DNA Genotyping in HIV Infection

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Case History

This is a 37 year old male with HIV infection diagnosed in 1996

- His CD4 nadir was in the 400s and he reports no prior opportunistic infections.
- He began antiretroviral therapy in August of 2006.
- He is unsure of the specific regimens but he recalls prior use of AZT, 3TC, tenofovir, DDI, ritonavir, lopinavir, nevirapine, and abacavir.
- He transferred care to our clinic in 2010.

Case History Continued

- Current regimen since 2008 is TDF/FTC + atazanavir/ritonavir.
- He presents for follow up in December of 2014.
- He has no known drug allergies.
- Current labs: CD4 946 cells/mm^3, HIV viral load < 20 copies/mL.
- Although he is tolerating ART, he is interested in changing to a once daily regimen.
- He is unsure of prior resistance testing and attempts to get his records were unsuccessful.

Can he be safely switched to one of our single tablet regimens?

Reasons to Consider Regimen Switching in the Setting of an Undetectable HIV Viral Load

- To simplify the regimen by reducing pill burden and dosing frequency to improve adherence
- To enhance tolerability and decrease short-term or long-term toxicity
- To change food or fluid requirements
- To avoid parenteral administration
- To minimize or address drug interaction concerns
- To allow for optimal use of ART during pregnancy or should pregnancy occur
- To reduce costs

Reasons Not to Switch

- “If it is not broken, don’t fix it!”
- “Don’t get off a winning horse!”
- “This has saved my life so why should I change?”
- Save newer options for later
- Avoid perceived and actual insurance barriers
- Concerns for new drug side effects and drug interactions

The Increasing number of Single Tablet Regimens

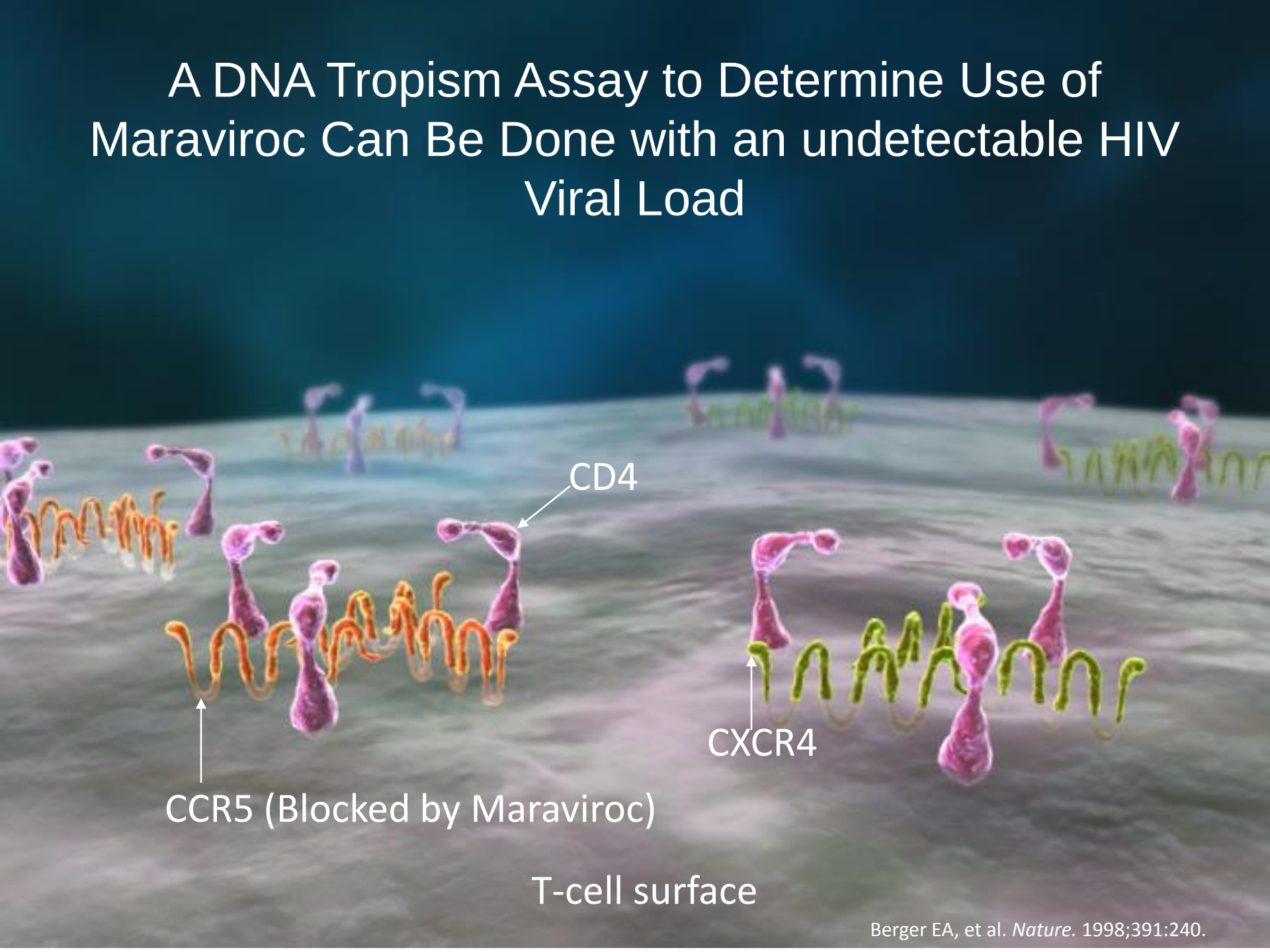
Currently Approved

- TDF/FTC/efavirenz
- TDF/FTC/rilpivirine
- TDF/FTC/cobicistat/elvitegravir
- TAF/FTC/cobicistat/elvitegravir
- ABC/3TC/dolutegravir

Potential approval in the future

- TAF/FTC/rilpivirine
- TAF/FTC/cobicistat/darunavir

A DNA Tropism Assay to Determine Use of Maraviroc Can Be Done with an undetectable HIV Viral Load



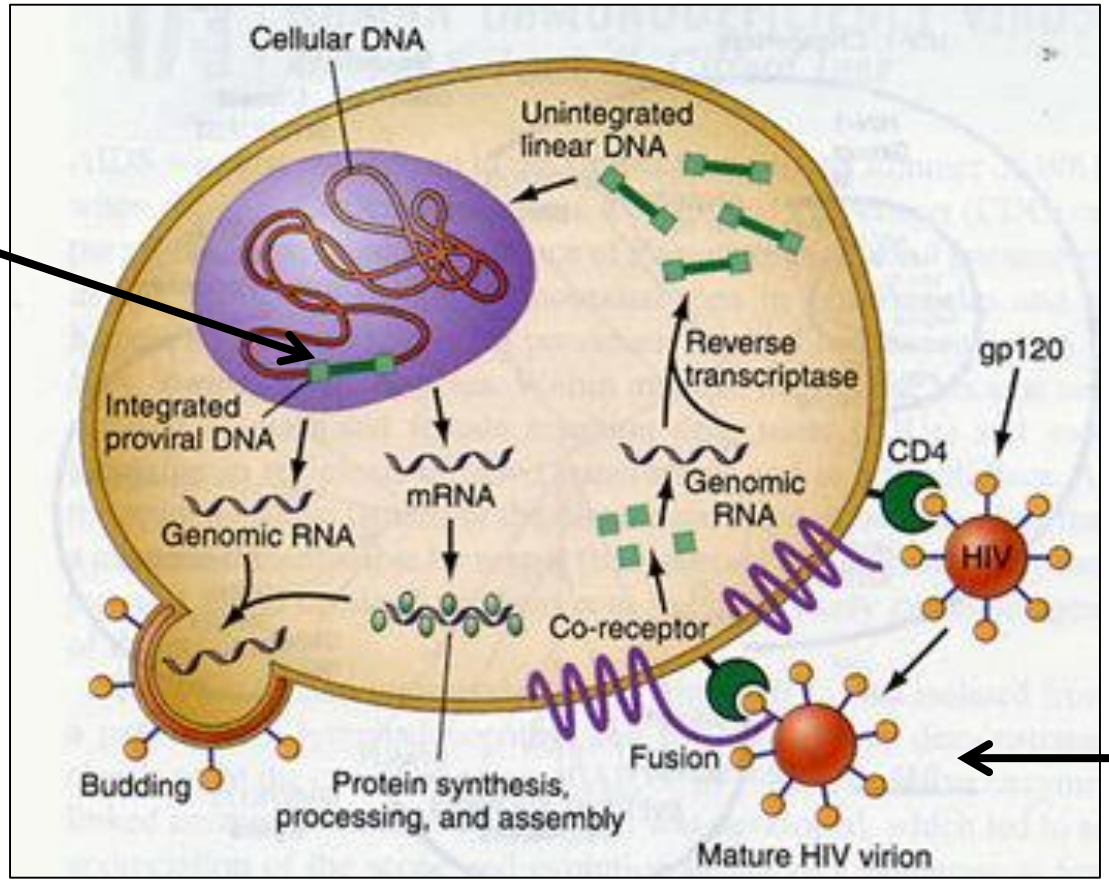
Berger EA, et al. *Nature*. 1998;391:240.

HIV DNA Genotyping

- Evaluates for “archived” mutations in HIV proviral DNA inside white blood cells.
- Studies show some correlation between archived genotyping results and historical genotypes.
- The test can be done in patients who have an undetectable HIV viral load on therapy.
- This technology also holds promise for resource-limited settings where prior changes in therapy were done without HIV resistance tests.

DNA Genotyping: Testing Resistance "Archived" in the White Blood Cell

DNA Resistance Tests



Standard Resistance Tests

HIV Drug Resistance Mutations in Proviral DNA from a Community Treatment Program

- 120 HIV+ patients
  - 38 patients with detectable viral loads had DNA and RNA samples
  - 82 patients without detectable viral loads had DNA samples only
- Concordance between RNA and DNA genotypes was seen in 84% of viremic patients
- Of the 82 patients with an undetectable viral load, 21 (26%) had drug resistance mutations to one (n = 16), two (n = 4), or three (n = 1) ARV classes

Derache et al. PLOS One. January 30, 2015. DOI:1371/journal.pone.0117430

Comparison of resistance mutation patterns in historical plasma HIV RNA genotypes with those in current proviral HIV DNA genotypes among extensively treated patients with suppressed replication.

- 169 patients from a clinical trial with prior exposure to NRTI, NNRTIs, and PIs
- All had HIV viral load < 400 copies/ml at time of study
- Historical genotypes (median of 4 per patient) were compared to HIV-1 DNA whole blood genotyping
- Median number of mutations HIV-1 RNA vs DNA
  - 5 versus 4 for NRTI, 3 versus 1 for NNRTI, 10 versus 8 for PI
  - Resistance to a drug found exclusively in RNA in 63%, 47% and 50% of patient for NRTI, NNRTI, and PI respectively

Deluagerre C, et al. HIV Med 2012;13:517-525

Genotypic Analyses of Pre-Existing HIV-1 Drug Resistance in Proviral HIV-1 DNA from PBMCs in Suppressed Patients Switching to RPV/FTC/TDF

- Analysis from the SPIRIT Study, a trial switching suppressed patients on PIs to RPV/FTC/TDF
- 81 samples chosen to compare DNA testing with historical genotypes
- DNA genotyping successful in 79% of these samples
- Additional mutations were found using the DNA assay

Table 4. PBMC DNA Genotype Identified Additional NRTI and NNRTI Resistance Mutations

Resistance Mutations at Baseline	Baseline PBMC DNA Genotype (n=56)	Historical RNA Genotype (n=56)
NRTI-Associated		
M184I/V	1*	0
D67N	2	1
V75I	1	1
V118I	0	1
K219E/N/Q/R	1	1
NNRTI-Associated		
K103N/S	21	21
Y181C/I/V	1*	0
E138A/G/K/Q/R	7	4
H221Y	2	1
V179D/F/I/L/T	10	8
V90I	4	3
G190A/E/Q/S	3	3
V106A/I/M	2	1
V108I	1	2
A98G	1	1
L100I	1	0
P225H	1	0
M230I/L	1	0

Yellow rows indicate mutations associated with RPV treatment
* M184I and Y181Y/C were present in the same sample (see Figure 4a).

White K, et al. International Workshop on HIV & Hepatitis Virus Drug Resistance and Curative Strategies. June 4-8, 2013. Toronto, Canada

Drug Resistance Mutations from Whole Blood Proviral DNA Among Patients on Antiretroviral Drugs in Zimbabwe

Methods

- 125 whole blood samples from patients on first-line ART were investigated for drug resistance mutations using an in-house genotypic testing method.
- Patients had been on HIV reverse transcriptase inhibitors only, with some having been on both HIV and TB treatment.
- DNA was extracted from whole blood; amplicons were generated by nested PCR and sequenced.

Chimukangara B, et al. Current HIV Research 2016; 12:309-316

Drug Resistance Mutations from Whole Blood Proviral DNA Among Patients on Antiretroviral Drugs in Zimbabwe

Results:

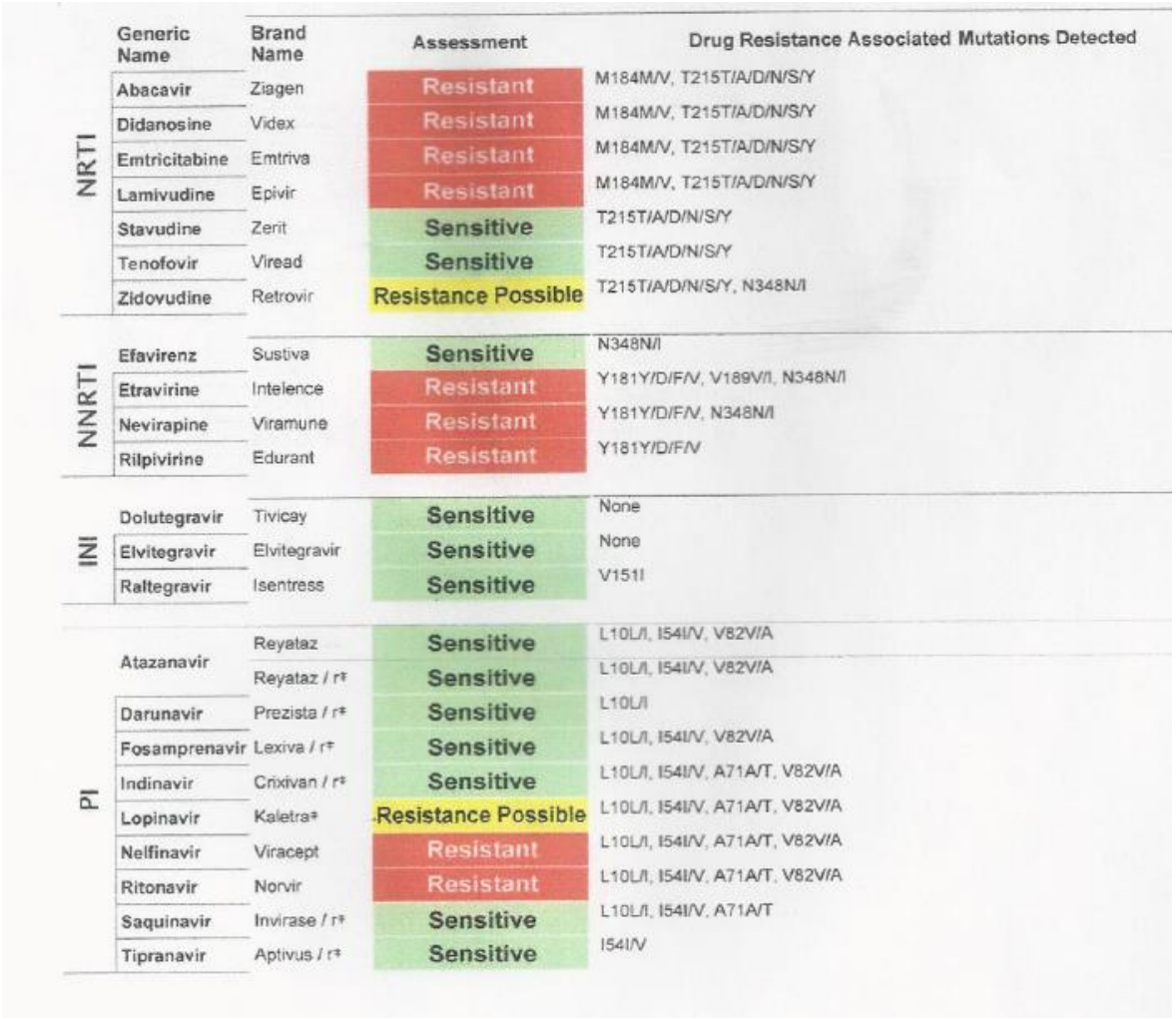
- From 125 samples, 108 were successfully analyzed for drug resistance mutations.
- 11 of the 108 sequences had drug resistance mutations; predominantly M184V and Y181C.
- For a 100-cell increase in CD4 count, the odds of being resistant were 61% lower than those with the baseline CD4 count (p=0.04, CI: 0.34-0.98).
- There was no association between concurrent HIV/TB treatment and drug resistance (p=0.41).

Chimukangara B, et al. Current HIV Research 2016; 12:309-316

Potential Issues with DNA Genotyping

- The test may not detect all mutations that have occurred over time.
- Consequently, positive test results are likely more helpful than negative test results.
- Are certain types of mutations more reliably found than others?
- If the test is not fully sensitive, could ART changes lead to treatment failure?
- If unexpected resistance to the current regimen is discovered by this test, does the current regimen need to be changed or intensified?

Case: DNA Genotyping Results



	Generic Name	Brand Name	Assessment	Drug Resistance Associated Mutations Detected
NRTI	Abacavir	Ziagen	Resistant	M184M/V, T215T/A/D/N/S/Y
	Didanosine	Videx	Resistant	M184M/V, T215T/A/D/N/S/Y
	Emtricitabine	Emtriva	Resistant	M184M/V, T215T/A/D/N/S/Y
	Lamivudine	Epivir	Resistant	M184M/V, T215T/A/D/N/S/Y
	Stavudine	Zerit	Sensitive	T215T/A/D/N/S/Y
	Tenofovir	Viread	Sensitive	T215T/A/D/N/S/Y
	Zidovudine	Retrovir	Resistance Possible	T215T/A/D/N/S/Y, N348N/I
NNRTI	Efavirenz	Sustiva	Sensitive	N348N/I
	Etravirine	Intelence	Resistant	Y181Y/D/F/V, V189V/I, N348N/I
	Nevirapine	Viramune	Resistant	Y181Y/D/F/V, N348N/I
	Rilpivirine	Edurant	Resistant	Y181Y/D/F/V
INI	Dolutegravir	Tivicay	Sensitive	None
	Elvitegravir	Elvitegravir	Sensitive	None
	Raltegravir	Isentress	Sensitive	V151I
PI	Atazanavir	Reyataz	Sensitive	L10L/I, I54I/V, V82V/A
		Reyataz / r*	Sensitive	L10L/I, I54I/V, V82V/A
	Darunavir	Prezista / r*	Sensitive	L10L/I
	Fosamprenavir	Lexiva / r*	Sensitive	L10L/I, I54I/V, V82V/A
	Indinavir	Crixivan / r*	Sensitive	L10L/I, I54I/V, A71A/T, V82V/A
	Lopinavir	Kaletra*	Resistance Possible	L10L/I, I54I/V, A71A/T, V82V/A
	Nelfinavir	Viracept	Resistant	L10L/I, I54I/V, A71A/T, V82V/A
	Ritonavir	Norvir	Resistant	L10L/I, I54I/V, A71A/T, V82V/A
	Saquinavir	Invirase / r*	Sensitive	L10L/I, I54I/V, A71A/T
	Tipranavir	Aptivus / r*	Sensitive	I54I/V

M184V
T215T/A/D/N/S/Y

Y181Y/D/F/V

I54/I/V, V82V/A

Case Conclusion

- DNA genotyping detected significant NRTI, NNRTI, and PI mutations.
- Given these mutations, it was not clear that switching to a single tablet regimen with either an NNRTI or an integrase inhibitor as an anchor would be adequate.
- We elected to continue the current regimen and may consider an upcoming single tablet regimen containing a boosted protease inhibitor.
- His regimen was not intensified, that is, additional drugs were not added.

Take Home Points

- Recently licensed medications and formulations have increased the number of safe and simple treatment options
- Switching medications to gain some benefit is an important part of HIV care but every clinical situation is unique
- Switches should be done only after review of treatment history, prior resistance tests, issues with the current regimen, and the pros and cons of the proposed new regimen
- DNA genotyping is a promising technique to help with HIV regimen changes in persons who are virologically suppressed