HIV Drug Resistance Testing & Interpretation

Brian R. Wood, MD
Medical Director, MWAETC Project ECHO
Professor of Medicine, University of Washington

Last Updated: October 9, 2025

Disclosures

No conflicts of interest or relationships to disclose.

Disclaimer

Funding for this presentation was made possible by 5 TR7HA53202-02-00 from the Human Resources and Services Administration HIV/AIDS Bureau. The views expressed do not necessarily reflect the official policies of the Department of Health and Human Services nor does mention of trade names, commercial practices, or organizations imply endorsement by the U.S. Government. *Any trade/brand names for products mentioned during this presentation are for training and identification purposes only.*

AETC AIDS Education & Training Center Mountain West

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

Resistance Test Steps & Comparison

Genotype*	Phenotype*
Amplify RNA, sequence *pol* gene (reverse transcriptase, protease, +/- integrase), compare to wild type, find amino acid changes (including those known to confer resistance to ARVs)	Amplify RNA, sequence *pol* gene, insert into lab virus, grow virus in culture, add ARVs 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 can be challenging	Helpful if complex resistance history, especially to protease inhibitors (PI)

*Both types: need circulating RNA; resistance only detected if >10-20% of virus population
**IC_{50} = drug concentration required to inhibit viral replication by 50%

Sources: HHS Adult & Adolescent Treatment Guidelines, National HIV Curriculum (hiv.uw.edu)

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

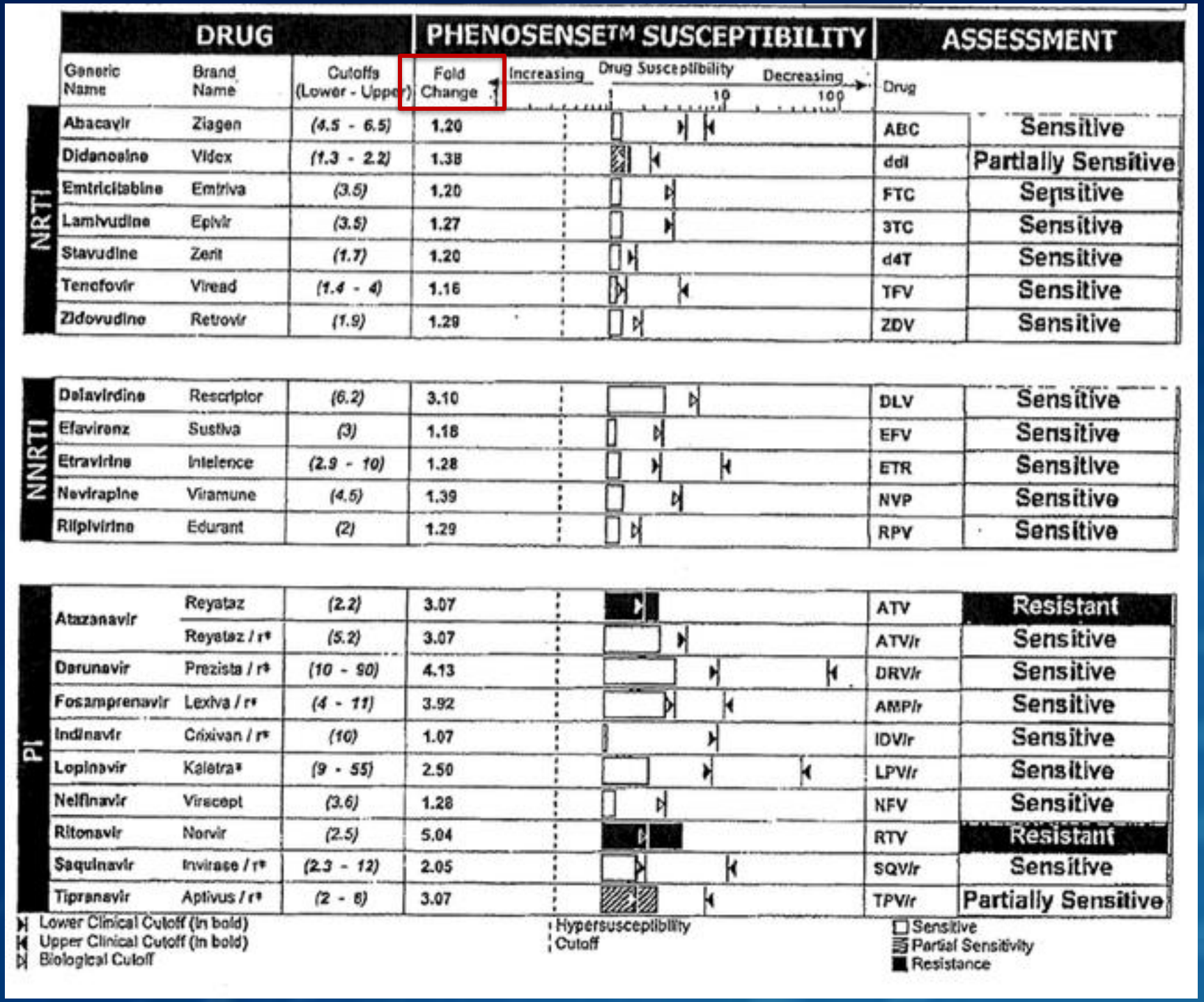
NNRTI associated resistance mutations found: K103N

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

MWAETC

Example phenotype report

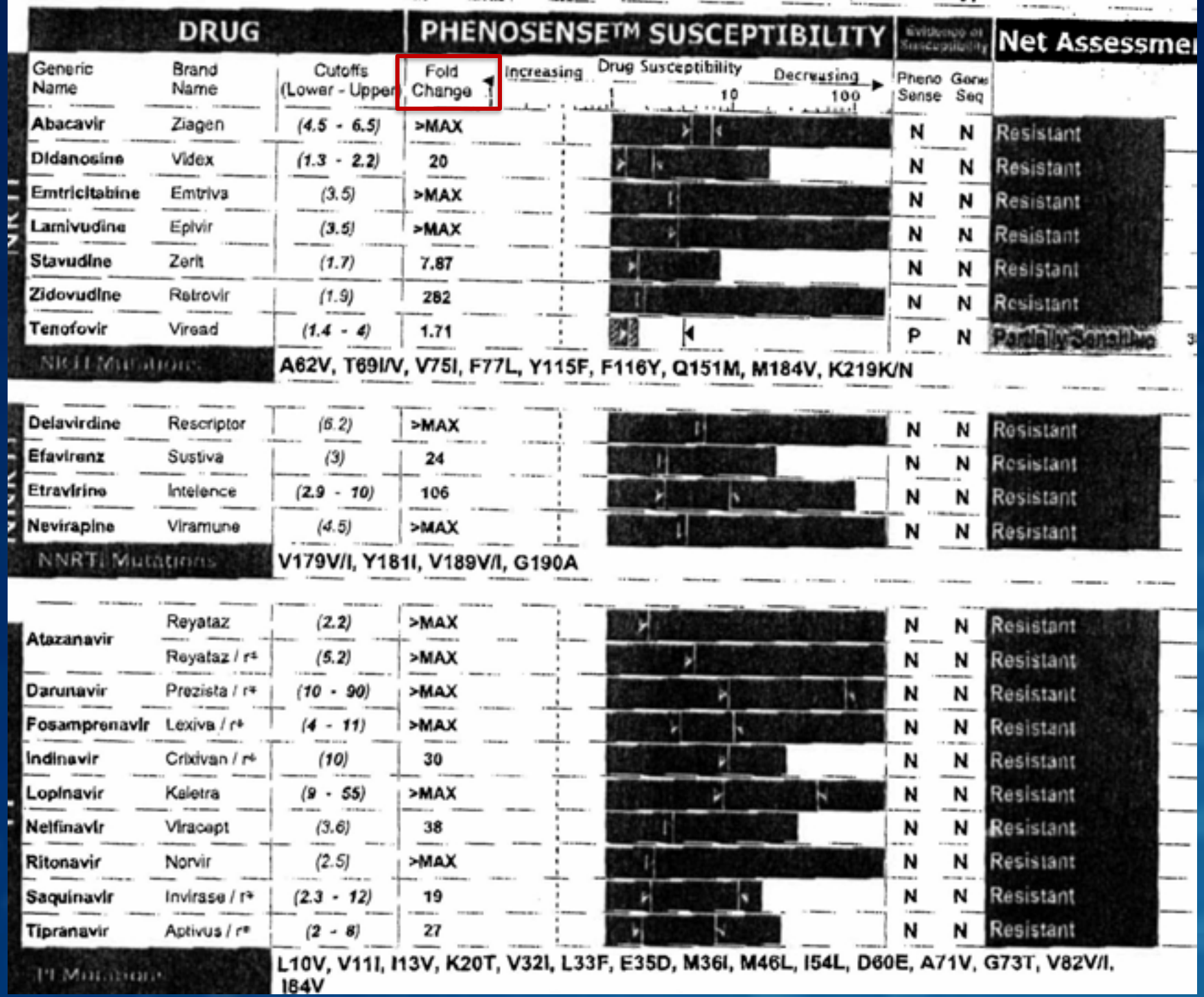


	DRUG			PHENOSENSE™ SUSCEPTIBILITY		ASSESSMENT	
	Generic Name	Brand Name	Cutoffs (Lower - Upper)	Fold Change	Increasing Drug Susceptibility Decreasing (1 / 10 / 100)	Drug	
NRTI	Abacavir	Ziagen	(4.5 - 6.5)	1.20		ABC	Sensitive
	Didanosine	Videx	(1.3 - 2.2)	1.38		ddI	Partially Sensitive
	Emtricitabine	Emtriva	(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

MWAETC

Example phenotype report



DRUG		PHENOSENSE™ SUSCEPTIBILITY				Net Assessment
Generic Name	Brand Name	Cutoffs (Lower - Upper)	Fold Change ↑	Increasing Drug Susceptibility Decreasing (1 / 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[a]	(5.2)	>MAX		N	N	Resistant
Darunavir	Prezista / r[a]	(10 - 90)	>MAX		N	N	Resistant
Fosamprenavir	Lexiva / r[a]	(4 - 11)	>MAX		N	N	Resistant
Indinavir	Crixivan / r[a]	(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[a]	(2.3 - 12)	19		N	N	Resistant
Tipranavir	Aptivus / r[a]	(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 Standard RNA Genotype Resistance Testing Reverse Transcriptase & Protease

- **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
  - Also public health purposes (surveillance, transmission networks, inform guidelines)

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

Indications for Standard RNA Genotype Resistance Testing Reverse Transcriptase & Protease

- **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 attempted)
  - Suboptimal virologic suppression: HIV RNA does not decrease to <200 copies/mL despite adherence to ART

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

Indications to Add Integrase Genotype Resistance Testing
Note: May Require Separate Lab Order

- **Indication #1**: virologic failure while taking an integrase inhibitor
- **Indication #2**: Check for integrase resistance at baseline if:
  - 1) Suspected transmitted integrase resistance, or
  - 2) History of integrase inhibitor use for PrEP or PEP

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

Another Genotype Option: PBMC DNA Resistance Testing (a.k.a. Archive, DNA, proviral, or PBMC genotype)

- **Use whole blood proviral DNA (integrated into viral chromosome)**
- Indication: need resistance data to make ART change and cannot get RNA data
- Advantage: available at any RNA level, including not detected
- Disadvantage: less sensitive than cumulative RNA genotype data because…
  - Mutations emerge first in circulating RNA; takes time to accumulate in PBMCs
  - High concordance only if high level of viral replication circulating for sufficient time
  - Reservoir is dynamic & mutations are lost with longer viral suppression time

Sources: 1) Delaugerre C et al. HIV Medicine. 2012;13:517–525. 2) Chu C, et al. Clin Microbiol Rev. 2022;35(4):e0005222. 3) Campagna R, et al. Viruses. 2024;16(11):1697.

Indications for Phenotype Drug Resistance Testing

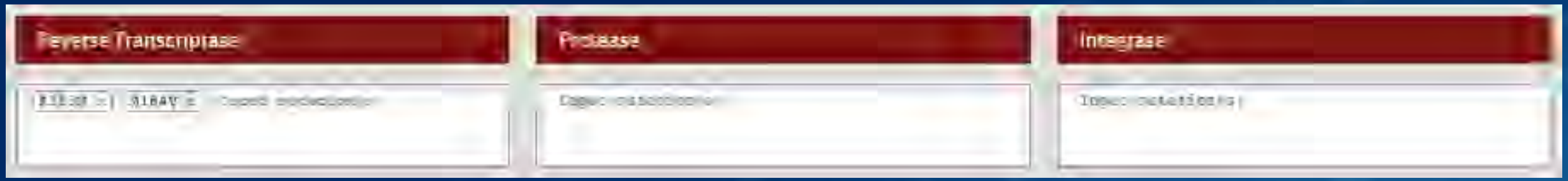
- Per guidelines: add to genotype if known or suspected complex mutation pattern
- In practice: almost never nowadays

How best to interpret genotype resistance results?

https://hivdb.stanford.edu/

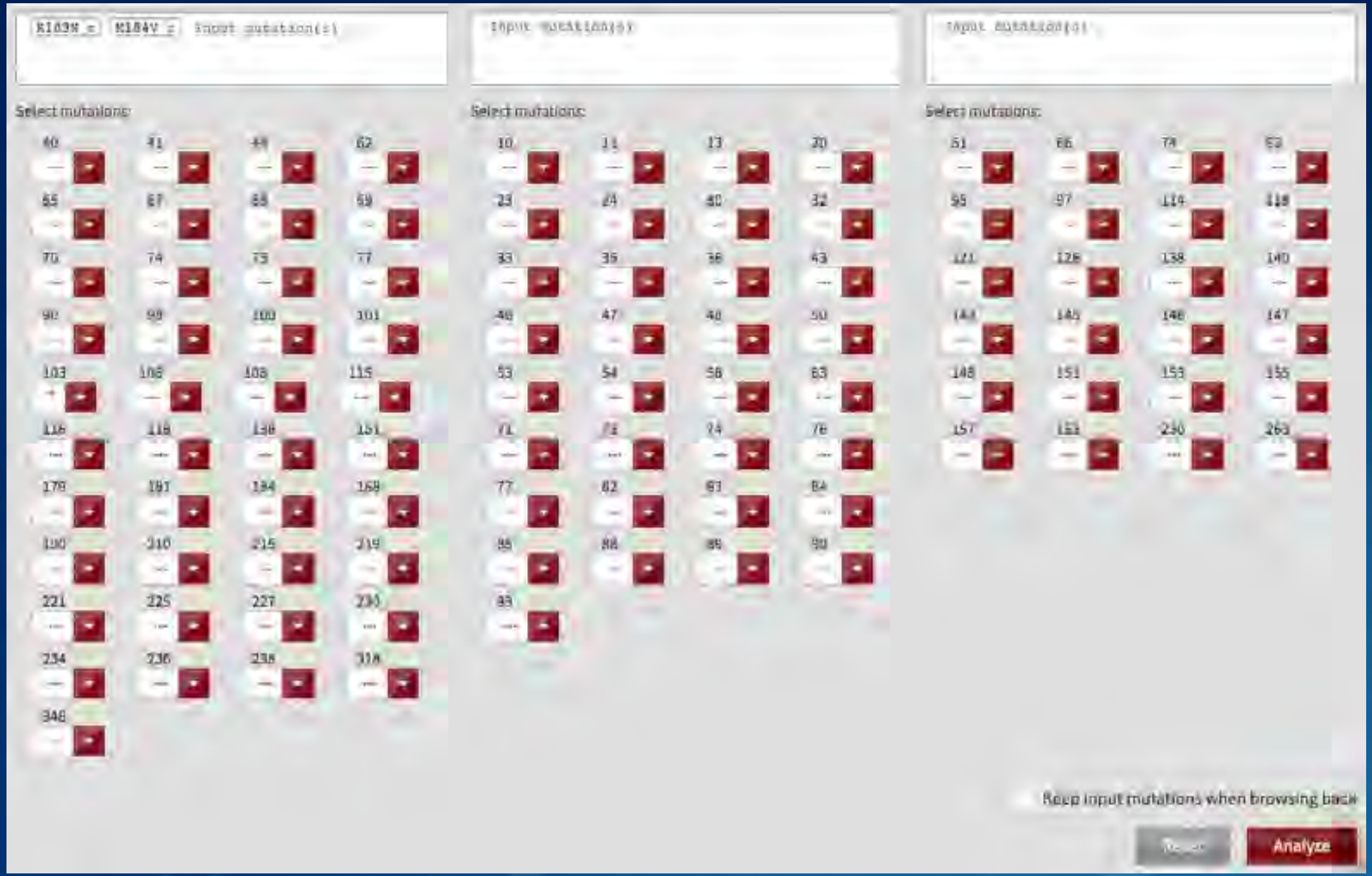
MWAETC


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
109
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
32
32
33
35
43
46
47
48
50
54
56
60
71
73
74
76
77
82
83
84
85
88
89
90
99
Integrase
Input mutation(s)
Select mutations:
97
98
114
118
121
128
138
140
143
145
146
147
148
151
153
155
157
166
230
263


Reverse Transcriptase
Protease
Integrase


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
316
348
Select mutations:
10
11
13
20
23
24
30
32
33
35
36
43
46
47
48
50
53
54
68
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
156
157
163
230
263


Select mutations:
40
41
44
62
65
67
68
69
70
74
75
77
90
98
100
101
103
106
108
115
110
138
151
181
184
188
210
215
219
225
227
230
236
238
318
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
148
147
148
151
153
155
157
163
230
263


M184V
Select mutations:
Select mutations:
Select mutations:
Keep input mutations when browsing back
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.

MWAETC

Mutation scoring: RT

Drug resistance mutation scores of NRTI: Copy to clipboard

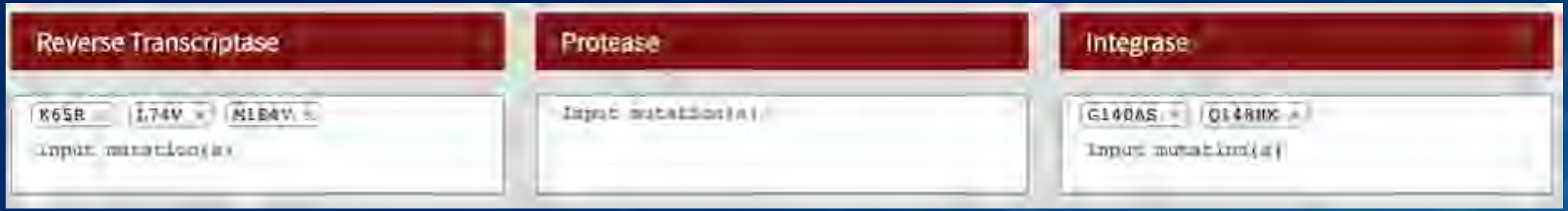
Rule	ABC	AZT	FTC	3TC	TDF
M184V	15	-10	60	60	-10

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.

MWAETC


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

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

MWAETC

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 is a powerful tool for interpreting & learning mutations
  - Remember to enter all resistance mutations from all past genotype tests!

Acknowledgment

This Mountain West AIDS Education and Training (MWAETC) program is supported by the Health Resources and Services Administration (HRSA) of the U.S. Department of Health and Human Services (HHS) as part of award 5 TR7HA53202-02-00 totaling $2,820,772 with 0% financed with non-governmental sources.

AETC AIDS Education & Training Center Mountain West