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# HIV Resistance Testing Consultation Service

## Consultation Report

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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

A 43-year-old Caucasian man with HIV/AIDS diagnosed in 1992 has a past medical history significant for a myocardial infarction (7/06), hypertension, depression, facial lipoatrophy, and non-Hodgkins lymphoma (diagnosed 2005). His prior antiretroviral (ARV) history is significant for zidovudine (AZT), lamivudine (3TC), stavudine (d4T), saquinavir (SQV), indinavir (IDV), ritonavir (RTV), nevirapine (NVP), efavirenz (EFV), abacavir (ABC), amprenavir (APV), fosamprenavir (fAPV), hydroxyurea (HU), and didanosine (ddI) in various combinations. Most recently he was enrolled in the DUET study where he received Truvada® (tenofovir/emtricitabine), enfurvirtide (T-20), ritonavir boosted darunavir (DRV/r), and placebo etravirine (ETV). His nadir CD4+ is 30 cells/mL which occurred before his enrollment in the DUET study (6/16/06). His viral load (VL) on therapy has ranged from <75 to >700,000 copies/mL. His most recent VL is 2110 copies/mL (9/20/07) and his most recent CD4 count was 142 cells/mL (6%). During the DUET study, his VL ranged from <50 to 3500 copies/mL and the CD4 count ranged from 133 to 192 cells/mL (6%).

His current medications include Truvada plus T-20 plus DRV/r, metoprolol, clopidogrel (Plavix®), ASA, atorvastatin (Lipitor®), and trimethoprim/sulfamethoxazole (TMP/SMX). His adherence is good and he denies missed doses. However, he works as a flight attendant and therefore has had some difficulty with the correct timing of some medications, particularly T-20. He also does not have a well-documented antiretroviral history due to changing providers and clinic sites several times in the past.

A Trofile® test obtained on 8/14/07 showed an R5 tropic virus (his viral load at the time of the assay was 1000 copies/mL). An entry inhibitor phenotype obtained on 7/31/07, showed a fold change of 12 for T-20 (99<sup>th</sup> percentile of susceptibility distribution). All other laboratory values are within normal limits except for a hemoglobin (Hgb) of 11.5 gm/dL and a platelet count of 110,000.

His antiretroviral history is as follows:

DATE	REGIMEN	CD4 range	VL range	COMMENTS
9/95-1/96	d4T/3TC	67 (15%) - 140 (19%)	-	
1/96-4/96	d4T/3TC/SQV	48 (12%) - 66 (11%)	-	
4/96- 10/97	d4T/3TC/IDV	185 (14%) - 263 (16%)	3000-32,000	
10/97-	RTV/SQV/AZT/NVP	240 (8%) -	<500-44,000	CD4% drops from 12% to 8%

2/99		318 (14%)		(up until 1/99 CD4% >12%)
2/99-7/99	d4T/EFV/RTV/SQV	178 (11%)	3000	
7/99-2/00	APV/ABC/HU/EFV	136 (10%) - 180 (10%)	2000-3000	
2/00-6/00	APV/ABC/RTV/EFV	242 (9%) - 291 (10%)	2000-3000	
6/00-4/05	APV/RTV/ABC/ddI	242 (9%) - 267 (9%)	2000-4000	Unknown how long patient was on this regimen
4/05-4/06	fAPV/RTV/ABC/ddI	30 (2%) - 61 (3%)	20,000- 30,000	
5/06	T20/Truvada®/DRV/ r/etravirine (TMC125)	133 (6%) - 192 (6%)	<50 - 3000	Placebo etravirine (TMC125); Pt had VL<50 three times while on this regimen (9/06-12/06)

## Resistance Test Findings

### 7/31/07 Phenotype (Monogram)

NRTI	FC: ABC (10), ddI (2.3), 3TC/FTC (>max), d4T (3.6), AZT (8.9), TDF (2.06)
NNRTI	FC: EFV (77), NVP (>max)
PI	FC: ATV (153), rATV (153), DRV (346), fos-APV (>max), IDV (36), rLPV (93), NFV (54), SQV (20), TPV (3)

### 7/31/07 Genotype (Monogram)

NRTI	41L, 44D, 67N, 69D, 118I, 184M/V, 210W, 215Y, 219D/N
NNRTI	98G, 101Q, 181C, 190A
PI	10I, 11I, 13V, 20K/R, 32I, 33F, 46I, 54M, 63P, 73A, 77I, 84V, 90M

### 5/26/06 Phenotype (VircoType)

NRTI	FC: ABC (2.5), ddI (2.1), 3TC/FTC (5.3), d4T (2.1), AZT (16), TDF (3.3)
NNRTI	FC: EFV (?), NVP (67.8)
PI	FC: fos-APV (42.4), IDV (44.7), rLPV (31.5), NFV (45.3), SQV (39.9), ATV (76.9), TPV (9.3)

5/25/06 Genotype (VircoType)

NRTI	41L, 44wt/D, 67N, 69D, 74wt/I, 118I, 203wt/K, 210W, 211wt/K, 215Y, 219N, 223wt/E, 228R
NNRTI	98G, 101Q, 179wt/D/F/Y, 181C, 190A
PI	10I, 11I, 13V, 33F, 46L, 54M, 63P, 73A, 77I, 84V, 90M, 93L

7/25/02 Phenotype (ViroLogic)

NRTI	FC: ABC (7), ddI (1.6), 3TC/FTC (7.3), d4T (2.4), AZT (85), TDF (3.4)
NNRTI	FC: EFV (242), NVP (>max)
PI	FC: APV (63), IDV (20), rLPV (28), NFV (38), SQV (32)

7/25/02 Genotype (ViroLogic)

NRTI	41L, 67N, 69D, 74L/I, 210W, 215Y, 219N
NNRTI	98G, 101E/Q, 181C, 190A
PI	10I, 33F, 46L, 54I/M, 63P, 73A, 77I, 84V, 90M

**Questions for discussion:**

- 1- Can we trust the tropism assay given his low viral load? What is the probability that the virus is, in fact, R5-tropic? What is the likelihood that the virus is dual-mixed (D/M) tropic?
- 2- What are the viable regimens to achieve an undetectable viral load?
- 3- What are the risks versus benefits of continuing his current ARV regimen?
- 4- If he were to continue his current ARV regimen (Truvada/rDRV/T-20), what are the risks versus benefits of using T-20 once daily for immunologic benefits?

## Interpretation/Implications for Treatment

This is a 43-year-old individual with a long-standing history of antiretroviral use. Due to a history of sequential suboptimal regimens (beginning in the pre-HAART era) and possible non-adherence, the patient has developed a multi-drug resistant virus. After multiple regimens, the patient in 2006 enrolled in the DUET study, which compared the efficacy and safety of darunavir/r with or without etravirine--a 2<sup>nd</sup> generation NNRTI. The patient was randomized to placebo and hence received enfuvirtide (Fuzeon®, T-20), darunavir (Prezista®, DRV)/r, Truvada® (combination of tenofovir and emtricitabine), and placebo etravirine. Also relevant to his current situation is a history of a myocardial infarction and non-Hodgkins lymphoma. It is important to keep in mind the risks associated with on-going viremia and non-AIDS defining illnesses as well as the toxicity of ARV medications.

Based on a combination of all three genotypes from 2002, 2006, and 2007, this patient demonstrates high-level resistance to all PIs, NNRTIs, and NRTIs (with some activity remaining for tipranavir and perhaps, tenofovir). The genotypes show five of the 11 DRV-specific mutations: V11I, V32I, L33F, I54M, and I84V.<sup>1</sup> There are also five of the 21 TPV-specific mutations: I13V, K20R, L33F, I54M, I84V.<sup>2</sup> Also, A98G, V179D, Y181C, and G190A of the 13 ETV-specific mutations confirm resistance to ETV.<sup>3</sup>

In retrospect, this patient should not have been enrolled in the DUET studies given the high level of phenotypic resistance to both darunavir and etravirine (the genotypic and phenotypic correlates of resistance to these drugs only recently became established). T-20 was the only active agent in the patient's regimen and as a consequence failure occurred rapidly. It is apparent that resistance to T-20 develops quickly and rebound viremia can occur as early as 12 weeks.<sup>4</sup> Thus, it is likely responsible for the quick development of T-20 resistance.

The tropism assay shows that the virus is CCR5-tropic and should respond to a CCR5 inhibitor. According to Monogram, the company who performs the tropism assay or Trofile®, this test is 100% sensitive when the CXCR4-tropic virus constitute at least 10% of the viral population and 85% sensitive when the CXCR4-tropic virus comprises at least 5% of the viral population. The lack of an X4 or dual/mixed tropic virus is encouraging, but low level variants may still be present (a more sensitive assay is in development).

The tropism assay requires an HIV RNA level of at least 1,000 copies/mL to perform this test accurately. The result of the tropism assay may be difficult to interpret due to the low HIV RNA level at the time of the testing, since at low viral loads it is more difficult to efficiently sample a large number of variants. The caller was correct in wondering if the low viral load at the time of the tropism test may have led to difficulties interpreting this test. Multiple studies have shown that X4 utilizing variants are more common in heavily pre-treated patients than those who were never treated, raising even greater concerns that this patient may harbor a virus which is naturally resistant to maraviroc. As

stated above, there is currently a more sensitive tropism assay in development that would detect populations of CXCR4-tropic viruses down to 1%.<sup>6</sup> This enhanced assay has been shown to optimize the D/M-tropic virus luciferase activity on CXCR4+ cells. Even at HIV RNA levels <1000 copies/mL, this assay can reveal D/M-tropic virus population at a higher rate than the current tropism assay. It is unknown what quantity of X4-tropic virus would be considered clinically irrelevant and therefore, would not interfere with the activity of CCR5 inhibitors.

The MOTIVATE 1 and 2 are pivotal trials that led to the FDA approval of maraviroc (Selzentry®, MVC),<sup>7,8</sup> the first CCR5 Inhibitor. In these trials, approximately 8% of subjects had a change in tropism between screening and baseline testing (within a 4-6 week timeframe), and this shift was associated with subsequent failure of a maraviroc-based regimen. In the SCOPE cohort, 10% of patients switched back and forth between R5- and X4-tropic viruses all the time (personal communication with Steven Deeks, MD). These switches appear to reflect the presence of low-level X4 utilizing viruses and will likely be more readily detected using the enhanced tropism assay.<sup>9</sup>

Raltegravir (Isentress®, RAL), the first FDA approved integrase inhibitor, is another fully active drug that can be utilized in this patient's next ARV regimen. BENCHMRK 1 and 2 are the major efficacy trials resulting in the FDA approval of raltegravir.<sup>10,11</sup> At 16 weeks, RAL demonstrated an HIV RNA reduction to <50 copies/mL in 61-62% of patients as compared to 33-36% in the placebo arm. In the RAL arm, 16% of subjects experienced treatment failure versus 51% in the placebo arm. This drug seems to be well tolerated and has adverse effects similar to placebo.

There are other investigational drugs in development, although most would not be viable options for this patient. Rilpivirine (TMC278) is a second generation NNRTI, currently in phase III trials, but little is known of its efficacy against a virus with numerous NNRTI mutations. Bevirimat (PA457) is the first drug in the Maturation Inhibitor class that is in the early phases of development.

Although tipranavir (Aptivus®, TPV) has a lower fold change on this patient's phenotype compared to other PIs, there are concerns about the use of this drug in this patient. TPV has a black-box warning for increased risk of intracranial hemorrhage<sup>12</sup>, especially in patients using anti-platelet agents. His use of clopidogrel and aspirin places him at a higher risk of bleeding. In addition, TPV can decrease RAL's Cmin (minimum concentration) by 55%, AUC (area under the curve concentration) by 24%, and Cmax (maximum concentration) by 18%; although these changes are currently believed to be acceptable as low dose RAL (200 mg bid) performed well in clinical trials.<sup>13</sup> Lastly, there are many unpredictable drug interactions with TPV that make it a less desirable drug to use in this patient, especially given the number of newly developed drugs that will be used in this patient's next ARV regimen.

The regimen options in this patient consist of continuing his current ARV regimen (Truvada/ritonavir/darunavir +/- enfuvirtide) or changing him to a fully suppressive ARV regimen. A fully suppressive regimen may include raltegravir, maraviroc plus a

nucleoside backbone (such as zidovudine/lamivudine/tenofovir) plus a ritonavir-boosted protease inhibitor (such as tipranavir or saquinavir).

The advantages of continuing the current regimen are the following: 1) a clinically significant rise in his CD4+ cell count, 2) maintain a low viral load, 3) low risk of developing new mutations, and 4) low pill-burden. However, the primary disadvantage of this option is that there are no novel ARV medications in the late phases of development and by waiting we may lose maraviroc as a viable option. Conversely, waiting may shed more light on data about these newly FDA approved drugs and we may gain more experience in utilizing these drugs.

The panel felt strongly about continuing a PI in treatment-experienced patients. Clinical experience and small pathogenesis oriented studies suggest that protease inhibitors have residual benefit even in absence of full viral suppression.<sup>14</sup> The panel also felt that continued NRTIs was indicated, as these drugs continue to exert significant antiviral activity even in presence of multiple mutations. As to whether to continue T20, the panel noted that there are data showing immunologic benefit associated with certain T20 associated mutations. In a study by Aquaro,<sup>4</sup> of subjects failing a T-20-based regimen, the presence of the V38A mutation was associated with a 4.5- and 6-fold CD4 increase at 24 and 36 weeks, respectively. Therefore, it may be possible to preserve the CD4+ response by preserving this mutation. Since this patient has achieved a clinically significant immunologic response and is resistant to T-20, one option is to continue T-20 subcutaneously. There are no data quantifying the minimum amount of T-20 needed to maintain the beneficial CD4+ effects; however, based on an abstract by Charpentier and colleagues,<sup>15</sup> persistence of T-20 resistance was associated with the duration of T-20 therapy. The disappearance of T-20 resistance was observed as early as 1 month after stopping therapy but patients who had received T-20 longer had prolonged persistence of the specific mutations.

## Regimen Options

### **Option 1:** Continue current regimen:

Truvada® (combination of tenofovir and emtricitabine): 1 tablet orally once daily;

Prezista® ( (darunavir): 2 tablets (2 x 300 mg) orally twice daily;

Norvir® ( (ritonavir): 1 capsule (100 mg) orally twice daily;

Fuzeon® ( (enfuvirtide): 1 injection (90 mg or 1 mL) subcutaneously once or twice daily

Pro: maintenance of the CD4+ cell count increase; maintenance of a low viral load; low pill-burden

Con: acquire new mutations, including the possible loss of CCR5 inhibitors as X4 variants emerge; also, although the current regimen is clearly

providing benefit, it is likely that the patient will eventually progress and this may reduce the chance of achieving durable suppression once a switch is required

**Option 2:** Change regimen to:

Combivir® (combination of zidovudine and lamivudine): 1 tablet orally twice daily;

Viread® (tenofovir): 1 tablet (300 mg) orally once daily;

Invirase® (saquinavir): 2 tablets (2 x 500mg) orally twice daily;

Norvir® (ritonavir): 1 capsule (100 mg) orally twice daily;

Selzentry® (maraviroc): 1 tablet (150 mg) orally twice daily;

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

Pro: high likelihood of achieving undetectable viral load; maintain increase in CD4+ cell count; decrease risk of further non-AIDS defining illnesses

Con: high pill burden (13 pills per day); if DM or X4- tropic virus with only raltegravir being active will increase raltegravir's risk of resistance

**Option 3:** Change regimen to:

Combivir® (combination of zidovudine and lamivudine): 1 tablet orally twice daily;

Viread® (tenofovir): 1 tablet (300 mg) orally once daily;

Kaletra® (lopinavir/ritonavir): 2 tablets orally twice daily;

Selzentry® (maraviroc): 1 tablet (150 mg) orally twice daily;

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

Pro: likelihood of achieving undetectable viral load; maintain increase in CD4+ cell count; decrease risk of further non-AIDS defining illnesses

Con: high pill burden (13 pills per day); if DM or X4- tropic virus with only raltegravir being active will increase raltegravir's risk of resistance

increased risk of resistance to raltegravir; Kaletra® may cause increase in cholesterol thereby increasing cardiovascular risk factors

**Option 4:** A final option is to consider the use of tipranavir/ritonavir (500/200 mg BID) as the protease inhibitor in the strategies outlined in Options 2 and 3. This approach is likely to result in the most effective antiretroviral regimen. However, the concurrent administration of clopidogrel (Plavix®) and aspirin with tipranavir may result in an even higher risk of intracerebral hemorrhage than when tipranavir is used in the

absence of these drugs. Consultation with a cardiologist regarding the long-term need for clopidogrel might be considered. Note that when tipranavir is co-administered with maraviroc, the dose of the latter is 300 mg BID.

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

Monitor CD4 count every 2-3 months and HIV RNA very frequently (every 1 month) until undetectable.

Monitor lipid panel, blood glucose, hemoglobin A1C, liver function tests

Monitor adverse effects of antiretrovirals:

Combivir: complete blood count, GI side effects

Tenofovir: serum creatinine, urinalysis, serum phosphorus

Protease Inhibitors (saquinavir, ritonavir, lopinavir): GI side effects, lipid panel, glucose, LFT

Maraviroc: jaundice, LFT

Raltegravir: creatinine kinase, serum creatinine

Monitor for drug-drug interactions (PIs can increase serum concentrations of atorvastatin with increased risk of myopathy or rhabdomyolysis).

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