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# HIV Resistance Testing Consultation Service

## Consultation Report

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**History/Clinical Course**

The patient is a 56-year-old Indian male with a past medical history significant for HIV infection (subtype B), diabetes mellitus, urticaria, and hearing loss. He has a CD4+ nadir of 5 cells/mm<sup>3</sup>, which occurred in 1997 when he was not taking antiretroviral (ARV) medications. His pre-ARV HIV viral load in 1997 was 200,000 copies/mL.

The patient's medical history is also notable for lichen planus and a recent diagnosis of chronic urticaria, which dermatology thought was the reason for an intermittent rash that has complicated his prior treatment regimens. This pruritic and erythematous rash usually affects his arms, legs, abdomen, and back and is not accompanied by any other symptoms.

The patient has a complex treatment history (see Table). The patient had evidence of durable viral suppression on several regimens between 1997 and 2005. His viral load became detectable on a nelfinavir-based regimen in 2005, and has since remained in the low intermittently detectable range on a series of protease inhibitor-based regimens. During this time, his self-reported adherence was consistently 100%, although a review of his pharmacy refill history suggests 90% adherence. He uses a pill box for his medications.

All of the patient's laboratory values are within normal limits. He is currently not taking any other medications, but uses Claritin or Sarna lotion as needed for allergy and pruritus. He is not co-infected with hepatitis B or C and has no other sexually transmitted diseases.

DATE	REGIMEN	CD4+	VL range	COMMENTS
8/97-4/98	d4T/3TC/NFV	100-200	VL<500	
4/98-3/00	ddI/NFV/NVP	200-600	VL<500	
3/00-7/05	ddI/ABC/NVP/NFV	500-700	VL<50	
7/05-10/05	LPV/r/Truvada	500-900	VL<75	
10/05-1/07	NFV/Truvada	600-800	VL<75 initially; 7/06-1/07 viremic w/ VL=90-1300	GT 8/24/06
1/07-1/07	Epzicom/TDF/ATV/r x2wks	900	150	patient had rash
1/07-2/07	Truvada/NFV	700-900	95-218 (VL<75 x1)	rash cont w/ some improvement
2/07- 4/07	Truvada/fos-APV/r			
4/07-8/08	Epzicom/TDF/LPV/r	640-920	<75 x4, intermittent viremia throughout	8/07 reports rash

			(VL=85-240)	
8/7/08-8/19/08	Off ARVs	870	93	GT 8/18/08
8/19/08-9/16/08	Epzicom/TDF/LPV/r			Continued ARVs until availability of GART
9/16/08-current	Epzicom/TDF/DRV/r	730-880	110-140	

d4t = stavudine (Zerit)

NVP = nevirapine (Viramune)

3TC = lamivudine (Epivir)

NFV = nelfinavir (Viracept)

ddl = didanosine ( Videx EC)

LPV/r = lopinavir/ritonavir (Kaletra)

ABC = abacavir (Ziagen)

ATV/r = atazanavir (Reyataz) with ritonavir

TDF= tenofovir (Viread)

Fos-APV/r = fosamprenavir (Lexiva) with ritonavir

Truvada = tenofovir plus emtricitabine

DRV/r = darunavir (Prezista) with ritonavir

Epzicom = abacavir plus lamivudine

### Resistance Test Findings

#### Genotype 8/24/06

NRTI	no mutations (35I, 83K, 123E, 166R, 207K, 276I)
NNRTI	
PI	10I, 93L (15V, 19T)

#### Genotype 8/18/08

NRTI	no mutations (35I, 83K, 123E, 166R, 207K, 276I, 293I/V)
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NNRTI	
PI	10I, 15V, 19T, 20K/R, 63L/P, 93L

### Questions Addressed by the Panel:

- 1- Why is it not possible to suppress patient's HIV viral load despite being on a regimen consisting of three nucleosides plus a ritonavir boosted PI?
- 2- Why do the genotypes not show more mutations, e.g. M184V?
- 3- What are the consequences of constant low-level viremia in this case?
- 4- How can the ARV regimen be changed to fully suppress the patient's viral load?

### Background, Panel Discussion, and Options:

In general, factors that can influence viral load include regimen potency, drug-resistance and/or antiretroviral (ARV) drug levels (with the latter influenced by several factors, including adherence, drug-drug interactions, drug-food interactions, renal insufficiency, hepatic impairment, pregnancy, host genetic differences, and/or malabsorption). In order to uncover potential reasons for this patient's low-level viremia and inability to achieve an undetectable viral load, we need to examine this list. Factors such as drug-drug interactions, other comorbidities, pregnancy, and malabsorption are not relevant in this case.

Medication adherence was assessed by pharmacy refill records and patient self-reports. Based on both of these methods, he has approximately 90-100% adherence. Other possible options for assessing adherence in a clinical setting would be to determine the protease inhibitor (PI) drug concentration, discuss adherence with family members who are involved in patient's care, and perform random pill-counts.

When questioned about dietary habits, the patient did state that he eats large quantities of garlic every day. One study by Piscitelli and colleagues<sup>1</sup> showed that concomitant use of saquinavir and garlic caplets resulted in a 51%, 49%, and 54% reduction in area under the curve (AUC), trough levels, and maximum concentrations (C<sub>max</sub>), respectively. In another study by Gallicano<sup>2</sup>, use of two 5 mg garlic capsules (equivalent to 1 g of fresh garlic) twice daily with ritonavir resulted in a 17% decrease in AUC and a 1% reduction in C<sub>max</sub>. However, discontinuation of garlic consumption when taking the DRV/r-based regimen did not result in further reduction of our patient's viral load.

Another possibility is that he has a genetic predisposition to metabolize protease inhibitors (PI) faster than the general population. Polymorphisms at CYP 450 enzymes can increase or decrease PI clearance and changes in the p-glycoprotein (P-gp) cell membrane efflux pump can influence

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intracellular concentration of some ARVs. P-gp is encoded by the multidrug resistance 1 (MDR1) gene<sup>3</sup>; and can affect the amount of ARVs in different tissues. Some polymorphisms at MDR1 have been shown to result in higher or lower NFV plasma levels.<sup>4,5</sup> Additionally, decreases in intracellular levels of ritonavir and saquinavir in cells overexpressing P-gp or multiresistance proteins (MRPs) have been shown in vitro.<sup>6</sup> These genetic changes can ultimately affect the efficacy and safety profile of ARVs. Currently, there are no methods of determining these genetic diversities in a clinical setting.

Even in context of complete adherence, some regimens may lack sufficient potency to fully suppress replication of wild-type HIV. This may explain in part the failure of his earlier nelfinavir-based regimen, but it is unlikely to explain persistent low-level viremia on his more recent regimens (including a regimen of three nucleoside analogues and ritonavir/darunavir).

A final and more complex factor is the presence or absence of drug resistance. An interesting point about this case is that despite ongoing low-level viremia for two years, no major drug-resistance mutations have emerged. Also, the viral genotype has not change significantly, suggesting a lack of evolution and a lack of ongoing viral replication. It is possible that the most recent genotype does not reveal all of the existing mutations because the patient's viral load was very low at this time; however, we would expect that a resistant virus would have a "fitness advantage" and that the potential mutations (at least the 184V mutation conferring resistance to lamivudine) to be present.

It is also interesting that the patient's viremia remained low, even after discontinuing ARV therapy for two weeks. This may show that viral replication is at such a low level that it did not accelerate in the few weeks that he was off therapy. This suggests a strong host-component to ongoing virus control.

Much of the panel discussion focused on new data presented at the 2009 Conference on Retroviruses and Opportunistic Infections in Montreal. Some of these studies evaluated the effects of intensification on low-level viremia. In a study by Palmer and Coffin,<sup>7</sup> they determined that at least 80% of patients on ARVs have low level viremia (< 50 copies/mL). There is no evidence to show new drug-resistance mutation in these viral particles, which may mean that there is no effective replication. In a recently presented series of studies in which patients with low but detectable viremia (generally 1 to 10 copies RNA/mL) added either efavirenz, atazanavir or raltegravir to their stable regimen, plasma HIV RNA levels did not change, suggesting that "complete" viral suppression had been achieved.<sup>8</sup> Whether these findings apply to a patient with viral loads in the 100 copy/RNA range remains unclear.

Low level viremia on his PI-based regimen may represent a steady state where small quantities of non-infectious, non-replicating virus particles are "seeping out" from the viral reservoir. If this is the case, then adding more drugs should not decrease the viral load to undetectable levels. Based on this theory, the addition of raltegravir to this patient's baseline ARV regimen should not make any difference on his viral load. An argument against this theory is that he has achieved a viral load of <75 copies/mL previously.

A question that was discussed by the panel was whether resistance to PIs can occur despite lack of mutations in the protease gene. A study by Nijhuis and colleagues has shown mutations in the GAG region causing PI resistance.<sup>9</sup> They investigated other mechanisms by which HIV could become less susceptible to PIs. They demonstrated that viruses that did not have any substitutions in the viral protease, showed a 4-8 fold resistance to PIs. Upon full genomic sequences, they revealed cleavage

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site substitutions in the GAG polyprotein (K436E and/or I437T/V). Additionally, they showed that these cleavage site substitutions were associated with PI resistance and virologic failure in the clinical isolates.

An undetectable viral load (<75 copies/mL) on a non-nucleoside reverse transcriptase inhibitor (NNRTI)-based regimen but not on a PI-based regimen may suggest that the virus is replicating on the PI-based regimen. Forward evolution implies replication; which can eventually lead to drug resistance. In this patient, if the virus is evolving, then drug resistance will eventually occur. Therefore, panel members agreed that this patient's regimen should be changed to avoid subsequent failure from ongoing viremia on his ARV regimen. Some options for change consisted of Truvada + Isentress (raltegravir, RAL) + Intelence (etravirine, ETR) or Truvada + Intelence (etravirine, ETR) + Prezista (Darunavir, DRV) + Norvir (ritonavir, RTV) or Atripla (efavirenz/tenofovir/emtricitabine) + Isentress (raltegravir, RAL).

## Regimen Options

### Option 1:

Truvada (tenofovir 300mg/emtricitabine 200mg): 1 tablet orally once daily

+

Isentress (raltegravir 400mg): 1 tablet orally twice daily

+

Intelence (etravirine 100mg): 2 tablets orally twice daily with food

**Pros:** This regimen does not contain a PI, therefore it may be a good option if we are worried about a certain genetic predisposition.

**Cons:** If the patient is non-adherent to ARVs, then he would be at risk of developing resistance to the integrase inhibitor and the second generation NNRTI class.

### Option 2:

Truvada (tenofovir 300mg/emtricitabine 200mg): 1 tablet orally once daily

+

Intelence (etravirine 100mg): 2 tablets orally twice daily with food

+

Prezista (darunavir 600mg) 1 tablet orally twice daily with food

+

Norvir (ritonavir 100mg): 1 capsule orally twice daily with food

**Pros:** possibly effective regimen with 3 active agents

**Cons:** If the patient is failing the current regimen, then we would be adding one active drug ETR to a failing regimen. Also, if we are concerned about genetic predisposition where patient would have lower PI drug levels, then this may not be a good regimen.

### Option 3:

Atripla (efavirenz 600mg/tenofovir 300mg/emtricitabine 200mg): 1 tablet orally once daily

+

Isentress (raltegravir 400mg): 1 tablet orally twice daily

**Pros:** This regimen has the lowest pill burden and is the simplest regimen of all the other options.

**Cons:** If patient is non-adherent, then he could quickly develop drug resistance to the NNRTI and the integrase inhibitor classes.

### **Monitoring and Follow-up Recommendation**

- 1- HIV RNA: every 3-4 weeks, until undetectable; then, every 3 months.
- 2- If patient is continued on tenofovir, monitor serum creatinine, BUN, urinalysis, phosphorus every 3 months.
- 3- If patient is continued on darunavir, monitor liver function tests, and cholesterol, glucose every 3 months.

### **References:**

- 1- Piscitelli SC, Burstein AH, Welden N, et al. The effect of garlic supplements on the pharmacokinetics of saquinavir. *CID* 2002;34:234-8.
- 2- Gallicano K, Foster B, Choudhri S. Effect of short-term administration of garlic supplements on single-dose ritonavir pharmacokinetics in healthy volunteers. *Br J Clin Pharmacol* 2003;55:199-202.
- 3- Juliano R, Ling V. A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. *Biochim Biophys Acta* 1976; 455:152–62.
- 4- Fellay J, Marzolini C, Meaden E, Back D, Buclin T, Chave J et al. Response to antiretroviral treatment in HIV-1-infected individuals with allelic variants of the multidrug resistance transporter 1: a pharmacogenetics study. *Lancet* 2002; 359: 30–36.
- 5- Zhu D, Taguchi-Nakamura H, Goto M, et al. Influence of single-nucleotide polymorphisms in the multidrug resistance-1 gene on the cellular export of nelfinavir and its clinical implication for highly active antiretroviral therapy. *Antivir Ther* 2004; 9: 929–935.
- 6- Jones K, Bray P, Khoo S, Davey R, Meaden E, Ward S et al. Pglycoprotein and transporter MRP1 reduce HIV protease inhibitor uptake in CD4 cells: potential for accelerated viral drug resistance? *AIDS* 2001; 15: 1353–1358.
- 7- Palmer S, Maldarelli F, Josefsson L, et al. Single-cell Analysis of HIV DNA from Infected Patients. CROI 2009. Montreal, Canada. Abstract # 442.
- 8- Jones J, McMahan D, Wiegand D, et al. No Decrease in Residual Viremia during Raltegravir Intensification in Patients on Standard ART. CROI 2009. Montreal, Canada. Abstract #423b.
- 9- Nijhuis M, van Maarseveen NM, Lastere S, et al. A Novel Substrate-Based HIV-1 Protease Inhibitor Drug Resistance Mechanism. *PLoS Med* 2007, 4:e36.