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Evolution of the gp41 *env* region in HIV-infected patients receiving T-20, a fusion inhibitor

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T-20 belongs to a new family of antiretroviral drugs that inhibits HIV entry. Resistance may develop *in vitro* as result of changes within the GIV motif of the HIV-1 gp41. However, none of four individuals who failed treatment with T-20 selected those changes. One developed a change (G→V) at position 36 not previously described. Therefore, other changes in the *env* gene might be responsible for the loss of susceptibility to T-20 *in vivo*.

T-20 is a 36 amino acid synthetic peptide that mimics the HR2 region of the HIV-1 gp41. Its mechanism of action is little understood but it seems to interact competitively with HR1 to prevent the fusion of viral and cellular membranes [1]. Few data are available on the evolution of the *env* gene in patients undergoing treatment with T-20. In-vitro studies have defined a highly conserved three amino acid motif, GIV, within the HR1 region of gp41 to be critical for the loss of susceptibility to T-20 [2]. In particular, two changes in this motif at positions 36 (G→S) and 38 (V→M) have been associated *in vitro* with resistance to T-20.

The purpose of this study was to analyse the possible changes in the gp41 env region potentially associated with resistance to T-20 in clinical samples collected from HIV-positive patients failing therapy with T-20. Four heavily pre-treated patients, with multiple mutations associated with resistance to both protease and reverse transcriptase (RT) inhibitors, who received T-20 were included in the study. Genotypic and phenotypic resistance tests (Virologic, San Francisco, CA, USA) were performed at baseline. The antiretroviral drugs planned to be used along with T-20 were chosen individually on the basis of the results of resistance testing. T-20 (Roche Pharmaceuticals, Nutley, NJ, USA) was administered subcutaneously twice a day at doses of 100 mg every 12 h. One patient (no. 2) discontinued T-20 at month 5 as a result of adverse events.

Genetic analyses were performed on HIV RNA extracted from the plasma from each individual. Specimens collected at baseline and at 2–3 month intervals while under T-20 were examined. A gp41 fragment (567 base pairs) that included HR1 and HR2 genomic regions was amplified by RT-nested polymerase chain reaction using E41ext1 5′–GAGAAGAGTGGTGCA GAGAG–3′ and E41ext2 5′–ATTCCTTCGGGCCT GTCGGG–3′ as outer primers, and E41int1 5′–GCAGCAGGAAGCACTATGGGCG–3′ and E41int2 5′–GGTGARTATCCCTGCTAACTC–3′ as inner primers. The amplicon was sequenced in both directions using the dRhodamine Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA), and sequences were aligned using Clustall X. Plasma HIV-RNA levels and CD4 cell counts were monitored monthly. All four patients experienced a significant decrease in viral load (>1 log) within the first month of therapy with T-20 (patient 1: -1.89 logs; patient 2: -1.66 logs; patient 3: -1.17 logs; and patient 4: -1.75 logs). However, between months 2 and 3, the viral loads rebounded up to baseline values in all cases. At the initiation of treatment with T-20, the mean CD4 cell count was 217 ± 86.5 cells/μl, and remained without significant change during the whole study period in all but one individual (no. 4), in whom a net gain of 199 cells/μl was recorded (Fig. 1).

DNA sequence analyses and the encoded protein sequences of gp41 revealed that all four patients carried a consensus GIV motif at baseline. The intrapatient variability in gp41 sequences during treatment with T-20 was low, ranging from 0 to 0.5%, and resulting almost always in synonymous substitutions. None of the sequences analysed harboured amino acid changes previously associated with resistance to T-20 *in vitro*, not even after 9 months of virological failure. However, one subject (no. 1) developed a change G→V at position 36 at month 7, although being on T-20. The viral loads and CD4 cell counts during follow-up did not differ in this patient in respect to the others. Another subject (no. 3) presented with three changes (Q110Q/E, E119E/Q and R122R/K) within the HR2 domain after 9 months on T-20. Sequences generated in this study have received the following GenBank accession numbers: AF500084–AF500093. A GenBank database search has so far not recorded the G36V change in the GIV motif of the HR1 region in any of the reported sequences. Moreover, to our knowledge no gp41 sequences from patients receiving T-20 have shown amino acid substitutions in the GIV motif. The recognition of non-conservative amino acid substitutions in the HR2 genomic region may equally be involved in the loss of susceptibility to T-20,

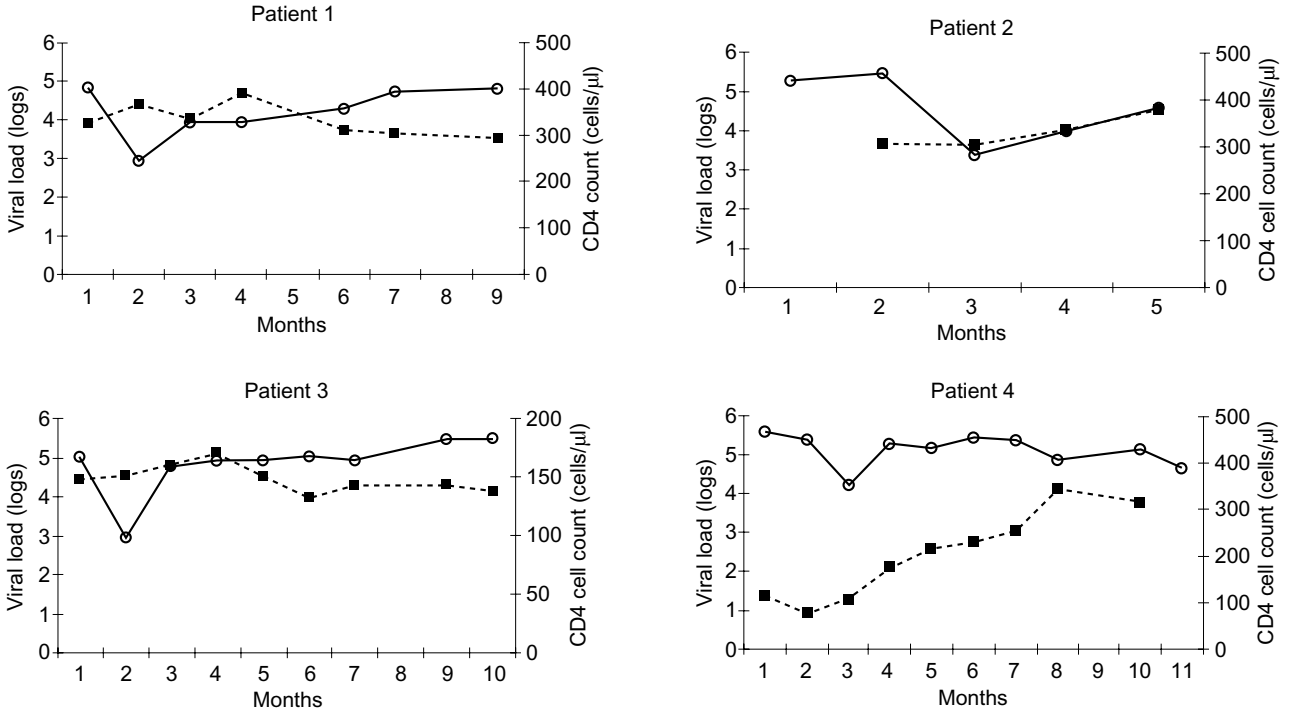

Fig. 1. Evolution of plasma HIV-1 RNA and CD4 T cells in patients on T-20. ——○—— Plasma HIV-1 RNA; – –■– – CD4 T cells.

affecting the molecular interactions between the heptad repeats [3]. Phenotypic studies are currently underway, using individual clones with genotypic differences found in HR1 and HR2, to characterize these mutations and determine their possible association with resistance to T-20.

All four patients showed a rapid and significant decline in plasma viraemia within one month of therapy with T-20. This dramatic drop may not be attributed to other antiretroviral drugs in the regimen taken along with T-20, because large and broad cross-resistance to the agents in use was present (data not shown). Nevertheless, all patients experienced a rebound in their viral load 2–3 months later. All but one subject have continued on T-20 despite virological failure for longer than 9 months.

The possible immunological benefit provided by T-20, despite virological failure in these patients, may be of interest. The mechanism involved is unknown. Although a net gain of CD4 T lymphocytes was only recorded in one patient (no. 4), a clear disconnection with the viral load was noticed in the remaining cases. So far, all patients are clinically asymptomatic and have not developed opportunistic infections.

Although virus susceptibility to T-20 seems to be greatly dependent on the GIV motif, co-receptor specificity defined by the V3 loop of gp120 modulates it [4]. Recent studies support the finding that gp120 interactions with CCR5 are more vigorous than interactions with CXCR4, suggesting that co-receptor influence on HR1-targeted peptide inhibitors is a general phenomenon [5]. This is in contrast to previous reports [6], which found no differences in T-20 susceptibility in viruses as a function of the distinct co-receptor use for cell entry.

Amino acid changes in other regions of the *env* gene, such as V2V3, might be involved in the loss of susceptibility to T-20. Primary CXCR4 syncytium-inducing (SI) isolates seem to be more sensitive to inhibition by T-20 than are CCR5 non-SI isolates [4]. The characteristics of HIV infection in our four patients (long duration of infection, multiple treatment failures, etc.) might have favoured the presence of CXCR4 SI viruses. Pharmacological pressure with T-20 could have resulted in a shift in virus co-receptor use, favouring the replication of CCR5 over CXCR4 isolates. Accordingly, V2V3 sequences from our patients after several months under T-20 showed critical domains associated with CCR5 co-receptor use [7]. Hypothetically, these changes in cell tropism might have conferred a biological advantage to viruses in the face of T-20 therapy. Besides, this fact might have resulted in an amelioration of the CD4 cell destruction rate in our patients.

In conclusion, this study shows that the HR1 and HR2 regions of the HIV-1 genome are highly conserved throughout long-term therapy with T-20, and that other *env* regions may be involved in the develop-

ment of T-20 resistance. A longer follow-up of these patients may provide additional insights to give a better understanding of the mechanisms of escape of HIV in response to T-20 inhibition.

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Biphasic decay of cell-associated HIV-1 DNA in HIV-1-infected children on antiretroviral therapy

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Cell-associated HIV-1 DNA was quantified in 33 HIV-1-infected children followed for 96 weeks after the initiation of antiretroviral therapy. HIV-1 DNA decay was biphasic, being 10-fold more rapid during the first 4 weeks, and was inversely associated with the baseline plasma HIV-1-RNA level, but unrelated to HIV-1-RNA suppression. HIV-1 DNA decay per absolute CD4 cell number was lower than per 10^6 CD4 cells, suggesting an ongoing infection of newly produced CD4 cells, despite HIV-1 suppression in plasma.

It is well established that despite the strong inhibition of viral replication in patients on antiretroviral therapy (ART), HIV-1 may persist in peripheral blood cells and lymphoid tissues. We assessed the relationship between changes in cell-associated HIV-1 DNA and plasma HIV-1 RNA in a subset of 33 ART-naive children initiating therapy in the PENTA 5 trial [1], with a median age of 7.1 years (range 0.3–15.5), CD4 cell percentage 17% (interquartile range 9–24%), and mean HIV-1-RNA level 5.01 $\log_{10}$ copies/ml (SD 0.76) at baseline. Children were randomly assigned to receive zidovudine/lamivudine (n = 8), zidovudine/abacavir (n = 13) and lamivudine/abacavir (n = 12). Sixteen children with more advanced disease also received nelfinavir; and 17 with asymptomatic disease received nelfinavir (n = 10) or placebo (n = 7) in a second randomization.

Cell-associated HIV-1 DNA was measured in peripheral blood mononuclear cells (PBMC) at baseline, and at 4, 12, 24, 48 and 96 weeks. HIV-1-DNA quantification was performed using real-time polymerase chain reaction [2]. HIV-1 DNA copy numbers were normalized to the number of β-actin genes, and expressed relative to 10^6 PBMC (2×10^6 β-actin copies), and to 10^6 CD4 cells, by attributing the HIV-1-DNA load to the CD4 cell fraction, given that these cells are the main target of HIV-1 infection. Children in PENTA 5 experienced substantial increases in CD4 cell counts during the trial [1], so HIV-1-DNA copy numbers per cell were also transformed to copy numbers per millilitre of blood by taking into account the total number of CD4 cells per millilitre. HIV-1-RNA levels were determined using the Roche Amplicor assay (version 1.5; Roche Diagnostic Systems, Inc., Branchburg, NJ, USA). Normal interval regression [3] was used to estimate the mean absolute levels of and change in $\log_{10}$ HIV-1 DNA and HIV-1 RNA, replacing undetectable values with the interval in which the true value could lie (the interval up to the cut-off), and adjusting for multiple measurements on each child [4].

Overall, there was a significant decrease in $\log_{10}$ HIV-1-DNA copies in PBMC and CD4 cells after baseline (both $P < 0.0001$, Fig. 1(a) and (b)). Declines in HIV-1 DNA per 10^6 PBMC and 10^6 CD4 cells were 0.06 [95% confidence interval (CI) 0.02–0.10; $P = 0.003$] and 0.09 (0.05–0.13; $P < 0.001$) $\log_{10}$ copies per week, respectively, to week 4; followed by slower declines of 0.005 (0.002–0.007; $P = 0.001$) and 0.008 (0.004–0.012; $P < 0.001$) $\log_{10}$ copies per week in PBMC and CD4 cells, respectively, from 4 to 96 weeks. Expressing HIV-1-DNA copies per millilitre of blood to incorporate changes in CD4 cells, decreases of 0.06 $\log_{10}$ copies per week to week 4 (0.02–0.10; $P = 0.005$) and 0.005 $\log_{10}$ copies per week from 4 to 96 weeks (0.002–0.008; $P < 0.001$) were observed (Fig. 1(c) and (d)), which were lower than that estimated per 10^6 CD4 cells.

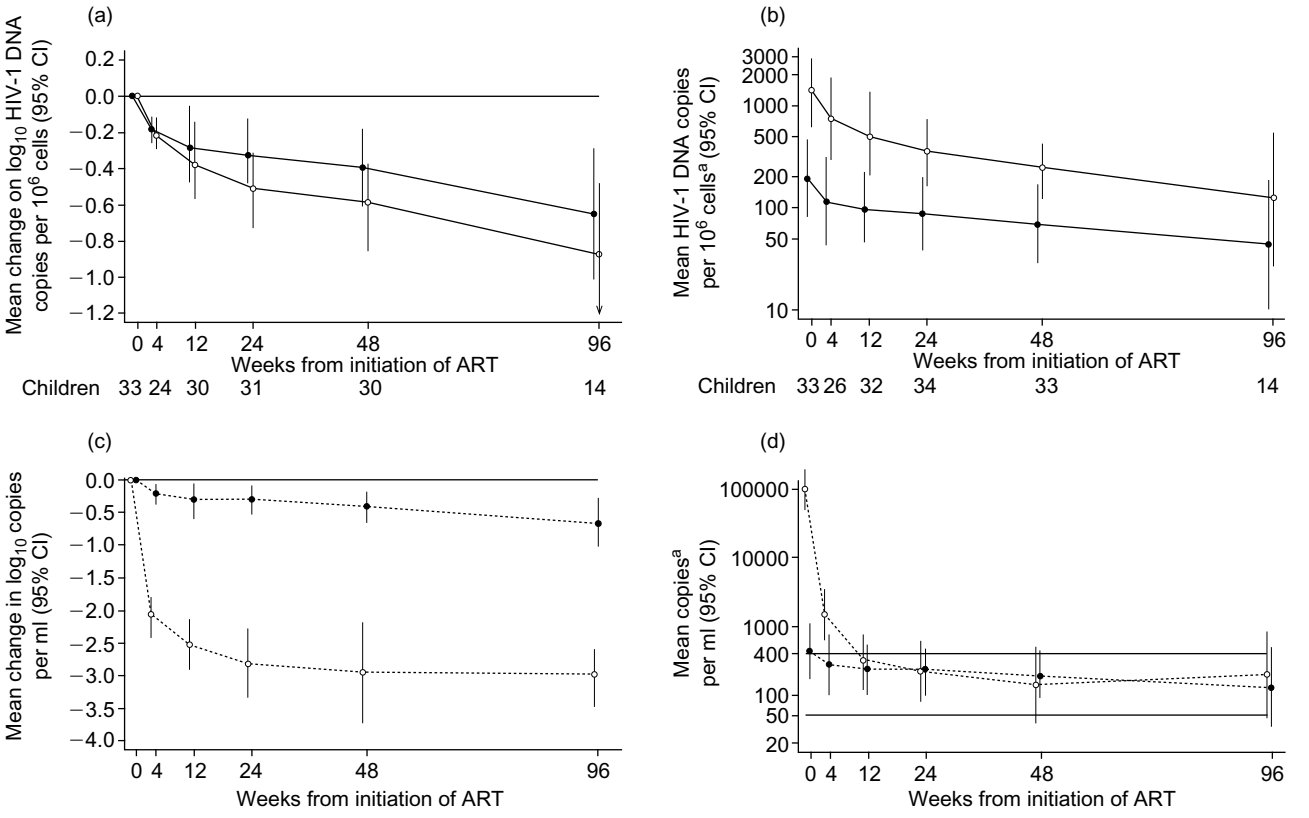

Fig. 1. HIV-1-DNA copies per 10^6 cells and per millilitre, and HIV-1-RNA copies per millilitre after initiation of antiretroviral therapy. (a) Change in $\log_{10}$ HIV-1-DNA copies per 10^6 cells. ——●—— Peripheral blood mononuclear cells (PBMC), ——○—— CD4 cells; (b) absolute HIV-1-DNA copies per 10^6 cells ——●—— PBMC, ——○—— CD4 cells; (c) change in $\log_{10}$ HIV-1-DNA and plasma HIV-1-RNA copies per millilitre. - - - ● - - - HIV-1 DNA, - - - ○ - - - plasma HIV-1 RNA; (d) absolute $\log_{10}$ HIV-1-DNA and plasma HIV-1-RNA copies per millilitre. - - - ● - - - HIV-1 DNA, - - - ○ - - - plasma HIV-1 RNA. [a]Calculated as mean (95% confidence interval) absolute $\log_{10}$ copies and back-transformed to absolute copies.

Higher baseline plasma HIV-1-RNA levels were associated with a faster decline in HIV-1 DNA in the first 4 weeks (0.06 steeper slope for 1 $\log_{10}$ higher baseline HIV-1-RNA level; $P = 0.04$), but not after 4 weeks ($P = 0.99$). At week 4, no child had a plasma HIV-1-RNA level of less than 50 copies/ml, and only eight children (33%) had HIV-1-RNA levels of less than 400 copies/ml. Thirty children (91%) achieved HIV-1-RNA levels of less than 400 copies/ml and 19 (58%) less than 50 copies/ml at some time during follow-up. There was no evidence of a greater decline in HIV-1-DNA levels at timepoints when children had HIV-1-RNA levels of less than 50 copies/ml; if anything, changes in the HIV-1-DNA level were 0.07 $\log_{10}$ copies per millilitre smaller at these assessments ($P = 0.63$; 95% CI 0.37 smaller to 0.22 greater change from baseline at timepoints with HIV-1-RNA levels < 50 copies/ml). A higher baseline CD4 cell percentage was not associated with a decline in HIV-1-DNA level per 10^6 CD4 cells ($P = 0.58$), but was marginally associated with a faster decline in the HIV-1-DNA level per millilitre in the first 4 weeks (0.01 steeper slope for 5% higher baseline CD4%; $P = 0.11$). This is consistent with smaller increases in CD4 cell counts during ART in children with higher baseline CD4 cell percentages [2].

The overall change in plasma HIV-1 RNA per millilitre was 0.60 $\log_{10}$ copies per week to 4 weeks (95% CI 0.51–0.68; $P < 0.0001$), and 0.007 $\log_{10}$ copies per week from 4 to 96 weeks (0.001–0.010; $P = 0.03$). Although higher baseline HIV-1-DNA levels were associated with less steep initial and subsequent slopes in plasma HIV-1-RNA levels, neither interaction was statistically significant (both $P = 0.16$). However, children with higher baseline HIV-1-DNA levels had a lower chance of subsequently achieving plasma HIV-1-RNA levels of less than 50 copies/ml (odds ratio 0.54 for 1 $\log_{10}$ higher baseline HIV-1-DNA level; 95% CI 0.29–1.00; $P = 0.05$), whereas there was no significant effect of the baseline plasma HIV-1-RNA level ($P = 0.25$).

In conclusion, our data show that after the initiation of

ART the decay of cell-associated HIV-1 DNA is biphasic, with a rapid decrease during the first 4 weeks followed by a slower decline, similar to that observed for HIV-1 RNA, and in contrast with previous observations in adults [5]. We found that the higher the baseline HIV-1-RNA level the faster the initial decline in the HIV-1-DNA level, but that there was no association between the suppression of plasma HIV-1-RNA and HIV-1-DNA levels during ART. The decrease in the HIV-1-DNA level per millilitre of blood was lower than that estimated per 10^6 CD4 cells, mostly because of ongoing increases in CD4 cells, which are the main target of HIV-1 infection, and did not differ significantly in children with or without HIV-1-RNA suppression. The persistence of HIV-1 DNA may result both from latently infected cells with a long half-life and newly infected cells [6,7]. Together with the evidence that the peripheral increase in CD4 cells mainly consists of naive cells [1,8], these data strongly suggest that viral replication and the infection of newly produced CD4 cells occur in children on ART, regardless of their apparent HIV-1 suppression in plasma.

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Impact of HIV genotyping and drug levels on the response to salvage therapy with saquinavir/ritonavir

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A total of 139 HIV-infected adults failing a protease inhibitor (PI) regimen were recruited in a multicentre, prospective, open trial. All began saquinavir soft gel 1000 mg and ritonavir 100 mg twice a day plus two nucleoside analogues. Ten discontinued treatment because of adverse events. Plasma HIV-RNA levels fell > 1 log at 24 weeks in 44.5% of patients in an intent-to-treat analysis. The number of PI resistance mutations and saquinavir plasma levels independently predicted the virological response.

The efficacy and safety of a rescue intervention based on saquinavir 1000 mg boosted by ritonavir 100 mg, both twice a day, plus two reverse transcriptase (RT) inhibitors, was examined in an open, multicentre, prospective trial conducted in Spain on patients failing a protease inhibitor (PI) triple combination. As a secondary goal, the predictive value of baseline genotyping as well as of plasma levels of saquinavir on the therapeutic response were assessed.

Patients were enrolled in 2000 at 20 clinical centres. HIV-infected adults with plasma HIV-RNA levels above 1000 copies/ml, while on two nucleoside analogues and at least one PI (excluding saquinavir) for longer than 6 months, were eligible for the study. In all instances, saquinavir/ritonavir was prescribed in combination with two RT inhibitors. Patients were assessed at inclusion (baseline) and every 3 months. The main endpoints for efficacy were plasma HIV-RNA and CD4 cell measurements. A virological response was considered to be significant when plasma HIV-RNA reductions were greater than 1 log or fell to below 50 HIV-RNA copies per ml. The presence of drug resistance mutations was examined in plasma HIV RNA using the ABI 3100 DNA sequencer (Applied Biosystems, Foster City, CA, USA). A set of eight codon substitutions at the protease (L10I/R/V, G48V, I54V/L, A71V/T, V77I, V82A, I84V, and L90M) were considered as saquinavir resistance mutations, according to the most recent guidelines [1,2]. Blood samples for measuring saquinavir concentrations were collected 12 weeks after beginning treatment (to ensure steady state) and just before the morning dose (to estimate trough concentrations or C_{min}). Trough concentrations of saquinavir less than 0.1 μg/ml were considered to be suboptimal [3–5].

A total of 139 patients were recruited into the study. The main patient characteristics are summarized in Table 1. The median time on previous antiretroviral therapies was 52 months; the mean time of exposure to PI was 28 months. Up to 86% of subjects had been exposed to two or more PI, indinavir and nelfinavir being the most frequently used. Finally, 60% of patients had been exposed to nevirapine or efavirenz.

Saquinavir/ritonavir was mostly prescribed with two nucleoside analogues, but 12 patients (8%) received one nucleoside analogue plus one non-nucleoside analogue. Overall, abacavir was the drug most frequently prescribed with saquinavir/ritonavir. There were no other combinations with saquinavir/ritonavir, such as those including an extra PI or three or more RT inhibitors.

The mean plasma HIV-RNA copy number before beginning saquinavir/ritonavir was 4.9 logs. The mean CD4 cell count was 368 cells/mm^3. Forty-one individuals did not complete 24 weeks on therapy. The reasons were: one died of HIV dementia, nine had voluntary withdrawal, eight complained of gastrointestinal symptoms, two had liver toxicity, and 21 were lost to follow-up.

Overall, 44.5% subjects (62/139) showed a significant virological response at 24 weeks in an intent-to-treat analysis. Considering only patients on treatment, 68% (67/98) showed a virological response at 24 weeks. Up to 50% obtained plasma HIV-RNA values below 50 copies/ml. The mean CD4 lymphocyte increase was +127 cells/μl at 24 weeks.

The median number of baseline resistance mutations was four at the *RT* gene (range 0–13), and three at the *protease* gene (range 0–10). More than four PI resistance mutations were recorded in 40.4% of patients. The most prevalent primary PI mutation at baseline was 82A/T/F (23%).

Saquinavir plasma concentrations were measured in 73

Table 1. Baseline characteristics of the study population.

No. of patients	139
Sex (%)	
Male	76
Female	24
Risk factor (%)	
Intravenous drug user	58.8
Heterosexual	25
Homosexual	15.2
Others	1
Length of antiretroviral therapy (months)	52
Length of treatment with PI (months)	28
Baseline plasma HIV RNA (logs)	4.9 ± 1.1
Baseline CD4 cell count (cells/μl)	368 ± 255

PI, Protease inhibitor.

patients with sufficient volume of plasma available 12 weeks after being on treatment. The median saquinavir plasma concentration was 1.43 μg/ml (range 0.2–6.3), and did not differ significantly when comparing virological responders and non-responders. Mean saquinavir plasma levels were approximately 30-fold the EC_{50} for wild-type viruses.

Overall, 83% of patients harbouring less than four saquinavir resistance mutations at baseline reached a significant virological response, whereas it occurred in only 28.6% of those harbouring four or more saquinavir mutations ($P = 0.009$). Moreover, an association between the lack of virological response and specific PI resistance mutations was recognized only for L90M or V82A. However, the influence of these mutations on the rate of virological response was much lower than taking into account the total number of saquinavir resistance mutations.

When the analysis was performed only considering those 73 patients for whom saquinavir plasma levels were available, a significant correlation between virological response and optimal levels of saquinavir (> 0.1 μg/ml) was noted. Fifty-one of the 59 patients (86.4%) with saquinavir plasma levels greater than 0.1 μg/ml reached a virological response. In contrast, it occurred in 50% of subjects (7/14) presenting with saquinavir plasma levels less than 0.1 μg/ml ($P = 0.006$).

In the multivariate analysis, both HIV genotyping and saquinavir levels independently predicted the virological response to saquinavir/ritonavir at 24 weeks (see Table 2). More than 97% of patients harbouring less than four saquinavir resistance mutations together with saquinavir plasma levels greater than 0.1 μg/ml showed a favourable virological response. In contrast, only 12% of subjects with more than four saquinavir resistance mutations and suboptimal saquinavir levels showed a satisfactory virological response.

Ritonavir-boosted PI therapy is currently widely used for treating patients who fail conventional highly active antiretroviral therapy regimens [6–10]. In contrast with what is seen with indinavir, amprenavir or lopinavir, the main effect of ritonavir on saquinavir pharmacokinetics is on the C_{max} rather than on the elimination half-life [4]. In our trial, the performance of saquinavir/ritonavir in heavily pre-treated patients was quite satisfactory: 68% of patients on treatment showed a significant virological response, and half reached less than 50 HIV-RNA copies/ml. Moreover, the combination of saquinavir/ritonavir was relatively well tolerated. Only 8% of subjects discontinued therapy because of adverse events, mainly gastrointestinal symptoms.

Patients who have failed multiple PI-containing regimens often accumulate a large number of resistance mutations at the *protease* gene. In this setting, cross-resistance to all current PI should be expected [1,2,11]. Several studies have shown that the presence of PI resistance mutations at baseline predicts the virological response to salvage therapy [12–17]. In our study, patients harbouring less than four saquinavir resistance mutations showed the best virological outcome. Similar studies have been conducted using other ritonavir-boosted PI combinations, and for example, for lopinavir/ritonavir (Kaletra), the breakpoint in the number of protease mutations over which the response is compromised seems to be five [18–20].

Interindividual variability in the exposure to antiretroviral drugs is widely recognized and could partly explain the different degrees of inhibition of viral replication provided by the same regimen in distinct patients [21,22]. In our trial, a significant relationship was clearly recognized between virological response and the attainment of adequate saquinavir plasma levels ($C_{min} > 0.1$ μg/ml). All together, these data suggest that adding low doses of ritonavir to saquinavir is the best way to guarantee the achievement of optimal saquinavir levels in most instances. Only through this enhancement in saquinavir plasma concentrations may the inhibition of wild-type and low-level resistant viruses be presumed.

In conclusion, our data support the integration of both therapeutic drug monitoring and resistance testing in clinical practice, particularly when managing antiretroviral-experienced patients who begin PI-containing regimens. These methods represent useful tools for optimizing therapy.

Appendix

Members of the Fortogene Spanish Study Group

Mercedes Gónzalez-Serrano, Hospital Virgen de la

Table 2. Predictive value of HIV genotyping and saquinavir drug levels on the virological response to a saquinavir/ritonavir salvage combination (multivariate analysis).

Variable	OR	CI (95%)	*P*
Less than four saquinavir resistance mutations	13	2.3–73.1	0.0036
Saquinavir plasma level > 0.1 μg/ml	8.5	1.6–44.3	0.0108

CI, Confidence interval; OR, odds ratio.

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Intermittent and sustained low-level HIV viral rebound in patients receiving potent antiretroviral therapy

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Low-level viral rebound (LLVR) was identified in 704 of 2055 patients on effective antiretroviral treatment. It was followed by a value of 50 copies/ml, a 'blip', in 490 patients, whereas two consecutive values between 51 and 500 copies/ml, a 'bump', were observed in 155. Hazard ratios of viral failure were 2.01 for blips and 5.80 for bumps. LLVR is frequent and generally resolves spontaneously, but is associated with an increased risk of viral failure.

The occurrence of viral rebound to a moderate level (51–500 copies/ml) raises concern regarding the durability of response to antiretroviral therapy (ART) as it has been correlated with sequence evolution [1–3], with failure to reduce the number of latently infected cells [4], and with drug resistance mutations in the latent reservoir [5]. However, low-level viral rebound (LLVR) could be caused by test variability [6], polymerase chain reaction contamination, non-compliance, intercurrent infection, or vaccination [7]. When evaluating the nature of 'blip', clinicians should be aware of the variability of the ultrasensitive amplification methods. Inter-assay and intra-assay coefficient variability of up to 35.4 and 40%, respectively, has been reported for this assay [6].

In most instances, the next viral load is again below the level of detection without a treatment change. Episodes of intermittent low-level viral load, so called 'blips', have been reported in up to 40% of patients receiving the combination of zidovudine, lamivudine, and indinavir [8]. It is of interest to assess the significance, prevalence, and predictive value of blips. To explore these issues, we analysed data on LLVR among 2055 individuals achieving viral suppression to below 50 RNA copies/ml and followed in two large observational cohort studies, the Swiss HIV Cohort Study and the Frankfurt HIV Clinic Cohort.

We included for analysis all participants who had, while being treated with potent ART, at least two consecutive values below 50 copies/ml within a 24 week interval on samples taken between 1 January 1998 and 31 December 1999, and a minimum of two additional viral determinations taken within 24 weeks after the previous viral load. The plasma HIV viral load was determined using Roche Amplicor (Roche, Basel, Switzerland), with a level of detection of 50 copies/ml or lower. Viral failure was defined by one HIV viral load value above 500 copies/ml. LLVR was defined as a viral rebound of 51–500 copies/ml. The next viral load taken within 24 weeks after a LLVR was used to classify the viral event into two categories: 'blip', the next viral load below or equal to 50 copies/ml; and 'bump', the next viral load between 51 and 500 copies/ml.

Logistic regression was used for the analysis of predictors of the next viral load level for patients after their first LLVR. The variables included in the model were age, sex, intravenous drug use, pretreatment (use of mono- or bitherapy before potent ART), CD4 cell count at the time of viral rebound, viral load at the initiation of potent ART, a non-nucleoside reverse transcriptase or protease inhibitor-containing regimen, treatment change, and the interruption of treatment between viral rebound and next viral load. Cox's regression for time-to-event analyses were used to assess the prognostic value of any episode of blip or bump. The model used the two most recent viral load values. The time to viral failure was measured since the fourth viral load. Observations were censored at the time of the last available viral load. The variables included in Cox's models were age, sex, intravenous drug use, AIDS at baseline, CD4 cell count, non-nucleoside reverse transcriptase or protease inhibitor-containing regimen, and previous treatment. The CD4 cell count was included as a time-dependent variable. SAS (version 6.12) and STATA (version 7.0) software were used for analysis.

A total of 2055 patients achieved HIV viral suppression. The median follow-up after the first viral load below 50 copies/ml was 17.7 months. HIV viral load values were available on average 11.6 times per subject during the study period. A total of 1175 subjects (57.2%) maintained viral suppression during the whole study period, 704 patients (34.3%) presented a LLVR (51–500 copies/ml) and 176 patients (8.6%) had an initial viral rebound greater than 500 copies/ml.

A blip was observed in 490 out of 704 subjects (69.6%) with a first LLVR, and a bump was observed in 155 of them (22.0%). A return to viral load below or equal to 50 copies/ml was also observed in 71 of 176 patients (40.3%) with a first rebound to more than 500 copies/ml (Fig. 1a).

The variables associated with an episode of viral rebound evolving into a blip were the level of initial viral rebound [odds ratios (OR) 0.85, 0.75–0.98, per additional 0.5 log copies/ml], and pretreatment with mono- or bitherapy before potent ART (OR 0.60, 0.38–0.94). Treatment change and the type of treatment were not associated with the probability of blip.

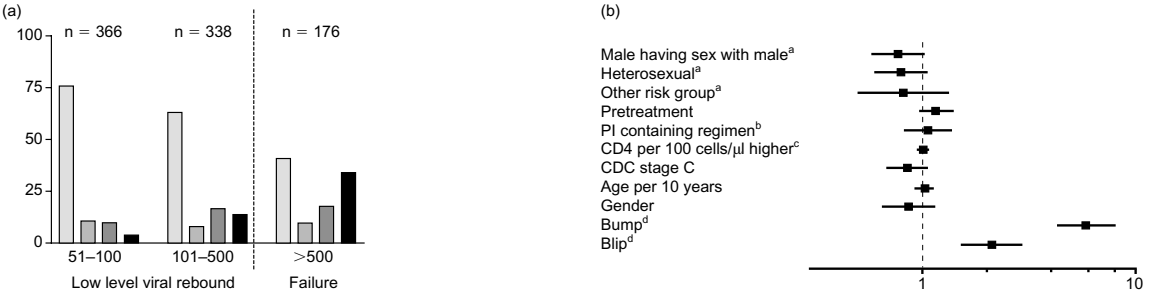

Fig. 1. (a) Next viral load (in copies/ml) for 704 patients presenting a low level viral rebound (51–100 copies/ml and 101–500 copies/ml), and 176 patients presenting a viral failure (500 copies/ml). □ 0–50; ▒ 51–100; ▓ 101–500; ■ > 500 copies/ml. (b) Hazard ratio of having a viral failure (one viral load greater than 500 copies/ml) adjusted for all listed variables. [a]Compared with intravenous drug user; [b]compared with protease inhibitor-sparing regimen; [c]time-dependent; [d]compared with optimally controlled subjects.

After a median follow up of 17.7 months, 157 out of 2055 patients (7.6%) experienced viral failure. In Cox's regression models, the hazard ratio (HR) of viral failure was 2.01 [95% confidence interval (CI) 1.51–2.91; $P < 0.0001$] for blip, and 5.80 (95% CI 4.26–7.90; $P < 0.0001$) for bump compared with viral suppression (Fig. 1b).

This cohort analysis thus identified a high incidence of LLVR. For most people, LLVR will resolve without treatment change. Nevertheless, it increases the risk of subsequent viral failure by approximately two-fold compared with patients with sustained viral suppression. The magnitude and persistence of the viral rebound predicted the viral outcome. In particular, the occurrence of bumps carried a worse prognosis than blips for the durability of viral suppression. The observed increased risk of viral failure associated with an episode of blip is in contradiction with the results of a recent analysis by Havlir *et al.* [8], in which the identification of a blip did not translate into different rates of virological failure (HR 1.28, 95% CI 0.59–2.79). This discrepancy could be explained by the smaller number of subjects included in the study by Havlir *et al.* [8] (n = 343), and the limited time of follow-up to detect failures in this group of patients with a very low risk of failure [9], or by differences in the study design and patient populations studied: a randomized controlled trial versus a prospective observational cohort study.

The main limit of the present study is the short follow-up of 17.7 months (as a result of the recent introduction of the ultrasensitive plasma HIV-RNA assay). The choice of a limit of detection of less than 50 viral RNA copies/ml employed in this study can also be challenged. This limit was chosen to allow the harmonization of results between cohorts and among various laboratories. The use of a lower limit of detection, i.e. less than five or less than 20 viral RNA copies/ml could have provided more insight into the various issues discussed above [10].

Future studies should aim at identifying the determinants for the diverse evolution after a viral rebound. This includes a detailed analysis of the immune determinants of the control of residual viral replication, an assessment of the role of resistance mutations by the development of more sensitive and rapid methods to assess drug resistance in samples with a low level of viraemia [5], and the determination of drug penetration in various compartments. Such studies may provide insight into the observed differences in the durability of optimal suppression in patients receiving potent ART.

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A prospective case–control survey of laboratory markers of skeletal muscle damage during HIV disease and antiretroviral therapy

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In a case–control prospective study of approximately 1000 HIV-infected patients, a 15% incidence of muscle laboratory abnormalities was found. No difference emerged between patients with altered creatine phosphokinase levels and controls in the duration of HIV infection, antiretroviral therapy and its duration, selected drug combinations, disease stage, mean CD4 cell count and viraemia, lipodystrophy syndrome, metabolic and bone abnormalities, and eventual myotoxic therapies, except a prevalence of male sex and a longer duration of stavudine administration.

Skeletal muscle abnormalities are emerging complications of HIV disease, but the causes and consequences are debated [1–4]. Altered serum creatine phosphokinase (CPK) levels are a clue to skeletal muscle disease, when other confounding conditions have been excluded [5]. Physical, instrumental, or histopathological examinations, and the concurrent evaluation of other laboratory enzymes, such as serum aldolase, transaminases, lactic dehydrogenase, and lactic acid, may contribute to a better assessment of the specificity, extent, and supporting factors. No controlled studies evaluating the frequency and features of skeletal muscle damage have been reported in HIV-infected individuals in the combined antiretroviral therapy era, except for a 1998 survey that identified nucleoside analogues as potential risk factors for peripheral neuropathy and myopathy, but failed to correlate mean CPK levels and the signs and symptoms of muscle involvement. In that study, the median CPK levels of patients on zidovudine–zalcitabine proved to be higher than those observed with other drug combinations [6].

A 1 : 3 case–control prospective study was performed in a cohort of approximately 1000 HIV-infected patients between June 2001 and February 2002. Among the 875 subjects who had had three or more laboratory examinations performed in the 9-month study period, and assured a greater than 90% adherence to antiretroviral therapy when prescribed (as assessed by patients' declarations and monthly drug accountability), the 131 patients who had one or more altered CPK tests were compared with 393 controls randomly selected from among the remaining 744 subjects, provided that they had three or more laboratory examinations in the same period. All patients with concurrent conditions potentially leading to abnormal CPK levels were ruled out. Automated assays determined serum CPK levels (normal range 0–195 U/l), aldolase (0.5–3.1 U/l), aspartate aminotransferase (0–37 U/l), alanine aminotransferase (0–40 U/l), lactate dehydrogenase (230–460 U/l), lactic acid (9–18 mg/dl), glucose (60–110 mg/dl), triglycerides (74–172 mg/dl), and cholesterol levels (< 200 mg/dl). A diagnosis of osteopenia, osteoporosis, or osteonecrosis was confirmed by the mineral metabolism study, X-ray examinations, and dual energy X-ray absorptiometry, whereas the definition of fat redistribution syndrome was ensured by physical examination, patients' self-assessment with specific questionnaires, dual energy X-ray absorptiometry, and bioelectric impedance assay.

A 14.97% crude frequency of CPK abnormalities was found among the 875 evaluable patients (131 overall subjects) (Table 1). CPK alteration occurred only once in 98 subjects (74.8%), but it was found two to three times in 2–9 months in the remaining 33 patients (25.2%). Abnormal CPK values varied from 196 to 3463 U/l (mean 256.2 ± 62.3), without significant differences between patients with transient (249.3 ± 54.2), and those with repeated (260.1 ± 41.7) CPK alteration. Concurrent high aldolase levels (3.1–10.8 U/l) were found in 25 patients, all suffering from repeated CPK elevations ($P < 0.0001$ compared with subjects with transient/absent CPK alterations). After the exclusion of concomitant chronic viral, alcoholic, or metabolic hepatitis, elevated serum transaminase levels (46–143 U/l), were found in 14 out of 26 patients who suffered from repeated CPK alterations (53.8%), compared with two cases among the 65 subjects with transient CPK elevation ($P < 0.0001$), and 22 cases in the control group ($P < 0.0001$). Increased lacticidaemia (20–25 mg/dl) was found in three patients with persistently altered CPK values (19–26 mg/dl) and an associated increase in aldolase levels, compared with eight controls ($P < 0.05$). In our case–control comparison, the male sex significantly prevailed

among patients with elevated CPK levels (125 cases out of 131: 95.4%; and 31 out of 33 with persistently altered CPK values: 93.9%; $P < 0.0001$), whereas no difference was found in the mean age of patients, HIV exposure, duration and stage of HIV disease, present anti-HIV therapy (taken by 121 patients and 355 controls), the length and type of previous and concurrent antiretroviral therapy, mean CD4 cell count and HIV-RNA levels throughout the study period, the frequency of an eventual lipodystrophy syndrome, the incidence of increased glucose, triglyceride and cholesterol levels, eventual bone disorders, concomitant therapies with potential muscle toxicity (i.e. hypolipidaemic drugs), and hepatitis B virus or hepatitis C virus co-infection. When considering the mean duration of administration of each nucleoside analogue, treatment with stavudine proved to be longer in patients who developed CPK abnormalities compared with controls ($P < 0.006$), although the frequency of stavudine use was no different between the two study groups. Mild fatigue or muscle weakness was reported by an appreciable number of patients in both study groups (mean 26.9%), but most signs and symptoms were undistinguishable from HIV-associated conditions. More severe clinical features suggestive of myopathy, and prompting further investigation (electromyography, scintigraphy, biopsy), were recognized in only five patients, all with repeatedly altered serum CPK levels ($P < 0.001$ versus patients with incidental serum CPK abnormalities, and $P < 0.0001$ versus controls). Histopathological and instrumental studies confirmed autoimmune polymyositis in one case; two subjects experienced associated myopathy and neuropathy, whereas two more patients suffered from moderate rhabdomyolisis, probably prompted by abacavir.

Muscle abnormalities have been incidentally reported since early in the HIV pandemic [1,4], and well before the awareness of metabolic abnormalities occurred during the HAART era [7,8], so the dispute about the causative role of antiretroviral therapy, HIV, and HIV-related illnesses, has lasted for more than 10 years. Myopathy was first associated with increased CPK levels, peripheral wasting, and mitochondrial abnormalities, although patients taking zidovudine did not show different median CPK levels compared with untreated controls [1]. The cellular and mitochondrial toxicity of nucleoside analogues on cultured muscle cells was demonstrated, although a multifactorial pathogenesis was postulated for in-vivo damage [9]. Frank myositis remains a rare event, described by anecdotal reports as an autoimmune disease associated with HAART-related immune recovery or a concurrent HCV infection [6,10,11]. Our case report of autoimmune polymyositis occurred in an AIDS patient who had been undergoing dual nucleoside analogue therapy for 6 years. After extensive HAART introduction, abnormalities involving muscles, nerves, and other organs and tissues became increasingly apparent, and most skeletal muscle disorders were related to mitochondrial damage probably associated with nucleoside analogue use, and potentially also responsible for lactic acidosis, intracellular fat accumulation and phosphocreatine depletion. These abnormalities seem to be responsible for muscle wasting, lipoatrophy, weight loss, myalgia, weakness, fatigue, elevated muscle enzyme levels, peripheral neuropathy, pancreatitis, and a broad spectrum of metabolic abnormalities [4,7–9,12,13]. We found an incidence of altered CPK levels approaching 15%, but this abnormality was usually transient, because only 3.8% of the 875 evaluable patients (25.2% of those with abnormal CPK levels), experienced two or more alterations in the 9-month period under examination. Serum aldolase and lactic acid measurements confirmed their specificity, because their alteration was limited to patients with repeatedly abnormal CPK values. When excluding an unexpected increased frequency of the male sex (observed in a series of osteoarticular diseases) [3], no significant difference emerged between patients and controls with regard to many investigated epidemiological, clinical, therapeutic, and laboratory features (including the use of hypolipidaemic drugs) [5]. Statins and fibrates are increasingly administered for HIV-associated dyslipidaemia, so that careful attention should be paid to this patient group, because a severe course has been reported during HIV infection [14]. In our series, the occurrence of muscle toxicity was associated with lactic acidosis only when repeatedly altered CPK and aldolase levels were present, but a possible relationship with the duration of stavudine administration was found, as previously suggested with stavudine and other nucleoside analogues [4,12,15]. Symptomatic [12] and asymptomatic [15] lacticidaemia associated with mitochondrial dysfunction, myopathy, and multiple metabolic abnormalities had a slightly increased rate in stavudine-treated subjects, or disappeared after stavudine withdrawal, whereas no relationship was found with both the frequency and severity of the lipodystrophy syndrome, and lipid metabolic abnormalities in another study [7]. The frequency of hepatitis B–hepatitis C virus co-infection was comparable in our two study groups. Interestingly, mitochondrial toxicity may be increased when hepatitis C virus co-infection is present [16], and this association might prompt polymyositis [10]. A clinically significant muscle disease was found to be rare in our series (3.8% of the 131 patients with one or more alteration of CPK levels), but it only considered patients with persistently abnormal muscle enzymes. Skeletal muscle damage, although frequently asymptomatic and transient, is an underestimated problem associated with HIV disease itself or antiretroviral therapy. Myositis should be taken into account when confronted with patients with repeated alterations of multiple skeletal muscle enzymes, regardless of the type and duration of antiretroviral therapy. The causative role of HIV, selected

antiretroviral drugs and their duration of use, related metabolic abnormalities, and all postulated pathogenetic pathways leading to muscle damage, deserve further investigation.

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Vascular disease in HIV-infected patients: a comparative study of two different therapeutic periods (1994–1997 versus 1998–2000)

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Highly active antiretroviral therapy (HAART) has been linked to increases in plasma lipids. To determine the risk of cardiovascular disease, 23 patients with a stroke or myocardial infarction (separated according to the year of event development; 1994–1997, n = 9 versus 1998–2000, n = 14) were identified and compared. 'Classic' risk factors were frequently detected, and seemed to be more deeply associated in the short term. HAART conferred longer survival, even after the development of cardiovascular complications.

The use of highly active antiretroviral therapy (HAART) for the treatment of HIV-1 infection has been linked to increases in plasma lipids. The risk of the development of premature atherosclerosis remains to be established. Increases in plasma concentrations of LDL and triglycerides may contribute to the appearance of coronary artery disease [1]. Triglycerides may increase the cardiovascular risk by increasing plasma viscosity [2]. 'Traditional' risk factors (e.g. cigarette smoking, alcohol consumption, dyslipemia) have frequently been described in the European HIV-infected population. Accordingly, there is an urgent need to assess the incidence of cardiovascular disease (CVD), to determine the relative contribution of various therapeutic regimens to its development, and to identify factors contributing to such side-effects.

We have identified all the HIV-infected adults who developed a vascular event (stroke or myocardial infarction; MI) in a tertiary centre located in Madrid, Spain, in a 7-year period. For the purpose of analysis, the patients were separated into two groups according to the year in which the vascular complication developed, and the availability of HAART (1994–1997 versus 1998–2000). In the second period, all the patients were receiving HAART, and therefore the event could have been treatment linked. The mean number of patients on follow-up were 1489 versus 1558 in the first and second periods, respectively.

A total of 23 patients were identified and their clinical charts were reviewed. The main characteristics of the study population can be seen in Table 1. Most patients were young, male, and current heavy smokers. Nine patients were diagnosed in the first period versus 14 patients in the HAART period. More strokes were detected in the pre-HAART era, with a well-balanced number of episodes of MI and central nervous system (CNS) complications in the second period. All patients

Table 1. Demographic, epidemiological, clinical, therapeutic, and laboratory features of the 131 HIV-infected patients who showed at least one altered creatine phosphokinase assay in a 9-month period, compared with those of 393 patients randomly selected from the same patient cohort, who did not show any muscle enzyme abnormality in the same time period.

Patients' features	Patients with altered serum CPK levels (n = 131)	Controls with normal CPK levels (n = 393)
Sex (men/women)	125/6	242/151
Mean age (years ± SD)	37.8 ± 6.7	38.3 ± 6.1
Intravenous drug addicts/heterosexuals/MSM	76/34/21	234/101/58
Duration of HIV infection (months ± SD)	42.6 ± 17.9	40.9 ± 18.6
Subjects with previous diagnosis of AIDS	19	55
Patients undergoing antiretroviral therapy (⩾ 6 months)	121	355
Mean duration of anti-HIV therapy (months ± SD)	29.6 ± 11.4	28.1 ± 14.7
Duration of HAART (months ± SD)	19.4 ± 8.9	18.9 ± 9.4
Duration of use of zidovudine (months ± SD)	9.3 ± 4.1	10.1 ± 4.4
Duration of use of didanosine (months ± SD)	8.8 ± 3.2	9.2 ± 4.1
Duration of use of zalcitabine (months ± SD)	4.1 ± 1.9	3.9 ± 2.2
Duration of use of stavudine (months ± SD)	18.9 ± 6.9	17.1 ± 6.1
Duration of use of lamivudine (months ± SD)	15.9 ± 7.2	16.8 ± 6.3
Duration of use of abacavir (months ± SD)	3.2 ± 1.3	3.4 ± 1.1
Mean CD4 lymphocyte count (cells/μl ± SD)	389.6 ± 108.8	378.1 ± 119.2
Mean plasma HIV-RNA levels (copies/ml ± SD)	4650 ± 2212	4977 ± 1975
Patients with fat redistribution syndrome	34	99
Patients with increased serum glucose levels	4	8
Patients with increased serum triglyceride levels	21	69
Patients with increased serum cholesterol levels	15	49
Patients with osteopenia/osteoporosis/osteonecrosis	7/1/0	24/4/2
Subjects taking hypolipidaemic drugs for ⩾ 3 months	13	41
Patients with concurrent chronic hepatitis C or B	29	65
Patients with concurrent altered serum aldolase levels	25	0
Patients with concurrent altered transaminase levels	16	22
Patients with concurrent abnormal lactic acid levels	3	8
Subjects complaining of fatigue or muscle weakness	37	104
Patients who developed myositis or rhabdomyolisis	5	0

CPK, Creatine phosphokinase; HAART, highly active antiretroviral therapy, including two nucleoside analogues together with one or two protease inhibitors, or one non-nucleoside reverse transcriptase inhibitor
MSM, men who have sex with men.

had at least one 'classic' risk factor, with a mean of 1.55 risk factors per patient (1994–1997 period) and 2.3 (1998–2000 period). Diabetes mellitus and overweight were only detected in the second period, as well as age greater than 65 years. These facts could be related to the use of HAART, which undoubtedly confers longer and better survival to HIV-infected individuals. In the second period, all patients received HAART. Eight patients started HAART (median time 29 months) before the vascular event occurred. Of note was the fact that HAART was administered after CVD in five patients, and none developed additional events. The survival in the group receiving HAART was higher. Only one patient died, as a result of a non HIV-related condition (a 74-year-old man with an extensive stroke).

The major finding of this study is that the risk of vascular disease in HIV-infected patients on HAART seems not to be increased with respect to that of untreated HIV-infected patients, at least in the short term. Moreover, the administration of HAART confers longer survival, even after the development of cardiovascular complications.

Since 1998, when anecdotal reports of premature CVD associated with new therapies were described [3–5], it has been debated whether the lipid perturbations that exist during HAART have a significant impact on the development of CVD in these patients. There are different reasons that support a presumably higher incidence of CVD in HIV-infected patients on HAART. First, the prolongation of survival may lead to an increased exposure to pre-existing cumulative cardiovascular risk factors, mainly smoking, the prevalence of which is particularly high in this population. Second, protease inhibitor (PI)-containing regimens are associated with a higher risk of dyslipemia and insulin resistance. Third, various inflammatory and immunological factors associated with HIV infection may contribute to accelerated atherosclerosis or thrombosis. It is not surprising that premature atherosclerosis within the femoral or carotid arteries, visualized with high resolution ultrasound imaging, has been de-

tected more frequently in the HIV-infected population [6]. A recent communication highlighted that the risk of MI appears to be increase by several-fold among HIV-infected patients being treated with PI [7].

Despite these arguments, controversy exists as to whether HAART is linked with CVD. In accordance with our results, one of the major findings in the above-mentioned study [6] was the lack of a link with the use of PI-based regimens, but rather with 'classic' cardiovascular risk factors. Similarly, PI were not associated with an increased risk of CVD in another case–control study [8]. Bozzette *et al.* [9] reported on a large group of contributing patients (n = 36 766, between January 1993 and June 2001), in which no association was found between an increased risk of any cardiovascular events and any antiretroviral therapy. They concluded that the large increase in antiretroviral use correlated with sharp declines in overall mortality and slight declines in cardiovascular events [9].

There are some limitations in our study. Most retrospective analyses are subject to ascertainment bias. The number of patients included is small. Longer exposures to new combination therapies could reveal an association with an increased risk of vascular complications. Moreover, the association of classic risk factors (e.g. dyslipemia, diabetes mellitus) might partly reflect the effects of PI, and their long-term consequences remain to be clarified.

In summary, our results suggest that 'traditional' risk factors are more deeply associated with cardiovascular complications in HIV-infected patients than new combination therapies. No significant association was found between HAART and the development of CVD in the short term. There is no doubt that the question is important and will receive much more attention in the near future. Additional larger longitudinal cohort studies will be needed in order to confirm or refute our findings.

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Prognostic impact of highly active antiretroviral therapy in HIV-related Hodgkin's disease

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To evaluate the influence of highly active antiretroviral therapy (HAART) on the outcome of HIV-related Hodgkin's disease (HD), two groups were studied: patients without previous HAART, and patients treated with HAART previously or concomitantly with HD therapy. In the second group, the complete response was higher and the disease-free survival and overall survival probabilities were significantly longer. HAART is thus associated with an improvement in response to therapy and survival in patients with HIV-related HD.

Recent data from AIDS-related non-Hodgkin's lymphomas (NHL) have shown a better response to chemotherapy and a longer survival in patients receiving highly active antiretroviral therapy (HAART) compared with those treated in the pre-HAART era [1–6]. However, to our knowledge no similar studies have been carried out in patients with HIV-related Hodgkin's disease (HD). The aim of this paper was to study the possible influence of HAART on the response to treatment and survival in a series of 45 HIV-related HD patients diagnosed in five Spanish institutions from 1987 to 2001.

Forty-five patients diagnosed with HIV-related HD from 1987 to 2001 at five hospitals from Catalonia, Spain, were analysed. The following clinical and biochemical parameters were recorded in each patient:

age, sex, risk activity, previous diagnosis of AIDS, B symptoms, complete blood cell counts, CD4 positive lymphocyte count, blood chemistry, histological subtype, stage of HD and extranodal involvement.

All pathological diagnoses of HD were reviewed for this study and classified according to the Revised European–American Classification of Lymphoid Neoplasms [7]. The Ann Arbor classification [8] was used to assess the stage of HD. The prognostic score proposed by the International Prognostic Factors Project on Advanced Hodgkin's Disease [9] was applied to patients with advanced HD. Standardized guidelines were applied for response assessment [10].

To evaluate the influence of HAART on response to therapy, the patients were grouped as follows: group 1, patients who did not receive HAART and patients who started HAART shortly after HD treatment was completed; and group 2, patients who were taking HAART at the time of HD diagnosis or those who started HAART after HD diagnosis (in those cases in which HD led to the diagnosis of HIV infection). For the analysis of disease-free survival (DFS) and overall survival (OS), patients who started HAART shortly after the HD treatment was completed were included in group 2. HAART consisted of one or two protease inhibitors (indinavir, saquinavir, ritonavir or nelfinavir) and two reverse transcriptase inhibitors (zidovudine, didanosine, zalzitabine, stavudine or lamivudine). Patients diagnosed before 1996 received zidovudine as the only antiretroviral agent.

Twenty-five HD patients did not receive HAART at any time, 12 were receiving HAART at the time of HD diagnosis, four started HAART at the time of the diagnosis of HD and the last four patients received HAART after completing HD therapy (range 1–6 months). Consequently, group 1 consisted of 25 patients and group 2, 20 patients. Patients from group 1 had a lower frequency of HD as a first AIDS-defining event and a higher frequency of lymphocyte depletion (LD) histological subtype (Table 1).

The response rate to therapy was significantly higher in group 2 patients (Table 1). Factors with influence on achievement of complete response (CR) in the univariate analysis included advanced stage, LD subtype, lower serum protein values and HAART. In the multivariate analysis, the only parameter associated with a higher probability of attaining CR was HAART [odds ratio (OR) 5.3, 95% confidence interval (CI) 1.2–23.2; $P = 0.026$].

OS (Fig. 1) and DFS were significantly better in group 2 patients (Table 1). The factors influencing OS in the univariate analysis were: LD subtype, leukopenia, lower serum protein levels, higher international prognostic score and HAART. Multivariate analysis identified HAART (OR 8.6, 95% CI 2.3–32.7; $P = 0.002$) as a protective factor, and a low serum protein level as prognostic variables. Prognostic factors for DFS in the univariate analysis were LD subtype, HAART and

Table 1. Baseline characteristics and outcome of 23 patients with vascular complications.

	Pre-HAART era (n = 9)	1998–2000 (n = 14)
Male/female	6/3	10/4
Median age in years (range)	32 (25–52)	35 (31–74)
Heart disease/CNS event	2/7	6/7
Both	0	1
Risk factors		
Current heavy smokers	9	11
Chronic liver disease	6	6
Hypertension	1	1
Dislypemia	1	5
Age > 65 years	0	2
Diabetes mellitus	0	1
Chronic renal failure	0	1
Overweight	0	1
Cocaine use	0	1
None	0	0
Antiretroviral therapy		
None	5	0
Before vascular event	3	9
With dual NRTI	3	1
With PI	NA	8
Median time (months) on HAART (range)	NA	29 (1–42)
After vascular event	1	5
Outcome		
Death	9	1
HIV related	8	0

CNS, Central nervous system; HAART, highly active antiretroviral therapy; NRTI, nucleoside reverse transcriptase inhibitors; PI, protease inhibitors; NA, not applicable.

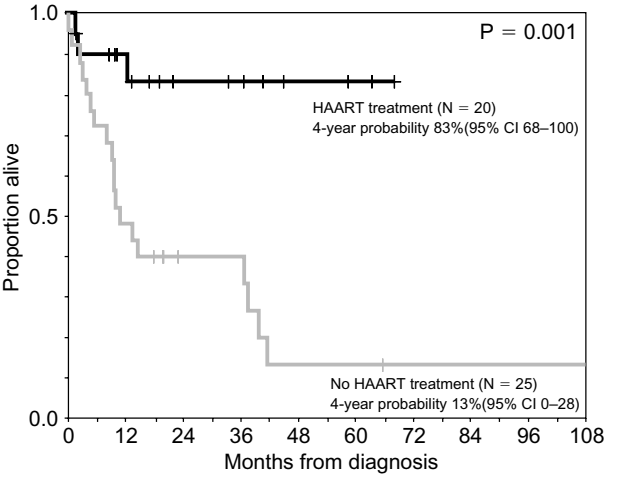

Fig. 1. Kaplan–Meier curves for overall survival of patients from groups 1 and 2. CI, Confidence interval; HAART, highly active antiretroviral therapy.

adriamycin–bleomycin–vinblastine–dacarbazine (ABVD) therapy. Multiple regression analysis identified LD and HAART as protective (OR 11.2, 95% CI 1.2–102; $P = 0.032$) to influence DFS.

The effect of the new potent antiretroviral therapy on the clinical–biological features and the response to treatment and survival of AIDS-related NHL has recently been studied, and a higher response to therapy and a better survival have been documented [8–13]. HD represents the most frequent subtype of non AIDS-defining HIV-related neoplasm [11]. Recent studies have demonstrated a significant increase in the incidence of HD in adults in the setting of HIV infection. This increase is especially prominent in the mixed cellularity and LD subtypes, the most frequent among HIV-infected patients [11]. The effect of HAART on the clinicopathological characteristics, the response to therapy and the prognosis has scarcely been studied, and we have not found any study specifically focused on this matter. In our study we found that HAART had a beneficial effect on response to therapy, DFS and OS in patients with HIV-related HD.

Although the main clinicopathological characteristics of HD patients were identical to those reported in other series [12–14], in our study some changes have been found in the clinicobiological characteristics of HIV-related HD since the introduction of HAART. The frequency of a previous AIDS diagnosis was lower in those patients receiving HAART, and a lesser number of cases had LD histology. The differences in the type of chemotherapy between the two groups reflect the trend towards a generalized use of ABVD in spite of cyclophosphamide–vincristine–procarbazine–prednisone (COPP) or COPP/adriamycin–bleomycin–vinblastine schedules that have been used in recent years in HD in immunocompetent patients as an attempt to reduce long-term toxicity.

The results of this study point to a favourable effect of HAART on an improvement of the response rate in patients with HIV-related HD. Similar findings have been observed in AIDS-related NHL [1–4,15]. This feature could support the theory that combined therapy for HD and HIV infection is also essential to improve the prognosis of HIV-related HD. Preliminary reports indicate that this approach is feasible in HD, as was demonstrated with the Stanford V regimen [16].

Although we cannot rule out a hypothetical antitumour effect of HAART to explain its favourable effect on the duration of response to chemotherapy, it seems more probable that the immune restoration induced by effective antiretroviral therapy could prevent the recurrence of HD. A similar feature has been observed in AIDS-related NHL [17–19], in which the best survival was observed in patients showing a virological or immunological response to HAART.

This is a retrospective study comparing historical groups. For ethical reasons, this is the only way to study the influence of HAART on the natural history of HIV-related HD. In spite of this limitation, our results show the important role of HAART in the management of HIV-related HD, being an independent prognostic factor for CR, OS and DFS. Although these findings should be confirmed in other studies, on the basis of our results and those from preliminary experience [16] the administration of HAART concomitantly and after chemotherapy should be recommended in the treatment of patients with HIV-related HD.

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High incidence of tuberculin skin test conversion among HIV-infected individuals who have a favourable immunological response to highly active antiretroviral therapy

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A total of 129 HIV-infected individuals who were tuberculin skin test (TST) negative in 1997 and started highly active antiretroviral therapy (HAART) after skin testing, received a second TST during 2000. Seven patients converted to a positive TST. In multivariate analysis, an increase in CD4 T lymphocyte count more than 200 × 10^6/l over baseline was significantly associated with TST conversion. TST should be repeated in TST-negative HIV-infected individuals who experience an immunological response to HAART.

The treatment of latent tuberculosis infection in HIV-infected individuals greatly reduces the risk of active tuberculosis [1]. Tuberculin skin test (TST) is at present the only standardized method of identifying individuals with latent tuberculosis infection [2]. However, the sensitivity of this test is greatly reduced in HIV-infected individuals [3]. Highly active antiretroviral therapy (HAART) may determine the restoration of in-vitro T cell response and of delayed-type hypersensitivity response to mycobacterial antigens [4,5]. Moreover, a conversion from negative to positive TST has been recorded in patients with active tuberculosis who initiated effective HAART [6]. On the basis of these observations, current guidelines recommend that clinicians should consider repeating TST for individuals whose initial skin test was negative and whose immune function has improved in response to HAART [7]. However, scant data are available on the rate of conversion from negative to positive TST reaction and on factors predictive of this conversion after the initiation of HAART in patients without active tuberculosis.

To address this issue, we performed a cohort study on 129 HIV-infected individuals who started HAART after a first negative TST in 1997 [8] and received a second TST during 2000. At the time of initial TST, delayed-type hypersensitivity to other recall antigens was also assessed. Baseline and follow-up data were collected from patients' charts and included: age, sex, HIV transmission category, diagnosis of AIDS-defining illnesses, CD4 lymphocyte count, plasma HIV-RNA level, and information on antiretroviral therapy. Differences in categorical variables were analysed with the use of the χ^2 test or Fisher's exact test, as appropriate. Continuous variables were compared by using the non-parametric Mann–Whitney U test. Logistic regression analysis was used to identify determinants of conversion to a positive TST.

The median age of the 129 patients included in the study was 39 years, 94 patients (73%) were men and 73 (57%) were injecting drug users. The median CD4 lymphocyte count was 295 × 10^6/l (range 5–963) at baseline and 461 × 10^6/l (range 54–1310) at follow-up. The median time between the initial and follow-up TST was 36.6 months (range 29.0–43.4), and the median duration of HAART was 31.3 months (range 13.9–42.5). Ninety-six patients (74%) received an antiretroviral regimen containing a protease inhibitor, whereas 33 patients (26%) were treated with a regimen including a non-nucleoside reverse transcriptase inhibitor. There was at least one change in antiretroviral regimen in 79 patients (61%). During follow-up there were no mycobacterial diseases among patients enrolled.

Overall, seven patients (5.4%) converted to a positive TST at follow-up. There were no significant differ-

Table 1. Demographic and clinical features of the patients and response to therapy.

Feature	Group 1	Group 2
Total no. of patients	25	20
Mean (SD) age (years)	34 (11)	37 (8)
Sex (M/F)	23/2	15/5
Risk group		
IVDU	13	7
Homosexual	7	7
Heterosexual	4	6
Transfusion	1	0
Previous AIDS	19 (76%)*	10 (50%)*
Haemoglobin (g/l) (mean SD)	101.6 (21.3)	107.2 (19.7)
Lymphocytes ($\times 10^6$/l) (mean SD)	898.9 (711.8)	1071.2 (1014.5)
CD4 lymphocytes ($\times 10^6$/l) (mean SD)	137.7 (120.9)[a]	194.7 (150.6)[a]
Serum proteins (g/l) (mean SD)	70.6 (12.0)	73.4 (8.5)
Albumin (g/l) (mean SD)	32.6 (7.5)	31.8 (7.7)
LDH (U/l) (mean SD)	398.9 (194.8)	327.1 (311.2)
Histological subtype**		
Nodular sclerosis	2	11
Mixed cellularity	6	4
Lymphocyte depletion	16	3
Unclassified	1	2
Stage		
I–II	4	4
III–IV	21 (84%)	16 (80%)
B symptoms	20	15
Extra lymph node disease	18 (75%)	11 (55%)
Bone marrow	12	6
International prognostic score[b]		
$\leqslant 3$	10	9
> 3	11	7
HIV viral load at HD diagnosis[c] ($\times 10^3$ copies/ml) (mean SD)	132 (188)	40 (106)
Patients treated	24	20
Type of treatment***		
No therapy	1	0
Radiotiotherapy	1	0
COPP	15	1
COPP/ABV	5	7
ABVD	2	12
ABVD plus radiotherapy	1	0
Evaluable for response[d]	28	16
CR†	12	12
NR	11	3
TD	5	1
Recurrences	5	0
Death in CR	2	1
DFS (median months)	11.2	NA
DFS at 5 years††	23%	87%
OS (median months)	10.9	NA
OS at 5 years†††	13%	83%

ABVD, Adriamycin–bleomycin–vinblastine–dacarbazine; COPP, cyclophosphamide–vincristine–procarbazine–prednisone; CR, complete response; DFS, disease-free survival; IVDU, intravenous drug users; LDH, lymphocyte depletion histology; NA, not achieved; NR, no response; OS, overall survival; TD, toxic death.

[a]Performed in 15 patients from group 1 and in 19 patients from group 2.

[b]Only for patients in stages III and IV.

[c]Performed in two patients from group 1 and 16 patients from group 2.

[d]For response comparisons patients who received highly active antiretroviral therapy after chemotherapy (n = 4) were included in group 1.

*$P = 0.02$, chi-square test.

**Global chi-square test 17.3, $P = 0.001$. Lymphocyte depletion: chi-square 11.9, $P = 0.001$.

***Global chi-square: 22.5, $P = 0.0001$. Chi-square for ABVD: 10.4, $P = 0.001$.

†$P = 0.02$.

††$P = 0.002$.

†††$P = 0.001$.

ences in the time elapsed between the initial and follow-up TST, in the duration of HAART and in the median CD4 lymphocyte count at baseline between patients converting to a positive TST and those remaining negative. In contrast, patients converting to a positive TST had a significantly higher median CD4 lymphocyte count at follow-up (592 versus 452×10^6/l; $P = 0.029$).

Table 1 summarizes TST conversions by the characteristics of the patients. There was no TST conversion among patients who had an increase in CD4 T lymphocyte count less than 100×10^6/l over baseline; one TST conversion (4.3%) among 23 patients whose increase was between 100 and 200×10^6/l, whereas there were six TST conversions (11.5%) among the 52 patients who had an increase in CD4 T lymphocyte count greater than 200×10^6/l ($P = 0.011$ versus patients with increases $< 100 \times 10^6$/l). In multivariate analysis (Table 1), an increase in CD4 T lymphocyte count greater than 200×10^6/l remained significantly associated with TST conversion.

The overall rate of conversion to a positive TST observed in this study is higher than that observed in previous studies conducted in industrialized countries before HAART came into widespread use [9,10] (E. Girardi, unpublished data). Moreover, all but one of the TST conversions were observed in patients who had an increase in CD4 T lymphocyte count greater than 200×10^6/l after HAART. These findings suggest that in our cohort conversion to a positive TST may be attributable to HAART-induced immune reconstitution. Previous studies have shown that HAART-treated patients frequently develop specific T cell reactivity and delayed-type hypersensitivity response to antigens that may be commonly encountered or may be present in the organism in a latent form (such as cytomegalovirus or mycobacterial antigens) [4]. It is therefore possible that our patients already had a latent tuberculosis infection at the time of first TST, and that HAART had restored their ability to mount a delayed-type hypersensitivity response to purified protein derivative. On the other hand, it has been shown that HAART can also induce a delayed-type hypersensitivity response to neoantigens administered after the initiation of antiretroviral therapy [11]. It is therefore also possible that some patients in our study acquired latent tuberculosis infection while on HAART, and those who had an efficient immune restoration were capable of mounting an immune response.

Table 1. Tuberculin skin test conversions by characteristics of 129 HIV-infected patients.

Variable	No. TST conversions/ total (%)	AOR (95% CI)[a]
Age range (years)		
> 39	4/60 (6.7)	ref
$\leqslant 39$	3/69 (4.3)	0.8 (0.1–5.3)
Sex		
Male	6/94 (6.4)	ref
Female	1/35 (2.9)	0.3 (0.1–3.6)
Injecting drug use		
No	1/56 (1.8)	ref
Yes	6/73 (8.2)	8.7 (0.8–9–94.1)
Positive delayed-type hypersensitivity testing at baseline		
Yes	1/48 (2.1)	ref
No	6/81 (7.4)	5.2 (0.4–65.9)
AIDS-defining illnesses before follow-up		
Yes	1/36 (2.8)	ref
No	6/93 (6.5)	3.4 (0.3–38.8)
Plasma HIV RNA < 80 copies/ml at follow-up		
No	2/70 (2.8)	ref
Yes	5/59 (8.5)	4.5 (0.6–31.8)
Increase in CD4 T lymphocyte over baseline		
$< 100 \times 10^6$/l	0/54 (0)	
$100–199 \times 10^6$/l	1/23 (4.3)	ref
$< 200 \times 10^6$/l	6/52 (11.5)[b]	10.7 (1.1–104.9)[c]

[a]Adjusted odds ratios (AOR) and their 95% confidence intervals (CI) were estimated in a logistic regression model including all the variables listed in the table and the time elapsed between initial and follow-up tuberculin skin test (TST).
[b]$P = 0.011$ versus increase $< 100 \times 10^6$/l.
[c]$P = 0.042$.

This study has a series of limitations. The small sample size of the study precluded a precise estimation of the rate of TST conversion and the study of the association with TST conversion of other potentially relevant determinants, such as a decrease in HIV viraemia [4]. Moreover, the study was performed in a single clinical centre and the observed cuticonversion rate may well be different in settings with a different prevalence or incidence of tuberculosis infection. Finally, our results cannot be entirely extrapolated to patients starting therapy at a very advanced stage of disease for whom immune recovery may frequently be incomplete even in presence of a marked increase in CD4 T lymphocyte count [12].

The risk of developing active tuberculosis is clearly reduced for HIV-infected patients taking HAART [13]. However, HIV-associated tuberculosis continues to occur in the context of the wide use of HAART, also among patients who have an immunological response to antiretroviral therapy [14]. Our results show that TST should be repeated in TST-negative HIV-infected individuals who experience an immunological response to HAART, in order to identify those at high risk of developing tuberculosis who would benefit from the treatment of latent tuberculosis infection.

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Plasmablastic lymphoma of the oral cavity and jaws

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Plasmablastic lymphoma is a recently described neoplasm arising in the oral cavity and jaws of HIV-infected patients, frequently related to Epstein–Barr virus, with a morphology and immunophenotype that can cause diagnostic difficulties to the pathologist. Although initially localized, it usually behaves aggressively. The use of highly active antiretroviral therapy in combination with chemo- and radiotherapy may provide a way of improving its prognosis.

Infection with HIV increases the risk of developing non-Hodgkin's lymphomas (NHL), the majority of which fall into the categories of Burkitt's and large B cell lymphomas (centroblastic or immunoblastic), and display a marked propensity to involve extranodal sites. Approximately 5–10% of HIV-infected patients will develop NHL.

It remains unclear whether the use of highly active antiretroviral therapy (HAART) will lead to a reduced incidence of systemic lymphomas, unlike primary brain lymphoma. An improved prognosis for NHL has been observed in the HAART era, suggesting a direct or indirect role of antiretroviral therapy against NHL [1]. A better response to chemotherapy and longer survival have also been observed in patients treated with HAART [2].

Lymphomas arising in HIV-positive patients can be classified [3] into those types also occurring in immunocompetent patients, such as Burkitt's lymphoma, those also occurring in other immunodeficient states (post-transplant-associated lymphoproliferative disease-like) and those more specifically seen in HIV-positive patients, that is, plasmablastic lymphoma (PL) and primary effusion lymphoma.

In 1997, Delecluse *et al.* [4] reported a series of 16 primary oral cavity and jaw-located large B cell lymphomas with a high proliferative index, and termed them 'plasmablastic lymphoma' (PL); 15 out of 16 patients were HIV positive. Since then there have been some reports of similar neoplasms arising in the same site [5,6] as well as in other organs such as the stomach [7] and lung [8].

PL typically involves the oral cavity and jaws (characteristically the gingiva and palate), and probably arises from a post-germinal centre B cell, related to Epstein–Barr virus (EBV) but not to human herpesvirus 8, as opposed to primary effusion lymphoma. The solid appearance of this tumour and its frequent areactivity with common lymphoid markers (LCA, CD20 or CD79α) can make it difficult to diagnose histopathologically. An immunohistochemical panel, including plasma cell antigens (such as CD138 and VS38c) and light chains as well as molecular studies, may be necessary to reach a diagnosis. Although initially being

limited to the mouth, it usually behaves aggressively, most patients dying within 2 years of the initial presentation. It is possible that, similar to other lymphomas, the use of HAART in combination with chemotherapy and radiotherapy may improve its prognosis.

A 36-year-old man, a drug addict, HIV and hepatitis C co-infected since 1988 and without medical follow-up, presented at our institution in July 2001 with a painful tumoral yellow mass with red wine margins in the hard palate, 4 cm in diameter. There was no history of fever nor systemic symptoms. A cervical body scan disclosed a large mass on the right maxillary sinus that infiltrated and destroyed the anterior and medial walls of the maxillary sinus, the right maxillary bone's alveolar border and part of the body of the sphenoid bone, infiltrating the lateral pterygoid muscle, the anterior pharyngeal pillar and the infratemporal fossa. A computed tomography scan of the chest and abdomen showed no abnormalities, and no enlarged lymph nodes were observed. A complete blood count revealed a haemoglobin level of 11.4 g/dl and a white blood cell count of 3700 (49.9% polynuclears, 41.1% lymphocytes), and a platelet count of 221 000/mm^3. β2-Microglobulin was 3.1 mg/l, alkaline phosphatase 321 U/mm^3, CD4 cell count 95 cells/μl and a viral load of 38 000 copies/ml.

A biopsy was performed, which revealed, under an ulcerated mucosa, a solid and diffuse cellular neoplasm with a focal starry-sky pattern. Neoplastic cells were large, with a central or eccentric nuclei, a single central nucleolus or several peripheral smaller nucleoli, abundant basophilic cytoplasm, and a high mitotic index (Fig. 1). An immunohistochemical study was performed, which showed no reactivity with either mesenchymal, epithelial (cytokeratins and epithelial membrane antigen) nor usual lymphoid markers (CD20, CD3, CD79α, CD43, CD30); just a focal staining pattern with leukocyte common antigen could be seen. A subsequent immunohistochemical study showed a positive cytoplasmic staining for lambda chains. The immunoglobulin heavy chain gene rearrangement was studied using specific primers, yielding a single amplification product, indicative of a clonal population. A hybridization study revealed most tumour cells expressing EBV encoded RNA. A diagnosis of PL was made. A subsequent bone marrow biopsy showed no medullar occupation by lymphoma cells.

Plasmablasts can be seen in several viral lymphadenopathies, e.g. caused by rubella virus or EBV (infectious mononucleosis). Also reported in HIV-infected patients, a plasmablastic variant of multicentric Castleman disease has been described as well as a lymphoma with plasmablastic features developing in these patients [9], all related to human herpesvirus 8.

A differential diagnosis of PB includes undifferentiated carcinoma, common diffuse large B cell lymphoma, Burkitt's lymphoma, myeloma multiple and plasmacytoma. Cellular morphology, immunohistochemical results, a high proliferative index and clinical presentation help distinguish these entities.

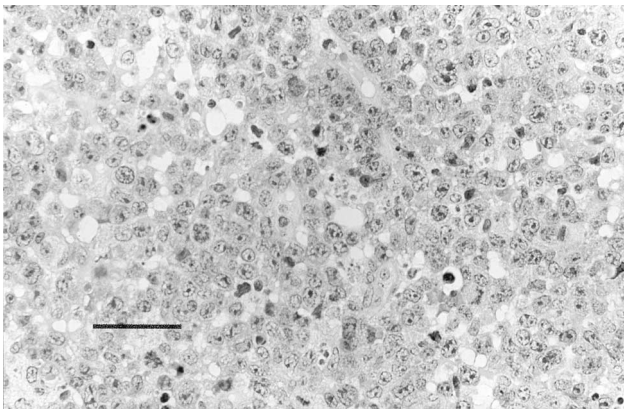

Fig. 1. Diffuse and solid neoplasm with many cells having a large central nucleolus. Frequent apoptotic cells as well as a few mitotic figures can be seen. Haematoxylin-eosin, original × 435; bar 50 micres.

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