NNRTI-associated resistance mutations, 12 PI-associated resistance mutations [including 3 major PI resistance mutations according to the 2008 International AIDS Soceity (IAS) list] and 1 FI-associated resistance mutation. Raltegravir was associated with abacavir, lamivudine, tenofovir, low-dose ritonavir, atazanavir and darunavir at standard doses, and the GSS of the optimized regimen was 0.5 excluding raltegravir [according to the 2008 Agence Nationale de Recherche sur le SIDA (ANRS) French resistance algorithm interpretation]. Of note, no new antiretroviral agent (such as etravirine or maraviroc) was available at that time except raltegravir. Plasma HIV-1 RNA reduced to <200 copies/mL as early as day 15 and to <50 copies/mL at week 4 (W4). Plasma HIV-RNA was monitored every 8 weeks thereafter and undetectability was subsequently sustained up to W84, but plasma HIV-RNA reached 82 copies/mL at W92. This low-level replication was confirmed at W94, with a plasma HIV-RNA of 66 copies/mL. In addition, a genotypic resistance test and phenotypic determination of HIV co-receptor tropism were performed and trough plasma concentrations measured. Amplification of the integrase gene from plasma HIV-RNA and HIV co-receptor tropism determination were not successful due to the very low-level replication. We then performed a genotypic resistance test on intracellular HIV-DNA extracted from peripheral blood mononuclear cells sampled at W94 as previously described,[4,5] with successful amplification of the integrase gene, and mutation E157Q was evident. Mutation E157Q confers resistance to raltegravir according to the 2008 resistance algorithm interpretation of the French National Agency for Research on AIDS (http://www.hivfrenchresistance.org). Interestingly, this mutation at position 157 of the HIV integrase gene was not evident in a pre-salvage treatment plasma HIV-RNA sample obtained in March 2006. The patient reported excellent adherence to his antiretroviral treatment combination and trough plasma concentrations at W94 were adequate: abacavir, <10 ng/mL; lamivudine, 96 ng/mL; tenofovir, 46 ng/mL; darunavir, 3965 ng/mL; atazanavir, 760 ng/mL; ritonavir, 379 ng/mL; and raltegravir, 638 ng/mL. Intensified treatment with etravirine durably resuppressed plasma HIV-RNA at <50 copies/mL.

As the lower detection limit of HIV-RNA decreases with improvements in quantification techniques, low-level viraemia is a growing issue for physicians managing HIV-infected patients. In some cases, this low-level viraemia does not allow amplification of plasma HIV-RNA in order to perform genotypic resistance tests, making the selection of an optimized subsequent regimen difficult. Thus, amplification of intracellular HIV-DNA might then prove useful. To the best of our knowledge, this is the first report of detection of a raltegravir resistance mutation in intracellular HIV-DNA. Unlike Katlama *et al.*,[6] who recently reported that the presence of raltegravir resistance mutations was only associated with high-level viral replication, we show that raltegravir-associated mutation E157Q was rapidly selected and archived in the presence of short-term low-level viral replication. Indeed, mutation at position 157 of the integrase gene was not present in baseline plasma HIV-RNA. In addition, unlike for subtypes A, CRF-01, CRF-03 and CRF-04, this mutation has not been reported to be a natural polymorphism in HIV-1 subtype B strains.[7] Thus, given that plasma HIV-RNA was permanently <50 copies/mL up to W84 and that the first HIV-RNA value above this threshold was measured at W92, and given that mutation E157Q was evident at W94, we show that mutation E157Q was rapidly selected and archived in intracellular HIV-DNA within a short term of 8 weeks of low-level replication (assuming that HIV replication started at a midpoint between W84 and W92). Of note, emergence of mutation E157Q in plasma HIV-RNA has already been reported after 8 weeks on a failing raltegravir-containing regimen, but with a much higher level of HIV-RNA (>5 $\log_{10}$ copies/mL).[8] More data are needed to better define guidelines for patients with low-level replication upon raltegravir drug-selective pressure, in order to minimize the risk of selection and addition of resistance mutations to avoid jeopardizing future therapeutic options with next-generation integrase inhibitors.

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A multicentre cohort experience with double-boosted protease inhibitors

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Sir,

Double-boosted protease inhibitor (DBPI) therapy has been an option in HIV-infected individuals requiring salvage therapy, including those who have exhausted treatment options, harbour complex resistance mutations or have intolerance to nucleoside reverse transcriptase inhibitors (NRTIs). Studies have shown a range of virological responses[1–5] and in a retrospective clinical cohort comparing DBPI versus single PI regimens no significant differences in the relative odds of viral suppression were observed.[6]

To further investigate the virological outcome in those exposed to DBPIs, we interrogated our prospective clinical databases at three major HIV treatment centres in the UK to reveal individuals with prior exposure to DBPIs over a 10 year period from 1997 to 2007. For this analysis, if patients had received more than one DBPI combination, the most recent was recorded. Those with <4 weeks of DBPI therapy were excluded as they tended to be patients recruited into short-term pharmacokinetic studies, rather than those requiring DBPIs as HIV therapy.

A total of 167 individuals had received DBPIs for >4 weeks. Over half (55%) were exposed to DBPIs in combination with at least one nucleoside analogue. Their average age was 44 years (range 26–81), most were Caucasian ($n=117$) and most were diagnosed in the pre-HAART ('highly active antiretroviral therapy') era. The mean CD4 nadir at diagnosis measured 81 cells/mm^3 (range 0–370). The mean exposure to DBPIs was 12 months (1–33) and patients had been exposed to a mean of 8 (1–18) previous regimens, with 119 patients (71%) exposed to all three main antiretroviral classes. For each individual, a mean of 4 (0–19) resistance tests had been performed revealing that 68 patients (41%) harboured at least 5 NRTI mutations, 52 (31%) had at least 2 non-NRTI (NNRTI) mutations and 77 (46%) had 1 major PI mutation. Of the 16 DBPI combinations used, ritonavir/lopinavir/saquinavir was prescribed most frequently.

At the start of DBPI therapy, the mean CD4 count measured 278 cells/mm^3 and 49 patients (29%) had an undetectable viral load. Of all patients commencing DBPI therapy, 47 individuals (28%) were found to have undetectable viraemia at 3 months compared with 63 patients at 6 months (38%) and 51 (31%) at 48 weeks. Thus, by intention-to-treat (where missing = failure) 31% of the starting population who received DBPIs for >4 weeks achieved a viral load <50 copies/mL at 48 weeks. The mean rise in the CD4 count for this small proportion of the cohort measured 228 cells/mm^3, this value being skewed by individuals who saw a vast increase in CD4 count >1000 cells/mm^3.

While regimens that spare antiretroviral classes have potential utility,[7] these data add to the previous cohort studies and suggest that DBPI regimens may not be suitable for patients with broad antiretroviral therapy experience or extensive resistance mutations. Following the introduction of more novel agents such as integrase and entry inhibitors, second-generation PIs and NNRTIs, an undetectable viral load has become a reality for many of our patients harbouring multiple resistance. Thus, it is likely that the practice of DBPI prescription will reduce.

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