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NNRTI plus PI Combinations in the Perspective of Nucleoside-Sparing or Nucleoside-Failing Antiretroviral Regimens

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Abstract

Although not yet recommended, regimens combining both a non-nucleoside reverse transcriptase inhibitor (NNRTI) and protease inhibitors (PI) can be used as first-line therapy, or as second-line or salvage therapy in patients who need to change antiretroviral treatment because of nucleoside reverse transcriptase inhibitors (NRTI) intolerance or virological failure with resistance to NRTI. Such combinations should not be used in patients infected with HIV-1 group O and HIV-2, due to the natural resistance to NNRTI of these subtypes. Dual NNRTI and PI combinations used as first-line therapy allow to spare NRTI, leaving a fully active class of drugs for later use, and delaying the risk of toxicity related to NRTI exposure, particularly mitochondrial toxicity. Several studies have shown that adding a NNRTI improves the efficacy of a second-line or salvage therapy based on a new combination of PI(s) and new or recycled NRTI(s). A possible explanation for the efficacy of NNRTI-containing regimens in NRTI-pretreated patients is that mutations conferring resistance to NRTI can increase the susceptibility of the viruses to the NNRTI. However, the decision to use a NNRTI in a salvage regimen needs to be weighed against the concern that subsequent failure will exhaust therapeutic options with any compound of this class, due the large degree of cross-resistance between the three available NNRTI. NNRTI and PIs are extensively metabolized in the liver through cytochrome P450, leading to pharmacokinetic interactions. The decrease in PIs plasma concentrations observed when they are combined with nevirapine or efavirenz is reduced when low doses of ritonavir, which strongly inhibits cytochrome P450, are associated with the combination of PI and NNRTI.

Key words

Non-nucleoside reverse transcriptase inhibitors. Protease inhibitors. HIV. Antiretroviral therapy.

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Introduction

The primary goals of antiretroviral therapy in the management of HIV infection are maximal and durable suppression of viral load, restoration and preservation of immunologic function,

improvement of quality of life and reduction of HIV-related morbidity and mortality. Eradication of HIV infection cannot be achieved with the available antiretroviral agents. Thus, once treatment has been initiated, it has to be maintained, exposing the patient to an increasing risk of long-term toxicity.

At least three antiretroviral agents are employed in highly active antiretroviral therapy (HAART), resulting from the magnitude of the effects seen in the early clinical trials of regimens combining the new protease inhibitors with the established nucleoside reverse transcriptase inhibitors (NRTI). As recommended by the guidelines established by different panels of experts[1,2], two NRTI provide the backbone of HAART, and will be associated with either a protease inhibitor, or with a non-nucleoside reverse transcriptase inhibitor (NNRTI), mainly efavirenz. The third alternative, a 3-NRTI regimen, has shown to be comparable to PI-containing regimens, except in patients with high baseline viral load (>100,000 copies/ml), in whom the antiviral activity might be suboptimal[3]. Cross-resistance within compounds of the same class has been reported for each class of drugs, but is particularly important for the NNRTI. This cross-resistance reduces the therapeutic efficacy of second-line and further regimens.

Dual NNRTI and PI combinations may be interesting as first-line therapy: 1) as sparing a complete class of drugs that will remain fully active for later use, and 2) in preventing the risk of mitochondrial toxicity related to exposure to NRTI. In patients failing antiretroviral therapy, NNRTI and PI combinations, with or without additional NRTI, have proved to be effective, providing that the virus remains susceptible to the NNRTI and, at least in part, to the PI used.

We will successively review the updated data on NRTI-related toxicity, NRTI cross-resistance, hypersusceptibility to NNRTI, particularly after exposure to RTIs and reduced activity of NNRTI on some HIV subtypes, the activity and tolerance of dual NNRTI-PI therapy in *naïve* and pretreated patients, the pharmacokinetic interactions between NNRTI and PI, the resistance profile in patients failing NNRTI/PI therapy and the limits of such combinations.

NRTI mitochondrial toxicity

NRTI are incorporated into the elongation viral DNA molecules being transcribed by HIV reverse transcriptase and thereby inhibit HIV replication. However, they also inhibit human DNA polymerase and consequently mitochondrial DNA (mtDNA) replication, resulting in mtDNA depletion and drug toxicity. Various adverse effects have been observed in patients treated with NRTI. These events, which can be ascribed to the mitochondrial toxicity induced by NRTI, include peripheral neuropathy, pancreatitis, diabetes, myopathy, hepatic steatosis and lactic acidosis.

Although cases of severe lactic acidosis with hepatomegaly and steatosis are rare (1.3 cases/1,000 person-years of NRTI-exposure), this syndrome is associated with a high mortality rate[4,5]. Eight cases of lactic acidosis during pregnancy have been reported, seven of which were in patients receiving the combination of stavudine and didanosine; there were three maternal deaths and three fetal deaths[6]. The initial clinical signs of patients with lactic acidosis syndrome are variable and include nonspecific gastrointestinal symptoms, myalgias and weight loss. Ascending neuromuscular weakness has been reported to be associated with lactic acidosis[6]; 22 out of the 25 patients were receiving stadudine. In addition to hyperlactatemia, laboratory evaluation might reveal an increased anion gap, elevated aminotransferases, lactic dehydrogenase and lipase. Because substantial technical problems are associated with lactate testing, routine monitoring of lactate level is not usually recommended. Clinicians must first rely on other laboratory abnormalities plus symptoms when lactic acidosis is suspected. Levels >5 mmol/dl are abnormal and levels >10 mmol/dl indicate serious and life-threatening situations. Antiretroviral treatment should be suspended in case of clinical and laboratory manifestations of lactic acidosis. Some patients tolerate administration of a revised NRTI-containing regimen[7,8], but insufficient data exist to recommend this strategy versus treatment with a NRTI-sparing regimen.

Certain features of lipodystrophy syndrome have been hypothesized as being tissue-specific mitochondrial toxicities caused by NRTI treatment[9-11]. Face and extremities lipoatrophy has been reported to increase with long-term NRTI exposure, and different studies argue for a preponderant role of stavudine among NRTI in the occurrence of lipoatrophy[11,12]. Different studies have shown that switching from stavudine to either zidovudine[13] or abacavir[13-15] was associated with an improvement in peripheral lipoatrophy, providing evidence that lipoatrophy was reversible with drug interruption.

NRTI cross-resistance

Until recently, resistance to nucleoside analogues was supposed to account for the emergence of specific mutations for each compound of this class. For example, mutation at codon L74V resulted in a reduction of sensitivity to didanosine, M184V to lamivudine and T215Y/F to zidovudine. Genotypic determinants to stavudine resistance remained unclear since V75T was rarely found *in vivo*. Recent observations have made cross-resistance to NRTI clearer. Actually, three patterns of multi-NRTI resistance have been identified. These include multi-nucleoside resistance (MNR) patterns and the set of zidovudine mutations. The first described pattern of MNR mutations was the association of A62V, V75I, F77L, F116Y and Q151M substitutions, the last one being critical for

this broad cross-resistance[16-18]. A second pathway is the presence of amino acids insertions, mostly serine, between codon 68 to 70 of the RT gene[19-21]. The third pattern involves zidovudine resistance mutations. Over the past few years, evidence has been accumulating that zidovudine resistance mutations can be selected by stavudine, another thymidine analogue[22-30]. Moreover, the presence of zidovudine mutations (M41L, D67N, K70R, L210W, T215Y/F and K219Q/E) has a negative impact on virological response to d4T-containing regimens[31-35] and up to 30% of subjects failing d4T select for the classical zidovudine mutations[18,25,26]. This set of mutations has been named thymidine analogue mutations (TAM). It has also been reported that zidovudine resistance mutations are selected after prolonged therapy with didanosine in the absence of zidovudine[36-38]. Therefore, the term nucleoside associated mutations (NAM) would apply more appropriately as they also contribute to resistance to non-thymidine nucleosides[39]. The mechanism by which the NAM are responsible for large cross-resistance among nucleoside analogues is an improvement of the excision of the chain terminator by phosphorolysis[40] rather than the decreased binding of the inhibitor to the target.

Hypersusceptibility to NNRTI

NNRTI bind to a hydrophobic pocket in the reverse transcriptase enzyme located close to the active site and inhibit HIV-1 allosterically by displacing the catalytic aspartate residue relative to the polymerase-binding site. A limitation of this class of antiretroviral compounds is that a single mutation in the RT enzyme may confer high-level resistance to one or all these agents[41-44]. NNRTI have been effective in suppressing HIV in combination with two NRTI in both *naïve* patients and some NRTI-experienced patients, despite a low resistance barrier[45-48]. A possible explanation for the efficacy of combination therapy with NRTI and NNRTI is that mutations conferring resistance to one class can increase the susceptibility of the viruses to the other class.

In 1992, Larder, et al. demonstrated that the presence of Y181C and L100I mutations associated with resistance to NNRTI increases the susceptibility to zidovudine in a given isolate[49]. More recently, different studies have reported that increasing numbers of NRTI mutations may enhance susceptibility to NNRTI (i.e., hypersusceptibility)[50-52]. NNRTI hypersusceptibility has been defined as a 50% inhibitory concentration (IC_{50}) of >2.5-fold less than that of the wild-type reference strain. This phenomenon has been described in association with multiple NRTI mutations, including the classical zidovudine mutations, conferring large cross-resistance among NRTI and in the absence of NNRTI associated mutations.

The prevalence of NNRTI hypersusceptibility varies among the studies. The first study by Whitcomb, et al. on 447 NRTI-experienced patients reported a prevalence of 29%[50]. Retrospectively, in a sub-study from 164 patients of the California Collaborative Treatment Group (CCTG) 575 trial who had received efavirenz as part of their treatment regimen, Haubrich reported a prevalence of 24%[51]. In the study from Shulman this prevalence was higher, reaching 40%, probably because of the highly treatment-experienced nature of the cohort[52]. The fold change susceptibility to efavirenz correlated inversely with the number of NRTI mutations in the two studies[50,52]. The pattern of mutations observed in subjects with EFV hypersusceptibility was M41L, M184V and T215Y, usually with L210W and, in a multivariate model, mutation at codon 215 significantly enhanced the EFV susceptibility[52]. Patients with baseline viruses exhibiting EFV hypersusceptibility achieved greater virological short-term response with EFV-containing salvage regimens than those who did not have hypersusceptibility. The same type of results was reported by Haubrich, et al. in patients enrolled in CCTG trials. The mean decrease in plasma HIV-RNA six months after starting a new NRTI-containing regimen was greater for the 21 patients with hypersusceptibility to NNRTI than in the 77 patients without hypersusceptibility to NNRTI. The difference persisted through 12 months of therapy. NNRTI hypersusceptibility in NRTI-experienced patients may explain the role of these compounds in salvage regimens[53].

Regarding NNRTI hypersusceptibility in a clinically significant way elevates this finding from merely an interesting *in vitro* phenomenon to a potential clinical tool. With a prevalence of approximately 20% for each of the three NNRTI agents, the occurrence of NNRTI-hypersusceptibility is relevant for a great number of patients who stand to gain enhanced antiretroviral efficacy. The confirmation that it is associated with prior use of NRTI may help to define exactly when the NNRTI class may best be used in treatment strategies: namely, in NRTI-experienced, NNRTI-*naïve* patients on failing regimens. This strategy of holding NNRTI for use in later rather than initial therapy regimens may seem to defy other findings that underline the usefulness of first-line NNRTI-based regimens.

The mechanism of NNRTI hypersusceptibility is still unknown and may be due to conformational changes occurring in the RT enzyme with NRTI mutations that increase the affinity of NNRTI to their binding pocket. *In vitro* phenotypic testing in different cell populations is needed to explore NNRTI hypersusceptibility further.

Reduced susceptibility to NNRTI in HIV-1 group O and HIV-2

To date, decreased susceptibility to NNRTI in HIV-1 non-B subtypes has not been demonstrated[54]. In contrast, HIV-1 group O, first identified in

Cameroon in 1994 and located mainly in Central Africa, is naturally resistant to NNRTI[55]. Genotypic analysis of HIV-1 group O reverse transcriptase gene revealed a tyrosine-to-cysteine substitution at residue 181[55]. Phylogenetic analysis of the *pol* gene showed that these isolates formed a separate cluster within group O[55]. The natural resistance to NNRTI for both HIV-1 group O and HIV-2 limits the use of this class of compounds in preventing vertical transmission, in particular in developing countries.

HIV-2 isolates appeared to be sensitive to most NRTI[56] and PI[57,58] although there are some reports showing diminished susceptibility to compounds of this class[59,60]. HIV-2 viruses are naturally resistant to at least all NNRTI licensed[61] probably due to mutations found in the NNRTI hydrophobic binding pocket of the reverse transcriptase enzyme. There is little overlap sequence identity between HIV-1 and HIV-2 viral genomes[62,63]; the *pol* gene of both viruses is highly conserved but there is only 60% identity between HIV-1 and HIV-2 residues that points toward the NNRTI binding pocket in the RT gene[61]. One of the key differences is the presence of an isoleucine instead of a tyrosine at codon 181.

Antiviral activity of NNRTI and PI combinations

Drug-*naïve* patients

In *naïve* individuals, initiation of an antiretroviral treatment combining a NNRTI and a PI would allow to spare the use of NRTI in first-line therapy. Although guidelines recommend the use a "triple-drug combination", the intrinsic potency of both NNRTI and PI would allow using them under this unusual combination of only two drugs. Few trials have evaluated the activity of this type of antiretroviral therapy. We will review the studies performed: 1) in truly *naïve* patients, and 2) in patients previously exposed to nucleosides, but *naïve* for NNRTI and PI, and for whom the residual antiviral activity of NRTI associated to PI and NNRTI could be considered to be low due to extensive prior exposure to this class of drugs.

A preliminary open-label study was conducted by Harris in 22 patients with advanced HIV-disease who had disease progression or virological failure while receiving NRTI-based combination therapy[64]. Patients received a combination of nevirapine 200 mg bid, indinavir 800 mg tid and lamivudine, without washout before starting therapy. All patients except three had previously demonstrated evidence of treatment failure with lamivudine, and likely carried lamivudine-resistant virus. At baseline, the median CD4 cell count was 30/mm^3 and the median plasma HIV-RNA was 5.16 log copies/ml. Nevirapine, indinavir and lamivudine given in combination had very substantial antiviral and immunologic effects, leading to a median reduction of plasma HIV-RNA > 3 log copies/ml which remained for the 24 weeks of the study, associated with a median increase in CD4 cell count of 95 cells/mm^3. These favorable results were observed in spite of the pharmacokinetic interaction between nevirapine and indinavir. In fact, the negative pharmacokinetic sub-study performed in 17 patients found that indinavir peak and trough levels were reduced compared to published levels for indinavir monotherapy, showing the necessity to increase the indinavir daily dosage when this drug is used in combination with nevirapine.

In a large, randomized, open-label trial, Staszewski, et al. compared three drug regimens: 1) efavirenz plus indinavir, 2) efavirenz plus zidovudine and lamivudine, and 3) indinavir plus zidovudine and lamivudine[46]. The indinavir dosage was increased from 800 mg tid to 1000 mg tid in the efavirenz plus indinavir group, to compensate for the increased metabolism of indinavir in the presence of efavirenz. Patients had not previously been treated with lamivudine, NNRTI or PI. Eighty five percent of the patients were *naïve* of any antiretroviral therapy. Baseline mean CD4 cell count was 345/mm^3 and mean baseline plasma HIV-RNA was 4.77 log copies/ml. A total number of 450 patients were randomized between the three arms. According to an intention-to-treat analysis, the percentages of patients with plasma HIV-RNA levels of less than 400 copies/ml at week 48 were 70% in the group assigned to efavirenz plus NRTI, 53% in the group assigned to indinavir and efavirenz, and 48% in the group assigned to indinavir plus NRTI. At week 48, mean increases of 201, 185 and 180 CD4 cells/mm^3 were found in the group given efavirenz plus NRTI, the group given indinavir plus NRTI, and the group given efavirenz plus indinavir, respectively. The rate of discontinuation as a result of adverse events was significantly higher in the indinavir plus NRTI group than in either of the efavirenz groups. These adverse events were largely gastrointestinal. The incidence of central nervous system symptoms was similar in the two arms containing efavirenz, i.e. 58% in the group given efavirenz and NRTI, 53% in the group given efavirenz plus indinavir. In this study, 200 mg capsules of indinavir were used. Thus, patients assigned to receive efavirenz and NRTI had to take far fewer pills than the other patients (four pills of indinavir taken three times daily without food and three pills of efavirenz taken once daily). The superior results of the arm without indinavir could therefore be in part due to better adherence of patients to the regimen. Furthermore, this mode of administration of indinavir is no longer used, and results may have been different with the combination of ritonavir at baby doses and indinavir, that allows a bid administration with a reduced daily number of pills, without restriction for food. It was possible to conclude from this study that a NNRTI/PI combination was as effective as a triple-drug therapy combining PI and 2 NRTI.

A randomized, double-blind placebo controlled trial compared among 327 NRTI-experienced pa-

tients, efavirenz plus indinavir to indinavir in combination with ≤2 concomitant NRTI[65]. Patients were PI and NNRTI *naïve*. The mean duration of prior nucleoside therapy was 2.8 years and the mean baseline plasma HIV-RNA load was 4.41 log copies/ml. According to an intention-to-treat analysis, the percentages of patients with plasma HIV-RNA levels of less than 400 copies/ml at week 24 were 68 and 52% in patients assigned to efavirenz plus indinavir and in patients assigned to efavirenz, respectively ($p < 0.04$). In the subgroup of lamivudine-experienced patients (246 subjects), the percentages of patients with virological response at week 24 were similar to those reported in the whole population of the study, for each treatment arm. Efavirenz-treated patients were encouraged to continue on the initially assigned regimen, allowing assessing the durability of antiviral effect beyond 24 weeks. Data demonstrated that virological and immunologic responses were well maintained at week 48. The increase in serum cholesterol and triglycerides was significantly higher in the efavirenz group at week 24. This study established the value of efavirenz plus indinavir, administered with concomitant NRTI, in the treatment of patients heavily pretreated by NRTI. The long duration of previous exposure to NRTI and the high percentage (75%) of patients pretreated with lamivudine suggest that most of the antiviral effect resulted from the potency of efavirenz and indinavir, and that the residual activity of concomitant NRTI was probably low. Although resistance to NRTI was not studied at baseline, one can expect that most patients had genotypic resistance to most NRTI, including those considered as "new NRTI", due to the large degree of cross-resistance between NRTI. In this study, there was an unexpected association of increasing duration of prior NRTI exposure with more favorable virological response at week 24. One possible explanation was that patients with more extensive prior NRTI experience may be more comfortable taking medications on a regular basis and therefore may be more adherent.

In ACTG protocol 370, patients who failed d4T/3TC or ddI/3TC were randomized to receive either AZT/3TC/indinavir or AZT/delavirdine/indinavir[66]. All patients were *naïve* for NNRTI and PI. Median plasma HIV-RNA level was 3.06 log copies/ml at entry in the study. At week 24, 66% of the patients in the delavirdine arm had plasma HIV-RNA <50 copies/ml, as compared to 42% taking 3TC ($p = 0.077$). At week 48, plasma HIV-RNA was <200 copies/ml in 83% of patients in the delavirdine arm as compared to 48% in the 3TC arm ($p = 0.007$). Time to virological failure, defined as 2 consecutive plasma HIV-RNA levels >200 copies/ml, was shorter for patients in the 3TC arm ($p = 0.044$). Steady-state plasma indinavir levels were higher among patients in the delavirdine arm as compared to the 3TC arm. It is possible that the superior outcome in the delavirdine arm was explained in part by the favorable pharmacokinetic interaction between delavirdine and indinavir. In this trial, the presence of TAM was associated with a greater likelihood of viral suppression. However, delavirdine susceptibility was not significantly associated with outcome, and better adherence to treatment in the subgroup of patients with NRTI-resistant virus at baseline was the most likely explanation of the association between the presence of TAM and improved virological outcome[67].

In the ACTG 364 study, 195 patients who had been treated with NRTI were randomly assigned to receive one of the three treatments in a double-blind fashion: nelfinavir 750 mg tid, efavirenz 600 mg od or nelfinavir plus efavirenz[48]. Each patient was also assigned to receive one of the three combinations of open-label NRTI: didanosine plus lamivudine, stavudine plus lamivudine, or stavudine plus lamivudine. Assignments of NRTI were made on the basis of the treatment the patient had previously received. These regimens were selected so that each patient received at least one and if possible two new NRTI. At baseline, the median CD4 cell count was 350/mm^3, median plasma HIV-RNA was 3.89 log copies/ml and the median duration of previous NRTI treatment was 5.6 years. One third of the patients were *naïve* for lamivudine. At week 16 and at weeks 40 and 48, the proportions of patients in whom plasma HIV-RNA levels <500 copies/ml was achieved were, respectively, 81 and 74% in the nelfinavir plus efavirenz group, 69 and 60% in the efavirenz group, and 64 and 35% in the nelfinavir group. Quadruple therapy achieved a higher rate of viral suppression than triple therapy with nelfinavir or efavirenz. Treatment with lamivudine as a new nucleoside was a significant independent predictor of viral suppression in multivariate analysis. Mutations in the reverse-transcriptase gene at baseline were examined retrospectively in 140 patients. Among the patients in whom genotyping was completed, the response rates within the treatment groups at weeks 40 and 48 were similar to those in the overall study population. In the group with five or more reverse transcriptase mutations, or a known genetic marker of multinucleoside resistance at baseline, HIV-RNA levels of less than 50 copies/ml were achieved at weeks 40 and 48 in 19, 30 and 75% of the patients included in the nelfinavir group, the efavirenz group and the nelfinavir plus efavirenz group, respectively. There were similar rates of viral suppression in patients with zero to four RT mutations as in those with five or more RT mutations or the presence of mutations known to confer multi-nucleoside resistance. These data argue for the low level of antiviral activity of the NRTI included in antiviral combination as "new NRTI" in these heavily NRTI-pretreated patients, except probably for lamivudine, the genotypic resistance of which is not related to the presence of thymidine associated mutations. In this study, a preliminary phenotypic analysis conducted at baseline in 130 patients showed that 37% of isolates of virus from these patients were hypersusceptible to efavirenz, defined as a concentration required to inhibit viral

replication by 50% (IC_{50}) <0.4 of the IC_{50} for a wild-type reference strain. Negative correlation was observed between the fold-change in susceptibility to zidovudine and efavirenz. There was a significant association with the RTI mutations at codon 215, 41 and 67. Mutations at codon 215 were present in 94% of hypersusceptible isolates. While the clinical significance of hypersusceptibility is uncertain, continued use of NRTs to maintain hypersusceptibility in salvage regimens utilizing NNRTI is a treatment strategy that warrants evaluation[68]. The main results obtained in NNRTI and PI *naïve* patients are summarized in table 1.

PI-experienced patients

Virological failure in the presence of PI may be associated with resistance mutations that confer cross-resistance to other PI. Switching to a second PI-containing regimen may fail to produce durable viral suppression. Several studies have evaluated the role of NNRTI in combination with second-line PI in patients for whom a PI-containing regimen had failed.

In an open-label study, Piketty, et al. assessed the safety and efficacy of a combination of ritonavir (100 mg bid), saquinavir (1,000 mg bid), efavirenz (600 mg od) and two recycled nucleosides in 32 patients who failed on a conventional triple-drug regimen including indinavir or ritonavir, but were *naïve* for efavirenz[69]. Peak and trough plasma levels of saquinavir were monitored throughout the study. Median CD4 cell count and median plasma HIV RNA at baseline were 258 cells/mm^3 and 4.31 log_{10} copies/ml, respectively. The plasma viral load decreased by a median of 1.20 log_{10} copies/ml and the CD4 cell count increased by a median of 60 cells/mm^3 at week 24 of therapy. Seventy-one per cent of the patients achieved a plasma viral load <500 copies/ml and 45% achieved a viral load <50 copies/ml. Patients exhibiting phenotypic resistance to saquinavir at baseline experienced a median decrease in HIV RNA of 0.91 log_{10} copies/ml at week 24 of therapy, as compared to a decrease of 1.52 log_{10} copies/ml in those exhibiting sensitive viral strains (p = 0.03).

ACTG study 359 compared antiretroviral activity among six salvage therapy regimens. The study was a prospective, randomized, 2 x 3 factorial, multicenter study and enrolled 277 HIV-infected patients *naïve* to NNRTI who had taken indinavir >6 months. Patients received saquinavir with ritonavir or nelfinavir, together with delavirdine and/or adefovir and were followed between baseline and week 16[70]. At baseline, the median plasma HIV-RNA was 4.50 log copies/ml and the median CD4 cell count was 229 cells/mm^3. The median length of previous indinavir use was 14.4 months. At week 16, 30% of patients had HIV-RNA <500 copies/ml. Virological response did not differ significantly between the ritonavir and nelfinavir groups (28 vs 33%, p = 0.5) or between pooled delavirdine or delavirdine/adefovir groups (40 vs 33%, p = 0.42). Pooled delavirdine groups had a greater virological response rate than did adefovir groups (40 vs 18%, p = 0.002). The superior virological effect shown in the delavirdine-containing arm likely results from the fact that patients had not taken any NNRTI before study entry. In addition, delavirdine is an inhibitor of cytochrome P450-mediated me-

Table 1. *Efficacy of NNRTI/PI combinations in NNRTI and PI naïve patients*

Reference	Previous treatment	Treatment studied	Baseline viral load	Antiviral effect
Harris[64]	NRTI (including 3TC)	3TC/IDV/NVP	5.16 log cps/ml	Decrease >3 log cps/ml in HIV-RNA at week 24
Staszewski[46]	15% of patients: NRTI 85% of patients: *naïve* All patients 3TC *naïve*			Percent of patients with HIV-RNA < 400 cps/ml at week 48:
		– EFV/IDV	4.77 log cps/ml	– 53% of patients
		– AZT/3TC/IDV		– 48% of patients
		– EFV/IDV		– 70% of patients
Haas[65]	NRTI (mean time = 2.8 yers)			Percent of patients with HIV-RNA < 400 cps/ml at week 24:
		– EFV/IDV/ 1 or 2 NRTI	4.41 log cps/ml	– 68% of patients
		– IDV/ 1 or 2 NRTI		– 52% of patients
Kuritzkes[66]	d4T/3TC or ddI/3TC			Percent of patients with HIV-RNA < 200 cps/ml at week 48:
		– AZT/3TC/IDV	3.06 log cps/ml	– 48% of patients
		– AZT/DLV/IDV		– 83% of patients
Albrecht[48]	NRTI (median time = 5.6 yers)	D4T or ddI and		Percent of patients with HIV-RNA < 500 cps/ml at week 16 and 40-48
		– 3TC/NFV		– 64 and 35%
		– 3TC/EFV	3.89 log cps/ml	– 69 and 60%
		– 3TC/NFV/EFV		– 89 and 74%

tabolism and can increase plasma concentrations of PI. An intensive pharmacokinetic study conducted in 37 of the study patients indicated that saquinavir plasma concentrations were higher in the delavirdine arms and lower in the delavirdine and adefovir dipivoxil combination arms[71]. There was also evidence for an interaction between delavirdine and adefovir dipivoxil because delavirdine plasma concentrations were significantly lower in the combination arms than in the delavirdine arms.

In a prospective, open-label study, 20 subjects experiencing virological failure of an indinavir or ritonavir-containing regimen after at least 24 weeks of continuous therapy were assigned to receive a combination of nelfinavir, saquinavir, abacavir, plus either a NRTI (10 patients), or nevirapine (10 patients)[72]. Only one patient, included in the NRTI group, had received prior therapy with NNRTI. All subjects had evidence of ongoing viral replication in the presence of indinavir or ritonavir for an extended period (median 12.4 months) before switching to the study medications. At week 24, the median decrease in virus load was 0.39 log in the NRTI group and 2.67 log in the nevirapine group ($p = 0.02$). Results from baseline phenotypic drug susceptibility testing were available in 15 subjects completing 24 weeks of therapy. The median change in plasma viral load at week 24 was –0.35 log in subjects with baseline virus sensitive to 0 or 1 drug and – 2.24 log in subjects with baseline virus sensitive to 2 or 3 drugs. This study shows clearly that a PI-containing salvage regimen in patients failing under PI is more efficient when including at least one agent from a class of antiretroviral agents to which the patient is *naïve*. It has to be stressed that, in this study, patients switched to a salvage regimen long after initial indinavir or ritonavir-containing regimen failed. Response rates to either treatment arm may have been better if patients had switched soon after treatment failure. Similar results with NNRTI-based salvage regimens were seen in a much larger observational study performed at San Francisco General Hospital (13 of the 20 subjects of the study contributed to that observational study)[73]. Patients studied were HIV-infected adults who had received at least 16 continuous weeks of therapy with a potent protease inhibitor (indinavir, ritonavir or nelfinavir)-based regimen, and who had had at least 48 weeks of follow-up. Of the 99 patients who experienced virological failure and switched to a salvage regimen, only 22 (22%) achieved an undetectable HIV RNA level 24 weeks after initiating salvage therapy. Independent predictors of failure with salvage therapy included an HIV-RNA greater than 4.0 $\log_{10}$ RNA copies/ml at the time of the switch and failure to use a non-nucleoside reverse transcriptase inhibitor (NNRTI) in the salvage regimen.

ACTG 373 trial was an open-label study designed to determine the antiviral activity and the safety of the four-drug regimen combining indinavir, nevirapine, stavudine and lamivudine in 56 patients previously exposed to amprenavir, either as monotherapy (36 subjects) or in combination with other antiretroviral agents (20 subjects)[74]. Seventy three percent of the subjects had HIV-RNA < 500 copies/ml at week 48. Prior treatment with amprenavir combination therapy, time on the amprenavir regimen and prior NNRTI use were associated with virological failure.

Benson, et al. compared the activity and safety of lopinavir 400 mg bid, administered with two different doses of ritonavir (100 mg bid and 200 mg bid) in patients failing on a first PI-containing regimen and *naïve* for NNRTI[75]. On day 15, nevirapine was added and the NRTI regimen was changed. A mean reduction of 1.14 log copies/ml of plasma HIV-RNA was observed at week 2. In a comparison between patients with <4-fold or ≥4-fold reduced susceptibility to lopinavir at baseline, there was no difference in the reduction in plasma HIV-RNA at week 2. At week 48, 70 and 60% of the patients had plasma HIV-RNA < 400 copies/ml and <50 copies/ml, respectively, in intent-to-treat analysis. The mean increase from baseline in CD4 cell count was 125 cells/mm^3 at week 48. Factors that likely contributed to these results are limited prior PI treatment, plasma HIV-RNA < 100,000 copies/ml at baseline and the use of a drug in a class not previously received. A ≥4-fold reduction in baseline phenotypic susceptibility to lopinavir was not associated with a diminished viral load response at week 24 or week 48. An insufficient number of isolates with higher levels of reduced phenotypic susceptibility to lopinavir prevented to delineate the clinically relevant breakpoint for phenotypic resistance. The ranges of lopinavir trough concentrations were 1.8-7.9 μg/ml for the 400/100 mg dose and 3.6-16.6 μg/ml for the 400/200 mg dose. Although the lowest trough concentrations observed were at or above the protein binding-corrected lopinavir IC_{50} for all baseline isolates tested, comparison of pharmacokinetic data from this study with data from patients receiving lopinavir/ritonavir without nevirapine suggests that nevirapine reduces trough concentrations of lopinavir in the presence of low doses of ritonavir. Consideration should be given to increasing the dose of lopinavir/ritonavir to 533/133 mg bid (4 coformulated capsules) in patients receiving lopinavir/ritonavir concomitantly with nevirapine when reduced susceptibility to lopinavir is clinically suspected by treatment history or resistance testing.

The same combination of lopinavir/ritonavir and nevirapine was studied in 30 antiretroviral-treated patients with plasma HIV-RNA < 80 copies/ml during at least nine months. Patients were switched to either lopinavir/ritonavir 400/100 mg plus nevirapine or lopinavir/ritonavir 400/100 mg plus the two previous NRTI[76]. At six months, viral suppression was maintained in both arms. There was a similar increase in mean cholesterol in both groups, whereas no significant changes were seen in triglycerides levels. Mean lopinavir C_{min} levels were similar between both arms at steady state conditions. Ongoing analysis of mitochondrial

DNA/nuclear DNA ratios will provide information on the benefit of NRTI interruption. In this study, lopinavir/ritonavir plus nevirapine seemed to be as safe and potent as lopinavir/ritonavir plus two NRTI; however, nevirapine was not able to counteract the lipid abnormalities related to lopinavir/ritonavir.

The efficacy and the safety of lopinavir/ritonavir, in combination with efavirenz, was investigated in multiple PI-experienced, NNRTI *naïve* patients[77]. The mean number of prior antiretrovirals was seven, and the mean number of prior PI was three. Two different dosages of lopinavir/ritonavir were studied: 400/100 mg bid and 533/133 mg bid. Seventy five percent of the patients did not receive a new NRTI in conjunction with lopinavir/ritonavir and efavirenz within the first eight weeks of study. A baseline viral isolate demonstrating a ≥10-fold increase in IC_{50} of lopinavir relative to wild-type virus was found in 43% of the patients. Lopinavir/ritonavir 533/133 mg dose with efavirenz provided similar lopinavir exposure to the 400/100 mg dose without efavirenz. All patients were converted to the 533/133 mg dose after week 24. At week 72, 67 and 61% of the patients had plasma HIV-RNA <400 copies/ml and <50 copies/ml, respectively, in intent-to-treat analysis. A response rate at <400 copies/ml was observed in 93% of patients whose baseline isolates displayed <10-fold reduced *in vitro* susceptibility to lopinavir, whereas it was observed in 73 and 25% of patients with 10-40 and >40-fold reduced susceptibility to lopinavir at baseline, respectively. Similarly, at 72 weeks, a response rate at 72 weeks at <400 copies/ml was observed in 91% of patients whose baseline isolates contained 0-5 mutations associated with reduced *in vitro* susceptibility to lopinavir, but in only 71 and 33% of patients with 6-7 and 8-10 resistance mutations to lopinavir, respectively. At week 72, the mean change from baseline in CD4 cell count was 126 cells/mm^3.

In a comparative randomized trial designed to assess whether adding a second PI improved antiviral efficacy of a 4-drug combination, including efavirenz, in patients with virological failure while taking a PI-containing regimen, Hammer, et al. showed that being *naïve* for NNRTI was associated with a favorable outcome[78]. In this trial, patients received a combination of efavirenz, abacavir, amprenavir and adefovir dipivoxil with either placebo, indinavir, nelfinavir or saquinavir. At week 24, a higher proportion of NNRTI-*naïve* patients had a viral load <200 copies/ml compared with NNRTI-experienced patients (43 vs 16%, $p < 0.001$). Baseline HIV hypersusceptibility to efavirenz

Table 2. *Efficacy of NNRTI/PI combinations in PI pretreated patients*

Reference	Previous treatment	Treatment studied	Baseline viral load	Antiviral effect
Piketty[69]	NRTI and IDV or RTV	2 NRTI/EFV/SQV/RTV	4.31 log cps/ml	1.2 log cps/ml decrease in HIV-RNA at week 24
Gulick[70]	NRTI and IDV	– SQV/RTV/DLV – SQV/RTV/ADF – SQV/NFV/DLV – SQV/NFV/ADF – SQV/RTV/DLV/ADF – SQV/NFV/DLV/ADF	4.50 log cps/ml	Percent of patients with HIV-RNA < 500 cps/ml at week 16: – Pooled DLV groups: 40% – Pooled without DLV groups: 18%
Deeks[72]	NRTI and IDV or RTV	– NFV/SQV/ABC/NRTI – NFV/SQV/ABC/NVP	4.50 log cps/ml 4.24 log cps/ml	Median decrease in HIV-RNA at week 24: – NRTI group: 0.39 log cps/ml – NVP group: 2.67 log cps/ml
Gulick[74]	APV ± NRTI ± NNRTI	IDV/NVP/d4T/3TC	4.19 log cps/ml	78% of patients with HIV-RNA < 500 cps/ml at week 48
Benson[75]	NRTI and PI (first line)	RTV/LPV/NVP/NRTI	4.1 log cps/ml	70% of patients with HIV-RNA < 400 cps/ml at week 48
Danner[77]	NRTI and PI (mean number = 3 PI)	RTV/LPV/EFV/NRTI	4.5 log cps/ml	67% of patients with HIV-RNA < 400 cps/ml at week 72
Hammer[78]	NRTI and PI ± NNRTI	EFV/APV/ABC/ADV and – placebo – or IDV – or NFV – or SQV	4.71 log cps/ml	Percent of patients with HIV-RNA < 200 cps/ml at week 24: – NNRTI-*naïve* patients: 43% – NNRTI-experienced patients: 16%

IDV = indinavir, RTV = ritonavir, NFV = nelfinavir, APV = amprenavir, SQV = saquinavir, LPV = lopinavir, NVP = nevirapine, EFV = efavirenz, DLV = delavirdine, ADF: adefovir, TNF = tenofovir, ABC = abacavir

(≤0.4-fold difference in susceptibility compared with reference virus) was associated with suppression of viral load at week 24 (OR = 3.49).

The decision to use a NNRTI in a salvage regimen needs to be weighed against the concern that subsequent failure will exhaust therapeutic options with any compound of this class, due the large degree of cross-resistance between the three available NNRTI. The main results obtained in pretreated patients are summarized in table 2.

Pharmacokinetic interactions

NNRTI and PI are extensively metabolized in the liver through cytochrome P450, leading to pharmacokinetic interactions. Nevirapine is an inducer of cytochrome P450 activity, efavirenz is a mixed inducer and inhibitor and delavirdine is an inhibitor of the cytochrome P450. Thus, compared to nevirapine, delavirdine has opposite interactions with compounds utilizing the same metabolic pathway, particularly PI, whose plasma concentrations are increased in the presence of delavirdine. Reciprocal kinetic interactions and recommended dosages of drugs are summarized in table 3. Plasma levels of the three available NNRTI are not significantly altered by available PI, except delavirdine whose AUC is decreased by 40% in the presence of nelfinavir and by 60% in the presence of amprenavir[79], and efavirenz whose plasma AUC is increased by 21% in the presence of ritonavir. The decrease in PI plasma concentrations observed when they are combined with nevirapine or efavirenz is reduced when low doses of ritonavir, which strongly inhibits cytochrome P450, are associated to the combination of PI and NNRTI[80-82].

Resistance profile in patients failing a NNRTI-PI containing regimen

Hoover, et al. studied genotypic and phenotypic resistance in 69 patients failing a regimen combining PI and NNRTI[84]. These patients had no previous NNRTI exposure and no NNRTI baseline genotypic or phenotypic resistance. NNRTI phenotypic and genotypic resistance were found in 83 and 78% of the failing patients, respectively. This high risk of NNRTI resistance was associated with the low number (≤2) of additional drugs being prescribed. In a retrospective cohort study evaluating resistance to NNRTI in patients failing a nevirapine plus PI-based regimen, prescribed as a rescue regimen, resistant isolates to nevirapine were found in 92% of patients at week 24. The development of nevirapine resistance was associated with baseline resistance to PI included in the regimen[85]. In the PACTG 377 study, in which children failing nucleosides were treated with d4T plus PI (nelfinavir or ritonavir) and 3TC, nevirapine or both, resistance mutations to nevirapine or 3TC were detected frequently at virological failure, whereas mutations associated with nelfinavir or ritonavir resistance were rarely detected[86].

Table 3. *Pharmacokinetic interactions between NNRTI and PI*

Protease inhibitor	Nevirapine	Delavirdine	Efavirenz
Indinavir	Indinavir decreases 28%, nevirapine no effect indinavir 1000 mg every 8 h, nevirapine standard dose	Indinavir increases >40%; delavirdine no effect indinavir 600 mg every 8 h, delavirdine standard dose	Indinavir decreases 31%; efavirenz no effect; indinavir 1000 mg every 8 h, standard dose efavirenz
Ritonavir	Ritonavir decreases 11%, nevirapine no effect standard dose	Ritonavir increases 70-100%, delavirdine no effect Monitor ritonavir levels and toxicity	Ritonavir increases 18%, efavirenz increases 21%
Saquinavir	Saquinavir decreases 25%, nevirapine no effect Co-administration not recommended without ritonavir boosting	Saquinavir increases 5 times, delavirdine no effect Allows administration of saquinavir hard gel without ritonavir boosting	Saquinavir decreases 62%, efavirenz decreases 12% Co-administration not recommended without ritonavir boosting
Nelfinavir	Nelfinavir increases 10%; nevirapine no effect Standard dose	Nelfinavir increases 2 times; delavirdine decreases 50%	Nelfinavir increases 20% Standard dose
Amprenavir	Potential decreases in amprenavir level	Amprenavir increases 2 times; delavirdine decreases 60%	Amprenavir decreases 36% Increase amprenavir dose or add ritonavir; efavirenz standard dose
Lopinavir Ritonavir	Lopinavir C_{min} decreases 55% Consider 533/133 mg bid in PI-experienced patients Nevirapine standard dose	Lopinavir levels expected to increase	Lopinavir blood AUC decreases 40%; efavirenz decreases 15% Increase lopinavir/ritonavir to 433/133 mg bid, efavirenz standard dose.
Atazanavir [Ref 83]			Atazanavir decreases 74% Add ritonavir, efavirenz standard dose

These data show that virological failure in the presence of a NNRTI-PI containing regimen is mainly associated with the emergence of resistance to NNRTI, in relation with the low genetic barrier of this class of compounds. The large degree of cross-resistance between the three available NNRTI then prevents the use of NNRTI in further rescue regimens. For these reasons, when NNRTI are prescribed as part of a salvage treatment, they must be part of a potent and fully active regimen, combining, if necessary, two PI at full dosage.

In conclusion, although experience remains limited, NNRTI/PI combination regimens can be used as first-line therapy to selectively delay the risk for certain side effects associated with NRTI, or as second-line or salvage therapy in patients who need to change therapy because of NRTI intolerance or virological failure with resistance to NRTI.

The choice to start antiretroviral therapy with a NNRTI/PI combination is not yet recommended[1,2] due to the limited number of clinical studies that compare the efficacy of NNRTI/PI combinations versus NRTI with either NNRTI or PI. Advantages would include targeting HIV at two different steps of viral replication, sparing NRTI's side effects, preserving NRTI class in case of virological failure and, most often, limiting and delaying resistance to PI in case of failure, since this resistance usually requires multiple mutations. In patients with advanced HIV disease, bone marrow suppression associated with zidovudine and the neuropathic effects of zalcitabine, didanosine or stavudine can combine with the direct effects of HIV to render the drugs intolerable, highlighting the value of NNRTI/PI combinations. The possible disadvantages of these combinations include drug interactions, including with non-antiretroviral drugs (rifampin, for example), regimens that might be difficult to use and adhere to, risk of PI-related long-term side effects such as hyperlipemia and insulin resistance and high risk of cross-resistance throughout the entire NNRTI class in case of failure. Since with a potent regimen the durable suppression of HIV replication reduces the risk of emergence of resistant variants, the therapy's goal should be to lower plasma HIV-RNA below detectable limits, thereby providing the strongest means to avoid the emergence of resistant viruses. Protease boosting by co-administering low dose ritonavir increases the trough levels of other PI, leading to more convenient regimens regarding pill burden, scheduling, elimination of food restriction and preventing efavirenz- or nevirapine-induced drug interactions.

The use of NNRTI/PI combinations as second-line or salvage therapy should remain limited to patients who have not failed previously a NNRTI-containing regimen and should be guided by results of resistance testing to define the best choice of PI. Several studies have shown that adding a NNRTI improves the efficacy of a second-line or salvage therapy based on a new combination of PI and new or recycled NRTI. The development of new NNRTI that remain active on viruses resistant to the three compounds available at the present time will extend the interest of NNRTI/PI combinations in the treatment of patients who have previously failed NNRTI.

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