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Virological and immunological response to Combivir and emergence of drug resistance mutations in a cohort of HIV-2 patients in The Gambia

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The virological and immunological responses of eight HIV-2 infected patients to zidovudine and lamivudine and the emergence of drug-resistance mutations were monitored. Most patients failed to suppress the virus to undetectable levels. Seven of the eight patients developed drug-resistance mutations; some HIV-1 resistance mutations were detected as natural polymorphisms and others were absent, suggesting that HIV-1 and 2 have both similar and different resistance pathways. For effective HIV-2 therapy, it is important to identify critical drug-resistance mutations.

The use of antiretroviral therapy (ART) has resulted in dramatic decreases in mortality in the developed world. Due to the conserved nature of the protease and reverse transcriptase (RT) enzymes, antiretroviral drugs developed for HIV-1 subtype B, were expected to have similar efficacy for all HIV viruses. However, HIV-1 group O and HIV-2 were later discovered to be naturally resistant to the non-nucleoside reverse transcriptase inhibitors (NNRTIs) [1].

Research on HIV-2 drug resistance is limited [2], due to the lower worldwide prevalence of HIV-2 and its restriction mainly to West Africa, where access to treatment was limited. However, with the Global Fund, a substantial number of HIV-2 patients will be treated and hence it is important to understand HIV-2 response to ART and the emergence of drug-resistance mutations.

We have monitored the emergence of drug-resistance mutations in HIV-2 infected patients treated with zidovudine and lamivudine. Although such treatment would now be considered sub-optimal, at the time of the study no other ART was available in The Gambia.

Between 1998 and 2001, before the advent of ART in the Gambia, eight treatment-naive HIV-2-infected patients were treated with Combivir (zidovudine + lamivudine). The Joint Gambian government–Medical Research Council Ethics Committee approved this study.

CD4 cell count measurements were done using flow cytometry (Becton-Dickinson Biosciences, Erembodegem, Belgium). Plasma HIV-2 RNA copies/ml were measured with an in-house viral load assay (100 copies/ml limit of detection), as previously described [3,4]. HIV-2 RNA was extracted from plasma [5], reverse transcribed and amplified to produce the entire HIV-2 protease and RT genes. Purified polymerase chain reaction products were directly sequenced on both strands and analysed with DNAstar software (Lasergene Software, DNAStar Inc., Madison, Wisconsin, USA). Phylogenetic analysis showed HIV-2 viruses from all patients to be subtype A. Sequences generated were assigned the accession numbers AM233873 to AM233900.

The response to therapy for each patient was computed with respect to reduction in viral load. In patients 1, 2 and 4, viral load dropped to undetectable levels. Patients 3, 5 and 7 had moderate responses of 1–2 logs drop in viral load whereas patients 6 and 8, had insignificant virologic responses (< 0.5 log drop in viral load). At the time of genotyping, all subjects had experienced viral load rebound (Fig. 1).

Analysis of the RT sequences for the presence of drug-resistance mutations showed four major HIV-1 nucleoside reverse transcriptase inhibitors (NRTI) mutations:

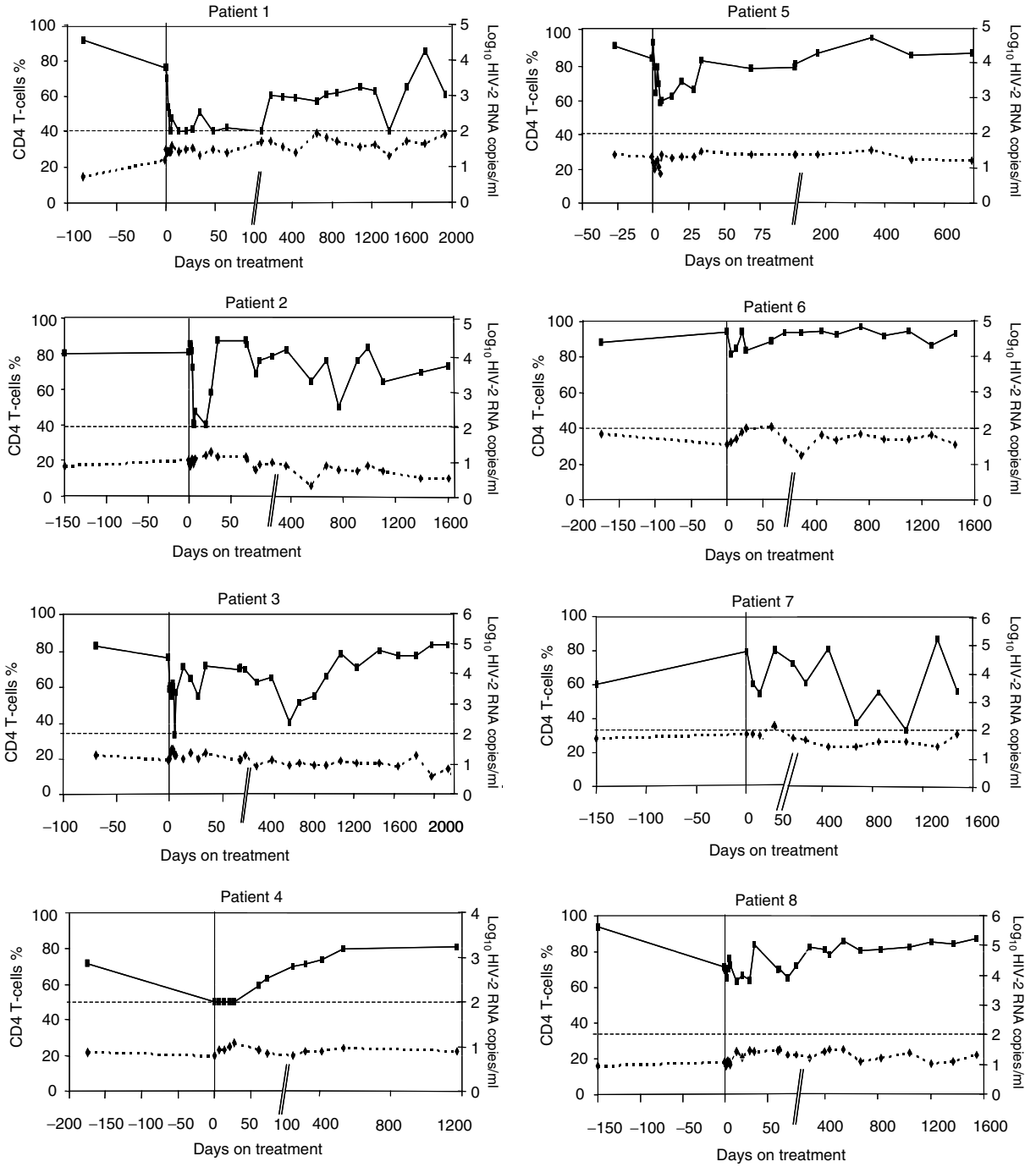

Fig. 1. Virological and immunological responses of HIV-2-infected patients treated with zidovudine and lamivudine. Time lines depicting changes in log plasma HIV-2 RNA copies/ml (continuous line with squares) and changes in percentage of CD4+ T-cells (dotted line with diamonds) over time. The horizontal dashed line at log 2 plasma HIV-2 RNA copies/ml represents the limit of detection of the viral load assay (i.e. 100 copies/ml).

K65R, Q151M, M184V and T215Y/F. The most common mutation, M184V, was found in seven of the eight patients, whereas the others were each found in one patient. Minor mutations, N69S and A62V were each found in one patient whereas V75I and K219Q/E occurred naturally in all patients.

Some commonly detected HIV-1 mutations were not observed in our study; K70R/S, which has previously been reported in HIV-2 patients [2,6,7] was absent. With the exception of S215Y and the K219Q/E polymorphism, the HIV-1 thymidine-analogue mutations, M41L, D67N, K70R, L210W, T215Y and K219Q/E, were also

absent. However, several new potential HIV-2 drug-resistance mutations including K20R, K40R, A62V, I118V, F214L and Q333L were detected.

Analysis of the protease sequences revealed several natural HIV-2 polymorphisms previously detected as drug-induced mutations in HIV-1. Several minor mutations, L10V, V32I, M36I, I47V, A71V and G73A were found in all the patients and the major mutation, M46I, was found in seven of the eight patients.

Several studies have shown that ART in HIV-1 infected individuals usually results in significant reduction in viral load [8,9], but we observed a range of responses, from good to insignificant, in our patients. Previous studies also show that HAART reduces HIV-1 viral load to undetectable levels, but in HIV-2 various HAART regimens failed to achieve this; instead, a drop of only 0.4–2 logs was observed [8,10].

The lamivudine resistance mutation, M184V, commonly observed in HIV-1-infected patients taking this drug, was the mutation most frequently observed (7/8) in this study. This mutation has been associated with phenotypic resistance to lamivudine in HIV-2 [8].

In contrast, the absence of the classic zidovudine resistance mutations suggests that HIV-2 may have a different zidovudine-resistance pathway from that observed in HIV-1, consistent with other findings [11]. Another possibility is the documented natural resistance of HIV-2 to zidovudine [12]. This study suggested that the lack of selective pressure by zidovudine on these viruses might explain the failure of zidovudine-related mutations to develop in the HIV-2 RT.

The multi-NRTI mutation, Q151M (1/8), although rare in HIV-1, has been reported to be more frequent in HIV-2 [1,8]. K65R together with A62V, N69S and M184V were observed in patient 6. This accumulation of A62V, K65R, N69S, Q151M and M184V, previously reported in HIV-2 [2] might represent a multi-NRTI mutation complex.

Apart from the M184V resistance pathway, HIV-2 may have another mechanism towards lamivudine resistance; which may explain the absence of M184V in patient 1 even after 5 years on Combivir. Even though the known HIV-1 mutations were absent, K20R, R22K, I181V, D195G, V201A and Q333L, which could represent an alternative resistance pathway, were present. In HIV-1, the G333D/E, present naturally in 50% of infected patients [13] facilitates dual resistance to both zidovudine and lamivudine [14]. Thus Q333L, also found naturally in HIV-2 subtype B and G might be the HIV-1 G333D/E equivalent and cause reduced susceptibility to zidovudine and/or lamivudine in HIV-2.

The presence of protease inhibitor mutations as natural polymorphisms may cause reduced sensitivity to some protease inhibitors in HIV-2 [15], especially M46I, which is associated with high-level resistance to indinavir in HIV-1. Therefore protease inhibitors should be selected with care in HIV-2 treatment.

We report for the first time the emergence of HIV-2 resistance mutations attributed to a single drug regimen, Combivir (zidovudine + lamivudine). We have shown that whereas HIV-2 has some equivalent HIV-1 mutations, others were either absent or pre-existed as natural polymorphisms. A phenotypic resistance assay is necessary to determine the clinical relevance of these new and previously documented mutations. It is clear from this and other studies that some antiretroviral drugs may not provide sufficient HIV-2 inhibition. Thus for effective and optimal HIV-2 treatment, the differences between HIV-1 and 2 should be taken into account in the development of new drugs.

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GB virus C genotype 1 is rarely transmitted vertically but acquired during infancy in West Africa

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Paired Ghanaian plasma and cord blood from pregnant women, alongside plasma samples from children aged 1 day to 70 months, were tested for GBV-C, HIV-1 RNA loads and anti-E2. Frequency of GBV-C vertical transmission in West Africa is significantly lower than in Europe, the USA or East Asia where genotype 2 or 3 is prevalent. While horizontal transmission appears predominant in West Africa, the lower viral load of African genotype 1 might explain limited vertical transmission.

In a previous study, prevalence and viral load of GBV-C and HIV-1 from West African populations of healthy blood donors, pregnant women and other adults were investigated [1]. Whether GBV-C was transmitted vertically as previously reported in other populations [2–10] or through other routes remained unknown. GBV-C viraemia and anti-E2 were tested in paired cord blood samples and pregnant women with or without co-existing HIV-1 infection and in young children to determine the mode of transmission and the influence of HIV infection in GBV-C natural history.

GBV-C and HIV-1 RNA were detected, confirmed and quantified according to previously described methods [1,11]. Anti-E2 was tested with the Roche kit (Roche Diagnostics, Penzberg, Germany) as described [1]. The GBV-C E2 fragment was amplified as described previously [12,13]. Genotyping was obtained by phylogenetic analysis including 19 available GBV-C references covering all five genotypes. GenBank accession numbers are: DQ430765–DQ430791.

Twenty-one of 75 HIV-1 infected (28%) and 34 of 136 HIV-1 non-infected pregnant women (25%) were GBV-C RNA positive. GBV-C and HIV-1 RNA were quantified in paired cord blood samples from 75 HIV-1 and 34 GBV-C infected pregnant women, respectively. Table 1 shows a low frequency of GBV-C vertical transmission (3.6%), and a higher frequency for HIV-1 (13.3%) ($P = 0.07$). There was no GBV-C RNA positive cord blood from HIV-1 infected mothers but two from HIV-1 non-infected mothers ($P = 0.26$) (Table 1). Median values of GBV-C RNA load in HIV-1 infected mothers and HIV-1 viral load in GBV-C infected mothers were not significantly lower than in the corresponding HIV-1 or GBV-C single-virus infected pregnant women. The viral load of two GBV-C-infected cord blood samples were 2.65E3 and 2.85E1 arbitrary units/ml (AU/ml) (Table 1). In the only pair where the viral load in the cord blood allowed sequencing, the phylogenetic clustering of the strains and 99.2% homology confirmed the transmission from mother to new born and excluded contamination. This contrasted with GBV-C vertical transmission rates ranging from 33% to 76.5% (median 60%) with the two lowest rates found in HIV-1 infected populations (42% and 50% in Italy, and 33% in Sweden) [2–4] while in other circumstances more than 50% transmission was observed [2,5–10]. The low frequency of vertical transmission observed in the Ghanaian population might be related to a relatively low viral load (1.7E0–5.6E5, Table 1 and [1]) when compared to published studies [6,8–10] indicating ranges of 1E4 to 5E8 copies [6], 10E3–10E8 and 10E3–10E7 titres [9,10], 10E5–10E8.3 genome equivalents [10]. In another study the median viral load titre was 10E4.7 ± 1.8 [5]. Considering the differences in methods and the absence of an international standard, it is difficult to make direct comparisons but in most studies viral loads appear higher