ELSEVIER

Antiviral Research 38 (1998) 153–179

Antiviral Research

Mini-review

The role of non-nucleoside reverse transcriptase inhibitors (NNRTIs) in the therapy of HIV-1 infection[1]

Erik De Clercq

Rega Institute for Medical Research, Katholieke Universiteit Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium

Received 2 April 1998; accepted 20 April 1998

Abstract

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) have, in addition to the nucleoside reverse transcriptase inhibitors (NRTIs) and protease inhibitors (PIs), gained a definitive place in the treatment of HIV-1 infections. Starting from the HEPT and TIBO derivatives, more than 30 structurally different classes of compounds have been identified as NNRTIs, that is compounds that are specifically inhibitory to HIV-1 replication and targeted at the HIV-1 reverse transcriptase (RT). Two NNRTIs (nevirapine and delavirdine) have been formally licensed for clinical use and several others are in preclinical or clinical development [thiocarboxanilide UC-781, HEPT derivative MKC-442, quinoxaline HBY 097 and DMP 266 (efavirenz)]. The NNRTIs interact with a specific 'pocket' site of HIV-1 RT that is closely associated with, but distinct from, the NRTI binding site. NNRTIs are notorious for rapidly eliciting resistance due to mutations of the amino acids surrounding the NNRTI-binding site. However, the emergence of resistant HIV strains can be circumvented if the NNRTIs, alone or in combination, are used from the start at sufficiently high concentrations. In vitro, this procedure has proved to 'knock-out' virus replication and to prevent resistance from arising. In vivo, various triple-drug combinations of NNRTIs (nevirapine, delavirdine or efavirenz) with NRTIs (AZT, 3TC, ddI or d4T) and/or PIs (indinavir or nelfinavir) have been shown to afford a durable anti-HIV activity, as reflected by both a decrease in plasma HIV-1 RNA levels and increased CD4 T-lymphocyte counts.

Keywords: Delavirdine; HIV; Nevirapine; NNRTIs (non-nucleoside reverse transcriptase inhibitors); Reverse transcriptase; Triple-drug combinations

[1] Presented at the Eleventh International Conference on Antiviral Research, San Diego, CA, 5–10 April 1998.

PII S0166-3542(98)00025-4

1. Introduction

The therapy of HIV (human immunodeficiency virus) infections has, since the advent of azidothymidine, been dominated by the 2′,3′-dideoxynucleoside (ddN) derivatives, five of which are now licensed for clinical use: azidothymidine (AZT, zidovudine, Retrovir®), dideoxyinosine (ddI, didanosine, Videx®), dideoxycytidine (ddC, zalcitabine, Hivid®), didehydrodideoxythymidine (d4T, stavudine, Zerit®) and 3′-thiadideoxycytidine (3TC, lamivudine, Epivir®). Four HIV protease inhibitors [i.e. saquinavir (Invirase®), ritonavir (Norvir®), indinavir (Crixivan®) and nelfinavir (Viracept®)] have joined the current anti-HIV drug armamentarium (De Clercq, 1995, 1997) and NNRTIs (non-nucleoside reverse transcriptase inhibitors) (De Clercq, 1996a,b) have recently gained an increasingly important role in the therapy of HIV infections. Several NNRTIs have proceeded onto clinical development (i.e. tivirapine, loviride, MKC-442, HBY 097, DMP 266) or are already licensed for clinical use [nevirapine (Viramune®), delavirdine (Rescriptor®)].

The era of the NNRTIs started about a decennium ago with the discovery of 1-(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) (Pauwels et al., 1990, Debyser et al., 1991) as specific HIV-1 inhibitors, targeted at the HIV-1 reverse transcriptase (Pauwels et al., 1990, Baba et al., 1991a,b, Debyser et al., 1991). These compounds excelled by their unique specificity for HIV-1: they were highly active against HIV-1, but inactive against HIV-2 or any other retrovirus, and, furthermore, their antiviral action could be attributed to a specific interaction with the viral reverse transcriptase (RT).

Following the HEPT and TIBO derivatives, nevirapine (BI-RG-587) (Merluzzi et al., 1990, Koup et al., 1991), pyridinone derivatives L-696,229 and L-697,661 (Goldman et al., 1991, 1992), and bis(heteroaryl)piperazine (BHAP) derivatives U-88204 and U-90152 (Romero et al., 1991, 1993) were identified as specific HIV-1 inhibitors. In contrast with HEPT and TIBO, which were first found to inhibit HIV-1 replication in cell culture before their target (HIV-1 RT) was unraveled, nevirapine, pyridinone and BHAP were discovered through an HIV-1 RT screening program before their antiviral activity in cell culture was established.

Following HEPT, TIBO, nevirapine, pyridinone and BHAP, yet other compounds, i.e. TSAO-T and TSAO-m^3T (Balzarini et al., 1992a,b,c), loviride [α-APA (R89439)] (Pauwels et al., 1993), PETT (LY 300046) (Ahgren et al., 1995), new derivatives from HEPT [i.e. I-EBU (MKC-442)] (Baba et al., 1994) and TIBO [i.e. 8-chloro-TIBO, tivirapine (R86183)] (Pauwels et al., 1994), and various other compounds were described as specific HIV-1 RT inhibitors, and all these compounds have been collectively referred as NNRTIs (De Clercq, 1996a,b) to distinguish them from the ddNs, now also referred to as NRTIs (nucleoside reverse transcriptase inhibitors) (De Clercq, 1995, 1997).

2. Non-nucleoside reverse transcriptase inhibitors (NNRTIs)

To qualify as an NNRTI, the compound should interact specifically with (a non-substrate binding site of) the RT of HIV-1, and inhibit the replication of HIV-1, but not HIV-2 (or any other retrovirus) at a concentration that is significantly lower than the concentration required to affect normal cell viability (De Clercq, 1996a). The potency and selectivity of the NNRTIs as specific inhibitors of HIV-1 should be evident from the EC_{50} (50% effective concentration, required to inhibit HIV-1 replication in cell culture) and the ratio of their CC_{50} (50% cytotoxic concentration) to the EC_{50}. The latter is termed selectivity index (SI). The interaction of the NNRTIs with HIV-1 RT should be evident from their K_i or IC_{50} (50% inhibitory concentration) for the enzyme.

Based on these premises, at least 30 different classes of NNRTIs could be considered (Figs. 1–5): TIBO derivatives [i.e. 8-chloro-TIBO (R86183, tivirapine)] (Pauwels et al., 1994), HEPT derivatives [i.e. I-EBU (MKC-442)] (Baba et al., 1994), dipyridodiazepinones [i.e. nevirapine (BI-RG-587)] (Merluzzi et al., 1990, Koup et al.,

1991), pyridinones (i.e. L-697,661) (Goldman et al., 1991), BHAP derivatives [i.e. delavirdine (U-90152)] (Dueweke et al., 1993a), TSAO derivatives (i.e. TSAO-m^{3}T) (Balzarini et al., 1992a,b), α-APA derivatives [i.e. loviride (R89439)] (Pauwels et al., 1993), PETT derivatives [i.e. trovirdine (LY 300046)] (Ahgren et al., 1995, Zhang et al., 1995), thiocarboxanilide derivatives (i.e. UC-781) (Balzarini et al., 1995a, Buckheit et al., 1997), quinoxaline derivatives (i.e. HBY 097) (Kleim et al., 1993, 1995), thiazolobenzimidazole (TBZ, NSC 625487) (Buckheit et al., 1993, Chimirri et al., 1997), thiazoloisoindolinone (BM + 51.0836) (Maass et al., 1993, Mertens et al., 1993), indole carboxamide L-737,126 (Williams et al., 1993), benzothiadiazine NSC

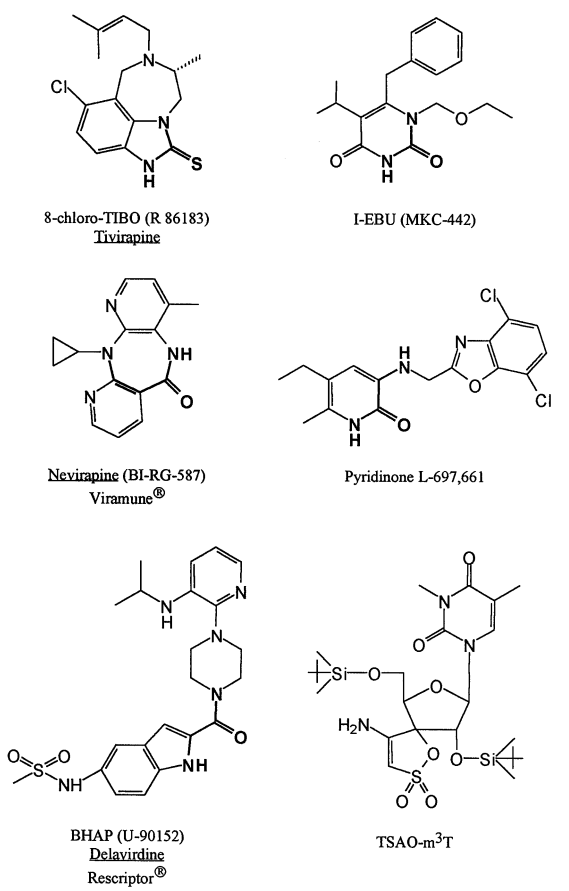

Fig. 1. Structural formulae of the non-nucleoside reverse transcriptase inhibitors (NNRTIs).

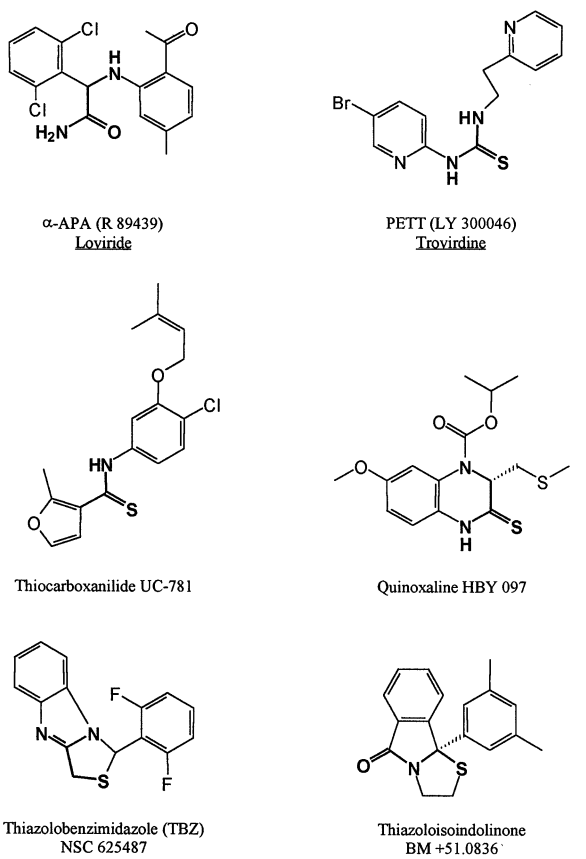

Fig. 2. Structural formulae of the non-nucleoside reverse transcriptase inhibitors (NNRTIs).

287474 (Buckheit et al., 1994a), quinazolinone (**13a**) (Tucker et al., 1994), benzoxazinone DMP 266 (efavirenz) (Young et al., 1995), calanolide A (Kashman et al., 1992, Zembower et al., 1997), pyrrolobenzodiazepinone (**4**) (De Lucca and Otto, 1992), imidazodipyridodiazepine UK-129,485 (Terrett et al., 1992), imidazopyridazine (**33**) (Livermore et al., 1993), thiadiazolyl dialkylcarbamate (TDA RD-4-2024) (Ijichi et al., 1995, 1996), arylpyridodiazepine and -thiodiazepine derivatives (i.e. MEN 10979) (Bellarosa et al., 1996), DABO derivatives (i.e. DABO, **12e**) (Artico et al., 1993, Massa et al., 1995), HEPT-pyridinone hybrids (i.e. **8a**) (Dollé et al., 1995), benzyloxymethylpyridinone (**18a**) (Jourdan et al., 1997), alkoxy(arylthio)uracil (**18**) (Kim et al., 1997), indolyldipyridodiazepinone (**7a**) (Kelly et al., 1997),

pyrrolobenzoxazepinone (**16e**) (Campiani et al., 1996), highly substituted pyrroles (Antonucci et al., 1995), the benzylthiopyrimidine U-31355 (Althaus et al., 1996) and pyridazinobenzoxazepinones (Barth et al., 1996). More recently, pyrrolobenzothiadiazepines (Di Santo et al., 1998), indolobenzothiazepines (Silvestri et al., 1998), and trioxothienothiadiazine (TTD) derivatives (Witvrouw et al., 1998) have also been described as specific HIV-1 RT inhibitors.

As shown in Table 1, the most potent and most selective congeners among the NNRTIs (i.e. tivirapine, loviride, thiocarboxanilide UC-781, quinoxaline HBY 097 and efavirenz) were found to inhibit HIV-1-induced cytopathicity at nanomolar concentrations, with selectivity indexes of 30000–200000. All the compounds listed in Table 1 were also found to inhibit HIV-1 RT activity, albeit at widely varying IC_{50} values,

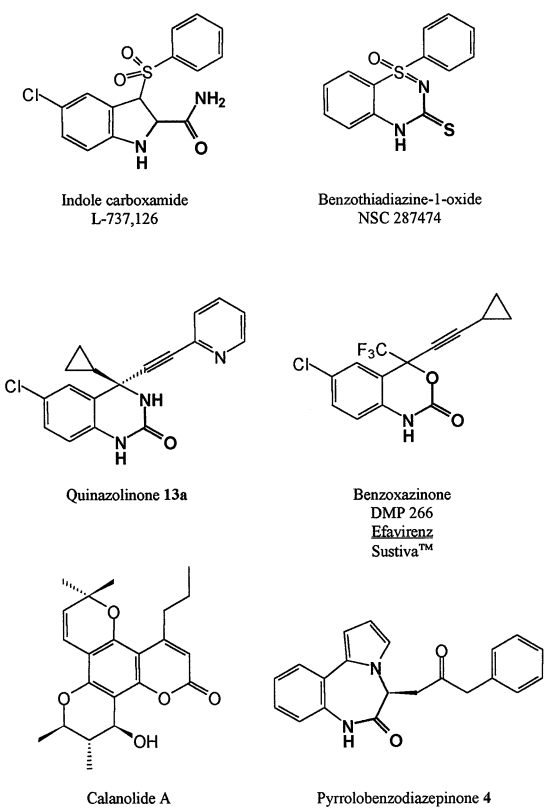

Fig. 3. Structural formulae of the non-nucleoside reverse transcriptase inhibitors (NNRTIs).

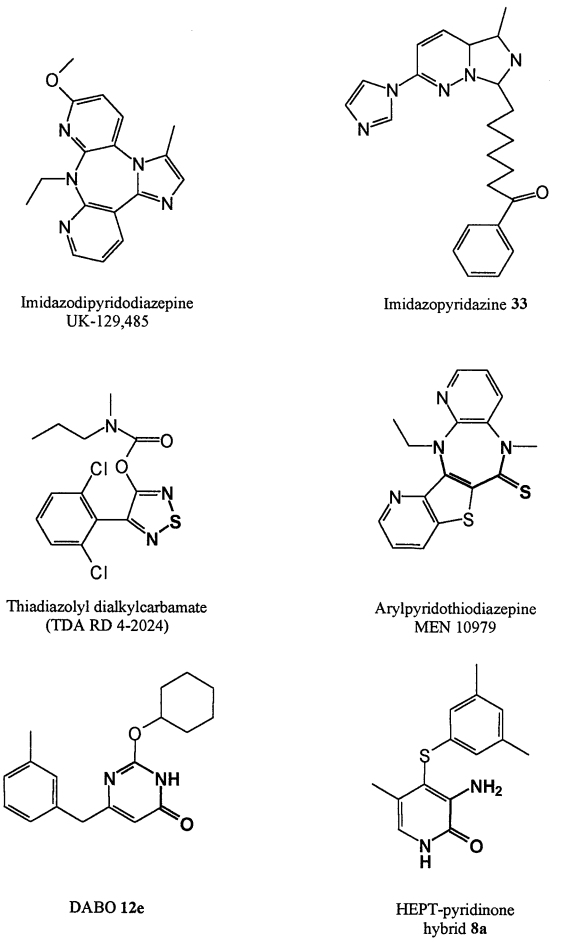

Fig. 4. Structural formulae of the non-nucleoside reverse transcriptase inhibitors (NNRTIs).

which may at least partially be related to the varying assay conditions (i.e. nature of the primer/template) used to monitor RT activity.

3. Interaction of NNRTIs with their pocket site at the HIV-1 RT

Whereas the ddN analogues (i.e. AZT, ddI, etc.), following their intracellular phosphorylation to the triphosphate form, interact with the substrate binding site of the HIV RT, the NNRTIs block the HIV-1 RT reaction through interaction with an allosterically located, non-substrate binding site (De Clercq, 1996a,b). This NNRTI-bind-

ing site ('pocket') is located at a close (about 10 Å) distance from the substrate-binding site (Tantillo et al., 1994). It is not only spatially but also functionally (Debyser et al., 1992, Dueweke et al., 1992) associated with the substrate-binding site. The cooperative interaction between these two sites (Spence et al., 1995) provides a means to increase the effectiveness of NRTIs and NNRTIs by using them in combination therapy.

Several studies have revealed a common mode of binding for the chemically diverse NNRTIs with their target site at the HIV-1 RT (Ren et al., 1995a). The NNRTIs cause a repositioning of the three-stranded β-sheet in the p66 subunit (containing the catalytic aspartic acid residues 110, 185 and 186) (Esnouf et al., 1995). This suggests that the NNRTIs inhibit HIV-1 RT by locking the active catalytic site in an inactive conformation, reminiscent of the conformation observed in the inactive p51 subunit (Esnouf et al., 1995). When bound into their pocket at the HIV-1 RT, the NNRTIs [i.e. α-APA R95846 (Ding et al., 1995a), TIBO R86183 (Ding et al., 1995b), 9-chloro-TIBO (R82913) (Ren et al., 1995b) and nevirapine (Kroeger Smith et al., 1995)] maintain a very similar conformational 'butterfly-like' shape. They roughly overlay each other in the binding pocket and appear to function as π-electron donors to aromatic side-chain residues surrounding the pocket (Kroeger Smith et al., 1995).

The bulky U-90152 (delavirdine) occupies the same pocket as other NNRTIs, but the complex is stabilized quite differently, in particular by hydrogen bonding to the main chain of Lys-103 and extensive hydrophobic contacts with Pro-236. When bound, part of U-90152 protrudes into the solvent creating a channel between Pro-236 and the polypeptide segments 225–226 and 105–106, thus providing evidence for the entry mode of NNRTIs (Esnouf et al., 1997a). The suggested model (Fig. 6) also allows predictions on resistance mutations (notably P236L, which occurs characteristically for BHAPs) and chemical modifications, which may make the binding more resilient to mutations in the HIV-1 RT (Esnouf et al., 1997a).

The binding of the thiocarboxanilide UC-781 in its HIV-1 RT pocket is shown in Fig. 7 (Esnouf et al., 1997b). The thiocarboxanilides bind to their pocket in a similar fashion as the other NNRTIs, i.e. through hydrogen binding with the main chain oxygen of Lys-101 and hydrophobic interactions with Leu-100, Val-106, Val-179, Tyr-188, Phe-227, Leu-234 and His-235. The thiocarboxanilide UC-781 also makes important hydrophobic interactions with Trp-229 (Esnouf et al., 1997b).

Fig. 8 shows how binding of an NNRTI, in this case UC-781, can affect parts of the structure of HIV-1 RT (Esnouf et al., 1997b). Binding of the NNRTI causes side-chain rearrangement of tyrosine residues 181 and 188, and repositioning of the catalytic aspartic acid residues, as compared to the unliganded RT structure (Esnouf et al., 1995).

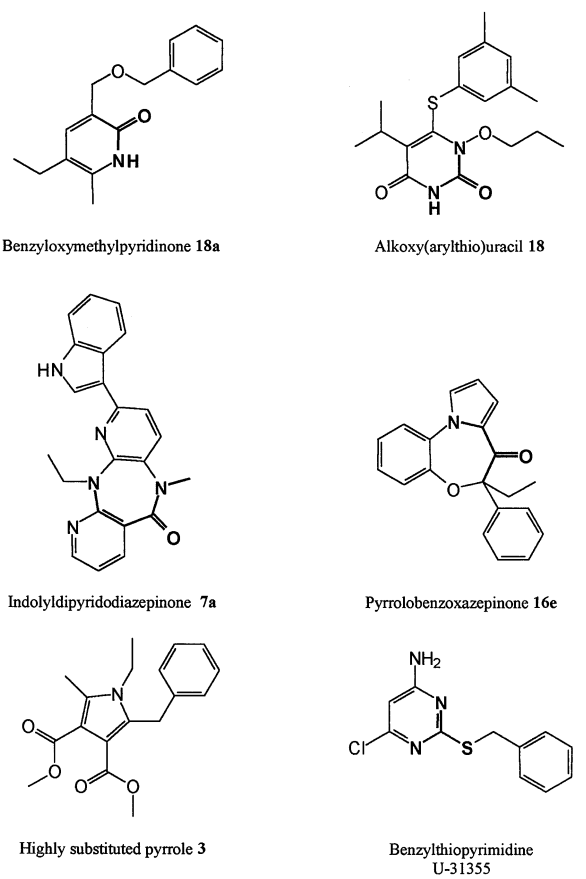

Fig. 5. Structural formulae of the non-nucleoside reverse transcriptase inhibitors (NNRTIs).

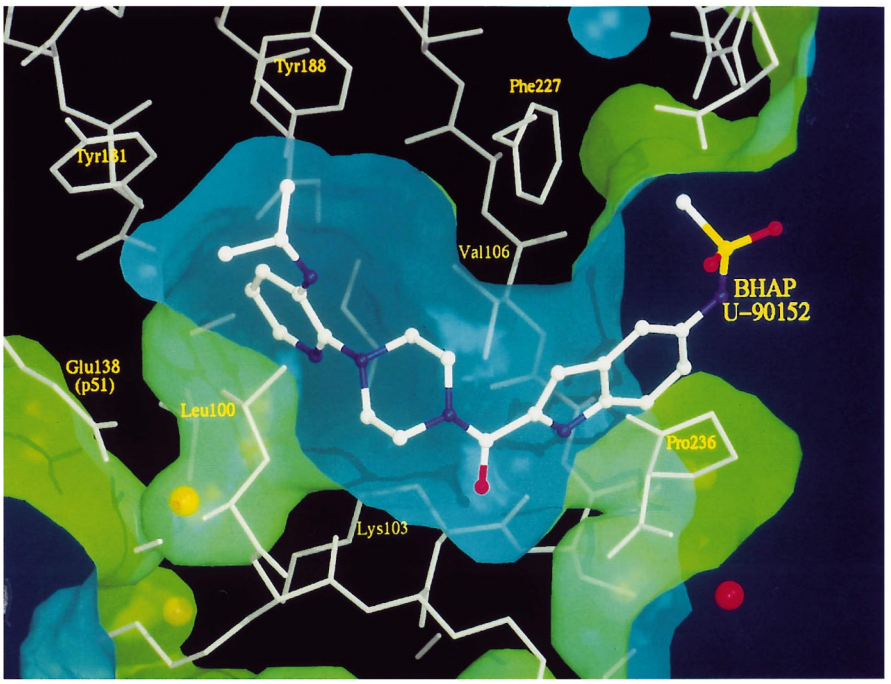

Fig. 6

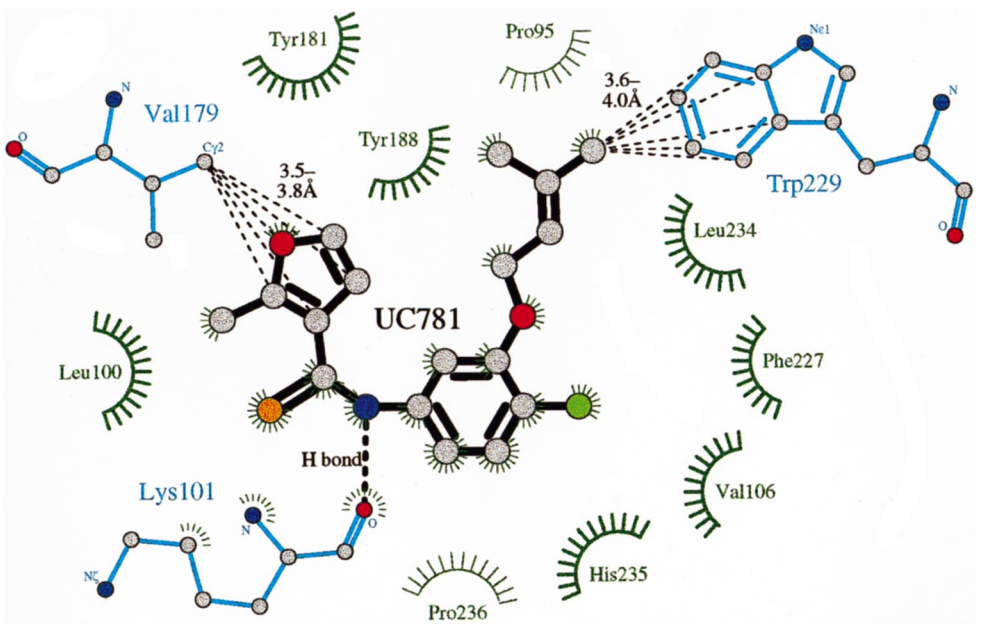

Fig. 7

Fig. 6. Positioning of U-90152 in the NNRTI-binding pocket (according to Esnouf et al., 1997a). The inhibitor is shown as an atom-colored ball-and-stick model with the surrounding protein structure as thin grey sticks. The green face of the surface points toward the protein, the blue face toward the solvent. This is the first RT–NNRTI complex structure to show the channel connecting the pocket to the solvent and may indicate the mode of entry into the (normally buried) pocket for all NNRTIs.

Fig. 7. Schematic diagram of the features stabilizing the HIV-1 RT complex with thiocarboxanilide UC-781 (according to Esnouf et al., 1997b). Thiocarboxanilide UC-781 is shown using atom-colored spheres. The hydrogen bond between UC-781 and the main chain of Lys-101 and the two methyl group–aromatic ring interactions are shown explicitly. Other major hydrophobic contacts are shown with bold green lines, minor ones with faint green lines. Modified from Esnouf et al. (1997b).

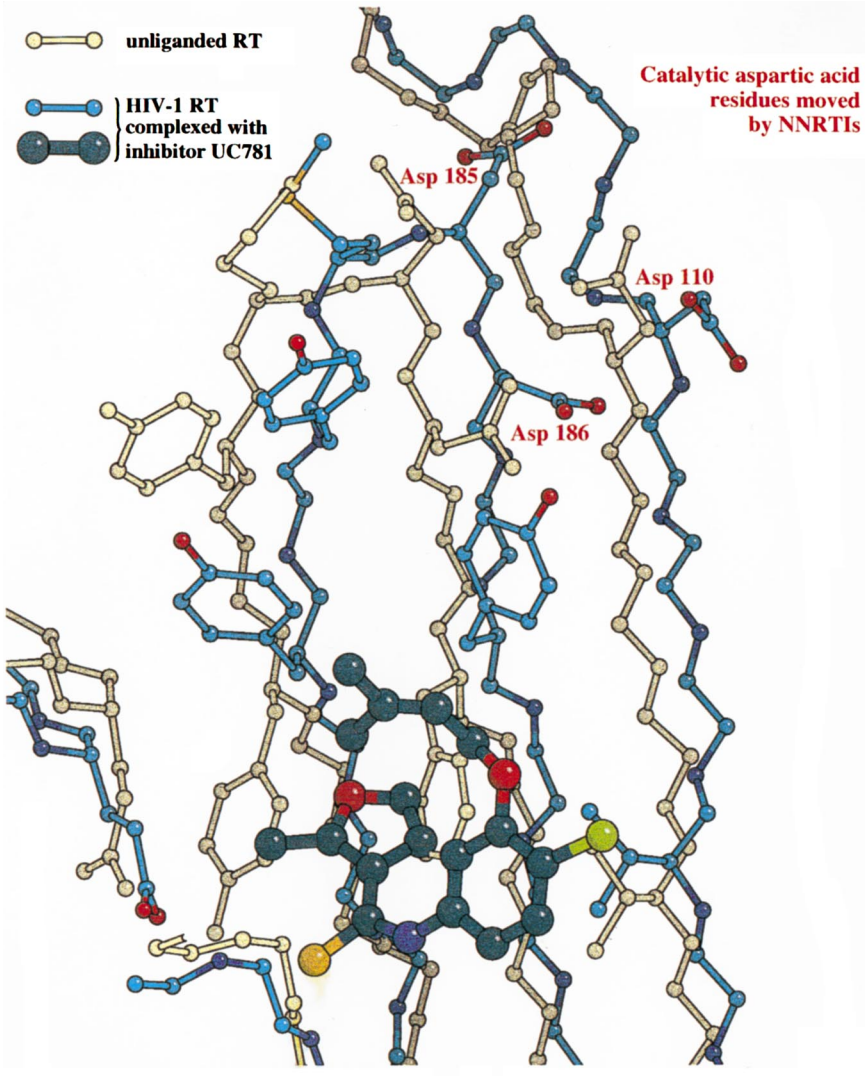

Fig. 8

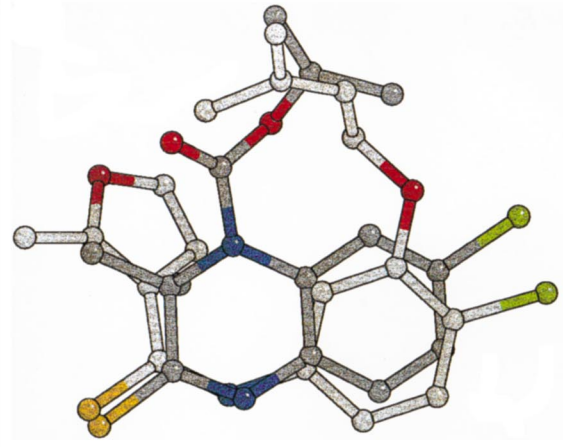

Fig. 9

Fig. 8. Unliganded versus liganded HIV-1 RT structure (Esnouf et al., 1995, 1997b). Thiocarboxanilide UC-781 is shown as an atom-colored ball-and-stick model with large atom spheres and the carbon atoms in dary cyan. The three β-strands of RT (left to right named $\beta 7$, $\beta 8$ and $\beta 4$, respectively) are shown as atom-colored ball-and-stick models with the carbon atoms in bright cyan; the equivalent structure from the unliganded RT structure (Esnouf et al., 1995) is shown in pale yellow. The figure was drawn using BobScript (Esnouf, 1997).

Fig. 9. Superposition of thiocarboxanilide UC-781 and quinoxaline S2720 within the HIV-1 RT 'pocket' site (Esnouf et al., 1997b). Atom-colored ball-and-stick models are shown for the thiocarboxanilide UC-781 (pale grey carbon atoms) and for the quinoxaline S2720 (dark grey carbon atoms). The positions of the sulfur and chlorine atoms and of the nitrogens involved in hydrogen bonding superimpose very well. The six-membered aromatic rings also occupy equivalent positions. Finally, the UC-781 ether group and the S2720 ester group adopt conformations that allow terminal methyl groups to interact strongly with the aromatic ring of Trp-229. The figure was drawn using BobScript (Esnouf, 1997).

Table 1
Inhibitory effects of NNRTIs on HIV-1 RT activity and HIV-1 cytopathicity

Compound	HIV-1 RT activity	HIV-1 cytopathicity			References
	IC_{50} (μM)[a]	EC_{50} (μM)[b]	CC_{50} (μM)[c]	SI[d]	
Tivirapine [8-chloro-TIBO (R86183)]	0.05	0.0046	138	30000	Pauwels et al., 1994
HEPT [I-EBU (MKC-442)]	0.012	0.014	>100	>7000	Baba et al., 1994
Nevirapine (BI-RG-587), Viramune®	0.084	0.048	>50	>1000	Merluzzi et al., 1990, Koup et al., 1991, De Clercq, 1996b
Pyridinone L-697,661	0.019	0.012	>60	>4800	Goldman et al., 1991
Delavirdine (BHAP U-90152), Rescriptor®	0.26	0.01	>100	>10000	Dueweke et al., 1993a
TSAO-m^3T	4.7	0.034	139	4088	Balzarini et al., 1992a,b
Loviride (α-APA R89439)	0.2	0.013	710	54615	Pauwels et al., 1993
Trovirdine (PETT LY 300046)	0.007	0.016	87	5438	Ahgren et al., 1995, Zhang et al., 1995
Thiocarboxanilide UC-781	0.02 [e]	0.002 [e]	>100 [e]	>50000	Balzarini et al., 1995a, Buckheit et al., 1997
Quinoxaline HBY 097	0.08	0.001	200	200000	Kleim et al., 1995
Thiazolobenzimidazole NSC 625487 (TBZ)	0.5	0.21	60	292	Buckheit et al., 1993, Chimirri et al., 1997
Thiazoloisoindolinone BM+51.0836	0.016	0.01	>50	>5000	Massa et al., 1995, Mertens et al., 1993
Indole carboxamide L-737,126	0.003	<0.003			Williams et al., 1993
Benzothiadiazine NSC 287474	1.2	4	>130	>32	Buckheit et al., 1994a
Quinazolinone (**13a**)	0.012	<0.025			Tucker et al., 1994
Efavirenz (Benzoxazinone DMP 266)	0.003	0.001	80	80000	Young et al., 1995
Calanolide A	0.07	0.1	20	200	Kashman et al., 1992, Zembower et al., 1997
Pyrrolobenzodiazepinone (**4**)	0.04 [e]	<0.3 [e]	10 [e]	>33	De Lucca and Otto, 1992
Imidazodipyridodiazepine UK-129,485	0.156	<0.002	>10	>5000	Terrett et al., 1992
Imidazopyridazine (**33**)	0.0006	0.01	26	2600	Livermore et al., 1993
TDA RD 4-2024	0.5	0.012	28	2280	Ijichi et al., 1995, 1996
MEN 10979	0.18	0.0025	38	15000	Bellarosa et al., 1996
DABO (**12e**)	1.8	0.8	>335	>418	Massa et al., 1995
HEPT-pyridinone hybrid (**8a**)	0.1	0.01	>10	>1000	Dollé et al., 1995
Benzyloxymethylpyridinone (**18a**)	12	100	8		Jourdan et al., 1997
Alkoxy(arylthio)uracil (**18**)	12.3	0.064	33	516	Kim et al., 1997
Indolyldipyridodiazepinone (**7a**)		0.028			Kelly et al., 1997
Pyrrolobenzoxazepinone (**16e**)	0.25	0.47	4.9	10	Campiani et al., 1996
Highly substituted pyrrole (**3**)	0.64	2.3	175	76	Antonucci et al., 1995
Benzylthiopyrimidine U-31355	50–80	0.3	>16	>50	Althaus et al., 1996

The data, as listed, were obtained under different assay conditions (i.e. with different templates, for determination of HIV-1 RT activity; and with different virus strains and cell types, for determination of HIV-1-induced cytopathicity). This makes a direct comparison of the individual compounds rather difficult.

[a] 50% Inhibitory concentration, or concentration required to inhibit HIV-1 RT activity by 50%.

[b] 50% Effective concentration, or concentration required to inhibit HIV-1-induced cytopathicity by 50%.

[c] 50% Cytotoxic concentration, or concentration required to reduce viability of the host cells by 50%.

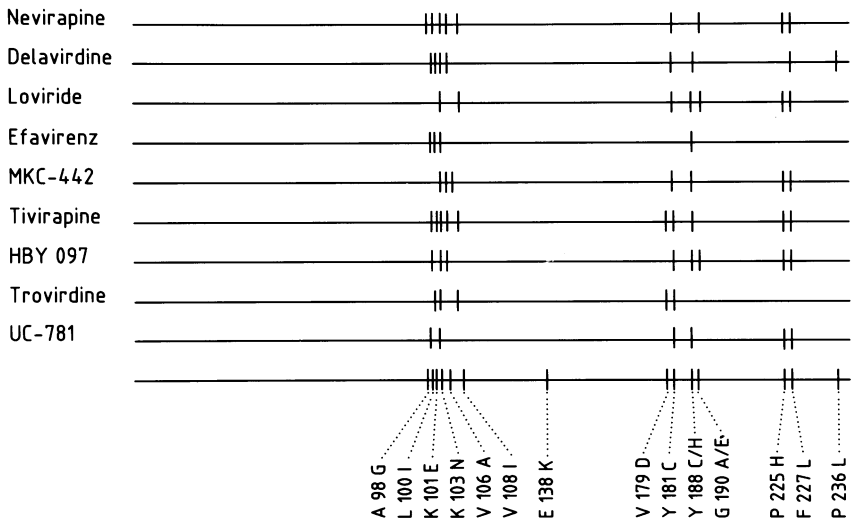

Fig. 10. Mutations in the HIV-1 RT that confer resistance to the non-nucleoside reverse transcriptase inhibitors (NNRTIs) (see also Schinazi et al., 1997).

Structural modelling studies have demonstrated that, despite their diverse structures, the conformations adopted by the NNRTIs, when bound to HIV-1 RT, lead to very similar features of binding. Fig. 9 shows superposition of the thiocarboxanilide UC-781 (Esnouf et al., 1997b) and the quinoxaline S2720, relative to the structure of the surrounding protein in the models of the NNRTI–RT complexes.

4. HIV-1 resistance to NNRTIs

Inevitably, the rapid replication of HIV and its inherent genetic variability must lead to the generation of viral variants that exhibit drug resistance (Schinazi et al., 1997). NNRTIs are notorious for rapidly triggering the emergence of drug-resistant HIV-1 variants. The first RT mutations shown to be associated with, and to account for, HIV-1 resistance to NNRTIs were the K103N and Y181C mutations, engendering resistance to pyridinone (Nunberg et al., 1991), nevirapine (Richman et al., 1991a) and TIBO R82150 (Mellors et al., 1992). In fact, the mutations K103N and Y181C have been observed with virtually all the NNRTIs (Fig. 10), except for the quinoxalines (i.e. HBY 097). The latter preferentially induce mutations at the RT position 190 (i.e. G190E) (Kleim et al., 1994), particularly under high selective pressure [whereas under low selective pressure, mutations L100I, K103N, V106A/I/L, Y181C and G190A/T/V are induced (Kleim et al., 1997)]. Concomitantly with the G190E mutation, HBY 097 induces the mutations L74V and V75I (Kleim et al., 1996, Boyer et al., 1998). Other 'specific' NNRTI mutations include L100I [TIBO 82150 (Mellors et al., 1993)], E138K [TSAO-T (Boyer et al., 1994, Jonckheere et al., 1994)], and P236L [BHAPs (Dueweke et al., 1993b)]. Resistance to the HEPT derivatives (i.e. MKC-442) can be associated with mutations K103N, V108I and Y181C (Seki et al., 1995) or yet others (Buckheit et al., 1995), although MKC-442 (I-EBU) still retains sufficient activity against the Y181C mutant (EC_{50}: 0.22 μM) as compared to the wild-type (EC_{50}: 0.002 μM) (Balzarini et al., 1995a).

The emergence of NNRTI resistance mutations (which, as a rule, are located at the amino acid residues aligning the NNRTI-binding 'pocket' site) is generally felt as compromising the clinical utility of the NNRTIs. Yet, it should be recognized that several NNRTI classes, viz. quinoxalines (i.e. S-2720, HBY 097) and thiocarboxanilides (i.e. UC-781), still retain pronounced activity against HIV-1 RT mutants containing the L100I, K103N, V106A and Y181C mutation (Table 2) (Balzarini et al.,

Table 2
Antiviral activity spectrum of NNRTIs [a]

NNRTI	Active against [b]						
	Wild-type (HIV-1 III_B)	Mutant					
		HIV-1 L100I	HIV-1 103N	HIV-1 106A	HIV-1 E138K	HIV-1 Y181C	HIV-1 Y188H
TIBO R82913	■	□	□	□	■	□	□
I-EBU (MKC-442)	■	■	□	■	■	■	□
Nevirapine	■	■	□	□	■	□	□
Pyridinone (L-697,661)	■	■	□	■	■	□	□
BHAP U-88204	■	□	□	■	■	■	■
BHAP U-90152 (Delavirdine)	■	□	■	■	■	■	■
TSAO-m^3T	■	■	■	□	□	□	□
Quinoxaline S-2720	■	■	■	■	■	■	■
Thiocarboxanilide UC-10	■	■	□	■	■	■	■
Quinoxaline HBY 097	■	■	■	■	■	■	■
Thiocarboxanilide UC-781	■	■	■	■	■	■	■
DMP 266 (Efavirenz)	■	■	■	■	■	■	■

[a] Data from Balzarini et al., 1995b,c, 1996b and unpublished.
[b] ■, $EC_{50} \leq 1$ μM; □, $EC_{50} > 1$ μM.

1995b, 1996b). Also DMP 266 (efavirenz) is equally active against the V108I, V179D, Y181C mutant wild-type HIV-1 (Young et al., 1995). The thiocarboxanilides are only 10- to 20-fold less active against those HIV-1 mutants (i.e. L100I, V106A, E138K and V179D) that they select for in vitro (Balzarini et al., 1996b). More remarkably, the P236L mutation that confers resistance to BHAP U-90152 (delavirdine) causes hypersensitivity to other NNRTIs such as nevirapine, TIBO and pyridinone (Dueweke et al., 1993b).

Recently, a novel mutation (P225H) was identified that consistently appeared in a V106A mutant background and conferred additional resistance to all NNRTIs, except for delavirdine, which actually showed hypersensitivity towards the P225H mutant (Pelemans et al., 1997). Another novel mutation (F227L) that arose in a V106A mutant background was found to confer high-level resistance to virtually all NNRTIs that were examined (Balzarini et al., 1998a). It has become increasingly clear that combinations of different RT mutations (i.e. L100I and K103N, or K101D and K103N, or K103N and Y181C) are required for engendering high-level resistance to NNRTIs. It is not clear, however, whether such double-mutants readily arise in vivo, in patients under NNRTI treatment.

With nevirapine, the most common resistance mutation observed in vivo is Y181C, and this mutation is prevented from emerging by coadministration of AZT (Richman et al., 1994). Vice versa, the Y181C mutation (Larder, 1992) or L100I mutation (Byrnes et al., 1994) in an AZT resistance background (T215Y) significantly suppress (phenotypic) resistance to AZT. Concomitant combination of 3TC with HBY 097 prevents the emergence of virus resistance to HBY 097 (Balzarini et al., 1997). In fact, an AZT-resistant HIV-1 strain was found to retain marked sensitivity to HBY 097 when subcultured in the combined presence of HBY 097 and 3TC (Balzarini et al., 1998b).

5. Optimization of NNRTI treatment regimens

The mutually antagonistic effects of different resistance mutations (i.e. Y181C or L100I versus T215Y), and the hypersensitivity that is seen under some conditions (i.e. with the P236L mutation towards some NNRTIs), argues in favor of the combined use of NNRTIs with NRTIs (nucleoside/nucleotide reverse transcriptase inhibitors), and different NNRTIs with one another. While achieving synergism in their anti-HIV action, different drugs combined may also reduce the risk of HIV drug resistance development and diminish toxic side-effects (through reduction of the individual doses). The compounds should not be given in sequential order (Wainberg et al., 1996), as such procedure may enable the virus to acquire resistance mutations to all the compounds (Balzarini et al., 1996c). Instead, concomitant combination therapy, as demonstrated particularly with 3TC and NNRTIs (Balzarini et al., 1996c), should be recommended.

Synergistic anti-HIV activity may be expected if NNRTIs are combined with NRTIs. For example, synergistic anti-HIV activity has been described for combinations of AZT with any of the NNRTIs, i.e. TIBO R82913 or R86183 (tivirapine) (Buckheit et al., 1994b), nevirapine (Richman et al., 1991b), delavirdine (Chong et al., 1994), or MKC-442 (Yuasa et al., 1993, Brennan et al., 1995). Synergistic anti-HIV activity has also been reported for the combination of delavirdine with protease inhibitors (i.e. U-75875) or interferon-α (Pagano and Chong, 1995) and for the combination of HEPT with interferon-α (Ito et al., 1991). Thiocarboxanilides (i.e. UC-781) show an additive inhibitory effect on HIV-1 replication when combined with other antiretroviral drugs (RT inhibitors or HIV protease inhibitors) (Balzarini and De Clercq, 1997). The different drug combinations that have proved to confer an additive/synergistic anti-HIV activity in vitro and/or in vivo (the latter, in patients) are illustrated in Fig. 11.

In contrast with the ddN analogues (AZT, ddI, etc.), the NNRTIs TIBO, BHAP and nevirapine were found to completely suppress HIV-1 infection, if added to the cells at a sufficiently high concentration (1–10 μM, or 100 times their EC_{50}) (Balzarini et al., 1993). The infected cells could

apparently be cleared from virus by the NNRTIs when used at these 'knocking-out' concentrations, and the resultant healthy cell culture could be subsequently maintained without drug with no evidence of latent proviral DNA (Vasudevachari et al., 1992, Balzarini et al., 1993). The more potent quinoxaline (Balzarini et al., 1994) and thiocarboxanilide (Balzarini et al., 1996b) derivatives were found to achieve this 'knocking out' effect at even lower concentrations (0.1–1 μM) (Fig. 12), that is concentrations that should be readily attainable in the plasma following systemic administration to patients. Also, DMP 266 (efavirenz) was found to completely suppress virus replication in peripheral blood mononuclear cells (PBMC) at a concentration of 0.96 μM, and no regrowth of virus occurred in the presence of compound after 10 weeks or in the absence of compound for 3 additional weeks (Winslow et al., 1996).

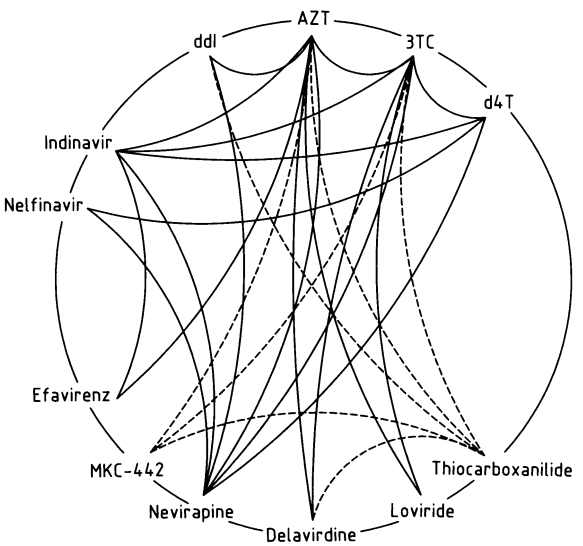

Fig. 11. Additive/synergistic anti-drug interactions shown in vitro (Richman et al., 1991b, Yuasa et al., 1993, Chong et al., 1994, Balzarini et al., 1995c, 1996a, Brennan et al., 1995, Okamoto et al., 1996, Balzarini and De Clercq, 1997) and in vivo (Staszewski et al., 1995, 1996b, Carr et al., 1996, D'Aquila et al., 1996, Davey et al., 1996, Schooley et al., 1996, CAESAR Coordinating Committee, 1997, Luzuriaga et al., 1997, Mayers et al., 1997; Skowron et al., 1998; M. Myers, personal communication, 1998; G. Tarpley, personal communication, 1998).

If, furthermore, the NNRTIs are used in combination with the ddN analogues, i.e. delavirdine combined with AZT (Dueweke et al., 1993a), or MKC-442 combined with AZT (Okamoto et al., 1996), or delavirdine combined with 3TC (Balzarini et al., 1996a), or MKC-442 combined with 3TC (Balzarini et al., 1996a), or thiocarboxanilides combined with other NNRTIs (Balzarini et al., 1995c), the drugs are able to suppress virus breakthrough for a much longer time, and at much lower concentrations than when the compounds are used individually (Fig. 13).

Thus, when optimizing the NNRTI-containing drug treatment regimens, the drugs, in combination, should be administered from the start at the highest possible doses so as to achieve complete virus suppression and prevent virus drug resistance from arising.

6. In vivo efficacy of NNRTIs

Given their high specificity as inhibitors of HIV-1 infection (for which there is no adequate animal model), NNRTIs have only rarely been studied in animal retrovirus models. When evaluated in HIV-1-infected hu-PBL-SCID mice, the NNRTIs delavirdine (BHAP U-90152) and thiocarboxanilide UC-781 protected the mice against HIV-1 infection (Balzarini et al., 1996b). Similarly, nevirapine proved effective in preventing HIV-1 infection in chimpanzees (Grob et al., 1997).

In humans, several NNRTIs have been the subject of short-term clinical studies: TIBO R82913 (Pialoux et al., 1991, De Wit et al., 1992), pyridinone L-697,661 (Davey et al., 1993, Saag et al., 1993), nevirapine (Havlir et al., 1995a), α-APA R089439 (loviride) (Staszewski et al., 1996a), MKC-442 (Moxham et al., 1997) and HBY 097 (Rübsamen-Waigmann et al., 1997). As a rule, the NNRTIs were very well tolerated [although rash developed in about half of the patients treated with nevirapine (Havlir et al., 1995a)]; they efficiently suppressed plasma viral load [up to 1.38 $\log_{10}$ (Rübsamen-Waig-

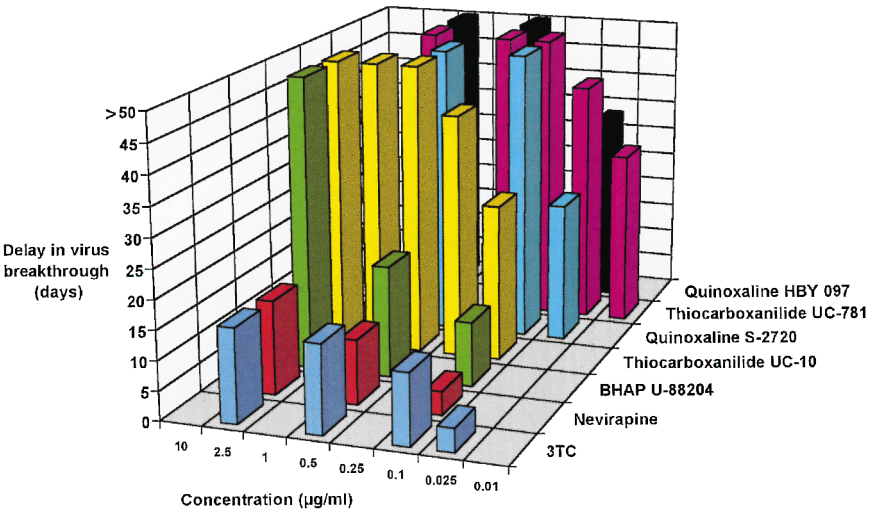

Fig. 12

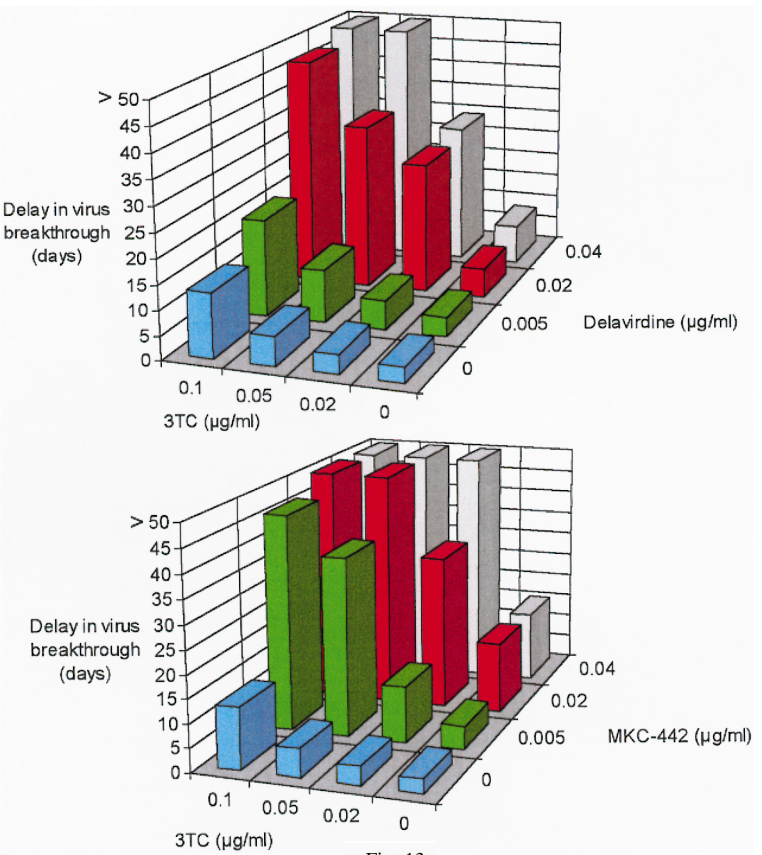

Fig. 13

Fig. 12. Suppression of virus breakthrough in CEM cells infected with HIV-1 III_B and treated with individual drugs at different concentrations. The delay in virus breakthrough corresponds to the number of days required for 50% viral cytopathicity to develop. Data taken from Balzarini et al. (1994, 1995c, 1996a,b).

Fig. 13. Suppression of virus breakthrough in CEM cells infected with HIV-1 III_B and treated with dual drug combinations at different concentrations: 3TC + delavirdine or 3TC + MKC-442. For some dual drug combinations [i.e. 3TC (at 0.1 μg/ml) with MKC-442 (at 0.02 or 0.04 μg/ml) or delavirdine (at 0.04 μg/ml)], the cell cultures remained p24 negative when the drugs were removed on day 52 and the cells were further passaged in the absence of the compounds. The delay in virus breakthrough corresponds to the number of days required for 50% viral cytopathicity to develop. Data taken from Balzarini et al. (1996a).

mann et al., 1997)], but could not prevent the emergence of drug-resistant virus strains (Davey et al., 1993, Havlir et al., 1995a). As shown for pyridinone L-697,661, suppression of virus replication was only transient: this suppressive effect disappeared coincidentally with the emergence of resistant virus (within 6-12 weeks) (Davey et al., 1993, Saag et al., 1993). Even following high-dose nevirapine (400 mg/day) therapy, nevirapine-resistant virus was isolated from all subjects tested at 12 weeks (Havlir et al., 1995a), and by that time plasma HIV-1 RNA load had returned to baseline values (De Jong et al., 1997). Although some patients treated with high-dose nevirapine (i.e. 400 mg daily) may experience sustained reduction in plasma HIV RNA despite the presence of resistant virus (Havlir et al., 1995b), this does not seem to hold true in previously untreated HIV-1-infected persons (De Jong et al., 1997).

Although monotherapy with NNRTIs may rapidly lead to the emergence of drug-resistant HIV strains (Davey et al., 1993, Saag et al., 1993), the rate of emergence of NNRTI-resistant virus can be markedly reduced in subjects receiving the NNRTI (i.e. pyridinone L-697,661) concomitantly with AZT (Staszewski et al., 1995, Schooley et al., 1996). As could be predicted from the in vitro studies (see above), NNRTIs such as nevirapine (Carr et al., 1996) and delavirdine (Davey et al., 1996) achieve higher efficacy in HIV-1-infected patients when combined with ddN analogues such as AZT than when used alone. If these combinations (AZT with nevirapine, or AZT with delavirdine) are extended by yet another drug (i.e. ddI), the efficacy (in both immunological and virological terms) is further increased (Davey et al., 1996, D'Aquila et al., 1996). Combined treatment of nevirapine with AZT and ddI has also proved efficacious in providing a sustained reduction of the plasma HIV-1 RNA load in infants (Luzuriaga et al., 1997). The latter authors also stated that "therapy with potent combinations of antiretroviral drugs should be started as early as possible in infants with maternally acquired infection (probably within the first 2 to 4 weeks), to minimize the likelihood that antiretroviral resistance will emerge and to maximize the opportunity for long-term control of HIV-1 replication" (Luzuriaga et al., 1997).

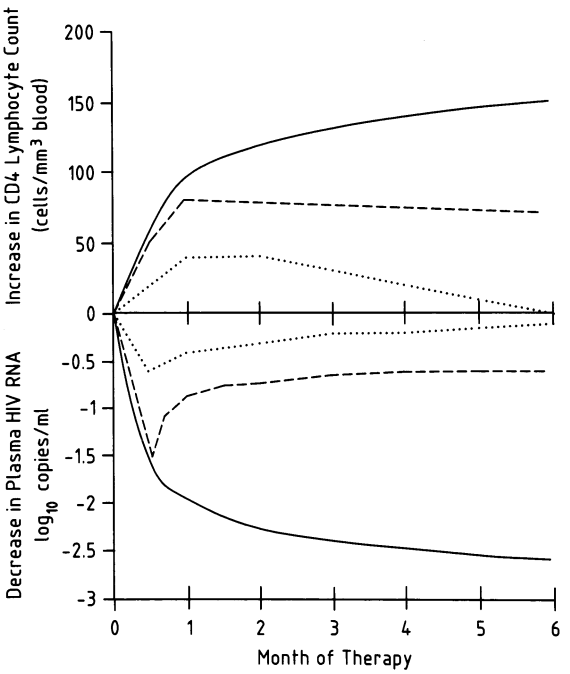

Fig. 14. Idealized response to tri-, di- and monotherapy, as exemplified for AZT (· · ·), AZT + 3TC (- - -) and AZT + 3TC + protease inhibitor (——). According to Havlir and Richman (1996), modified.

From a comprehensive study of 1330 HIV-1-infected patients enrolled in several antiretroviral treatment trials (Marschner et al., 1998), it appeared that having either a reduction in HIV-1 RNA level or an increase in CD4 lymphocyte count, or both, are associated with a delay in clinical disease progression. This implies that patient prognosis can be assessed using both HIV-1 RNA and CD4 cell responses to therapy (Marschner et al., 1998). In fact, the characteristic responses of CD4 cell counts (increase) and HIV RNA plasma levels (decrease) following therapy could be depicted as a mirror image (Havlir and Richman, 1996): the magnitude of the CD4 cell response tends to mirror the mag-

nitude of the viral RNA response (although the peak response in CD4 cell counts lags behind the maximum reduction in plasma HIV RNA levels) (Fig. 14). Pictured here are the responses of CD4 cell counts and plasma HIV RNA levels towards monotherapy (AZT), bitherapy (AZT + 3TC) and tritherapy (AZT + 3TC + protease inhibitor), but it is obvious that similar patterns may also be seen in response to NNRTI-containing drug regimens.

From Fig. 15 it is clear that extension of the dual-drug (AZT + ddI) therapy to the triple-drug (AZT + ddI + nevirapine) regimen resulted in higher CD4 cell counts paralleled by lower plasma HIV-1 RNA levels, thus attesting to the immunologic and virologic benefit afforded by the addition of nevirapine to the combination of AZT and ddI (D'Aquila et al., 1996).

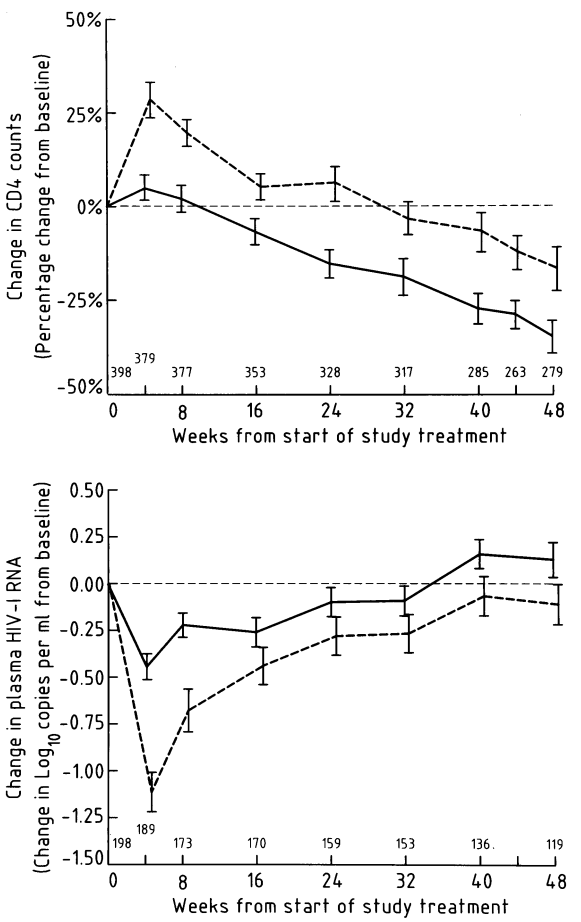

Fig. 15. Triple-drug combination (AZT + ddI + nevirapine; · · ·) versus double-drug combination (AZT + ddI; ——). According to D'Aquila et al. (1996).

As already mentioned above, the triple-drug combination AZT + ddI + delavirdine proved more potent than either two-drug combinations or monotherapy (Davey et al., 1996). Similarly, the triple-drug combination AZT + 3TC + delavirdine proved superior to the double-drug combination AZT + 3TC, which in turn, proved more efficacious than the double-drug combination AZT + delavirdine, when monitored either virologically (decrease of HIV RNA plasma levels) or immunologically (increase of CD4 cell counts) (Fig. 16) (G. Tarpley, personal communication, 1998).

In the CAESAR Trial (a large randomized trial, involving more than 3000 patients) the addition of 3TC, or 3TC + loviride, to AZT-containing treatment regimens (AZT monotherapy, or AZT + ddC, or AZT + ddI combination therapy),was found to slow the progression of HIV disease and improve survival (CAESAR Coordinating Committee, 1997); and again the incremental benefit resulting from the addition of 3TC and loviride was reflected by the increase in CD4 cell counts and decrease of plasma HIV RNA levels (Fig. 17).

In another study, (VAN Study, 22 patients enrolled), HIV patients were treated with the combination 3TC + indinavir + nevirapine (M. Myers, personal communication, 1998): this triple-drug combination resulted in a pronounced ($3 \log_{10}$) reduction in plasma HIV RNA levels, which, again, was virtually mirrored by an equivalent increase in CD4 cell counts (Fig. 18). In yet another small study (dNN Study, 25 patients enrolled), the combination d4T + nelfinavir + nevirapine was investigated (Skowron et al., 1998); and this, again, resulted in a marked reduction in viral load mirrored by a substantial increase in CD4 cell counts (Fig. 19).

Also efavirenz (DMP 266) has been the subject of combination studies with AZT and 3TC,

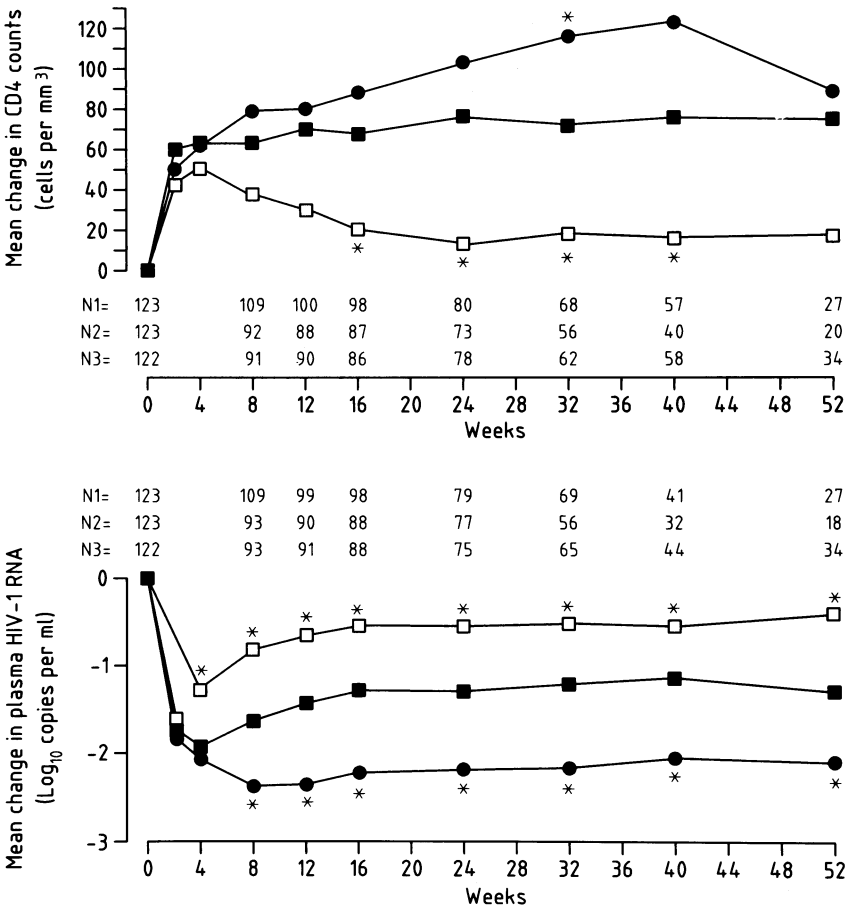

Fig. 16. Protocol 0021 Pt 2 (G. Tarpley, personal communication, 1998). ■, AZT + 3TC; □, AZT + delavirdine; ●, AZT + 3TC + delavirdine. $*P \leq 0.05$.

and with indinavir. In the latter study (101 patients enrolled), the patients receiving indinavir were allowed to add d4T + efavirenz from week 12 onwards (Mayers et al., 1997). The combination of indinavir with efavirenz conferred a more pronounced immunologic and virologic response than indinavir alone, and this response was sustained for the whole follow-up period (48 weeks) (Fig. 20).

As a rule, triple-drug therapy may thus be considered as superior to dual-drug therapy, which, in turn, can be considered as superior to single-drug therapy. It could a priori be expected that quadruple-drug therapy may even be more effective than triple-, and quintuple- more effective than quadruple-, and sextuple- more so than quintuple-drug therapy. Such multiple-drug regimens should, of course, be carefully monitored for toxic side effects and drug interactions. While possible interactions between NNRTIs and ddN analogues are neither known nor anticipated, interactions between NNRTIs and protease inhibitors are likely to occur, since they are both hepatically metabolized (Sahai, 1996). In this respect, delavirdine acts as a cytochrome P450 CYP3A4 inhibitor and may thus increase the plasma levels of the protease inhibitors (i.e. saquinavir). Vice versa, nevirapine rather acts as

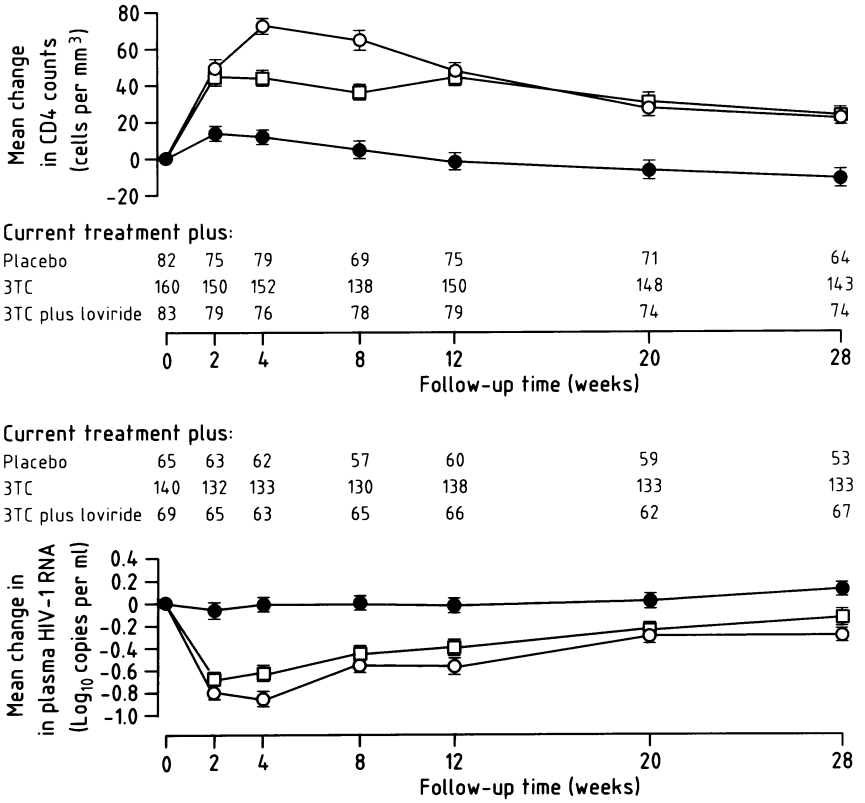

Fig. 17. CAESER trial (CAESAR Coordinating Committee, 1997). Median (± S.E.) changes from baseline in CD4 counts, and $\log_{10}$ copies per ml HIV RNA, per treatment group. Current treatment (AZT, or AZT + ddC, or AZT + ddI) to which was added either placebo (●), or 3TC (□), or 3TC + loviride (○).

an inducer of the P450 enzyme and may thus decrease the plasma levels of the hepatically metabolized drugs (Sahai, 1996).

7. Conclusion

HIV-1 viremia, the hallmark of HIV infection is sustained by a highly dynamic process involving continuous rounds of de novo virus replication and cell turnover (Ho et al., 1995, Wei et al., 1995). Productively infected cells have, on average, a life-span of 2.2 days (half-life = 1.6 days) and plasma virions have a mean life-span of 0.3 days (half-life = 0.24 days). The average total HIV-1 production is approximately 1010 virions/day, and the minimum duration of the HIV-1 life-cycle in vivo is 1.2 days on average (Perelson et al., 1996). Virus replication is the driving force in the progression to AIDS. Low viral load is associated with long-term non-progression to AIDS (Cao et al., 1995, Pantaleo et al., 1995), and, while both plasma HIV RNA levels and CD4 cell counts are valid predictors of the clinical progression of HIV disease (O'Brien et al., 1996, Saag et al., 1996), plasma viral load is a more accurate predictor of progression to AIDS than the number of CD4 cells (Mellors et al., 1996). Furthermore, the clinical course of HIV-1 infection may already be determined at the earliest phase of the disease, and this necessitates initia-

tion of definitive treatment very early in HIV-1 infection (Craib et al., 1997).

In view of all these considerations, and the remarks made above on the optimization of drug treatment regimens, a few recommendations could be formulated so as to ensure a successful treatment of HIV infections. These recommendations (Table 3) concern NNRTIs as well as any other anti-HIV drugs. It would be of paramount importance that the NNRTI-containing drug regimens should be started as soon as possible after HIV infection, and that the individual compounds should be used at the highest possible doses so as to completely suppress virus replication (and prevent drug-resistant virus strains from emerging) for as long a time as necessary. How long the treatment should be continued, and whether it could be discontinued or adjusted at certain time points, remain unsettled issues. Fact is that, despite prolonged suppression of plasma viremia, replication-competent virus could still be recovered from resting CD4 T-lymphocytes (Finzi et al., 1997, Wong et al., 1997), even after 30 months of highly active anti-retroviral therapy. It thus appears that with the current drug treatment regimes, virus replication may be suppressed, resistance development prevented, and progression to AIDS arrested, albeit without eradication of the virus from its reservoir(s).

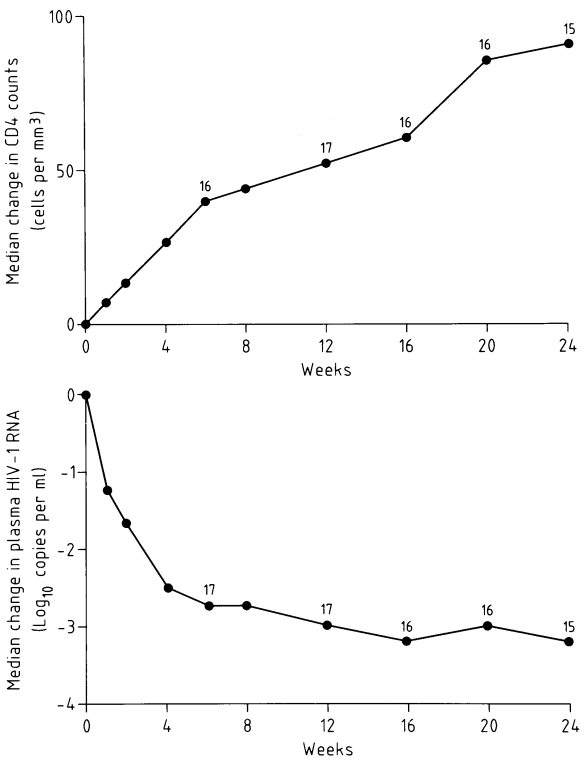

Fig. 18. VAN Study. One-year follow-up of HIV patients treated with 3TC, indinavir and nelfinavir in combination ($N = 22$). According to Harris et al. (M. Myers, personal communication, 1998).

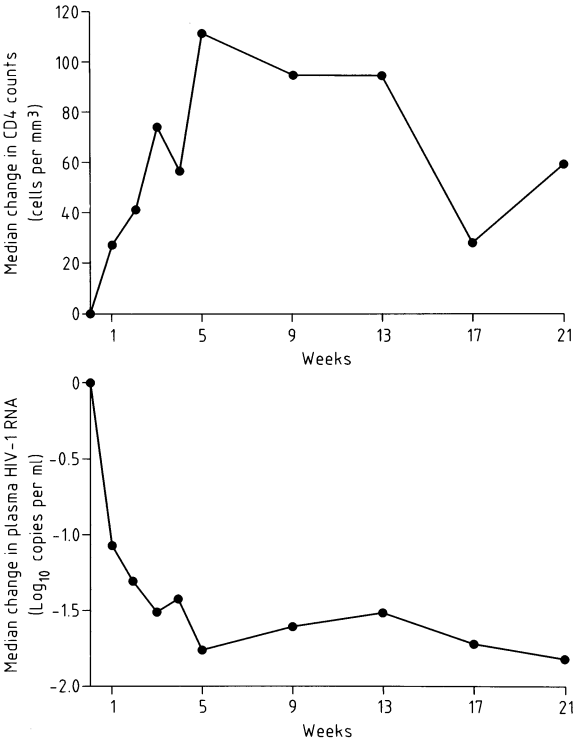

Fig. 19. ddN Study. d4T, nelfinavir and nevirapine in combination ($N = 25$). According to Skowron et al. (1998).

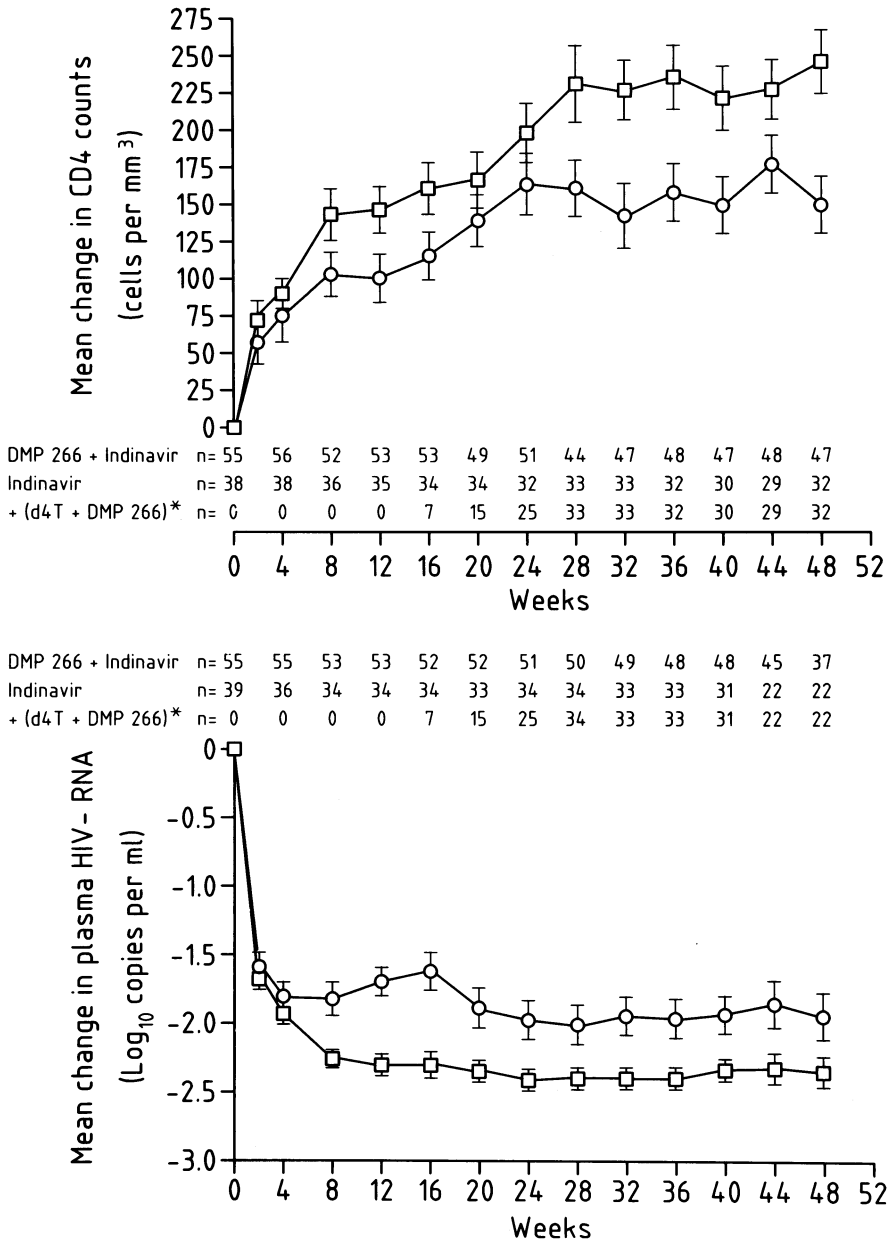

Fig. 20. Combination of efavirenz (DMP 266) with indinavir. Patients receiving indinavir were allowed to add d4T + DMP 266 from week 12 onwards. □, efavirenz 200 mg qd + indinavir 1000 mg q 8 h; ○, indinavir 800 mg/1000 mg q 8 h. * Number of patients receiving d4T + DMP 266 in addition to indinavir. According to Mayers et al. (1997).

Acknowledgements

The dedicated editorial assistance of Christiane Callebaut is gratefully acknowledged. E. De Clercq is titular of the Professor P. De Somer Chair for Microbiology.

Table 3
Recommendations for clinical use of anti-HIV drugs, including NNRTIs

1. Use different compounds in multiple (double, triple, quadruple, etc.) drug combinations
2. At sufficiently high (but subtoxic) doses
3. Starting as soon as possible after the HIV infection
4. With the aim to achieve complete suppression of virus replication (viral load below detection limit)
5. And to prevent the development of virus–drug resistance
6. Ensuring full compliance (patient taking his/her medicine)
7. While improving on the convenience of drug dosing (preferably once daily)
8. Minimizing adverse side-effects of the drug
9. Continuing drug treatment as long as required for a sustained suppression (and, ideally, eradication of the virus from the organs and from the organism)
10. Making the anti-HIV drugs widely available (at affordable costs)

References

Ahrgren, C., Backro, K., Bell, F.W., Cantrell, A.S., Clemens, M., Colacino, J.M., Deeter, J.B., Engelhardt, J.A., Högberg, M., Jaskunas, S.R., Johansson, N.G., Jordan, C.L., Kasher, J.S., Kinnick, M.D., Lind, P., Lopez, C., Morin, J.M. Jr., Muesing, M.A., Noreen, R., Öberg, B., Paget, C.J., Palkowitz, J.A., Parrish, C.A., Pranc, P., Rippy, M.K., Rydergard, C., Sahlberg, C., Swanson, S., Ternansky, R.J., Unge, T., Vasileff, R.T., Vrang, L., West, S.J., Zhang, H., Zhou, X.-X., 1995. The PETT series, a new class of potent nonnucleoside inhibitors of human immunodeficiency virus type 1 reverse transcriptase. Antimicrob. Agents Chemother. 39, 1329–1335.

Althaus, I.W., Chou, K.-C., Lemay, R.J., Franks, K.M., Deibel, M.R., Kezdy, F.J., Resnick, L., Busso, M.E., So, A.G., Downey, K.M., Romero, D.L., Thomas, R.C., Aristoff, P.A., Tarpley, W.G., Reusser, F., 1996. The benzylthio-pyrimidine U-31,355, a potent inhibitor of HIV-1 reverse transcriptase. Biochem. Pharmacol. 51, 743–750.

Antonucci, T., Warmus, J.S., Hodges, J.C., Nickell, D.G., 1995. Characterization of the antiviral activity of highly substituted pyrroles: a novel class of non-nucleoside HIV-1 reverse transcriptase inhibitor. Antiviral Chem. Chemother. 6, 98–108.

Artico, M., Massa, S., Mai, A., Marongiu, M.E., Piras, G., Tramontano, E., La Colla, P., 1993. 3,4-Dihydro-2-alkoxy-6-benzyl-4-oxopyrimidines (DABOs): a new class of specific inhibitors of human immunodeficiency virus type 1. Antiviral Chem. Chemother. 4, 361–368.

Baba, M., Tanaka, H., De Clercq, E., Pauwels, R., Balzarini, J., Schols, D., Nakashima, H., Perno, C.-F., Walker, R.T., Miyasaka, T., 1989. Highly specific inhibition of human immunodeficiency virus type 1 by a novel 6-substituted acyclouridine derivative. Biochem. Biophys. Res. Commun. 165, 1375–1381.

Baba, M., De Clercq, E., Tanaka, H., Ubasawa, M., Takashima, H., Sekiya, K., Nitta, I., Umezu, K., Nakashima, H., Mori, S., Shigeta, S., Walker, R.T., Miyasaka, T., 1991a. Potent and selective inhibition of human immunodeficiency virus type 1 (HIV-1) by 5-ethyl-6-phenylthiouracil derivatives through their interaction with the HIV-1 reverse transcriptase. Proc. Natl. Acad. Sci. USA 88, 2356–2360.

Baba, M., De Clercq, E., Tanaka, H., Ubasawa, M., Takashima, H., Sekiya, K., Nitta, I., Umezu, K., Walker, R.T., Mori, S., Ito, M., Shigeta, S., Miyasaka, T., 1991b. Highly potent and selective inhibition of human immunodeficiency virus type 1 by a novel series of 6-substituted acyclouridine derivatives. Mol. Pharmacol. 39, 805–810.

Baba, M.M., Shigeta, S., Yuasa, S., Takashima, H., Sekiya, K., Ubasawa, M., Tanaka, H., Miyasaka, T., Walker, R.T., De Clercq, E., 1994. Preclinical evaluation of MKC-442, a highly potent and specific inhibitor of human immunodeficiency virus type 1 in vitro. Antimicrob. Agents Chemother. 38, 688–692.

Balzarini, J., De Clercq, E., 1997. The thiocarboxanilides UC-10 and UC-781 have an additive inhibitory effect against human immunodeficiency virus type 1 reverse transcriptase and replication in cell culture when combined with other antiretroviral drugs. Antiviral Chem. Chemother. 8, 197–204.

Balzarini, J., Pérez-Pérez, M.-J., San-Félix, A., Schols, D., Perno, C.-F., Vandamme, A.-M., Camarasa, M.-J., De Clercq, E., 1992a. 2′,5′-Bis-*O*-(tert.-butyldimethylsilyl)-3′-spiro-5″(4″amino-1″,2″oxathiole-2″,2″-dioxide)pyrimidine (TSAO) nucleoside analogues: novel highly selective inhibitors of human immunodeficiency virus type 1 that are targeted at the viral reverse transcriptase. Proc. Natl. Acad. Sci. USA 89, 4392–4396.

Balzarini, J., Perez-Perez, M.-J., San-Felix, A., Velazquez, S., Camarasa, M.-J., De Clercq, E., 1992b. [2′,5′-Bis-*O*-(tert-butyldimethylsilyl)]-3′-spiro-5″-[4″-amino-1″,2″-oxathiole-2″,2″-dioxide) (TSAO) derivatives of purine and pyrimidine nucleosides as potent and selective inhibitors of human immunodeficiency virus type 1. Antimicrob. Agents Chemother. 36, 1073–1080.

Balzarini, J., Pérez-Pérez, M.-J., San-Félix, A., Camarasa, M.-J., Barr, P.J., De Clercq, E., 1992c. Kinetics of inhibition of human immunodeficiency virus type 1 (HIV-1) reverse transcriptase by the novel HIV-1-specific nucleoside analogue [2′,5′-bis-*O*-(tert-butyldimethylsilyl)-β-D-ribofuranosyl]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide)thymine (TSAO-T). J. Biol. Chem. 267, 11831–11838.

Balzarini, J., Karlsson, A., Pérez-Pérez, M.-J., Camarasa, M.-J., De Clercq, E., 1993. Knocking-out concentrations of HIV-1-specific inhibitors completely suppress HIV-1 infection and prevent the emergence of drug-resistant virus. Virology 196, 576–585.

Balzarini, J., Karlsson, A., Meichsner, C., Paessens, A., Riess, G., De Clercq, E., Kleim, J.-P., 1994. Resistance pattern of human immunodeficiency virus type 1 reverse transcriptase to quinoxaline S-2720. J. Virol. 68, 7986–7992.

Balzarini, J., Brouwer, W.G., Felauer, E.E., De Clercq, E., Karlsson, A., 1995a. Activity of various thiocarboxanilide derivatives against wild-type and several mutant human immunodeficiency virus type 1 strains. Antiviral Res. 27, 219–236.

Balzarini, J., Baba, M., De Clercq, E., 1995b. Differential activities of 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio) thymine derivatives against different human immunodeficiency virus type 1 mutant strains. Antimicrob. Agents Chemother. 39, 998–1002.

Balzarini, J., Pérez-Pérez, M.-J., Vélazquez, S., San-Félix, A., Camarasa, M.-J., De Clercq, E., Karlsson, A., 1995c. Suppression of the breakthrough of human immunodeficiency virus type 1 (HIV-1) in cell culture by thiocarboxanilide derivatives when used individually or in combination with other HIV-1-specific inhibitors (i.e. TSAO derivatives). Proc. Natl. Acad. Sci. USA 92, 5470–5474.

Balzarini, J., Pelemans, H., Pérez-Pérez, M.-J., San-Félix, A., Camarasa, M.-J., Karlsson, A., 1996a. Marked inhibitory activity of non-nucleoside reverse transcriptase inhibitors against human immunodeficiency virus type 1 when combined with (-)2′,3′-dideoxy-3′-thiacytidine. Mol. Pharmacol. 49, 882–890.

Balzarini, J., Pelemans, H., Aquaro, S., Perno, C.-F., Witvrouw, M., Schols, D., De Clercq, E., Karlsson, A., 1996b. Highly favorable antiviral activity and resistance profile of the novel thiocarboxanilide pentenyloxy ether derivatives UC-781 and UC-82 as inhibitors of human immunodeficiency virus type 1 replication. Mol. Pharmacol. 50, 394–401.

Balzarini, J., Pelemans, H., Karlsson, A., De Clercq, E., Kleim, J.-P., 1996c. Concomitant combination therapy for HIV infection preferable over sequential therapy with 3TC and non-nucleoside reverse transcriptase inhibitors. Proc. Natl. Acad. Sci. USA 93, 13152–13157.

Balzarini, J., Pelemans, H., Riess, G., Roesner, M., Winkler, I., De Clercq, E., Kleim, J.-P., 1997. Zidovudine-resistant human immunodeficiency virus type 1 strains subcultured in the presence of both lamivudine and quinoxaline HBY 097 retain marked sensitivity to HBY 097 but not to lamivudine. J. Infect. Dis. 176, 1392–1397.

Balzarini, J., Pelemans, H., Esnouf, R., De Clercq, E., 1998a. A novel mutation (F227L) arises in the reverse transcriptase of human immunodeficiency virus type 1 on dose-escalating treatment of HIV type 1-infected cell cultures with the nonnucleoside reverse transcriptase inhibitor thiocarboxanilide UC-781. AIDS Res. Hum. Retroviruses 14, 255–260.

Balzarini, J., Pelemans, H., Riess, G., Roesner, M., Winkler, I., De Clercq, E., Kleim, J.-P., 1998b. Retention of marked sensitivity to (*S*)-4-isopropoxycarbonyl-6-methoxy-3-(methylthiomethyl)-3,4-dihydroquinoxaline-2(1*H*)-thione (HBY 097) by an azidothymidine (AZT)-resistant human immunodeficiency virus type 1 (HIV-1) strain subcultured in the combined presence of quinoxaline HBY 097 and 2′,3′-dideoxy-3′-thiacytidine (lamivudine). Biochem. Pharmacol. 55, 617–625.

Barth, B., Dierich, M., Heinisch, G., Jenny, V., Matuszczak, B., Mereiter, K., Planer, R., Schöpf, I., Stoiber, H., Traugott, T., von Aufschnaiter, P., 1996. Pyridazino[3,4-*b*][1,5]benzoxazepin-5(6*H*)-ones: synthesis and biological evaluation. Antiviral Chem. Chemother. 7, 300–312.

Bellarosa, D., Antonelli, G., Bambacioni, F., Giannotti, D., Viti, G., Nannicini, R., Giachetti, A., Dianzani, F., Witvrouw, M., Pauwels, R., Desmyter, J., De Clercq, E., 1996. New arylpyrido-diazepine and -thiodiazepine derivatives are potent and highly selective HIV-1 inhibitors targeted at the reverse transcriptase. Antiviral Res. 30, 109–124.

Boyer, P.L., Ding, J., Arnold, E., Hughes, S.H., 1994. Subunit specificity of mutations that confer resistance to nonnucleoside inhibitors in human immunodeficiency virus type 1 reverse transcriptase. Antimicrob. Agents Chemother. 38, 1909–1914.

Boyer, P.L., Gao, H.-Q., Hughes, S.H., 1998. A mutation at position 190 of human immunodeficiency virus type 1 reverse transcriptase interacts with mutations at positions 74 and 75 via the template primer. Antimicrob. Agents Chemother. 42, 447–452.

Brennan, T.M., Taylor, D.L., Bridges, C.G., Leyda, J.P., Tyms, A.S., 1995. The inhibition of human immunodeficiency virus type 1 in vitro by a non-nucleoside reverse transcriptase inhibitor MKC-442, alone and in combination with other anti-HIV compounds. Antiviral Res. 26, 173–187.

Buckheit, R.W. Jr., Hollingshead, M.G., Germany-Decker, J., White, E.L., McMahon, J.B., Allen, L.B., Ross, L.J., Decker, W.D., Westbrook, L., Shannon, W.M., Weislow, O., Bader, J.P., Boyd, M.R., 1993. Thiazolobenzimidazole: biological and biochemical anti-retroviral activity of a new nonnucleoside reverse transcriptase inhibitor. Antiviral Res. 21, 247–265.

Buckheit, R.W. Jr., Fliakas-Boltz, V., Decker, W.D., Roberson, J.L., Pyle, C.A., White, E.L., Bowdon, B.J., McMahon, J.B., Boyd, M.R., Bader, J.P., Nickell, D.G., Barth, H., Antonucci, T.K., 1994a. Biological and biochemical anti-HIV activity of the benzothiadiazine class of nonnucleoside reverse transcriptase inhibitors. Antiviral Res. 25, 43–56.

Buckheit, R.W. Jr., White, E.L., Germany-Decker, J., Allen, L.B., Ross, L.J., Shannon, W.M., Janssen, P.A.J., Chirigos, M.A., 1994b. Cell-based and biochemical analysis of the anti-HIV activity of combinations of 3′-azido-3′-deoxythymidine and analogues of TIBO. Antiviral Chem. Chemother. 5, 35–42.

Buckheit, R.W. Jr., Fliakas-Boltz, V., Yeagy-Bargo, S., Weislow, O., Mayers, D.L., Boyer, P.L., Hughes, S.H., Pan, B.-C., Chu, S.-H., Bader, J.P., 1995. Resistance to 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine derivatives is generated by mutations at multiple sites in the HIV-1 reverse transcriptase. Virology 210, 186–193.

Buckheit, R.W. Jr., Snow, M.J., Fliakas-Boltz, V., Kinjerski, T.L., Russell, J.D., Pallansch, L.A., Brouwer, W.G., Yang, S.S., 1997. Highly potent oxathiin carboxanilide derivatives with efficacy against nonnucleoside reverse transcriptase inhibitor-resistant human immunodeficiency virus isolates. Antimicrob. Agents Chemother. 41, 831–837.

Byrnes, V.W., Emini, E.A., Schleif, W.A., Condra, J.H., Schneider, C.L., Long, W.J., Wolfgang, J.A., Graham, D.J., Gotlib, L., Schlabach, A.J., Wolanski, B.S., Blahy, O.M., Quintero, J.C., Rhodes, A., Roth, E., Titus, D.L., Sardana, V.V., 1994. Susceptibilities of human immunodeficiency virus type 1 enzyme and viral variants expressing multiple resistance-engendering amino acid substitutions to reverse transcriptase inhibitors. Antimicrob. Agents Chemother. 38, 1404–1407.

CAESAR Coordinating Committee, 1997. Randomised trial of addition of lamivudine or lamivudine plus loviride to zidovudine-containing regimens for patients with HIV-1 infection: the CAESAR trial. Lancet 349, 1413–1421.

Campiani, G., Nacci, V., Fiorini, I., De Filippis, M.P., Garofalo, A., Greco, G., Novellino, E., Altamura, S., Di Renzo, L., 1996. Pyrrolobenzothiazepinones and pyrrolobenzoxazepinones: novel and specific non-nucleoside HIV-1 reverse transcriptase inhibitors with antiviral activity. J. Med. Chem. 39, 2672–2680.

Cao, Y., Qin, L., Zhang, L., Safrit, J., Ho, D.D., 1995. Virologic and immunologic characterization of long-term survivors of human immunodeficiency virus type 1 infection. New Engl. J. Med. 332, 201–208.

Carr, A., Vella, S., de Jong, M.D., Sorice, F., Imrie, A., Boucher, C.A., Cooper, D.A., 1996. A controlled trial of nevirapine plus zidovudine versus zidovudine alone in p24 antigenaemic HIV-infected patients. AIDS 10, 635–641.

Chimirri, A., Grasso, S., Molica, C., Monforte, A.-M., Monforte, P., Zappalà, M., Bruno, G., Nicolò, F., Witvrouw, M., Jonckheere, H., Balzarini, J., De Clercq, E., 1997. Structural features and anti-human immunodeficiency virus (HIV) activity of the isomers of 1-(2′,6′-difluorophenyl)-1*H*,3*H*-thiazolo[3,4-*a*]benzimidazole, a potent non-nucleoside HIV-1 reverse transcriptase inhibitor. Antiviral Chem. Chemother. 8, 363–370.

Chong, K.-T., Pagano, P.J., Hinshaw, R.R., 1994. Bisheteroarylpiperazine reverse transcriptase inhibitor in combination with 3′-azido-3′-deoxythymidine or 2′,3′-dideoxycytidine synergistically inhibits human immunodeficiency virus type 1 replication in vitro. Antimicrob. Agents Chemother. 38, 288–293.

Craib, K.J.P., Strathdee, S.A., Hogg, R.S., Leung, B., Montaner, J.S.G., O'Shaughnessy, M.V., Schechter, M.T., 1997. Serum levels of human immunodeficiency virus type 1 (HIV-1) RNA after seroconversion: a predictor of long-term mortality in HIV infection. J. Infect. Dis. 176, 798–800.

National Institute of Allergy and Infectious Diseases AIDS Clinical Trials Group Protocol 241 Investigators, D'Aquila, R.T., Hughes, M.D., Johnson, M.D., Johnson, V.A., Fischl, M.S., Sommadossi, J.-P., Liou, S.-H., Timpone, J., Myers, M., Myers, M., Basgoz, N., Niu, M., Hirsch, M.S., 1996. Nevirapine, zidovudine, and didanosine compared with zidovudine and didanosine in patients with HIV-1 infection. A randomized, double-blind, placebo-controlled trial. Ann. Intern. Med. 124, 1019–1030.

Davey, R.T. Jr., Dewar, R.L., Reed, G.F., Vasudevachari, M.B., Polis, M.A., Kovacs, J.A., Falloon, J., Walker, R.E., Masur, H., Haneiwich, S.E., O'Neill, D.G., Decker, M.R., Metcalf, J.A., Deloria, M.A., Laskin, O.L., Salzman, N., Lane, H.C., 1993. Plasma viremia as a sensitive indicator of the antiretroviral activity of L-697,661. Proc. Natl. Acad. Sci. USA 90, 5608–5612.

Davey, R.T. Jr., Chaitt, D.G., Reed, G.F., Freimuth, W.W., Herpin, B.R., Metcalf, J.A., Eastman, P.S., Falloon, J., Kovacs, J.A., Polis, M.A., Walker, R.E., Masur, H., Boyle, J., Coleman, S., Cox, S.R., Wathen, L., Daenzer, C.L., Lane, H.C., 1996. Randomized, controlled phase I/II trial of combination therapy with delavirdine (U-90152S) and conventional nucleosides in human immunodeficiency virus type 1-infected patients. Antimicrob. Agents Chemother. 40, 1657–1664.

Debyser, Z., Pauwels, R., Andries, K., Desmyter, J., Kukla, M., Janssen, P.A.J., De Clercq, E., 1991. An antiviral target on reverse transcriptase of human immunodeficiency virus type 1 revealed by tetrahydroimidazo-[4,5,1-*jk*][1,4]benzodiazepin-2(1*H*)-one and -thione derivatives. Proc. Natl. Acad. Sci. USA 88, 1451–1455.

Debyser, Z., Pauwels, R., Baba, M., Desmyter, J., De Clercq, E., 1992. Common features in the interaction of tetrahydroimidazo[4,5,1-*jk*][1,4]benzodiazepin-2(1*H*)-one and -thione and 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio) thymine derivatives with the human immunodeficiency virus type 1 reverse transcriptase. Mol. Pharmacol. 41, 963–968.

De Clercq, E., 1995. Antiviral therapy for human immunodeficiency virus infections. Clin. Microbiol. Rev. 8, 200–239.

De Clercq, E., 1996a. Non-nucleoside reverse transcriptase inhibitors (NNRTIs) for the treatment of human immunodeficiency virus type 1 (HIV-1) infections: strategies to overcome drug resistance development. Med. Res. Rev. 16, 125–157.

De Clercq, E., 1996b. What can be expected from non-nucleoside reverse transcriptase inhibitors (NNRTIs) in the treatment of human immunodeficiency virus type 1 (HIV-1) infections? Rev. Med. Virol. 6, 97–117.

De Clercq, E., 1997. In search of a selective antiviral chemotherapy. Clin. Microbiol. Rev. 10, 674–693.

De Jong, M.D., Vella, S., Carr, A., Boucher, C.A.B., Imrie, A., French, M., Hoy, J., Sorice, S., Pauluzzi, S., Chiodo, F., Weverling, G.J., van der Ende, M.E., Frissen, Ph.J., Weigel, H.M., Kauffmann, R.H., Lange, J.M.A., Yoon, R., Moroni, M., Hoenderdos, E., Leitz, G., Cooper, D.A., Hall, D., Reiss, P., 1997. High-dose nevirapine in previously untreated human immunodeficiency virus type 1-infected persons does not result in sustained suppression of viral replication. J. Infect. Dis. 175, 966–970.

De Lucca, G.V., Otto, M.J., 1992. Synthesis and anti-HIV activity of pyrrolo-[1,2-*d*]-(1,4)-benzodiazepin-6-ones. Bioorg. Med. Chem. Lett. 2, 1639–1644.

De Wit, S., Hermans, P., Sommereijns, B., O'Doherty, E., Westenborghs, R., Van De Velde, V., Cauwenbergh, G.F.M.J., Clumeck, N., 1992. Pharmacokinetics of R 82913 in AIDS patients: a phase I dose-finding study of oral administration compared with intravenous infusion. Antimicrob. Agents Chemother. 36, 2661–2663.

Ding, J., Das, K., Tantillo, C., Zhang, W., Clark, A.D. Jr., Jesen, S., Lu, X., Hsiou, Y., Jacobo-Molina, A., Andries, K., Pauwels, R., Moereels, H., Koymans, L., Janssen, P.A.J., Smith, R.H. Jr., Kroeger Koepke, M., Michejda, C.J., Hughes, S.H., Arnold, E., 1995a. Structure of HIV-1 reverse transcriptase in a complex with the non-nucleoside inhibitor α-APA R 95845 at 2.8 Å resolution. Structure 3, 365–379.

Ding, J., Das, K., Moereels, H., Koymans, L., Andries, K., Janssen, P.A.J., Hughes, S.H., Arnold, E., 1995b. Structure of HIV-1 RT/TIBO R86183 complex reveals similarity in the binding of diverse nonnucleoside inhibitors. Struct. Biol. 2, 407–415.

Di Santo, R., Costi, R., Artico, M., Massa, S., Marongiu, M.E., Loi, A.G., De Montis, A., La Colla, P., 1998. 1,2,5-Benzothiadiazepine and pyrrolo[2,1-*d*][1,2,5]benzothiadiazepine derivatives with specific anti-human immunodeficiency virus type 1 activity. Antiviral Chem. Chemother. 9, 127–137.

Dollé, V., Nguyen, C.H., Aubertin, A.M., Kirn, A., Andreola, M.L., Jamieson, G., Tarrago-Litvak, L., Bisagni, E., 1995. A new series of pyridinone derivatives as potent non-nucleoside human immunodeficiency virus type 1 specific reverse transcriptase inhibitors. J. Med. Chem. 38, 4679–4686.

Dueweke, T.J., Kézdy, F.J., Waszak, G.A., Deibel, M.R. Jr., Tarpley, W.G., 1992. The binding of a novel bisheteroarylpiperazine mediates inhibition of human immunodeficiency virus type 1 reverse transcriptase. J. Biol. Chem. 267, 27–30.

Dueweke, T.J., Poppe, S.M., Romero, D.L., Swaney, S.M., So, A.G., Downey, K.M., Althaus, I.W., Reusser, F., Busso, M., Resnick, L., Mayers, D.L., Lane, J., Aristoff, P.A., Thomas, R.C., Tarpley, W.G., 1993a. U-90152, a potent inhibitor of human immunodeficiency virus type 1 replication. Antimicrob. Agents Chemother. 37, 1127–1131.

Dueweke, T.J., Pushkarskaya, T., Poppe, S.M., Swaney, S.M., Zhao, J.Q., Chen, I.S.Y., Stevenson, M., Tarpley, W.G., 1993b. A mutation in reverse transcriptase of bis(heteroaryl)piperazine-resistant human immunodeficiency virus type 1 that confers increased sensitivity to other nonnucleoside inhibitors. Proc. Natl. Acad. Sci. USA 90, 4713–4717.

Esnouf, R.M., 1997. An extensively modified version of MolScript that includes greatly enhanced coloring capabilities. J. Mol. Graphics 15, 132–134.

Esnouf, R., Ren, J., Ross, C., Jones, Y., Stammers, D., Stuart, D., 1995. Mechanism of inhibition of HIV-1 reverse transcriptase by non-nucleoside inhibitors. Struct. Biol. 2, 303–308.

Esnouf, R.M., Ren, J., Hopkins, A.L., Ross, C.K., Jones, E.Y., Stammers, D.K., Stuart, D.I., 1997a. Unique features in the structure of the complex between HIV-1 reverse transcriptase and the bis(heteroaryl)piperazine (BHAP) U-90152 explain resistance mutations for this nonnucleoside inhibitor. Proc. Natl. Acad. Sci. USA 94, 3984–3989.

Esnouf, R.M., Stuart, D.I., De Clercq, E., Schwartz, E., Balzarini, J., 1997b. Models which explain the inhibition of reverse transcriptase by HIV-1-specific (thio)carboxanilide derivatives. Biochem. Biophys. Res. Commun. 234, 458–464.

Finzi, D., Hermankova, M., Pierson, T., Carruth, L.M., Buck, C., Chaisson, R.E., Quinn, T.C., Chadwick, K., Margolick, J., Brookmeyer, R., Gallant, J., Markowitz, M., Ho, D.D., Richman, D.D., Siliciano, R.F., 1997. Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. Science 279, 1295–1300.

Goldman, M.E., Nunberg, J.H., O'Brien, J.A., Quintero, J.C., Schleif, W.A., Freund, K.F., Gaul, S.L., Saari, W.S., Wai, J.S., Hoffman, J.M., Anderson, P.S., Hupe, D.J., Emini, E.A., Stern, A.M., 1991. Pyridinone derivatives: specific human immunodeficiency virus type 1 reverse transcriptase inhibitors with antiviral activity. Proc. Natl. Acad. Sci. USA 88, 6863–6867.

Goldman, M.E., O'Brien, J.A., Ruffing, T.L., Nunberg, J.H., Schleif, W.A., Quintero, J.C., Siegl, P.K.S., Hoffman, J.M., Smith, A.M., Emini, E.A., 1992. L-696,229 specifically inhibits human immunodeficiency virus type 1 reverse transcriptase and possesses antiviral activity in vitro. Antimicrob. Agents Chemother. 36, 1019–1023.

Grob, P.M., Cao, Y., Muchmore, E., Ho, D.D., Norris, S., Pav, J.W., Shih, C.-K., Adams, J., 1997. Prophylaxis against HIV-1 infection in chimpanzees by nevirapine, a nonnucleoside inhibitor of reverse transcriptase. Nat. Med. 3, 665–670.

Havlir, D.V., Richman, D.D., 1996. Viral dynamics of HIV: implications for drug development and therapeutic strategies. Ann. Intern. Med. 124, 984–994.

Havlir, D., Cheeseman, S.H., McLaughlin, M., Murphy, R., Erice, A., Spector, S.A., Greenough, T.C., Sullivan, J.L., Hall, D., Myers, M., Lamson, M., Richman, D.D., 1995a. High-dose nevirapine: safety, pharmacokinetics, and antiviral effect in patients with human immunodeficiency virus infection. J. Infect. Dis. 171, 537–545.

Havlir, D., McLaughlin, M.M., Richman, D.D., 1995b. A pilot study to evaluate the development of resistance to nevirapine in asymptomatic human immunodeficiency virus-infected patients with CD4 cell counts of $> 500/mm^3$: AIDS clinical trials group protocol 208. J. Infect. Dis. 172, 1379–1383.

Ho, D.D., Neumann, A.U., Perelson, A.S., Chen, W., Leonard, J.M., Markowitz, M., 1995. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. Nature 373, 123–126.

Ijichi, K., Fujiwara, M., Hanasaki, Y., Watanabe, H., Katsuura, K., Takayama, H., Shirakawa, S., Sakai, S.-I., Shigeta, S., Konno, K., Yokota, T., Baba, M., 1995. Potent and specific inhibition of human immunodeficiency virus type 1 replication by 4-(2,6-dichlorophenyl)-1,2,5-thiadiazol-3-Y1 *N*,*N*-dialkylcarbamate derivatives. Antimicrob. Agents Chemother. 39, 2337–2340.

Ijichi, K., Fujiwara, M., Nagano, H., Matsumoto, Y., Hanasaki, Y., Ide, T., Katsuura, K., Takayama, H., Shirakawa, S., Aimi, N., Shigeta, S., Konno, K., Matsushima, M., Yokota, T., Baba, M., 1996. Anti-HIV-1 activity of thiadiazole derivatives: a structure–activity relationship, reverse transcriptase inhibition, and lipophilicity. Antiviral Res. 31, 87–94.

Ito, M., Baba, M., Shigeta, S., De Clercq, E., Walker, R.T., Tanaka, H., Miyasaka, T., 1991. Synergistic inhibition of human immunodeficiency virus type 1 (HIV-1) replication in vitro by 1-[(2-hydroxyethoxy)methyl]-6-phenylthiothymine (HEPT) and recombinant alpha interferon. Antiviral Res. 15, 323–330.

Jonckheere, H., Taymans, J.-M., Balzarini, J., Velázquez, S., Camarasa, M.-J., Desmyter, J., De Clercq, E., Anné, J., 1994. Resistance of HIV-1 reverse transcriptase against [2′,5′-bis-*O*-(tert-butyldimethylsilyl)-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide)] (TSAO) derivatives is determined by the mutation $Glu^{138} \rightarrow Lys$ on the p51 subunit. J. Biol. Chem. 269, 25255–25258.

Jourdan, F., Renault, J., Fossey, C., Bureau, R., Ladurée, D., Robba, M., Aubertin, A.M., Kirn, A., 1997. Design, synthesis and antiviral activity of new pyridinone derivatives. Antiviral Chem. Chemother. 8, 161–172.

Kashman, Y., Gustafson, K.R., Fuller, R.W., Cardellina II, J.H., McMahon, J.B., Currens, M.J., Buckheit, R.W. Jr., Hughes, S.H., Cragg, G.M., Boyd, M.R., 1992. The calanolides, a novel HIV-inhibitory class of coumarin derivatives from the tropical rainforest tree, *Calophyllum lanigerum*. J. Med. Chem. 35, 2735–2743.

Kelly, T.A., McNeil, D.W., Rose, J.M., David, E., Shih, C.-K., Grob, P.M., 1997. Novel non-nucleoside inhibitors of human immunodeficiency virus type 1 reverse transcriptase. 6. 2-Indol-3-yl- and 2-azaindol-3-yl-dipyridodiazepinones. J. Med. Chem. 40, 2430–2433.

Kim, D.-K., Gam, J., Kim, Y.-W., Lim, J., Kim, H.-T., Kim, K.H., 1997. Synthesis and anti-HIV-1 activity of a series of 1-alkoxy-5-alkyl-6-(arylthio)uracils. J. Med. Chem. 40, 2363–2373.

Kleim, J.-P., Bender, R., Billhardt, U.-M., Meichsner, C., Riess, G., Rösner, M., Winkler, I., Paessens, A., 1993. Activity of a novel quinoxaline derivative against human immunodeficiency virus type 1 reverse transcriptase and viral replication. Antimicrob. Agents Chemother. 37, 1659–1664.

Kleim, J.-P., Bender, R., Kirsch, R., Meichsner, C., Paessens, A., Riess, G., 1994. Mutational analysis of residue 190 of human immunodeficiency virus type 1 reverse transcriptase. Virology 200, 696–701.

Kleim, J.-P., Bender, R., Kirsch, R., Meichsner, C., Paessens, A., Rösner, M., Rübsamen-Waigmann, H., Kaiser, R., Wichers, M., Schneweis, K.E., Winkler, I., Riess, G., 1995. Preclinical evaluation of HBY 097, a new nonnucleoside reverse transcriptase inhibitor of human immunodeficiency virus type 1 replication. Antimicrob. Agents Chemother. 39, 2253–2257.

Kleim, J.-P., Rösner, M., Winkler, I., Paessens, A., Kirsch, R., Hsiou, Y., Arnold, E., Riess, G., 1996. Selective pressure of a quinoxaline nonnucleoside inhibitor of human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (RT) on HIV-1 replication results in the emergence of nucleoside RT-inhibitor-specific (RT Leu-74 → Val or Ile and Val-75 → Leu or Ile) HIV-1 mutants. Proc. Natl. Acad. Sci. USA 93, 34–38.

Kleim, J.-P., Winkler, I., Rösner, M., Kirsch, R., Rübsamen-Waigmann, H., Paessens, A., Riess, G., 1997. In vitro selection for different mutational patterns in the HIV-1 reverse transcriptase using high and low selective pressure of the nonnucleoside reverse transcriptase inhibitory HBY 097. Virology 231, 112–118.

Koup, R.A., Merluzzi, V.J., Hargrave, K.D., Adams, J., Grozinger, K., Eckner, R.J., Sullivan, J.L., 1991. Inhibition of human immunodeficiency virus type 1 (HIV-1) replication by the dipyridodiazepinone BI-RG-587. J. Infect. Dis. 163, 966–970.

Kroeger Smith, M.B., Rouzer, C.A., Taneyhill, L.A., Smith, N.A., Hughes, S.H., Boyer, P.L., Janssen, P.A.J., Moereels, H., Koymans, L., Arnold, E., Ding, J., Das, K., Zhang, W., Michejda, C.J., Smith, R.H. Jr., 1995. Molecular modeling studies of HIV-1 reverse transcriptase nonnucleoside inhibitors: total energy of complexation as a predictor of drug placement and activity. Protein Sci. 4, 2203–2222.

Larder, B.A., 1992. 3′-Azido-3′-deoxythymidine resistance suppressed by a mutation conferring human immunodeficiency virus type 1 resistance to nonnucleoside reverse transcriptase inhibitors. Antimicrob. Agents Chemother. 36, 2664–2669.

Livermore, D.G.H., Bethell, R.C., Cammack, N., Hancock, A.P., Hann, M.M., Green, D.V.S., Lamont, R.B., Noble, S.A., Orr, D.C., Payne, J.J., Ramsay, M.V.J., Shingler, A.H., Smith, C., Storer, R., Williamson, C., Willson, T., 1993. Synthesis and anti-HIV-1 activity of a series of imidazo[1,5-*b*]pyridazines. J. Med. Chem. 36, 3784–3794.

Luzuriaga, K., Bryson, Y., Krogstad, P., Robinson, J., Stechenberg, B., Lamson, M., Cort, S., Sullivan, J.L., 1997. Combination treatment with zidovudine, didanosine, and nevirapine in infants with human immunodeficiency virus type 1 infection. New Engl. J. Med. 336, 1343–1349.

Maass, G., Immendoerfer, U., Koenig, B., Leser, U., Mueller, B., Goody, R., Pfaff, E., 1993. Viral resistance to the thiazolo-iso-indolinones, a new class of nonnucleoside inhibitors of human immunodeficiency virus type 1 reverse transcriptase. Antimicrob. Agents Chemother. 37, 2612–2617.

Marschner, I.C., Collier, A.C., Coombs, R.W., D'Aquila, R.T., DeGruttola, V., Fischl, M.A., Hammer, S.M., Hughes, M.D., Johnson, V.A., Katzenstein, D.A., Richman, D.D., Smeaton, L.M., Spector, S.A., Saag, M.S., 1998. Use of changes in plasma levels of human immunodeficiency virus type 1 RNA to assess the clinical benefit of antiretroviral therapy. J. Infect. Dis. 177, 40–47.

Massa, S., Mai, A., Artico, M., Sbardella, G., Tramontano, E., Loi, A.G., Scano, P., La Colla, P., 1995. Synthesis and antiviral activity of new 3,4-dihydro-2-alkoxy-6-benzyl-4-oxopyrimidines (DABOs), specific inhibitors of human immunodeficiency virus type 1. Antiviral Chem. Chemother. 6, 1–8.

Mayers, D., Riddler, S., Bach, M., Stein, D.F., Havlir, M.D., Kahn, J., Ruiz, N., Labriola, D.F. and the DMP 266 Clinical Development Team, 1997. Durable clinical anti-HIV-1 activity and tolerability for DMP 266 in combination with indinavir (IDV) at 24 weeks. 37th Annual Meeting of the Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), Toronto, 30 September 1997, abstract I-175.

Mellors, J.W., Dutschman, G.E., Im, G.-J., Tramontano, E., Winkler, S.R., Cheng, Y.-C., 1992. In vitro selection and molecular characterization of human immunodeficiency virus-1 resistant to non-nucleoside inhibitors of reverse transcriptase. Mol. Pharmacol. 41, 446–451.

Mellors, J.W., Im, G.-J., Tramontano, E., Winkler, S.R., Medina, D.J., Dutschman, G.E., Bazmi, H.Z., Piras, G., Gonzalez, C.J., Cheng, Y.-C., 1993. A single conservative amino acid substitution in the reverse transcriptase of human immunodeficiency virus-1 confers resistance to (+)-(5*S*)-4,5,6,7-tetrahydro-5-methyl-6-(3-methyl-2-butenyl)-imidazo[4,5,1-*jk*][1,4]benzodiazepin-2(1*H*)-thione (TIBO R82150). Mol. Pharmacol. 43, 11–16.

Mellors, J.W., Rinaldo, C.R. Jr., Gupta, P., White, R.M., Todd, J.A., Kingsley, L.A., 1996. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. Science 272, 1167–1170.

Merluzzi, V.J., Hargrave, K.D., Labadia, M., Grozinger, K., Skoog, M., Wu, J.C., Shih, C.-K., Eckner, K., Hattox, S., Adams, J., Rosenthal, A.S., Faanes, R., Eckner, R.J., Koup, R.A., Sullivan, J.L., 1990. Inhibition of HIV-1 replication by a nonnucleoside reverse transcriptase inhibitor. Science 250, 1411–1413.

Mertens, A., Zilch, H., Konig, B., Schafer, W., Poll, T., Kampe, W., Seidel, H., Leser, U., Leinert, H., 1993. Selective non-nucleoside HIV-1 reverse transcriptase inhibitors. New 2,3-dihydrothiazolo[2,3-*a*]isoindol-5(9*H*)-ones and related compounds with anti-HIV-1 activity. J. Med. Chem. 36, 2526–2535.

Miyasaka, T., Tanaka, H., Baba, M., Hayakawa, H., Walker, R.T., Balzarini, J., De Clercq, E., 1989. A novel lead for specific anti-HIV-1 agents: 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine. J. Med. Chem. 32, 2507–2509.

Moxham, C.P., Borroto-Esoda, K., Noel, D., Furman, P.A., Szczech, G.M., Barry, D.W., 1997. Preliminary efficacy, pharmacokinetics and safety of repeated multiple doses of MKC-442 in HIV-infected volunteers. Abstracts of the Tenth International Conference on Antiviral Research, Atlanta, GA, 6–11 April 1997. Antiviral Res. 34 (2), A44.

Nunberg, J.H., Schleif, W.A., Boots, E.J., O'Brien, J.A., Quintero, J.C., Hoffman, J.M., Emini, E.A., Goldman, M.E., 1991. Viral resistance to human immunodeficiency virus type 1-specific pyridinone reverse transcriptase inhibitors. J. Virol. 65, 4887–4892.

Veterans Affairs Cooperative Study Group on AIDS, O'Brien, W.A., Hartigan, P.M., Martin, D., Esinhart, J., Hill, A., Benoit, S., Rubin, M., Simberhoff, M.S., Hamilton, J.D., 1996. Changes in plasma HIV-1 RNA and CD4+ lymphocyte counts and the risk of progression to AIDS. New Engl. J. Med. 334, 426–431.

Okamoto, M., Makino, M., Yamada, K., Nakade, K., Yuasa, S., Baba, M., 1996. Complete inhibition of viral breakthrough by combination of MKC-442 with AZT during a long-term culture of HIV-1-infected cells. Antiviral Res. 31, 69–77.

Pagano, P.J., Chong, K.-T., 1995. In vitro inhibition of human immunodeficiency virus type 1 by a combination of delavirdine (U-90152) with protease inhibitor U-75875 or interferon-α. J. Infect. Dis. 171, 61–71.

Pantaleo, G., Menzo, S., Vaccarezza, M., Graziosi, C., Cohen, O.J., Demarest, J.F., Montefiori, D., Orenstein, J.M., Fox, C., Schrager, L.K., Margolick, J.B., Buchbinder, S., Giorgi, J.V., Fauci, A.S., 1995. Studies in subjects with long-term nonprogressive human immunodeficiency virus infection. New Engl. J. Med. 332, 209–216.

Pauwels, R., Andries, K., Desmyter, J., Schols, D., Kukla, M.J., Breslin, H.J., Raeymaeckers, A., Van Gelder, J., Woestenborghs, R., Heykants, J., Schellekens, K., Janssen, M.A.C., De Clercq, E., Janssen, P.A.J., 1990. Potent and selective inhibition of HIV-1 replication in vitro by a novel series of TIBO derivatives. Nature 343, 470–474.

Pauwels, R., Andries, K., Debyser, Z., Van Daele, P., Schols, D., Stoffels, P., De Vreese, K., Woestenborghs, R., Vandamme, A.-M., Janssen, C.G.M., Anné, J., Cauwenbergh, G., Desmyter, J., Heykants, J., Janssen, M.A.C., De Clercq, E., Janssen, P.A.J., 1993. Potent and highly selective human immunodeficiency virus type 1 (HIV-1) inhibition by a series of α-anilinophenylacetamide derivatives targeted at HIV-1 reverse transcriptase. Proc. Natl. Acad. Sci. USA 90, 1711–1715.

Pauwels, R., Andries, K., Debyser, Z., Kukla, M.-J., Schols, D., Breslin, H.J., Woestenborghs, R., Desmyter, J., Janssen, M.A.C., De Clercq, E., Janssen, P.A.J., 1994. New tetrahydroimidazo[4,5,1-*jk*][1,4]-benzodiazepin-2(1*H*)-one and -thione derivatives are potent inhibitors of human immunodeficiency virus type 1 replication and are synergistic with 2′,3′-dideoxynucleoside analogs. Antimicrob. Agents Chemother. 38, 2863–2870.

Pelemans, H., Esnouf, R., Dunkler, A., Parniak, M.A., Vandamme, A.-M., Karlsson, A., De Clercq, E., Kleim, J.-P., Balzarini, J., 1997. Characteristics of the Pro225His mutation in human immunodeficiency virus type 1 (HIV-1) reverse transcriptase that appears under selective pressure of dose-escalating quinoxaline treatment of HIV-1. J. Virol. 71, 8195–8203.

Perelson, A.S., Neumann, A.U., Markowitz, M., Leonard, J.M., Ho, D.D., 1996. HIV-1 dynamics in vivo: virion clearance rate, infected cell life-span, and viral generation time. Science 271, 1582–1586.

Pialoux, G., Youle, M., Dupont, B., Gazzard, B., Cauwenbergh, G.F.M.J., Stoffels, P.A.M., Davies, S., De Saint Martin, J., Janssen, P.A.J., 1991. Pharmacokinetics of R 82913 in patients with AIDS or AIDS-related complex. Lancet 338, 140–143.

Ren, J., Esnouf, R., Garman, E., Somers, D., Ross, C., Kirby, I., Keeling, J., Darby, G., Jones, Y., Stuart, D., Stammers, D., 1995a. High resolution structures of HIV-1 RT from four RT-inhibitor complexes. Struct. Biol. 2, 293–302.

Ren, J., Esnouf, R., Hopkins, A., Ross, C., Jones, Y., Stammers, D., Stuart, D., 1995b. The structure of HIV-1 reverse transcriptase complexed with 9-chloro-TIBO: lessons for inhibitor design. Structure 3, 915–926.

Richman, D., Shih, C.-K., Lowy, I., Rose, J., Prodanovich, P., Goff, S., Griffin, J., 1991a. Human immunodeficiency virus type 1 mutants resistant to nonnucleoside inhibitors of reverse transcriptase arise in tissue culture. Proc. Natl. Acad. Sci. USA 88, 11241–11245.

Richman, D., Rosenthal, A.S., Skoog, M., Eckner, R.J., Chou, T.-C., Sabo, J.P., Merluzzi, V.J., 1991b. BI-RG-587 is active against zidovudine-resistant human immunodeficiency virus type 1 and synergistic with zidovudine. Antimicrob. Agents Chemother. 35, 305–308.

Richman, D.D., Havlir, D., Corbeil, J., Looney, D., Ignacio, C., Spector, S.A., Sullivan, J., Cheeseman, S., Barringer, K., Pauletti, D., Shih, C.-K., Myers, M., Griffin, J., 1994. Nevirapine resistance mutations of human immunodeficiency virus type 1 selected during therapy. J. Virol. 68, 1660–1666.

Romero, D.L., Busso, M., Tan, C.-K., Reusser, F., Palmer, J.R., Poppe, S.M., Aristoff, P.A., Downey, K.M., So, A.G., Resnick, L., Tarpley, W.G., 1991. Nonnucleoside reverse transcriptase inhibitors that potently and specifically block human immunodeficiency virus type 1 replication. Proc. Natl. Acad. Sci. USA 88, 8806–8810.

Romero, D.L., Morge, R.A., Genin, M.J., Biles, C., Busso, M., Resnick, L., Althaus, I.W., Reusser, F., Thomas, R.C., Tarpley, W.G., 1993. Bis(heteroaryl)piperazine (BHAP) reverse transcriptase inhibitors: structure–activity relationships of novel substituted indole analogues and the identification of 1-[(5-methanesulfonamido-1*H*-indol-2-yl)carbonyl]-4-[3-[(1-methylethyl)amino]pyridinyl]piperazine monomethanesulfonate (U-90152S), a second-generation clinical candidate. J. Med. Chem. 36, 1505–1508.

Rübsamen-Waigmann, H., Huguenel, E., Paessens, A., Kleim, J.-P., Wainberg, M.A., Shah, A., 1997. Second-generation non-nucleoside reverse transcriptase inhibitor HBY 097 and HIV-1 viral load. Lancet 349, 1517.

L-697,661 Working Group, Saag, M.S., Emini, E.A., Laskin, O.L., Douglas, J., Lapidus, W.I., Schleif, W.A., Whitley, R.J., Hildebrand, C., Byrnes, V.W., Kappes, J.C., Anderson, K.W., Massari, F.E., Shaw, G.M., 1993. A short-term clinical evaluation of L-697,661, a non-nucleoside inhibitor of HIV-1 reverse transcriptase. New Engl. J. Med. 329, 1065–1072.

Saag, M.S., Holodniy, M., Kuritzkes, D.R., O'Brien, W.A., Coombs, R., Poscher, M.E., Jacobsen, D.M., Shaw, G.M., Richman, D.D., Volberding, P.A., 1996. HIV viral load markers in clinical practice. Nat. Med. 2, 625–629.

Sahai, J., 1996. Risks and synergies from drug interactions. AIDS 10 (Suppl. 1), S21–S25.

Schinazi, R.F., Larder, B.A., Mellors, J.W., 1997. Mutations in retroviral genes associated with drug resistance. Int. Antiviral News 5, 129–142.

ACTG 184 Protocol Team, Schooley, R.T., Campbell, T.B., Kuritzkes, D.R., Blaschke, T., Stein, D.S., Rosandich, M.E., Phair, J., Pottage, J.C., Messari, F., Collier, A., Kahn, J., 1996. Phase 1 study of combination therapy with L-697,661 and zidovudine. J. Acquired Immune Defic. Syndr. Hum. Retrovirol. 12, 363–370.

Seki, M., Sadakata, Y., Yuasa, S., Baba, M., 1995. Isolation and characterization of human immunodeficiency virus type-1 mutants resistant to the non-nucleotide reverse transcriptase inhibitor MKC-442. Antiviral Chem. Chemother. 6, 73–79.

Silvestri, R., Artico, M., Bruno, B., Massa, S., Novellino, E., Greco, G., Marongiu, M.E., Pani, A., De Montis, A., La Colla, P., 1998. Synthesis and biological evaluation of 5*H*-indolo [3,2-*b*][1,5]benzothiazepine derivatives, designed as conformationally constrained analogues of the human immunodeficiency virus type 1 reverse transcriptase inhibitor L-737,126. Antiviral Chem. Chemother. 9, 139–148.

Skowron, G., Leoung, G., Dusek, A., Anderson, R., Grosso, R., Jacobs, M., Lamson, M., Kerr, B., Beebe, S., 1998. dNN study: stavudine (d4T), nelfinavir (NFV), and nevirapine (NVP): preliminary safety, activity, and pharmacokinetic (PK) interactions. 5th Conference on Retroviruses and Opportunistic Infections, Chicago, IL, 1–5 February 1998, abstract 350.

Spence, R.A., Kati, W.M., Anderson, K.S., Johnson, K.A., 1995. Mechanism of inhibition of HIV-1 reverse transcriptase by nonnucleoside inhibitors. Science 267, 988–993.

Staszewski, S., Massari, F.E., Kober, A., Gohler, R., Durr, S., Anderson, K.W., Schneider, C.L., Waterbury, J.A., Bakshi, K.K., Taylor, V.I., Hildebrand, C.S., Kreisl, C., Hoffstedt, B., Schleif, W.A., von Briesen, H., Rübsamen-Waigmann, H., Calandra, G.B., Ryan, J.L., Stille, W., Emini, E.A., Byrnes, V.W., 1995. Combination therapy with zidovudine prevents selection of human immunodeficiency virus type 1 variants expressing high level resistance to L-697,661, a non-nucleoside reverse transcriptase inhibitor. J. Infect. Dis. 171, 159–165.

Members of the Loviride Collaborative Study Group, Staszewski, S., Miller, V., Kober, A., Colebunders, R., Vandercam, B., Delescluse, J., Clumeck, N., Van Wanzeele, F., De Brabander, M., De Creé, J., Moeremans, M., Andries, K., Boucher, C., Stoffels, P., Janssen, P.A.J., 1996a. Evaluation of the efficacy and tolerance of R 018893, R 089439 (loviride) and placebo in asymptomatic HIV-1-infected patients. Antiviral Ther. 1, 42–50.

Staszewski, S., Miller, V., Rehmet, S., Stark, T., De Creé, J., De Brabander, M., Peeters, M., Andries, K., Moeremans, M., De Raeymaeker, M., Pearce, G., Van Den Broeck, R., Verbiest, W., Stoffels, P., 1996b. Virological and immunological analysis of a triple combination pilot study with loviride, lamivudine and zidovudine in HIV-1-infected patients. AIDS 10, F1–F7.

Tantillo, C., Ding, J., Jacobo-Molina, A., Nanni, R.G., Boyer, P.L., Hughes, S.H., Pauwels, R., Andries, K., Janssen, P.A.J., Arnold, E., 1994. Locations of anti-AIDS drug binding sites and resistance mutations in the three-dimensional structure of HIV-1 reverse transcriptase. J. Mol. Biol. 243, 369–387.

Terrett, N.K., Bojanic, D., Merson, J.R., Stephenson, P.T., 1992. Imidazo[2′,3′:6,5]dipyrido[3,2-*b*:2′,3′-*e*]-1,4-diazepines: non-nucleoside HIV-1 reverse transcriptase inhibitors with greater enzyme affinity than nevirapine. Bioorg. Med. Chem. Lett. 2, 1745–1750.

Tucker, T.J., Lyle, T.A., Wiscount, C.M., Britcher, S.F., Young, S.D., Sanders, W.M., Lumma, W.C., Goldman, M.E., O'Brien, J.A., Ball, R.G., Homnick, C.F., Schleif, W.A., Emini, E.A., Huff, J.R., Anderson, P.S., 1994. Synthesis of a series of 4-(arylethynyl)-6-chloro-4-cyclopropyl-3,4-dihydroquinazolin-2(1*H*)-ones as novel non-nucleoside HIV-1 reverse transcriptase inhibitors. J. Med. Chem. 37, 2437–2444.

Vasudevachari, M.B., Battista, C., Lane, H.C., Psallidopoulos, M.C., Zhao, B., Cook, J., Palmer, J.R., Romero, D.L., Tarpley, W.G., Salzman, N.P., 1992. Prevention of the spread of HIV-1 infection with nonnucleoside reverse transcriptase inhibitors. Virology 190, 269–277.

Wainberg, M.A., Drosopoulos, W.C., Salomon, H., Hsu, M., Borkow, G., Parniak, M.A., Gu, Z., Song, Q., Manne, J., Islam, S., Castriota, G., Prasad, V.R., 1996. Enhanced fidelity of 3TC-selected mutant HIV-1 reverse transcriptase. Science 271, 1282–1285.

Wei, X., Ghosh, S.K., Taylor, M.E., Johnson, V.A., Emini, E.A., Deutsch, P., Lifson, J.D., Bonhoeffer, S., Nowak, M.A., Hahn, B.H., Saag, M.S., Shaw, G.M., 1995. Viral dynamics in human immunodeficiency virus type 1 infection. Nature 373, 117–122.

Williams, T.M., Ciccarone, T.M., MacTough, S.C., Rooney, C.S., Balani, S.K., Condra, J.H., Emini, E.A., Goldman, M.E., Greenlee, W.J., Kauffman, L.R., O'Brien, J.A., Sardana, V.V., Schleif, W.A., Theoharides, A.D., Anderson, P.S., 1993. 5-Chloro-3-(phenylsulfonyl)indole-2-caboxamide: a novel, non-nucleoside inhibitor of HIV-1 reverse transcriptase. J. Med. Chem. 36, 1291–1294.

Winslow, D.L., Garber, S., Reid, C., Scarnati, H., Baker, D., Rayner, M.M., Anton, E.D., 1996. Selection conditions affect the evolution of specific mutations in the reverse transcriptase gene associated with resistance to DMP 266. AIDS 10, 1205–1209.

Witvrouw, M., Arranz, M.E., Pannecouque, C., Declercq, R., Jonckheere, H., Schmit, J.-C., Vandamme, A.-M., Diaz, J.A., Ingate, S.T., Desmyter, J., Esnouf, R., Van Meervelt, L., Vega, S., Balzarini, J., De Clercq, E., 1998. 1,1,3-Trioxo-2*H*,4*H*-thieno[3,4-*e*][1,2,4]thiadiazine (TTD) derivatives: a new class of nonnucleoside human immunodeficiency virus type 1 (HIV-1) reverse transcriptase inhibitors (NNRTIs) with anti-HIV-1 activity. Antimicrob. Agents Chemother. 42, 618–623.

Wong, J.K., Hezareh, M., Günthard, H.F., Havlir, D.V., Ignacio, C.C., Spina, C.A., Richman, D.D., 1997. Recovery of replication-competent HIV despite prolonged suppression of plasma viremia. Science 278, 1291–1295.

Young, S.D., Britcher, S.F., Tran, L.O., Payne, L.S., Lumma, W.C., Lyle, T.A., Huff, J.R., Anderson, P.S., Olsen, D.B., Carroll, S.S., Pettibone, D.J., O'Brien, J.A., Ball, R.G., Balani, S.K., Lin, J.H., Chen, I.-W., Schleif, W.A., Sardana, V.V., Long, W.J., Byrnes, V.W., Emini, E.A., 1995. L-743,726 (DMP-266): a novel, highly potent nonnucleoside inhibitor of the human immunodeficiency virus type 1 reverse transcriptase. Antimicrob. Agents Chemother. 39, 2602–2605.

Yuasa, S., Sadakata, Y., Takashima, H., Sekiya, K., Inouye, N., Ubasawa, M., Baba, M., 1993. Selective and synergistic inhibition of human immunodeficiency virus type 1 reverse transcriptase by a non-nucleoside inhibitor, MKC-442. Mol. Pharmacol. 44, 895–900.

Zembower, D.E., Liao, S., Flavin, M.T., Xu, Z.-Q., Stup, T.L., Buckheit, R.W. Jr., Khilevich, A., Mar, A.A., Sheinkman, A.K., 1997. Structural analogues of the calanolide anti-HIV agents. Modification of the *trans*-10,11-dimethyldihydropyran-12-ol ring (ring C). J. Med. Chem. 40, 1005–1017.

Zhang, H., Vrang, L., Backbro, K., Lind, P., Sahlberg, C., Unge, T., Öberg, B., 1995. Inhibition of human immunodeficiency virus type 1 wild-type and mutant reverse transcriptases by the phenyl ethyl thiazolyl thiourea derivatives trovirdine and MSC-127. Antiviral Res. 28, 331–342.