HIV Drug Resistance Testing Basics

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Disclaimer

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Learning Objectives

- Understand the process of genotype versus phenotype resistance testing and why one is preferred over the other
- Describe the indications for a traditional genotype (RT/PR), an integrase (IN) genotype, and a phenotype
- Know the resources for help with interpretation of resistance-associated mutations

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Poll

You are seeing a patient recently diagnosed with HIV for their first clinic visit. They have no history of PrEP use. Which of the following is recommended as part of the baseline laboratory evaluation?

A) Genotype resistance assay (integrase resistance testing not necessary)

B) Genotype resistance assay with integrase resistance testing

C) Phenotype resistance assay with integrase resistance testing

D) Genotype and phenotype resistance assays (integrase not necessary)

Resistance Test Comparison

Genotype	Phenotype
Sequence reverse transcriptase (RT) and protease (PR) genes, +/- integrase (IN) gene (or, rarely, envelope gene)	Grow virus in culture, add ARV drugs in various amounts, compare IC_{50} to IC_{50} of wild type virus ("fold change")
Quicker, lower cost, more sensitive	Takes longer, more expensive
Interpretation challenging if numerous mutations	Helpful if complex resistance history, especially to protease inhibitors (PI)

*Both types: need circulating RNA; resistance only detected if >10-20% of virus population

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Example genotype report

HIV-1 Genotyping

See Note

NRTI DRUGS

EPIVIR, (lamivudine, 3TC)	None
EMTRIVA, (emtricitabine, FTC)	None
RETROVIR, (zidovudine, AZT)	None
VIDEX, (didanosine, ddI)	None
ZERIT, (stavudine, d4T)	None
ZIAGEN, (abacavir, ABC)	None
VIREAD, (tenofovir, TDF)	None

NRTI associated resistance mutations found: None

NNRTI DRUGS

RESCRIPTOR, (delavirdine, DLV)	Resistance
SUSTIVA, (efavirenz, EFV)	Resistance
VIRAMUNE, (nevirapine, NVP)	Resistance
INTELENCE, (etravirine, ETR)	None

NNRTI associated resistance mutations found: K103N

Protease inhibitors

AGENERASE, (amprenavir, APV)	None
LEXIVA, (fosamprenavir, FOS)	None
CRIXIVAN, (indinavir, IDV)	None
FORTOVASE / INVIRASE, (saquinavir, SQV)	None
KALETRA, (lopinavir + ritonavir, LPV)	None
PREZISTA, (darunavir, DRV)	None
VIRACEPT, (nelfinavir, NFV)	None
REYATAZ, (atazanavir, ATV)	None
APTIVUS, (tipranavir, TPV)	None

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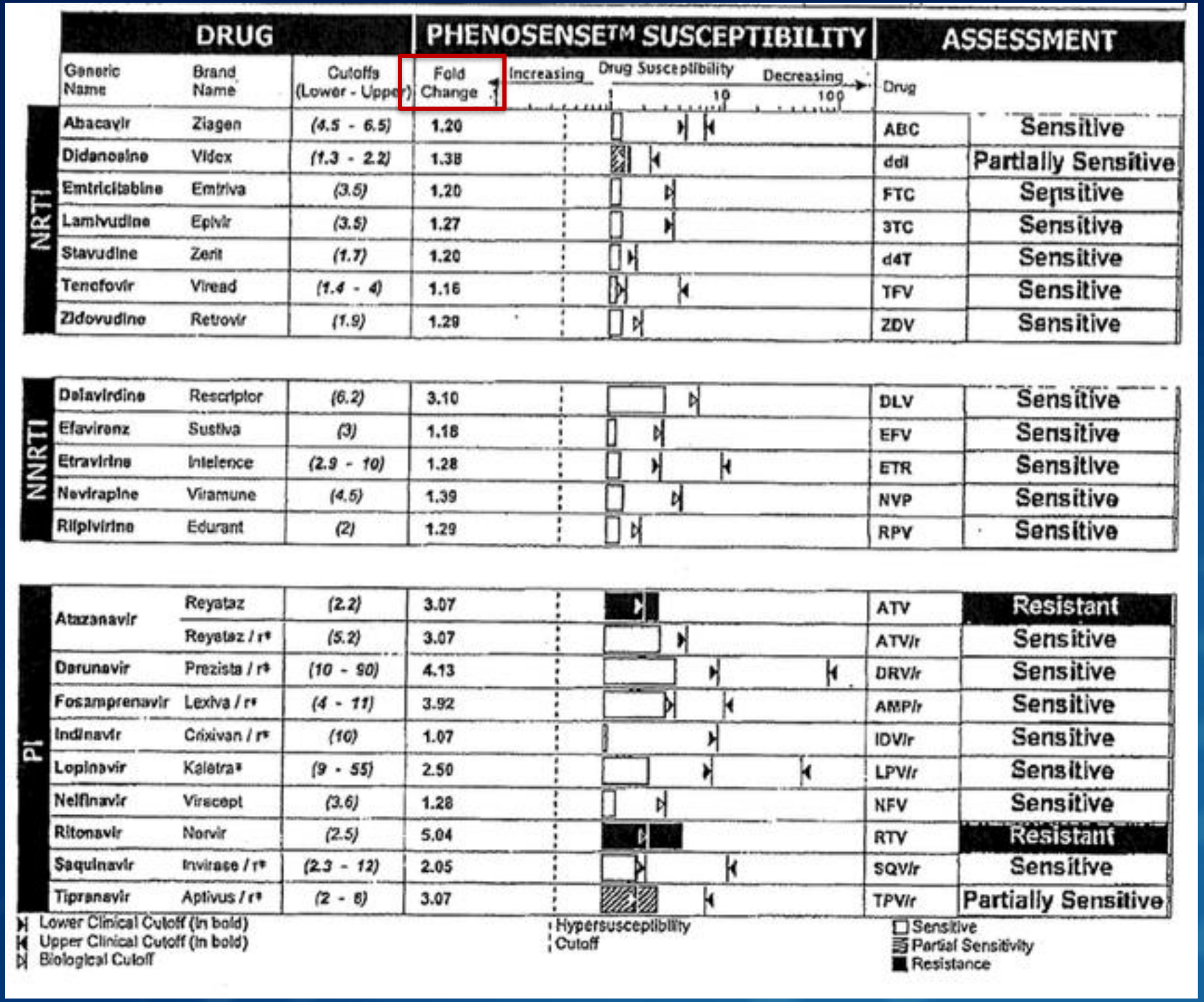
Example phenotype report



	DRUG			PHENOSENSE™ SUSCEPTIBILITY		ASSESSMENT	
	Generic Name	Brand Name	Cutoffs (Lower - Upper)	Fold Change	Increasing Drug Susceptibility Decreasing (1, 10, 100)	Drug	
NRTI	Abacavir	Ziagen	(4.5 - 6.5)	1.20		ABC	Sensitive
	Didanosine	Videx	(1.3 - 2.2)	1.38		ddI	Partially Sensitive
	Emtricitabine	Emtriva	(3.5)	1.20		FTC	Sensitive
	Lamivudine	Epivir	(3.5)	1.27		3TC	Sensitive
	Stavudine	Zerit	(1.7)	1.20		d4T	Sensitive
	Tenofovir	Viread	(1.4 - 4)	1.16		TFV	Sensitive
	Zidovudine	Retrovir	(1.9)	1.29		ZDV	Sensitive
NNRTI	Delavirdine	Rescriptor	(6.2)	3.10		DLV	Sensitive
	Efavirenz	Sustiva	(3)	1.18		EFV	Sensitive
	Etravirine	Intelence	(2.9 - 10)	1.28		ETR	Sensitive
	Nevirapine	Viramune	(4.5)	1.39		NVP	Sensitive
	Rilpivirine	Edurant	(2)	1.29		RPV	Sensitive
PI	Atazanavir	Reyataz	(2.2)	3.07		ATV	Resistant
		Reyataz / r*	(5.2)	3.07		ATV/r	Sensitive
	Darunavir	Prezista / r*	(10 - 90)	4.13		DRV/r	Sensitive
	Fosamprenavir	Lexiva / r*	(4 - 11)	3.92		AMP/r	Sensitive
	Indinavir	Crixivan / r*	(10)	1.07		IDV/r	Sensitive
	Lopinavir	Kaletra*	(9 - 55)	2.50		LPV/r	Sensitive
	Nelfinavir	Viracept	(3.6)	1.28		NFV	Sensitive
	Ritonavir	Norvir	(2.5)	5.04		RTV	Resistant
	Saquinavir	Invirase / r*	(2.3 - 12)	2.05		SQV/r	Sensitive
	Tipranavir	Aptivus / r*	(2 - 8)	3.07		TPV/r	Partially Sensitive

Lower Clinical Cutoff (in bold)
Upper Clinical Cutoff (in bold)
Biological Cutoff
Hypersusceptibility Cutoff
Sensitive
Partial Sensitivity
Resistance

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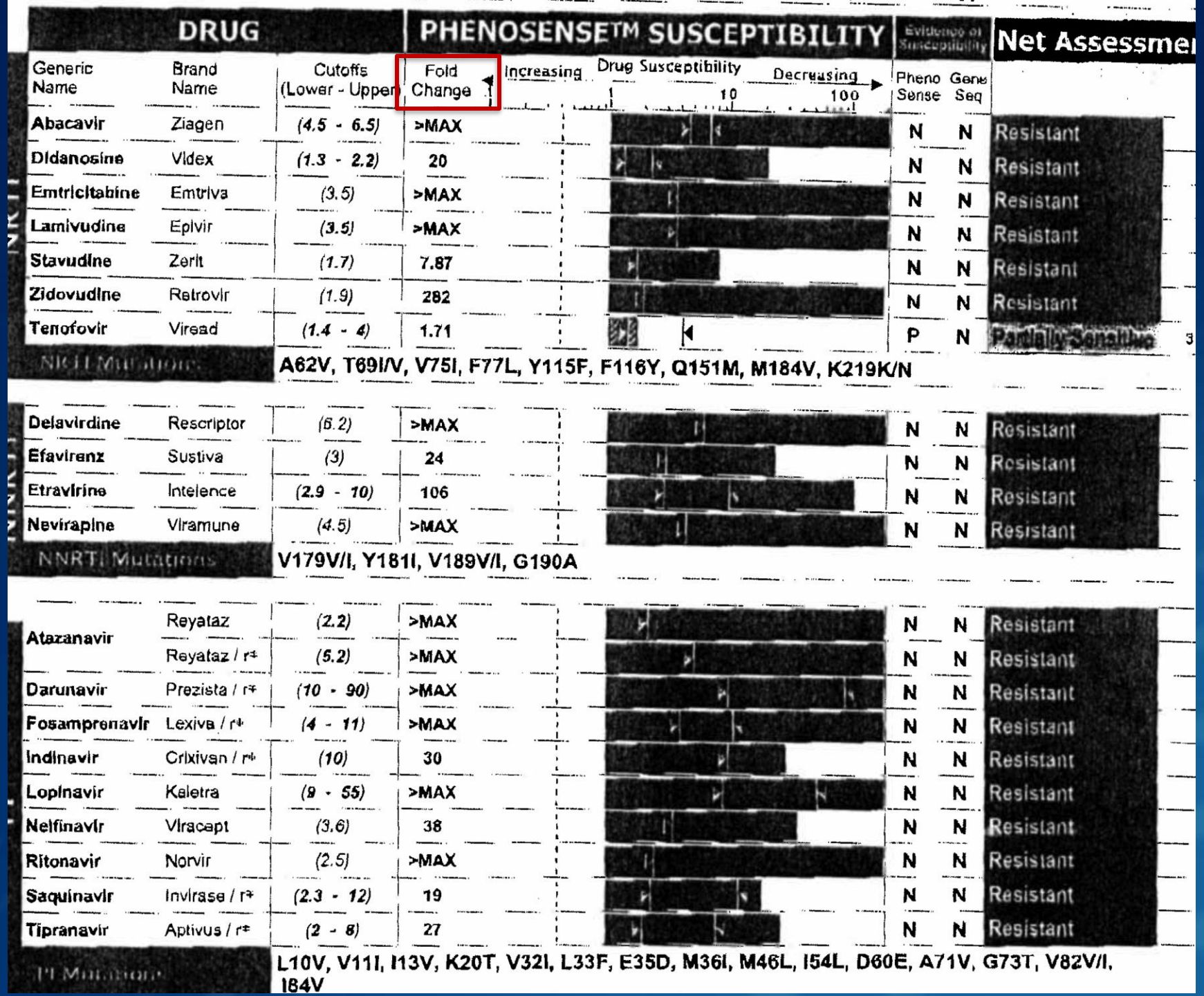
Example phenotype report



DRUG		PHENOSENSE™ SUSCEPTIBILITY				Net Assessment
Generic Name	Brand Name	Cutoffs (Lower - Upper)	Fold Change	Increasing Drug Susceptibility Decreasing (10 – 100)	Pheno Sense	Gene Seq	
Abacavir	Ziagen	*(4.5 - 6.5)*	>MAX		N	N	Resistant
Didanosine	Videx	*(1.3 - 2.2)*	20		N	N	Resistant
Emtricitabine	Emtriva	*(3.5)*	>MAX		N	N	Resistant
Lamivudine	Epivir	*(3.5)*	>MAX		N	N	Resistant
Stavudine	Zerit	*(1.7)*	7.87		N	N	Resistant
Zidovudine	Retrovir	*(1.9)*	282		N	N	Resistant
Tenofovir	Viread	*(1.4 - 4)*	1.71		P	N	Partially Sensitive
NRTI Mutations		**A62V, T69I/V, V75I, F77L, Y115F, F116Y, Q151M, M184V, K219K/N**					
Delavirdine	Rescriptor	*(6.2)*	>MAX		N	N	Resistant
Efavirenz	Sustiva	*(3)*	24		N	N	Resistant
Etravirine	Intelence	*(2.9 - 10)*	106		N	N	Resistant
Nevirapine	Viramune	*(4.5)*	>MAX		N	N	Resistant
NNRTI Mutations		**V179V/I, Y181I, V189V/I, G190A**					
Atazanavir	Reyataz	*(2.2)*	>MAX		N	N	Resistant
	Reyataz / r+	*(5.2)*	>MAX		N	N	Resistant
Darunavir	Prezista / r+	*(10 - 90)*	>MAX		N	N	Resistant
Fosamprenavir	Lexiva / r+	*(4 - 11)*	>MAX		N	N	Resistant
Indinavir	Crixivan / r+	*(10)*	30		N	N	Resistant
Lopinavir	Kaletra	*(9 - 55)*	>MAX		N	N	Resistant
Nelfinavir	Viracept	*(3.6)*	38		N	N	Resistant
Ritonavir	Norvir	*(2.5)*	>MAX		N	N	Resistant
Saquinavir	Invirase / r+	*(2.3 - 12)*	19		N	N	Resistant
Tipranavir	Aptivus / r+	*(2 - 8)*	27		N	N	Resistant
PI Mutations		**L10V, V11I, I13V, K20T, V32I, L33F, E35D, M36I, M46L, I54L, D60E, A71V, G73T, V82V/I, I84V**					

Indications for Genotype Resistance Testing

- **Indication #1: all treatment-naïve patients at entry into care**
  - Frequency of transmitted mutations: 5-15% (mostly NNRTI)
  - Check even if deferring ART
  - Ok to start ART before results return
  - Integrase resistance testing not routinely indicated at baseline

Source: DHHS Adult & Adolescent Treatment Guidelines, https://clinicalinfo.hiv.gov/en/guidelines

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Indications for Genotype Resistance Testing

- **Indication #2: Virologic failure or suboptimal virologic suppression**
  - Virologic failure: HIV RNA rebound to >200 copies/mL (genotype may be unsuccessful if RNA 200-500 copies/mL, but should be considered)
  - For non-long-acting ART, ideally perform genotype within 4 weeks of stopping ART (not always realistic)

Source: DHHS Adult & Adolescent Treatment Guidelines, https://clinicalinfo.hiv.gov/en/guidelines

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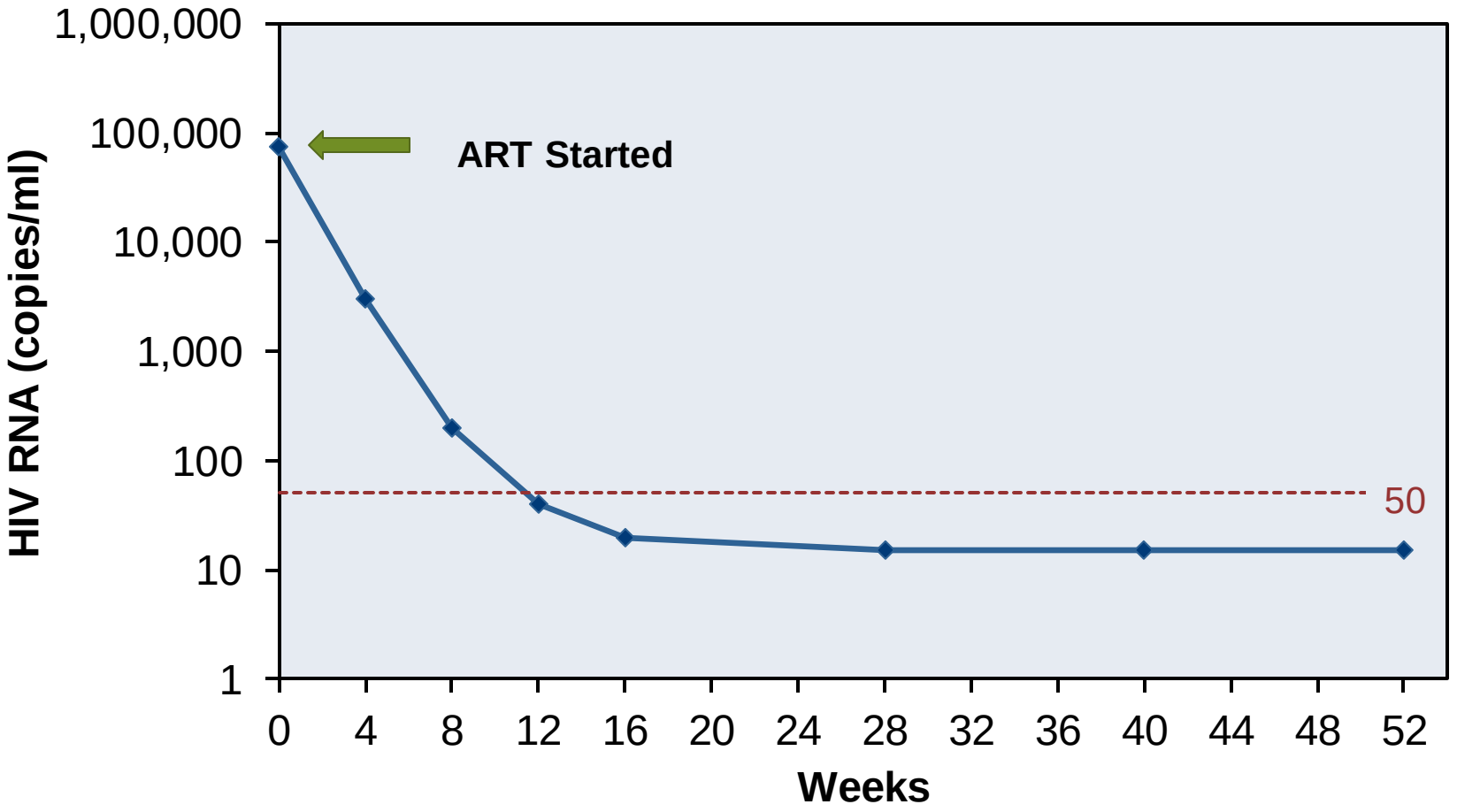
Virologic Responses on Antiretroviral Therapy

Virologic Suppression



A confirmed HIV RNA level below the lower limit of assay detection.

Slide courtesy of Dr. David Spach

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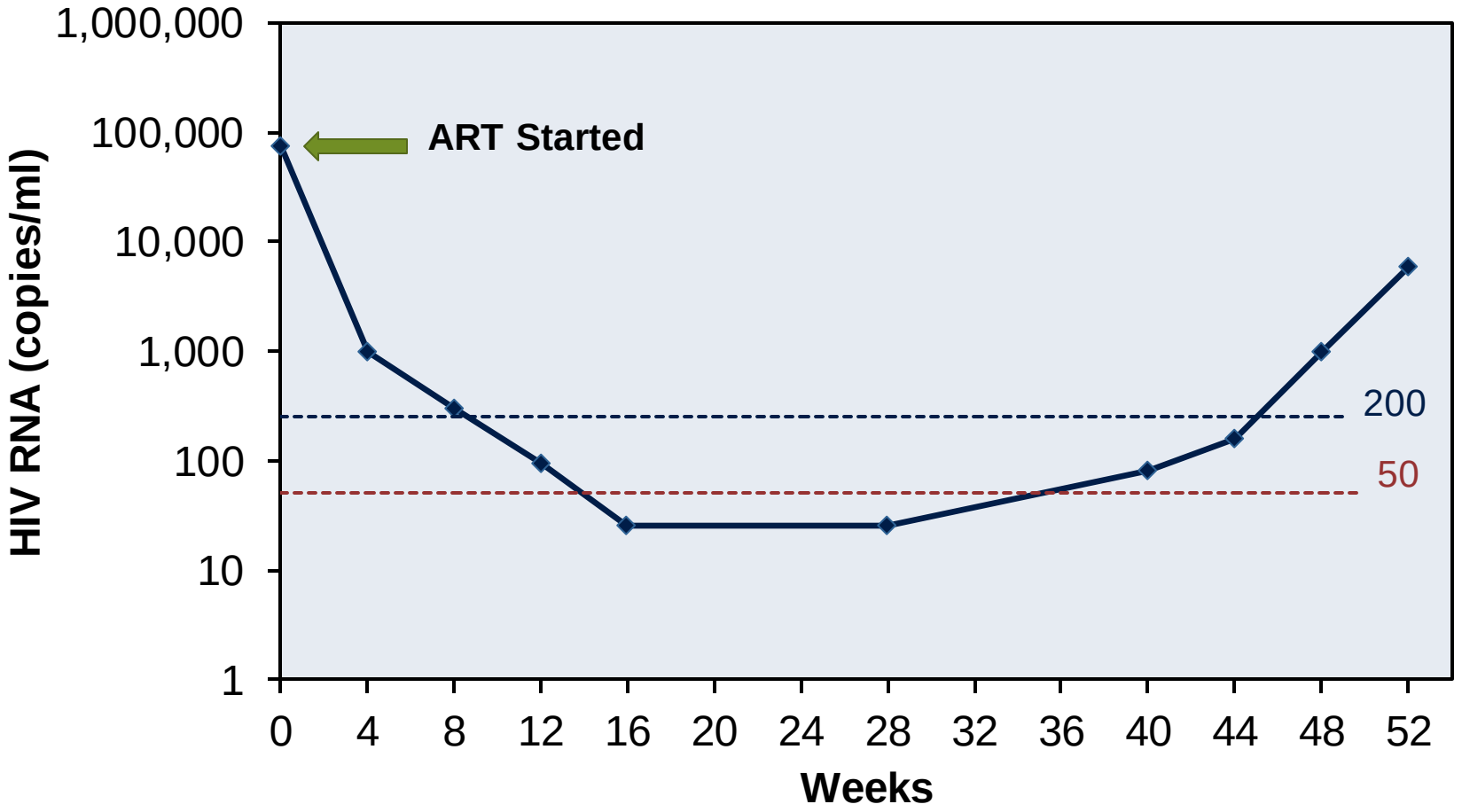
Virologic Responses on Antiretroviral Therapy

Virologic Failure



Confirmed HIV RNA ≥200 copies/mL after virologic suppression

Slide courtesy of Dr. David Spach

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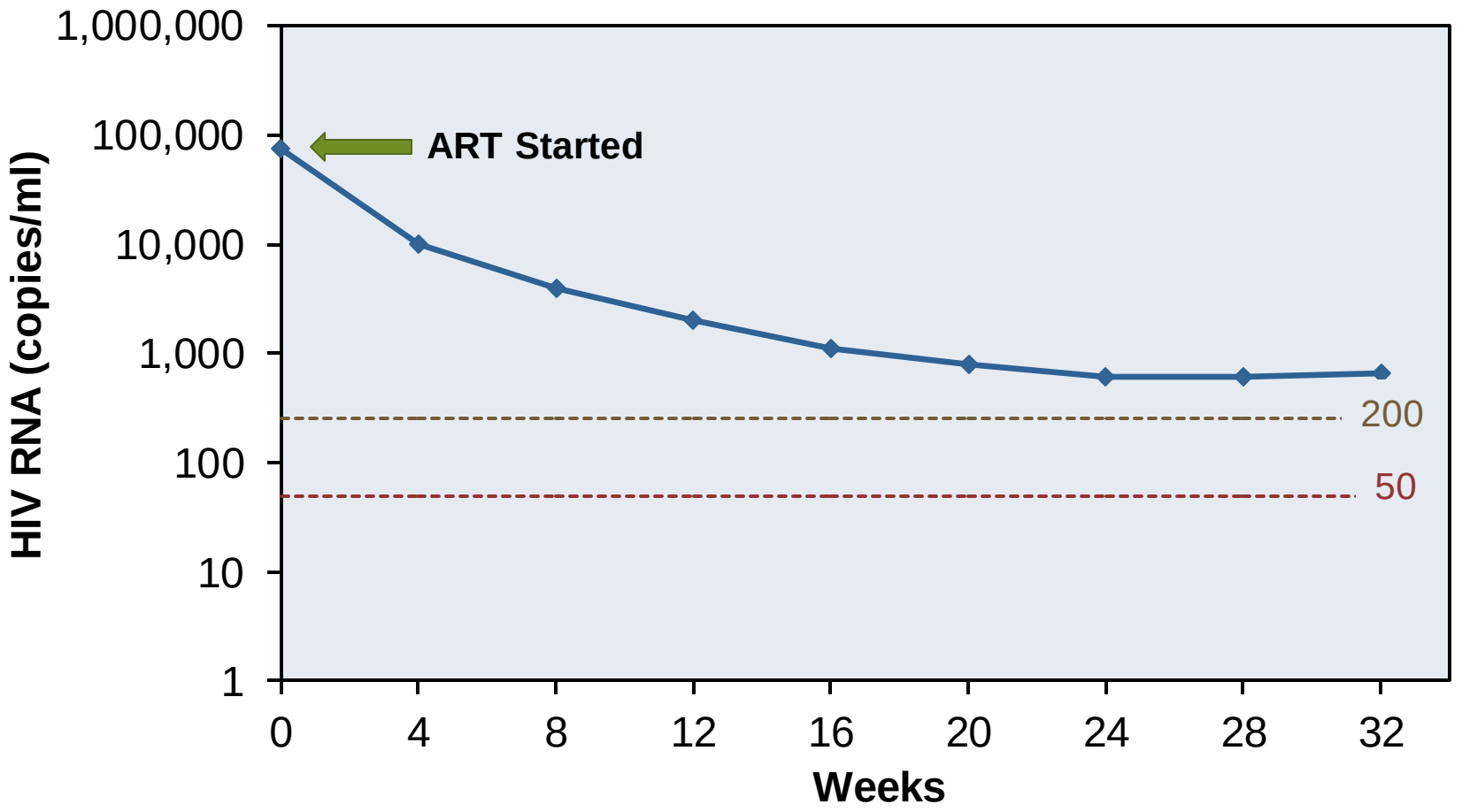
Virologic Responses on Antiretroviral Therapy

Incomplete or Suboptimal Virologic Response



Two consecutive plasma HIV RNA levels >200 copies/mL after 24 weeks on an ARV regimen.

Baseline HIV RNA may affect the time course of response, and some regimens will take longer than others to suppress HIV RNA levels.

Slide courtesy of Dr. David Spach

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Indications for Integrase (IN) Genotype

- **Indication #1**: virologic failure while taking an integrase inhibitor
- **Indication #2**: add to RT/PR genotype at baseline if past integrase inhibitor exposure (prior cabotegravir for PrEP) or integrase inhibitor resistance exposure

*Remember, integrase resistance testing may require a separate order!

Source: DHHS Adult & Adolescent Treatment Guidelines, https://clinicalinfo.hiv.gov/en/guidelines

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Another Genotype Option: PBMC DNA Resistance Testing (also called: archive, DNA, proviral, or PBMC genotype)

- What is it? Sequence mutations in **proviral DNA, instead of plasma RNA**
- Advantage: available at any RNA level, including undetectable
- Disadvantage: less sensitive than cumulative RNA genotypes
  - Why? Takes weeks to months for mutations to accumulate in PBMCs, especially if low HIV RNA levels or periods of virologic failure brief
- **Indication:** taking salvage ART, need resistance data in order to change or simplify regimen, and cannot obtain past RNA genotype results

1. Delaugerre C et al. HIV Medicine. 2012;13:517–525.
2. Chu C, et al. Clin Microbiol Rev. 2022 Dec 21;35(4):e0005222.

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Indications for Phenotype Resistance Testing

- Per guidelines: add to genotype if known or suspected complex mutation pattern
- In practice: almost never

Case

- A 55-year-old patient, who was prescribed rilpivirine/tenofovir alafenamide/emtricitabine, presents after an absence from care. They report missed doses of ART over the prior 3 months. The prior HIV RNA levels were suppressed but a repeat level returns at just over 1,000 copies/mL and an RT/PR genotype resistance assay shows the RT mutations K103N and M184V.
- How can you obtain help interpreting the effects of these mutations?

Stanford University

HIV DRUG RESISTANCE DATABASE

A curated public database to represent, store and analyze HIV drug resistance data.

HOME GENOTYPE-RX GENOTYPE-PHENO GENOTYPE-CLINICAL HIVDB PROGRAM

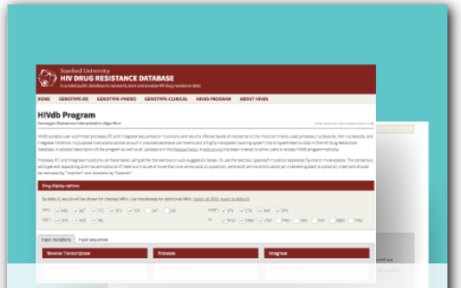
ABOUT HIVDB SUPPORT HIVDB!

SIERRA

Sierra 3.4.2

release notes / web service

Dec 14, 2022



HIVDB Algorithm Version 9.4

Dec 7, 2022

HIV in vitro selection

HIV in vitro selected PR, RT, IN and CA mutations

Mar 13, 2023

JavaScript-based Algorithm Specification Interface (ASI) editor

Aug 18, 2022

Stanford Coronavirus Antiviral & Resistance Database

Experimental data on SARS-CoV-2 variants to monoclonal antibodies, convalescent plasma, and vaccinee plasma

CPR Calibrated Population Resistance

HIVDB released on January 10, 2023

Query / Download

HIVdb Program

https://hivdb.stanford.edu/

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Reverse Transcriptase

Input mutation(s)

Select mutations:

40 ---	41 ---	44 ---	62 ---
65 ---	67 ---	68 ---	69 ---
70 ---	74 ---	75 ---	77 ---
90 ---	98 ---	100 ---	101 ---
103 ---	106 ---	108 ---	115 ---
116 ---	118 ---	138 ---	151 ---
179 ---	181 ---	184 ---	188 ---
190 ---	210 ---	215 ---	219 ---
221 ---	225 ---	227 ---	230 ---
234 ---	236 ---	238 ---	318 ---

Protease

Input mutation(s)

Select mutations:

10 ---	11 ---	13 ---	20 ---
23 ---	24 ---	30 ---	32 ---
33 ---	35 ---	36 ---	43 ---
46 ---	47 ---	48 ---	50 ---
53 ---	54 ---	58 ---	63 ---
71 ---	73 ---	74 ---	76 ---
77 ---	82 ---	83 ---	84 ---
85 ---	88 ---	89 ---	90 ---
93 ---			

Integrase

Input mutation(s)

Select mutations:

51 ---	66 ---	74 ---	92 ---
95 ---	97 ---	114 ---	118 ---
121 ---	128 ---	138 ---	140 ---
143 ---	145 ---	146 ---	147 ---
148 ---	151 ---	153 ---	155 ---
157 ---	163 ---	230 ---	263 ---

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Reverse Transcriptase	Protease	Integrase
K103N x M184V x Input mutation(s)	Input mutation(s)	Input mutation(s)

Select mutations:

40 ---	41 ---	44 ---	62 ---
65 ---	67 ---	68 ---	69 ---
70 ---	74 ---	75 ---	77 ---
90 ---	98 ---	100 ---	101 ---
103 ---	106 ---	108 ---	115 ---
116 ---	118 ---	138 ---	151 ---
179 ---	181 ---	184 ---	188 ---
190 ---	210 ---	219 ---	
221 ---	225 ---	230 ---	
234 ---	236 ---	238 ---	318 ---
348 ---			

184 dropdown options: I, V, *

Select mutations:

10 ---	11 ---	13 ---	20 ---
23 ---	24 ---	30 ---	32 ---
33 ---	35 ---	36 ---	43 ---
46 ---	47 ---	48 ---	50 ---
53 ---	54 ---	58 ---	63 ---
71 ---	73 ---	74 ---	76 ---
77 ---	82 ---	83 ---	84 ---
85 ---	88 ---	89 ---	90 ---
93 ---			

Select mutations:

51 ---	66 ---	74 ---	92 ---
95 ---	97 ---	114 ---	118 ---
121 ---	128 ---	138 ---	140 ---
143 ---	145 ---	146 ---	147 ---
148 ---	151 ---	153 ---	155 ---
157 ---	163 ---	230 ---	263 ---

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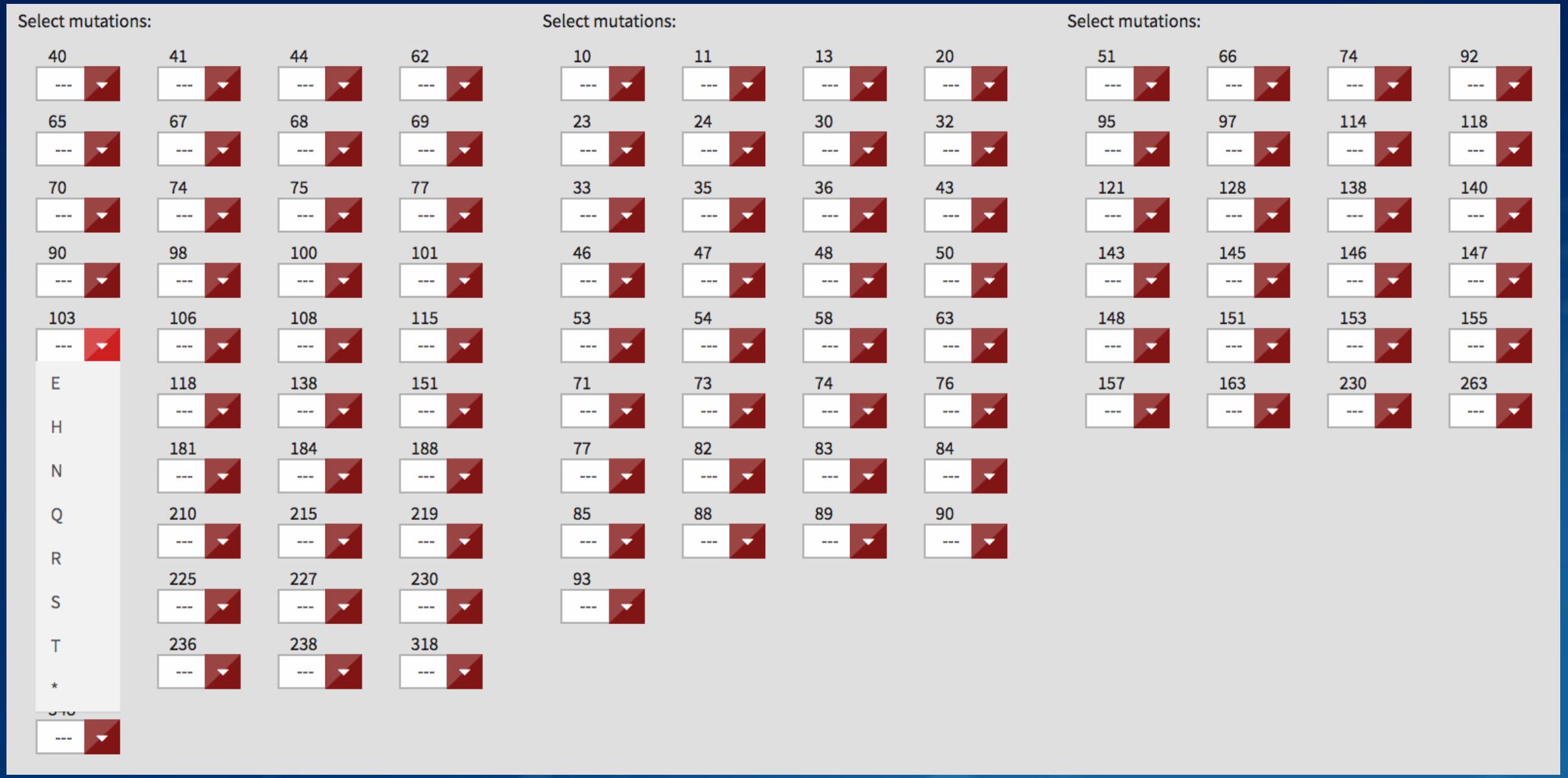
Select mutations:
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Select mutations:
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Select mutations:
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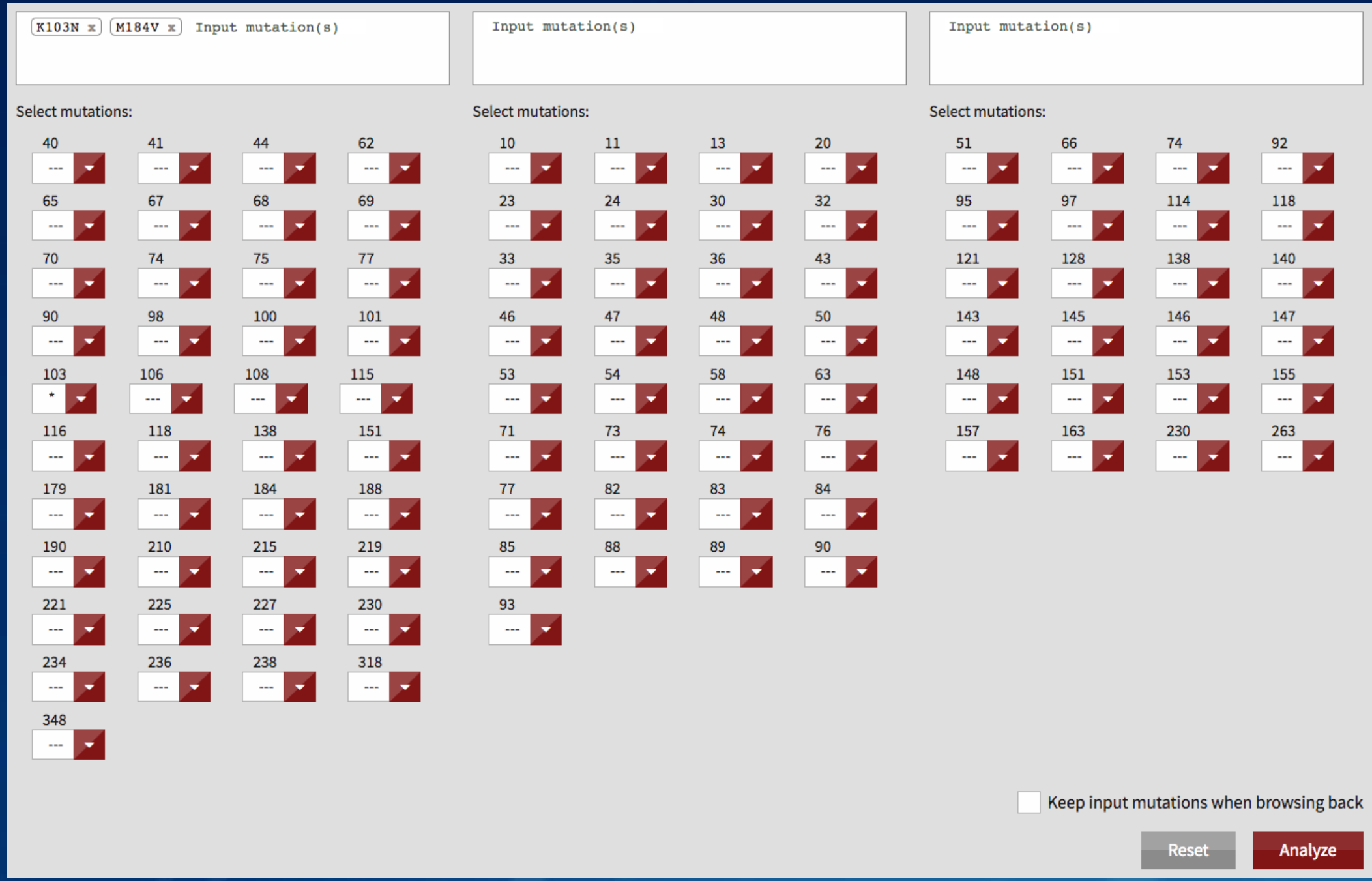
K103N x
M184V x
Input mutation(s)
Input mutation(s)
Input mutation(s)
Select mutations:
40 41 44 62
65 67 68 69
70 74 75 77
90 98 100 101
103 106 108 115
116 118 138 151
179 181 184 188
190 210 215 219
221 225 227 230
234 236 238 318
348
Select mutations:
10 11 13 20
23 24 30 32
33 35 36 43
46 47 48 50
53 54 58 63
71 73 74 76
77 82 83 84
85 88 89 90
93
Select mutations:
51 66 74 92
95 97 114 118
121 128 138 140
143 145 146 147
148 151 153 155
157 163 230 263
Keep input mutations when browsing back
Reset
Analyze

Nucleoside Reverse Transcriptase Inhibitors

abacavir (ABC)	Low-Level Resistance
zidovudine (AZT)	Susceptible
emtricitabine (FTC)	High-Level Resistance
lamivudine (3TC)	High-Level Resistance
tenofovir (TDF)	Susceptible

Non-nucleoside Reverse Transcriptase Inhibitors

doravirine (DOR)	Susceptible
efavirenz (EFV)	High-Level Resistance
etravirine (ETR)	Susceptible
nevirapine (NVP)	High-Level Resistance
rilpivirine (RPV)	Susceptible

RT comments

NRTI

- **M184V/I** cause high-level in vitro resistance to 3TC and FTC and low-level resistance to ddI and ABC. However, **M184V/I** are not contraindications to continued treatment with 3TC or FTC because they increase susceptibility to AZT, TDF and d4T and are associated with clinically significant reductions in HIV-1 replication.

NNRTI

- **K103N** is a non-polymorphic mutation that causes high-level reductions in NVP and EFV susceptibility.

Mutation scoring: RT

Drug resistance mutation scores of NRTI: Copy to clipboard

Rule	ABC	AZT	FTC	3TC	TDF
M184V	15	-10	60	60	-10

Drug resistance mutation scores of NNRTI: Copy to clipboard

Rule	DOR	EFV	ETR	NVP	RPV
K103N	0	60	0	60	0

*Scores <10 indicate susceptible; scores 10-14 indicate potential low-level resistance; scores 15-29 indicate low-level resistance; scores 30-59 indicate intermediate resistance; scores 60 or higher indicate high-level resistance.

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Case

- A 31-year-old patient, who was prescribed elvitegravir/cobicistat/tenofovir alafenamide/emtricitabine, presents for a first visit after transferring care. They report that due to a move and other factors they were taking their medication every other day for about 3 months and then were completely out for about 3 months. Lab testing is performed, including an RT/PR genotype and IN genotype. These demonstrate the RT mutations M184V, K65R, and L74V, plus IN mutations Q148H/K and G140A/S.
- How would you interpret these resistance mutations?

Reverse Transcriptase	Protease	Integrase
K65R ✕ L74V ✕ M184V ✕ Input mutation(s)	Input mutation(s)	G140AS ✕ Q148HK ✕ Input mutation(s)

Drug resistance interpretation: RT

NRTI Resistance Mutations:	**K65R, L74V, M184V**
NNRTI Resistance Mutations:	None
Other Mutations:	None

Nucleoside Reverse Transcriptase Inhibitors

abacavir (ABC)	High-Level Resistance
zidovudine (AZT)	Susceptible
emtricitabine (FTC)	High-Level Resistance
lamivudine (3TC)	High-Level Resistance
tenofovir (TDF)	Intermediate Resistance

Non-nucleoside Reverse Transcriptase Inhibitors

doravirine (DOR)	Susceptible
efavirenz (EFV)	Susceptible
etravirine (ETR)	Susceptible
nevirapine (NVP)	Susceptible
rilpivirine (RPV)	Susceptible

RT comments

NRTI

- **K65R** causes intermediate/high-level resistance to TDF, ddI, ABC and d4T and low/intermediate resistance to 3TC and FTC. **K65R** increases susceptibility to AZT.
- **L74V/I** cause high-level resistance to ddI and intermediate resistance to ABC.
- **M184V/I** cause high-level in vitro resistance to 3TC and FTC and low-level resistance to ddI and ABC. However, **M184V/I** are not contraindications to continued treatment with 3TC or FTC because they increase susceptibility to AZT, TDF and d4T and are associated with clinically significant reductions in HIV-1 replication.

Mutation scoring: RT

Drug resistance mutation scores of NRTI:

Copy to clipboard

Rule	ABC	AZT	FTC	3TC	TDF
K65R	45	-10	30	30	50
L74V	30	0	0	0	0
L74V + M184V	15	0	0	0	0
M184V	15	-10	60	60	-10
Total	105	-20	90	90	40

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Drug resistance interpretation: IN

IN Major Resistance Mutations:	**G140AS, Q148HK**
IN Accessory Resistance Mutations:	None
Other Mutations:	None

Integrase Strand Transfer Inhibitors

bictegravir (BIC)	Intermediate Resistance
dolutegravir (DTG)	Intermediate Resistance
elvitegravir (EVG)	High-Level Resistance
raltegravir (RAL)	High-Level Resistance

IN comments

IN Major

- **G140S/A/C** are non-polymorphic mutations that usually occur with Q148 mutations. Alone, they have minimal effects on INSTI susceptibility. However, in combination with Q148 mutations they are associated with high-level resistance to RAL and EVG and intermediate reductions in DTG and BIC susceptibility.
- **Q148H/K/R** are non-polymorphic mutations selected by RAL, EVG, and rarely DTG. **Q148H/R/K** are associated with high-level reductions in RAL and EVG susceptibility particularly when they occur In combination with E138 or G140 mutations. Alone, **Q148H/K/R** have minimal effects on DTG and BIC susceptibility. But in combination with E138 and G140 mutations they cause moderate and occasionally high-level reductions in DTG and BIC susceptibility.

Dosage Considerations

- There is evidence for intermediate **DTG** resistance. If **DTG** is used, it should be administered twice daily.

Mutation scoring: IN

Drug resistance mutation scores of INSTI:

Copy to clipboard

Rule	BIC	CAB	DTG	EVG	RAL
G140AS	10	10	10	30	30
G140AS + Q148HK	10	20	10	0	0
Q148HK	30	50	30	60	60
Total	50	80	50	90	90

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Take-Home Points

- Genotype is the principal resistance test used in clinical care
  - Indicated for all at baseline (integrase testing not routinely indicated)
  - Also indicated for virologic failure or incomplete virologic response
  - If virologic failure occurs while taking integrase inhibitor, add integrase testing
  - Genotype of proviral DNA in PBMC (aka, archive genotype) rarely indicated
- Stanford Database (db) is a powerful tool for interpreting & learning mutations
  - Remember to enter all resistance mutations from all past genotype tests!

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Other Resources for Learning Key Mutations

- National HIV Curriculum module Evaluating and Managing Virologic Failure: https://www.hiv.uw.edu
- Project ECHO video archive: https://www.youtube.com/@MWAETCProjectECHO/videos
- Prior relevant ECHO talks:
  - Introduction to HIV Resistance Testing (Spach)
  - NNRTI Resistance (Wood)
  - NNRTI Resistance 2015 (Spach)
  - NRTI Resistance (Wood)
  - Resistance to Integrase Strand Transfer Inhibitors (Spach)
  - Recent Trials of Second-Line ART (Wood)
  - Management of NRTI Resistance (Spach)

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