The use of tenofovir disoproxil fumarate for the treatment of nucleoside-resistant HIV-1

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Resistance background

Nucleoside reverse transcriptase inhibitors (NRTIs) were the first drugs introduced for the treatment of human immunodeficiency virus-1 (HIV-1) infection. Six NRTIs have been approved for use: zidovudine, stavudine, lamivudine, didanosine, abacavir and zalcitabine. HIV-1 reverse transcriptase (RT) copies the viral RNA genome into the double-stranded DNA required for integration; NRTIs are incorporated into the growing DNA chain and act as chain terminators of this process. Current antiretroviral therapy (ART) for HIV combines one or more NRTIs with a protease inhibitor (PI) and/or a non-nucleoside reverse transcriptase inhibitor (NNRTI), and can achieve high-level suppression of HIV-1 RNA in most HIV-1-infected patients. This has resulted in a dramatic decrease in AIDS mortality.1 Despite successful ART, HIV-1 resistance to NRTIs (as well as to PIs and NNRTIs) can emerge during therapy and result in treatment failure.2 The reasons for this are complex, but fundamentally the RT enzyme of HIV-1 cannot proof-read errors in deoxynucleotide incorporation, and thus mutations occur at a rate of about one base error per replication cycle.3 Some of these variant forms of HIV-1 may have reduced susceptibility to antiretroviral drugs. Thus incomplete suppression of viral replication, in the presence of antiretroviral agents, can lead to the selection of highly resistant virus.

A recent addition to the antiretroviral armamentarium is the nucleotide analogue tenofovir disoproxil fumarate (Viread), approved for use in the USA and the European Union. Tenofovir is unique among the NRTIs in that it is an acyclic nucleoside phosphonate, analogous to the monophosphate form of the other NRTIs.4 Tenofovir disoproxil fumarate is an oral prodrug of tenofovir that is rapidly converted into tenofovir upon absorption.5,6 Tenofovir has activity in vitro against both HIV-1 and HIV-2,7,8 and in resting and activated T cells, monocytes and macrophages.8,9 Cross-resistance within the NRTI class of drugs has important clinical consequences for patients who are highly treatment experienced, or for those patients in whom primary HIV infection is associated with the transmission of a resistant virus.10 In this article, we will discuss some of the features of tenofovir disoproxil fumarate that highlight its utility in the treatment of NRTI-resistant HIV-1.

Resistance to the six previously approved NRTIs has been observed both in vitro and in vivo. Each NRTI induces a relatively defined set of resistance mutations that are located in or near the substrate-binding pocket of RT. Two mechanisms of resistance have been defined: the first mechanism involves steric hindrance, in which the resistance mutation directly interferes with the binding and incorporation of the NRTI, as observed for lamivudine and its signature mutation M184V.11 The second mechanism involves ATP-mediated excision of the newly incorporated NRTI that is removed by the RT, in a reaction that is the reverse of the incorporation reaction.12 The resistance mutations [known as ‘thymidine analogue mutations’ or TAMs (M41L, D67N, K70R, L210W, T215F/Y and K219Q/E/N)] that accumulate with continuing zidovudine or stavudine exposure appear to mediate resistance via this mechanism.13 In addition to their effects on zidovudine and stavudine susceptibility, the TAMs can mediate cross-resistance to a number of other NRTIs. Cross-resistance to didanosine and zalcitabine, due to the TAMs, has been observed, even though these NRTIs do not usually select for TAMs. Cross-resistance to lamivudine, in the presence of TAMs, has also been documented in lamivudine-naive patients, despite the absence of the M184V mutation.14 Susceptibility to abacavir is also reduced in the presence of TAMs, and resistance rises notably with the addition of M184V.15 The clinical significance of these reductions in abacavir susceptibility has been confirmed in multiple clinical trials.16,17 TAMs can therefore affect susceptibility to all six NRTIs. Other mutations, such as the Q151M multidrug resistance complex and T69 insertions, although rare, cause high-level resistance to all six NRTIs.18,19

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From analyses in vitro, tenofovir appears active against a wide variety of NRTI-resistant strains, including viruses with some TAMs (D67N + K70R + T215Y), didanosine (L74V) or zalcitabine (T69D) resistance mutations.20,21 Susceptibility to tenofovir can also be enhanced by the presence of the M184V mutation induced by lamivudine.20,22 Unlike other NRTIs, tenofovir retains activity against the Q151M complex of mutations, whereas isolates carrying the T69SS insertion mutations show high-level resistance to tenofovir.23 Tenofovir can select for the K65R mutation in vitro, as can zalcitabine, didanosine and abacavir, and this mutation results in a three- to four-fold decreased susceptibility to tenofovir.15,20,24,25 However, K65R has a very low prevalence (<2%) in the antiretroviral-experienced population.26

Clinical virology analyses of tenofovir disoproxil fumarate: study 902

The clinical efficacy of tenofovir disoproxil fumarate has been shown in Phase II (GS-98-902) and III (GS-99-907) clinical trials, in highly treatment-experienced patients, and is undergoing further evaluation in treatment-naive patients. The clinical and virological results of GS-98-902 have recently been described.37,28 Study 902 was a randomized, double-blind, placebo-controlled, multicentre intensification study of tenofovir disoproxil fumarate. Patients had plasma HIV-1 RNA levels between 400 and 100,000 copies/mL, and had been administered a failing antiretroviral regimen for ≥8 weeks prior to entry. In total, 186 patients were enrolled, and assigned randomly 2:2:2:1 to add tenofovir disoproxil fumarate at one of three doses (75, 150 or 300 mg once daily), or placebo, in addition to their existing regimen. At baseline, the mean treatment experience of these patients was 4.6 years, and baseline genotyping analyses revealed that 94% of the patients had plasma HIV-1 expressing one or more primary NRTI-associated mutations. Many of the patients (57%) also expressed primary PI-associated mutations and primary NNRTI-associated resistance mutations (32%). The majority of patients (74%) had at least one TAM at positions 41, 67, 70, 210, 215 or 219, and 66% of patients had the M184V/I mutation. These high levels of resistance mutations were somewhat surprising, given that the mean baseline viral load in these patients was ~5000 copies/mL, indicating that the patients were only partially failing their therapies. Overall, among the patients adding 300 mg tenofovir disoproxil fumarate once daily to their existing failing regimen, there were significant decreases in HIV-1 RNA. These patients had an average decrease in HIV-1 RNA from week 0 to week 24 (DAVG24) of -0.58 log10 copies/mL (P < 0.001 versus placebo), which was durable through week 48 (DAVG48, -0.62 log10 copies/mL).

In a virology substudy,27 patients with HIV-1 containing TAMs, or the M184V mutation at baseline, demonstrated statistically significant reductions in HIV-1 RNA, compared with the placebo group. Patients carrying the M184V mutation showed stronger responses to tenofovir disoproxil fumarate 300 mg than patients without M184V (mean DAVG24s were -0.64 log10 and -0.35 log10 copies/mL, respectively, but subtracting the response of the placebo group negated this effect, suggesting it was not specific to tenofovir disoproxil fumarate therapy. The HIV RNA response in patients with TAMs (~0.52 log10 copies/mL) was notable as these patients had a mean of 2.8 TAMs. Statistically significant reductions in HIV-1 RNA were also observed in those patients whose HIV-1 carried the TAM that is associated with the highest level of zidovudine resistance, T215Y/F.21

Patients were monitored for the emergence of new resistance mutations during the trial. Post-baseline genotypic analyses obtained on 159 patients suggested that background therapy, and not tenofovir disoproxil fumarate, was driving the appearance of the majority of new mutations during the trial. Seventy-nine patients developed new NRTI-associated mutations by week 48, and the majority were TAMs (n = 63, 34%) in patients taking the thymidine analogues zidovudine or stavudine. Four patients (2%) developed K65R by week 48; these four patients were also receiving didanosine+ stavudine (n = 2), didanosine (n = 1) or abacavir (n = 1). It was unclear which drug drove the selection of K65R, but phenotypic analysis confirmed a decrease in susceptibility to tenofovir (2.8–3.9-fold). Phenotypic analyses were performed for all patients originally treated with tenofovir disoproxil fumarate 300 mg. For the 53 patients for whom baseline results were obtained, the mean susceptibility to tenofovir was 1.9-fold above wild-type. In contrast, mean susceptibility at baseline to zidovudine and lamivudine was 13.8-fold and greater than 24-fold above wild-type, respectively, indicating significant resistance to zidovudine and lamivudine. Patients with up to four-fold reduced susceptibility to tenofovir at baseline had reductions in HIV-1 RNA from ~0.55 to ~0.71 log10 copies/mL. Greater than a four-fold reduction in tenofovir susceptibility at baseline was rare, occurring in four patients, but these patients did not appear to respond to tenofovir disoproxil fumarate. One of these patients had K65R and the others had extensive TAMs and other mutations. Post-baseline phenotypic analyses confirmed that only development of K65R was associated with decreased susceptibility to tenofovir by 48 weeks.

Study 907

A recently completed trial in treatment-experienced patients, study GS-98-907, has confirmed the utility of tenofovir disoproxil fumarate therapy.29 This study was a randomized, double-blind, placebo-controlled intensification study similar to study 902, in which patients added tenofovir disoproxil fumarate therapy (300 mg once daily) in addition to their...
existing regimen. Patient entry criteria were HIV-1 RNA levels between 400 and 10,000 copies/mL (median HIV-1 RNA 2340 copies/mL), and stable antiretroviral therapy for at least 8 weeks previously (mean prior therapy 5.4 years). Patients were randomly assigned 2:1 into either tenofovir disoproxil fumarate (n = 368) or placebo arms (n = 182). Half of the patients were randomly assigned to a genotyping sub-study and 25% to a phenotyping substudy. As expected, most patients had HIV-1, with resistance mutations at baseline, including NRTI-associated mutations (94%), PI-associated mutations (58%) and NNRTI-associated mutations (48%). Nevertheless, patients in the tenofovir disoproxil fumarate therapy arm showed an average decline in HIV-1 RNA levels of −0.59 log_{10} copies/mL at week 24 (DAVG_{24}), compared with −0.03 log_{10} copies/mL for placebo patients (P < 0.0001 versus placebo), which was maintained at week 48 (DAVG_{48} −0.56 log_{10} copies/mL).

Analysis of responses by baseline genotype showed that patients with HIV carrying one to two TAMs, or three or more TAMs without M41L or L210W, responded similarly to those without TAMs. There was reduced susceptibility to tenofovir disoproxil fumarate in those patients with HIV-1 expressing three or more TAMs at baseline, which included M41L or L210W. As in study 902, development of new NRTI mutations was predominantly due to the background therapy, and tenofovir disoproxil fumarate did not appear to select for the development of TAMs. Over the 48 week course of the trial, eight patients out of 253 (3%) analysed developed the K65R mutation. In contrast, 79 patients (31%) developed one or more TAM while taking a thymidine analogue in their background regimen. In addition to either a PI or NNRTI, the patients who developed K65R maintained a significant reduction in response. The mechanisms leading to this reduced response in the presence of M41L or L210W is currently unknown, but is likely to involve chain terminator removal. However, given that tenofovir does not select for the appearance of new TAMs; the lack of viral load rebound when it does develop, is reduced enzymic and viral fitness of the K65R mutant. Given the low-level phenotypic changes observed for tenofovir, there is also the possibility of residual tenofovir disoproxil fumarate activity in these patients.

These studies have shown that tenofovir disoproxil fumarate does not select for the appearance of new TAMs; however, evidence was noted for a reduced response to tenofovir disoproxil fumarate therapy if three or more TAMs with mutations M41L or L210W were present at baseline. In patients whose HIV had three or more TAMs, but which did not include the mutations M41L or L210W, there was no significant reduction in response. The mechanisms leading to this reduced response in the presence of M41L or L210W is currently unknown, but is likely to involve chain terminator removal. However, given that tenofovir does not select for TAMs, it provides a rationale for using tenofovir in a ‘thymidine analogue-sparing’ regimen. A thymidine analogue-sparing regimen may be beneficial for both treatment-experienced and treatment-naive patients where use of tenofovir disoproxil fumarate will not result in the development of TAMs, thereby providing further therapy options to both patients and physicians.

Conclusions and commentary

Studies 902 and 907 demonstrated that tenofovir disoproxil fumarate is a useful treatment option for highly treatment-experienced patients. In this patient population, in which existing regimens were not providing maximal HIV-1 RNA suppression, tenofovir disoproxil fumarate demonstrated the ability to maintain long-term viral suppression in the vast majority of patients. An important observation from these studies was a high rate of development of new TAMs, due to the background therapy. This is of particular importance because it shows that in patients whose regimen is providing partial suppression new mutations can still continue to accumulate, which may subsequently lead to high-level resistance. Tenofovir disoproxil fumarate itself did not select for the TAM pathway, and there was a low incidence of the development of the K65R mutant. Interestingly, among those patients who developed K65R, there was no evidence of viral load rebound and often low-level changes in tenofovir susceptibility (two- to three-fold). The relatively low frequency of the K65R mutation in antiretroviral-treated patients suggests that HIV-1 with this mutation may have decreased fitness in vivo. Recombinant RT carrying the K65R mutation shows decreased processivity and decreased catalytic activity, and single-cycle virus replication capacity assays have also demonstrated a decreased fitness of viruses carrying the K65R mutation relative to wild-type. Thus, the simplest explanation for the low frequency of K65R development, and the lack of viral load rebound when it does develop, is reduced enzymic and viral fitness of the K65R mutant. Given the low-level phenotypic changes observed for tenofovir, there is also the possibility of residual tenofovir disoproxil fumarate activity in these patients.

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