
HIV Resistance Testing Consultation Service

Consultation Report

Co-Chairs: Steven G. Deeks, MD
Betty J. Dong, Pharm.D

Panel Members: Richard Aranow, MD
Teri Liegler, PhD
Brad Hare, MD
Amy Kindrick, MD, MPH
Jason Tokumoto, MD

Project Director: Ronald H. Goldschmidt, MD

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

The patient is a 40-year-old Caucasian male, known to be HIV-positive since 2000. He denies any history of taking antiretrovirals (ARVs) and has never had an opportunistic infection. He has a long history of severe depression, schizophrenia, and a remote history of substance abuse, but denies current drug use. He expressed a strong preference to delay ARVs until his CD4 declined to 200 cells/mm³. Once his CD4 reached 200 cells/mm³, the patient continued to decline ARVs despite strong recommendations to start by his provider. Subsequently, he was lost to care for eight months, during which time he was non-compliant with prophylactic medications.

January 2008: Developed thrush and Pneumocystis pneumonia. Agreed to start ARVs and a genotype was obtained, which demonstrated some drug resistance (see below).

February 2008: Prescribed Combivir® (CBV: zidovudine plus lamivudine) + tenofovir (TDF, Viread®) + ritonavir (r, Norvir®) plus darunavir (DRV, Prezista®).

April 2008: Viral load increased. Repeat resistance testing performed. Patient later admitted he took only a few doses of ARVs that were initially prescribed. He noted side effects of headache, nausea, fatigue and constipation.

Patient expressed strong preference for once-daily regimen with few pills and minimal side effects.

Other medical conditions: Genital herpes; Hypogonadism.

Current meds: Paroxetine, Aripiprazole, Acyclovir, Azithromycin, Fluconazole, TMP-SMX, testosterone

DATE	REGIMEN	CD4 cells/mm ³	VL COPIES/ML	RESISTANCE TEST FINDINGS	CLINICAL COURSE
2000	Reported first HIV+ test				
2000-04	No data available				
9/21/04	Naïve, by patient report	390 (23%)	20,049		Establishes care at current facility
12/20/04		409 (22%)	19,660		
3/7/05		416 (22%)	31,844		
8/8/05		357 (19%)	31,844		
11/22/05		296 (22%)	46,306		
2/21/06		276 (16%)	50,400		

CASE NUMBER
PANEL CLINICIAN:

DATE

6/12/06		279 (18%)	70,853		
8/21/06		255 (16%)	55,461		
3/23/07	Patient declines ARV	165 (13%)	79,062		
4/9/07		183 (11%)			
1/7/08		44 (5%)	281,889	Baseline Genotype	Diagnosed with thrush and PCP
2/08	CBV + TDF + DRV/r				
4/3/08		72 (5%)	1,176,731		
4/14/08			1,160,437	PhenoSenseGT Trofile	HLA B57*01 positive
5/12/08		33 (4%)	1,636,176		

Resistance Test Findings

SFGH Baseline Genotype (1/8/08)

RT	M41L, L210W, T215S
PR	I13V, D30N, L33F, M46L, I62V, L63P, V77I, N88D, L89M, I93L
Polymorphisms	RT: T39A, V60I, E122K, I135A, Q174H, T200A, Q207E, R211K, L214F, V245K PR: I15L, S37N

Monogram PhenoSenseGT (4/14/08)

(See Attached report)

RT	M41L, L210W, T215S
PR	I13V, D30N, L33F, M46L, L63P, V77I, N88D, L89M
Polymorphisms	RT: V35V/I, T39A, V60I, Q102K, E122K/E, I135A, C162S, Q174H, T200A, Q207Q/E, R211K, V245K, A272P, R277K, V293I PR: I15L, I62V, I93L

Fold changes:

NRTI: ABC (0.82); ddI (0.63); FTC (1.08); 3TC (1.12); d4T (0.60); AZT (0.48); TDF (0.75)

NNRTI: DLV (0.60); EFV (0.47); ETR (0.59); NVP (0.46)

PI: ATV (1.38); DRV (1.22); FPV (2.38); IDV (0.84); LPV (1.18); NFV (1.13); RTV (0.78); SQV (1.00); TPV (0.91)

Trofile (4/14/08)

R5 only

Interpretation/Implications for Treatment

The panel found many interesting aspects to this case to address:

Do the mutations on the patient's first genotype represent persistence of transmitted resistance or prior undisclosed ARV therapy in this patient?

The resistance mutations present on both genotypes demonstrate common thymidine analog mutations (TAMS) at positions 41 and 210 as well as a revertant or "shadow" mutation at position 215. These mutations would be consistent with previous exposure of the virus to thymidine analogs (e.g. zidovudine or stavudine). It is curious that the M184V mutation is not detected; however that mutation, while common among patients with treatment-associated resistance, is under-represented among transmitted resistance. This is either because it is less likely to be transmitted (due to its affect on viral fitness) or because it rapidly reverts after transmission (again due to the effect of the mutation on fitness; also, only as only a single amino acid change is necessary for this reversion).

The presence of the D30N mutation in the protease gene commonly results from exposure to nelfinavir (NFV, Viracept®). The other protease mutations may represent evolution of resistance in the ongoing presence of the drug. Some of these compensatory mutations may serve to increase the fitness of the resistance virus after the development of the D30N. Thus, contrary to reversion of M184V, multiple "back mutation" steps may be necessary for a transmitted virus to lose these protease mutations. Importantly, some of the reversion steps may actually make the virus less fit, thus creating a barrier to back mutation and explaining why transmitted mutations may have persisted for several years in this patient.

Thus, on the whole, the panel members believed that this resistance pattern likely represents transmitted mutations, with persistence of those mutations for several years. It is likely that the individual from whom this patient acquired HIV had been treated with Combivir and nelfinavir in the 1990s (which was apparently when the patient became infected). This observation supports the Department of Health and Human Services (DHHS) recommendations to perform resistance testing on every patient, even those who are chronically infected, prior to initiation of first treatment.

Other observations of the panel members:

- The relative lack of phenotypic resistance to the nucleoside reverse transcriptase inhibitors (NRTIs) (particularly zidovudine, stavudine, and tenofovir) is notable, given the presence of TAMS on the genotype. The PhenoSenseGT does note this discordance and defers to the phenotype. Members of the panel felt slightly uncomfortable with this, and believed that drugs affected by the NRTI mutations detected on genotype may not have full activity and should be considered as such.
- This patient tested positive for HLA-B57*01, which is associated with a high risk of abacavir hypersensitivity reaction.¹ Interestingly, the genotype also showed mutation V245K in reverse transcriptase gene, which is associated with the presence of HLA-B57*01.
- The initial regimen of Combivir + ritonavir-boosted darunavir selected by the provider in February 2008 is a reasonable choice, and is supported by the TITAN study.² The panel believed that darunavir was likely to have full activity based on the genotype at that time, so the use of three NRTIs may not have been necessary and Truvada may have been sufficient.

- Avoiding the zidovudine may have reduced the risk of medication intolerance. The group noted that the patient's stated side effects of headache, nausea, and fatigue could all be attributable to zidovudine.
- The group noted that the laboratory values between 9/04 and 1/08 showed a typical "natural history" of untreated HIV. The relatively accelerated pace of CD4 decline and viral load increase seen over the last year is often associated with the emergence of X4 virus, which was not detected in this patient. The group noted that the Trofile assay used in this patient had a sensitivity of detecting 10% of X4 population, whereas the recently-launched version has a sensitivity of 0.3%.³ So, there is some trepidation as to whether this patient was truly R5-only tropic.

Recommendations: Regimen Options

Several potential regimens were discussed by the panel. The panel approached this case by first designing regimens that may optimally inhibit the patient's virus, and then considered how the patient's co-morbid conditions and personal preferences might affect the appropriateness of those regimens.

- **Nucleosides.** While abacavir would be effective against this virus, the presence of HLA-B57*01 in this patient precludes its use.¹ The regimens discussed by the panel preferred tenofovir as part of the nucleoside backbone. In other patients who are HLA-B57*01 negative, abacavir could be an appropriate substitution for tenofovir.
- **Atripla®** (tenofovir, emtricitabine, efavirenz) one tablet daily. Based on the phenotype, this would be a highly active regimen. It has been studied only in naïve patients, and while this patient is technically naïve, he does harbor resistant virus. The panel also had reservations about the presence of TAMs, and believed that using Atripla alone would incur a high risk of virologic failure with non nucleoside reverse transcriptase inhibitor (NNRTI) resistance. It was pointed out, however, that in ACTG 384, subjects who failed an initial randomized regimen of didanosine, stavudine and nelfinavir (not unlike the virus seen in this patient), subsequently did very well on a regimen of Combivir and efavirenz. There were also concerns of starting efavirenz in a patient with depression and schizophrenia. Of note, Atripla® would optimally address the patient's stated preferences of once daily dosing and low pill burden.
 - The panel did not endorse this regimen.
- **2 NRTIs + a ritonavir boosted PI.** Depending on the agents chosen, this would be consistent with DHHS guidelines as a "preferred" first line regimen. Based on the phenotypic fold change to fosamprenavir (Lexiva®) being slightly higher, the group would prefer ritonavir boosted atazanavir (based on the CASTLE study⁴), lopinavir/ritonavir (based on 703 study and others⁵), or boosted darunavir (based on the ATREMIS study⁶). The group did note that all of these studies were in naïve patients, as this patient technically is. However, the group framed the discussion around treating resistant virus. Each of the protease inhibitors listed could be dosed once daily, which is the patient's preference. Note that once daily dosing of darunavir is currently off-label. The preferred NRTI combination in all cases was Truvada® (tenofovir plus emtricitabine).
 - The panel generally was comfortable with this option; however, there were still some concerns that the NRTI backbone may be slightly compromised.
- **Truvada + ritonavir boosted darunavir + etravirine.** This regimen is well-supported by the DUET studies^{7,8} and the panel believed that adding etravirine (ETR, Intelence®) offered the

- patient an improved chance of virologic control when compared to regimens with a boosted PI only. The downside would be the requirement for BID dosing of etravirine, along with a relatively significant increase in pill burden of four pills per day. Drug interactions with the patient's current medications would also have to be considered.
- The panel endorsed this as their preferred regimen to treat the virus; however, it doesn't meet the patient's preferences of once-daily dosing and a low pill burden.
 - **Maraviroc (MVC, Selzentry®).** Based on the tropism assay, maraviroc should be active against this virus. The group again expressed some concern over the sensitivity of the tropism assay used in this patient and considered that there may be low levels of X4 tropic virus not detected by this assay. The newer version of the Trofile assay would have improved sensitivity to detect these low-level X4 variants. Approved dosing of maraviroc is BID. The panel noted that the once-daily arm of MOTIVATE study, while inferior to the BID arm, did not perform terribly. Some panelists entertained the idea of dosing maraviroc once daily – off label – based on this data, in this particular situation where the patient may only be able to adhere to once-daily dosing.
 - The panel ultimately did not endorse once-daily dosing of maraviroc in this situation.
 - **Raltegravir (RAL, Isentress®).** Raltegravir, an integrase inhibitor, should be fully active against this virus and would be a good choice in an optimally effective regimen. However, raltegravir requires BID dosing and was thus not supported by the panel for this patient.
 - **Enfuvirtide (T-20, Fuzeon®).** Enfuvirtide should be fully active against this virus. The requirement for subcutaneous injections, BID dosing and difficulty with patient tolerability would preclude its use in this case.
 - **Additional regimens.** The group noted that designing once-daily regimens for treatment-experienced patients remains difficult, emphasizing the need for new drugs and new formulations for this population. Some panelists considered modifying one of the above BID regimens such that only one drug needed by BID dosing. For example, Truvada + ritonavir boosted darunavir (once daily) + either etravirine or maraviroc dosed BID. While clearly off-label and not evidence-based, such a regimen would provide a solid once-daily regimen with the potential for additional potency with the BID drug, acknowledging that high levels of adherence are unlikely with this part of the regimen. The group did not endorse this strategy, but suggested that it might be an alternative to consider when individualizing regimens to patients' needs.

REGIMEN CHOSEN: Dosing, Monitoring, and Follow-up Recommendations

The patient's provider chose a regimen of Atripla® one tablet daily + darunavir (900mg once daily) + ritonavir (100mg once daily). Although this would likely be a potent regimen and addressed the patient's request of low pill burden and once-daily dosing, the panel had three primary concerns about this regimen: 1) tolerability of efavirenz in a patient with depression and schizophrenia; 2) toxicity of efavirenz in combination with a boosted PI (note: the combination of efavirenz and lopinavir/ritonavir had significant metabolic toxicities in ACTG 5142); and 3) drug interactions with off-label once-daily darunavir and efavirenz, as well as with the patient's other medications. Some panel members suggested monitoring levels of darunavir.

The patient started the above regimen on May 22, 2008 with the most recent laboratory values from May 12, 2008 demonstrating CD4 = 33 (4%) and viral load = 1,636,176 copies/mL. The patient tolerated the regimen well and reported good adherence (he was enrolled in a community adherence

program that provided observed dosing on many days). Repeat labs done June 5, 2008 showed CD4 = 102 (12%) and viral load = 3,451 copies/mL.

Further follow-up is ongoing.

Selected References:

1. [Mallal S et PREDICT-1 Study Team.](#) HLA-B*5701 screening for hypersensitivity to abacavir. *N Engl J Med.* 2008 Feb 7;358(6):568-79
2. Madruga JV et Titan study group. Efficacy and safety of darunavir-ritonavir compared with that of lopinavir-ritonavir at 48 weeks in treatment-experienced, HIV-infected patients in TITAN: a randomised controlled phase III trial. *Lancet.* 2007 Jul 7;370(9581):49-58.
3. Trofile): Lan Trinh, Dong Han, Wei Huang, Terri Wrin, Jeffrey Larson, Linda Kiss, Eoin Coakley, Christos Petropoulos, Neil Parkin, Jeannette Whitcomb, and Jacqueline Reeves. Technical Validation of an Enhanced Sensitivity Trofile HIV Co-receptor Tropism Assay for Selecting Patients for Therapy with Entry Inhibitors Targeting CCR5. XVII HIV Drug Resistance Workshop, 2008. Sitges, Spain.
4. [AIDS Patient Care STDS.](#) 2008 Mar;22(3):253. CASTLE study showed similar efficacy
5. Gathe J, da Silva B, Loutfy M, et al. Study M05-730 primary efficacy results at Week 48: phase 3, randomized, open-label study of lopinavir/ritonavir tablets once daily vs twice daily, co-administered with tenofovir DF + emtricitabine in ARV-naive HIV-1-infected subjects. Program and abstracts of the 15th Conference on Retroviruses and Opportunistic Infections; February 3-6, 2008; Boston, Massachusetts. Abstract 775.
6. Ortiz R, DeJesus E et al (ARTEMIS) Efficacy and safety of once-daily darunavir/ritonavir versus lopinavir/ritonavir in treatment-naive HIV-1-infected patients at Week 48. *AIDS* 2008 ;22 :1389-97.
7. [DUET-2 study group.](#) Efficacy and safety of TMC125 (etravirine) in treatment-experienced HIV-1-infected patients in DUET-2: 24-week results from a randomised, double-blind, placebo-controlled trial. *Lancet.* 2007 Jul 7;370(9581):39-48.
8. DUET-1 study Group: Efficacy and safety of TMC125 (etravirine) in treatment-experienced HIV-1-infected patients in DUET-1: 24-week results from a randomised, double-blind, placebo-controlled trial. *Lancet.* 2007 Jul 7;370(9581):29-38

PhenoSENSE GT[™]

COMBINATION HIV DRUG RESISTANCE ASSAY

Positive Health, Ward 86
 995 Potrero Avenue, Bldg 80, Ward 86
 RM 627
 San Francisco, CA 94110
 USA

monogram

Formerly ViroLogic, Inc.
 Patrick Joseph, MD, Medical Director - 345 Oyster Point Blvd
 South San Francisco, CA 94080 - Tel: (800) 777-0177

Client: 00128 Project: 00218
 Phone: (415)626-2348 Fax: (415)206-8374

Patient Name: [REDACTED] DOB: [REDACTED] Patient ID: [REDACTED] Gender: M Monogram Accession #: [REDACTED]
 Date Collected: 04/14/2008 14:37 Date Received: 04/17/2008 21:08 Date Reported: 05/13/2008 11:11 Mode: F,M,W Report Status: FINAL
 Referring Physician: [REDACTED] Reference Lab ID: 31220083698

HIV-1 Subtype: B

DRUG		PHENOSENSE [™] SUSCEPTIBILITY				Evidence of Susceptibility		Net Assessment	
Generic Name	Brand Name	Cutoffs (Lower - Upper)	Fold Change	Increasing Drug Susceptibility	Decreasing	Pheno Sense	Gene Seq		
NRTI	Abacavir	Ziagen	(4.5 - 6.5)	0.82	[Graph]	100	Y	Y	Sensitive
	Didanosine	Videx	(1.3 - 2.2)	0.63	[Graph]	100	Y	Y	Sensitive
	Emtricitabine	Emtriva	(3.5)	1.08	[Graph]	100	Y	Y	Sensitive
	Lamivudine	Epivir	(3.5)	1.12	[Graph]	100	Y	Y	Sensitive
	Stavudine	Zerit	(1.7)	0.60	[Graph]	100	Y	Y	Sensitive
	Zidovudine	Retrovir	(1.9)	0.48	[Graph]	100	Y	N	Sensitive
	Tenofovir	Viread	(1.4 - 4)	0.75	[Graph]	100	Y	Y	Sensitive
NRTI Mutations		M41L, L210W, T215S							

NNRTI	Delavirdine	Rescriptor	(6.2)	0.60	[Graph]	100	Y	Y	Sensitive
	Efavirenz	Sustiva	(3)	0.47	[Graph]	100	Y	Y	Sensitive
	Etravirine	Intelence [™]	(2.9)	0.59	[Graph]	100	Y	Y	Sensitive
	Nevirapine	Viramune	(4.5)	0.46	[Graph]	100	Y	Y	Sensitive
NNRTI Mutations		none							

PI	Atazanavir	Reyataz	(2.2)	1.38	[Graph]	100	Y	Y	Sensitive
		Reyataz / r*	(5.2)	1.38	[Graph]	100	Y	Y	Sensitive
	Darunavir	Prezista / r*	(10 - 90)	1.22	[Graph]	100	Y	Y	Sensitive
	Fosamprenavir	Lexiva	(2)	2.38	[Graph]	100	N	Y	Resistant
		Lexiva / r*	(4 - 11)	2.38	[Graph]	100	Y	Y	Sensitive
	Indinavir	Crixivan / r*	(10)	0.84	[Graph]	100	Y	Y	Sensitive
	Lopinavir	Kaletra	(9 - 55)	1.18	[Graph]	100	Y	Y	Sensitive
	Nelfinavir	Viracept	(3.6)	13	[Graph]	100	N	N	Resistant
	Ritonavir	Norvir	(2.5)	0.78	[Graph]	100	Y	Y	Sensitive
	Saquinavir	Invirase	(1.7)	1.00	[Graph]	100	Y	Y	Sensitive
Invirase / r*		(2.3 - 12)	1.00	[Graph]	100	Y	Y	Sensitive	
Tipranavir	Aptivus / r*	(2 - 8)	0.91	[Graph]	100	Y	Y	Sensitive	
PI Mutations		I13V, D30N, L33F, M46L, L63P, V77I, N88D, L89M							

[H] Lower Clinical Cutoff (in bold) [H] Hypersusceptibility Cutoff [] Sensitive [Y] Evidence of Drug Sensitivity
 [H] Upper Clinical Cutoff (in bold) [H] Partially Sensitive [P] Evidence of Partial Drug Sensitivity
 [H] Biological Cutoff [H] Resistant [N] Evidence of Drug Resistance

For more information on interpreting this report, please visit www.MonogramHIV.com or call Customer Service at 800-777-0177 between the hours of 6:30am to 5:00pm PST Monday through Friday.