Drug Susceptibility in HIV Infection After Viral Rebound in Patients Receiving Indinavir-Containing Regimens

Diane V. Havlir, MD
Nick S. Hellmann, MD
Christos J. Petropoulos, PhD
Jeannette M. Whitcomb, PhD
Ann C. Collier, MD
Martin S. Hirsch, MD
Pablo Tebas, MD
Jean-Pierre Sommadossi, PhD
Douglas D. Richman, MD

Context Loss of viral suppression in patients infected with human immunodeficiency virus (HIV), who are receiving potent antiretroviral therapy, has been attributed to outgrowth of drug-resistant virus; however, resistance patterns are not well characterized in patients whose protease inhibitor combination therapy fails after achieving viral suppression.

Objective To characterize drug susceptibility of virus from HIV-infected patients who are failing to sustain suppression while taking an indinavir-containing antiretroviral regimen.

Design and Setting Substudy of the AIDS Clinical Trials Group 343, a multicenter clinical research trial conducted between February 1997 and October 1998.

Patients Twenty-six subjects who experienced rebound (HIV RNA level ≥200 copies/mL) during indinavir monotherapy (n = 9) or triple-drug therapy (indinavir, lamivudine, and zidovudine; n = 17) after initially achieving suppression while receiving all 3 drugs, and 10 control subjects who had viral suppression while receiving triple-drug therapy.

Main Outcome Measure Drug susceptibility, determined by a phenotypic assay and genotypic evidence of resistance assessed by nucleotide sequencing of protease and reverse transcriptase, compared among the 3 patient groups.

Results Indinavir resistance was not detected in the 9 subjects with viral rebound during indinavir monotherapy or in the 17 subjects with rebound during triple-drug therapy, despite plasma HIV RNA levels ranging from 10^2 to 10^5 copies/mL. In contrast, lamivudine resistance was detected by phenotypic assay in rebound isolates from 14 of 17 subjects receiving triple-drug therapy, and genotypic analyses showed changes at codon 184 of reverse transcriptase in these 14 isolates. Mean random plasma indinavir concentrations in the 2 groups with rebound were similar to those of a control group with sustained viral suppression, although levels below 50 ng/mL were more frequent in the triple-drug group than in the control group (P = .03).

Conclusions Loss of viral suppression may be due to suboptimal antiviral potency, and selection of a predominantly indinavir-resistant virus population may be delayed for months even in the presence of ongoing indinavir therapy. The results suggest possible value in assessing strategies using drug components of failing regimens evaluated with resistance testing.

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COMPLETE AND PROLONGED suppression of human immunodeficiency virus (HIV) replication is a primary objective of antiretroviral therapy.[1] Rates of viral suppression achieved by potent combination therapies exceed 90% in select clinical trial groups, but these rates are less with the same regimens outside research settings.[2-4] Rebound of plasma viremia also may occur after having suppression below level of detectability. Major factors contributing to loss of suppression include suboptimal drug potency, inadequate drug exposure, and insufficient regimen adherence. A large increase in CD4 cells with therapy, providing more target cells for virus replication, has been proposed[5] and observed[6] to contribute to loss of suppression.

Resistance to protease inhibitor (PI) monotherapy is characterized by sequential acquisition of mutations conferring stepwise reductions in drug susceptibility.[7,8] Early mutant virus appears to be fitness-disadvantaged vs wild-type virus, but later mutations in protease and gag cleavage sites appear to compensate for this.[9-12] Early reports of PI resistance featured patients failing monotherapy or having a PI added to their regimen. Multiple protease-resistance mutations were present in virus isolated from these patients.[7,8] How-

See also pp 205 and 250.

Author Affiliations and Financial Disclosures are listed at the end of this article.
Corresponding Author: Diane V. Havlir, MD, University of California, San Diego, 2760 Fifth Ave, Suite 300, San Diego, CA 92103 (e-mail: dhavlir@ucsd.edu).

ever, these patients' regimens had not suppressed the virus fully, thus providing opportunity for selection of virus with resistance mutations. Resistance patterns in patients failing PI combination therapy following suppression are less well characterized.

We describe drug susceptibility in 26 trial participants achieving suppression with indinavir, zidovudine, and lamivudine followed by loss of suppression. Nine patients were receiving only indinavir when rebound was observed.

METHODS

Subjects

Subjects were a subset of the AIDS Clinical Trials Group 343 (ACTG 343) participants (for whom eligibility criteria were CD4 cells $\geq 200 \times 10^6$/L, HIV RNA level $\geq$1000 copies/mL, limited treatment [<7 days] with PIs, and no prior treatment with lamivudine or abacavir).[6] The goal for ACTG 343 was to assess whether suppression achieved by potent triple-drug therapy could be sustained with less intensive therapy. Subjects (n = 509) were prescribed 6 months of open-label induction therapy with indinavir, 800 mg every 8 hours, lamivudine, 150 mg twice daily, and zidovudine, 300 mg twice daily. Levels of HIV RNA were assayed at 4-week intervals. Treatment discontinuation was recorded but detailed adherence studies were not performed. Subjects with HIV RNA levels less than 200 copies/mL after 16, 20, and 24 weeks of induction therapy were randomized (blinded) in the maintenance phase to receive indinavir monotherapy (n = 100), zidovudine plus lamivudine (n = 104), or all 3 drugs (n = 105). Loss of suppression (plasma HIV RNA levels of $\geq$200 copies/mL) was the primary study end point. Subjects reaching a study end point had the option to resume triple-drug therapy. Of those receiving indinavir and those receiving zidovudine plus lamivudine, 23% in each arm had rebound early during maintenance vs 3% of those continuing triple-drug therapy.[6] The first available specimens were assessed, as per resource constraints, from 9 of 23 subjects (indinavir group) with rebound after switching to indinavir, 17 of 75 subjects (triple-drug therapy group) with at least 1 HIV RNA level of less than 200 copies/mL during induction and 10 of 178 subjects (control group) receiving triple therapy with sustained suppression throughout the trial. Subjects provided written informed consent. Given the expectation that more than 95% of subjects would have indinavir-resistant virus at virologic rebound, there was a greater than 99.9% probability that at least 1 subject in the group of 9 patients and 99.9% probability that at least 1 subject in the group of 17 would have indinavir-resistant virus at rebound.

Phenotypic Resistance Testing

Resistance was evaluated using a phenotypic assay for drug susceptibility (PhenoSense, ViroLogic Inc, San Francisco, Calif) on baseline and follow-up plasma samples from all patients in the indinavir, triple-drug, and control groups as previously reported.[13] Drug susceptibility was quantified by determining the 50% inhibitory concentration (IC_{50}) of drug assayed in vitro of a recombinant test strain incorporating protease and reverse transcriptase gene segments from patient isolates in the presence of protease and reverse transcriptase inhibitors compared with a control (NL4-3) strain. The IC_{50} values greater than 2.5-fold those of the drug-susceptible reference strain indicated reduced susceptibility based on assay validation studies.[14]

Genotypic Resistance Testing

Sequence analysis of drug-resistance mutations in reverse transcriptase and protease genes was done using population-based sequence analysis (PE Biosystems, Foster City, Calif) on all resistance test-vector plasmid pools evaluated for evidence of resistance by phenotypic assay. Amino acid substitutions identified via comparison with NL4-3 were reported. As with the PhenoSense assay, the sequencing results represent the majority species of the HIV RNA amplified from plasma, except in 1 case involving 1 subject. In a prior study evaluating the sensitivity of this method for detecting mixtures of virus pools with 5 HIV polymerase gene polymorphisms, we found that the majority population was readily detectable.[15] Minority species may not be uniformly detected by this method. Resistance mutations were classified as primary or secondary based on recent consensus guidelines.[16]

Indinavir Concentrations

Indinavir concentrations were measured in a central laboratory using high-pressure liquid chromatography on plasma from 29 subjects with available banked plasma samples from time points coinciding with ACTG 343 protocol visits. After extracting indinavir with ethyl t-butyl ether, indinavir and an internal standard were back-extracted from the organic layer following acidification. Repeat extraction of indinavir and the internal standard with methyl t-butyl ether was performed after basification and the final organic was decanted and evaporated. The residue was dissolved with a phosphate buffer and acetonitrile mixture, and the extract was analyzed using high-pressure liquid chromatography with column switching. Chromatograph peaks were monitored by assessing absorbance at 210 nm.

The standard curve range for the indinavir assay ranged from 5 to 500 ng/mL. Precision and inaccuracy were 5.0% and 5.8%, respectively, at the low standard, and 1.6% and 0.7% at the high standard. The indinavir concentration was weighted by number of indinavir measures (range, 4-8) for each subject. Mean and median of indinavir values for each subject were used to generate weighted mean and median indinavir concentrations for each group. These values were compared using Kruskall-Wallis analysis of variance. The proportion of subjects with at least 1 indinavir value of less than 50 ng/mL was compared among the 3 groups using Fisher exact test.

RESULTS

Drug Susceptibility With Maintenance Therapy

During induction of triple-drug therapy, suppression below a plasma HIV RNA level of 50 copies/mL was achieved in the 9 subjects subsequently randomized to indinavir maintenance monotherapy

(mean baseline HIV RNA level, 46 109 copies/mL). Four subjects had HIV RNA levels of less than 50 copies/mL by 8 weeks, 2 by 12 weeks, and 3 by 16 weeks. Rebound was detected 2 to 8 weeks after subjects switched to maintenance therapy. Peak HIV RNA levels during rebound ranged from 10^3 to 10^5 copies/mL.

Viral isolates were assayed for drug susceptibility and drug-resistance mutations 3 to 14 weeks after the switch to indinavir monotherapy, and for 3 subjects were assessed at 2 sequential time points (TABLE 1). Levels of HIV RNA ranged from 10^2 to 10^5 copies/mL in the samples collected at the same time points used for drug susceptibility testing. Viral isolates at baseline and during rebound showed no reduction in susceptibility to indinavir or to PIs nelfinavir, ritonavir, and saquinavir.

Nucleotide sequencing detected no primary mutations known to be associated with indinavir resistance (codons 46 and 82). Changes at codons 10, 20, 24, 32, 54, 63, 71, 73, and 90 were reported in patients with indinavir resistance and classified as secondary mutations.[16] Persons infected with HIV may have changes at these codons prior to therapy. Subjects 4 and 7 had L63P at baseline, and subjects 1 and 2 had this substitution identified in rebound isolates. Subject 3 had L10I at baseline.

After loss of suppression was confirmed, subjects were encouraged to change therapy. Five of the 9 subjects discontinued study participation after rebound was detected. Four subjects resumed open-label, triple-drug therapy with indinavir, zidovudine, and lamivudine. At the time zidovudine and lamivudine were added back to the indinavir monotherapy regimen, the viral loads had been greater than 200 copies/mL for 2 to 8 weeks. Suppression was achieved by 4 weeks in 3 subjects and sustained for 7 to 10 months. Initial suppression was lost 4 months after all 3 drugs were resumed in the fourth subject.

Drug Susceptibility With Triple-Drug Therapy

No significant changes in indinavir susceptibility were detected during rebound in the 17 patients receiving triple-drug therapy despite peak HIV RNA levels during rebound of 1864 to 138 989 copies/mL (TABLE 2) (a representative subject's experience is illustrated in the FIGURE). The primary indinavir-resistance mutation M46L was identified in subject 24 at week 35 but not week 41. Antiretroviral therapy interruption with reduction of selective pressure shortly after the week 35 visit may explain the reappearance of wild-type virus at week 41. Secondary-resistance mutations were present at baseline at codon 63 (9 subjects), codon 10 (5 subjects), and codon 71 (2 subjects), but no new secondary indinavir-resistance mutations appeared in any rebound isolate. Duration of observation during rebound (mean, 6 months; range, 1-12 months) was longer in patients failing triple-drug therapy vs those with rebound when receiving indinavir maintenance therapy (mean, 1 month; range, 0.5-2.5). Although encouraged to switch to alternative antiretroviral regimens, patients chose to continue taking this triple-drug therapy due in part to the limited number of other regimens available at that time.

In 14 of the 17 subjects, lamivudine resistance was detected with the phenotypic assay in viral isolates obtained during rebound. Sequencing confirmed that the methionine to valine substitution at codon 184 of reverse transcriptase, known to confer high-level resistance to lamivudine, was present in all 14 isolates. In 13 of the 14 subjects with lamivudine resistance, lamivudine susceptibility decreased by more than 100-fold at rebound vs the control isolate. In 1 of the 14 subjects, a mixture of isolates with methionine and valine were present, and susceptibility to lamivudine was 7-fold less than that in the control group. Rebound isolates were sensitive to lamivudine in 3 subjects. In analyses from a separate pharmacokinetic study (J-P.S., unpublished data, 1999), indinavir concentrations were undetectable at weeks 12, 20, and 35 in subject 24, who had no resistance to lamivudine, suggesting prescribed medications were not taken.

Random Indinavir Levels

Detectable indinavir concentrations were present in 98% of samples from the indinavir group, 72% from the triple-drug group, and 82% from the control group. At least 4 samples per patient were assessed (mean, 5.4 per patient). Indinavir levels were obtained both during suppression and rebound in 7 patients in the triple-therapy group and 2 patients in

Table 1. Drug Susceptibility in Subjects With Viral Rebound Receiving Indinavir Maintenance Therapy*

Subject	HIV RNA, copies/mL: Baseline	HIV RNA, copies/mL: During Viral Rebound†	Weeks of Indinavir Maintenance at Assay‡	Secondary Indinavir-Resistance Mutations§
1	168 035	2386	4, 12	L63P
2	4570	37 607	8	L63P
3	58 586	155 000	8, 14	None
4	8491	13 411	8	None
5	9065	1107	12	None
6	3956	1852	4	None
7	28 870	16 545	3, 8	None
8	44 513	62 012	6	None
9	65 582	2430	3	None

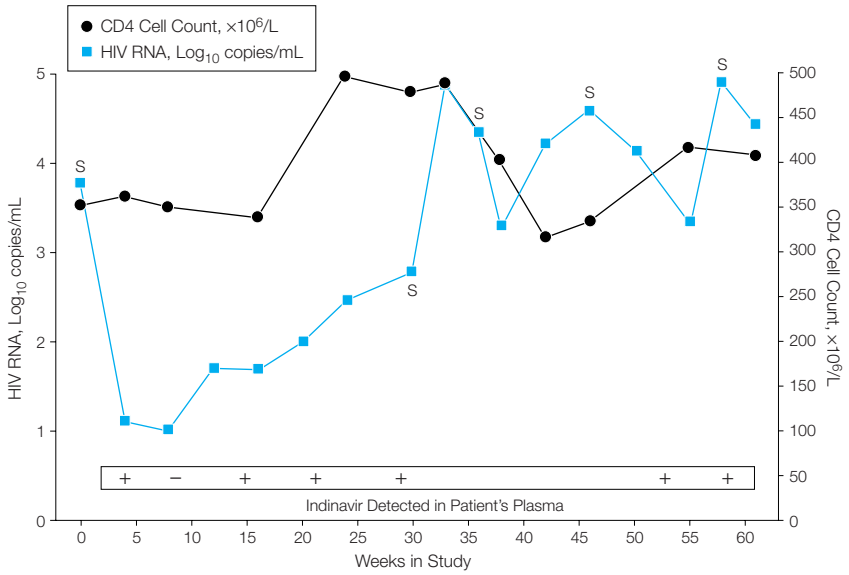
*There were no primary indinavir-resistance mutations with substitutions at codons 46 and 82.
†Peak level of human immunodeficiency virus (HIV) RNA during rebound when the viral isolates were assayed for drug susceptibility.
‡Each isolate assayed at these time points was sensitive for indinavir susceptibility. Sensitive was defined by 50% inhibitory concentration (IC_{50}) of drug assayed in vitro less than or equal to 2.5-fold that of control isolate; resistance was defined as IC_{50} greater than 2.5-fold those of control.
§Substitutions at the following codons have been associated with indinavir resistance: 10, 20, 24, 32, 54, 63, 71, 73, and 90.[16] Some patients had polymorphisms at these codons prior to therapy initiation. Only those substitutions present during rebound but not at baseline are shown here.

Table 2. Drug Susceptibility in Subjects Receiving Indinavir, Zidovudine, and Lamivudine With Viral Rebound*

Subject	Maximum Weeks of Viral Rebound at Assay	Peak HIV RNA Level During Viral Rebound, copies/mL	Weeks of Triple-Drug Therapy at Assay	Susceptibility†		Genotype of Codon 184V of Reverse Transcriptase
				Indinavir‡	Lamivudine	
10	12	32 962	32, 36, 44	Sensitive	Resistant	M184V
11	17	22 426	24, 31	Sensitive	Resistant	M184V
12	37	68 659	30, 36, 46, 61	Sensitive	Resistant	M184V
13	22	10 320	16, 24, 38	Sensitive	Resistant§	M184V/wild-type mixture
14	25	6220	20, 45	Sensitive	Resistant	M184V
15	20	2996	24, 36, 44	Sensitive	Resistant	M184V
16	4	1864	16, 20	Sensitive	Resistant	M184V
17	8	2460	29, 38	Sensitive	Resistant	M184V
18	19	3967	24, 35	Sensitive	Resistant	M184V
19	28	19 487	20, 48	Sensitive	Sensitive	Wild type
20	16	57 917	20, 28	Sensitive	Resistant	M184V
21	36	10 061	26, 62	Sensitive	Sensitive	Wild type
22	29	6240	49	Sensitive	Resistant	M184V
23	46	11 493	66	Sensitive	Resistant	M184V
24	25	49 697	35, 41	Sensitive	Sensitive	Wild type
25	24	138 989	24, 40	Sensitive	Resistant	M184V
26	49	27 172	65	Sensitive	Resistant	M184V

*The baseline isolates contained polymorphisms considered secondary indinavir-resistance mutations in 16 subjects and are listed in the "Results" section. HIV indicates human immunodeficiency virus.
†Sensitive defined as 50% inhibitory concentration (IC_{50}) of drug assayed in vitro less than twice that of control isolate; resistance defined as IC_{50} 2.5-fold greater than that of control.
‡There were no primary or secondary indinavir-resistance mutations except for subject 24, who had a primary mutation at M46L.
§This isolate had a 7-fold reduction in susceptibility vs all the other resistant isolates (which had >100-fold reductions).

Figure. Indinavir Susceptibility During Viral Rebound of Subject 12 While Receiving Triple-Drug Therapy of Indinavir, Zidovudine, and Lamivudine



The baseline and 4 isolates tested during rebound at weeks 30, 36, 46, and 61 remained sensitive (S) to indinavir. Indinavir levels were detectable at all time points tested except week 8, which immediately preceded loss of viral suppression.

the indinavir group. Levels were obtained during suppression for the other subjects. Weighted mean indinavir concentrations were 1486, 1429, and 1627 ng/mL for indinavir, triple-drug, and control groups, respectively, and were not significantly different (TABLE 3). In the triple-therapy group, mean indinavir concentration was 990 ng/mL during suppression and 1280 ng/mL during rebound ($P = .74$). Although weighted mean indinavir concentrations did not differ significantly among groups, the proportion of patients with at least 1 indinavir level below 50 ng/mL was higher in the group failing triple therapy vs the control group ($P = .03$; Table 3).

COMMENT

In earlier studies of PI resistance in patients receiving combination antiretroviral therapy, patients received sequential therapy and plasma HIV RNA levels were only partially suppressed.[17] Under these conditions, PI-resistant virus emerged rapidly. These observations and similar ones involving PI monotherapy[18] led to the generally held assumption that when suppression failure occurs with a regimen containing a PI, PI-resistant virus accounts for HIV RNA rebound. Failure to detect resistance in some patients was attributed to regimen nonadherence.[19,20] The results from this study and others challenge this view and suggest that suboptimal antiviral potency permits rebound, and that selection of a predominantly PI-resistant virus population may be delayed for months.[21,22]

The patients in this study had suppressed viral load to below 50 copies/mL while taking triple-drug therapy. Suppression was then lost either when continuing triple therapy or when switching to indinavir maintenance therapy. In both groups, indinavir levels were detectable in most samples tested and indinavir-sensitive virus was the predominant population identified during rebound. In most patients continuing to receive lamivudine as part of triple-drug therapy, virus was lamivudine-resistant phenotypically and genotypically at the time of rebound. Outgrowth of indinavir-sensitive, lamivudine-

resistant virus with continuing treatment pressure may be explained by viral fitness and antiviral potency.

By definition, the predominant virus replicating under a set of selective pressures is the most fit. For lamivudine or non–nucleoside reverse transcriptase inhibitors such as nevirapine or efavirenz, a single nucleotide change can confer a 20- to 1000-fold reduction in susceptibility.[23-26] In the presence of drugs, the mutant virus is so much more fit that it will predominate. Clinical data confirm that when antiviral potency of a regimen containing one of these drugs is insufficient to suppress replication, drug-resistant virus rapidly emerges.[27-29] Most patients failing triple therapy herein had lamivudine resistance. In a study of isolates from patients with rebound when taking an efavirenz and indinavir combination regimen, most isolates were resistant to efavirenz.[30]

Why did indinavir-sensitive virus appear in patients continuing therapy? Possible factors include impaired fitness of early indinavir-resistant mutant virus, reduced antiviral potency, and an increase in target cells. In contrast to lamivudine and non–nucleoside reverse transcriptase inhibitors, development of high-level resistance to PIs and zidovudine requires the accumulation of multiple mutations.[9,10,31,32] For PIs, the first mutation confers only limited reduction in susceptibility, usually less than 10-fold.[33] Also, the first mutations adversely affect protease function and virus replication.[9-11,34] Thus, a virus with 1 or 2 mutations is less fit than wild type, even in the presence of drugs.

In those receiving indinavir maintenance therapy, reduction in antiviral potency (ie, discontinuation of zidovudine and lamivudine) allowed increased viral replication. Because the wild-type virus had a fitness advantage over early mutant virus, it was the predominant population for months. Replication may also have been enhanced by an increase in target cells. In patients randomized to maintenance therapy in ACTG 343, loss of suppression was most likely in those with the greatest increment in CD4 cell number,[6] supporting predator-prey models proposed to explain viral dynamics in patients receiving zidovudine.[35] The models were later extended to induction-maintenance treatment strategies.[5] In these models, increased numbers of target cells resulting from treatment provide better conditions for the virus when suppression is incomplete.

Table 3. Random Plasma Indinavir Concentrations*

Group	No. of Subjects	No. of Measures	Range, ng/mL	Indinavir Concentration, ng/mL		No. of Subjects With at Least 1 Indinavir Value <50 ng/mL
				Mean (SE)	Median	
Indinavir	9	51	114-4928	1486 (584)	522	5
Triple drug	10	55	22-3585	1429 (370)	1084	9
Control†	10	50	375-3047	1627 (309)	1585	4‡

*Indinavir levels were obtained both during suppression and viral rebound for 7 patients in the triple-therapy group and 2 patients in the indinavir group. For the other subjects, levels were obtained during suppression.
†Patients maintaining suppression who were receiving indinavir, zidovudine, and lamivudine.
‡The proportion of subjects with at least 1 indinavir value less than 50 ng/mL is significantly higher in the triple-drug group vs the control group ($P = .03$).

Based on prior studies of indinavir monotherapy, one would expect that had patients failing indinavir maintenance therapy not been switched back to more potent regimens, indinavir-resistant virus would have become the predominant population. Continuing growth of the breakthrough virus in the presence of drug selects for an accumulation of mutations conferring both reduced susceptibility and compensation for the adverse impact of resistance mutations on protease function and virus replication. Compensatory mutations have been well characterized both in protease outside the substrate binding site and in protease cleavage sites in gag.[9-11,34] The maximum period of observation of indinavir maintenance failures was 3 months. It is probable that selection of early indinavir-resistant mutant virus occurred, but that the prevalence remained below the limit of detection of the assay used to assess drug susceptibility.

In patients failing triple-drug therapy, diminished antiviral potency (as a result of suboptimal adherence or drug delivery) undoubtedly contributed to rebound. Although the specimen collection schedule was not designed to assess indinavir exposure, evaluation of random samples for indinavir levels suggested that patients taking triple-drug therapy that was failing had more dosing interruptions than the indinavir maintenance group (data not shown). Brief periods of low or undetectable drug levels may have allowed unabated replication and the fitness disadvantage of early indinavir-resistant mutant virus may have allowed sensitive virus to predominate for months.

In terms of alternative hypotheses to explain outgrowth of virus wild type in protease with indinavir, the presence of p7/p1 or p1/p6 gag cleavage-site mutations were ruled out by the sequencing, which also excluded the theoretical possibility of a gag-pol frameshift mutation resulting in increased expression of protease. Also, drug efflux transporters could have diminished indinavir's effect and not been detected via measure of indinavir levels. This possibility is supported by the recent recognition of P glycoprotein transporters that can serve as protease efflux pumps in vitro.[36,37]

Our findings have several clinical implications. First, in patients failing suppressive antiretroviral combination regimens, the predominant virus population may be resistant to 1 (ie, lamivudine), but not all (ie, PI) components of the regimen. Second, not all drugs in a failing regimen (defined as a rebound in HIV RNA levels) may be lost options. Third, these data suggest that drug-resistance testing early after loss of suppression may be useful in identifying components of a failing regimen that might be useful in a new combination regimen. These results suggest value in assessing strategies using drug components of a failing combination evaluated by resistance testing.

However, systematic studies are needed to address concerns that retaining part of a regimen that appears sensitive on resistance testing could lead to selection of resistant minority species that may contribute to virologic failure of the new regimen and reduced treatment options. Finally, it must be acknowledged that PI-sensitive virus in patients taking a failing regimen is not necessarily evidence of nonadherence.

Author Affiliations: University of California, San Diego (Drs Havlir and Richman), San Diego Veterans Affairs Medical Center (Dr Richman); ViroLogic Inc, San Francisco, Calif (Drs Hellmann, Petropoulos, and Whitcomb); Harvard Medical School, Boston, Mass (Dr Hirsch); University of Washington School of Medicine, Seattle (Dr Collier); Washington University, St Louis, Mo (Dr Tebas); and the University of Alabama, Birmingham (Dr Sommadossi).

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REFERENCES

1. Carpenter CCJ, Fischl MA, Hammer SM, et al. Antiretroviral therapy for HIV infection in 1998. *JAMA.* 1998;280:78-86.

2. Fätkenheuer G, Thiesen A, Rockstroh J, et al. Virological treatment failure of protease inhibitor therapy in an unselected cohort of HIV-infected patients. *AIDS.* 1997;11:F113-F116.

3. Mocroft A, Gill MJ, Davidson W, Phillips AN. Predictors of a viral response and subsequent virological treatment failure in patients with HIV starting a protease inhibitor. *AIDS.* 1998;12:2161-2167.

4. Gulick RM, Mellors JW, Havlir D, et al. Treatment with indinavir, zidovudine, and lamivudine in adults with human immunodeficiency virus infection and prior antiretroviral therapy. *N Engl J Med.* 1997;337:734-739.

5. Wein LM, D'Amato RM, Perelson AS. Mathematical analysis of antiretroviral therapy aimed at HIV-1 eradication or maintenance of low viral loads. *J Theor Biol.* 1998;192:81-98.

6. Havlir DV, Marschner IC, Hirsch MS, et al. Maintenance antiretroviral therapies in HIV-infected subjects with undetectable plasma HIV RNA after triple-drug therapy. *N Engl J Med.* 1998;339:1261-1268.

7. Condra JH, Schleif WA, Blahy OM, et al. In vivo emergence of HIV-1 variants resistant to multiple protease inhibitors. *Nature.* 1995;374:569-571.

8. Molla A, Korneyeva M, Gao Q, et al. Ordered accumulation of mutations in HIV protease confers resistance to ritonavir. *Nat Med.* 1996;2:760-766.

9. Ho DD, Toyoshima T, Mo H, et al. Characterization of human immunodeficiency virus type 1 variants with increased resistance to a C_2-symmetric protease inhibitor. *J Virol.* 1994;68:2016-2020.

10. Gulnik SV, Suvorov LI, Liu B, et al. Kinetic characterization and cross-resistance patterns of HIV-1 protease mutants selected under drug pressure. *Biochemistry.* 1995;34:9282-9287.

11. Nijhuis M, Schuurman R, de Jong D, et al. Selection of HIV-1 variants with increased fitness during ritonavir therapy. In: International Workshop on HIV Drug Resistance, Treatment Strategies and Eradication; June 25-28, 1997; St Petersburg, Fla. Abstract 92.

12. Mammano F, Petit C, Clavel F. Resistance-associated loss of viral fitness in human immunodeficiency virus type 1. *J Virol.* 1998;72:7632-7637.

13. Little SJ, Daar ES, D'Aquila RT, et al. Reduced antiretroviral drug susceptibility among patients with primary HIV infection. *JAMA.* 1999;282:1142-1149.

14. Hellmann N, Johnson P, Petropoulos C. Validation of the performance characteristics of a novel, rapid phenotypic drug susceptibility assay: PhenoSense HIV. In: Programs and abstracts of the 3rd International Workshop on Drug Resistance and Treatment Strategies; June 23-26, 1999; San Diego, Calif. Abstract 51.

15. Gunthard HF, Wong JK, Ignacio CC, Havlir DV, Richman DD. Comparative performance of high-density oligonucleotide sequencing and dideoxynucleotide sequencing of HIV type 1 pol from clinical samples. *AIDS Res Hum Retroviruses.* 1998;14:869-876.

16. Hirsch MS, Conway B, D'Aquila RT, et al. Antiretroviral drug resistance testing in adults with HIV infection. *JAMA.* 1998;279:1984-1991.

17. Shafer RW, Winters MA, Palmer S, Merigan TC. Multiple concurrent reverse transcriptase and protease mutations and multidrug resistance of HIV-1 isolates from heavily treated patients. *Ann Intern Med.* 1998;128:906-911.

18. Condra JH, Holder DJ, Schleif WA, et al. Genetic correlates of in vivo viral resistance to indinavir, a human immunodeficiency virus type 1 protease inhibitor. *J Virol.* 1996;70:8270-8276.

19. Young B, Johnson S, Bahktiari M, et al. Resistance mutations in protease and reverse transcriptase genes of human immunodeficiency virus type 1 isolates from patients with combination antiretroviral therapy failure. *J Infect Dis.* 1998;178:1497-1501.

20. Mayers DL, Gallahan DL, Martin GJ, et al. Drug resistance genotypes from plasma virus of HIV-infected patients failing combination drug therapy. In: International Workshop on HIV Drug Resistance, Treatment Strategies and Eradication; June 25-28, 1997; St Petersburg, Fla. Abstract 80.

21. Holder DJ, Condra JH, Schleif WA, Chodakewitz J, Emini EA. Virologic failure during combination therapy with Crixivan and RT inhibitors is often associated with expression of resistance-associated mutations in RT only. In: 6th Conference on Retroviruses and Opportunistic Infections; January 31 to February 4, 1999; Chicago, Ill. Abstract 492.

22. Descamps D, Peytavin G, Calvez V, et al. Virologic failure, resistance and plasma drug measurements in induction maintenance therapy trial (Anrs 072, Trilege). In: 6th Conference on Retroviruses and Opportunistic Infections; January 31 to February 4, 1999; Chicago, Ill. Abstract 493.

23. Richman D, Shih CK, Lowy I, et al. Human immunodeficiency virus type 1 mutants resistant to non–nucleoside inhibitors of reverse transcriptase arise in tissue culture. *Proc Natl Acad Sci U S A.* 1991;88:11241-11245.

24. Schinazi RF, Lloyd RM Jr, Nguyen MH, et al. Characterization of human immunodeficiency viruses resistant to oxathiolane-cytosine nucleosides. *Antimicrob Agents Chemother.* 1993;37:875-881.

25. Tisdale M, Kemp SD, Parry NR, Larder BA. Rapid in vitro selection of human immunodeficiency virus type 1 resistant to 3′-thiacytidine inhibitors due to a mutation in the YMDD region of reverse transcriptase. *Proc Natl Acad Sci U S A.* 1993;90:5653-5656.

26. Boucher CA, Cammack N, Schipper P, et al. High-level resistance to enantiomeric 2′-deoxy-3′-thiacytidine in vitro is due to one amino acid substitution in the catalytic site of human immunodeficiency virus type 1 reverse transcriptase. *Antimicrob Agents Chemother.* 1993;37:2231-2234.

27. Schuurman R, Nijhuis M, van Leeuwen R, et al. Rapid changes in human immunodeficiency virus type 1 RNA load and appearance of drug-resistant virus populations in persons treated with lamivudine (3TC). *J Infect Dis.* 1995;171:1411-1419.

28. Wei X, Ghosh SK, Taylor ME, et al. Viral dynamics in human immunodeficiency virus type 1 infection. *Nature.* 1995;373:117-122.

29. Havlir DV, Eastman S, Gamst A, Richman DD. Nevirapine-resistant human immunodeficiency virus. *J Virol.* 1996;70:7894-7899.

30. Bacheler L, Weislow O, Snyder S, Hanna G, D'Aquila R. The Sustiva Resistance Study Team: virological resistance to efavirenz. In: 12th World AIDS Conference; June 28-July 3, 1998; Geneva, Switzerland. Abstract 41213.

31. Larder B. Nucleosides and foscarnet mechanisms. In: Richman DD, ed. *Antiviral Drug Resistance.* Chichester, England: John Wiley & Sons; 1996:169-190.

32. de Jong MD, Veenstra J, Stilianakis NI, et al. Host-parasite dynamics and outgrowth of virus containing a single K70R amino acid change in reverse transcriptase are responsible for the loss of human immunodeficiency virus type 1 RNA load suppression by zidovudine. *Proc Natl Acad Sci U S A.* 1996;93:5501-5506.

33. Markowitz M, Ho DD. Protease inhibitors—mechanisms and clinical aspects. In: Richman DD, ed. *Antiviral Drug Resistance.* Chichester, England: John Wiley & Sons; 1996:261-278.

34. Zennou V, Mammano F, Paulous S, Mathez D, Clavel F. Loss of viral fitness associated with multiple gag and gag-pol processing defects in human immunodeficiency virus type 1 variants selected for resistance to protease inhibitors in vivo. *J Virol.* 1998;72:3300-3306.

35. McLean AR, Nowak MA. Competition between zidovudine-sensitive and zidovudine-resistant strains of HIV. *AIDS.* 1992;6:71-79.

36. Lee CG, Gottesman MM, Cardarelli CO, et al. HIV-1 protease inhibitors are substrates for the MDR1 multidrug transporter. *Biochemistry.* 1998;37:3594-3601.

37. Kim RB, Fromm MF, Wandel C, et al. The drug transporter P-glycoprotein limits oral absorption and brain entry of HIV-1 protease inhibitors. *J Clin Invest.* 1998;101:289-294.