Minority K65R variants and early failure of antiretroviral therapy in HIV-1-infected Eritrean Immigrant

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The article is dedicated to the memory of Carole Fitzsimons, who was instrumental in planning this study and collecting the worms, but who died before the project was complete.

Richard P. Bendall, Maggie Barlow, Martha Betson, J. Russell Stothard, and Peter Nejsum

Author affiliations: Royal Cornwall Hospital, Truro, UK (R.P. Bendall); Health Protection Agency, St. Austell, UK (M. Barlow); Natural History Museum, London, UK (M. Betson, J.R. Stothard); Liverpool School of Tropical Medicine, Liverpool, UK (M. Betson, J.R. Stothard); and University of Copenhagen, Copenhagen, Denmark (P. Nejsum)

Address for correspondence: Richard P. Bendall, Royal Cornwall Hospital, Clinical Microbiology, Treliske, Truro TR1 3LJ, UK; email: richard.bendall@rcht.cornwall.nhs.uk

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 Minority K65R Variants and Early Failure of Antiretroviral Therapy in HIV-1–infected Eritrean Immigrant

To the Editor: Genotypic drug resistance testing before initiation of first-line antiretroviral therapy (ART) is recommended to detect drug-resistant viruses and to avoid treatment failure caused by preexisting drug-resistant viruses (1). However, standard resistance testing cannot detect drug-resistant HIV-1 minority variants unless they represent 20%–25% of the population (2). Approximately 15% of those who underwent seroconversion in the acute phase in industrialized settings harbor drug-resistant HIV-1 minority variants, while standard resistance testing did not detect drug-resistant viruses in those patients (3). We report the case of a treatment-naive HIV-1–infected patient with early treatment failure because of preexisting minority K65R-harboring HIV-1 variants.

A 32-year-old immigrant to Switzerland from Eritrea with a recently diagnosed HIV-1 subtype C infection was seen at University Hospital, Zurich. On the basis of the low CD4+ T-cell count of 69 cells/μL (15%) and high HIV-1 viral load of 980,000 copies/mL plasma, we started directly observed ART with tenofovir and emtricitabine plus nevirapine. Genotypic resistance testing showed no evidence of resistance. Within the first 4 weeks of ART, the viral load decreased to 540; however, 4 weeks later it increased to 15,000, and then 12 days later to 71,000 HIV-1 RNA copies/mL (Figure). Resistance testing at this time revealed the reverse transcriptase (RT) mutations K65R, K103N, and M184V, which confer resistance to all prescribed drugs. ART was changed to lamivudine/zidovudine, darunavir/ritonavir, and etravirine, and subsequently viremia decreased and remained undetectable.

We hypothesized that preexisting drug-resistant HIV-1 minority variants might have caused this early treatment failure. Thus, we performed clonal analysis of the RT gene before and during ART. At baseline, 17/222 clones (7.7%) carried the K65R mutation, synonymous to ≈51,000 HIV-1 RNA copies. Later, the K65R mutation was comprised in all clones. Further preexisting drug-resistance mutations have been detected in single, K65 wild-type, separate clones: K70R, V106A, and V108I. However, neither the K103N nor the M184V mutation was detected in any of those 222 clones, but both mutations were rapidly selected during early treatment failure.

Thus, the presence of the K65R mutation in a substantial fraction
of the virus population and the rapid acquisition of the K103N and M184V mutations led eventually to early treatment failure. We cannot formally rule out that K103N mutants were present before ART at a very low frequency <0.5% and that K65R/K103N double mutants were potentially selected. The M184V mutation was acquired later because only 12.5% of K65R/K103N viruses carried the M184V mutation at the first time point during treatment failure.

Because of the considerable absolute number of less replication-competent K65R-harboring viruses in the patient, we assume that this variant has been transmitted. The additional presence of isolated, nonnucleoside RT inhibitor (NNRTI)/nucleoside RT inhibitor (NRTI) resistance mutations is another indication for the transmission of drug-resistant viruses, although they and the K65R mutation were not found in the same viral genomes. Presumably, the index patient was treated with nevirapine, stavudine, and lamivudine, the commonly prescribed first-line ART in resource-limited settings.

The prevalence of K65R-harboring drug-resistant HIV-1 minority variants is not negligible in treatment-naive patients. We have shown that 2.7% of HIV-1–infected patients, mainly those infected with HIV-1 subtype B, harbor the K65R mutation as a minority variant (5), which is comparable with the prevalence of minority K65R-harboring variants (4%) in patients from South Africa who are infected with HIV-1 subtype C (6). A meta-analysis showed that limited or unavailable HIV-1 RNA monitoring in combination with ART regimens with a low genetic barrier to resistance in resource-limited settings is associated with high rates of NNRTI/NRTI resistance in patients for whom ART fails (7). Although ritonavir-boosted protease inhibitors containing ART regimens have a higher genetic barrier to resistance and would be preferable in such settings, they are generally not part of first-line therapy in resource-limited settings. Thus, proper monitoring and drug-resistance testing would be desirable when using NNRTI-based ART regimens. Moreover, it could be beneficial to apply sensitive assays for the detection of drug-resistant HIV-1 minority variants in clinical practice. However, clinical cut-off levels for those minority variants need to be defined.

Immigration from resource-limited settings is increasing, and data suggest that minority variants harboring the K65R mutation are quite prevalent in those infected with HIV-1 subtype C, who are treatment naive (6). The case described here demonstrates that these drug-resistant HIV-1 minority variants can quickly accumulate further drug-resistance mutations and lead to early treatment failure, especially in the context of an ART regimen with low genetic barriers to resistance. This case was the first among immigrants treated in our center, but, given the potential for considerable transmission rates of resistance in countries that lack virologic monitoring, this mutation could become a larger problem.

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**Figure.** Kinetics of viremia, CD4+ T-cell count, and drug resistance mutations in a treatment-naive person from Eritrea, infected with HIV-1 subtype C, who was experiencing early antiretroviral therapy (ART) failure. Viral load (circles) was measured by using the Cobas AmpliPrep TaqMan HIV-1 test version 2.0 (Roche Diagnostics, Rotkreuz, Switzerland) with a detection limit of 20 HIV-1 RNA copies/mL plasma (dotted line). CD4+ T-cell count is depicted in crosses. Genotypic resistance testing (GRT) based on population sequencing was performed at time points indicated by black arrows. Duration of different ART regimens is shown in shades of gray. Part of the HIV-1 reverse transcriptase (codons 52–218) gene was cloned and sequenced at time points before ART and during virologic failure: n = 222 clones at –2 days before ART, n = 24 clones, and n = 38 clones during virologic failure, respectively. The dynamics of the selection of the K65R, K103N, and M184V mutations are depicted by percentages and 95% confidence intervals. TDF, tenofovir; FTC, emtricitabine; NVP, nevirapine; AZT, zidovudine; 3TC, lamivudine; DRV/r, darunavir in combination with ritonavir; ETV, etravirine.
Diagnosis of Rickettsioses from Eschar Swab Samples, Algeria

To the Editor: Tick-borne rickettsioses are zoonoses caused by intracellular bacteria belonging to the spotted fever group rickettsiae (J). The main clinical signs are high fever, maculopapular rash, and an inoculation eschar at the site of the tick bite (corresponding to the portal of entry of rickettsiae into the host). Recently, several rickettsioses were diagnosed by using swab samples from skin lesions (2,3). In an animal model, as long as eschars were present, rickettsial DNA was detected (2). Our aim was to evaluate the advantage of skin swab samples for diagnosis of rickettsial diseases in a country where rickettsioses are endemic (4).

From July 2009 through October 2010, a total of 39 patients in the infectious disease department of Oran Teaching Hospital, Algeria (27 men, 12 women; median age 46.5 years) were included in a prospective study. The mean (± SD) interval between onset of lesions and consultation was 7 ± 1.8 days. Underlying conditions were present in 13 (33%) patients: diabetes (4 patients, 10.2%), hypertension (2 patients, 5%), chronic renal failure (1 patient, 2.5%), cervical cancer (1 patient, 2.5%), bronchial cancer (1 patient, 2.5%), and tobacco consumption (6 patients, 15%). Fever and generalized maculopapular rash (also on palms and soles) were found for 38 (97.4%) patients, including 5 (12.8%) with purpuric rash. One (2.5%) patient had 2 eschars on the back. Eschars were frontal (1 [2.5%] patient), cervical (3 [7%]), axillary (4 [10.2%]), on the back (6 [15%]), on the abdomen (2 [5%]), on the thorax (1 [2.5%]), inguinal (6 [15%]), on the leg (8 [20.5%]), on the arm (1 [2.5%]), on the buttock (2 [5%]), on the breast (1 [2.5%]), on the nipple (1 [2.5%]), on the penis (1 [2.5%]), and on the scrotum (2 [5%]). Conjunctivitis was reported for 24 (61.5%) patients and myalgia for 34 (87.8%). Lymphadenopathy was found near the eschar for 8 (20.5%) patients. Antimicrobial drug therapy (doxycycline 200 mg 1×d) for 3.5 ± 1.4 days was empirically prescribed for all case-patients before diagnoses were confirmed.

A dry sterile swab (Copan, Brescia, Italy) sample was collected from the inoculation eschar of each patient by the same person (N.M.). Two patients had 2 swab samples collected from the same eschar. Only 1 swab sample was collected from the patient with 2 eschars. Scabs were removed from eschars before swabbing. The swabs, while being rotated vigorously, were directed to the base of the eschar at a 50°–60° angle for 5–6 times. For 4 patients, an eschar biopsy sample was collected under sterile conditions. Swabs were then placed back in their tubes and stored at –20°C before transportation to Unité des Rickettsies, Marseille, France.

In the laboratory, each sample was placed in 2 mL of culture medium. DNA was extracted from...