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Review

Non-nucleoside reverse transcriptase inhibitors (NNRTIs), their discovery, development, and use in the treatment of HIV-1 infection: A review of the last 20 years (1989–2009)

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ABSTRACT

It is almost 20 years since NNRTIs were identified as a new class of antiretroviral drugs for the treatment of HIV-1 infection. Although they belong to different and diverse chemical families, they share a common and unique mechanism of action: their interaction with HIV-1 reverse transcriptase induces conformational changes that inhibit the catalytic activities of the enzyme. They are characterized by their specificity for HIV-1, which makes them very selective inhibitors of the virus. First generation NNRTIs nevirapine and efavirenz, in combination with other antiretroviral drugs, have become a cornerstone for the treatment of HIV-1 infection, in patients initiating antiretroviral therapy. Further research has led to the discovery and development of next generation NNRTIs with an increased genetic barrier to the development of resistance. Etravirine is the first NNRTI to show sustained virologic efficacy in patients with NNRTI resistant HIV-1. This review covers the NNRTI class of anti-HIV-1 drugs, from the initial discovery of the class in 1990 to the current compounds in clinical development, i.e. around 20 years of research and development efforts. It describes the characteristics of the NNRTIs, their mechanisms of action, HIV-1 resistance to the inhibitors, and the drugs that have been approved for the treatment of HIV-1 infection, or are currently in clinical development. The role of NNRTIs in prevention of HIV transmission is also addressed. This article forms part of a special issue of *Antiviral Research* marking the 25th anniversary of antiretroviral drug discovery and development, vol. 85, issue 1, 2010.

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1. Introduction

We are nearing the end of the third decade of the Acquired Immune Deficiency Syndrome (AIDS) pandemic, and in the developed countries at least, the times when diagnosis of human immunodeficiency virus (HIV) infection represented a death sentence (Rothenberg et al., 1987) are over. Researchers around the world have worked hard, and nowadays 25 antiretroviral drugs have been approved by regulatory authorities for the treatment of HIV infection. Treatment guidelines recommend that patients be administered a combination of at least two to three active drugs from at least two different classes, and that the goal of therapy for all patients, regardless of the number of therapies they have been subjected to, should be to control virus replication, as measured by a plasma viral load below the quantification limit of current available assays, i.e. 50 or 40 HIV RNA copies/mL (DHHS, 2007; Gazzard, 2008). Available drugs belong to 6 different classes: eight nucleoside (nucleotide) reverse transcriptase (RT) inhibitors (N[t]RTIs), four non-nucleoside RT inhibitors (NNRTIs), ten protease inhibitors (PIs) and one integrase inhibitor, which are targeted at viral enzymes; a fusion inhibitor, which prevents the fusion of the virus envelope with the host-cell membrane; and a CCR5 inhibitor, which blocks the interaction of the virus with one of its receptors on the host cell (De Clercq, 2009). Although some of these drugs are minimally used, because of their side effect profiles, high pill burden and/or inconvenient administration schedules, or difficult to manage drug-drug interactions, patients and their treating physicians still have at their disposition many options to construct the most appropriate combination regimen, the one that will best fit the requirements of the individual patient.

NRTIs were the first class of antiretroviral drugs to gain regulatory approval: in 1987 zidovudine (AZT) was the first drug to be licensed for the treatment of HIV infection (Ezzell, 1987), a mere four years after the identification of HIV as the etiology agent for AIDS (Barre-Sinoussi et al., 1983; Gallo et al., 1983). In those days, AZT was administered as monotherapy and as such would prolong the lives of patients for 6 to 18 months. The introduction of a second class of antiretroviral drugs, i.e. the PIs, with the approval of saquinavir in 1995, changed the picture of treatment of HIV infection. This opened the era of Highly Active Antiretroviral Therapy, or HAART. The recommended treatment regimens should combine three drugs, from at least two different classes, in order to keep virus replication under control, and to observe a concomitant increase in CD4 cells, and thus an immunologic improvement. HAART drastically reduced the incidence of opportunistic infections and death in AIDS patients. Shortly after the approval of the first protease inhibitors, a new class of antiretroviral drugs was introduced: the NNRTIs. Nevirapine was approved by the FDA in 1996, followed by delavirdine in 1997, and efavirenz in 1998. However, a significant proportion of patients did not fully benefit from HAART, as their virus had accumulated resistance to the available drugs, as a consequence of monotherapy, and/or suboptimal combination therapy regimens. In recent years, several molecules with better resistance profiles have been developed to address this issue. Among those, etravirine was the first NNRTI to demonstrate clinical efficacy in patients with NNRTI-resistant HIV-1 infection, and was approved by the FDA in 2008. Other NNRTIs with an improved resistance profile are currently being developed.

This review covers the NNRTI class of anti-HIV-1 drugs, from the initial discovery of the class in 1990 to the current compounds in clinical development, i.e. around 20 years of research and development efforts. It describes the characteristics of the NNRTIs, their mechanisms of action, HIV-1 resistance to the inhibitors, and the drugs that have been approved for the treatment of HIV-1 infection, or are currently in clinical development. The role of NNRTIs in prevention of HIV transmission is also addressed.

2. The HIV-1 RT enzyme

The Pol gene of HIV encodes three enzymes: the protease, the RT with embedded ribonuclease H (RNaseH) activity and the integrase. The HIV-1 RT is an asymmetric heterodimer, comprising a p66 subunit (560 amino acids) and a p51 subunit (440 amino acids) (Kohlstaedt et al., 1992). Both subunits are encoded by the same sequence in the viral genome. RNAseH consists of the last (carboxy terminal) 120 amino acids of the p66 subunit, which correspond to the p15 fragment cleaved from the p66 subunit by the viral protease to generate the p51 subunit.

Several crystal structures of free, unliganded HIV-1 RT have been solved (Esnouf et al., 1995; Hsiou et al., 1996; Rodgers et al., 1995). The three-dimensional structure of the p66 subunit is often compared to a right hand (Fig. 1), with a fingers (amino acids 1–85 and 118–155), a palm (amino acids 86–117 and 156–237) and a thumb (amino acids 238–318) domain (Kohlstaedt et al., 1992). The palm domain contains the polymerase active site with its three aspartic acids (110, 185 and 186) and the YMDD characteristic motif. Co-crystals of RT with a modified oligonucleotide and a dNTP (Huang et al., 1998) or double-stranded DNA (Jacobo-Molina et al., 1993) have revealed that the nucleic acid passes in the cleft behind the fingers and in front of the thumb domain. The catalytic pocket is formed by the fingers folding down into the palm domain, as observed in the

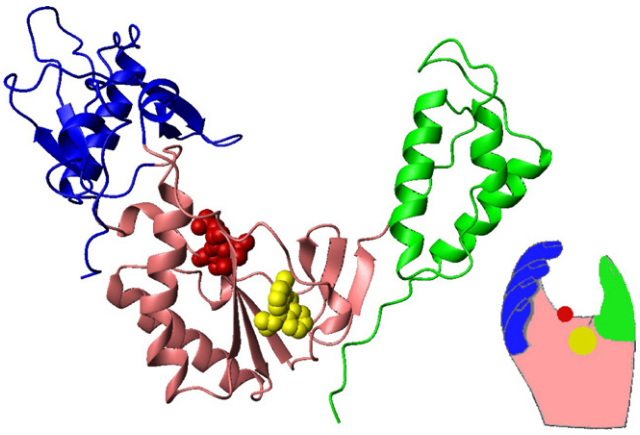

Fig. 1. Ribbon representation of the active domain of RT, accessed on June 14, 2009 at http://www.psc.edu/science/Madrid/getting_a_grip_on_aids.html . This ribbon representation of the RT active domain illustrates its hand-like structure, showing fingers (blue), palm (pink) and thumb (green). The active site (red atoms), where DNA is elongated, is in the palm region. Also shown is an RT-inhibitor drug (yellow) in the pocket where it binds.

RT-dNTP complex (Huang et al., 1998). In this structure, the nucleic acid is located in front of both the fingers and the thumb.

Next to the catalytic domain, the p66 subunit also contains the RNaseH domain (amino acids 427–560), linked to the former by the connection domain (amino acids 319–426). The connection domain is also involved in interactions with the nucleic acid and the p51 subunit.

Despite their sequence homology, the p66 subunit assumes a flexible and open structure, whereas the p51 subunit is rather compact, and seems to play a structural role, devoid of catalytic activity, with the three aspartic acids buried inside (Kohlstaedt et al., 1992).

3. The reverse transcription reaction

Reverse transcription is a complex process that requires different catalytic activities. A tRNA3Lys functions as a primer and hybridizes to the primer-binding site located near the 5′ end of the viral genome. This results in the synthesis of a short stretch of single stranded DNA, which then relocates and hybridizes to the repeat sequence at the 3′-end of the RNA genome. After this strand transfer, the synthesis of the first DNA strand can continue. The synthesis of the first DNA strand requires multiple enzymatic activities: RNA-primed RNA-dependent DNA polymerization (RDDP), DNA-primed RDDP, strand-transfer, and RNaseH activity. The RNaseH hydrolyzes the copied RNA template prior to strand-transfer, it is embedded in the RT enzyme. The synthesis of the second DNA strand uses as primers RNA fragments still hybridized to the first DNA strand like, e.g. the polypurine tract, because of the RNaseH-resistant conformation of those RNA–DNA duplexes. The second DNA strand synthesis also requires a strand transfer reaction.

4. Reverse transcriptase inhibitors

During its replication HIV-1 uses four different viral enzymes, all encoded by the Pol gene: the RT, the RNAseH, which is embedded in the RT, the integrase and the protease. Protease, RT and integrase are targets for antiretroviral drugs. Two classes of antiretroviral drugs inhibit the reverse transcriptase reaction: the N(t)RTIs and the NNRTIs. Table 1 summarizes the characteristics of the two classes of HIV-1 RT inhibitors, and highlights the differences between N(t)RTIs and NNRTIs.

N(t)RTIs are structural analogs of the natural substrates of the enzyme (the deoxyribonucleosides), which lack the 3′-hydroxyl group on their sugar moiety. Like their natural counterparts, they need to be converted by host-cell kinases in 5′-triphosphate nucleotides, in order to be incorporated in the nascent DNA. In those reactions they compete with the analogous deoxy-nucleotides-tri-phosphate (dNTPs), and their incorporation into the newly synthesized DNA results in termination of the DNA synthesis (Balzarini and De Clercq, 1998). N(t)RTIs can thus be considered as pro-drugs. N(t)RTIs are active against HIV-1 RT, and also against the RTs of other retroviruses, e.g. HIV-2, SIV, murine leukemia virus, visna virus, etc. (Balzarini and De Clercq, 1996, 1998). For a recent review on this class of inhibitors see Cihlar and Ray (2010).

The NNRTI class is characterized by a high chemical diversity, with more than 50 families of molecules (Balzarini, 2004; De Clercq, 1998, 2004; Jochmans, 2008; Jochmans et al., 2009). As a matter of fact the vast majority of hits observed in screening campaigns for anti HIV-1 compounds using cell-based assays are NNRTIs. The first NNRTIs to be identified were the 1-(2-2-hydroxyethoxymethyl)-6-(phenylthio)thymine (HEPT) (Baba et al., 1989; Miyasaka et al., 1989) and tetrahydroimidazo[4,5,1-jkj][1,4]benzodiazepin-2(1*H*)-one and -thione (TIBO) compounds (Debyser et al., 1991; Pauwels et al., 1990). NNRTIs inhibit RT by binding to the enzyme in a hydrophobic pocket located at a distance of around 10 Å from its catalytic site. The interaction of the compounds with the RT induces conformational changes that impact the catalytic activities of the enzyme (Sluis-Cremer et al., 2004). Unlike the N(t)RTIs, NNRTIs are active as such, and do not need intracellular metabolisation. NNRTIs are highly specific inhibitors of HIV-1, they are not active against other retroviruses (Balzarini and De Clercq, 1996, 1998). This specificity results in high selectivity indexes (ratio of in vitro cytotoxicity over antiviral activity) for this class of compounds (Balzarini, 2004).

5. The NNRTI binding pocket

Despite the chemical heterogeneity of NNRTIs, they all bind at the same site in the RT. This binding site is located in the palm domain of the p66 subunit of the heterodimeric protein, between the β6–β10–β9 and β12–β13–β14 sheets, and at the basis of the β4–β7–β8 sheet, at a distance of approximately 10 Å from the catalytic site of the enzyme. This pocket is hydrophobic in nature and is lined by the aromatic (Y181, Y188, F227, W229, and Y232), hydrophobic (P59, L100, V106, V179, L234, and P236), and hydrophilic (K101, K103, S105, D132, and E224) amino acids of

Table 1
Characteristics of HIV RT inhibitors: N(t)RTIs and NNRTIs.

Characteristic	N(t)RTIs	NNRTIs
Chemical structure	Analogs of the natural substrates, i.e. nucleosides	Chemically diverse, non-nucleoside
Active form	Metabolic conversion to 5′-triphosphates by host-cell enzymes	No metabolic conversion
Mechanism of action	Incorporate into growing DNA chain, terminate chain synthesis	Induce conformational changes in RT, reducing catalytic activities
Type of inhibition	Competitive with the natural substrates (dNTPs)	Non-competitive/uncompetitive
Binding site on the RT	Catalytic site	Allosteric (non-substrate) hydrophobic pocket
Spectrum	Broad spectrum antiretrovirals	HIV-1 specific RT inhibitors
Selectivity	Low to moderate	Very high

the p66 subunit, and two amino acids of the p51 subunit (I135 and E138). In the crystal structures of unliganded RT, the NNRTI binding pocket is not observed, but it is created when an inhibitor binds to the enzyme (Kohlstaedt et al., 1992). Indeed, the binding of an NNRTI induces a conformational change that rotates the side chains of the Y181 and Y188 amino acids up toward the catalytic site (Hsiou et al., 1996). This results in a concomitant shift of the β4–β7–β8 sheet and the three catalytic aspartic acid residues of ~2 Å (Esnouf et al., 1995; Ren et al., 1995). These conformation changes, induced by the binding of the NNRTIs to the RT are thought to be at the basis of their inhibitory action against the enzyme. Noteworthy, the overall shape of the pocket does not vary significantly, even if the ligands are chemically very different.

6. Mechanisms of inhibition of HIV-1 replication by NNRTIs

The discovery of the TIBO compounds and the unexpected finding that they were RT inhibitors (Kukla et al., 1991; Pauwels et al., 1990) triggered research to define the mechanism of action of this family of compounds. This led to the definition of the NNRTI class. Indeed, even if the HEPT compounds had been described earlier (Baba et al., 1989), they had been originally designed as NRTIs (Miyasaka et al., 1989), and it is only later that it was suggested that they would share a common mechanism of action with the TIBO compounds (Baba et al., 1991; Debyser et al., 1992; Pauwels, 1993).

The fact that TIBO compounds effectively were RT inhibitors was derived from a series of experimental observations: (1) time of addition experiments showed a profile compatible with the inhibition of the reverse transcription reaction (Pauwels et al., 1990); (2) they were active only against HIV-1 RT, and not other RTs, ruling out an aspecific inhibitory mechanism like is the case for suramin and other poly-anionic substances (Debyser et al., 1991); (3) they exhibited a template-primer dependency, with a lower IC_{50} value when poly(C)/oligo(dG) was used, as compared to poly(A)/oligo(dT) (Debyser et al., 1991). The specificity of the NNRTIs for HIV-1 remains a hallmark of the NNRTI drug class.

Over the years different types of studies have been performed to gain a better understanding of the mechanisms by which NNRTIs inhibit the HIV-1 RT, and HIV-1 replication: resolution of co-crystals of HIV-1 RT with different NNRTIs, enzyme inhibition kinetics, RT dimerization, and others. For a more detailed review, see Sluis-Cremer and Tachedjian (2008).

6.1. Structural biology studies with HIV-1 RT

Numerous co-crystal structures of HIV-1 RT unliganded, complexed with primer/template and dNTPs, or with bound NNRTIs have been resolved, allowing for computational studies of the structural changes associated with NNRTI binding (for a review, see Zhou et al., 2006).

The conformation of the catalytic site of RT is impacted by NNRTI binding: the position of the YMDD motif is affected, especially of the D185 and D186 residues that coordinate the Mg^{2+} ions in the active site (Esnouf et al., 1995), as well as that of the structural elements that constitute the "primer grip", i.e. a hairpin in the palm domain (amino acids 227–235) of the p66 subunit of RT that positions the DNA primer in the active site (Hsiou et al., 1996).

Another major change observed upon NNRTI binding is the position of the thumb region in the p66 subunit. This change could result in a decreased thumb mobility (the "arthritic thumb" model), thus slowing or even preventing the translocation of the primer/template, and the elongation of the nascent DNA strand (Kohlstaedt et al., 1992; Tachedjian and Goff, 2003; Tantillo et al., 1994). Alternatively, thumb mobility would not be affected by NNRTI binding, but rather the direction of movement of the thumb and other RT domains involved in primer/template translocation (Temiz and Bahar, 2002).

The various mechanisms proposed are not mutually exclusive. However, because of the lack of crystal structures of RT complexed with the primer/template, dNTP and bound NNRTI, it is not possible to determine in how much each of the proposed mechanisms contribute to the inhibition.

6.2. Enzyme kinetics studies with HIV-1 RT

Initial steady-state kinetic studies have shown that the TIBO compounds are non-competitive RT inhibitors with regard to the substrate, i.e. they bind to a site, often called allosteric, different from the catalytic site of the enzyme without changing the apparent binding affinity of the enzyme for the substrate, and uncompetitive relative to the primer/template, i.e. that the inhibitors bind only to the enzyme-substrate complex (Debyser et al., 1991). This inhibition mode was confirmed in later studies with other NNRTIs: the inhibitors bind independent of the substrate (Althaus et al., 1993; Ren et al., 2000). Some NNRTIs however, show increased inhibitor binding in the presence of the dNTP (uncompetitive inhibition) and are designated mixed noncompetitive/uncompetitive inhibitors (Fletcher et al., 1995; Maga et al., 2000). Those studies also observed an uncompetitive inhibition relative to the primer/template, with a minor non-competitive component. Those different inhibition modes appear to take place at different concentrations ranges of the inhibitors assessed in the assays.

Pre-steady state kinetic experiments showed that in general, NNRTI binding does not inhibit and even seems to improve the binding of dNTP and primer/template to the RT (Rittinger et al., 1995). This could be a consequence of the repositioning of the thumb upon NNRTI binding. Alternatively, in silico studies suggest that NNRTI binding would trigger a decreased flexibility of almost all RT amino acids, which would allow higher affinity for both primer/template and dNTP (Zhou et al., 2005, 2006). Further pre-steady kinetic experiments confirmed that NNRTIs do not directly inhibit the phosphodiester bond formation but rather, as already suggested from the structural data, prevent the correct positioning of the dNTP relative to the primer/template terminus (Spence et al., 1995; Xia et al., 2007). This would yet be an additional mechanism by which NNRTIs inhibit RT. The increase in binding affinity of dNTPs, and hence NRTI-TPs, for RT in the presence of bound NNRTIs could account for the strong synergy observed between the two classes of RT inhibitors, and explain the potent efficacy of treatment regimens combining two NRTIs and an NNRTI, typically used as first line therapy.

6.3. RNaseH and strand transfer reactions

Although NNRTIs bind to RT around 60 Å away from the RNaseH active site, several studies have demonstrated that they can either partially inhibit or accelerate RNaseH activity depending on the RNA/DNA template and the inhibitor used (Gopalakrishnan and Benkovic, 1994; Hang et al., 2007; Palaniappan et al., 1995; Radzio and Sluis-Cremer, 2008; Shaw-Reid et al., 2005). Other studies showed that both DNA polymerase and RNase H inhibition efficiencies of NNRTIs influenced their inhibitory potencies of strand transfer reactions (Hang et al., 2006). The repositioning of the primer/template upon binding of NNRTIs to RT may explain their influence on the RNaseH activity. Plus strand synthesis initiation in particular is sensitive to NNRTIs, probably because of a stabilization of the RT in an RNaseH cleavage mode upon NNRTI binding. This in turn inhibits polymerization, and would be an additional mechanism by which NNRTIs inhibit the reverse transcription reaction (Grobler et al., 2007).

6.4. RT dimerization and late stages of HIV-1 replication

Some potent NNRTIs have been shown to enhance RT heterodimerization: this was the case for efavirenz, less so for nevirapine, and not at all for delavirdine (Tachedjian et al., 2001). This effect does not seem to play a role in the inhibition of the reverse transcription reaction by the NNRTIs (Xia et al., 2007). RT dimerization while the protein is still embedded in the GagPol polyprotein triggers protease dimerization, and the protease in turn processes the Gag and GagPol polyproteins. If this happens at a high rate intracellularly, the supply of polyproteins to be incorporated in the budding virions is decreased, and less viral particles can be produced. This is what has been observed in cells treated with efavirenz, dapivirine or etravirine (Figueiredo et al., 2006). However, the significance of this proposed mechanism of action for particular NNRTIs is unknown, as the effect is seen at much higher inhibitor concentrations than those required to inhibit RT.

6.5. Conclusion

NNRTIs appear to interfere with different steps in the reverse transcriptase reaction. Their binding to the HIV-1 RT triggers conformational changes in the enzyme, which seem responsible for the observed effects on the catalytic activity of the RT, even if the exact molecular mechanisms are still hypothetical. Crystals of the HIV-1 RT complexed with the primer/template, dNTP and bound inhibitors should help evaluate the validity of the proposed mechanisms. Moreover, the enhancement of RT dimerization by some NNRTIs, and its subsequent effect on viral particle production are interesting models for the discovery of drugs targeted at the GagPol polyprotein.

7. Mechanisms of HIV-1 resistance to NNRTIs

Resistance is the cause and/or the consequence of treatment failure. HIV infection is characterized by a very high replication rate, with the production of 1 to 10 billion new virus particles per day in an untreated infected individual (Perelson et al., 1996). Moreover, HIV-1 RT lacks exonucleolytic proof-reading functionality, and this results in an average error rate per detectable nucleotide incorporated of 1/1700 (Roberts et al., 1988). Combining these two factors with the length of the viral genome (~10,000 nucleotides), it can be calculated that a mutant at each nucleotide position in the viral genome is produced every day. As a consequence, suboptimal treatments, like monotherapy regimens, will readily select the mutants in the replicating population that are resistant to the administered drug(s). Moreover, the selected drug resistant viruses will compromise the efficacy of subsequent HAART regimens, as extensive cross-resistance was rapidly observed within each class of antiretroviral drugs (Hertogs et al., 2000; Miller et al., 1998; Shulman and Winters, 2003). For a recent review on HIV-1 drug resistance, see Menendez-Arias (2010).

In this section, the genotypic and phenotypic determinants of NNRTI-resistance are covered, and the underlying structural mechanisms, as well as considerations on the fitness of resistant viruses, and the clinical relevance and significance of those observations. Specific resistance determinants are addressed in the respective sections devoted to the different NNRTIs described in this review (see Section 8).

7.1. Determinants of genotypic NNRTI-resistance

Table 2 presents a list of mutations associated with resistance to NNRTIs. Originally, mutations described to be associated with resistance to NNRTIs, both in vitro and in vivo, were all located in the NNRTI binding pocket. Those mutations are mainly observed in domains spanning amino acids 98–108, 178–190 and 225–238 of the p66 subunit (Soriano and de Mendoza, 2002). The most prevalent substitutions observed in viruses from patients who have been on a failing NNRTI containing regimen are K103N and Y181C (Cheung et al., 2004; Tambuyzer et al., 2009). Another mutation, Y318F, outside the originally identified domains has also been reported, which was missed by several genotyping assays, as they did not extend far enough in the 5′ part of the pol gene (Harrigan et al., 2002; Pelemans et al., 1998). Additionally, mutations at the E138 position cause resistance to NNRTIs, even when they are present in the p51 subunit only (Jonckheere et al., 1994). Over 40 amino acid substitutions have been identified to be associated with NNRTI resistance, in vitro and in vivo (Table 2) (Ceccherini-Silberstein et al., 2007; Tambuyzer et al., 2009).

More recently, other mutations, outside the NNRTI binding pocket have been reported in NRTI-treated patients: they are located in the connection domain of the p66 subunit, between the thumb and the RNaseH domains (Nikolenko et al., 2007; Santos et al., 2008; Waters et al., 2009). The N348I substitution has been associated with resistance to both AZT and nevirapine (Yap et al., 2007). A session was devoted to this topic at the XVIII International HIV Drug Resistance Workshop—basic principles and clinical implications, held at Sanibel Harbour Resort, Fort Myers, FL, USA, June 9–13, 2009: presented results indicated that HIV-1 susceptibility to nevirapine, but not to efavirenz nor to etravirine was reduced by the presence of mutations in the connection domain (Brehm et al., 2009; Gupta et al., 2009b; Nikolenko et al., 2009; Vingerhoets et al., 2009).

NNRTI resistance mutations impact the binding of the molecules in the NNRTI binding pocket (see Section 7.4), but some of those mutations have also been described to influence functionalities of RT other than DNA polymerisation: the V106A and P236L mutations cause a slowing in the RNA 5′-end and DNA 3′-end directed RNaseH cleavage activities (Archer et al., 2000; Gerondelis et al., 1999); in contrast, the Y181C mutation shows an acceleration of RNaseH cleavage activities (Archer et al., 2000); in the presence of the Y188L mutation, resistance to efavirenz was 30-fold higher in strand transfer assays than in polymerase or RNaseH assays (Hang et al., 2006).

Most of the genotypic analyses of NNRTI resistance have been conducted in patients infected with clade B HIV-1. Because of the differences in nucleotide sequences among the clades of HIV-1, different resistance associated mutations and mutation patterns can be expected. This was indeed the case with the identification of the V106 M substitution in clade C viruses exposed to efavirenz (Brenner et al., 2003). The prevalence of mutations also varies among different clades (Grossman et al., 2004). These observations are not restricted to the NNRTI class of antiretroviral drugs, and call for the upscaling of genotypic interpretation systems to non-B clades, which are most prevalent in developing countries where the vast majority of HIV patients are found.

7.2. Phenotypic susceptibility to NNRTIs

In vitro phenotypic assays can determine susceptibility, and hence resistance of HIV-1 to antiretroviral drugs. They have been key in the correlation between the presence of mutations, single or multiple, and decreased (or increased) susceptibility of the virus to the drugs. A virus is considered resistant to a particular drug if the decrease in susceptibility, generally expressed as fold change in EC_{50} value as compared with a susceptible, well characterized laboratory strain, is above the biological cut-off for that drug. Biological cut-offs are usually determined by the 95% confidence interval (CI) of the distribution of EC_{50} values observed for a large number of wild-type, drug susceptible viruses. Any EC_{50} value outside this range will return a resistance (value is above the 97.5% limit of

Table 2
Common NNRTI resistance associated mutations, and their impact on the susceptibility of HIV-1 to NNRTIs[a].

NNRTI RAM	Prevalence in Samples for RCRT (%)	Prevalence in NNRTI-Resistant Samples (%)	Number of Nucleotide Substitutions (Codon Change)	FC[b] in SDMs							Reference(s) in which the association with NNRTI resistance was demonstrated
				EFV	NVP	ETR	TMC278[d]	RDEA806[e]	IDX899[f]	UK453061[g]	
V90I	4.68	6.84	1 (GTT → ATT)	1.7	3.8	1.5	NA	NA	0.9	NA	Vingerhoets et al. (2005, 2007)
A98G	3.46	7.76	1 (GCA → GGA)	2.2	8.1	2.5	NA	NA	NA	NA	Byrnes et al. (1993), Bacheler et al. (2000)
L100I	2.96	6.92	1 (TTA → ATA)	13.1	NA	1.8	0.8	NA	0.4	2.9	Johnson et al. (2007), Mellors et al. (1993)
K101E	3.89	9.01	1 (AAA → GAA)	2.9	4.3	1.7	2.5	NA	NA	8.7	Byrnes et al. (1993), Bacheler et al. (2000)
K101P	0.87	2.04	2 (AAA → CCA)	97.4	>733.4	6.2	46.5	NA	NA	NA	Rhee et al. (2004)
K101Q	3.02	5.95	1 (AAA → CAA)	3.8	NA	3.4	1.4	NA	NA	NA	Bacheler et al. (2000), Kleim et al. (1999), Ceccherini-Silberstein et al. (2007)
K103H	0.08	0.18	2 (AAA → CAC)	15.6	17.8	1.6	NA	NA	NA	NA	Harrigan et al. (2005)
K103N	24.34	56.96	1 (AAA → AAC)	26.7	56.2	0.7	0.8	0.5	1.1	NA	Johnson et al. (2007), Nunberg et al. (1991)
K103S	1.11	2.6	2 (AAA → AGC)	4.9	36.2	0.9	1.2	NA	NA	NA	Rhee et al. (2004), Kleim et al. (1999)
K103T	0.07	0.17	1 (AAA → ACA)	1.4	>37.0	1.3	NA	NA	NA	NA	Rhee et al. (2004)
V106A	1.01	2.37	1 (GTA → GCA)	1.8	86.1	0.5	0.7	NA	NA	3.1	Johnson et al. (2007), Larder (1992)
V106I	2.62	3.47	1 (GTA → ATA)	NA	NA	NA	NA	NA	NA	NA	Vingerhoets et al. (2007), Kleim et al. (1997), Taylor et al. (2000)
V106M	0.49	1.15	2 (GTA → ATG)	2.3	6.9	0.8	1	NA	NA	NA	Johnson et al. (2007), Loemba et al. (2002)
V108I	4.81	10.68	1 (GTA → ATA)	1.2	2.7	0.5	NA	NA	NA	4.9	Johnson et al. (2007), Byrnes et al. (1993)
E138G	0.42	0.8	1 (GAG → GGG)	2.3	NA	3.8	NA	NA	NA	NA	Pelemans et al. (2001)
E138K	0.34	0.5	1 (GAG → AAG)	1.8	1.7	2.4	2.2	NA	0.8	5.8	Balzarini et al. (1994)
E138Q	0.42	0.91	1 (GAG → CAG)	7.1	NA	5.1	NA	NA	NA	NA	Pelemans et al. (2001), McCreedy et al. (1999)
V179D	1.75	3.25	1 (GTT → GAT)	6.2	5.7	2.6	1.6	NA	NA	NA	Byrnes et al. (1993), Palmer et al. (2003)
V179E	0.75	1.47	2 (GTT → GAG)	4	2.6	1.1	1.1	NA	NA	NA	Byrnes et al. (1993), Vingerhoets et al. (2006)
V179F	0.15	0.34	1 (GTT → TTT)	<0.4	1.6	0.1	0.1	NA	NA	NA	Vingerhoets et al. (2005, 2006)
V179G	0.08	0.15	1 (GTT → GGT)	0.6	NA	0.6	NA	NA	NA	NA	Miller et al. (2003)
V179I	8.55	11.82	1 (GTT → ATT)	0.9	1.3	0.8	NA	NA	NA	NA	Turner et al. (2004)
Y181C	10.66	24.95	1 (TAT → TGT)	2.2	207.6	3.9	2.6	3.2	2.7	2.2	Johnson et al. (2007), Nunberg et al. (1991)
Y181I	0.45	1.05	2 (TAT → ATT)	1.6	>55.5	12.5	14.3	NA	11.5	0.8	Johnson et al. (2007), Shih et al. (1991)
Y181V	0.27	0.63	2 (TAT → GTT)	2.8	2155.9	17.4	12.6	NA	NA	NA	Vingerhoets et al. (2006), Shih et al. (1991)
Y188C	0.18	0.43	1 (TAT → TGT)	2.1	36.5	0.2	NA	NA	NA	0.3	Johnson et al. (2007), Richman (1993)
Y188H	0.44	1.02	1 (TAT → CAT)	7.6	5.5	0.3	NA	NA	NA	NA	Johnson et al. (2007), Sardana et al. (1992)
Y188L	2.72	6.37	2 (TAT → CTT)	31.9	173.4	0.9	2.8	6.3	NA	NA	Johnson et al. (2007), Shih et al. (1991)
V189I	1.73	2.79	1 (GTA → ATA)	1.2	2.9	0.8	NA	NA	NA	NA	Vingerhoets et al. (2007), Kleim et al. (1996)
G190A	8.16	19.09	1 (GGA → GCA)	6.8	105	0.8	1	NA	0.4	NA	Johnson et al. (2007), Bacolla et al. (1993)
G190C	0.07	0.17	2 (GGA → TGC)	NA	NA	NA	NA	NA	NA	NA	Huang et al. (2003)
G190E	0.29	0.67	1 (GGA → GAA)	NA	NA	NA	NA	NA	NA	NA	Bacheler et al. (2000), Kleim et al. (1993)
G190Q	0.2	0.47	2 (GGA → CAA)	NA	NA	NA	NA	NA	NA	NA	Huang et al. (2003), Kleim et al. (1994)
G190S	1.66	3.89	2 (GGA → AGC)	94.4	177.1	0.2	0.2	NA	NA	NA	Johnson et al. (2007), Kleim et al. (1994)
H221Y	3.75	8.34	1 (CAT → TAT)	2.4	4.4	2.5	NA	NA	NA	NA	Ceccherini-Silberstein et al. (2007), Gonzales et al. (2003), Saracino et al. (2006), Perno et al. (2006)
P225H	2.34	5.38	1 (CCT → CAT)	2.2	2.8	1	NA	NA	0.3	NA	Johnson et al. (2007), Bacheler et al. (2000), Kleim et al. (1999)
F227C[c]	0.01	0.02	1 (TTC → TGC)	27.2	24.3	3.6	NA	NA	NA	56.4	Andries et al. (2004)
F227L	0.97	2.24	1 (TTC → CTC)	0.7	2.9	0.4	NA	NA	NA	6.3	Balzarini et al. (1994), Parkin et al. (2000)
M230I	0.08	0.19	1 (ATG → ATA)	4.7	13.1	2.4	NA	NA	NA	NA	Kleim et al. (1999)
M230L	0.5	1.16	1 (ATG → CTG)	5.7	13.9	3.4	2.5	NA	1.5	NA	Rhee et al. (2004), Huang et al. (2003)
P236L	0.09	0.21	1 (CCT → CTT)	2.4	4.6	1.3	NA	NA	NA	0.3	Johnson et al. (2007), Dueweke et al. (1993a,b)
K238N	0.35	0.76	1 (AAA → AAC)	2.7	NA	1.7	NA	NA	NA	NA	The Stanford University HIV Drug Resistance Database (2007)
K238T	1.79	4.11	1 (AAA → ACA)	3.4	NA	2.4	NA	NA	NA	NA	Rhee et al. (2004), Demeter et al. (1998)
Y318F	0.85	1.97	1 (TAT → TTT)	0.6	1.5	1.4	NA	NA	NA	NA	Pelemans et al. (1998), Harrigan et al. (2002)

Abbreviations: NNRTI: non-nucleoside reverse transcriptase inhibitor; RAM: resistance-associated mutation; RCRT: routine clinical resistance testing; FC: fold change in 50% effective concentration; SDM: site-directed mutant; EFV: efavirenz; NVP: nevirapine; ETR: etravirine; NA: not available.

[a] Adapted from Tambuyzer et al. (2009).

[b] FC values were determined for EFV, NVP, ETR and TMC278 as described in Vingerhoets et al. (2005). Results were reported as median values from 2 or more measurements.

[c] This SDM also contained the L234I mutation in the RT.

[d] FC values for TMC278 were extracted from Rimsky et al. (2009).

[e] FC values for RDEA806 were calculated from data presented in Girardet et al. (2007).

[f] FC values for IDX899 were calculated from data presented in Jakubik et al. (2008).

[g] FC values for UK453061 were extracted from Corbau et al. (2007).

the CI) or hypersusceptibility (value is below the 2.5% limit of the CI) call. Phenotypic testing has been used to confirm the role and importance of mutations observed either in viruses selected in the presence of the drugs in vitro, or in viruses emerging in patients on failing NNRTI containing regimens.

Interestingly, phenotypic studies have shown that certain combinations of drug resistance associated mutations can make the virus hypersusceptible to certain antiretroviral drugs. In the case of NNRTIs, particular NRTI resistance associated mutations in combination with NNRTI mutations cause hypersusceptibility to NNRTIs (Clark et al., 2006; Shulman et al., 2001). The patterns of mutations involved in hypersusceptibility to NNRTIs are complex and involve multiple mutations (Whitcomb et al., 2002). Noteworthy, it has also been observed that certain NNRTI mutations cause hypersusceptibility to NRTIs (Ambrose et al., 2009; Larder, 1992). The clinical relevance of hypersusceptibility to NNRTIs has been assessed in the clinic: several studies show increased virologic response associated with hypersusceptibility (Demeter et al., 2008; Haubrich et al., 2002; Katzenstein et al., 2003; Shulman et al., 2001), although one study showed this better virologic response to be transient (Tozzi et al., 2004). Although interesting, the clinical use of hypersusceptibility to NNRTIs awaits further guidance (Tachedjian and Mijch, 2004; Delgado and Shulman, 2005).

7.3. Clinical relevance of phenotypic resistance testing for NNRTIs

The interpretation of phenotypic resistance testing requires the availability of cut-off values that are correlated to virologic outcome in the clinic. Cut-off values are expressed as a fold change in susceptibility of the patient's virus to a particular drug above which the activity of that drug starts decreasing (lower cut-off), or is lost (upper cut-off). The definitions of decrease in activity and loss of activity vary among the different phenotypic resistance testing providers. Typically, these clinical cut-offs are derived from clinical studies assessing the efficacy of an investigational drug, or of a new regimen. Clinical cut-offs are best determined in treatment-experienced patient populations infected with viruses with varying levels of resistance to the studied drugs. First generation NNRTIs (nevirapine, delavirdine and efavirenz) have been studied essentially in treatment-naïve patients, and are indicated for use in combination therapy in this population. It is thus not possible to determine valid clinical cut-offs for those. Moreover, clinical experience has shown that a first generation NNRTI should not be used after virologic failure of another first generation NNRTI, even if the emerging virus remains susceptible to the former (Antinori et al., 2002; Lecossier et al., 2005). The presence of one NNRTI resistance associated mutation is an indicator of cross-resistance to first generation NNRTIs, and is used as such in clinical practice. Genotypic resistance testing remains the best tool to guide the use of these drugs. In contrast, clinical cut-offs could be determined for etravirine (see Section 8.2.1), the only NNRTI so far studied in patients with NNRTI resistance, and phenotypic resistance testing is a valuable tool for guiding the use of this drug.

7.4. Structural basis for mechanisms of resistance to NNRTIs

The structural basis for resistance mechanisms to NNRTIs comes from numerous crystallography, docking and computational modeling studies with RT containing NNRTI resistance associated mutations (see review by Ren and Stammers, 2008). Nevirapine and delavirdine rely heavily on ring stacking interactions with Y181 and Y188 for their binding to the enzyme, it is therefore not surprising that substitutions by non-aromatic amino acids at those positions drastically reduce the binding affinity of these inhibitors for RT. This is not the case for efavirenz, which does not show extensive contact regions with Y181. The most prevalent NNRTI resistance associated mutation is K103N. Several models have been proposed to explain the mechanism by which this substitution causes resistance to the first generation NNRTIs, including the formation of an H-bond between the asparagine at position 103 and the tyrosine at position 188 preventing the interaction of the inhibitors with the mutated RT (Das et al., 2007; Hsiou et al., 2001). However, interaction kinetic studies of NNRTIs with mutant HIV-1 RTs contradict this hypothesis (Geitmann et al., 2006). Further studies are needed to fully elucidate the structural role of the K103N mutation in HIV-1 resistance to first generation NNRTIs, and its lack of effect on the susceptibility of the virus to next generation NNRTIs, e.g. etravirine (Ren and Stammers, 2008) (see also Section 8.2.1).

7.5. Fitness and persistence of NNRTI resistant HIV-1

Numerous in vitro studies have assessed the fitness and/or the replication capacity of HIV-1 strains harbouring NNRTI resistance associated mutations (see reviews by Buckheit Jr., 2004, and Martinez-Picado and Martínez, 2008). The K103N mutation has little impact on the fitness of HIV-1: it showed only slightly reduced replication capacity compared to the wild-type in competition experiments, but outgrew rapidly the wild-type in the presence of NNRTIs (Gerondelis et al., 1999; Collins et al., 2004; Gatanaga et al., 2006). Other mutations (L100I, V179D, Y181C/I, and P225H) have a little to moderate impact on replication capacity (Antinori et al., 2001; Archer et al., 2000; Huang et al., 2002). In contrast, the mutations V106A, G190S/C/Q/E/T/V, but not G190A, and P236L all have a serious impact on replication capacity (Huang et al., 2002, 2003). The compensatory mutation L74V is often observed in viruses harbouring those mutations (Huang et al., 2003). In general, viral fitness decreases as the number of mutations increases (Collins et al., 2004).

In patients, NNRTI resistant HIV-1 tends to persist over long periods of time after discontinuation of NNRTI therapy. This is most probably related to the little to moderate impact on viral fitness of many NNRTI resistance associated mutations (Bangsberg et al., 2006; Gianotti et al., 2005; Joly et al., 2004; Koval et al., 2006). This might also account for the fact that transmitted NNRTI resistant HIV-1 is more prevalent than PI resistant HIV-1, as PI resistance associated mutations have a more severe impact on viral fitness (Booth et al., 2007; Gonsalez et al., 2007; Little et al., 2002, 2008; Ross et al., 2007; SPREAD Programme, 2008).

7.6. Prevention of NNRTI resistance development

Resistance associated mutations accumulate readily in vitro under selection pressure of NNRTIs, increasing the level of resistance to the drugs. A similar accumulation of mutations has been observed in treated patients who remain on an NNRTI containing failing regimen. The viruses from those patients show high levels of resistance to the NNRTIs, and also to the underlying therapy, thus reducing the number of options for subsequent HAART regimens (Deeks, 2001). This is particularly the case in developing countries, where patient monitoring is less frequent than in the developed world (Chetchotisakd et al., 2006; Ferradini et al., 2006, 2007; Gupta et al., 2009a; Hamers et al., 2008). It is thus recommended to change the HAART regimen very rapidly when virologic failure happens during therapy with an NNRTI based treatment regimen to prevent the selection of viruses with resistance to all the drugs in the regimen, and the loss of future treatment options (Bartlett et al., 2006; DHHS, 2007; Gazzard, 2008).

Clinically used NNRTIs are characterized by long plasma half-lives. Hence, upon treatment interruptions, voluntary, or involuntary as a result of insufficient drugs supply in resource limited settings, detectable plasma levels of NNRTIs are observed for several days or weeks after the last intake, while this is not the

case for the concomitant drugs. Patients on treatment interruption end up being on NNRTI monotherapy and this in turn results in the appearance of NNRTI resistance (Darwich et al., 2008). Therefore, treatment guidelines advise to continue the administration of the other components of HAART, or to replace the NNRTI by a boosted PI, for some time after discontinuing the NNRTI (DHHS, 2007; Gazzard, 2008).

8. Clinical use of NNRTIs: treatment of HIV-1 infection

NNRTIs in combination with other antiretroviral drugs have been used for the treatment of HIV-1 infection for over a decade. So far, four drugs in this class have been approved by regulatory authorities, although several others have entered clinical development, but were discontinued for efficacy, pharmacokinetic and/or safety reasons. This review covers the approved NNRTIs, as well as those currently in clinical development. For an overview of other NNRTIs see Balzarini (2004), De Clercq (2004), Jochmans (2008), Jochmans et al. (2009), and Pedersen and Pedersen (1999).

The first generation NNRTIs were approved by the FDA in 1996 (nevirapine), 1997 (delavirdine), and 1998 (efavirenz). Two of them, nevirapine and efavirenz, are cornerstones of first line HAART, whereas delavirdine is barely used nowadays. They are characterized by a low genetic barrier to the development of resistance: they need to be combined with at least two other fully active non-NNRTI antiretroviral drugs, and resistance to one of them precludes subsequent use of the other first generation NNRTIs. They are generally safe and well tolerated, although hepatotoxicity and severe rash are associated with the use of nevirapine (Podzamczer and Fumero, 2001), whereas efavirenz causes CNS side effects that are sometimes difficult to overcome, especially in the first days of administration (Best and Goicoechea, 2008). They have a long plasmatic half life and are both given as a once daily regimen, which adds to the convenience of therapy, especially in treatment-naïve patients.

The confinement of these drugs to first line therapy triggered the research for next generation NNRTIs that would have a better resistance profile, in order to offer treatment experienced patients the option to benefit from the convenience and good safety profile of the NNRTI class. Etravirine was the first next generation NNRTI to be approved by the FDA, in combination with other antiretroviral agents for the treatment of HIV-1 infection in treatment-experienced adult patients, who have evidence of viral replication and HIV-1 strains resistant to an NNRTI and other antiretroviral agents (Tibotec Inc., 2008). The first presentation of in vitro data on the resistance profile of etravirine by Andries et al. (2000), and de Béthune et al. (2000) at ICAAC in 2000 revived the interest for the class, by showing that it was possible to design NNRTIs, which retained activity against HIV-1 strains with one or more NNRTI resistance associated mutations. There are currently four next generation NNRTIs in clinical development: rilpivirine, IDX-899, RDEA-428, and lersivirine. Fig. 2 shows the chemical structures of the NNRTIS described below.

8.1. First generation NNRTIs

8.1.1. Nevirapine (BI-RG-587, Viramune®)

Nevirapine is a dipyridodiazepinone inhibitor of HIV-1, discovered by researchers at Boehringer Ingelheim (Merluzzi et al., 1990). It is efficacious in treatment naïve patients when used as part of a fully suppressive regimen, as measured by both the control of virus replication and immunologic improvement (Lange, 2003). The most commonly selected resistance mutation in vivo is Y181C, but substitutions at positions 103, 106, 108, 188 and 190 are also frequently observed (Richman et al., 1994; Wainberg, 2003). When AZT is used in combination with nevirapine, an alternative pattern of mutations is observed, that does not include substitutions at position 181, once more illustrating the interplay between NRTI and NNRTI resistance (Richman et al., 1994). Nevirapine was the first NNRTI for which co-crystals with HIV-1 RT were obtained and resolved, helping to better understand the mechanism of action of this class of molecules (Smerdon et al., 1994).

At the time nevirapine was developed the concept of HAART was not yet established, and the drug was often studied in combination with a single NRTI, mostly AZT, a regimen that could not prevent the emergence of resistance (Havlir et al., 1995). Nevirapine was also assessed in treatment experienced patients, with results that nowadays would not be acceptable (Harris, 2003).

Nevirapine, in combination with two NRTIs is the recommended NNRTI for fist line therapy in resource limited countries. Being non teratogenic, it is also used for the prevention of mother to child transmission in developing countries. Nevirapine was shown to be superior to AZT, when given as single dose intrapartum (Guay et al., 1999). However, as administration of a single dose of nevirapine results in the selection of resistance in the treated mothers, which can negatively influence the efficacy of subsequent anti-HIV-1 treatment with NNRTIs, new strategies for the prevention of mother to child transmission need to be developed and evaluated (Mellors and Chow, 2009).

8.1.2. Delavirdine (U-90152, Rescriptor®)

Delavirdine belongs to the family of bis(hetero-aryl)piperazine compounds discovered by researchers at Upjohn Laboratories (subsequently Pharmacia, now Pfizer) (Dueweke et al., 1993a). It is bulkier than the other NNRTIs, and crystal structures of delavirdine with HIV-1 RT have shown that it protrudes outside the NNRTI binding pocket, which explains its particular resistance profile (Esnouf et al., 1997). The mutation mostly selected by this family of compounds is P236L, located at the mouth of the NNRTI binding pocket. This mutation causes hypersusceptibility to other NNRTIs (Dueweke et al., 1993b). Like nevirapine, delavirdine was originally assessed in suboptimal regimens, where emergence of resistance could not be prevented (Davey et al., 1996; Scott and Perry, 2000). Nowadays, delavirdine is very rarely used.

8.1.3. Efavirenz (DMP-266, Sustiva™, Stocrin™)

Efavirenz is a benzoxazinone discovered by the researchers at Merck (Young et al., 1995), and developed jointly by DuPont and Merck. Unlike nevirapine and delavirdine, efavirenz could be studied in Phase III trials as part of appropriate HAART regimens, and showed sustained efficacy (Staszewski et al., 1999). The most frequently selected mutation in efavirenz failures is K103N. Other substitutions observed in Phase II trials were V108I, P225H or L100I, K101E, K101Q, Y188H, Y188L, G190S, G190A, and G190E (Bacheler et al., 2000, 2001).

Efavirenz is the most used NNRTI in treatment naïve patients, in combination with two NRTIs. Recently it was co-formulated with tenofovir disoproxil fumarate and emtricitabine in one tablet marketed as Atripla™, providing the easiest and most convenient HAART regimen in one pill once a day (Goicoechea and Best, 2007).

8.2. Next generation NNRTIs

8.2.1. Etravirine (TMC125, Intelence™)

Etravirine belongs to the family of di-aryl-pyrimidine (DAPY) compounds, and is the result of a long lead optimization campaign conducted by researchers at the Janssen Research Foundation and Tibotec, aiming at identifying new NNRTIs with a better resistance profile and an increased genetic barrier to the development of resistance (Ludovici et al., 2001a,b,c). The screening process included the profiling of compounds against both wild type and selected single

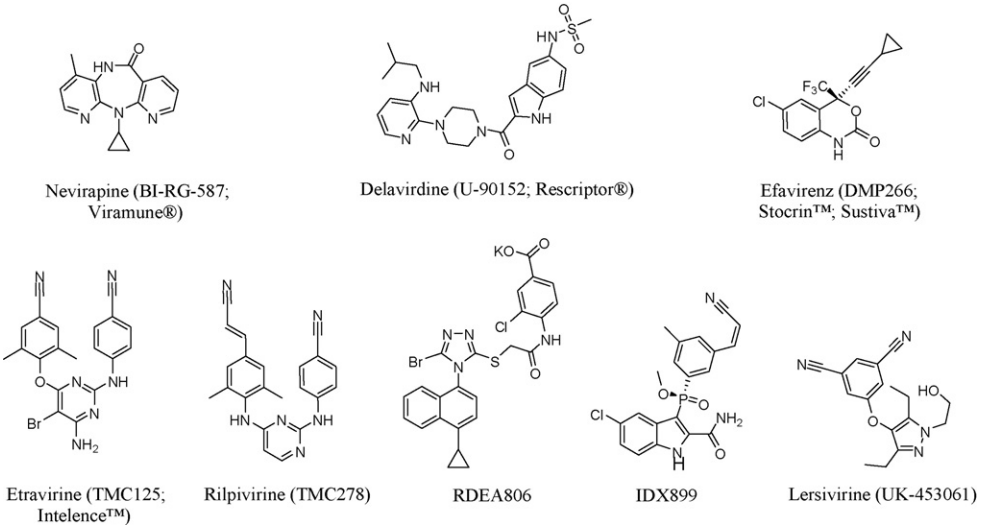

Fig. 2. Chemical structures of first generation NNRTIs (nevirapine, delavirdine, and efavirenz) and next generation NNRTIs (etravirine, rilpivirine, RDEA806, IDX899, and lersivirine).

and double mutant NNRTI resistant HIV-1 strains, as well as the assessment of their metabolic stability (Andries et al., 2004). The resistance profile of etravirine was further confirmed by testing the molecule against thousands of NNRTI resistant HIV-1 clinical isolates, representing the diversity of mutations patterns encountered in the clinic (de Béthune et al., 2000; Rimsky et al., 2009). In vitro, etravirine shows a higher genetic barrier to the development of resistance as compared to nevirapine and efavirenz (Vingerhoets et al., 2005). Co-crystals of etravirine with the K103N mutant RT helped study the binding mode of the molecule to the enzyme: it is hypothesized that the inhibitor can adopt different conformations in the NNRTI binding pocket, thanks to its flexibility, and can thereby accommodate the mutations better than first generation NNRTIs, which are more rigid molecules (Das et al., 2004, 2005).

The clinical development of etravirine was conducted exclusively in treatment experienced patients. The Phase III clinical trials (DUET-1 and DUET-2) enrolled patients with extensive NRTI and PI resistance, and documented evidence of NNRTI resistance, either at screening, or in previous resistance assessments (Lazzarin et al., 2007; Madruga et al., 2007). Those were patients with little to no available treatment options. The results of the week 96 analysis have been recently presented, and show a sustained superior response of the etravirine arms over placebo, as well as a safety profile comparable to placebo, except for rash (Trottier et al., 2009).

Further analyses of the DUET data have allowed to determine phenotypic and genotypic resistance determinants associated with virologic outcome of etravirine treatment. Phenotypic clinical cutoffs were identified (Peeters et al., 2008), and a weighted mutation score was established (Vingerhoets et al., 2008) to help in the interpretation of resistance testing results. Noteworthy, the mutations with the highest weight, Y181I, Y181V, K101P, L100I, Y181C, and M230L, were observed in less than 10% of the patients in the DUET trials, with the exception of Y181C (Vingerhoets et al., 2009). Confirming the in vitro data, K103N did not impact the antiviral efficacy of etravirine. Mutations emerging most frequently in the DUET trials were V108I, V179I, V179F, and Y181C, all in the background of NNRTI mutations present at baseline (Vingerhoets et al., 2007). Because etravirine was not studied in treatment naïve patients, its exact resistance profile cannot be determined.

The DUET trials confirmed the hypothesis that an NNRTI with a better resistance profile can be efficacious in patients with NNRTI resistant HIV-1. This represents a shift in treatment paradigm (Geretti, 2008), as sequential therapy with first generation NNRTIs is not recommended.

8.2.2. Rilpivirine (TMC278)

Rilpivirine is another DAPY compound, that is the result of further optimization within this family of NNRTIs, aiming at establishing interactions with the conserved amino acids of the NNRTI binding pocket, in this case W229 (Guillemont et al., 2005; Janssen et al., 2005). In vitro rilpivirine shows a resistance profile and a genetic barrier to the development of resistance comparable to those of etravirine (Rimsky et al., 2009). High resolution crys-

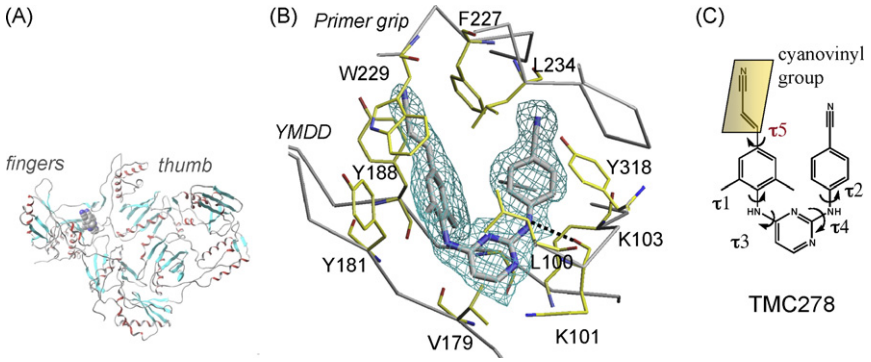

Fig. 3. Structure of HIV-1 in complex with TMC278. (A) Overall structure of the wildtype HIV-1 RT/TMC278 complex determined at 1.8 Å resolution. (B) The position and conformation of TMC278 were defined by the difference (|Fo| − |Fc|) electron density calculated at 1.8 Å resolution (3.5σ contours). (C) Chemical structure of TMC278. The τ angles define the torsional flexibility of TMC278 (reproduced from Das et al. (2008), with permission of the author).

tal structures of rilpivirine complexed with HIV-1 RT show that the molecule reaches deeper in the NNRTI binding pocket toward amino acid W229 than etravirine (Das et al., 2008) (Fig. 3). Both inhibitors exhibit a similar flexibility in adapting to the resistance mutations.

Rilpivirine has so far been studied only in treatment naïve patients. Unlike etravirine, which due to its poor solubility and poor bioavailability in crystalline form, has required extensive chemical formulation work, rilpivirine shows very good bioavailability, and is easier to formulate. The pharmacokinetics of rilpivirine allow for once a day dosing (de Béthune et al., 2005). The week 96 analysis of the dose finding Phase IIB trial (TMC278-C204) has shown sustained efficacy for all three doses studied, comparable to efavirenz in the control group (Molina et al., 2008). In the same analysis, TMC278 appeared to be generally better tolerated than efavirenz, with lower incidences of neuropsychiatric AEs, rash and fewer lipid disturbances than efavirenz (Pozniak et al., in press). Rilpivirine is currently studied at a dose of 25 mg once a day in Phase III confirmatory trials, in treatment naïve patients, and in double blind comparison with efavirenz.

With a daily dose of 25 mg, rilpivirine is an excellent candidate for co-formulation with other antiretroviral drugs. Recently Gilead Sciences and Tibotec Pharmaceuticals announced a license and collaboration agreement for the development and commercialization of a new once-daily fixed-dose antiretroviral regimen containing Gilead's Truvada® (emtricitabine and tenofovir disoproxil fumarate) and Tibotec's investigational NNRTI TMC278 (rilpivirine hydrochloride, 25 mg) for treatment-naïve HIV-infected individuals. Additionally, long-acting injectable formulations of the drug are investigated, that would have the advantage of increasing adherence to therapy (Baert et al., 2009).

8.2.3. RDEA806

A new family of triazole NNRTIs was presented at the 47th ICAAC in 2007 by researchers from Ardea Biosciences. The screening strategy was similar to the one used for the discovery of etravirine and rilpivirine. The selected clinical candidate is RDEA806, it belongs to the family of triazoles (De La Rosa et al., 2006). The compound exhibits a resistance profile against selected NNRTI resistant HIV-1 strains comparable to that of other next generation NNRTIs (Hamatake et al., 2008; Zhang et al., 2007). Crystal structures of HIV-1 RT complexed with an analog of RDEA806 reveal that the compound extends deep in the pocket to form interaction with W229, Y181 and Y188 (Girardet et al., 2007). Phase IIA data were presented at the IAS meeting in 2008: monotherapy with once daily RDEA806 in treatment naïve patients for 7 days showed both good antiviral activity and tolerability (Moyle et al., 2008). RDEA806 should enter dose finding Phase IIB trials in treatment naïve patients, provided Ardea Bisosciences finds a partner for its development. Additionally, RDEA806, as a prodrug of RDEA594 is currently assessed for the treatment of gout in Phase IIB trials.

8.2.4. IDX899

IDX899 is another next generation NNRTI, of the family of 3-phosphoindoles, developed by Idenix Pharmaceuticals. In vitro, IDX899 shows a resistance profile comparable to that of the other next generation NNRTIs (Richman et al., 2008). Preliminary data of a Phase IIA trial were presented at the IAS meeting in 2008: monotherapy with once daily IDX899 in treatment naïve patients for 7 days showed both good antiviral activity and tolerability at the studied doses (Zala et al., 2008). The trial has been extended to study a lower dose. In February 2009, Glaxo-Smith-Kline obtained an exclusive worldwide license on IDX899, and will assume all development responsibilities and associated costs for the molecule.

8.2.5. Lersivirine (UK-453061)

Lersivirine is a next generation NNRTI belonging to the pyrazole family, being developed by Pfizer. Again, the in vitro resistance profile is comparable to that of the other next generation NNRTIs described here (Mori et al., 2008; Thornberry et al., 2009), and the molecule has shown good antiviral activity and tolerability in a proof of concept monotherapy with once and twice daily administration in treatment naïve patients for 7 days (Fätkenheuer et al., 2007). It is being studied in Phase IIB dose finding, with once and twice daily administration.

8.2.6. Conclusion

In conclusion, the first generation NNRTIs nevirapine in the developing world and efavirenz in the developed world remain a cornerstone of first line HAART. The approval of etravirine represents a shift in treatment paradigm: for the first time an NNRTI shows durable efficacy in patients with NNRTI resistant HIV-1 infection (Jayaweera et al., 2008; Pecora Fulco and McNicholl, 2009; Seminari et al., 2008). Although efavirenz and nevirapine are generally safe and well tolerated, there is a need for drugs in this class that would be devoid of the CNS side effects and hepatotoxicity associated with their use. Additionally, new antiretroviral drugs should have an increased genetic barrier to the development of resistance, and have a convenient dosing schedule, with potential to co-formulation with other antiretroviral drugs. Rilpivirine is a next generation NNRTI, which is studied in treatment naïve patients, and appears to fulfill those criteria, while showing comparable efficacy with efavirenz (Garvey and Winston, 2009). Other next generation NNRTIs are being developed, however the currently available data are too scarce to predict their usefulness in the treatment of HIV-1 infection as of today.

9. Other potential clinical uses of NNRTIs

Although the armamentarium of antiretroviral drugs for the treatment of HIV infection nowadays answers most of the needs of patients and their physicians in terms of long term control of virus replication, HAART remains heavy and cumbersome for patients, even if progresses have been made on the convenience and tolerability of treatment regimens. Moreover, the vast majority of HIV infected individuals live in the developing world, and despite recent large scale initiatives it is estimated that in those countries only around 25% of those who need treatment have access to a very limited number of drugs. Access to second line HAART, after first line failure, is even more problematic in resource limited settings.

In both the developed and the developing world, prevention of HIV infection remains the best approach, also in light of the recently released disappointing data of vaccine trials. Different prevention strategies are evaluated: condom use, male circumcision, vaginal microbicides, prevention of mother to child transmission, and others. Antiretroviral drugs are investigated in some of those strategies.

9.1. Vaginal microbicides

Women are more susceptible to sexual HIV transmission than men, for physiological, but also sociologic reasons. Providing women with prevention means they can control themselves, unlike male condoms, should help decrease the spread of the virus. Initial studies of potential vaginal microbicides yielded disappointing results. Those were conducted with surfactants/membrane disruptors, vaginal milieu protectors, and molecules that inhibited entry of the virus into cells in non specific ways (for a review, see Cutler and Justman, 2008). It was then hypothesized that specific anti-HIV molecules with mechanisms of action that interfere with the virus

replication prior to the integration of proviral DNA in the host cell genome, could be potential vaginal microbicide candidates. Several anti-HIV compounds are currently evaluated for this purpose: CCR5 inhibitors, NRTIs, NNRTIs and integrase inhibitors.

Several NNRTIs are currently under evaluation as candidate microbicide agents (D'Cruz and Uckun, 2006). The most advanced is dapivirine (TMC120), a member of the DAPY family, and is investigated by the International Partnership for Microbicides (Nuttall et al., 2007). Dapivirine has shown inhibition of vaginal HIV-1 transmission in a hu-SCID mice model where nevirapine was inactive (Di Fabio et al., 2003). A common characteristic of the NNRTIs as candidate vaginal microbicide agents is their in vitro increased genetic barrier and better resistance profile, as compared to first generation NNRTIs. Indeed, it remains to be seen whether the use of antiretroviral drugs for this purpose will select for resistance, especially when used by women who are unaware of their infection status, as is the case for the vast majority in the developing world (Martinez et al., 2006). If this were to happen, it would put a heavy burden on the treatment of those patients, for which the standard first line therapy would be of no use anymore. Emergence of resistance will need to be monitored very closely in the efficacy trials.

9.2. Prevention of mother to child transmission

As indicated above (Section 8.1.1), nevirapine has been used in the prevention of mother to child transmission, with an improvement on the rates of virus transmission to the infant, but with the selection of NNRTI resistance in the mother. Whereas single dose nevirapine is not anymore recommended, there might be a role to play for next generation NNRTIs in this prevention strategy, in combination with other antiretroviral drugs.

10. Conclusions

In 1994, Kilby and Saag asked the question: "Is there a role for non-nucleoside reverse transcriptase inhibitors in the treatment of HIV infection?" (Kilby and Saag, 1994). The answer 15 years later is definitely "yes, there is".

Although originally studied in suboptimal conditions, efavirenz and nevirapine have demonstrated over the years that, despite their low genetic barrier to the development of resistance, they afford long and sustained virologic efficacy, when combined with two other fully active antiretroviral drugs. They have become a cornerstone in first line HAART: efavirenz is listed as preferred NNRTI in the treatment guidelines for the developed countries, whereas nevirapine is the most used NNRTI in the developing world. Biological, biochemical and structural studies have helped understand the mechanisms by which NNRTIs inhibit HIV-1 replication, and have shed light on how the virus builds up resistance to the molecules. The specificity of NNRTIs for HIV-1 might be a factor contributing to their generally good safety and tolerability profile. However, the side effects associated with the use of nevirapine and efavirenz, e.g. severe rash, hepatotoxicity, and/or CNS effects, could in the mid to long term negatively impact their use. Whereas most combination therapy regimens nowadays show comparable efficacy, the long term success of new regimens will increasingly rely on their safety and tolerability profiles. Therefore, next to their improved resistance profile as compared to first generation NNRTIs, novel NNRTIs currently in clinical development are thoroughly evaluated for safety and tolerability. Safer and better tolerated drugs should increase patient compliance, and hence long term efficacy of the new regimens.

The discovery, development, and approval of etravirine has shown that, contrary to the belief that virologic failure on an NNRTI containing treatment regimen rendered the class useless for further therapy, next generation NNRTIs were an option for some treatment experienced patients. Moreover, the safety and tolerability of etravirine was shown to be comparable to placebo in the Phase III trials, with the exception of rash, indicating an improvement over efavirenz and nevirapine. This has revived interest for this class of drugs, and might result in the approval of next generation NNRTIs, with a better safety and tolerability profile than efavirenz and nevirapine, and a similar long term efficacy.

Finally, NNRTIs are also to play a role in the prevention of HIV-1 transmission, which would favorably impact the spread of the pandemic, especially in resource limited countries, where the vast majority of people living with HIV/AIDS are found, and the need highest is.

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