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Sequential transmission and long-term persistence of an HIV strain partially resistant to protease inhibitors

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SUMMARY

Transmission of drug-resistant HIV-1 variants has been increasingly documented. The most commonly observed resistance-associated mutations are thymidine analogue mutations as well as non-nucleoside reverse transcriptase inhibitor mutations. We report on a case of secondary transmission of a protease inhibitor (PI) primary mutation from an infected untreated subject to his sexual partner. Sequences isolated from the 2 patients showed a high level of identity (>99%), both carrying the major IAS PI mutation M46I. The latter mutation persisted in the bloodstream of the female partner 1 year after its first detection.

KEY WORDS: HIV, Resistance testing, Sequential transmission of resistance

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Selection of drug resistance mutations is the major cause of virological failure to combination antiretroviral therapy (cART) for HIV infection (Clevenbergh *et al.,* 2002). Although most mutant quasispecies do not persist in the bloodstream in the absence of selective pressure, becoming undetectable by standard genotype resistance testing (GRT) assays in patients on long-term interruptions of treatment, in some cases they can be transmitted as majority quasispecies whilst present, showing remarkable stability in the new host (Little *et al.,* 2008, Devereux *et al.,* 2000). Patients infected with HIV strains carrying resistance mutations have reduced treatment options, a higher probability of failing first line treatments and fewer probabilities of long-term suppression of HIV (<50 c/mL), as sub-optimal responses to therapy allow for viral replication under ineffective drug pressure and promote further evolution of resistance (Little *et al.,* 2002, Both *et al.,* 2007).

Surveys of naïve patients with recent seroconversion revealed prevalence rates of HIV resistance mutations very similar in the USA (8,3%) and in Europe, where 1 of 10 antiretroviral-naïve patients carried quasispecies with ≥1 drug-resistance mutations (Weinstock *et al.,* 2004, Wensing *et al.,* 2005). For this reason, genotype-resistance testing of chronically infected, antiretroviral-naïve patients, as well as resistance testing at the time of first diagnosis of HIV, are now considered cost-effective and are widely recommended, as they may well improve clinical outcomes (Saw *et al.,* 2005).

The most commonly observed reverse transcription (RT) resistance-associated mutations among naïve patients were the TAM1 mutations M41L, L210W, K219E/Q, all conferring various degrees of resistance to all NRTIs, the revertant T215D/S/A mutations, as well as the K103N mutation, leading to resistance to both Nevirapine and Efavirenz (Weinstock *et al.,* 2004). Major protease inhibitor

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(PI) resistance mutations are far more rarely reported than RT-associated mutations among naïve patients. The L90M and M46I mutations were the most frequently reported (Weinstock *et al.,* 2004), the latter being the most stable, due to their limited effect on viral fitness (Little *et al.,* 2008). For this reason, the M46I mutation may persist in the newly infected host as the dominant strain, jeopardizing the success of PI-based antiretroviral regimens (Little *et al.,* 2008).

This report describes for the first time a case of sequential sexual transmission of the M46I PI mutation, detected as the majority quasispecies in both members of a Romanian heterosexual couple, both followed at the Infectious Disease Unit of Pescara General Hospital since 2007, recently diagnosed with clade B HIV-1 infection and naïve to antiretrovirals.

Patient 1

A 20 year old Romanian male was enrolled at our Institution on 6th September 2007, due to HIV infection. He had been lately tested for HIV elsewhere due to fever, malaise, anorexia, weight loss, oral rash and generalized lymphoadenopathy. Symptoms had resolved after approximately one month with persistence of lymphoadenopathy. He reported being diagnosed with acute pre-B lymphoblastic leukemia at the age of 13 in Romania. The disease had been effectively treated with appropriate chemotherapy. At that time he reported a negative HIV test. Before and during chemotherapy he also reported multiple blood transfusions, without further HIV testing. In more recent years, he reported a few exposures to Italian heterosexual partners before engaging in a long-lasting relationship with his present Romanian partner. At presentation, CD4 T-cells were 331/mm^3, HIV viremia 75,200 copies/mL. Genotype resistance testing was performed and analyzed by the Stanford algorithm (last accessed as of July, 2008), revealing a clade B HIV sequence carrying the M46I IAS major PI mutation as well as the L63P and I93L polymorphisms in the HIV protease gene. The strain also carried the K103R polymorphism in the RT region, in the absence of any oth-

TABLE 1 - *GRTs of the HIV strains from the 2 observed patients, as interpreted by the Stanford HIV database algorithm.*

	HIV resistance mutations Patient 1 as of July, 2007	*HIV resistance mutations Patient 2 as of October, 2007*	*HIV resistance mutations Patient 2 as of August, 2008*
PI Major Resistance Mutations:	M46I	M46I	M46I
PI Minor Resistance Mutations:	None	None	None
PI Other Mutations:	K14V, L33V, N37C, R41K, R57K, I62V, L63P, I64V, E65D, I72V, I93L	K14V, L33V, N37C, R41K, R57K, I62V, L63P, I64V, E65D, I72V, I93L	K14V, L33V, N37C, R41K, R57K, I62V, L63P, I64V, E65D, I72V, I93L
NRTI Resistance Mutations:	None	None	None
NNRTI Resistance Mutations:	K103R	K103R	K103R
RT Other Mutations:	V35L, E36N, I37F S48E, W88C, D123E I178M, T200I, R211G L234H/L	V35L, E36N, I37F S48E, W88C, D123E I178M, T200I, R211G L234H/L	V35L, E36N, I37F S48E, W88C, D123E I178M, T200I, R211G

er known RT resistance mutation. Low grade resistance to Atazanavir and Nelfinavir was predicted, as well as a possible minor reduction of sensitivity to other PIs (fos-Amprenavir, Indinavir and Lopinavir, Table 1). On 27th September 2007, HAART was first started with Lopinavir, Tenofovir and Emtricitabine; 4 week viremia was 347 copies/mL, 8 week viremia was undetectable (<50 copies/mL). The patient was still on the same successful regimen as of August 2008, with an unremarkable follow-up.

Patient 2

A 20-year-old Romanian female, steady partner of patient 1 since early 2006, was first checked for HIV at patient 1's enrollment. Her medical history was unremarkable. The last negative HIV test was in September 2007. In October 2007, she reported unprotected vaginal sex with her partner on a few occasions. HIV viremia was 430 copies/mL; the p24 antigen was positive, whereas no HIV specific antibodies were detected. CD4 T-cells were 743/mm^3. GRT revealed a clade B HIV strain >99% identical to her partner's sequence at blast analysis (Los Alamos DB), with a perfectly overlapping mutation profile including the M46I PI mutation (Table 1). The patient was asymptomatic, and refused any early treatment for primary HIV infection. Her immune status remained satisfactory during follow-up until the last check in August 2008. At that time, CD4 T-cells were 800/mm^3 and HIV viremia 232 copies/mL; the patient was still out of treatment. A repeated GRT confirmed the presence of the M46I mutation as the majority quasispecies (Table 1).

Our experience highlights the sequential transmission of a mutant clade B HIV strain from a patient with likely recent sexual infection to his partner through unprotected sexual intercourse. Sequences isolated from the 2 patients displayed a very high level of identity (>99%), both carrying the PI major mutation M46I, associated with other polymorphisms not associated with drug resistance. This dominant drug resistant variant persisted in the bloodstream of patient 1 from the time of his primary infection to his first positive HIV test, that is likely for >1 year. In patient 2, its persistence as the dominant quasispecies after sexual transmission was documented on 2 separate occasions, 44 weeks apart.

A chronic remote transfusional infection in our index case (patient 1), related to the multiple blood transfusions received in Romania in 2000, cannot be conclusively excluded, as a test for HIV was not performed after transfusions until 2007. In this case, interestingly, persistence of a M46I carrying quasispecies for as long as 7 years might be postulated. This is quite unlikely, however, as blood units used in Romania are considered safe from 1990, as a consequence of strict control procedures introduced in the wake of the HIV epidemic in Romanian children between 1980 and 1989. Furthermore, sexual transmission has been demonstrated as the most frequent route of HIV transmission in Romania recent years (Kozinetz *et al.,* 2001), subtype F being the most prevalent. On the other hand, our index patient reported at risk sexual behavior in the 3 years preceding presentation, after moving to Italy, and sexual transmission of mutant HIV strains has been more frequently reported in patients with recent HIV infection (Little *et al.,* 2008, Smith *et al.,* 2007). As a consequence, a relatively recent sexual HIV infection is the most likely hypothesis to be considered for our index patient. Our report therefore provides, to our best knowledge, the first evidence of secondary transmission of an HIV strain stably carrying the M46I major IAS PI resistance mutation, associated with partial resistance to Atazanavir and Nelfinavir and minor reduction of sensitivity to other PIs.

Romano *et al.* demonstrated for the first time, by phylogenetic analysis, a case of sequential, secondary heterosexual transmission of an HIV quasispecies partially resistant to NRTIs, from a recently infected individual to his partner. GRTs from the 2 patients revealed the D67N and K219Q mutations in the RT gene, conferring resistance to Zidovudine, as well as of the V118I and T69N mutations, conferring partial resistance to other NRTIs (Romano *et al.,* 2002). Sequential transmission of an HIV strain highly resistant to antiretrovirals has been also reported by Zaccarelli *et al.* in 2007. They described heterosexual transmission from an HIV patient naïve to antiretrovirals to his partner. GRTs from both patients revealed a clade B HIV strain carrying the K103N and the L100I mutations, conferring resistance to both Efavirenz and Nevirapine, as well as partial resistance to Etravirine, and the L210W e T215D/Y mutations, reducing viral sensitivity to

Zidovudine and Stavudine. Their experience reinforces the view that some mutant strains may persist for as long as 2 years in patients never exposed to cART after infection and that these strains may be sexually transmitted (Zaccarelli *et al.,* 2008).

These experiences and ours, taken together, would also suggest that sexual exposure may play a major role in the spread of mutant HIV strains. The slower kinetics of viral reversion to wild type in the genital tract of drug-experienced patients during treatment interruptions may add to the understanding of such a durable risk of transmission in naïve patients (Ghosn *et al.,* 2004).

In conclusion, our observation documents for the first time the sequential sexual transmission of a major PI mutation to a new host, stably harboring it as her dominant quasispecies after infection. We also provide further evidence of long-term persistence of the M46I mutation in these 2 patients naïve to antiretrovirals. The need for mutational analysis of HIV strains in patients with recent HIV diagnosis as well as in patients with chronic untreated infection appears to be reinforced accordingly.

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