretroviral regimen in patients self-injecting twice daily enfuvirtide. Plasma tipranavir concentrations for patients receiving enfuvirtide rarely rose above concentrations associated with an increased risk of grade 3/4 transaminase elevations. Analyses of the RESIST population did not show higher rates of grade 3/4 transaminase elevation in patients receiving tipranavir/ritonavir plus enfuvirtide, compared with those receiving tipranavir/ritonavir without enfuvirtide. The rates were actually significantly lower in the tipranavir plus enfuvirtide group than in the tipranavir without enfuvirtide group ($P < 0.05$). Overall rates of clinical hepatic events were low, even with concomitant enfuvirtide administration. These results indicate that the observed increases in $C_{min,ss}$ among RESIST participants who received enfuvirtide were not associated with an increased risk of hepatotoxicity. Finally, on the basis of these results from a large clinical trial, the authors discourage any recommendation to alter ritonavir or tipranavir dosing in patients receiving tipranavir/ritonavir plus enfuvirtide, as this may result in clinically significant underexposure to tipranavir and loss of the antiretroviral efficacy observed with this combination of therapeutic agents.

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Cytokine pattern in Kaposi's sarcoma associated with immune restoration disease in HIV and tuberculosis co-infected patients

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We analysed the evolution of different cytokines (IL-4, IL-6, tumour necrosis factor alpha and vascular endothelial growth factor; VEGF) involved in the development of Kaposi's sarcoma in two patients in whom HIV infection presented with disseminated *Mycobacterium tuberculosis* infection. They simultaneously developed tuberculosis-associated immune restoration disease and Kaposi's sarcoma shortly after the initiation of HAART. Analysis of VEGF and pro-inflammatory cytokines led us to hypothesize that Kaposi's sarcoma could be promoted by the tuberculosis immune response.

In the post HAART era, Kaposi's sarcoma remains the most common AIDS-associated neoplasm in HIV-infected patients, although its incidence has significantly decreased [1]. HAART-induced viral suppression is associated with an improvement in immune function. Immune restoration may, however, also result in the paradoxical development of inflammatory reactions to preexistent infections including *Mycobacterium tuberculosis* [2]. These clinical responses, known as immune restoration disease (IRD) have been reported in a very few cases of patients with Kaposi's sarcoma [3]. We analysed the progress of different cytokines, involved in the development of Kaposi's sarcoma, in two patients in whom HIV infection was revealed by disseminated *M. tuberculosis* infection, who developed tuberculosis-associated IRD and Kaposi's sarcoma shortly after the initiation of HAART.

Case reports

The first patient was a 33-year-old woman who presented with tuberculosis involving the lymph nodes, peritoneum and lungs. On admission, no Kaposi' sarcoma lesion was noted. HIV-1 antibodies were detected in her serum. The CD4 cell count was 19 cells/μl and the plasma HIV-1-RNA level was 380 000 copies/ml. Tuberculosis treatment was started. Two months later, antiretroviral treatment was started. Two months later, the patient was readmitted for gastrointestinal bleeding. Kaposi's sarcoma skin lesions and mucosal lesions had developed. Esophagogastroduodenoscopy revealed Kaposi's sarcoma bleeding lesions involving her stomach. Simultaneously, a cervical lymphadenopathy that had recently appeared was biopsied, and histological study showed caseating granulomas and acid-fast bacilli. Deep lymphadenopathies were discovered on computed tomography scans of the thorax and abdomen, and were also compatible with *M. tuberculosis*-associated IRD successfully treated with steroids. Lowenstein–Jensen media cultures remained negative. The human herpesvirus 8 (HHV8) load in peripheral blood mononuclear cells (PBMC) was 9600 copies/150 000 cells, the CD4 cell count was 154 cells/μl and the plasma HIV-1-RNA level was less than 200 copies/ml. Six courses of chemotherapy consisting of doxorubicin, bleomycin and vincristine were undertaken, and the outcome was favourable. Four months after beginning chemotherapy, HHV8 DNA was negative in PBMC (see Table 1).

The second patient was a 44-year-old woman admitted to our department for disseminated tuberculosis. HIV-1 antibodies were detected in her serum. Her CD4 cell count was 13 cells/μl and the plasma HIV-1-RNA level was 146 000 copies/ml. Tuberculosis treatment was started. No skin or mucosal lesions were noted and oesophagogastroduodenoscopy was normal. One month later, the patient was readmitted with fever and abdominal pain. Abdominal computed tomography scan revealed enlarged deep celiomesenteric lymph nodes concordant with *M. tuberculosis*-associated IRD. The patient's status improved with a short course of steroids. One month later, HAART was initiated. She back-presented one month later with stridor, and endoscopy revealed diffuse Kaposi's sarcoma lesions involving the larynx, trachea, oesophagus and stomach. At this time, the CD4 cell count was 76 cells/μl, the plasma HIV-1-RNA level was less than 200 copies/ml and the HHV8 load in PBMC

Table 1. Characteristics of patients at diagnosis and at the onset of Kaposi's sarcoma.

	Patient 1	Patient 2
At diagnosis		
Age (years)	33	44
Previous antiretroviral treatment	No	No
History of tuberculosis	No	No
Tuberculosis[a]	Lung, peritoneum, lymph nodes	Bone marrow, liver, spleen, lymph nodes
CD4 cell count/μl (%)	19 (5)	13 (5)
HIV-1-RNA plasma (copies/ml)	380 000	146 000
Kaposi's sarcoma lesions	No	No
At onset of Kaposi's sarcoma		
HAART[b,c]	56	53
IRD tuberculosis[b]	109	30
Visceral Kaposi's sarcoma[b]	109	86
Kaposi's sarcoma localizations	Skin, mouth, stomach	Mouth, larynx, trachea, oesophagus, stomach
CD4 cell count/μl (%)	154 (23)	76 (13)
HIV-1-RNA plasma (copies/ml)	< 200	< 200

IRD, Immune restoration disease.
[a]Tuberculosis treatment was started with pyrazinamide, ethambutol, isoniazid and rifampicin (2 months) followed by isoniazid and rifabutin (7 months).
[b]Days after diagnosis of tuberculosis.
[c]Lamivudine, zidovudine, indinavir and low-dose ritonavir for both patients.

was 40 copies/150 000 cells. She received chemotherapy with doxorubicin, bleomycin and vincristine and steroids and her outcome was favourable. The HHV8 DNA in PBMC was negative 2 months after beginning chemotherapy.

As the clinical evolution of these two patients was unusual, we aimed to describe the clinical evolution of cytokines involved in Kaposi's sarcoma, in order to make a pathophysiological hypothesis.

Quantification of the plasma HIV-1 RNA was performed using the Amplicor monitor assay (Cobas 1.5 test; Roche Diagnostics, Basel, Switzerland) with a detection limit of 200 copies/ml. HHV8 DNA was extracted in PBMC using the QIAamp system, assayed using real-time polymerase chain reaction, and quantified using fluorescent TaqMan methods on an ABI Prism 7700 sequence detection system (PE Applied Biosystems, Foster City, California, USA). The HHV8 load in PBMC was expressed as the absolute number of HHV8 genome copies/1.5×10^5 human diploid cells, with a positive threshold of 10 copies/1.5×10^5 PBMC.

Commercially available kits for enzyme-linked immunosorbent assay were used in duplicate to detect IL-4, IL-6, tumour necrosis factor alpha (TNF-α; R&D Systems, Minneapolis, Minnesota, USA) and vascular endothelial growth factor (VEGF; Pierce Chemical Co., Rockford, Illinois, USA). In our laboratory, the usual detection thresholds for IL-4, IL-6 and TNF-α are, respectively, 10.0, 0.15 and 0.12 pg/ml and the normal value for VEGF is less than 55 pg/ml.

Figure 1 summarizes the dynamic pattern of plasma cytokine levels of both patients. IL-4 levels were always undetectable for both patients. At diagnosis, the patients' plasma levels of IL-6 and TNF-α were elevated (148 and 23 pg/ml, respectively, for patient 1, and 15 and 12 pg/ml for patient 2). Two months after starting tuberculosis treatment, they were reduced to 5 and 5 pg/ml and 7 and 6 pg/ml, respectively. When IRD and Kaposi's sarcoma developed, they increased to 19 and 45 pg/ml and 46 and 16 pg/ml, respectively. After 4 months of chemotherapy, the levels decreased markedly to 13 and 4 pg/ml, respectively, for patient 1, and 19 and 5 pg/ml for patient 2. The course of VEGF plasma levels differed from the other cytokines. They were high at diagnosis: 312 pg/ml for patient 1, and 81 pg/ml for patient 2. After 2 months of antituberculosis treatment, VEGF plasma levels remained high, with a slight decrease to 178 pg/ml for patient 1, and a slight increase to 128 pg/ml for patient 2. In both cases, when visceral Kaposi's sarcoma developed, they dramatically decreased to 24 and 39 pg/ml, respectively, and then increased again to 192 and 297 pg/ml after 4 months of Kaposi's sarcoma treatment.

IRD are a heterogeneous group of inflammatory symptoms that follow HIV viraemia suppression by HAART. IRD probably reflects deregulated immune responses against preexisting infections by opportunistic pathogens [2]. It has been hypothesized that improvements in CD4 and CD8 cell numbers and function after HAART led to an enhanced cytotoxic response to HHV8 in previously unnoticed lesions as described with other opportunistic pathogens [3].

The pathophysiology of Kaposi's sarcoma includes the interaction between stromal cells that support neoangiogenesis, spindle cells that are usually clonal in Kaposi's sarcoma lesions, HHV8, which could infect spindle cells, and many cytokines that are secreted either by spindle cells, inflammatory cells, stromal cells or HHV8 [4]. In PBMC from patients experiencing IRD, both plasma

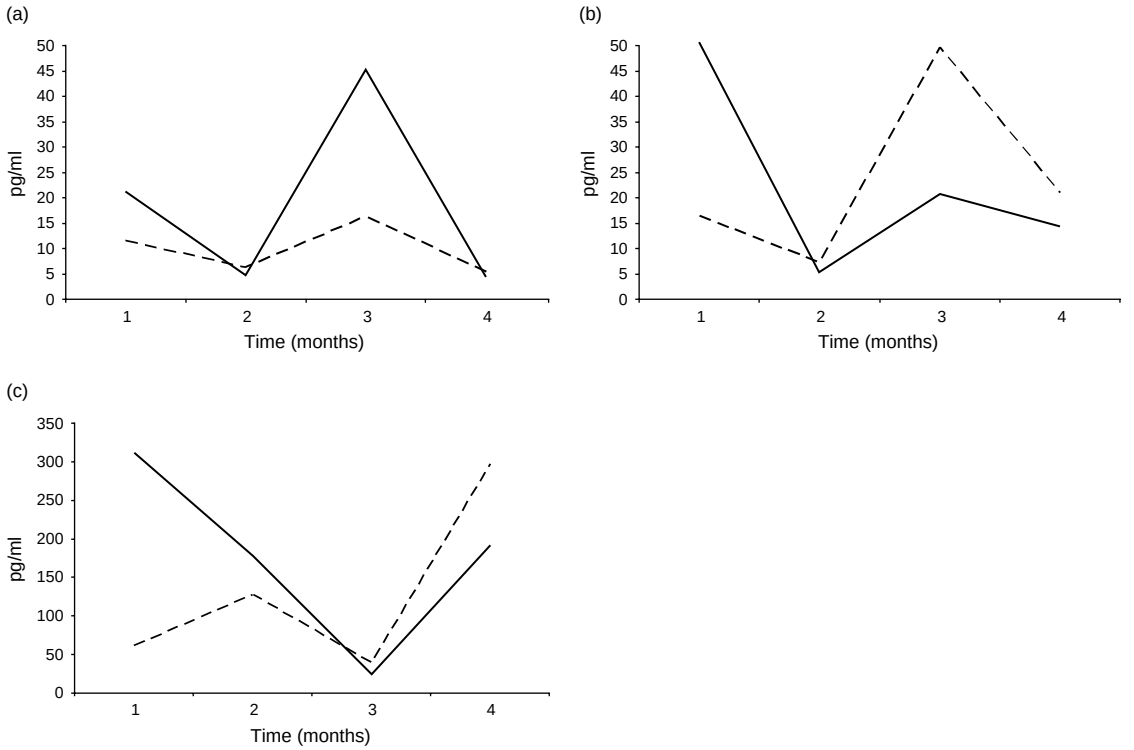

Fig. 1. Evolution of pro-Kaposi's sarcoma cytokines during immune restoration disease, in patients 1 and 2. Results are presented in pg/ml. Panels (a), (b) and (c) present tumour necrosis factor alpha (TNFα), IL-6 and vascular endothelial growth factor (VEGF) plasma level variations, respectively, after the onset of tuberculosis treatment, in months. ——— Patient 1; – – – patient 2.

IL-6 and soluble IL-6 receptor are elevated compared with non-IRD patients [5].

VEGF is a mediator of potent angiogenic, mitogenic and vascular permeability-enhancing activities that are specific for endothelial cells. In active pulmonary tuberculosis, increased serum VEGF levels and intense angiogenesis in tuberculosis lesions have been found [6]. VEGF may be central in the development of Kaposi's sarcoma: plasma VEGF levels are higher in HIV-1-infected patients with Kaposi's sarcoma than in those without, and the antiangiogenic therapy fenretide inhibits Kaposi's sarcoma tumour growth *in vivo* in nude mice xenograft models, and induces a rapid reduction in VEGF and VEGF receptor on Kaposi's sarcoma and endothelial cells [7,8].

These two patients experienced *M. tuberculosis*-associated IRD and visceral Kaposi's sarcoma after starting HAART. Proinflammatory cytokine courses were similar in both patients. At diagnosis, IL-6 and TNF-α were elevated, although their levels were lower in patient 2. After an initial reduction of IL-6 and TNF-α levels under tuberculosis treatment, a second peak was observed when visceral Kaposi's sarcoma occurred, contrary to the usual evolution of those cytokines after tuberculosis treatment, but as reported in cases of IRD [5,9]. VEGF levels were elevated at tuberculosis diagnosis, as previously described [6]. Interestingly, VEGF levels decreased dramatically when visceral Kaposi's sarcoma occurred in the two patients. VEGF levels are, however, usually elevated in extensive Kaposi's sarcoma [7].

We hypothesize that persistent high level of circulating VEGF related to tuberculosis may have caused the development of Kaposi's sarcoma lesions in the two patients. The IRD-associated peak of pro-inflammatory cytokines could have been the decisive factor leading to the development of life-threatening visceral Kaposi's sarcoma. The decrease in VEGF plasma levels remains unclear, however; it could be caused by a downregulation of VEGF production in response to extensive Kaposi's sarcoma. Other cytokines could support Kaposi's sarcoma growth, acting on the tumour microenvironment or neoangiogenesis. In this setting, tuberculosis may be a determinant of Kaposi's sarcoma growth, as has been reported in two kidney allograft recipients with extensive cutaneous Kaposi's sarcoma, which disappeared without any reduction in immunosuppressive therapy once their pulmonary tuberculosis had been treated [10].

Analysis of VEGF and pro-inflammatory cytokine courses led us to hypothesize that Kaposi's sarcoma could be promoted by a tuberculosis immune response.

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