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# HIV Resistance Testing Consultation Service

## Consultation Report

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**Disclaimer:**

This information has been developed solely as an educational resource for health care professionals interested in HIV care and research. The information presented represents the views of the Panel members only and not necessarily those of the National HIV/AIDS Clinicians' Consultation Center's HIV Telephone Consultation Service (Warmline), the Positive Health Program at San Francisco General Hospital, or sponsoring organizations. Resistance testing can help identify whether certain drugs or classes of drugs might be ineffective, but cannot establish which drugs will be effective. Furthermore, test results can be inaccurate and interpretation of tests is not yet standardized. Because of the many factors involved in treatment decisions when resistant virus is present, the antiretroviral regimens and the therapeutic strategies discussed are not the only possible options and might be different from current Practice Guidelines. Other sources of information on resistance testing, such as clinical HIV websites, can be of help. Health care professionals should consult the HIV Telephone Consultation Service (Warmline) or HIV experts in their community before using any of the recommended therapeutic regimens or strategies in this document.

Consultation is available to California AIDS Drug Assistance Program providers through the California State Office of AIDS Voucher Program by calling the HRSA/ AIDS ETC National HIV Telephone Consultation Service (Warmline) at 1/800/933-3413. The HIV Resistance Testing Consultation Service is supported by a grant from the California State Office of AIDS through the Pacific AIDS Education and Training Center.

## History/Clinical Course

This is a 44-year-old man who was first diagnosed to be HIV+ in 1985. His course has been complicated by esophageal candidiasis, oral HSV, cryptogenic seizures and reactive PPD (INH prophylaxis for unclear duration). He was treated with AZT monotherapy at some point, the dose and duration are unclear.

He presented to his current provider in 1/95 on no antiretrovirals with a CD4 count of 53 cells/mm<sup>3</sup>. Dideoxycytidine (ddC) monotherapy was begun in 2/95 and his CD4 count rose to 83 in 3/95. His ddC was stopped in 12/95 when his CD4 was 31 cells/mm<sup>3</sup>. AZT/3TC were started but stopped a month later for anemia and "other problems".

In 2/96, the patient developed vacuolar myelopathy, severe degenerative joint disease, and active tuberculosis (smear and culture positive for *Mycobacterium TB*). Tuberculosis treatment was initiated and patient was admitted to hospice. By 3/96, off all antiretrovirals, his VL was 1,900,000 and his CD4 count was 53 cells/mm<sup>3</sup>. His subsequent treatment course is summarized in the table below.

DATE	REGIMEN *	CD4 cells/mm <sup>3</sup>	VL COPIES/ML	RESISTANCE TEST FINDINGS	CLINICAL COURSE
3/96	None	53	1,900,000		M.Tb, vacuolar myelopathy
4/96	D4T/3TC				
7/96			54,000		
8/96	D4T/3TC/SQV				
9/96		150			Discharged from hospice
10/96			213,000		
10/96	AZT/3TC/IDV				
11/97				Mutations noted at RT positions: 67, 184, 219	
2/98		75	49,000		
8/98				"NRTI pan-sensitive, multiple. PI mutations"	
2/99	ABC/EFV/RTV/SQV				RTV/SQV "intolerant"
3/99	ABC/EFV/NLF				Didn't take meds; ? ABC hypersensitivity
4/99	DDI/D4T/EFV/NLF				
5/99		75	15,000		
6/99			18,000	NRTI: 67,69,70 PI: 10I, 46L, 54L/V, 64I/V, 71V, 82A	
7/99		87	83,000		
7/99	RTV/SQV/EFV/DDI/D4T (+/- HU)				
9/99		128	50,000		
10/99				Phenotype 1 (see below)	

DATE	REGIMEN *	CD4 cells/mm <sup>3</sup>	VL COPIES/ML	RESISTANCE TEST FINDINGS	CLINICAL COURSE
11/99		146	35,000		
2/00		121	35,000		Gabapentin for Peripheral Neuropathy (PN)
1/01		146	35,000		
6/01		125		Phenotype 2 (see below)	PN worse, fatigue, depression, HSV outbreak

\* See below for abbreviations of antiretroviral agents

## Resistance Test Findings

### Genotype (6/4/99)

### Key Mutations

NRT	67, 69, 70
NNRT	None
PI	10I, 46L, 54L/V, 64I/V, 71V, 82A

### Phenotype 1 (10/29/99)

Nucleoside Reverse Transcriptase Inhibitors (NRTI)	Fold Change in IC50
Abacavir (ABC)	6.7
Didanosine (ddI)	1.3
Lamivudine (3TC)	2.6
Stavudine (d4T)	2.3
Zalcitabine (ddC)	1.1
Zidovudine (AZT)	200
Nonnucleoside Reverse Transcriptase Inhibitors (NNRTI)	
Delavirdine (DLV)	1.3
Efavirenz (EFV)	34.5
Nevirapine (NVP)	150.7
Protease Inhibitors (PI)	
Amprenavir (APV)	0.9
Indinavir (IDV)	2.6
Nelfinavir (NFV)	4.7
Ritonavir (RTV)	11.0
Saquinavir (SAQ)	1.9

## Phenotype 2 (6/5/01)

Nucleoside Reverse Transcriptase Inhibitors (NRTI)	Fold Change in IC50
Abacavir	2.6
Didanosine	1.4
Lamivudine	2.2
Stavudine	3.3
Zalcitabine	1.4
Zidovudine	28.0
Nonnucleoside Reverse Transcriptase Inhibitors (NNRTI)	
Delavirdine	0.04
Efavirenz	132
Nevirapine	183
Protease Inhibitors (PI)	
Amprenavir	4.0
Indinavir	14.0
Lopinavir	15.0
Nelfinavir	11.0
Ritonavir	49.0
Saquinavir	29.0

## Interpretation/Implications for Treatment

In summary, this 44-year-old man with AIDS was treated initially with dual nucleoside therapy, to which one, then two protease inhibitors were subsequently added. Over the course of 5 years, he received combination regimens containing agents from each of the drug classes, with variable adherence, largely due to drug intolerance.

This patient has benefited from antiretroviral therapy, even though he has never achieved complete viral suppression. In the 2 years that he has been on his current regimen (RTV/SQV/EFV/ddI/D4T) his viral load has remained stable in the range of 35,000 to 50,000 copies/mL, and he has developed no new HIV-related opportunistic complications. His CD4 count has ranged between 120 and 150 cells/mm<sup>3</sup>. His chief problems now are fatigue and peripheral neuropathy, the latter requiring increasing doses of gabapentin for relief. This symptom is almost certainly related to ddI and/or d4T toxicity and soon may limit their use. Importantly, while on abacavir, he developed signs and symptoms suggestive of hypersensitivity. Abacavir was discontinued and cannot be considered for future use.

The series of resistance tests obtained on a variety of treatment regimens reflect cumulative viral resistance in the face of incomplete viral suppression. Initially, inadequate drug potency was probably responsible for virologic failure. Subsequently, viral resistance and imperfect adherence probably have been the primary problems. The first genotype, obtained in 11/97, showed the 184 mutation associated with high level 3TC resistance, as well as one accessory AZT mutation and an NRTI polymorphism not generally associated with diminished clinical response to AZT. These findings are consistent with incompletely suppressive dual nucleoside therapy with D4T and 3TC. After adding hard gel saquinavir to this regimen the CD4 count tripled and the patient felt well enough to be discharged from hospice, even though the viral load remained high. Replacing D4T and saquinavir with AZT and indinavir respectively did reduce the viral load by a log over the

course of about a year, but its failure to completely suppress viral replication was probably related to a combination of already-present NRTI resistance and ongoing poor adherence. Details of the second genotype, obtained in 8/98, are not available, but by report, multiple protease inhibitor mutations had appeared. These results make sense in the context of this patient's treatment history, in which 2 different single PIs were added in sequence to a failing dual NRTI regimen.

Between 2/99 and 6/99 the patient received 3 separate regimens containing agents from all 3 antiretroviral classes. He tolerated these regimens poorly and his adherence was not optimal. His viral load over this period remained measurable (15,000–18,000 copies), but was low relative to his predicted off therapy "set-point". His CD4 count did not maintain its initially robust rise, but after falling from a high of 150 in 9/96 to 75 in 2/98, it remained stable.

In 6/99, while on ddl/d4T/EFV/NFV, a third genotype was obtained. As before, it demonstrated minimal to moderate AZT resistance. The 184 mutation was not detected, probably because 3TC had not been a part of the patient's regimen for several years. Although it is unlikely to have disappeared, without the selective pressure of ongoing 3TC treatment, the viral quasispecies containing the 184 mutation does not comprise a sufficiently large proportion of the total viral population to be detectable by the genotype test. Surprisingly, no NNRTI mutations were present, even though adherence on efavirenz-containing regimens was erratic. However, quite consistent with the clinical history was the appearance of multiple PI mutations, suggesting primary resistance to saquinavir and indinavir as well as possibly diminished clinical response to other PIs as well.

In 7/99 the viral load more than quadrupled (to 83,000 copies/mL) and the patient's regimen was changed to RTV/SQV/EFV/ddl/d4T. Hydroxyurea (HU) was added for several months, and then discontinued when it appeared not to be adding any significant benefit. In 10/99, with a CD4 of 128 and a viral load of 50,000 copies/ml, a phenotype resistance test was obtained. Consistent with the treatment history, the results suggested that additional NRTI resistance had accumulated. A 200-fold increase in IC50 compared with control indicated high-level AZT resistance. A moderately increased IC50 for ABC was also present, while IC50s for the remaining NRTIs were increased 3-fold or less, suggesting some degree of susceptibility to these agents was still present. Predictably, NNRTI resistance had emerged, as well, with 150-fold and 35-fold increases in NVP and EFV IC50, respectively. However, the virus did demonstrate susceptibility to DLV.

No changes were made to the patient's regimen and his condition remained stable. Since beginning this regimen, the patient's CD4 has ranged between 120 and 150, and the viral load has remained around 35,000 copies/mL. His adherence reportedly has been excellent. His peripheral neuropathy symptoms have gradually worsened and recently he has become fatigued. Otherwise he is tolerating his antiretrovirals well.

Worsening fatigue prompted another phenotype resistance test in June 2001. The results were largely similar to those from 10/99, with several notable exceptions. In the NRTI class, AZT resistance was less prominent (28-fold increase in IC50 compared with 200-fold in 10/99), as was ABC resistance (2.6-fold compared with 6.7-fold). It's difficult to know how clinically relevant these changes are, although taken together with the antiretroviral history and prior resistance test results it seems reasonable to suppose that ABC might provide some clinical benefit while AZT probably would not. Fold-changes in IC50 for ddl and ddC remained in the "sensitive" range, although it's important to note that reliable and clinically-significant cut-off points defining resistance to these agents have yet to be determined (current estimates place the cut-off for diminishing susceptibility to d4T, ddl and perhaps ddC at 1.7 fold).

In the PI class, the 6/01 phenotype demonstrates more prominent resistance to all currently available agents, compared with the 10/99 test. Such results would be expected in the context of ongoing viral replication despite treatment with a PI-based HAART regimen. Even so, the 4-fold and 15-fold IC50 increases for APV and LPV/r, respectively suggest that this patient's virus may have retained some susceptibility to these agents, especially if their pharmacokinetics are optimized with RTV.

**NNRTI hypersusceptibility.** In the NNRTI class, high-level resistance to EFV and NVP persists in the 6/01 phenotype compared with the 10/99 test. In addition, the 0.04-fold change in IC50 for DLV suggests that “hypersusceptibility” to this agent has developed. This phenomenon was first reported by Whitcomb, et al. at the 7<sup>th</sup> Conference on Retroviruses and Opportunistic Infections in 2/2000<sup>i</sup>. They described enhanced phenotypic susceptibility to NNRTIs in some patients that were NRTI-experienced and NNRTI naïve. In early 2001, Shulman, et al. published a retrospective study of 30 patients receiving salvage therapy containing efavirenz<sup>ii</sup>. All were efavirenz-naïve, but some previously had received NVP or DLV. In patients with pre-existing NNRTI resistance mutations, the presence of multiple NRTI mutations was associated with less prominent EFV resistance than would have been expected. In other words, the NRTI mutations seemed to be “restoring” partial EFV susceptibility to a virus that had mutations predictive of high-level resistance. This “reduced” resistance to EFV was not associated with improved response to the efavirenz-containing salvage regimen, arguing against the clinical significance of this finding. However, in the group of patients without preexisting NNRTI resistance mutations, NNRTI hypersusceptibility was associated with improved virologic outcomes after 24 weeks of therapy, suggesting that including EFV in a regimen for this subgroup of patients may be beneficial.

Further work on NNRTI hypersusceptibility was presented at the recent (7/01) meeting of the International AIDS Society in Buenos Aires. Katzenstein, et al., reported a positive association between prior NRTI experience and NNRTI hypersusceptibility in an NNRTI-naïve salvage treatment cohort and further identified an association between NNRTI hypersusceptibility and NRTI mutations at codons 41, 67, and 215<sup>iii</sup>. Keiser, et al, addressed the clinical relevance of these findings at the same meeting<sup>iv</sup>. They compared response to an efavirenz-containing salvage regimen in comparable groups of heavily NRTI experienced, but NNRTI naïve patients who did and did not exhibit pre-treatment NNRTI hypersusceptibility. Their study suggested that NNRTI hypersusceptibility in this group was associated with the number of mutations present at codons 41, 69, and 215. Furthermore, patients with NNRTI hypersusceptibility experienced virologic failure on their new regimen more rapidly than did patients with virus that was normally susceptible to NNRTIs.

Taken together, these preliminary clinical studies, and others, do indicate that complex interactions between NRTI and NNRTI mutations occur. However, the clinical implications of these interactions remain unclear and currently there is insufficient evidence to suggest that NNRTI hypersusceptibility should be considered when making salvage treatment decisions.

**Summary.** In summary, this highly ARV-experienced patient who is virologically and immunologically stable on a partially suppressive HAART regimen has multiply resistant virus that is not likely to suppress completely with currently available antiretroviral agents. This patient has tolerated his current antiretroviral regimen reasonably well and has been able to adhere to it, but progressive fatigue and peripheral neuropathy raise the question of whether a new regimen might be better at optimizing the benefits and minimizing the risks of treatment. Resistance testing suggests that some susceptibility to ABC, APV, and LPV/r (Kaletra) may be present, although it's impossible to predict what the clinical response to a regimen containing these agents might be. In addition, there is evidence of hypersusceptibility to DLV, despite high-level EFV and NVP resistance. The clinical significance of this finding is unclear.

## Recommendations

### Regimen Options

The Panel was evenly split in its preference for each of the following two options, with some members advocating for Option 2 immediately, and others preferring Option 1 with a plan to move to Option 2 if Option 1 does not successfully maintain the patient's stable condition. All members were concerned about the risk of irreversible neurologic injury if drug-related peripheral neuropathy was not relieved. All members also supported additional evaluation of the patient's fatigue and depression if these symptoms did not resolve after efavirenz was discontinued.

- Option 1: Continue partially suppressive regimen to avoid immunologic decline, but modify to minimize toxicity.

This strategy would continue the dual PI regimen the patient currently is tolerating, but to minimize neuropathy symptoms, would replace ddI and d4T with AZT and 3TC. ABC would be likely to contribute to the efficacy of this regimen, but its use is precluded by the history of ABC hypersensitivity in the past.

Efavirenz should be discontinued, since it increases the regimen's complexity and is not likely to be adding benefit. In addition, it may be contributing to this patient's worsening depression.

The Panel suggested obtaining another genotype resistance test when the patient is stable on the modified regimen. While the test is unlikely to be immediately useful, it may provide information about this patient's virus that will be helpful in planning future regimens.

In addition to discontinuing ddI and d4T, there is preliminary evidence that treatment with L-carnitine 330 mg, 2-3 tablets BID-TID may help with drug-induced peripheral neuropathy. It may be worth considering a trial of this supplement, if symptoms persist after adjusting the antiretroviral regimen.

➤ **Advantages**

Familiar and likely to maintain partial viral suppression without additional toxicity

Saves drugs with partial activity for future use in combination with new agents

Reduces risk of accumulating additional NNRTI mutations that may limit usefulness of newer agents in this class

Minimizes risk of clinically disadvantageous complex drug-drug interactions

➤ **Disadvantages**

May miss opportunity to capitalize on DLV hypersusceptibility

Very unlikely to suppress viral load completely

Unlikely to improve immunologic status significantly

Ongoing risk of developing GI intolerance, body habitus changes and/or hyperlipidemia

- Option 2: Attempt complete viral suppression with all available drugs likely to have activity

This strategy would overhaul the entire regimen and would use the resistance test results and the clinical history to guide selection of agents that would be most likely to have activity. Such a regimen might include 3TC and tenofovir, the recently approved nucleotide reverse transcriptase inhibitor expected to have activity against highly NRTI resistant viral species, along with delavirdine, and lopinavir/ritonavir, +/- amprenavir. Data concerning the clinical benefit of using

NNRTIs in NRTI-experienced patients with phenotypic NNRTI hypersusceptibility are conflicting. Available data suggest that there may be some benefit for patients who have not previously received NNRTIs. In this case, the patient has prior EFV experience and a documented K103N mutation, as well as extensive prior NRTI experience and DLV hypersusceptibility. There is no way to predict whether including DLV in his regimen would improve his clinical outcome. Use of DLV in this context may be considered if the hypothetical benefit of hypersusceptibility (i.e., enhanced DLV efficacy) is thought to outweigh the potential risks (i.e., no efficacy or only temporary efficacy, additional toxicity, drug-drug interactions).

➤ **Advantages**

May achieve complete viral suppression

May capitalize on DLV hypersusceptibility

➤ **Disadvantages**

Unknown risk of dangerous drug-drug interactions

Risk of unfamiliar toxicity

Moderately large pill burden with TID dosing requirement

Possible difficulty in obtaining tenofovir

Minimal information about tenofovir toxicity and potential for disadvantageous drug-drug interactions

Moderately high probability of rash, GI intolerance, body habitus changes, and/or hyperlipidemia

***The combination of ritonavir, amprenavir and lopinavir has not been studied, and should be used with caution. Pharmacokinetic drug interactions are expected to be complex and studies are underway. If used, usual dosages of lopinavir/r (3 capsules bid) are recommended with 600 mg to 750 mg of amprenavir bid. The tolerability and long-term safety of regimens containing three protease inhibitors are not known.***

***Including delavirdine (a CYP450 3A inhibitor) in a regimen containing two or three protease inhibitors is expected to substantially increase the complexity of pharmacokinetics drug interactions. Protease inhibitor concentrations and possible toxicity can be increased. The tolerability and long-term safety of regimens containing two or three protease inhibitors and delavirdine are not known.***

## Dosing, Monitoring, and Follow-up Recommendations

In the absence of appropriate pharmacokinetics interaction studies, consider using delavirdine and lopinavir/ritonavir together, with or without amprenavir, at the following doses:

- Delavirdine 400 mg, (Two X 200 mg tablets) po TID.
- Lopinavir/ritonavir 133/33.3 mg/capsule, (Kaletra), 3 capsules po BID
- Amprenavir 600-750 mg (4 to 5 X 150 mg tablets) po BID

The panel recommends close follow up for this patient, with CD4 and HIV RNA monitoring monthly until the patient is determined to be stable. If Option 1 leads to immunologic decline, Option 2 should be pursued as quickly as possible (preferably within weeks) after the falling CD4 count is confirmed, to avoid the risk of accumulating additional resistance.

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<sup>i</sup> Whitcomb J, Deeks S, Huang W, Wrin T, Paxinos E, Limoli K, Hoh R, Hellmann N, Petropoulos C. Reduced susceptibility to NRTI is associated with NNRTI hypersensitivity in virus from HIV-1-infected patients. 2000. 7<sup>th</sup> CROI, Abstract 234.

<sup>ii</sup> Shulman N, Zolopa A, Passaro D, Shafer R, Huang W, Katzenstein D, Israelski D, Hellmann N, Petropoulos C, Whitcomb J. Phenotypic hypersusceptibility to non-nucleoside reverse transcriptase inhibitors in treatment-experienced HIV-infected patients: impact on virological response to efavirenz-based therapy. *AIDS* 2001, 15:1125-1132.

<sup>iii</sup> Katzenstein D, Shulman N, Bosch R, Liou S, Whitcomb J, Hellmann N, Albrecht M. Genetic correlates of phenotypic hypersusceptibility to efavirenz in highly nucleoside-experienced subjects in ACTG 364. 2001. First International AIDS Society Conference, Buenos Aires, Argentina. Abstract 594.

<sup>iv</sup> Keiser P, Evans L, Haubrich R, O'Brien W, Skiest D. Genotypic predictors of efavirenz hypersusceptibility and clinical response to efavirenz in patients with hypersusceptibility. 2001. First International AIDS Society Conference, Buenos Aires, Argentina. Abstract 601.