Correspondence

AIDS 2011, 25:871–881

Rebound of plasma viremia following cessation of antiretroviral therapy despite profoundly low levels of HIV reservoir: implications for eradication

We read with great interest the article by Chun *et al.* [1]. Their paper addresses one of the great challenges in HIV treatment. The authors demonstrated that, despite starting antiretroviral therapy (ART) soon after seroconversion, having undetectable plasma RNA for more than 10 years and having undetectable level of proviral DNA still did not eradicate HIV in the peripheral blood CD4⁺ T cells. HIV replication was demonstrated *in vitro* by a high-input quantitative co-culture assay and *in vivo* by the plasma viral rebound 50 days after ART was stopped in one patient. The clear implication here is that novel and integrated approaches remain essential for the goal of HIV eradication.

There are some limitations that deserve further discussions in their paper. First, the authors did not report how antiretroviral drugs were stopped in the patient in this study. This information is critical to interpret the first plasma viral rebound. This is especially true when the antiretroviral regimen includes a nonnucleoside analogue (efavirenz) with a much longer half-life than the nucleoside backbone [2], potentially giving resistant viral quasispecies in the reservoirs a replication advantage in the setting of monotherapy with a drug that has low genetic resistance barrier for a significant amount of time. Population or clonal sequencing of the reverse transcriptase gene during viral rebound episodes would have been informative. The ethnicity of the patient is also relevant as this may further extend the half-life of efavirenz.

Second, the authors postulated but did not illustrate that the second viral rebound was due to the emergence of escape mutants. Although this was not the main study aim, data on the nature, kinetics, and fitness of immune escape quasispecies are limited [3] and provide a window of opportunity to study the adaptive immune responses during and between the viral rebound episodes of this unique patient. Understanding how the rebound viral quasispecies are clonally different from the transmitted virus, and how the adaptive immune system controls and then loses control of viremia, would provide further insights for HIV eradication research.

Finally, although the level of infectious virus was profoundly low in this patient, viral rebound could reasonably have been expected as a recent study by Carter *et al.* [4] has demonstrated that HIV infects hematopoietic progenitor cells and is capable of causing active as well as

latent infection. Perhaps, it is more interesting to characterize the kinetics of and the adaptive immune responses to viral rebound in this patient: whether a lower 'viral setpoint' is achieved because of better adaptive immune response to early ART initiation and whether the new viral setpoint achieves a 'functional cure' in such a patient. To answer these questions, the authors would need to *a priori* determine a threshold at which ART should be restarted while observing the patient off ART for a longer period of time, as compared to restarting ART reactively as in this case. Although the findings of the Strategies for Management of Antiretroviral Therapy (SMART) trial, in which patients who interrupted therapy had higher risks of HIV progression and death, are widely accepted [5], this patient with a CD4 cell count of 1060 cells/ml is clearly very different from the patients enrolled in the SMART trial whose median nadir CD4 cell count was 253 cells/ml.

The authors are to be congratulated for this important finding. Our main comment on their paper is that it did not attempt to probe deeper into the intricate interplay between the viral and immune pathogenesis of HIV persistence in this unique patient.

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Authors' reply to correspondence by Le and Farrar

We appreciate the interest shown by Drs Thuy Le and Jeremy Farrar [1] regarding our paper entitled 'Rebound of plasma viremia following cessation of antiviral therapy despite profoundly low levels of HIV reservoir: implications for eradication' [2].

The authors inquired about the antiretroviral regimen of our study patient at the time of discontinuation of antiretroviral therapy (ART). Given a relatively long half-life of efavirenz [3], the patient's drug regimen was switched 2 weeks prior to discontinuation of ART, from abacavir, lamivudine, and efavirenz to abacavir, lamivudine, and lopinavir/ritonavir. We did so in order to prevent a possible emergence of efavirenz-resistance virus and to minimize the possibility that the initial lack of detectable plasma viremia following cessation of therapy may be due to the presence of low levels of efavirenz. We agree with the authors that it would be useful to carefully examine the reverse transcriptase gene of rebounding HIV. However, it is highly unlikely that non-nucleoside reverse transcriptase-resistant virus emerged nearly 64 days after efavirenz was withdrawn from the patient's drug regimen. The ethnicity of the patient we studied is Caucasian.

Drs Le and Farrar commented that additional information regarding the nature, kinetics, and fitness of immune-escaping HIV during the second viral rebound would have been interesting. However, as the authors pointed out, the main aim of this study was to examine the relationship between the size of persistent viral reservoirs and plasma viral rebound upon discontinuation of long-term ART. We can only speculate at this point that the host immune response, such as cytotoxic CD8⁺ T cells and neutralizing antibodies, may have played a role in suppressing plasma viremia following the first viral rebound after cessation of ART [4]. It is clear that the host immune system ultimately failed to contain HIV replication after a brief period of aviremia. However, it will take a much larger cohort of long-term ART-treated infected individuals with extraordinarily low levels of viral reservoirs to better understand the interplay between re-emerging HIV following cessation of ART and the host immune responses. Such analyses could have important implications for research aimed at addressing the role of the immune response in controlling and ultimately eradicating virus in HIV-infected individuals.

Finally, Drs Le and Farrar pointed out that rebound of plasma viremia in our patient was inevitable as HIV may persist in multiple cellular and anatomical sites that may include hematopoietic progenitor cells [5,6]. In fact, we were able to detect replication-competent HIV in CD4⁺ T cells in the blood of our patient prior to discontinuation of ART. What was unique about this individual was that the sizes of his viral reservoir in blood and tissue were the lowest we had ever recorded in our laboratory. Drs Le and Farrar suggested that it would have been helpful to extend the duration of the off-ART period in order to study the kinetics of viral rebound and associated immunologic responses. Although our patient had a relatively high CD4⁺ T-cell count prior to discontinuation of ART and it is possible that his viremia could have been transiently controlled following the second viral rebound, previous studies involving interruption of ART have demonstrated that immunologic control of plasma viremia ultimately fails [7] and does not clinically benefit infected individuals [8]. Furthermore, the decision to reinitiate ART was ultimately up to the patient and his primary care physician.

Identification of infected individuals with unique profiles, including initiation of ART during the early/acute phase of HIV infection, receiving therapy for extended periods of time, and carrying extraordinarily low levels of replication-competent virus, remains a major challenge to the field. We plan to continue examining such infected individuals and to conduct systematic analyses, such as those recommended by the authors in their correspondence, in order to better understand the pathogenesis of HIV disease and to possibly identify individuals who may have achieved a 'functional cure' [9].

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Vitamin D deficiency in HIV-infected patients: associated with non-nucleoside reverse transcriptase inhibitor or efavirenz use?

We read with interest recent articles by Mueller *et al.* [1] and Welz *et al.* [2] on the prevalence and factors associated with vitamin D deficiency in HIV-infected patients. As far as exposure to specific antiretroviral drugs are considered, Mueller *et al.* [1] demonstrated that non-nucleoside reverse transcriptase inhibitor (NNRTI) use is associated with 25-hydroxy-vitamin D [25(OH)D] deficiency. Welz *et al.* [2] demonstrated that the current use of efavirenz but not nevirapine is associated with severe 25(OH)D deficiency. Although the number of studies stating that NNRTI use is associated with vitamin D deficiency is growing it is unclear whether this association is related to NNRTI class or to efavirenz use [3,4].

We assessed factors associated with vitamin D deficiency in HIV-infected patients in our Tourcoing Clinical Cohort [5] in Northern France and more specifically studied the impact of NNRTI use on 25(OH)D level. HIV-infected patients included in this study were those followed by three physicians of the clinic in whom 25(OH)D serum levels were prospectively evaluated from 1 December 2008 to 1 April 2009. Vitamin D deficiency was defined by a level of 25(OH)D below 30 nmol/l. We used univariate linear regression models to evaluate the correlation between patients characteristics and 25(OH)D levels. Moreover, a multivariate linear regression model was used to assess the correlation between NNRTI and 25(OH)D levels adjusted on variables associated with 25(OH)D level at a *P* value 0.20 or less in the univariate analysis.

Overall, 395 patients were included. The median age was 45 years [interquartile range (IQR) 40–52], 68% were men, 14% were from sub-Saharan Africa, the median

body mass index (BMI) was 24 (IQR 21–26), 10% were hepatitis C-positive and the median CD4 cell count was 575/ μ l (IQR 430–757). Three hundred and fifty-two patients (89%) were on combination antiretroviral therapy (cART) of whom 88% had a viral load less than 50 copies/ml. Among those receiving cART, 125 (35%) had an NNRTI-containing regimen of whom 58 (46.4%) on efavirenz and 58 on nevirapine (46.4%); 231 (66%) were on a protease inhibitor-containing regimen; and 117 (33%) were receiving tenofovir. The median 25(OH)D level was 38 nmol/l (IQR 20–52), and 160 patients (41%) had a 25(OH)D deficiency. In the univariate analysis, current exposure to NNRTI tend to be correlated with vitamin D deficiency (*P* = 0.06) (model 1; Table 1), but not current exposure to efavirenz (*P* = 0.17) or nevirapine (*P* = 0.20) (model 2; Table 1). However, the crude coefficient estimate for NNRTI in the linear regression model (–5.66) was close to the coefficient estimate for efavirenz (–5.30) and nevirapine (–5.04) (Table 1) in a second model with four classes (efavirenz use, nevirapine use, etravirine use versus no NNRTI use). There was a trend toward a correlation between time under tenofovir and vitamin D deficiency (*P* = 0.09). In the multivariate analysis, when adjusted on factors associated with 25(OH)D level in the univariate analysis, current exposure to NNRTI remained significantly correlated (coefficient = –5.94, *P* = 0.05) with vitamin D deficiency in a first model but not efavirenz and nevirapine in a second model. However, coefficient estimates for NNRTIs and efavirenz or nevirapine were again comparable.

In this study, performed in winter/spring season, likewise in other studies, severe vitamin D deficiency was found to

Table 1. Factors associated with vitamin D deficiency in 352 HIV-infected patients on combination antiretroviral therapy (1 December 2008 to 1 April 2009; Tourcoing clinical cohort, France).

	Univariate analysis			Multivariate analysis		
	Coefficient	95% CI	P	Coefficient	95% CI	P
Model 1						
Age	-0.20	-0.47 to 0.08	0.16	-0.22	-0.49 to 0.06	0.11
BMI	-0.60	-1.30 to 0.12	0.10	-0.59	-1.30 to 0.13	0.11
Hepatitis B status positive	9.11	-4.69 to 22.93	0.20			
Tenofovir use	3.04	-2.84 to 8.93	0.31			
Time under tenofovir (months)	0.11	-0.02 to 0.25	0.09			
Mode of HIV transmission						
Heterosexual	1		0.07			
MSM	-4.38	-9.69 to 1.81	0.13			
Intravenous drug users	-5.59	-20.42 to 9.36	0.46			
Transfused/hemophilia	16.08	-0.69 to 32.99	0.06			
PI use ^a	3.77	-2.05 to 9.60	0.20			
NNRTI use	-5.66	-11.55 to 0.22	0.06	-5.94	-11.81 to -0.07	0.05
Model 2						
Age	-0.20	-0.47 to 0.08	0.16	-0.23	-0.51 to 0.05	0.11
BMI	-0.60	-1.30 to 0.12	0.10	-0.59	-1.31 to 0.13	0.11
Hepatitis B status positive	9.11	-4.69 to 22.93	0.20			
Mode of HIV transmission						
Heterosexual	1		0.07			
MSM	-4.38	-9.69 to 1.81	0.13			
Intravenous drugs users	-5.59	-20.42 to 9.36	0.46			
Transfused/hemophilia	16.08	-0.69 to 32.99	0.06			
NNRTI use versus none						
None	1		0.36	1		0.25
Efavirenz	-5.30	-12.95 to 2.34	0.17	-6.84	-14.68 to 1.00	0.09
Nevirapine	-5.04	-12.69 to 2.61	0.20	-4.80	-12.53 to 2.93	0.22
Etravirine	-6.57	-24.24 to 11.09	0.47	-7.63	-25.32 to 10.04	0.40

BMI, Body mass index; MSM, men who have sex with men; NNRTI, non-nucleoside reverse transcriptase inhibitors; PI, protease inhibitors.

^aNot included into the model because collinear with NNRTI.

be highly prevalent [3,6]. NNRTI current use was associated with lower levels of 25(OH)D as described by Mueller *et al.* [1] but not efavirenz and nevirapine use. However, the lack of correlation between efavirenz and nevirapine use and 25(OH)D level in our study is probably related to the lack of the statistical power of our analysis. Comparable coefficient estimates of efavirenz, nevirapine, and NNRTIs in linear regression models are in favour of the NNRTI class impact on vitamin D deficiency and not only efavirenz. It has been postulated that this correlation is related to an increased catabolism of 25(OH)D through induction of CYP450, CYP4A, CYP24, and CYP 2C9 [7,8]. Both efavirenz and nevirapine have been described as acting on those cytochromes [7,8]; thus, if this is the only underlying mechanism of vitamin deficiency, it is not understood why only efavirenz and not NNRTI class is associated with vitamin deficiency.

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Do nevirapine and efavirenz affect vitamin D homeostasis similarly?

Pasquet and colleagues observed, in keeping with previous studies [1–5], an association between exposure to non-nucleoside reverse transcriptase inhibitor (NNRTI)-containing antiretroviral therapy and vitamin D deficiency in their cross-sectional cohort study [6]. Their study was insufficiently powered to look at individual NNRTI, and no significant association between vitamin D deficiency and either efavirenz or nevirapine was observed. However, comparable crude and adjusted coefficients for efavirenz and nevirapine led the authors to question whether the observed association between vitamin D deficiency and NNRTI reflects an NNRTI class effect rather than a phenomenon restricted to efavirenz as reported by ourselves [2].

An increasing body of evidence supports an association between efavirenz and 25-hydroxy-vitamin D [25(OH)D] deficiency [2,7,8]. Initiation of efavirenz appears to be consistently associated with reductions in 25(OH)D levels [5,6,9], and preliminary data suggest that efavirenz and efavirenz may have similar effects on 25(OH)D homeostasis [10]. Although we found an association between efavirenz use and severe 25(OH)D deficiency [adjusted odds ratio (aOR) 2.0 (1.5, 2.7)] as well as raised alkaline phosphatase levels [aOR 1.6 (1.02, 2.4)], nevirapine use appeared to be protective against 25(OH)D deficiency [aOR 0.6 (0.3, 1.5)] and raised alkaline phosphatase [aOR 0.5 (0.3, 0.9)] in multivariate models adjusted for sex, ethnicity, season and CD4 cell count [2], and no association between nevirapine and 25(OH)D deficiency or insufficiency was observed in the SUN cohort [7]. Whilst comparable reductions in 25(OH)D have been observed in patients initiating efavirenz and nevirapine [5], the small sample size of this study precluded inclusion of individual NNRTI in multivariate analyses. Furthermore, no significant reductions in 25(OH)D from baseline were observed in 18 patients who initiated zidovudine, lamivudine and nevirapine [11] and in 27 patients who commenced nevirapine together with ritonavir-boosted lopinavir [12]. Whereas the effects of antiretroviral treatment-associated reductions in 25(OH)D on bone remain to be defined, a trend toward lower bone mineral density (BMD) with efavirenz and higher BMD with nevirapine has been reported in HIV infected children [13].

In clinical practice, efavirenz and nevirapine are preferentially used in some patients and avoided in others; associations with either NNRTI in observational cohort studies may thus be subject to channeling bias. For example, nevirapine may be the drug of choice for premenopausal African women with low CD4 cell counts and efavirenz for white men with preserved CD4 cell counts. As black ethnicity, female sex and low nadir CD4 cell count were all

independently associated with severe vitamin D deficiency in our study [2], an inability to adjust for these factors may lead to a spurious association between nevirapine and vitamin D deficiency in cross-sectional studies. On the contrary, 61% of our patients were black, and it is possible that the effects of nevirapine on 25(OH)D homeostasis differ by ethnicity [14]. Of note, the two studies that reported a decline in 25(OH)D with nevirapine or an association between nevirapine and vitamin D deficiency predominantly included white men with preserved CD4 cell counts, did not adjust for sex, ethnicity and nadir CD4 cell count, and showed that 25(OH)D levels in black patients were minimally affected by antiretroviral treatment [5,6].

The consistent finding that vitamin D deficiency is very common in HIV-infected patients raises more important questions than which antiretroviral drug(s) may be implicated, namely what is the clinical significance of low 25(OH)D and is there any benefit from vitamin D supplementation? Vitamin D deficiency in the general population has been associated with numerous adverse health outcomes including cardiovascular disease, cancer, infection, osteopenia and fractures [15]. HIV infection is associated with an increased risk of (opportunistic) infections, cancer [16] and low BMD [17], and exposure to antiretroviral therapy with an increased risk of cardiovascular events [18] and further reductions in BMD [19]. As the greatest reductions in BMD are observed in patients who initiate antiretroviral therapy [12,20–21], the contribution of vitamin D deficiency and the potential of vitamin D supplementation to reduce this initial bone loss deserve further study. An effect of ethnicity and specific antiretrovirals, especially efavirenz and tenofovir, should be considered in the design of these studies.

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Is lower serum 25-hydroxy vitamin D associated with efavirenz or the non-nucleoside reverse transcriptase inhibitor class?

Several studies have associated non-nucleoside reverse transcriptase inhibitor (NNRTI) use with lower serum 25-hydroxy vitamin D [25(OH)D] levels [1–3]. Some investigators have attributed this effect to efavirenz (EFV) [4]. The question of whether lower 25(OH)D levels represent an NNRTI class effect or can be related to a specific drug is of clinical importance, as the population at risk may merit more rigorous monitoring and supplementation. Furthermore, more data are needed to judge whether reported statistically significant differences are clinically relevant.

In response to the study by Pasquet *et al.* [1] and in order to evaluate the specific effects of EFV and nevirapine (NVP), we performed a secondary analysis of our

longitudinal study published in a recent issue of *AIDS* [2]. Furthermore, we tried to confirm our findings in an independent, cross-sectional study.

Both analyses were performed in the context of the Swiss HIV Cohort Study with patients' and Ethical committees' approval. In the longitudinal multicenter study, we measured 25(OH)D levels either during spring and fall season immediately before and 1 year after the initiation of combined antiretroviral treatment (cART). The actual analysis included 209 patients (75% men, 88% Caucasian, median age 37 years). Data from February to April (spring) and August to October (fall) were pooled after the exclusion of season-related differences in the NNRTI effects (data not shown).

The cross-sectional study was performed between March and May 2009 in Berne and included 262 unselected, consecutive individuals attending the HIV outpatient clinic (69% men, 79% Caucasian, median age 46 years). We compared 25(OH)D values in patients treated with EFV, NVP and boosted protease inhibitors (PI/rs) and calculated rates of 25(OH)D deficiency (<30 nmol/l) and insufficiency (<75 nmol/l). For the comparison of continuous variables we used *t*-tests (paired for the longitudinal study); for categorical variables χ^2 tests. In the cross-sectional study, comparisons between treatment groups were adjusted for black ethnicity and IDU by linear regression, both of which have been associated with lower 25(OH)D values. All analyses were conducted with Stata 10 software (StataCorp LP, College Station, Texas, USA) using two-sided *P* values.

The longitudinal study provided 25(OH)D values before and 1 year after the initiation of cART (Fig. 1a). Whereas EFV was associated with a reduction of the median 25(OH)D from 46 to 34 nmol/l ($P < 0.001$), NVP and PI/r use correlated with small and nonsignificant increases. After 1 year of cART, 25(OH)D deficiency in EFV and NVP-treated patients occurred in 40.6 and 25.0%, respectively ($P = 0.4$). Consistently, EFV use was associated with an insignificantly higher proportion of 25(OH)D insufficiency (91.3 vs. 83.3%; $P = 0.3$).

An association of EFV with lower 25(OH)D levels was also seen in the cross-sectional study: compared with EFV-treated individuals, 25(OH)D levels were higher with NVP ($P = 0.04$) and PI/r ($P = 0.048$) (Fig. 1b). NVP-treated patients were more likely to have sufficient 25(OH)D levels than individuals exposed to EFV (17.9 vs. 5.4%; $P = 0.05$). Consistently, 25(OH)D deficiency was more prevalent in EFV than NVP-exposed individuals, yet without reaching statistical significance (41.3 vs. 32.1%; $P = 0.5$). Whereas treatment duration did not differ between groups ($P = 0.7$), there was an under-representation of black patients in the NVP as compared with the other groups ($P = 0.05$), and there were more IDU in the protease inhibitor as compared with the NNRTI groups ($P = 0.005$). Adjusting for these factors, 25(OH)D values remained significantly lower in the EFV than the protease inhibitor group ($P = 0.006$), but not in the EFV compared with the NVP group ($P = 0.2$).

In accordance with Welz *et al.* [5], 25(OH)D levels were consistently lower in EFV than in protease inhibitor-treated patients in both our studies. This difference could not be explained by an unequal distribution of black and IDU patients. Differences were small and unlikely to be clinically relevant. These findings are consistent with a case report that described severe 25(OH)D deficiency after starting EFV. EFV is thought to stimulate 25(OH)D catabolism through induction of cytochrome P450.

Fig. 1. Associations of efavirenz (EFV), nevirapine (NVP) and boosted protease inhibitors (PI/rs) with 25(OH) vitamin D. (a) Longitudinal multicenter analysis comparing values immediately before (white boxes) and 1 year after initiation of combined antiretroviral treatment (i.e. same season; grey boxes). (b) Cross-sectional single-center analysis. Number indicates median, box interquartile ranges.

Several enzymes of this family are involved in the anabolism (CYP27A1, CYP2R1, CYP3A4) and catabolism (CYP3A4 rather than CYP24) of 25(OH)D [6].

NVP use was not associated with lower 25(OH)D values in the longitudinal analysis, suggesting a specific effect of EFV rather than an NNRTI class phenomenon. This is in accordance with an earlier publication reporting no interaction between NVP and 25(OH)D [7]. NVP also was associated with higher 25(OH)D values than EFV in the cross-sectional analysis. After adjusting for the unequal distribution of black ethnicity and IDU among the subgroups, however, this difference lost statistical significance, which at least partially is explained by the small number of NVP-treated patients.

The somewhat higher 25(OH)D values in the longitudinal analysis are explained by the fact that 50% (vs. 0%

in the cross-sectional study) of the measurements were performed in fall after peak sun exposure.

In our analyses, EFV treatment was associated with lower 25(OH)D levels compared with both protease inhibitors and NVP. Part of the difference between the NNRTIs was due to important confounders that must rigorously be controlled for. Still, our data rather support the hypothesis of an EFV-specific effect. Considering the relevant proportion of patients with 25(OH) deficiency in all treatment groups and the postulated benefits of vitamin D, we believe that screening and correction of 25(OH)D deficiency should not be restricted to EFV-treated individuals but offered to all patients. Specific CYP polymorphisms and classical risk factors, such as black ethnicity and IDU, may define patients in whom EFV will result in clinically relevant 25(OH)D deficiency [5].

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Lenalidomide in treating AIDS-related Kaposi's sarcoma

Kaposi's sarcoma is the most common AIDS-associated malignancy [1]. Highly active antiretroviral therapy (HAART) and/or specific chemotherapy are the gold standard to manage Kaposi's sarcoma, with a response rate varying between 20% and 59% and with various adverse effects [2–4]. More efficient and well tolerated drugs are needed.

Lenalidomide is an immunomodulatory drug, thalidomide analogue, with antiangiogenic, direct antitumoral and immune stimulatory properties [5,6]. We describe three cases of HIV-associated Kaposi's sarcoma successfully treated by lenalidomide.

First, in 2005, a 28-year-old African woman presented with cutaneous exophytic lesions in the context of HIV infection. Physical examination revealed numerous purple lesions predominantly in the legs, with genital and rectal invasion (Fig. 1a). Biopsies confirmed Kaposi's sarcoma. In August 2005, CD4 cell counts were 248 cells/ μ l and HIV viral load was 10 000 copies/ml. The patient was treated by HAART (emtricitabine/tenofovir/atazanavir/ritonavir) and received pegylated liposomal doxorubicin for four

cycles with a partial response (PR) on Kaposi's sarcoma. Patient was lost to follow-up during 10 months. After recovering and according to Kaposi's sarcoma progression, six new cycles were performed with PR. In November 2007, Kaposi's sarcoma increased despite a CD4 cell counts of 283 cells/ μ l and an HIV viral load of less than 40 copies/ml. Lenalidomide was introduced at the dosage of 25 mg once daily on days 1–21 and repeated every 28 days for 24 months. The patient reported no side-effect. At 2 months, PR can be objective with complete flattening of exophytic lesions and has been maintained until now (Fig. 1b).

Second, a 50-year-old man with HIV infection since 1991 had a first diagnosis of skin and lung Kaposi's sarcoma in 1992 with a relapse in 2002. Different therapies were used without benefits: interferon, radiotherapy, daunorubicin and bleomycin. Docetaxel induced PR after 6 months of therapy, but had clinical and biological grade 3 adverse effects. Since 2008, Kaposi's sarcoma increased progressively with more than 50 skin lesions and painful lymphedema of the legs. In March 2009, CD4 cell counts were 706 cells/ μ l and HIV viral

Fig. 1. Treatment and imaging for Kaposi's sarcoma (a) Exophytic lesion of Kaposi's sarcoma of the leg before therapy. (b) Complete flattening of the exophytic lesion of the leg after one cycle of lenalidomide. (c) Before therapy, intense diffuse hypermetabolism of the leg corresponding to cutaneous lesions. (d) Total reduction of hypermetabolic abnormalities of the leg after one cycle of lenalidomide.

load was less than 40 copies/ml under HAART. PET scan showed intense diffuse metabolism of the legs (Fig. 1c). Lenalidomide was introduced according to the same schedule. After the first cycle, the patient's condition improved and a PET scan showed a total reduction of hypermetabolic abnormalities (Fig. 1d). Therapy did not result in side-effects, with the exception of asthenia. The patient underwent eight cycles of lenalidomide and we observed an 85% reduction of the Kaposi's sarcoma. Fourteen months after discontinuation of therapy, no relapse had appeared, CD4 cell counts were 589 cells/ μ l and HIV-1 viral load remained undetectable.

Third, in May 2006 a 37-year-old man was diagnosed HIV with cutaneous Kaposi's sarcoma. At this time, CD4 cell counts were 70 cells/ μ l and HIV viral load was 367 671 copies/ml. Six weeks after HAART introduction (tenofovir/emtricitabine/lopinavir/ritonavir/enfuvirtide), Kaposi's sarcoma increased with skin, genital, pulmonary

and bone lesions considered as an immune reconstitution inflammatory syndrome. Biopsies confirmed Kaposi's sarcoma. Pegylated liposomal doxorubicin (12 cycles) induced complete disappearance of visceral localization, but progression of other lesions was seen. New therapy including bleomycin/vindesine/pegylated liposomal doxorubicin was used for six cycles with no effect, followed by 10 cycles of docetaxel. Despite chemotherapy, Kaposi's sarcoma progressed with painful and exophytic mucocutaneous lesions (more than 60) and lymphedema. In January 2010, CD4 cell counts were 700 cells/ μ l and HIV viral load was less than 40 copies/ml. PET scan showed inguinal adenopathies and skin lesion in the left testicle and the lower limbs. Oral lenalidomide has been introduced for 12 months and is still ongoing. After 1 month of treatment, we observed a 75% PR on cutaneous lesions and lymphoedema and a PET scan revealed only small skin lesions with strong reduced metabolism. After 1 year, the patient showed PR

of skin lesions in size and colour and no significant side-effects have been reported.

In these three patients, Kaposi's sarcoma was successfully treated with lenalidomide after failure of HAART and several chemotherapies.

Lenalidomide has been reported in two HIV-infected patients for the treatment of hemopathies [7,8]. Thalidomide, an analogue of lenalidomide, was used in HIV and non-HIV patients with a variable efficacy on Kaposi's sarcoma (47% and 27%) [9,10]. Use of lenalidomide is easy owing to its oral course and few side-effects in our patients. Indeed, we have detected the disappearance or reduction to a minimum of 85% of skin lesions during treatment that is maintained after drug withdrawal. The PET scan appears to be an interesting method for assessing the involvement of Kaposi's sarcoma after the first month of therapy.

In conclusion, although our results are based on a small number of patients, they show that lenalidomide in HIV-associated Kaposi's sarcoma could be effective and well tolerated. Quick flattening of exophytic lesions and clearing of the PET scan may reflect the antiangiogenic effects of lenalidomide. New data on efficacy and tolerability are necessary to consider lenalidomide as a new drug for HIV-associated Kaposi's sarcoma.

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Genital tract HIV-1 RNA shedding among women with below detectable plasma viral load

We thank Dr Chaudry for her comment on our article regarding the possibility that the HIV RNA from the genital tract sample may result from cell-associated RNA and not purely from cell free RNA [1].

The author is correct that the assay we used will amplify both cell-free and cell-associated RNA but not proviral DNA. The use of the Sno-Strip filter paper, in comparison to other methods such as cervicovaginal lavage, endocervical cytobrush, endocervical swab, or aspiration, is

thought to absorb undiluted genital secretion (approximately 7 ul each) while minimizing the inclusion of cellular material. However, there may be few cells that can adhere to the Sno-Strip filter paper. In the future, it would be interesting to assess the contribution of cell-associated versus cell-free RNA from each Sno-Strip.

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