Antiviral Drug Resistance and the Need for Development of New HIV-1 Reverse Transcriptase Inhibitors.

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Abstract

Highly active antiretroviral therapy (HAART) consists of a combination of drugs to achieve maximal virological response and reduce the potential for the emergence of antiviral resistance. Despite being the first effective antivirals described to be effective against HIV, reverse transcriptase inhibitors remain the cornerstone of HAART. There are two broad classes of reverse transcriptase inhibitor, the nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs).

Since the first such compounds were developed, viral resistance to them has inevitably been described; this necessitates the continuous development of novel compounds within each class. In this review we consider the NRTIs and NNRTIs currently in both pre-clinical and clinical development or approved for second line therapy, and describe the patterns of resistance associated with their use, as well as the underlying mechanisms that have been described. Due to reasons of both affordability and availability, some reverse transcriptase inhibitors with low genetic barrier are more commonly used in resource limited settings. Their use results to the emergence of specific patterns of antiviral resistance and so may require specific actions to preserve therapeutic options for patients in such settings.
Introduction

The standard treatment for patients infected with human immunodeficiency virus (HIV), referred to as highly active antiretroviral therapy (HAART), consists of three or more HIV drugs, most commonly two nucleoside reverse transcriptase inhibitors (NRTIs) in combination with either a non-nucleoside reverse transcriptase inhibitor (NNRTI), a protease inhibitor (PI) or, more recently, an integrase inhibitor (INI) (65). The goal of HAART is to optimally suppress HIV replication during long-term therapy and to maintain immune function (92). Rational drug selection is essential to maximize potency, minimize side effects and cross resistance, preserve future treatment options, and increase overall duration of viral suppression [reviewed in (23)]. Although numerous antiretroviral combinations may provide potent suppression of viral replication, therapeutic choices necessitate careful consideration of the potential impact of viral resistance on subsequent treatment options.

Advances in antiretroviral therapy have improved HIV management and the control of the spread of regional epidemics (64). However, resistance to antiretroviral drugs is largely unavoidable, due to the error-prone nature of HIV reverse transcriptase (RT), and its lack of a proofreading function (77). In addition, the sheer number of replication cycles occurring in an infected individual and the high rate of RT-mediated recombination events, facilitate the selection of drug resistant mutant strains of HIV (13, 28). Furthermore, certain tissue compartments seem able to select for resistance mutations due to the presence of low drug concentrations (33). These mutations are located on the genes that encode antiretroviral targets such as RT, resulting in the production of RT that is different than its wild-type (wt) counterpart in both structure and function. Although this protein is still able to play its role in HIV replication, it is not inhibited as effectively as wt protein by the ARV drugs.
The number of mutations required for resistance to occur varies from drug to drug. Many factors determine the relative rate of resistance selection with different drugs and drug combinations, and this is reflected in the 'genetic barrier' to resistance which refers to the number of mutations that must occur within a given target in order for resistance to be present against a particular drug. Interactions between mutations, the effects of individual resistance mutations on viral replication capacity, and viral fitness all influence mutational pathways and the overall impact of resistance mutations on viral phenotype. Many different mechanisms through which HIV-1 escapes from drug pressure have been described; these mechanisms differ from one drug class to another and can even differ between drugs of the same class.

**Reverse Transcriptase (RT) Inhibitors**

Two classes of RT inhibitors exist; the nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs). NRTIs incorporate into nascent viral DNA, resulting in DNA chain termination and blocking further extension of DNA. The NNRTIs stop HIV-1 replication by binding to the hydrophobic pocket within the p66 subunit of the RT enzyme thus preventing it from converting viral RNA into DNA (17, 74). NNRTIs are non-competitive inhibitors of HIV-1 RT and do not require activation. The low fidelity of HIV-1 RT, high level of HIV-1 replication and the high rate of RT mediated recombination collectively contribute to the emergence of resistance to RT inhibitors (10, 28).

**Early NRTIs**

HIV can become resistant to NRTIs via two distinct mechanisms. The first is discrimination, whereby the mutated viral RT can selectively avoid incorporating NRTIs in favor of natural
dNTPs; this mechanism is typified by such mutations as K65R, L74V, Q151M and M184V (37). The second mechanism of resistance allows a mutated RT to enact the phosphorolytic excision of NRTIs from the 3’ end of the viral DNA chain that extends from the primer, a process referred to as ‘primer unblocking’. Examples of mutations involved in this process are those selected by zidovudine (ZDV) and stavudine (d4T), that are termed thymidine analogue mutations (TAMs), e.g. M41L, D67N, K70R, L210W, T215Y/F and K219Q/E (48, 68). TAMs confer resistance to all NRTIs except lamivudine (3TC) and emtricitabine (FTC). Although, there is a degree of cross-resistance associated with TAMs, ultimate levels of resistance depend on the specific NRTI and the number of TAM mutations found in the viral RT (reviewed in (48)).

The two NRTI resistance mechanisms of discrimination and excision can also influence each other. For example, the M184V/I mutation that is selected by 3TC and FTC is a discrimination mutation but viruses that contain M184V/I are less likely to quickly develop TAMs under selective pressure with such drugs as ZDV. Viruses containing M184V/I are also more susceptible to ZDV and d4T than wild-type viruses (reviewed in (48)).

Antiretroviral therapy with abacavir (ABC) leads to the selection of such mutations as K65R, L74V, Y115F and M184V (53) and the combination of L74V and M184V is commonly observed. The K65R mutation is also selected by tenofovir (TFV) and reduces susceptibility by 3 to 6-fold against this drug (8, 67). Usually, the selection of K65R precludes the occurrence of TAMs while the presence of the latter mutations prevents the selection of K65R due to the fact that viruses that contain both K65R and TAMs are not viable. The gold standard in patients initiating therapy involves a combination of TFV/FTC or ABC/3TC together with a NNRTI (reviewed in (75)).
Early NNRTIs

Nevirapine (NVP) and Efavirenz (EFV) are FDA approved NNRTIs and have become the cornerstone of therapy within both developed and under-developed countries. However, the low genetic barrier to resistance of these earlier NNRTIs serves as a major limitation for prolonged antiretroviral therapy and sequential use of inhibitors of this class (2, 7, 42). Notably, a single amino acid substitution in the RT enzyme is often adequate to yield high-level clinically relevant resistance. Additionally, high-level cross-resistance among early NNRTIs has been reported (7) and this can also have an impact by decreasing virologic response in patients with transmitted resistance (44). The prevalence rate of transmitted antiretroviral drug resistance in treatment-naïve patients with HIV-1 has been estimated to be 5-15% for resistance mutations to at least one antiretroviral class (6). In this US drug resistance survey, the NNRTI class showed the highest prevalence at a rate of 6.9% compared to NRTIs and PIs at 3.6% and 2.4% respectively. The increasing use of NNRTIs in clinical practice and the fact that NNRTI resistance mutations do not severely impair viral replication capacity but remain part of the dominant viral variant may explain the high prevalence of NNRTI resistance [reviewed in (20)].

The NNRTI binding pocket is located largely in the p66 subunit of RT and consists of the following residues; 95, 100, 101, 103, 106, 108, 179, 181, 188, 190, 227, 229, 234, 236 and 318 (36, 74). Some residues from p51, such as 138, also contribute to the NNRTI binding. NNRTI mutations confer resistance by disrupting the interactions between the inhibitor and enzyme. This can occur through three mechanisms: 1. they can block the entry of inhibitor into the NNRTI binding pocket (e.g K103N); 2. they can affect contacts between the inhibitor and residues that line the NNRTI binding pocket (e.g Y181C), or 3. they can alter the conformation or size of the NNRTI binding pocket so that it becomes less specific for the
inhibitor (e.g Y188L) (20). Some resistance mutations can affect the binding of NNRTIs through more than one mechanism.

**Newer NRTIs**

**Elvucitabine**

Elvucitabine (Table 1) is a novel NRTI currently in late phase II study and was developed by Achillion Pharmaceuticals. In an *in vitro* selection study, only two amino acid substitutions, M184I and D237E, were identified in the resultant variant (21). The double mutation conferred moderate resistance to elvucitabine (about 10 fold) and cross-resistance to lamivudine but not to other nucleoside inhibitors tested. Elvucitabine has also demonstrated potent antiviral activity in HIV-infected patients with resistance to 3TC and other NRTIs. The drug has good oral bioavailability and an intracellular half-life of >24 hours (15).

**Apricitabine**

Apricitabine (ATC) (Table 1) is a novel deoxycytidine NRTI currently in clinical development for the treatment of HIV infection. *In vitro* selection for resistance with ATC selected for M184V, V75I and K65R (25). The resulting mutants from this selection conferred low-level resistance of less than 4 fold. Others showed that continuous passage of HIV-1 already containing M184V, K65R or combinations of M41L, M184V and T215Y did not result in any additional mutations (62). *In vitro* ATC has shown favorable antiviral activity against HIV-1 wt strains and clinical isolates containing NRTI mutations including M184V, L74V, and TAMs (11). ATC yielded virological response in treatment-naïve and treatment-experienced HIV-1 infected patients whose viruses contained M184V and up to 5 TAMs. Resistance to ATC was reported to be slow to develop *in vitro*, and there is little evidence of the development of resistance to this drug in patients (11, 25, 62).
4'-Ethynyl-2-fluoro-2'-deoxyadenosine triphosphate (EFdA-TP) (Table 1) is a new NRTI, now in pre-clinical development, that retains the 3'-OH group and has excellent antiviral properties i.e EC\textsubscript{50} of 0.07 nM against wt virus (31, 35) compared to approved NRTIs that range in EC\textsubscript{50} between 17-89 nM (38). This robust antiviral activity is due to a mechanism of action that is different from approved NRTIs. Notably, EFdA-TP acts by binding through its 3'-primer terminus to RT; the addition of subsequent nucleotides is then prevented by blocking the translocation of the primer strand on the viral polymerase (52). Thus, EFdA-TP is termed a “translocation-defective reverse transcriptase inhibitor (TDRTI)”. Modeling studies have confirmed the binding of EFdA-TP to a hydrophobic pocket of RT residues consisting of A114, Y115, F160 and M184 and the aliphatic chain of D185 (52).

In an \textit{in vitro} pre-steady-state kinetics study to determine toxicity, it was shown that EFdA-TP is a poor human mitochondrial DNA polymerase (Pol) substrate, suggesting that Pol-mediated toxicity might be minimal (82). Resistant variants including those containing the K65R/L74V/Q151M complex did not affect the susceptibility of this compound while the HIV-1 clone containing M184V alone conferred low to moderate level resistance to EFdA-TP (31). \textit{In vitro}, a parental compound of EFdA-TP, 2'-deoxy-4'-C-ethynyl-adenosine (EdA) selected for resistant variants after 58 passages with novel combination of mutations, I142V/T165R/M184V (31). Site directed mutagenesis of clones containing the mutations showed that either I142V or T165R alone did not affect the antiviral activity of EFdA-TP while M184V alone or in combination with I142V or T165R demonstrated moderate resistance to EFdA-TP. The triple mutant I142V/T165R/M184V had the highest resistance amongst clones tested (31).
GS-9131

GS-9131 (Table 1) is a prodrug of the nucleotide reverse transcriptase analogue GS-9148, which belongs to the same family as TFV. GS-9131 demonstrated potent antiviral activity against a variety of HIV-1 subtypes i.e. EC50 of 37 nM (12). In vitro, the parent drug (GS-9148) caused only low-level cytotoxicity compared to TFV (12). GS-9131 also demonstrated synergy in combination with other antiretrovirals as well as potent antiviral activity against multi-NRTI resistant strains, including K65R, M184V, and L74V, and only a minimal increase in EC50 in regard to viruses carrying four or more TAMs (12). The use of GS-9131 was shown to result in 76-290 times more of the di-phosphorylated form of GS-9148 compared to GS-9148 itself (73).

GS-7340

GS-7340 (Table 1) is a prodrug of TFV that exhibits anti-HIV activity and that possesses a favorable resistance profile. The EC50 of GS-7340 against HIV-1 in MT-2 cells was 0.005 µM compared to 5 µM for the parent drug TFV (40). In HIV-I infected patients, GS-7340 demonstrated enhanced antiviral activity, with no TFV mutations identified, and yielded higher intracellular concentrations of TFV-diphosphate than did TFV itself (69).

CMX157

CMX157 (Table 1) is a lipid moiety prodrug of TFV that has activity against wt viruses of major HIV-1 subtypes with EC50 ranging from 0.2 to 7.2 nM (38). In contrast TFV has EC50 against HIV-1 group M and O that range between 500-2200 nM in PBMCs (24). In an in vitro study, CMX157 demonstrated potent activity against NRTI-resistant strains, including multidrug-resistant viruses against which it showed >300-fold activity compared to TFV (38). The higher potency and lower EC50 of CMX157 is due to better cellular uptake.
than TFV and the fact that it is not a substrate for organic anion transporters. This permits it
to maintain high concentrations inside cells, in contrast to TFV that is actively metabolized
by organic anion transporters, leading to decreased intracellular concentrations (72). In vitro
and pre-clinical studies in rats showed that CMX157 also has a favorable cytotoxicity profile
in PBMCs and that it does not lead to nephrotoxicity as has been reported for TFV (38, 63).
No information is available on the selection of resistance mutations with CMX157.

**Amdoxovir**

Amdoxovir (AMDX) is a prodrug of β-D-dioxolane guanosine (DXG) that is currently in
phase II clinical trials (Table 1). In vitro phenotypic analyses have shown that DXG is
effective against HIV-1 variants that are resistant to lamivudine and emtricitabine (M184V/I)
as well as against viruses that contain TAMs, while selection studies in MT-2 cells resulted in
the appearance of K65R and L74V (5, 51). The combination of AMDX together with
zidovudine (ZDV) in HIV-1 infected patients was shown to be synergistic, resulting in
reduced viral loads (57). AMDX thus represents a new NRTI that possesses potent antiviral
activity against NRTI-resistant viruses.

**Festinavir (OBP-601)**

Festinavir (OBP-601) (Table 1) is a new NRTI in the same family as stavudine (d4T) but that
has an improved safety profile. In vitro, it shows potent antiviral activity against wt HIV-1 of
multiple subtypes with EC_{50}s ranging from 0.76-5.8 µM, compared to 1.57-6.06 µM for d4T
(90). In a phenotypic susceptibility assay, viruses carrying the K65R and Q151M resistance
mutations were shown to be hypersusceptible to this compound. In contrast, a slightly
decreased antiviral response was observed against viruses carrying either TAMs or TAMs
together with K103N and M184V (90). More importantly, a strong synergistic effect of
OBP-601 with several approved NRTIs and NNRTIs was observed against wt and resistant viruses (90).

**Newer NNRTIs**

**Etravirine**

Etravirine (ETR) (Table 2), formerly known as TMC125, is a diarylpyrimidine (DAPY)-based NNRTI that possesses potent antiviral activity against both wt HIV-1 of multiple subtypes as well as against some viruses containing NNRTIs resistance mutations (1, 88). Specifically, ETR retains full activity against viruses containing the most prevalent NNRTI mutation, K103N (1). In *vitro*, ETR is more difficult to generate resistance against compared to initial NNRTIs (88). In clinical studies of ETR in combination with potent background regimens that included NRTIs, integrase and protease inhibitors, it was observed that viral loads became significantly decreased in patients with resistance to older NNRTIs and some PIs (9, 45, 58). In *vitro* and clinical studies have described 20 ETR resistance-associated mutations (RAMs) (V90I, A98G, L100I, K101E/H/P, V106I, E138A/K/G/Q, V179D/F/T, Y181C/I/V, G190A/S, and M230L) and have allowed a weighted score to be assigned to each mutation (3, 83, 89). Of these ETR RAMs, three or more are required for high level resistance to occur, thus demonstrating a high genetic barrier to resistance compared to older NNRTIs. The structure of ETR allows it to bind to the RT enzyme, such that mutations in the NNRTI-binding pocket do not compromise binding and, thus, activity is maintained. ETR can rotate within the pocket allowing multiple interactions despite the presence of mutations in the binding pocket. (19). Because of its unique characteristics, ETR is the only NNRTI approved for treatment of NNRTI experienced patients.
Only a few studies have prospectively studied the efficacy of ETR in combination with other background regimens in NNRTI experienced patients (30, 49). In the phase III DUET-1 and DUET-2 studies, 57% of patients in the ETR arm versus 36% in the placebo had a viral load <50 copies/ml after 96 weeks of treatment (30).

In the DUET-1 and DUET-2 clinical studies, it was found that patients who experienced virologic failure had greater numbers of ETR resistance mutations at baseline than treatment successes. Second, patients who experienced virologic failure were often found to have received background regimens that were less potent than the drugs given to patients who did not fail therapy (84). In this study, the V179F, V179I and Y181C mutations in RT were commonly associated with treatment failure alongside changes at positions K101 and E138 (84). The authors concluded that these mutations usually emerge in a background of other multiple NNRTI mutations and were, in most cases, associated with a decrease in phenotypic sensitivity to ETR.

Another sub-analysis of the DUET trial studied the impact of background regimen on virologic response to ETR, and the authors further confirmed that a higher virologic response rate was observed in patients who demonstrated an increased activity of the background regimen, with the highest responses being achieved in patients who used more than two active agents in addition to ETR (85). In the TMC125-C227 (ETR) trial, ETR was inferior to a protease inhibitor (PI) in PI-naïve patients with a history of previous NNRTI failure (78). In a post-hoc analysis of baseline resistance data for the TMC125-C227 trial, a diminished virological response was observed in patients who possessed the following characteristics at baseline; the presence of Y181C, a baseline ETR fold change of \( \geq 10 \), and a higher number of ETR resistance mutations (78). In another study, the authors studied 42 NNRTI treatment-experienced patients for 6 months on an ETR containing regimen (49). At failure, 12 of 42
patients developed at least one new NNRTI mutation. The most frequently selected mutations included V179I, Y181C and V179F.

In contrast, several studies have researched the theoretical potential of ETR, based on the resistance patterns of patients who previously failed NNRTI therapy and accumulated ETR RAMs. These studies have observed a prevalence of more than three ETR RAMs among viral isolates from patients experiencing NNRTI treatment failure, ranging from 4.6% to 10%, while the prevalence of isolates with single ETR RAMs was 17.4% to 35.9% (41, 61, 70, 71). These studies concluded that there is a low prevalence of ETR resistance at baseline and that patients with prior failure to NNRTIs could potentially benefit from ETR rescue therapy. However, these analyses focused on patients in developed countries that have full access to the most potent antiretroviral drugs and patients are constantly monitored for viral load and the development of resistance.

In contrast, patients in countries with limited resources developed resistance faster due to a lack of potent antiretroviral drugs and drug resistance testing. Some studies in resource limited settings have observed a high prevalence of NNRTI resistance mutations associated with ETR resistance among patients failing an NNRTI containing regimen (32, 34, 39). Using NVP in the failing regimen was associated with intermediate and reduced response to ETR while use of EFV and co-administration of 3TC reduced the risk of ETR resistance (39). The authors concluded that the frequent occurrence of NNRTI mutations in resource limited settings in which drug resistance testing is rare might compromise the continuous use of ETR and also its use in second line therapy. The Y181C mutation, associated with NVP therapy, has been reported with a high prevalence in NNRTI experienced patients and shown to decrease susceptibility to ETR (39, 41, 46, 47). This demonstrates cross-resistance of both
NVP and ETR as confirmed by our group (3). Thus, the widespread use of NVP in resource-limited settings without resistance testing casts doubt as to whether ETR could be effective in NNRTI experienced patients in poorer countries. One of these studies suggested that ETR should be avoided in salvage regimens in the setting of first-line NVP failure where drug resistance testing is not performed (47). Another study analyzed the prevalence of minority variants in treatment-naïve and NNRTI experienced patients by ultradeep pyrosequencing: while such variants were not identified in any of 13 drug-naive patients, it was shown that 7 of 20 patients who had failed an NNRTI-containing regimen possessed minority variants as well as ETR associated-NNRTI resistance mutations (86). This suggests that minority variants in NNRTI experienced patients may lead to decreased ETR activity and virologic failure (66).

In vitro selection and clinical trials results with ETR have identified amino acid substitutions at position 138 in RT (3, 83). Mutations at position 138 are not associated with resistance to older NNRTIs. Although these mutations conferred only low-level resistance to ETR, E138K is also associated with resistance to most newer NNRTIs (Table 2); its emergence may also facilitate the development of additional ETR mutations (3). The connection domain mutation N348I has been identified and implicated in reduced susceptibility to NVP and EFV as well as to the nucleoside analogue ZDV (26). Two independent studies have assessed and confirmed that this mutation also decreases susceptibility to ETR, either alone or in combination (27, 50). This effect was reversed when M184V was co-expressed with N348I. Additional connection domain mutations found to be associated with impaired susceptibility to ETR were T369I and E399G.
Two independent genotypic scores have been established to predict ETR resistance. The first, developed by Janssen, has been correlated with treatment response in the DUET studies and, when combined with *in vitro* selection studies, identified 17 (recently updated to 20) ETR RAMs (83, 89). In this analysis, any three or more of these mutations were required to cause resistance to ETR. A second score by Monogram is based on a correlation of phenotype and genotype results of 4,923 samples containing at least one NNRTI mutation (54). The Monogram genotypic score identified 30 mutations associated with ETR resistance with the weighted score for each mutation being slightly higher than the 20 mutation-based Janssen score. However, the final interpretation of the two scores seems to be similar.

**Rilpivirine (TMC278)**

Rilpivirine (RPV) (Table 2), also known as TMC278, is another DAPY compound that was recently approved for treatment of NNRTI-naïve patients. The structure and binding of RPV in the NNRTI binding pocket is similar to that of ETR, which allows reorientation of both compounds within RT. *In vitro*, RPV possesses subnanomolar activity against wt HIV-1 of multiple subtypes and shows antiviral activity against viruses containing many NNRTI resistance-associated mutations (4). NNRTI RAMs emerging in culture under RPV selective pressure included combinations of V90I, L100I, K101E, V106A/I, V108I, E138G/K/Q/R, V179F/I, Y181C/I, V189I, G190E, H221Y, F227C, and M230I/L. The resistance profile and genetic barrier to the development of resistance of RPV are comparable to those of ETR. High-resolution crystal structures of RPV in complex with HIV-1 RT reveal that the cyanovinyl group of TMC278 is positioned in a hydrophobic tunnel connecting the NNRTI-binding pocket to the nucleic acid-binding cleft (18). Both RPV and ETR exhibit similar flexibility in adapting to resistance mutations. In the ECHO and THRIVE phase 3 trials (14, 55), resistance analysis showed a slightly higher proportion of treatment failures in the RPV...
arm compared to the EFV arm. The most frequent NNRTI mutation in the RPV arm was E138K in addition to mutations such as Y181C, K101E, H221Y, V901, E138Q, and V189I (76). Also, the proportion of NRTI mutations that emerged in the study was higher in the RPV arm than the EFV arm. The NRTI mutations selected included M184I/V, K65R, K219E and Y115F.

The IAS-USA recently published a total of 15 mutations (K101E/P, E138A/G/K/Q/R, V179L, Y181C/I/V, H221Y, F227C, and M230I/L) associated with decreased susceptibility to RPV (29). These mutations have been described based on in vitro studies and in patients in whom RPV was failing. The quantitative impact of each of these mutations on RPV resistance differs.

Dapivirine (TMC 120)

Dapivirine (TMC 120) (Table 2) is another DAPY compound that can accommodate some mutations within the NNRTI binding site without significant loss of activity (42, 43). TMC 120 has shown potent antiviral activity against both wt and NNRTI-resistant HIV-1 strains (22, 79). In 2004, Janssen officially licensed the further development of TMC 120 for use as a vaginal microbicide to the International Partnership for Microbicides (IPM) to help prevent sexual transmission of HIV-1.

The results of both phase I and II studies have shown that TMC 120 was widely distributed through the lower genital tract with low systemic absorption when administered as a vaginal gel formulation for up to 42 days (59, 60). The gel was safe and well tolerated. In vitro selection studies have identified drug resistance mutations in the presence of TMC 120, notably L100I, K101E, V108I, E138K/Q, V179M/E, Y181C and F227Y (80, 81). Most of
these TMC 120 resistance-associated mutations occur at exactly the same position as many of
the mutations associated with ETR and RPV resistance (3, 4, 89). However, in one of these
studies, it was shown that sub-optimal concentrations of TMC120 alone facilitated the
emergence of common NNRTI resistance mutations while sub-optimal concentrations of
TMC120 plus tenofovir (TFV) gave rise to fewer mutations (80). Due to the likelihood of
transmitted resistant strains in HIV-1 infected individuals, resistance mutations might impact
the ability of a single drug in preventing HIV-1 as a microbicide. Using a combination of
antiviral drugs of different classes may be useful. Another study showed in an in vitro model
that using TMC 120 in combination with TFV as a microbicide was more potent and
exhibited synergy in comparison with either drug alone (79).

Lersivirine

Lersivirine (Table 2) is a new NNRTI belonging to the pyrazole family and is being
developed by Pfizer. In an in vitro resistance study, lersivirine selected for the amino acid
substitutions; V108I, E138K, V179D, F227L and M230I (16). In a phase II b trial, Pozniak
and colleagues reported better responses in patients with EFV compared to lersivirine, i.e.
86% versus 79% respectively (87). Amongst patients who failed lersivirine, the mutations
identified included K101E, V106M, V108I, H221Y, Y188H, F227C/L and L234I.

RDEA806

RDEA806 (Table 2) is a novel NNRTI being developed by Ardea Biosciences. In a genotypic
and phenotypic analyses of mutant viruses selected by RDEA806, the K104E, E138K, T240I,
V179D and F227L substitutions were identified (91). Phenotypic analysis of these mutations
demonstrated that RDEA806 requires at least 3 mutations for greater than 10 fold loss of
susceptibility. In a phase 2a trial, RDEA806 was well tolerated and exhibited robust antiviral activity with no genotypic or phenotypic changes (56).

Conclusion

An ideal new HIV inhibitor should possess a high genetic barrier in regard to potential development of resistance for this same drug class as well as a unique resistance profile in both B and HIV-1 non-B subtypes. NRTI resistance mutations are widespread and there is currently no approved NRTI compound that possesses activity against all NRTI mutations. However, a number of new NRTIs with potent activity against NRTI-resistant viruses are now in preclinical and clinical development. In some cases, these compounds possess the ability to maintain high concentrations inside cells (e.g. CMX157, GS-9131 and GS-7340) or may have a different mechanism of action than older NRTIs, such seems to be the case for EFdA-TP. Although M184V is a common NRTI mutation that confers high-level resistance to 3TC and FTC and cross-resistance to other NRTIs, the levels of resistance conferred against newer NRTIs such as ELV, ATC, and EFdA-TP is low-level, suggesting that these compounds may be useful against M184V-containing viruses. It will be important to determine whether selection of M184V in patients receiving ELV, ATC or EFdA-TP may result in elevated viral loads and treatment failure. Hopefully, their potential to synergize either together or with currently approved drugs will make them important components of future antiretroviral strategies.

ETR is the only NNRTI approved for treatment of HIV-1 NNRTI experienced patients. In a limited number of studies, ETR has proven to be effective in patients with treatment failure who harbor viruses that are resistant to initial NNRTIs and that carry several resistance mutations. A number of studies have shown that patients who fail NVP therapy are more
prone to develop ETR resistance mutations faster than those who fail EFV therapy. Considering the wide-spread use of NVP in developing countries, the use of ETR in NNRTI experienced patients in these settings is under debate. RPV that is now approved for treatment naïve patients shows high level cross-resistance with ETR. Therefore, the use of RPV in first-line therapy may jeopardize the future of ETR as a second line NNRTI and the sequential use of these drugs is not recommended. The emergence of E138K as a signature mutation for almost all new NNRTIs (both approved and under clinical development) is another limitation of these compounds.

TMC120, Lersivirine and RDEA806 are in still in phase II clinical trial and large scale phase III trials are required to exploit their potential use in the clinics. Some mutations have been selected in the presence of these three compounds together with ETR and RPV. The fact that TMC120 in combination with TFV as a microbicide is more potent and decreases the possibility of selecting for resistance mutations than either compound alone shows potential for such an antiviral based microbicide in preventing HIV-1 infection. The search for novel NNRTIs should now focus on compounds with different resistance profiles, so as to broaden treatment options for patients who have experienced NNRTI therapy failure. Overall, new NRTI and NNRTI agents can provide a welcome therapy option for patients with existing NRTI or NNRTI resistance and also for patients who are naïve to therapy.

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Table 1: New NRTIs undergoing pre-clinical or clinical development

<table>
<thead>
<tr>
<th>Drug Candidate</th>
<th>ELV</th>
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<th>EFdA-TP</th>
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<td>Chemical Structure</td>
<td><img src="image1" alt="ELV Chemical Structure" /></td>
<td><img src="image2" alt="ATC Chemical Structure" /></td>
<td><img src="image3" alt="EFdA-TP Chemical Structure" /></td>
<td><img src="image4" alt="GS-9131 Chemical Structure" /></td>
</tr>
<tr>
<td>Phase of Development</td>
<td>IIb</td>
<td>IIb</td>
<td>Pre-clinical development</td>
<td>I</td>
</tr>
</tbody>
</table>
Table 1: Cont.

<table>
<thead>
<tr>
<th>Drug Candidate</th>
<th>GS-7340</th>
<th>CMX157</th>
<th>Amdoxovir</th>
<th>Festinavir</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical Structure</td>
<td><img src="image1" alt="Structure of GS-7340" /></td>
<td><img src="image2" alt="Structure of CMX157" /></td>
<td><img src="image3" alt="Structure of Amdoxovir" /></td>
<td><img src="image4" alt="Structure of Festinavir" /></td>
</tr>
<tr>
<td>Phase of Development</td>
<td>II</td>
<td>II</td>
<td>II</td>
<td>II</td>
</tr>
</tbody>
</table>
Table 2: New NNRTIs that approved or are undergoing clinical development.

<table>
<thead>
<tr>
<th>Drug candidate</th>
<th>ETR</th>
<th>RPV</th>
<th>TMC 120</th>
<th>RDEA806</th>
<th>Lersivirine (UK-453061)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical structure</td>
<td><img src="ETR" alt="Chemical structure" /></td>
<td><img src="RPV" alt="Chemical structure" /></td>
<td>![Chemical structure](TMC 120)</td>
<td><img src="RDEA806" alt="Chemical structure" /></td>
<td><img src="Lersivirine" alt="Chemical structure" /></td>
</tr>
<tr>
<td>Phase of development</td>
<td>Approved (2008)</td>
<td>Approved (2011)</td>
<td>License for HIV-1 microbicide development</td>
<td>IIb</td>
<td>IIb</td>
</tr>
</tbody>
</table>
References


reverse transcriptase (RT) genotype and susceptibility to RT inhibitors during abacavir monotherapy and combination therapy. AIDS 14:163-71.


69. Peter Ruane, E. D., D Berger, M Markowitz, F Bredeek, C Callebaut, L Zhong, S Ramanathan, M Rhee, and K Yale. 2012. GS-7340 25 mg and 40 mg Demonstrate Superior Efficacy to Tenofovir 300 mg in a 10-day Monotherapy Study of HIV-1+ Patients., 19th Conference on Retroviruses and Opportunistic Infections Seattle, WA.


