Introduction to HIV Drug Resistance

Kevin L. Ard, MD, MPH
Massachusetts General Hospital
Harvard Medical School

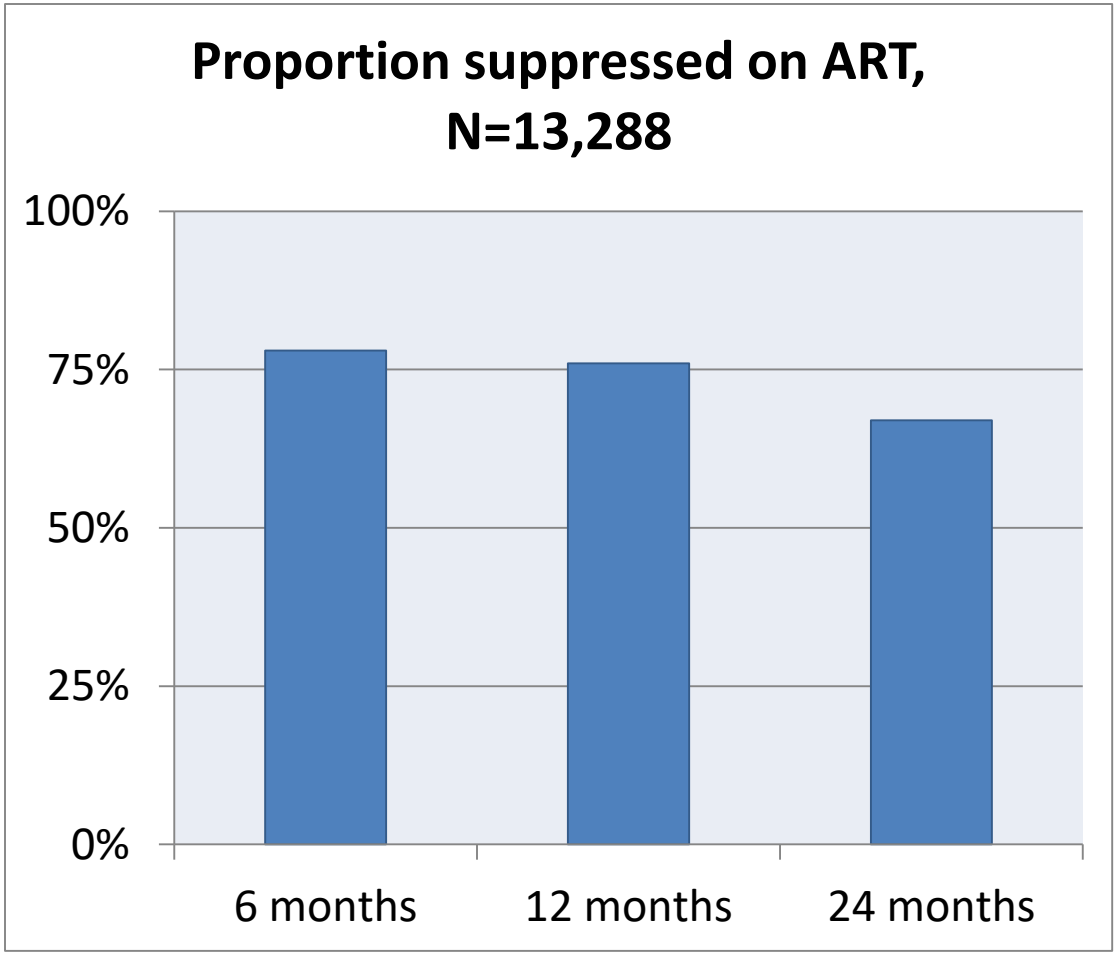
Objectives

1. Describe the epidemiology of HIV drug resistance in sub-Saharan Africa.
2. Summarize important principles in the management of patients with drug resistance.
3. Review resistance assays and features of NRTI, NNRTI, PI, and INSTI resistance.
4. Discuss common approaches to the management of patients failing antiretroviral therapy with resistance.

I have used a resistance test to select ART for a patient failing treatment.

A. Yes
B. No

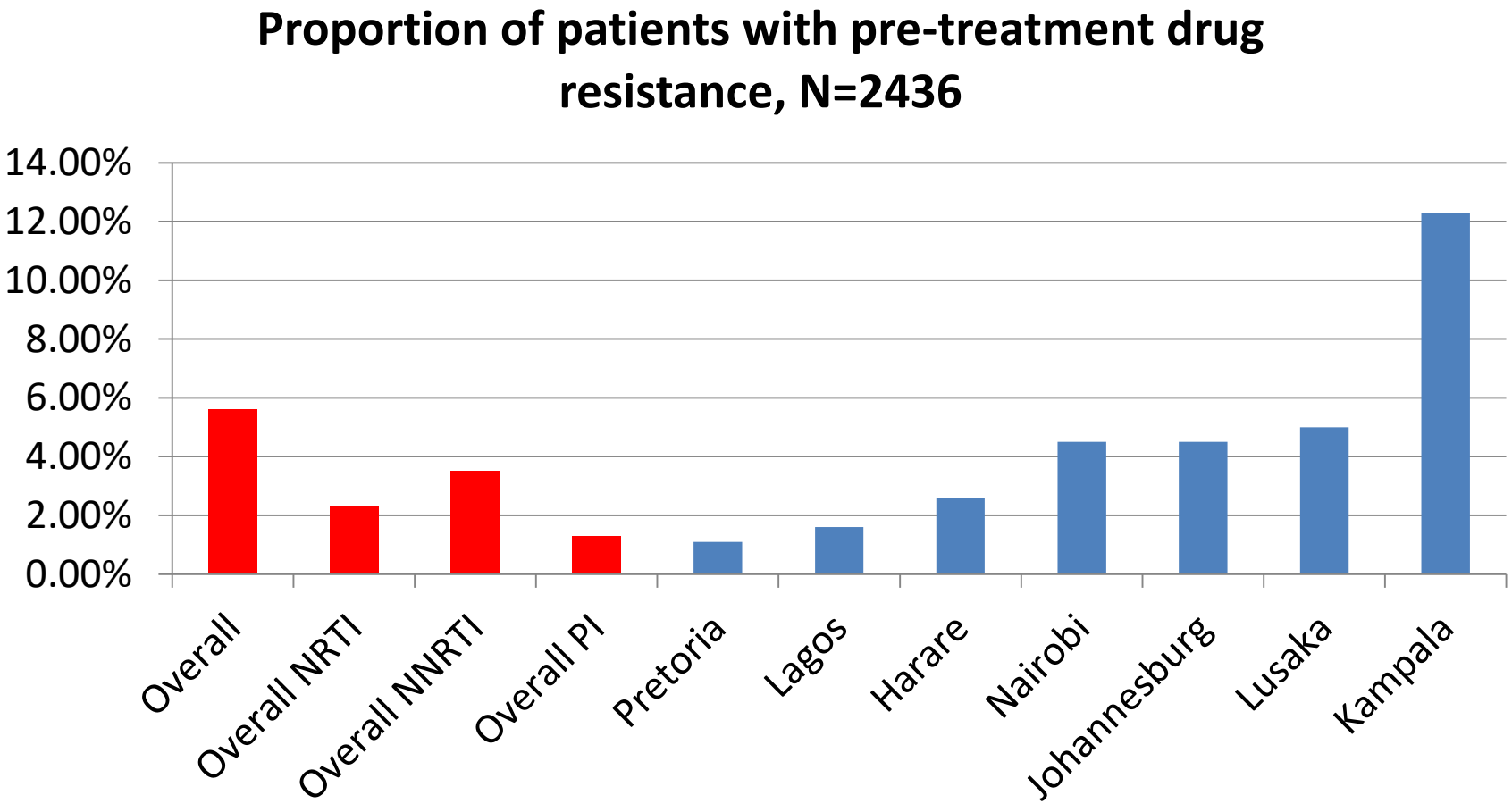
67% of people remain suppressed after 24 months of ART.



Barth RE, van der Loeff MF, Schurrman R, Hoepelman AI, Wensing AM. Virologic follow-up of adult patients in antiretroviral treatment programs in sub-Saharan Africa: a systematic review. Lancet Infect Dis. 2010;10(3):155.

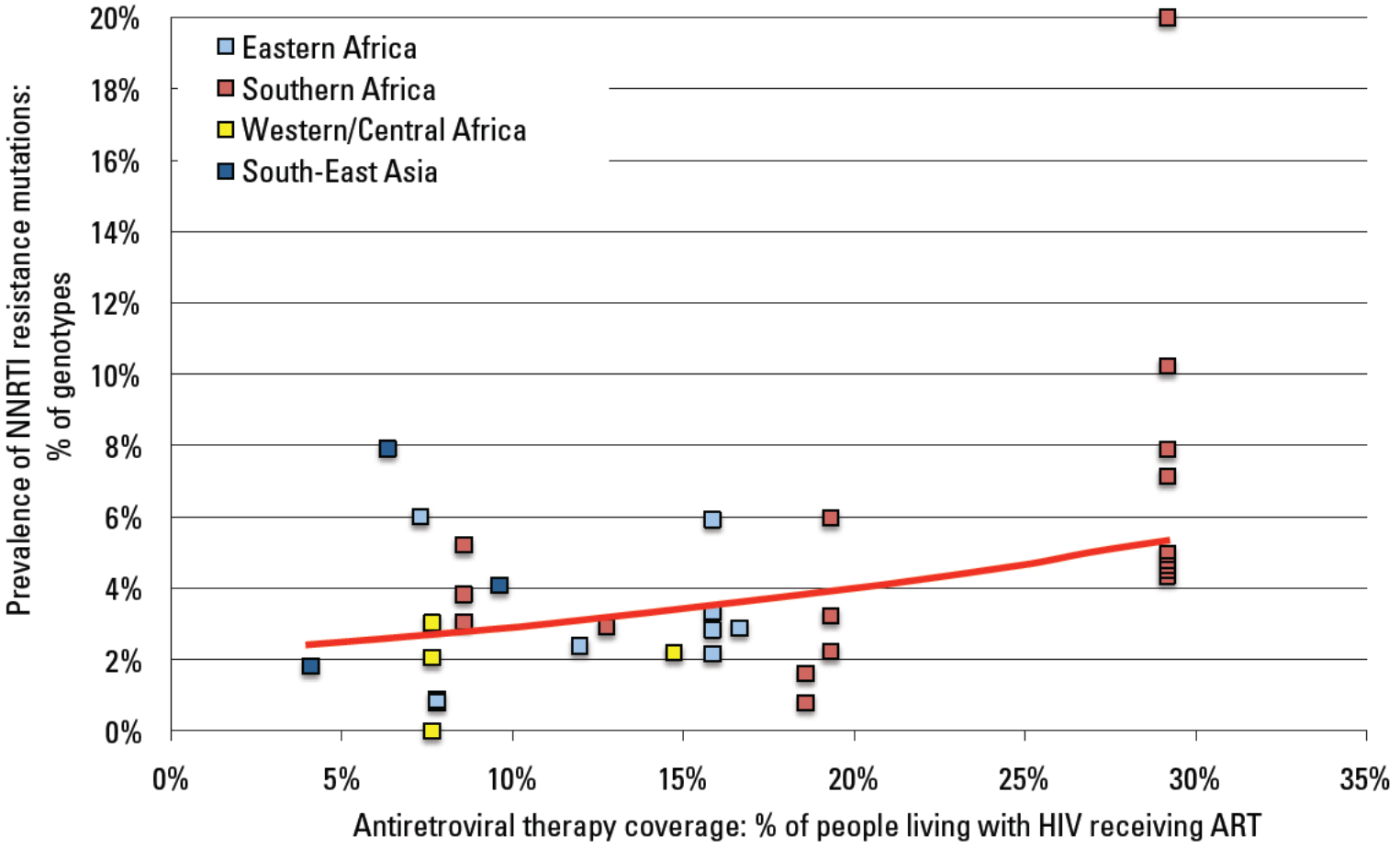
Pre-treatment resistance

The frequency of pre-treatment drug resistance varies across SSA.



Hamers RL, Wallis CL, Kityo C, et al. HIV-1 drug resistance in antiretroviral-naïve individuals in sub-Saharan Africa after rollout of antiretroviral therapy: a multicentre observational study. Lancet Infect Dis. 2011;11(10):750.

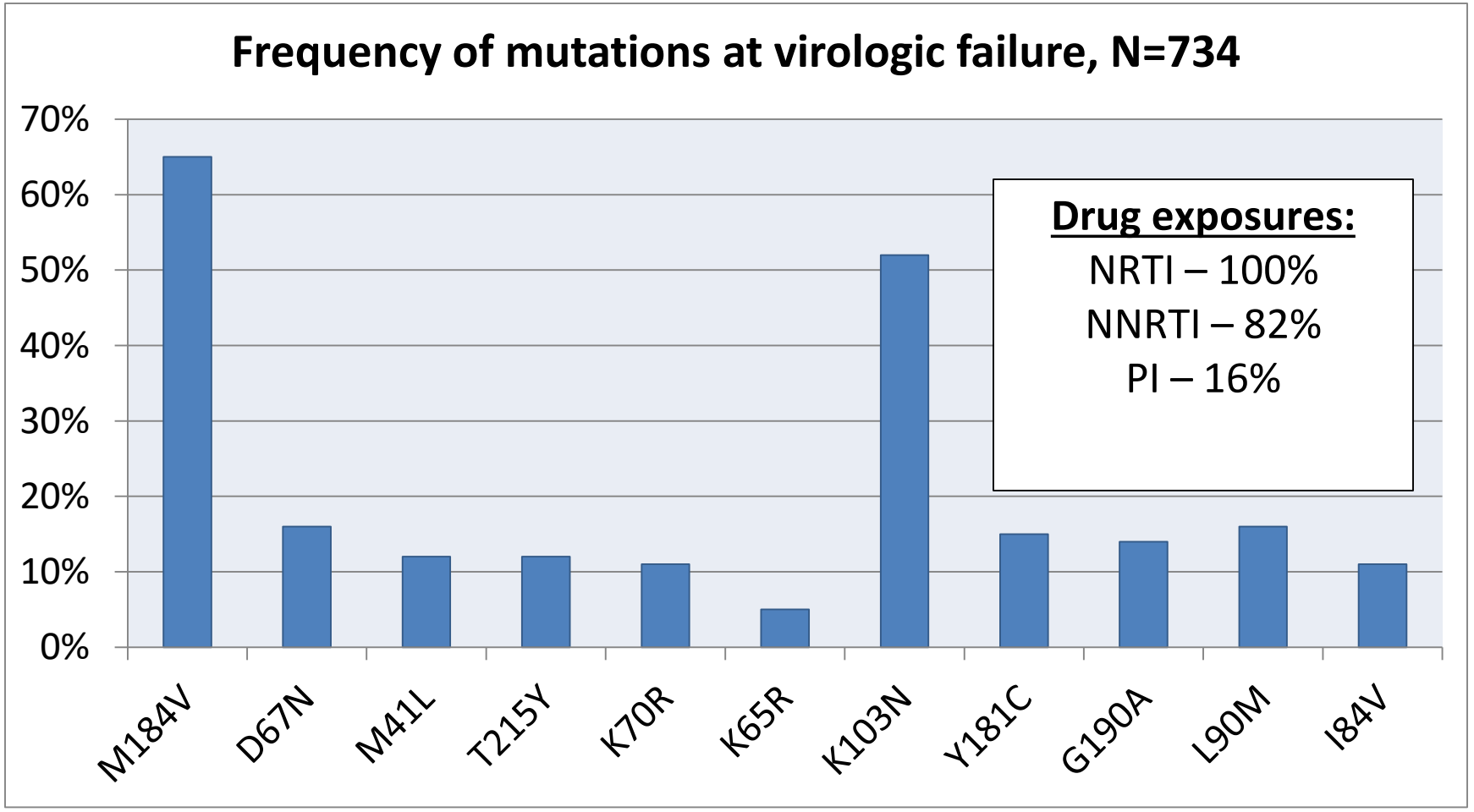
Pre-treatment resistance increases modestly with ART roll-out.



WHO HIV drug resistance report 2012. WHO. 2012. Available at: http://apps.who.int/iris/bitstream/10665/75183/1/9789241503938_eng.pdf?ua=1.

Resistance upon virologic failure

A majority of those with virologic failure have drug resistance.



Barth RE, van der Loeff MF, Schurrman R, Hoepelman AI, Wensing AM. Virologic follow-up of adult patients in antiretroviral treatment programs in sub-Saharan Africa: a systematic review. Lancet Infect Dis. 2010;10(3):155.

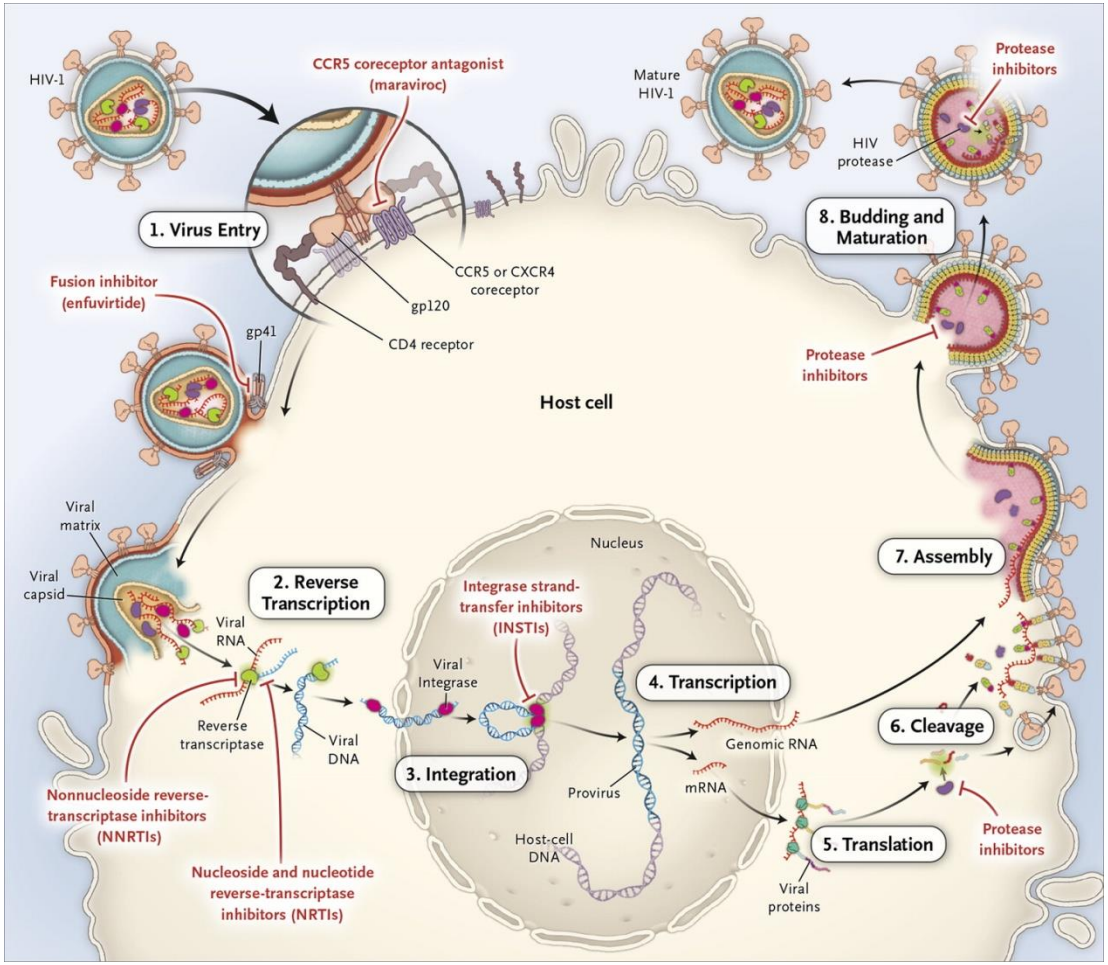
Resistance basics

HIV is a mutation-prone virus.

- HIV has a high intrinsic mutation rate.
- In an untreated patient, there are multiple viral populations.
- Drug-resistant variants can emerge when:
  - Antiretroviral (ART) adherence is poor
  - Drug-drug or drug-food interactions hamper ART effectiveness
  - ART is insufficiently potent
  - A person contracts a resistance virus (transmitted resistance)

Cuevas JM, Geller R, Garijo R, Lopez-Aldeguer J, Sanjuan R. Extremely high mutation rate of HIV-1 in vivo. PLoS Biol. 2015;13(9):e1002251.

Mutations consist of changes in the amino acid chain of ART targets.



Gandhi M, Gandhi RT. Single-pill combination regimens for treatment of HIV-1 infection. N Engl J Med. 2014;371:248.

Mutations are named with a number and two letters.

Note: M184V,I or M184V/I indicates substitution of either V or I for M.

Resistance testing

Which is true about genotypic resistance testing?

A. The viral load must be at least ~1000 for the test to be performed.
B. The test can detect viral populations that make up 1% of the sample.
C. Genotypic resistance testing measures resistance directly.
D. A patient should stop a failing ART regimen before resistance testing is performed.

Genotypic resistance testing

- Genotypic resistance testing is most common.
- It involves sequencing HIV genes to detect resistance-causing mutations.
- It requires HIV RNA of > 500-1000 copies/mL and detects species constituting > 20% of the sample.
- Results are reported as amino acid changes (e.g., M184V) with an accompanying interpretation (e.g., “high-level resistance”).

HIV Drug Resistance Genotyping Report:

Method	In-house assay
Reason for testing	2nd line failure
Result	HIV-1 resistance mutation(s) detected

Drug Resistance Interpretation: PR

PI Major resistance mutations	M46L	I50V	I54V	V82A
PI Minor resistance mutations	L23I	A71V		

Protease Inhibitors:

Atazanavir/r (ATV/r)	High-level resistance
Darunavir/r (DRV/r)	Low-level resistance
Fosamprenavir/r (FPV/r)	High-level resistance
Indinavir/r (IDV/r)	High-level resistance
Lopinavir/r (LPV/r)	High-level resistance
Nelfinavir/r (NFV)	High-level resistance
Saquinavir/r (SQV/r)	High-level resistance
Tipranavir/r (TPV/r)	Low-level resistance

Drug Resistance Interpretation: RT

NRTI resistance mutations	M41L	M184V	T215F
NNRTI resistance mutations	K101H	K103N	G190A

Nucleoside RTI:

Lamivudine (3TC)	High-level resistance
Abacavir (ABC)	Intermediate resistance
Zidovudine (AZT)	High-level resistance
Stavudine (D4T)	High-level resistance

Phenotypic resistance testing

- Assesses the IC50 (concentration of ART required to inhibit 50% of viral growth).
- May not detect minority variants amounting to less than 20% of the sample.
- Is more expensive and has a longer turnaround time than genotypic assays.
- May be helpful when many mutations are present.
- Generates a fold change comparing the IC50 of a patient's virus with a reference virus.

DRUG			PHENOSENSE® SUSCEPTIBILITY		Evidence of Susceptibility		NET ASSESSMENT	
Generic Name	Brand Name	Cutoffs (Lower - Upper)	Fold Change	◄ Increasing Drug Susceptibility Decreasing ► (.1 / 1 / 10 / 100)	Pheno Sense	Gene Seq		
PI								
Atazanavir	Reyataz	*(2.2)*	**11**		**N**	**N**	Resistant	1
	Reyataz / r†	*(5.2)*	**11**		**N**	**N**	Resistant	1
Darunavir	Prezista / r†	*(10 - 90)*	**5.89**		**Y**	**N**	**Sensitive**	
Fosamprenavir	Lexiva / r‡	*(4 - 11)*	**14**		**N**	**N**	Resistant	
Indinavir	Crixivan / r†	*(10)*	**2.20**		**Y**	**N**	Resistant	1
Lopinavir	Kaletra	*(9 - 55)*	**7.43**		**Y**	**N**	**Sensitive**	
Nelfinavir	Viracept	*(3.6)*	**4.77**		**N**	**N**	Resistant	1
Ritonavir	Norvir	*(2.5)*	**15**		**N**	**N**	Resistant	1
Saquinavir	Invirase / r‡	*(2.3 - 12)*	**1.77**		**Y**	**N**	Resistant	1
Tipranavir	Aptivus / r‡	*(2 - 8)*	**1.56**		**Y**	**N**	**Sensitive**	
PI Mutations		**L10F, V32I, L33F, M36M/L, M46M/L, I62I/V, L63C/S/T, A71V, I84V**						

Key resistance concepts

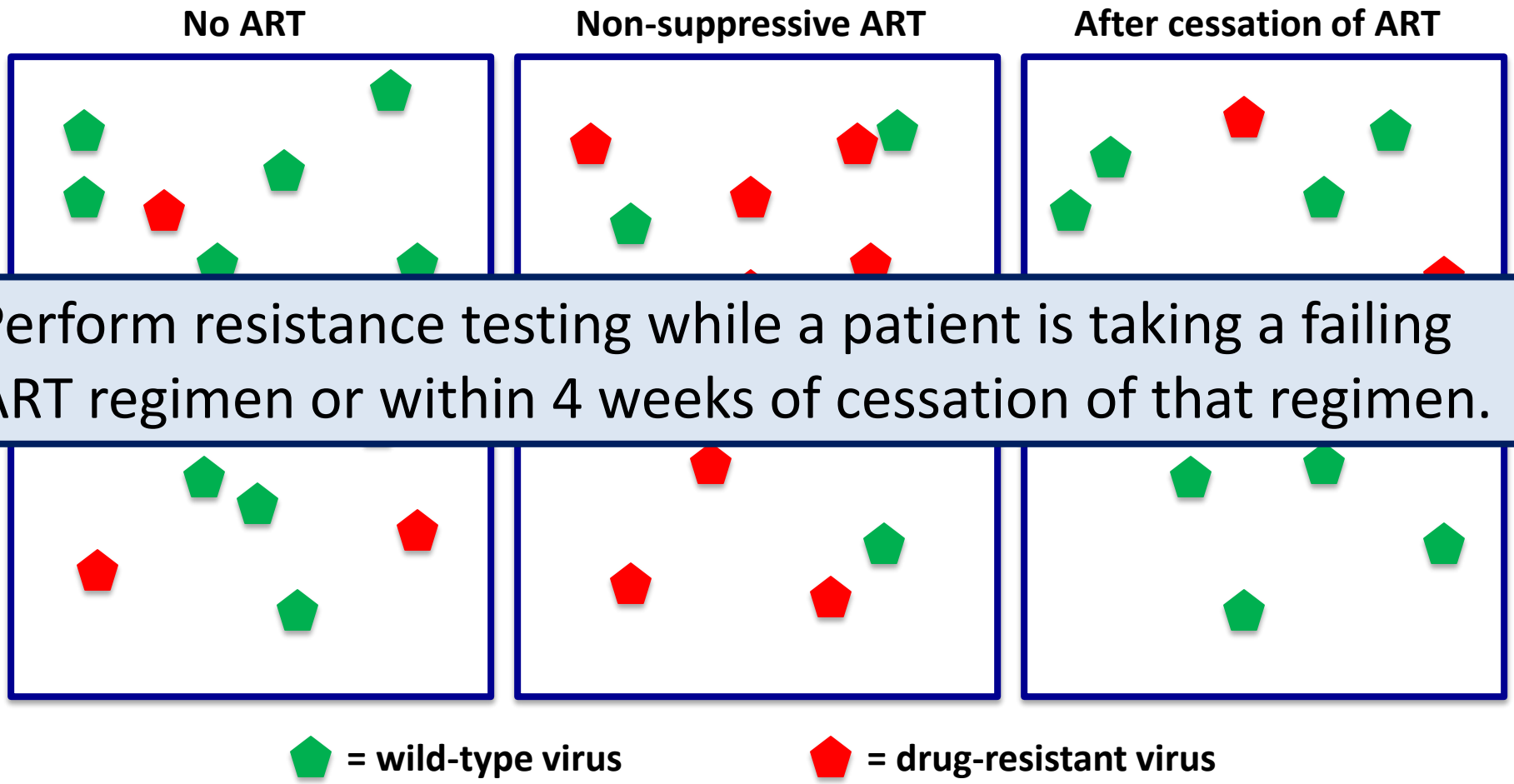
Some resistance mutations reduce viral fitness.

- M184V confers resistance to lamivudine and emtricitabine.
- This mutation also reduces viral fitness.

Maintaining lamivudine or emtricitabine in the ART regimens of patients with M184V helps lower viral load despite resistance.

Campbell TB, Shulman NS, Johnson SC, et al. Antiviral activity of lamivudine in salvage therapy for multidrug-resistant HIV-1 infection. Clin Infect Dis. 2005;41(2):236.

Resistance mutations can be "archived."



Perform resistance testing while a patient is taking a failing ART regimen or within 4 weeks of cessation of that regimen.

Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. Department of Health and Human Services. 2016. Available at: https://aidsinfo.nih.gov/guidlines.

Mutations can have multiple, disparate effects on drug activity.

- **Cross resistance:** A mutation may confer resistance to more than one drug.
  - Example: K103N causes resistance to both efavirenz and nevirapine.

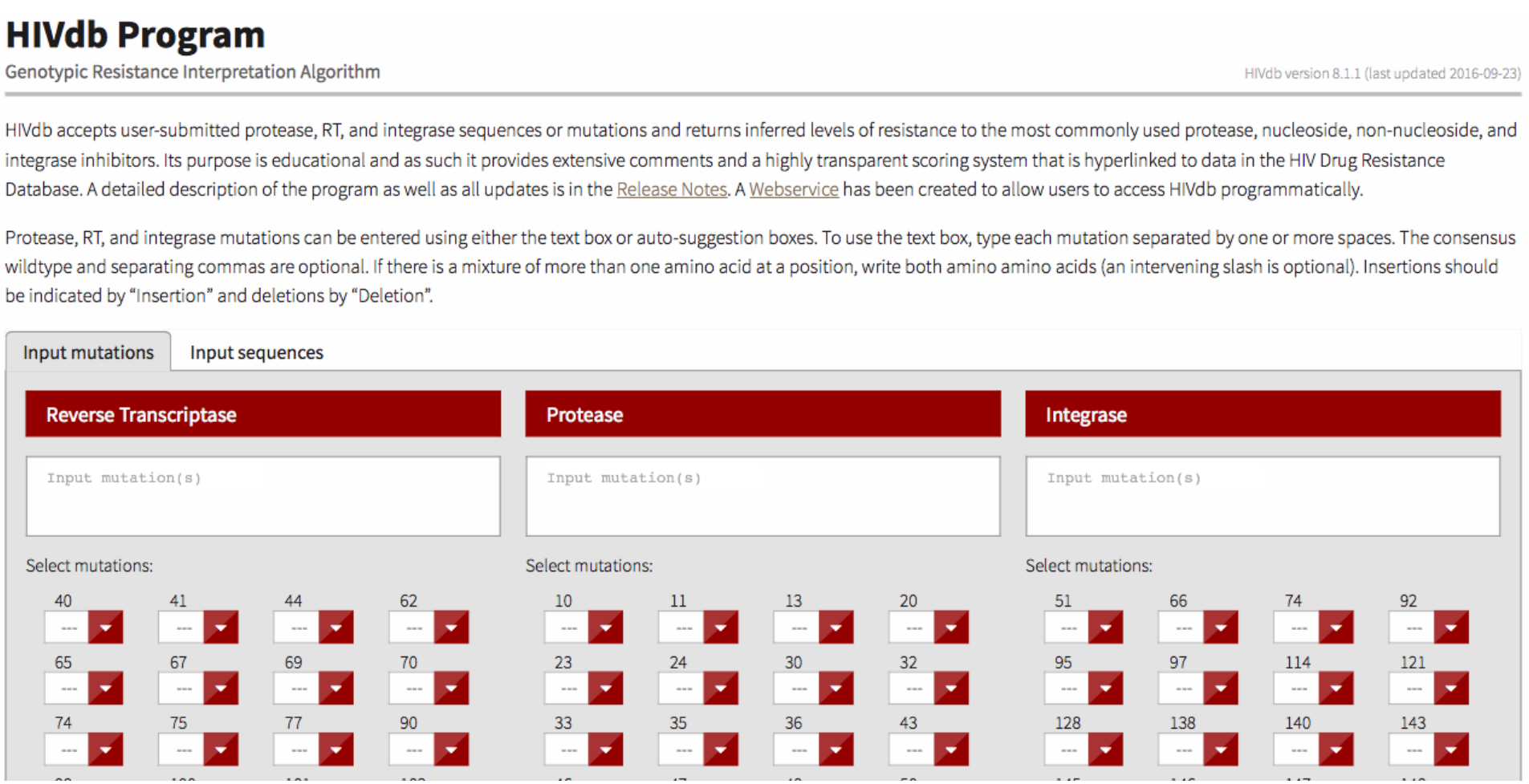
Selection of ART for a patient with drug resistance relies upon knowledge of the effects (cross resistance or hyper-susceptibility) of each mutation on the patient's resistance report.

tenofovir.

Rhee SY, Gonzales MJ, Kantor R, Betts BJ, Ravela J, Shafer RW. Human immunodeficiency virus reverse transcriptase and protease sequence database. Nucleic Acids Research. 2003;31(1):298.

Resistance by drug class

Stanford drug resistance database (hivdb.stanford.edu)



Stanford drug resistance database (hivdb.stanford.edu)

Drug Resistance Interpretation: RT

NRTI Resistance Mutations:	None
NNRTI Resistance Mutations:	K103N
Other Mutations:	None

Nucleoside Reverse Transcriptase Inhibitors	
abacavir (ABC)	Susceptible
zidovudine (AZT)	Susceptible
stavudine (D4T)	Susceptible
didanosine (DDI)	Susceptible
emtricitabine (FTC)	Susceptible
lamivudine (3TC)	Susceptible
tenofovir (TDF)	Susceptible

Non-nucleoside Reverse Transcriptase Inhibitors	
efavirenz (EFV)	High-Level Resistance
etravirine (ETR)	Susceptible
nevirapine (NVP)	High-Level Resistance
rilpivirine (RPV)	Susceptible

IAS-USA drug resistance mutations list (iasusa.org)

MUTATIONS IN THE PROTEASE GENE ASSOCIATED WITH RESISTANCE TO PROTEASE INHIBITORS[p,q,r]

Atazanavir +/- ritonavir[s]

	10	16	20	24	32	33	34	36	46	48	**50**	53	54	60	62	64	71	73	82	**84**	85	**88**	90	93
Wild type	L	G	K	L	V	L	E	M	M	G	I	F	I	D	I	I	A	G	V	I	I	N	L	I
Mutations	I F V C	E	R M I T V	I	I	I F V	Q	I L V	I L	V	L	L Y	L V M T A	E	V	L M V	V I T L	C S T A	A T F I	V	V	S	M	L M

Darunavir/ ritonavir[t]

	11	32	33	**47**	**50**	**54**	74	**76**	**84**	89
Wild type	V	V	L	I	I	I	T	L	I	L
Mutations	I	I	F	V	V	M L	P	V	V	V

Fosamprenavir/ ritonavir

	10	32	46	47	**50**	54	73	76	82	**84**	90
Wild type	L	V	M	I	I	I	G	L	V	I	L
Mutations	F I R V	I	I L	V	V	L V M	S	V	A F S T	V	M

Indinavir/ ritonavir[u]

	10	20	24	32	36	**46**	54	71	73	76	77	**82**	**84**	90
Wild type	L	K	L	V	M	M	I	A	G	L	V	V	I	L
Mutations	I R V	M R	I	I	I	I L	V	V T	S A	V	I	A F T	V	M

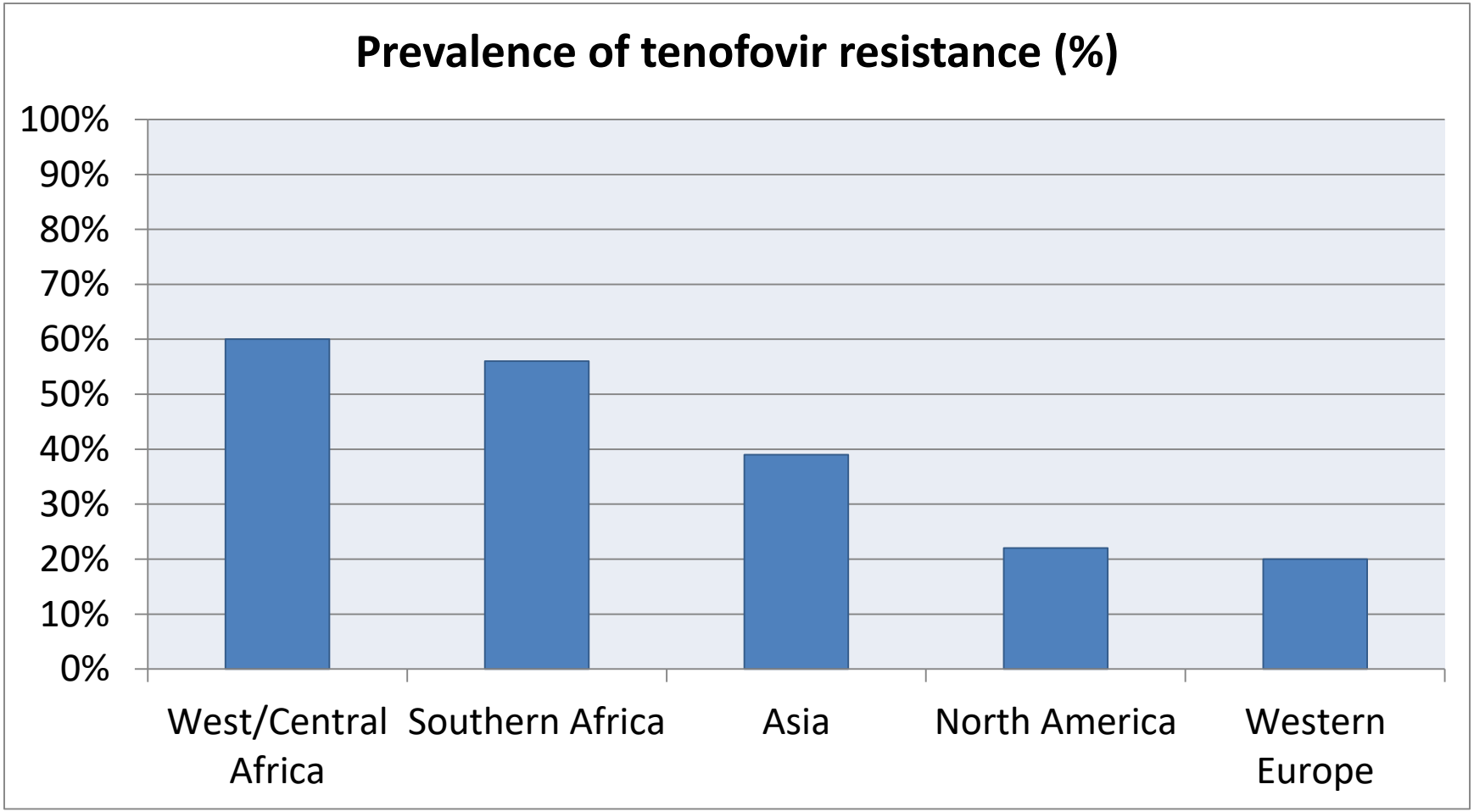
Lopinavir/ ritonavir[v]

	10	20	24	**32**	33	46	**47**	50	53	54	63	71	73	**76**	**82**	84	90
Wild type	L	K	L	V	L	M	I	I	F	I	L	A	G	L	V	I	L
Mutations	F I R V	M R	I	I	F	I L	V A	V	L	V L A M T S	P	V T	S	V	A F T S	V	M

NRTI resistance

- Common upon failure of first-line NRTI + NNRTI treatment.
- Many NRTI mutations reduce viral fitness.
- Common mutations:
  - **M184V/I:** Resistance to 3TC and FTC
  - **K65R:** Resistance to tenofovir and abacavir

Tenofovir resistance is more common in Africa than elsewhere.



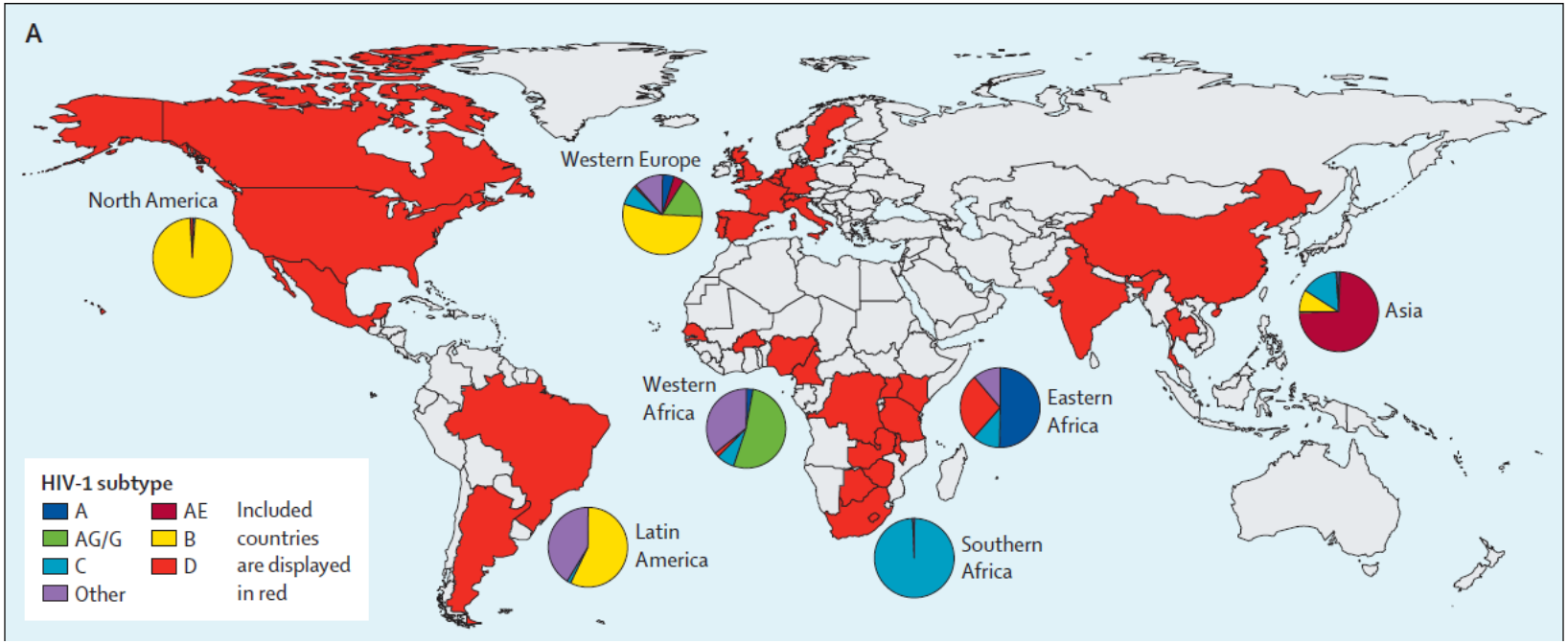
TenoRes Study Group. Global epidemiology of drug resistance after failure of WHO recommended first-line regimens for adult HIV-1 infection: a multicenter retrospective cohort study. Lancet Infect Dis. 2016;16:565.

Factors associated with tenofovir resistance at virologic failure

FACTOR	OR (95% CI) FOR TENOFOVIR RESISTANCE
CD4 cell count < 100 (versus > 100)	1.5 (1.27-1.77)
Lamivudine (versus emtricitabine)	1.48 (1.2-1.82)
Nevirapine (versus efavirenz)	1.46 (1.28-1.67)
Subtype C (versus non-C, non-B)	2.44 (1.66-3.59)

TenoRes Study Group. Global epidemiology of drug resistance after failure of WHO recommended first-line regimens for adult HIV-1 infection: a multicenter retrospective cohort study. Lancet Infect Dis. 2016;16:565.

Global distribution of HIV subtypes



TenoRes Study Group. Global epidemiology of drug resistance after failure of WHO recommended first-line regimens for adult HIV-1 infection: a multicenter retrospective cohort study. Lancet Infect Dis. 2016;16:565.

NRTI resistance

- Common upon failure of first-line NRTI + NNRTI treatment.
- Many NRTI mutations reduce viral fitness.
- Common mutations:
  - **M184V/I:** Resistance to 3TC and FTC
  - **K65R:** Resistance to tenofovir and abacavir
  - **L74V/I:** Resistance to abacavir
  - **TAMs:** Impact all NRTIs; the more TAMs there are, the worse the resistance tends to be

Pathway 1 TAMs

Drug Resistance Interpretation: RT

NRTI Resistance Mutations:	M41L, L210W, T215Y
NNRTI Resistance Mutations:	None
Other Mutations:	None

Nucleoside Reverse Transcriptase Inhibitors

abacavir (ABC)	Intermediate Resistance
zidovudine (AZT)	High-Level Resistance
stavudine (D4T)	High-Level Resistance
didanosine (DDI)	High-Level Resistance
emtricitabine (FTC)	Low-Level Resistance
lamivudine (3TC)	Low-Level Resistance
tenofovir (TDF)	Intermediate Resistance

Pathway 2 TAMs

Drug Resistance Interpretation: RT

NRTI Resistance Mutations:	D67N, K70R, K219Q
NNRTI Resistance Mutations:	None
Other Mutations:	None

Nucleoside Reverse Transcriptase Inhibitors

abacavir (ABC)	Low-Level Resistance
zidovudine (AZT)	High-Level Resistance
stavudine (D4T)	Intermediate Resistance
didanosine (DDI)	Intermediate Resistance
emtricitabine (FTC)	Potential Low-Level Resistance
lamivudine (3TC)	Potential Low-Level Resistance
tenofovir (TDF)	Low-Level Resistance

NNRTI resistance

- Common upon failure of first-line NRTI + NNRTI treatment
- Common mutations:
  - **K103N:** Resistance to efavirenz and nevirapine
  - **Y181C:** Resistance to all available NNRTIs

PI resistance

- Multiple mutations are often required to significantly reduce PI/r activity.
- Mutations are classified as major or minor.
- Darunavir may remain active in the setting of resistance to other PIs.

INSTI resistance

- More common with RAL and EVG than DTG.
- DTG is active against some viruses that harbor RAL/EVG resistance.
- Common mutations:
  - **N155H:** Resistance to RAL and EVG
  - **Y143C/R:** Resistance to RAL
  - **Q148H/K/R:** Resistance to RAL and EVG; also affects DTG when other mutations are present

Management of patients with drug resistance

General principles

1. Avoid adding a single agent to a failing ART regimen (i.e., “virtual monotherapy”).
2. Create a regimen with at least 2, and preferably 3, fully active drugs.
3. Change regimens as soon as possible to avoid the accumulation of resistance mutations.
4. Check for drug-drug interactions within the new regimen.

Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. Department of Health and Human Services. 2016. Available at http://aidsinfo.nih.gov/contentfiles/lvguidelines/AdultandAdolescentGL.pdf.

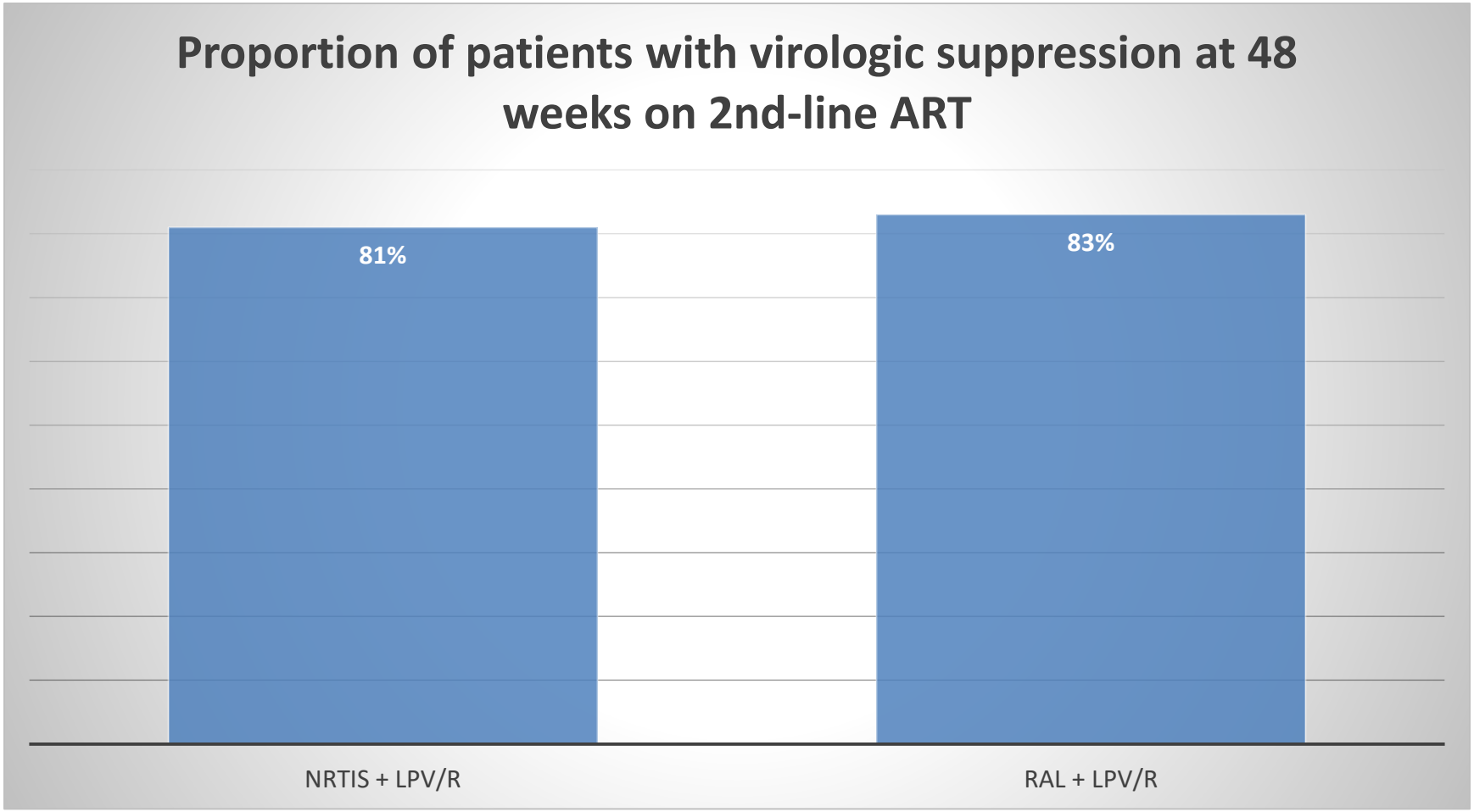
Case : Failure of 1st-line ART

- A 35 year-old woman has been taking TDF, 3TC, and EFV for 1.5 years.
- Her viral load is 12,000.
- A resistance report shows:
  - NRTI mutations: K65R, M184V
  - NNRTI mutations: K103N

What is the best second-line regimen for her?

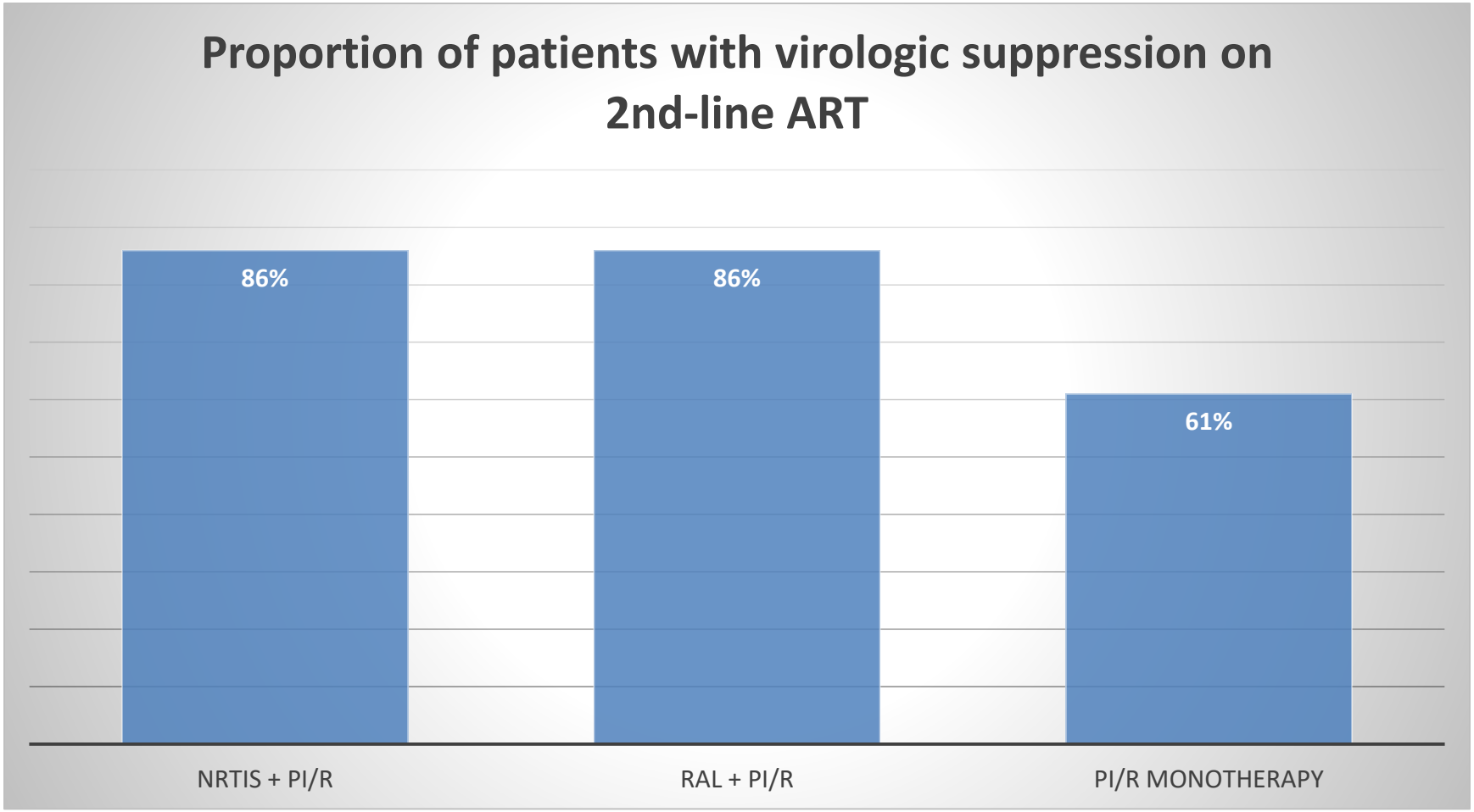
A. AZT, 3TC, LPV/r
B. AZT, 3TC, RAL
C. TDF, 3TC, LPV/r
D. TDF, 3TC, RAL

PI/r-based ART constitutes potent second-line therapy.



SECOND-LINE Study Group. Ritonavir-boosted lopinavir plus nucleoside or nucleotide reverse transcriptase inhibitors versus ritonavir-boosted lopinavir plus raltegravir for treatment of HIV-1 infection in adults with virological failure of a standard first-line ART regimen (SECOND-LINE): a randomised, open-label, non-inferiority study. Lancet. 2013;381:2091.

Additional evidence for PI/r-based second-line therapy.



1. Paton NI, Kityo C, Hoppe A, et al. Assessment of second-line antiretroviral therapy regimens for HIV in Africa. N Engl J Med. .2014;371:234.

Treatment of the patient with multi-class resistance may involve:

- Darunavir/ritonavir
- Raltegravir or dolutegravir
- NRTIs
- Etravirine
- Maraviroc

1. Arasteh K, Yeni P, Pozniak A, et al. Efficacy and safety of darunavir/ritonavir in treatment-experienced HIV type-1 patients in the POWER 1, 2, and 3 trials at week 96. Antivir Ther. 2009;14(6):859.
2. Steigbigel RT, Cooper DA, Kumar PN, et al. Raltegravir with optimized background therapy for resistant HIV-1 infection. N Engl J Med. 2008;359:339.
3. Castagna A, Maggiolo F, Penco G, et al. Dolutegravir in antiretroviral-experienced patients with raltegravir and/or elvitegravir-resistant HIV-1: 24 week results of the phase III Viking-3 study. J Infect Dis. 2014;210(3):354.
4. Tashima KT, Smeaton LM, Fichtenbaum CJ, et al. HIV salvage therapy does not require nucleotide reverse transcriptase inhibitors: a randomized, controlled trial. Ann Intern Med. 2015;163(12):908.
5. Katlama C, Haubrich R, Lalezari J, et al. Efficacy and safety of etravirine in treatment-experienced, HIV-1 patients: pooled 48-week analysis of two randomized, controlled trials. AIDS. 2009;23(17):2289.

Take-home points

- Drug resistance is common upon failure of first-line ART.
- Genotypic resistance testing aids in ART selection for individuals with resistant virus.
- Resistance mutations can have multiple effects, including reducing viral fitness and increasing susceptibility to other ART drugs.
- Most patients with NRTI and NNRTI resistance can be treated effectively with NRTIs and a PI/r.