Diagnosis, Antiretroviral Therapy, and Emergence of Resistance to Antiretroviral Agents in HIV-2 Infection: a Review

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Human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2) are the causative agents of AIDS. HIV-2 is prevalent at moderate to high rates in West African countries, such as Senegal, Guinea, Gambia, and Cape Verde. Diagnosis of HIV-2 is made with a positive HIV-1/HIV-2 ELISA or simple/rapid assay, followed by one or two confirmatory tests specific for HIV-2. Following CD_4^+ T cell counts, HIV-2 viral burden and clinical signs and symptoms of immunodeficiency are beneficial in monitoring HIV-2 disease progression. Although non-nucleoside reverse transcriptase inhibitors are ineffective in treating HIV-2, nucleoside reverse transcriptase inhibitors and protease inhibitors can be effective in dual and triple antiretroviral regimens. Their use can decrease HIV-2 viral load, increase CD_4^+ T cell counts and improve AIDS-related symptoms. HIV-2 resistance to various nucleoside reverse transcriptase inhibitors and protease inhibitors, including zidovudine, lamivudine, ritonavir and indinavir, has been identified in some HIV-2 infected patients on antiretroviral therapy. The knowledge of HIV-2 peculiarities, when compared to HIV-1, is crucial to helping diagnose and guide the clinician in the choice of the initial antiretroviral regimen and for monitoring therapy success.

Key Words: HIV-2, antiretroviral treatment, review.

Human Immunodeficiency Virus Type 2 (HIV-2) and Human Immunodeficiency Virus Type 1 (HIV-1) are the causative agents of AIDS. HIV-2 was first identified in 1986 from a West African patient with AIDS, two years after the identification of HIV-1. HIV-2 is prevalent at moderate to high rates in West African countries, such as Senegal, Guinea, Gambia, and Cape Verde and is the leading cause of AIDS in Guinea-Bissau [1]. It is relatively rare outside of West Africa [2], although individual cases have been described in other parts of Africa, Europe, the Americas, and Asia, with most cases of HIV-2 infection having some epidemiological link to West Africa [3]. Recently, HIV-2 transmission in Brazil was documented in a Brazilian woman who had been sexually exposed to a man from Guinea-Bissau [4]. It is now known that at least seven phylogenetic subtypes of HIV-2 exist, including HIV-2 subtype A, predominant in Guinea-Bissau, and HIV-2 subtype B, predominant in the Ivory Coast [5-9].

HIV-2 is transmitted the same way as HIV-1, mainly through sexual contact, IV drug use, perinatally, and by contact with contaminated blood products[2]. HIV-2 appears to be less virulent than HIV-1, with a longer latent period before progression to AIDS[10], lower plasma viral loads, slower decline in CD_4^+ T cell count, lower mortality, lower heterosexual transmission, is rarely transmitted vertically, and is possibly protective against infection by HIV-1 [11-19]. The data regarding HIV-2 as a protective factor against infection by HIV-1 is being revisited as subsequent studies have shown conflicting results [20, 21]. A recent meta-analysis concluded that HIV-2 is a risk factor, not a protective factor, for HIV-1 infection [22].

A genetic comparison of HIV-1 and HIV-2 reveals a significant difference in amino acid sequences. For

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instance, the two viruses share only about 60% of the predicted amino acid sequence for the entire *pol* gene. Despite these genetic differences, the HIV-1 and HIV-2 reverse transcriptase (RT) proteins are similar in overall structure and functionality [23]. This finding has led to the suggestion that at least some of the drugs found to be effective against HIV-1 could also be effective against HIV-2 [24]. Drug classes effective against HIV-1 that may be effective against HIV-2 include protease inhibitors (PIs), nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs). In this review, the diagnosis and monitoring of HIV-2 infection are briefly discussed, followed by an in depth evaluation of the mechanism of action, *in vitro* studies and case reports for each drug class that may be useful for the treatment of HIV-2 infection.

Diagnosis of HIV-2 Infection

Most diagnostic and therapeutic strategies available to monitor and treat HIV were developed specifically for clade B of HIV-1. This poses unique therapeutic challenges for the diagnosis, monitoring, and therapy of patients infected with genetically diverse HIV viruses, including HIV-2. In 1991, the United States Food and Drug Administration licensed the first combination HIV-1/HIV-2 ELISA screening test[25]. Currently available HIV-1/HIV-2 combination tests incorporate antigens from both viruses. The World Health Organization guidelines for the diagnosis of an HIV infection recommend various strategies based on the presence of clinical signs or symptoms and prevalence of HIV infection. A positive HIV-1/HIV-2 ELISA or simple/rapid assay is followed by one or two confirmatory tests specific for either HIV-1 or HIV-2 [26]. Western blot is normally used as a confirmatory test, although combinations of simple/rapid and ELISA tests can be used with reported >99% specificity and sensitivity [27]. Guidelines for HIV-2 Western blot vary by organization. The US Center for Disease Control recommends that each Western blot test be interpreted by the criteria suggested by the kit manufacturer [25]. The World Health Organization guidelines require reactivity to at least two HIV-2 envelope antigens to be considered a positive result [28]. Other organizations require reactivity to a combination of HIV-2 *gag* (p26, gp34) and *env* (gp105) antigens.

Monitoring HIV-2 Infection

Monitoring patients with HIV-2 is more difficult than monitoring those with HIV-1. A small-scale retrospective analysis of an HIV-2 cohort showed that a proportional increase in HIV-2 viral load burden results in an increased rate of CD_4 T cell decline [29]. When equal plasma viral loads are compared between individuals infected with HIV-1 and HIV-2, both groups are found to have a similar rate of CD_4^+ T cell decline [30] . This data suggests that similar to the HIV-1 model, serial CD_4^+ T cell count and HIV-2 viral burden are useful for monitoring HIV-2 disease progression. Although there are a number of commercially available assays for the quantification of HIV-1 RNA in plasma, none are able to detect HIV-2 or group O HIV-1 [31]. In the absence of viral load monitoring, the U.S. Centers for Disease Control suggest that physicians use CD_4^+ T cell counts and other indicators of immune suppression to monitor disease progression and response to treatment.

Therapy

Although HIV-1 and HIV-2 are closely related when compared to other retroviruses, the differences that have been detected in the reverse transcriptase and protease genes result in diverse susceptibility to antiretroviral agents, particularly the non-nucleoside reverse transcriptase inhibitors. We have made a detailed review of the mechanisms of action of each class of antiretroviral agent and the mechanism of resistance, focussing on the HIV-2 strains.

Nucleoside Reverse Transcriptase Inhibitors

HIV-1 and HIV-2 reverse transcriptase (RT) enzyme regulates the formation of viral DNA prior to integration into the host cell genome. This RT catalyzes

the incorporation of deoxynucleoside triphosphate (dNTPs), forming a negative-sense DNA strand, by using HIV-positive sense RNA as a template. The RT RNase activity catalyzes the degradation of the positive-sense RNA from the negative sense DNA. This enzyme's DNA polymerase activity generates a second positive-sense DNA strand to form double-stranded proviral DNA [32]. Nucleoside reverse transcriptase inhibitors (NRTIs) structurally resemble endogenous dNTPs, inhibiting the formation of viral DNA through two mechanisms. First, they competitively inhibit dNTPs for the RT enzyme. Second, once incorporated into the HIV DNA strand, their modified 3' hydroxyl group causes chain termination of DNA synthesis.

In vitro studies of antiretroviral activity against HIV-2 show NRTIs to have inhibitory effects against wild type and mutant HIV-2 clones [33]. The effective concentrations of zidovudine (AZT), lamivudine (3TC), didanosine (ddI), zalcitabine (ddC) and stavudine (d4T) needed to inhibit wild type HIV-2 are very similar to what is effective against wild type HIV-1 [34-38]. *In vitro* data and the relative safety profile of NRTIs have made them attractive first line agents against HIV-2 infection. Based on a limited number of case reports, antiretroviral therapy against HIV-2 with dual NRTI and triple NRTI/PI-based therapy appears to decrease HIV-2 viral load, increase CD_4^+ T cell counts and improve AIDS-related symptoms [39-42].

Nucleoside Reverse Transcriptase Inhibitor Resistance

HIV-1 NRTI resistance results from base changes within the RT genome, provoking amino acid substitutions in the transcribed enzyme, which in turn confer structural changes at the enzyme active site or at associated functional areas. Each NRTI induces a predictable set of genetic alterations in a step-wise fashion. Primary mutations generally arise first, with secondary mutations developing during continued therapy [32]. Data from modified HIV-2 mutants show that HIV-2 RT resistance can be conferred by mutations at sites homologous to those for HIV-1 RT NRTI resistance. In a cell-free system, site-directed mutagenesis of HIV-2 RT amino acid residues homologous to residues in HIV-1 RT reduces the sensitivity of mutated HIV-2 to NRTIs [33]. The HIV-1 RT mutation T215Y confers resistance to zidovudine. Based on further analysis of HIV-2 mutants, it appears that the homologue S215Y mutant in HIV-2 RT confers similar resistance to zidovudine by repositioning the template-primer [43].

Contrary to data from genetically modified HIV-2 clones, clinical studies of HIV-2 antiviral resistance suggest that the pattern of HIV-2 RT mutations after exposure to NRTIs is less well defined than for HIV-1. Van der Ende et al. conducted a genotypic and phenotypic analysis of HIV-2 clones obtained from individuals before and after initiation of NRTI therapy. [41,44] HIV-2 clones obtained from two individuals who were treated with zidovudine therapy alone were 10 to 20 times less sensitive to inhibition by zidovudine then HIV-2 clones from individuals who were naive to zidovudine. One of the clones had a methionine at position 151 [44]. In HIV-1, the Q151M mutation is associated with multiple drug resistance and generally develops in individuals after more than a year of combined therapy with zidovudine and zalcitabine (ddC) or ddI.[45] No other mutations associated with resistance to zidovudine in HIV-1 infection were found. HIV-2 clones obtained from two HIV-2 infected patients after dual therapy with NRTIs (AZT + ddI; AZT + 3TC) were 10 to 45 times less sensitive to 3TC therapy. Genetic analysis of both clones showed a M184V mutation [44]. In HIV-1, this mutation is associated with strongly reduced sensitivity to 3TC in phenotypic assays [46]. Based on this latter study, it appears that some mutations associated with HIV-1 RT resistance also play a role in HIV-2 resistance. However, the genetic basis of HIV-2 NRTI resistance may not be limited to or include every region homologous to HIV-1 NRTI resistance [44].

Rodes et al. conducted genotypic analysis of 12 HIV-2 infected individuals who had been exposed to antiretroviral drugs for longer than six months [40]. Four individuals were found to carry virus genotypes with amino acid substitutions associated with NRTI resistance in HIV-1. Two patients carried the K70R

mutation, which is associated with zidovudine resistance. Two others carried the M184V mutation. Notably, these two individuals also carried the Q151M mutation. Both of these individuals had experienced a decline in CD_4^+ lymphocyte counts and high viral load values, based on measurements made with two different techniques. The first patient had been treated with ZDV and 3TC for 26 months while the other had been treated with AZT, 3TC, and indinavir (IDV) for 12 months, followed by nevirapine (NVP), saquinavir (SQV), and d4T therapy for one month. This clinical picture supports the idea that they were experiencing NRTI-based treatment failure [40]. Though these two studies have limited data the studies of Smith et al. support these findings; in their study of seven patients with HIV-2, two patients did not respond to antiviral therapy [42]. One patient failed to respond to dual NRTI therapy (AZT and ddI) and the other did not respond to dual ddC/SQV therapy, followed by sequential single-drug switches. Phenotypic resistance studies performed on viruses isolated from this patient after the start of antiretroviral therapy suggested high 3TC and SQV resistance [42].

The clinical findings from these three studies support the conclusion that the mechanism of HIV-2 RT resistance to NRTIs is similar to that of HIV-1. Amino acid changes associated with HIV-1 NRTI resistance at M184 and Q151M also seem to occur in HIV-2, and have been associated with NRTI treatment failure (Table 1). The reasons for the presence of these mutations remain unclear. The significance of other mutations in the HIV-2 RT gene after exposure to NRTI is also unknown. It is also not known if mutations in the NRTI gene accumulate in a step-wise fashion, conferring increasing resistance to NRTI therapy. Further genetic and phenotypic analysis of NRTI drug resistance in HIV-2 is needed.

Non-nucleoside Reverse Transcriptase Inhibitors (NNRTIs)

The NNRTIs are a structurally diverse group of compounds, with an aromatic structure, that bind to a hydrophobic pocket near the polymerase site of HIV-1 RT. *In vitro* studies that measure HIV-2 susceptibility to inhibition by NNRTIs have supported the conclusion that these types of drugs have no effect against HIV-2 [47]. This may be due to differences in the amino acid sequence around the two RT NNRTI binding pockets, conferring resistance to NNRTI drugs [47]. Natural resistance of HIV-2 RT to NNRTIs is thought to be conferred by a single amino acid, Leu-188. A single amino acid change at this site renders HIV-2 RT sensitive to some NNRTIs, including efavirenz and delavirdine [48]. Recent *in vitro* studies have found that the newer NNRTIs, including delavirdine, inhibit HIV-2 at effective concentrations at least 50 fold higher than those that inhibit HIV-1 [24,49]. The majority of these more powerful NNRTIs do not inhibit HIV-2 replication at sub toxic concentrations. Although NNRTIs are considered very safe, with few cytotoxic effects at concentrations effective against HIV-1, the higher concentrations needed to suppress HIV-2 make NNRTIs a questionable therapeutic choice for HIV-2 infected patients.

Protease Inhibitors (PI)

HIV-1, HIV-2 and SIV proteases belong to the aspartyl protease family. They are responsible for post-translational processing and cleavage of the polyprotein products Pr^{GAG} and $Pr^{GAG\text{-}POL}$ into functional core proteins and essential enzymes, including reverse transcriptase, integrase, and protease. Proteases are required to produce a mature retrovirus. Protease inhibitors competitively bind to the protease substrate site, resulting in the production of immature, non-infectious particles [50]. Kinetic studies of protease inhibitor binding activity against wild type HIV-2 protease show PIs to be effective against HIV-2, yet they bind with a 10 to 100 times weaker affinity to HIV-2 protease, depending on the inhibitor [51, 52]. Studies of individual PIs show nelfinavir and saquinavir to exert the same inhibitory activity against HIV-2 as against HIV-1, whereas ritonavir and indinavir are one to two orders of magnitude less inhibitory against HIV-2 [53-56]. A limited number of case reports have confirmed that antiretroviral therapy against HIV-2 with

Table 1. Mutations that appear after treatment with ART of HIV-2-infected patients who experienced clinical decline or phenotypic resistance, possibly associated with drug resistance

Antiretroviral drug	Resistance Mutation	Reference
Nucleoside analog reverse transcriptase inhibitors		
ZDV	Q151M	[40,44]
	K70R	[40]
3TC	M184V	[40,44]
Protease Inhibitors		
Ritonavir	M46I	[40]
Indinavir	M46I	[40]
	V82F	[40]

dual PI or triple NRTI/PI based therapy results in decreased HIV-2 viral load, increased CD_4^+ T cell count and an improvement in AIDS related symptoms [42]. There are no published studies that indicate whether the increased concentrations of ritonavir and indinavir needed to suppress HIV-2 in cell culture are clinically significant. Differences in the protease ligand binding mechanism may be the reason for the difference in inhibitory activity between these two retroviruses.

Mechanism of Protease Ligand Binding

HIV-1 and HIV-2 proteases have a 45% similarity in their primary protein structure. However, the protease active site residues differ at only three amino acids: 32 (Val—Ile), 47 (Ille—Val), and 82 (Val—Ile). Comparative studies of HIV-1 and HIV-2 protease crystallography structures complexed with identical synthetic protease inhibitors indicate minimal differences in the secondary and tertiary structure of these two proteases [57,58]. The largest structural differences between the HIV-1 and HIV-2 proteases are located at residues 15-20, 34-40, and 65-73, away from the substrate binding sites [58]. It has been suggested that since these regions are not near the binding site region it is unlikely that they have any direct effect on inhibitory activity [58]. However, other studies have suggested that residues outside the active site cavity can confer differences in HIV-1 and HIV-2 structural and kinetic properties [59].

Studies on HIV-1, HIV-2 and SIV protease crystal structures bound to a tripeptide analogue protease inhibitor SB203386 have provided additional information on differences in retroviral protease ligand specificity and structure. SB203386 is a potent competitive inhibitor of HIV-1 (Ki=18nM), however it shows a 2nd order magnitude decreased affinity for HIV-2 protease (Ki=1280nM) and SIV (Ki=960nM) [59]. Comparative analysis of the crystal structures of HIV-1 and HIV-2 proteases complexed with SB203386 reveals different modes of inhibitory binding. In HIV-1 protease, a single inhibitor molecule is found bound to the protease active site. The HIV-2 protease binds two inhibitor molecules, each occupying half of the active site and they bind with decreased affinity. It has been suggested that this difference in the inhibitory binding mechanism is due to differences in the active site residues of HIV-1 and HIV-2/SIV proteases. However, mutating these three residues in HIV-1 protease to their HIV-2 and SIV counterparts does not reduce the PI binding affinity to that of HIV-2 and SIV, nor does it invoke the SIV protease-like inhibitor-binding mode [59,60]. This finding has lead to the hypothesis that inhibitor specificity and mode of binding are partially conferred by residues outside the active site cavity [60]. Studies of an HIV-1 strain with HIV-2 amino acid substitutions at positions 31-37, an HIV-1 and -2 chimera protease named HIV-1 (2:31-37), suggest that decreased affinity to SB203386 is conferred by the amino acids in the 31-37 position of the HIV-2 protease region

("the 30's loop") [61]. Crystallographic analysis of the HIV-1 (2:31-37) chimera reveals that the 30's loop renders the chimera similar to HIV-2 protease, both in terms of inhibitory binding affinity and the two inhibitory molecules per protease in the dimer mode of binding [62]. Differences in ligand binding between HIV-1 and HIV-2 protease may explain kinetic differences between these two proteases. What remains unclear is if differences in the mechanism of SB203386 ligand binding are the sole explanation for differences in protease inhibitory kinetic and *in vitro* studies.

Protease Inhibitor Resistance

PI resistance to HIV-1 is not completely understood. Mutations that confer drug resistance have been identified in protease genes. Mutations of the HIV-1 protease gene that confer resistance to specific protease inhibitors include L90M induced by saquinavir, M46I and V82A/P induced by ritonavir and indinavir, and D30N induced by nelfinavir [63]. As mentioned previously, the HIV-2 protease active site differs at 82 (Val—Ile), which may confer natural HIV-2 resistance to ritonavir and indinavir. It has been suggested that the presence of the D30N mutation as a natural polymorphism in HV-2 reduces the efficacy of nelfinavir against HIV-2 [42]. A genotypic analysis of four HIV-2 individuals exposed to PI therapy demonstrated the M46I mutation in all four individuals. Minor resistance mutations, as well as new mutations not previously reported to be associated with PI resistance in HIV-1, were also found. One patient was also found to harbor the V82F mutation. This patient showed a progressive decline in CD_4^+ lymphocytes, despite being treated with indinavir (Table 1) [40]. In a case report of two patients infected with both HIV-1 and HIV-2, antiretroviral therapy successfully suppressed HIV-1, but failed to suppress HIV-2 [64]. One of the patients was being treated with ritonavir and saquinavir, while the other was treated with AZT, lamivudine and nelfinavir. No genotypic analysis results were available. The first patient never experienced a decline in HIV-2 viral load. The second patient experienced an initial decline in HIV-2 viral load, followed by a rebound to pre-antiretroviral therapy levels. The available comparative studies of HIV-1 and HIV-2 antiretroviral effectiveness have not evaluated coinfections with HIV-1 and HIV-2. Based on what is know about these two viruses, we predict that the first patient would have suppressed HIV-2, though perhaps less than for HIV-1. The lack of HIV-2 suppression when there is adequate HIV-1 response to therapy suggests preferential HIV-1 ligand binding. This possibility is supported by the findings for the second patient. The initial HIV-2 viral suppression followed by a rebound suggests the development of multi-NRTI resistance when there is an inadequate PI response, or the acquisition of PI resistance mutation(s) during the course of therapy.

Conclusions

In conclusion, many questions regarding the epidemiology, diagnosis, monitoring and treatment of HIV-2 remain unanswered. Epidemiological data must always be considered to provide insights for an early diagnosis of HIV-2 infection. The currently available data supports initiation of HIV-2 therapy with an NRTI and PI based triple antiretroviral regimen, considering the described natural resistance to NNRTI. As observed in HIV-1 infection, dual NRTI therapy alone may be associated with the development of NRTI resistance by HIV-2. Cohort studies of patients infected with HIV-2 have been extremely limited and have not addressed central questions regarding therapy for this virus, mostly due to the small number of publications and case series. No large clinical trials investigating the most effective types of antiretroviral therapies for HIV-2 are available. Therefore, clinicians treating HIV-2-infected patients should use the experience accumulated when treating HIV-1, and available HIV-2 *in vitro* susceptibility data, as well as case series, to guide therapeutic decisions. As developing counties in Africa increase the utilization of antiretroviral therapy as part of their overall HIV/AIDS prevention and treatment program and HIV-2 infection is more frequently found outside of Africa, clinical studies that test for effective treatment options are needed.

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References

1. Schim van der Loeff M.F., Aaby P. Towards a better understanding of the epidemiology of HIV-2. Aids **1999**;13:S69-84.
2. Markovitz D.M. Infection with the human immunodeficiency virus type 2. Ann Intern Med **1993**;118:211-8.
3. Grant A.D., De Cock K.M. ABC of AIDS. HIV infection and AIDS in the developing world. Bmj **2001**;322:1475-8.
4. Kallas E., Caruso S., Lopez D., et al. First identification of human immunodeficiency virus type 2 (HIV-2) transmission in Brazil. Submitted for publication [**2002**].
5. Gao F., Yue L., Robertson D.L., et al. Genetic diversity of human immunodeficiency virus type 2: evidence for distinct sequence subtypes with differences in virus biology. J Virol **1994**;68:7433-47.
6. Chen Z., Luckay A., Sodora D.L., et al. Human immunodeficiency virus type 2 (HIV-2) seroprevalence and characterization of a distinct HIV-2 genetic subtype from the natural range of simian immunodeficiency virus-infected sooty mangabeys. J Virol **1997**;71:3953-60.
7. Yamaguchi J., Devare S.G., Brennan C.A. Identification of a new HIV-2 subtype based on phylogenetic analysis of full-length genomic sequence. AIDS Res Hum Retroviruses **2000**;16:925-30.
8. Esteves A., Parreira R., Piedade J., et al. Genetic characterization of HIV type 1 and type 2 from Bissau, Guinea- Bissau (West Africa). Virus Res **2000**;68:51-61.
9. Pieniazek D., Ellenberger D., Janini L.M., et al. Predominance of human immunodeficiency virus type 2 subtype B in Abidjan, Ivory Coast. AIDS Res Hum Retroviruses **1999**;15:603-8.
10. Marlink R., Kanki P., Thior I., et al. Reduced rate of disease development after HIV-2 infection as compared to HIV-1. Science **1994**;265:1587-90.
11. Most J., Spotl L., Sarcletti M., et al. Differences in heterosexual transmission of HIV-1 and HIV-2. Lancet **1994**;344:1572-3.
12. Andreasson P.A., Dias F., Naucler A., et al. A prospective study of vertical transmission of HIV-2 in Bissau, Guinea-Bissau. Aids **1993**;7:989-93.
13. Travers K., Mboup S., Marlink R., et al. Natural protection against HIV-1 infection provided by HIV-2. Science **1995**;268:1612-5.
14. Kanki P.J., Travers K.U.S.M.B., Hsieh C.C., et al. Slower heterosexual spread of HIV-2 than HIV-1. Lancet **1994**;343:943-6.
15. Simon F., Matheron S., Tamalet C., et al. Cellular and plasma viral load in patients infected with HIV-2. Aids **1993**;7:1411-7.
16. The HIV Infection in Newborns French Collaborative Study Group. Comparison of vertical human immunodeficiency virus type 2 and human immunodeficiency virus type 1 transmission in the French prospective cohort. Pediatr Infect Dis J **1994**;13:502-6.
17. Adjorlolo-Johnson G., De Cock K.M., Ekpini E., et al. Prospective comparison of mother-to-child transmission of HIV-1 and HIV- 2 in Abidjan, Ivory Coast. Jama **1994**;272:462-6.
18. Prazuck T., Yameogo J.M., Heylinck B., et al. Mother-to-child transmission of human immunodeficiency virus type 1 and type 2 and dual infection: a cohort study in Banfora, Burkina Faso. Pediatr Infect Dis J **1995**;14:940-7.
19. Popper S.J., Sarr A.D., Travers K.U., et al. Lower human immunodeficiency virus (HIV) type 2 viral load reflects the difference in pathogenicity of HIV-1 and HIV-2. J Infect Dis **1999**;180:1116-21.
20. Norrgren H., Andersson S., Biague A.J., et al. Trends and interaction of HIV-1 and HIV-2 in Guinea-Bissau, west Africa: no protection of HIV-2 against HIV-1 infection. Aids **1999**;13:701-7.
21. van der Loeff M.F., Aaby P., Aryioshi K., et al. HIV-2 does not protect against HIV-1 infection in a rural community in Guinea-Bissau. Aids **2001**;15:2303-10.
22. Greenberg A.E. Possible protective effect of HIV-2 against incident HIV-1 infection: review of available epidemiological and *in vitro* data. Aids **2001**;15:2319-21.
23. Hizi A., Tal R., Hughes S.H. Mutational analysis of the DNA polymerase and ribonuclease H activities of human immunodeficiency virus type 2 reverse transcriptase expressed in Escherichia coli. Virology **1991**;180:339-46.
24. Witvrouw M., Pannecouque C., Van Laethem K., et al. Activity of non-nucleoside reverse transcriptase inhibitors against HIV- 2 and SIV. Aids **1999**;13:1477-83.
25. O'Brien T., George J., Epstein J., et al. Recommendations and Reports: Testing for antibodies to human immunodeficiency virus type 2 in the United States. MMWR Morb Mortal Wkly Rep **1992**;41:1-9.
26. Joint United Nations Programme on HIV/AIDS (UNAIDS)-WHO. Revised recommendations for the selection and use of HIV antibody tests. Wkly Epidemiol Rec **1997**;72:81-7.

27. Meda N., Gautier-Charpentier L., Soudre R.B., et al. Serological diagnosis of human immuno-deficiency virus in Burkina Faso: reliable, practical strategies using less expensive commercial test kits. Bull World Health Organ **1999**;77:731-9.
28. Acquired immunodeficiency syndrome (AIDS). Proposed WHO criteria for interpreting results from western blot assays for HIV-1, HIV-2, and HTLV-I/HTLV-II. Wkly Epidemiol Rec **1990**;65:281-3.
29. Ariyoshi K., Jaffar S., Alabi A.S., et al. Plasma RNA viral load predicts the rate of CD_4 T cell decline and death in HIV-2-infected patients in West Africa. Aids **2000**;14:339-44.
30. Gottlieb G.S., Sow P.S., Hawes S.E., et al. Equal Plasma Viral Loads Predict a Similar Rate of CD_4^+ T Cell Decline in Human Immunodeficiency Virus (HIV) Type 1- and HIV-2-Infected Individuals from Senegal, West Africa. J Infect Dis **2002**;185:905-14.
31. WHO, UNAIDS. Guidance module on antiretroviral treatments.
32. Loveday C. International perspectives on antiretroviral resistance. Nucleoside reverse transcriptase inhibitor resistance. J Acquir Immune Defic Syndr **2001**;26 Suppl 1:S10-24.
33. Perach M., Rubinek T., Hizi A. Resistance to nucleoside analogs of selective mutants of human immunodeficiency virus type 2 reverse transcriptase. J Virol **1995**;69:509-12.
34. Coates J.A., Cammack N., Jenkinson H.J., et al. (-)-2'-deoxy-3'-thiacytidine is a potent, highly selective inhibitor of human immunodeficiency virus type 1 and type 2 replication *in vitro*. Antimicrob Agents Chemother **1992**;36:733-9.
35. Balzarini J., Kruining J., Wedgwood O., et al. Conversion of 2',3'-dideoxyadenosine (ddA) and 2',3'-didehydro-2',3'- dideoxyadenosine (d4A) to their corresponding aryloxyphosphoramidate derivatives markedly potentiates their activity against human immunodeficiency virus and hepatitis B virus. FEBS Lett **1997**;410:324-8.
36. Balzarini J., Wedgwood O., Kruining J., et al. Anti-HIV and anti-HBV activity and resistance profile of 2',3'-dideoxy- 3'-thiacytidine (3TC) and its arylphosphoramidate derivative CF 1109. Biochem Biophys Res Commun **1996**;225:363-9.
37. Korba B.E., Milman G. A cell culture assay for compounds which inhibit hepatitis B virus replication. Antiviral Res **1991**;15:217-28.
38. Cox S.W., Aperia K., Albert J., Wahren B. Comparison of the sensitivities of primary isolates of HIV type 2 and HIV type 1 to antiviral drugs and drug combinations. AIDS Res Hum Retroviruses **1994**;10:1725-9.
39. Clark N.M., Dieng Sarr A., Sankale J.L., et al. Immunologic and virologic response of HIV-2 infection to antiretroviral therapy. Aids **1998**;12:2506-7.
40. Rodes B., Holguin A., Soriano V., et al. Emergence of drug resistance mutations in human immunodeficiency virus type 2-infected subjects undergoing antiretroviral therapy. J Clin Microbiol **2000**;38:1370-4.
41. van der Ende M.E., Schutten M., Ly T.D., et al. HIV-2 infection in 12 European residents: virus characteristics and disease progression. Aids **1996**;10:1649-55.
42. Smith N.A., Shaw T., Berry N., et al. Antiretroviral therapy for HIV-2 infected patients. J Infect **2001**;42:126-33.
43. Perach M., Rubinek T., Hughes S.H., Hizi A. Analysis of HIV-2 RT mutants provides evidence that resistance of HIV-1 RT and HIV-2 RT to nucleoside analogs involves a repositioning of the template-primer. J Mol Biol **1997**;268:648-54.
44. van der Ende M.E., Guillon C., Boers P.H., et al. Antiviral resistance of biologic HIV-2 clones obtained from individuals on nucleoside reverse transcriptase inhibitor therapy. J Acquir Immune Defic Syndr **2000**;25:11-8.
45. Shafer R.W., Kozal M.J., Winters M.A., et al. Combination therapy with zidovudine and didanosine selects for drug- resistant human immunodeficiency virus type 1 strains with unique patterns of pol gene mutations. J Infect Dis **1994**;169:722-9.
46. Schuurman R., Nijhuis M., van Leeuwen R., et al. Rapid changes in human immunodeficiency virus type 1 RNA load and appearance of drug-resistant virus populations in persons treated with lamivudine (3TC). J Infect Dis **1995**;171:1411-9.
47. Hizi A., Tal R., Shaharabany M., et al. Specific inhibition of the reverse transcriptase of human immunodeficiency virus type 1 and the chimeric enzymes of human immunodeficiency virus type 1 and type 2 by nonnucleoside inhibitors. Antimicrob Agents Chemother **1993**;37:1037-42.
48. Isaka Y., Miki S., Kawauchi S., et al. A single amino acid change at Leu-188 in the reverse transcriptase of HIV-2 and SIV renders them sensitive to non-nucleoside reverse transcriptase inhibitors. Arch Virol **2001**;146:743-55
49. Buckheit R.W., Jr., Watson K., Fliakas-Boltz V., et al. SJ-3366, a unique and highly potent nonnucleoside reverse transcriptase inhibitor of human immunodeficiency virus type 1 (HIV-1) that also inhibits HIV-2. Antimicrob Agents Chemother **2001**;45:393-400.
50. Lewis J.S., 2nd, Terriff C.M., Coulston D.R., Garrison M.W. Protease inhibitors: a therapeutic breakthrough for the treatment of patients with human immunodeficiency virus. Clin Ther **1997**;19:187-214.
51. Tomasselli A.G., Hui J.O., Sawyer T.K., et al. Specificity and inhibition of proteases from human immunodeficiency viruses 1 and 2. J Biol Chem **1990**;265:14675-83.

52. Pichova I., Weber J., Litera J., et al. Peptide inhibitors of HIV-1 and HIV-2 proteases: a comparative study. Leukemia **1997**;11 Suppl 3:120-2.
53. Martin J.A., Mobberley M.A., Redshaw S., et al. The inhibitory activity of a peptide derivative against the growth of simian immunodeficiency virus in C8166 cells. Biochem Biophys Res Commun **1991**;176:180-8.
54. Patick A.K., Mo H., Markowitz M., et al. Antiviral and resistance studies of AG1343, an orally bioavailable inhibitor of human immunodeficiency virus protease. Antimicrob Agents Chemother **1996**;40:292-7.
55. Vacca J.P., Dorsey B.D., Schleif W.A., et al. L-735,524: an orally bioavailable human immunodeficiency virus type 1 protease inhibitor. Proc Natl Acad Sci U S A **1994**;91:4096-100.
56. Kempf D.J., Marsh K.C., Denissen J.F., et al. ABT-538 is a potent inhibitor of human immunodeficiency virus protease and has high oral bioavailability in humans. Proc Natl Acad Sci U S A **1995**;92:2484-8.
57. Tong L., Pav S., Pargellis C., et al. Crystal structure of human immunodeficiency virus (HIV) type 2 protease in complex with a reduced amide inhibitor and comparison with HIV-1 protease structures. Proc Natl Acad Sci USA **1993**;90:8387-91.
58. Mulichak A.M., Hui J.O., Tomasselli A.G., et al. The crystallographic structure of the protease from human immunodeficiency virus type 2 with two synthetic peptidic transition state analog inhibitors. J Biol Chem **1993**;268:13103-9.
59. Hoog S.S., Towler E.M., Zhao B., et al. Human immunodeficiency virus protease ligand specificity conferred by residues outside of the active site cavity. Biochemistry **1996**;35:10279-86.
60. Sardana V.V., Schlabach A.J., Graham P., et al. Human immunodeficiency virus type 1 protease inhibitors: evaluation of resistance engendered by amino acid substitutions in the enzyme's substrate binding site. Biochemistry **1994**;33:2004-10.
61. Towler E.M., Thompson S.K., Tomaszek T., Debouck C. Identification of a loop outside the active site cavity of the human immunodeficiency virus proteases which confers inhibitor specificity. Biochemistry **1997**;36:5128-33.
62. Swairjo M.A., Towler E.M., Debouck C., Abdel-Meguid S.S. Structural role of the 30's loop in determining the ligand specificity of the human immunodeficiency virus protease. Biochemistry **1998**;37:10928-36.
63. Temesgen Z., Wright A.J. Antiretrovirals. Mayo Clin Proc **1999**;74:1284-301.
64. Schutten M., van der Ende M.E., Osterhaus A.D. Antiretroviral therapy in patients with dual infection with human immunodeficiency virus types 1 and 2. N Engl J Med **2000**;342:1758-60.