

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

The patient is a 17 year old male HIV-infected at birth. Both parents are deceased from HIV infection; most recently his mother died of PML in late 2004. He is a high school senior who lives with his grandmother. His medical history is significant for depression following his mother's death, oral candidiasis, bacterial pneumonias, and a recent episode of Herpes zoster. The Herpes zoster infection was particularly frightening for the patient as his father's initial HIV index diagnosis was Herpes zoster and his father's health spiraled quickly downward after that diagnosis.

This adolescent has had great difficulty taking medications (including opportunistic infection prophylaxis) due to lack of motivation and possible lack of family support structure. He does not report any adverse effects to the nurse practitioners who follow him either weekly or bi-weekly. He takes his medications sporadically, stating that he often misses the morning doses on his way to school. His self-reported adherence is greater than what is documented by pharmacy refill records.

He has only been treated with 3 antiretroviral regimens - dual nucleosides (NRTIs), then highly active antiretroviral therapy (HAART) with didanosine (ddI) plus stavudine (d4T) plus ritonavir (RTV), which was then changed to nelfinavir (NFV). It is unclear why the patient was changed to NFV. The most recent regimen was a study regimen (PACTG 1038) that consisted of ddI plus efavirenz (EFV) and a double dose of lopinavir/ritonavir (/Kaletra). The second phase of the study would have added on a double dose of saquinavir (SAQ). The patient never reached this phase because he was discontinued from the study for non-adherence as documented by non-detectable lopinavir plasma levels. The patient has now been off medications for 2 weeks.

Date	CD4	%	VL	Meds	Notes
2/25/92	700	19%			
5/92	295	14%		ddl/ZDV	PACTG 152
10/92					anemia
6/93					pneumonia
6/94					Varicella – hospital admit x 2 wks
7/95					PACTG 254 (PCP prophylaxis)
2/97	218	17%	1000	ddl/d4T/RTV	PACTG 338 (off study 8/12/97)
1/99			2068	ddl/d4T/NFV	
9/99	281	13%	16208		
12/00	249	15	25563		
7/12/00	298	14	7402		
10/26/01	198	13	4648		
8/16/02			2560		
10/28/02					Protocol 402: Admit for interleukin-2 (IL-2) study Q8 weeks x 6 cycles – got fever, chills, nausea, vomiting (all grade one reactions) but by day 6 had developed grade III-IV rash over 50% of body.
8/11/03	820	28%	5204		Last IL-2 cycle. Declined to participate in Phase II of the study.
1/04	673	25	11693		
4/04	437	23	9156		
8/04	189	15	13075		
9/04	121	12	12919		Start trimethoprim/sulfamethoxazole for PCP prophylaxis
11/04					Mother passes away
12/04	104	10	13714		Missing 2 doses/week
2/05	83	7	7334		Defer changes until improved adherence?

					Pharm.D. suggest change to NFV 625mg tablets
3/05	78	7	8485		Consent PACTG 1045
6/05	43	4	35746		Consent PACTG 1038 GT/PT drawn
7/05	33	4			GT/PT drawn
8/05	31	4	21204	ddl/EFV/Kaletra (6 caps)	Rash to EFV – resolved with symptomatic treatment
8/23/05	46	6%	165		Suggest change to Kaletra liquid
9/05	47	5			Non adherent by pill count and intensive PK studies – start Kaletra liquid
10/5/05	26	4			NP begins daily phone calls to patient to improve adherence
10/18/05					Herpes Zoster infection, shortly after pt reports increased adherence
11/2/05	35	6	15295		
11/16/05	22	4			Medications stopped. Pt removed from study protocol PACTG 1038 due to undetectable ARV levels of EFV and Kaletra.
11/20/05					GT/PT drawn

Resistance Test Findings

6/1/05: Quest Diagnostics

NRT	M41L, L210W, T215Y, V118I/V, R211K
PI	L10V, L21I, M46L, I54V, L63P, A71V, V77I, V82A,

Phenotype: 6/1/05: Virologic

	Fold Change	Sensitivity
NRTI		
ABC	1.99	Sens
TFV	1.85	Reduced Sens
ddl	1.00	Sens
3TC	2.03	Sens
D4T	1.44	Sens
AZT	56	Reduced Sens
FTC	2.27	Sens
NNRTI		
DLV	0.31	Sens
NVP	0.55	Sens
EFV	0.64	Sens
PI		
FAPV	8.59	Reduced susc
ATZ	20	Reduced susc
IDV	19	Reduced susc
LPV	52	Reduced susc
NFV	38	Reduced susc
RTV	80	Reduced susc

SQV	5.49	Reduced susc
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Phenotype: 7/12/05: Virologic

	Fold Change	Sensitivity
NRTI		
ABC	1.73	Sens
TFV	1.85	Reduced Sens
ddl	1.01	Sens
3TC	2.07	Sens
D4T	1.30	Sens
AZT	21	Reduced Sens
FTC	2.06	Sens
NNRTI		
DLV	0.36	Sens
NVP	0.50	Sens
EFV	0.69	Sens
PI		
FAPV	4.39	Reduced susc
ATZ	17	Reduced susc
IDV	15	Reduced susc
LPV	33	Reduced susc

CASE NUMBER

DATE: 12/7/05

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NFV	20	Reduced susc
RTV	54	Reduced susc

SQV	3.64	Reduced susc
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11/05: Monogram (Virologic) Phenosense GT

NRT	M41L, L210W, T215Y, V118I/V, K103N
PI	L10V, L24I, K43T, M46L, I54V, L63P, A71V, V77I, V82A,

Phenotype: 11/05: Virologic (Monogram)

	Fold Change	Sensitivity	PT?	GT?
NRTI				
ABC	1.10	Sensitive	Y	Y
TFV	1.56	Reduced Sens	N	N
ddI	0.83	Sensitive	Y	Y
3TC	1.18	Reduced Sens	Y	N
D4T	1.01	Reduced Sens	Y	N
AZT	5.98	Reduced Sens	N	N
FTC	1.44	Reduced Sens	Y	N
NNRTI				
DLV	28	Reduced Sens	N	N
NVP	46	Reduced Sens	N	N
EFV	23	Reduced Sens	N	N
PI				
FAPV	5.46	Reduced susc	N	Y
ATZ	16	Reduced susc	N	N
IDV, IDV/r	26	Reduced susc	N	N
LPV	43	Reduced susc	N	N
NFV	34	Reduced susc	N	N
RTV	93	Reduced susc	N	N
SQV	5.46	Reduced susc	N	N
TPV/r	1.43	Sensitive	Y	Y

Replication Capacity = 42%

Interpretation/Implications for Treatment

Adherence is a major determinant in the development of antiretroviral resistance. Non-adherence may be prevalent amongst particular sub-groups of patients including, in this case, adolescents. In a study of 231 adolescents in 13 U.S. Cities, only 69% reported baseline adherence to therapy.¹ For 65 patients who initially reported being adherent to therapy that were followed longitudinally, the median time to non-adherence was approximately 12 months, and only 50% of patients who achieved an undetectable viral load remained undetectable. In the REACH cohort, only 28.3% of the 114 adolescents included in the study reported 100%

¹ Murphy DA, Belzer M, Durako SJ, Sarr M, Wilson CM, Muenz LR. Longitudinal antiretroviral adherence among adolescents infected with human immunodeficiency virus. Arch Pediatr Adolesc Med. 2005 Aug;159(8):764-70.

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adherence to their medications within the last month.² The reported barriers to adherence in this group were medication adverse effects and disruption of daily routines.

This patient had not been exposed to many antiretrovirals until 2005. Similar to many HIV infected children, the patient's initial treatment was dual nucleoside therapy with didanosine and lamivudine (ddI/3TC). HIV therapy then progressed to un-boosted protease- based HAART containing nelfinavir (NFV). The panel noted that the patient had been maintained on the NFV-based regimen for quite some time despite inadequate viral suppression. This was presumably due to a combination of non-adherence to medications and resistance. The goal of antiretroviral therapy, if possible, should be to achieve maximal virologic suppression. In certain cases such as non-adherence, intolerance, or severe resistance there may still be some benefit to maintaining a less optimal ARV regimen. Immunologic, virologic, and clinical benefits for these non-suppressive regimens have been demonstrated.^{3,4}

The risk to maintaining a non-suppressive regimen is the development of new antiretroviral resistance mutations. In 98 HIV-positive patients on non-suppressive ARV therapy with at least two resistance tests available, Napravnik et al. found that the main predictors of resistance mutation accumulation included average HIV RNA level and slope and number of mutations on the first resistance test.⁵ Accumulation of resistance mutations, while undesirable, may affect the "fitness" or ability of the virus to replicate. Barbour et al. observed a rise in phenotypic resistance with a simultaneous decrease in replicative capacity in 248 patients with virologic failure on continued protease inhibitor therapy.⁶

This patient, maintained for many years on a non-suppressive single protease inhibitor containing regimen, appears to have accumulated many mutations. In the reverse transcriptase gene the patient has the M41L, L210W, T215Y, V118I/V, and R211K mutations. The mutations 41-215-210 often arrive as a consecutive pattern that confers high level resistance to the thymidine analogues zidovudine (ZDV) and stavudine (d4T), and particularly to tenofovir. (TDF)^{7,8,9,10} V118I is sometimes present in patients who have been treated with dual-nucleoside therapy and is likely to confer low-level NRTI resistance. R211K is thought to be a

² Murphy DA, Sarr M, Durako SJ, Moscicki AB, Wilson CM, Muenz LR. Barriers to HAART adherence among human immunodeficiency virus-infected adolescents. *Arch Pediatr Adolesc Med.* 2003 Mar;157(3):249-55.

³ Ledergerber B, Egger M, Opravil M, et al. Clinical progression and virological failure on highly active antiretroviral therapy in HIV-1 patients: a prospective cohort study. *Lancet.* 1999;353:863-868

⁴ Deeks SG, Wrin T, Liegler T, et al. Virologic and immunologic consequences of discontinuing combination antiretroviral-drug therapy in HIV-1 infected patients with detectable viremia. *N Engl J Med.* 2001;344: 472-480.

⁵ Napravnik S, Edwards D, Stewart P, Stalzer B, Matteson E, Eron JJ Jr. HIV-1 drug resistance evolution among patients on potent combination antiretroviral therapy with detectable viremia. *J Acquir Immune Defic Syndr.* 2005 Sep 1;40(1):34-40.

⁶ Barbour JD, Wrin T, Grant RM, Martin JN, Segal MR, Petropoulos CJ, Deeks SG. Evolution of phenotypic drug susceptibility and viral replication capacity during long-term virologic failure of protease inhibitor therapy in human immunodeficiency virus-infected adults. *J Virol.* 2002 Nov;76(21):11104-12.

⁷ Yahi N, Tamalet C, Tourres C, Tivoli N, Ariasi F, Volot F, Gastaut JA, Gallais H, Moreau J, Fantini J. 1999. Mutation patterns of the reverse transcriptase and protease genes in human immunodeficiency virus type 1-infected patients undergoing combination therapy: Survey of 787 sequences. *J Clin Microbiol* 37:4099-4106.

⁸ Hanna GJ, Johnson VA, Kuritzkes DR, Richman DD, Brown AJ, Savara AV, Hazelwood JD, D'Aquila RT. 2000. Patterns of resistance mutations selected by treatment of human immunodeficiency virus type 1 infection with zidovudine, didanosine, and nevirapine. *J Infect Dis* 181:904-911.

⁹ Marcelin AG, Delaugerre C, Wiriden M, Viegas P, Simon A, Katlama C, Calvez V. Thymidine analogue reverse transcriptase inhibitors resistance mutations profiles and association to other nucleoside reverse transcriptase inhibitors resistance mutations observed in the context of virological failure. *J Med Virol.* 2004 Jan;72(1):162-5.

¹⁰ Shafer RW. Genotypic testing for HIV-1 drug resistance (HIV InSite Knowledge Base Chapter). Available at: <http://www.hivinsite.com/InSite?page=kb-03&doc=kb-03-02-07>. Accessed on 12/22/05.

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polymorphism.¹⁰ The panel noted that it was interesting to follow the decreasing phenotypic AZT fold-change from 56 to 21 to 5.98. The panel also commented that although the phenotypic fold change for ddl is reported to be <1, this seems like it would be inaccurate because the patient had been on ddl for years. Another interesting note is that the patient does not have the M184V mutation (associated with lamivudine and emtricitabine) because the patient had never been treated with these medications before.

In the protease gene the patient has the primary resistance mutations L24I, M46L, I54V, V82A and the minor mutations at L10V, L63P, A71V, V77I. The primary resistance mutations result in multiple PI resistance. Of note, it is interesting that the patient had been treated with nelfinavir for approximately 8 years but does not show evidence of one or both of the main NFV associated protease mutations: the D30N or the L90M. V77I is a secondary PI mutation that is associated with NFV treatment. The patient has developed the I54V mutation, which causes multi-PI resistance, but is a major mutation for amprenavir/fosamprenavir.¹¹

Severe resistance in combination with non-adherence is what likely resulted in NNRTI failure during the last ddl/EFV/LPV/RTV regimen. Based on the available genotype, NRTIs and PIs were only partially active, while efavirenz was the only fully active drug in the regimen. With a regimen in which only one drug is fully active and the others are partially active, full adherence is needed to support the active drug to provide the best chance for virologic success. The double doses of lopinavir in the study regimen were likely difficult to adhere to for a patient with pre-existing adherence problems. There was an initial suppressive response (down to 165 copies/mL) but without support of the partially active drugs, the NNRTI failed, and the K103N mutation emerged. K103N confers resistance to all NNRTIs.

In summary, this patient has developed triple class resistance to antiretrovirals. His available options are in the NRTI class (partially active), PI class (partially active), and fusion inhibitor class (fully active). The non-nucleoside reverse transcriptase inhibitor class is no longer active. His low CD4 count makes antiretroviral treatment necessary. The panel agreed that psychosocial counseling, treatment of his depression, and continued adherence monitoring would be key to his success.

One option includes treating the patient with the most aggressive antiretroviral therapy. This strategy has the benefit of maximal virologic suppression and immunologic restoration. One such regimen that could be constructed include a fusion inhibitor (T-20, enfuvirtide) plus a protease inhibitor for multi-PI resistant viruses (tipranavir/ritonavir) in combination with as many partially active nucleosides as tolerated. Lamivudine or emtricitabine should be included in the regimen as the patient had never been exposed to these before. The patient's last phenotype suggested sensitivity to tipranavir, and he lacks some of the major mutations to this drug (33, 84, L90M). Investigational agents such as TMC-114 (PI) and or TMC-125, a second-generation NNRTI manufactured by Tibotec, would also be ideal for this patient, though most studies require a minimum age of 18, and maintainance on a failing regimen prior to study entry. The major disadvantages to this strategy include toxicity and pill burden.

Another option is to place the patient back on a non-suppressive ARV regimen with the goal of immunologic boosting/maintenance and incomplete virologic suppression while treating the depression and working on adherence. If this approach is taken, the panel believed that the lowest pill burden and frequency and least toxic drugs should be used. Suggestions included two possible once daily regimens abacavir plus tenofovir plus boosted atazanavir (ABC/TDF/ATV/r) (5 pills) or ABC/ddl/ATV/r (6 pills). This option saves boosted tipranavir, lamivudine, and T-20 for future use. Both of these suggested regimens are once daily, although ddl and atazanavir should technically be taken separately (one on an empty stomach and the other with food). Risks of this strategy include the CD4 remaining under 200cells/mm³ and the patient becoming at greater risk of opportunistic infections. Despite these risks, the panel members were in favor of the approach to work on adherence before risking failure of the few remaining active antiretrovirals for this patient.

Recommendations

Regimen Options

OPTION #1: Non-suppressive but manageable regimen

How to “wait” has not been prospectively studied. There are many options available, including the use of a well-tolerated protease inhibitor (e.g., atazanavir) with well-tolerated NRTIs (of note, the patient should not go on 3TC or FTC until a fully suppressive approach is pursued). A non-suppressive regimen will likely result in delayed disease progression but might select for additional drug-resistance mutations.

OPTION #2: Maximally suppressive regimen

Based on current data, one possible fully suppressive regimen would be ritonavir/tipranavir (or ritonavir/darunavir), enfuvirtide (T-20), tenofovir, zidovudine, abacavir, lamivudine. Alternatively, as the patient is not likely to adhere or tolerate T20, enrollment in an integrase inhibitor or R5 inhibitor study might be considered.

Dosing, Monitoring, and Follow-up Recommendations

OPTION #2:

Tipranavir (250mg) 2 capsules orally twice daily with food
Ritonavir (100mg) 2 capsules orally twice daily with food
Enfuvirtide (90mg): 1 injection subcutaneously twice daily
Tenofovir (300mg): 1 tablet orally once daily with/without food
Trizivir (300/150/300mg): 1 tablet orally twice daily with food
Monitor CD4 and VL in one month

OTHER Recommendations:

Monitor adherence
Treat depression with counseling and behavioral therapy plus/minus antidepressants
Ensure appropriate opportunistic prophylaxis with Septra DS 1 PO daily (PCP prophylaxis) and azithromycin 1200mg PO Q week (MAC prophylaxis)