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Resistance against Nucleoside Reverse Transcriptase Inhibitors Subtitle: Antiviral Drug resistance

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Abstract
The response to antiretroviral therapy in human immunodeficiency virus-infected patients is limited by the emergence of drug resistance. This resistance is a consequence of the high rate of HIV mutation, the high rate of viral replication (especially when potent multidrug therapies are not used or taken reliably), and the selective effect of these drugs, which favors emergence of mutations that can establish clinical drug resistance. The introduction of highly active antiretroviral therapy, which typically includes at least 2 nucleoside reverse transcriptase inhibitors and a protease inhibitor or a nonnucleoside reverse transcriptase inhibitors, for most treatment-naive patient's results in a reduction of viral load below the limit of detection determined by currently available HIV RNA assays. It is this marked reduction that results in durable viral suppression, usually only possible by the simultaneous use of 3 or 4 drugs. The reverse transcriptase inhibitors components of highly active antiretroviral therapy are crucial for these benefits of combination therapy. Specific amino acid changes are associated with resistance to several reverse transcriptase inhibitors, but new mutation complexes have been observed that can confer broad cross-resistance within this class. Genotypic and phenotypic resistance assays to measure drug resistance are being developed, but refinements in both methodology and our ability to interpret results of these assays are necessary before they are introduced into widespread clinical use.

Keywords: Human immunodeficiency virus type 1, reverse transcriptase, nucleoside reverse transcriptase inhibitors, DNA polymerization, chain termination, antiviral drug resistance, phosphorolysis, pyrophosphorolysis

Introduction
Almost fifteen years ago, the first non-nucleoside reverse transcriptase (RT) inhibitor (NNRTI) lead compounds have been discovered. Nowadays, three NNRTIs are approved for treatment of HIV-1-infected individuals and several others are subject of (advanced) clinical trials. Although the NNRTIs target HIV-1 RT, they are clearly different from the nucleoside RT inhibitors (NRTIs). They are highly selective for HIV-1 and do not inhibit HIV-2 or any other (retro) virus. They target HIV-1 RT by a direct interaction without the need to be metabolized by cellular enzymes, and they interact at a site on the HIV-1 RT that is near to, but distant from, the substrate-binding site. The majority of NNRTIs share common conformational properties and structural features that let them fit in a hydrophobic pocket at the HIV-1 RT, which is nowadays well-characterized [1]. A wide variety of crystal structures of RT complexed with NNRTIs have been obtained. They provide detailed insights in the molecular interaction of the NNRTIs with the amino acids lining the pocket in HIV-1 RT. Due to their unprecedented specificity, the NNRTIs are relatively non-toxic in cell culture, and the most potent compounds reach selectivity indices that exceed 100,000 or more. However, inherent to their high specificity, the NNRTIs easily select for mutant virus strains with several degrees of drug resistance. The first-generation NNRTIs such as nevirapine and delavirdine easily lose their inhibitory potential against mutant virus strains that contain single amino acid mutations in their RT. The second-generation NNRTIs such as efavirenz, capravirine and etravirine usually require two or more mutations in the HIV-1 RT before significantly decreasing their antiviral potency. Evidently, it requires a markedly longer time to obtain significant resistance against second-generation NNRTIs. The resistance spectrum

of NNRTIs is entirely different from the NRTI resistance spectrum, and, as a rule, NRTI-resistant mutant virus strains keep full sensitivity to the inhibitory effects of NNRTIs, and vice versa NNRTI-resistant and mutant virus strains keep full sensitivity to the inhibitory effects of NRTIs. NNRTIs have proven beneficial when included in drug combination (triple or quadruple) therapy, preferably in the presence of protease inhibitors and NRTIs [2].

Nucleoside reverse transcriptase inhibitors (NRTIs), such as 3′-azido-3′-deoxythymidine, 2′, 3′-dideoxyinosine and 2′, 3′-dideoxy-3′-thiacytidine, are effective inhibitors of human immunodeficiency type 1 (HIV-1) replication. NRTIs are deoxynucleotide triphosphate analogs, but lack a free 3′-hydroxyl group. Once NRTIs are incorporated into the nascent viral DNA, in reactions catalyzed by HIV-1 reverse transcriptase, further viral DNA synthesis is effectively terminated. NRTIs should therefore represent the ideal antiviral agent. Unfortunately, HIV-1 inevitably develops resistance to these inhibitors, and this resistance correlates with mutations in RT. To date, three phenotypic mechanisms have been identified or proposed to account for HIV-1 RT resistance to NRTIs [3]. These mechanisms include alterations of RT discrimination between NRTIs and the analogous dNTP (direct effects on NRTI binding and/or incorporation), alterations in RT-template/primer interactions, which may influence subsequent NRTI incorporation, and enhanced removal of the chain-terminating residue from the 3′ end of the primer. These different resistance phenotypes seem to correlate with different sets of mutations in RT.

This review discusses the relationship between HIV-1 drug resistance genotype and phenotype, in relation to our current knowledge of HIV-1 RT structure.

Mechanism of NRT1 Resistance

One mechanism for resistance to NRTIs is discrimination, whereby the reverse transcriptase enzyme is able to avoid binding of the NRTI, while retaining the ability to recognize the analogous natural deoxynucleotide triphosphate (dNTP) substrate. Examples include virus with the point mutations K65R, L74V, Q151M and M184V, which cause diminished affinity of RT for specific NRTIs with little or no change in affinity for the corresponding dNTP substrate. The consequence is a diminished incorporation of drugs into the DNA chain [4].

The other mechanism is the enhanced phosphorolytic removal of the chain-terminating NRTI from the 3′-terminus of the primer after it has been incorporated into the viral DNA. NRTI-associated mutations may affect the phosphorolytic activity of RT, in some cases overcoming chain termination in a mechanism called `primer unblocking'. Mutations that enhance primer unblocking activity include those selected by zidovudine (ZDV) and stavudine (d4T), and are known as thymidine analogue mutations (TAMs): M41L, D67N, K70R, L210W, T215Y/F and K219Q/E. TAMs are involved in resistance to all NRTIs, except lamivudine (3TC), but the degree of cross-resistance depends on the NRTI considered and the number of TAMs on the virus. Some interactions exist between the different mechanisms of resistance. The M184V/I mutations, selected by 3TC and emtricitabine (FTC), delay the appearance of TAMs and increase the in vitro susceptibility to ZDV and d4T [5].

Principles of Resistance to Nucleoside Reverse Transcriptase Inhibitors

The nucleoside reverse transcriptase inhibitor (NRTI) classremain a key component of the backbone of most antiretroviral regimens used in current HIV clinical practice. The medications include abacavir (*Ziagen*), didanosine (*Videx*), stavudine (*Zerit*), tenofovir (*Viread*), zidovudine (*Retrovir*), and zalcitabine (*Hivid*). The NRTI drugs exert their action by inhibiting HIV reverse transcription, the key step that generates the conversion of HIV RNA to HIV DNA [6,7]. Specifically, the NRTIs are incorporated by HIV into the elongating DNA strand, but act as chain terminators because the NRTIs lack the 3'-hydroxyl group on the deoxyribose moiety, which is present on the naturally occurring deoxynucleotides and critical for the binding of the next incoming deoxynucleotide. The reverse transcription process is generated by the enzyme reverse transcriptase, but this enzyme does not have proof-reading functionality, a property that lead to error prone DNA synthesis and increased drug-resistant mutation frequency. Drugs in the NRTI class include a heterogenous group, but all are considered competitive inhibitors (via competition with the natural deoxynucleotides). The mechanism of action and mechanisms of resistance with the NRTI class are distinct from those with the non-nucleoside reverse transcriptase inhibitors (NNRTIs). The NRTI resistance involves one of two biochemical mechanisms: (i) decreased incorporation (discriminatory) and (ii) excision (primer unblocking) [8, HYPERLINK "http://www.hivwebstudy.org/cases/antiretrovirals-resistance/resistance-nucleoside-reverse-transcriptase-inhibitors" HYPERLINK "http://www.hivwebstudy.org/cases/antiretrovirals-resistance/resistance-nucleoside-reverse-transcriptase-inhibitors"9,10,11]. The discriminatory mutations allow the reverse transcriptase enzyme to preferentially select the naturally occurring deoxynucleotides present in the cell, thereby decreasing the incorporation of the NRTI-triphosphate into the elongation HIV DNA strand. Excision mutations enhance the phosphorolytic excision of the NRTI-triphosphate that had been added to the elongation HIV RNA DNA, resulting in unblocking the primer. Examples of mutations that cause decreased NRTI incorporation include: M184I/V, K65R, L74V, and the Q151M complex (Q151M followed by the accessory mutations A62V, V75I, F77L, and F116Y). Characteristic mutations that occur via the primer unblocking pathway include the M41L, D67N, K70R, L210W, T215Y/F, and K219Q/E.

M184I/V Mutation

The M184I/V mutation is the signature mutations that develops with resistance to the medications lamivudine (*Epivir*) and emtricitabine (*Emtriva*). The M184I mutation typically develops first and usually is rapidly replaced by the M184V, primarily because the M184I mutation causes a greater impairment in viral fitness than does the M184V [12,13]. Accordingly, the M184V is identified much more frequently in genotypic resistance testing than the M184I mutation and overall the M184V mutation is the most frequently identified NRTI mutation. The M184I/V mutations develop via the discriminatory pathway. One early study has shown that patients treated with lamivudine

monotherapy develop virologic failure within 4 weeks of starting lamivudine and the increase in HIV RNA levels correlates with the emergence of the M184V mutation; even with development of the M184V and high-level resistance, lamivudine continues to exert an approximately 0.5 $\log_{10}$ decrease in HIV level [14]. In clinical trials involving combination antiretroviral therapy, the M184V mutation was the most common mutation to develop with initial virologic failure [15, HYPERLINK "http://www.hivwebstudy.org/cases/antiretrovirals-resistance/resistance-nucleoside-reverse-transcriptase-inhibitors" HYPERLINK "http://www.hivwebstudy.org/cases/antiretrovirals-resistance/resistance-nucleoside-reverse-transcriptase-inhibitors"16]. In vitro data demonstrates the the M184V mutation cause high-level resistance to emtricitabine and lamivudine, low-level resistance to abacavir and didanosine, and enhanced susceptibility to stavudine, tenofovir, and zidovudine. Several studies have suggested that treatment with lamivudine in the presence of an M184V mutation may confer clinical benefit, potentially through residual antiviral activity, resultant decreased viral count, and hypersensitivity to some other NRTIs, and perhaps delaying development of mutations in other NRTIs [17, 18, 19]. In the absence of drug pressure from either emtricitabine or lamivudine, the M184V mutation rapidly disappears, reflecting the overall negative impact of the 184V on viral count [20]. Once the M184V mutation develops, there are no further cascading mutations that develop that would negatively impact other antiretroviral medications. The M184V mutation is not known to impact mediations outside of the NRTI class, but the M184I mutation augments resistance to rilpivirine (*Edurant*) resistance in conjunction with a E138K mutation [20, 21, 22].

Thymidine Analog Mutations (Tam Mutations)

The TAM mutations which include: M41L, D67N, K70R, L210W, T215Y/F, and K219Q/E, develop in the setting of virologic failure with a regimen that include the thymidine analog medications stavudine or zidovudine [23]. Although these medications are infrequently used in current clinical practice, patients with long-standing HIV may have acquired TAM mutation in the past. In addition, patients from resource limited regions who have immigrated to the United States recently may have received stavudine or zidovudine in recent years, or may be currently taking these medications. In the United States, thymidine analog mutations infrequently develop in patients on modern antiretroviral regimens that have tenofovir-emtricitabine (*Truvada*) or abacavir-lamivudine (*Epzicom*) as the NRTI backbone of the regimen. Although the TAM mutations are selected by stavudine and zidovdine, the accumulation of multiple TAMs can also have significant impact on HIV susceptibility to abacavir, didanosine, and tenofovir. The TAM mutations tend to accumulate in one of two characteristic, but overlapping patterns: (a) the type I pattern that has M41L, L210W, and T215Y or (b) type II pattern consisting of D67N, K70R, T215F, and K219Q/E [23]. In general, type I TAM mutations result in higher levels of phenotypic resistance to stavudine and zidovdine, as well as greater cross resistance to abacavir, didanosine, and tenofovir [24]. Indeed, if all three type I TAM mutations are detected, clinical response to abacavir, didanosine, and tenofovir is markedly reduced. Some patients develop the D67N mutation will the type I cluster. The presence of a M184V mutation reduces the impact of the TAM mutations to some degree [25], but the favorable impact of the M184V is negligible with high numbers of TAM mutations.

K65R Mutations

Selection of the K65R mutation can occur with exposure to abacavir, didanosine, stavudine, and tenofovir. In clinicaltrials, the development of the K65R mutation has primarily occurred in patients who were taking an antiretroviral regimen that did not include a thymidine analog (stavudine or zidovudine) [26]. In early trials of abacavir monotherapy, approximately 10% of patients developed the K5R mutation [27]. Even higher rates (greater than 50%) of K65R mutation were observed in patients treated with the triple nucleoside regimen of abacavir plus lamivudine plus tenofovir [28]. Addition of a drug from a class other than NRTI appears to markedly reduce the likelihood of developing the K65R mutation in patients receiving tenofovir-emtricitabine. Development of the K65R can have variable impact on NRTI medications, including intermediate-level resistance (abacavir, didanosine, emtricitabine, lamivudine, and tenofovir), low-level resistance (stavudine), and hypersusceptibility (zidovudine). The mechanism of resistance with the K65R is decrease incorporation and the K65R mutation shows bilateral antagonism with the primer unblocking (excision) activity of the reverse transcriptase enzyme that contains TAMS [29, 30]. In clinical trials and clinical practice, it is very uncommon to observe the K65R mutation in conjunction with multiple TAMs. The 65R and M184V double mutation causes higher-level resistance to abacavir than either mutation alone and the K65R mutation reverses the hypersusceptibility effect of the M184V on stavudine, tenofovir, and zidovudine [31]. With abacavir resistance, the M184V typically precedes the K65R [32].

L74V Mutation

The L74V mutation was first identified with didanosine and abacavir monotherapy; this mutation alone causes high-level resistance to didanosine and intermediate-level resistance to abacavir. Similar to the M184V mutation, the L74V mutation causes in *in vitro* hypersusceptibility to tenofovir, zidovudine, and possibly stavudine. The L74V in combination with M184V has been seen in patients treated with an abacavir plus lamivudine or didanosine plus lamivudine NRTI backbone. Overall, the L74V mutation is an uncommon NRTI mutation, but is identified in approximately 25% of samples that contain HIV with K101E plus G190S mutations and in approximately 50% of samples L100I plus K103N mutations [33].

Multi Nucleosid-Resistance Mutations

The multi-nucleoside resistance mutations occur relatively infrequently, but may have a major impact on the NRTIs. The T69-insertion mutation consists of double amino acid (diserine) insertion between codons 69 and 70 in the reverse transcriptase enzyme. The T69 occurs only in the setting of existing TAM-1 mutations and together the T69-insertion and TAM-1 generate high-level resistance to all of the NRTI medications, except for lamivudine and emtricitabine, which have intermediate resistance [34].The

Q151M mutation complex usually occurs with several accessory mutations (A62V, V75I, F77L, and F116Y) and these mutations in tandem cause high-level resistance to abacavir, didanosine, and zidovudine, as well as intermediate resistance to emtricitabine, lamivudine, and tenofovir. The Q15M mutation complex develops only in the setting of prolonged viremia while on therapy.

Conclusion

In case of treatment failure, especially in early regimens, clinicians should intervene quickly with a strategy to prevent viral evolution and the accumulation of resistance mutations. Further studies are needed to elucidate the mutational pathways associated with various NRTI-containing regimens to determine differences in resistance patterns, and the implications of these differences for future options and sequencing therapy.

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