AETC AIDS Education & Training Center Program
Southeast Regional Conference 2023

Resistance 101: Basics of HIV Antiretroviral Therapy Resistance

Todd Hulgan, MD, MPH
Department of Medicine, Division of Infectious Diseases
Vanderbilt University Medical Center
Veterans Affairs – Tennessee Valley Veterans Healthcare System
Nashville, TN
todd.hulgan@vumc.org

Disclosures

- No financial disclosures
- The views and opinions expressed herein do not necessarily state or reflect those of the U.S. Government or the Veterans Affairs Administration
- *This program is supported by the Health Resources and Services Administration (HRSA) of the U.S. Department of Health and Human Services (HHS) under grant number U1OHA30535 as part of an award totaling $4.2m. The contents are those of the author(s) and do not necessarily represent the official views of, nor an endorsement, by HRSA, HHS, or the U.S. Government. For more information, please visit HRSA.gov.*
- *"Funding for this presentation was made possible by cooperative agreement U1OHA30535 from the Health 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."*

Learning Objectives

- By the end of this session, each participant will be able to:
  - **Define** HIV drug resistance
  - **List** 2-3 reasons why HIV drug resistance develops
  - **Describe** the difference between HIV genotype & phenotype testing
  - **Recognize** 1-2 key resistance mutations
  - **Identify & access** online HIV resistance resources

Outline

- Resistance **concepts**
- Types of **resistance tests**
- DHHS resistance testing **recommendations**
- **Specific mutations** of note
- **Miscellaneous**
  - (1) HIV replication "fitness" – M184V example
  - (2) Integrase inhibitor resistance
  - (3) Resources

How can we characterize ART?

- **Potency**
- **Tolerability**
- **Adverse effects**
- **Resistance**

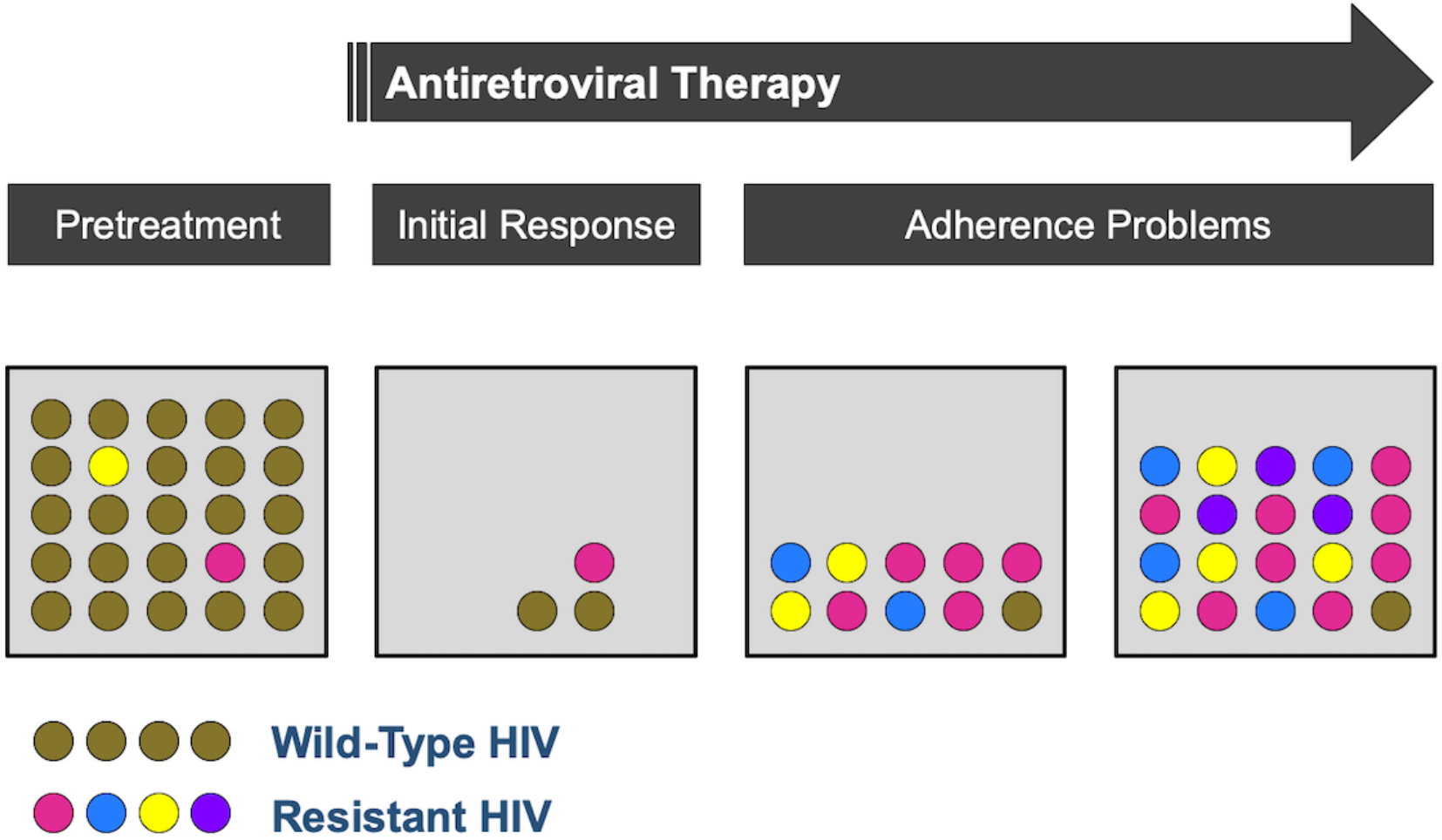
<u>Definition</u>: Development of mutations in an HIV genome that makes that strain less sensitive to a drug or drug class

Drugs or classes have different genetic *barriers to resistance*

Higher barrier = several steps (or mutations) need to occur for clinically relevant resistance to develop and/or high fitness cost on viral replication

ART drugs with a ***lower*** barrier should always be combined with other potent active agents

Causes of HIV drug resistance



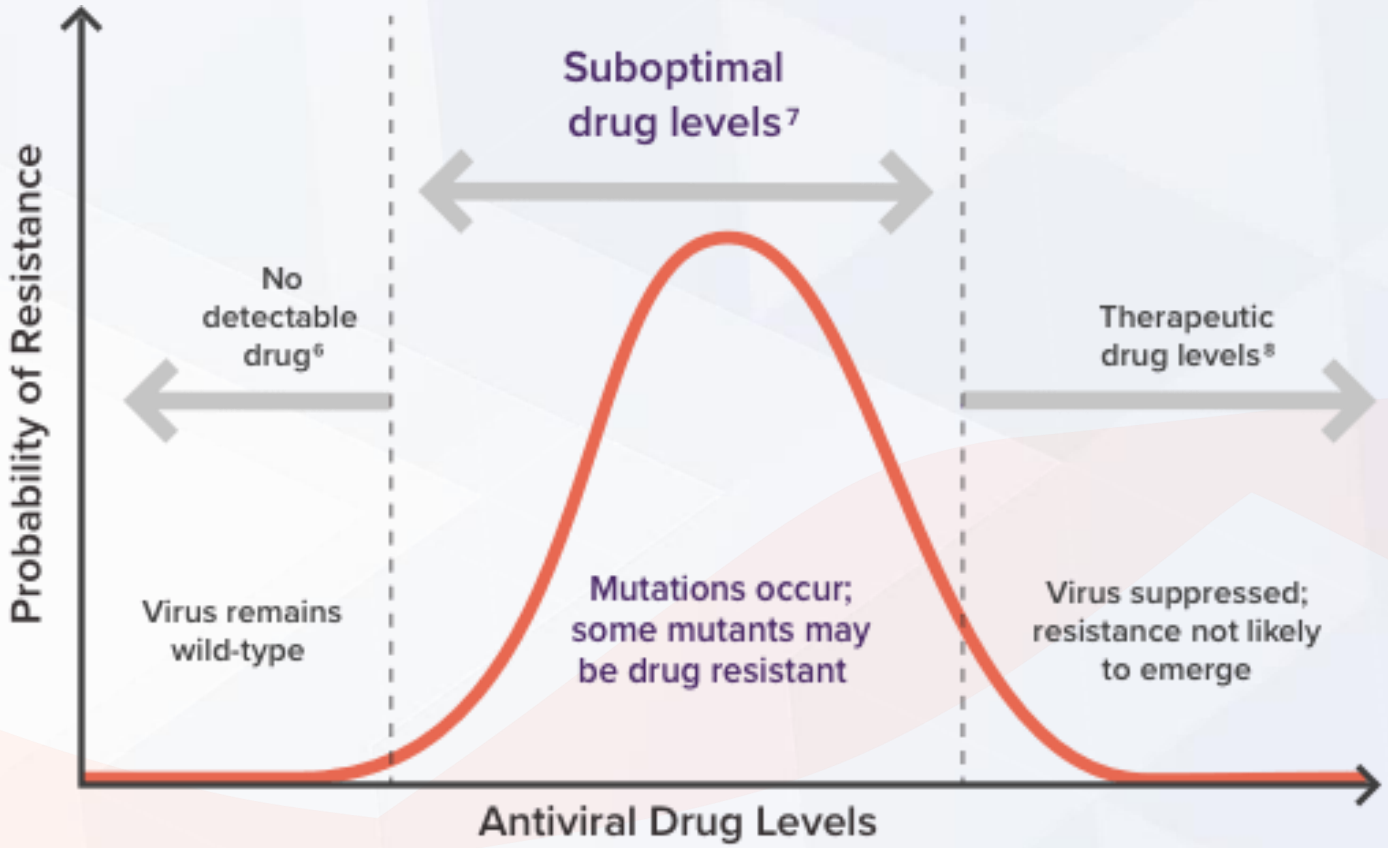
Other reasons:

- ↓ potency
- ↓ drug absorption
  - Drug interactions
  - Clearance/induction
  - Host genetics

https://www.hiv.uw.edu/go/antiretroviral-therapy/evaluation-management-virologic-failure/core-concept/all

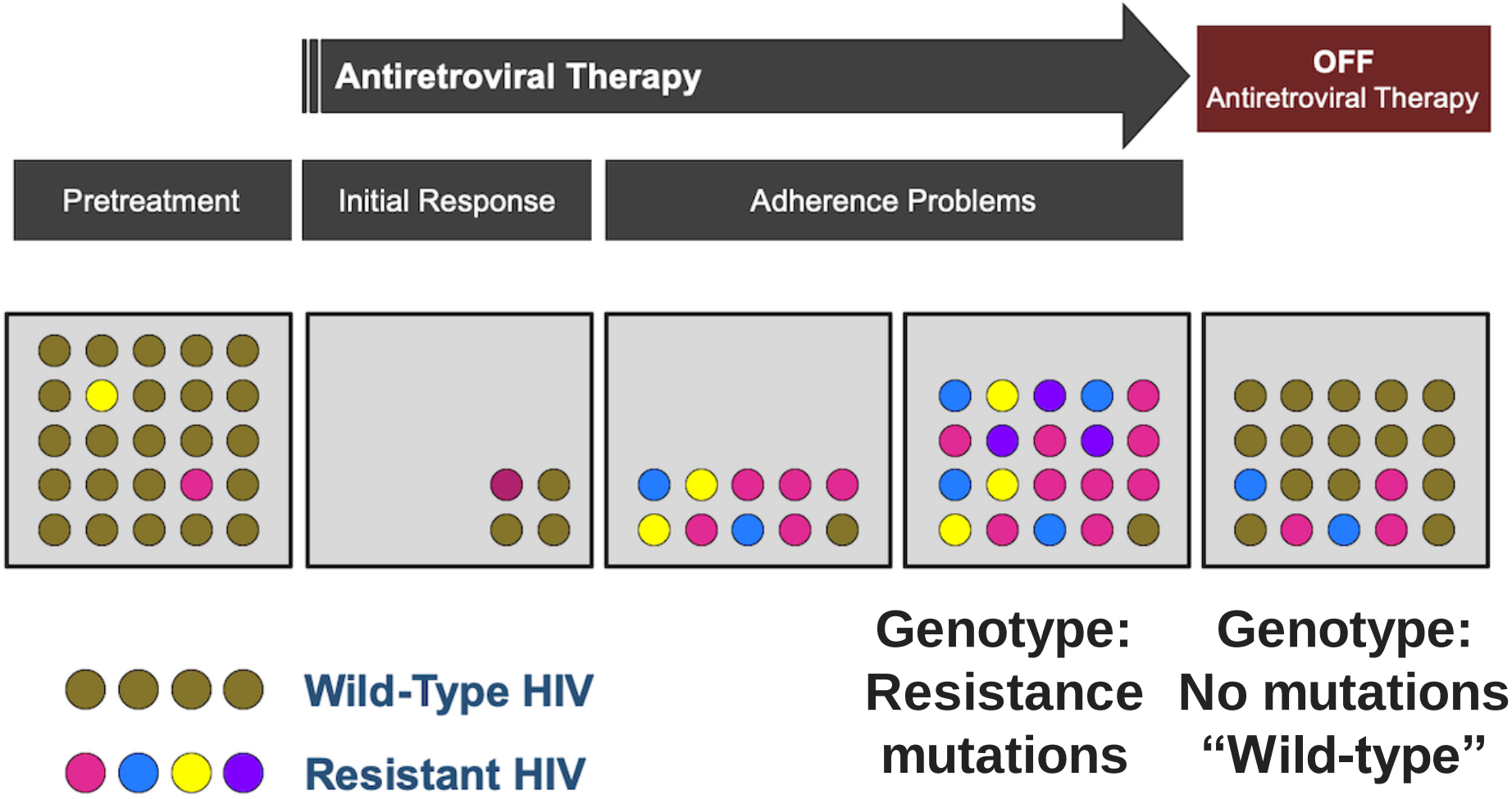
True or false: Patients with the **lowest** ART adherence have the **greatest** risk of HIV resistance

FALSE!



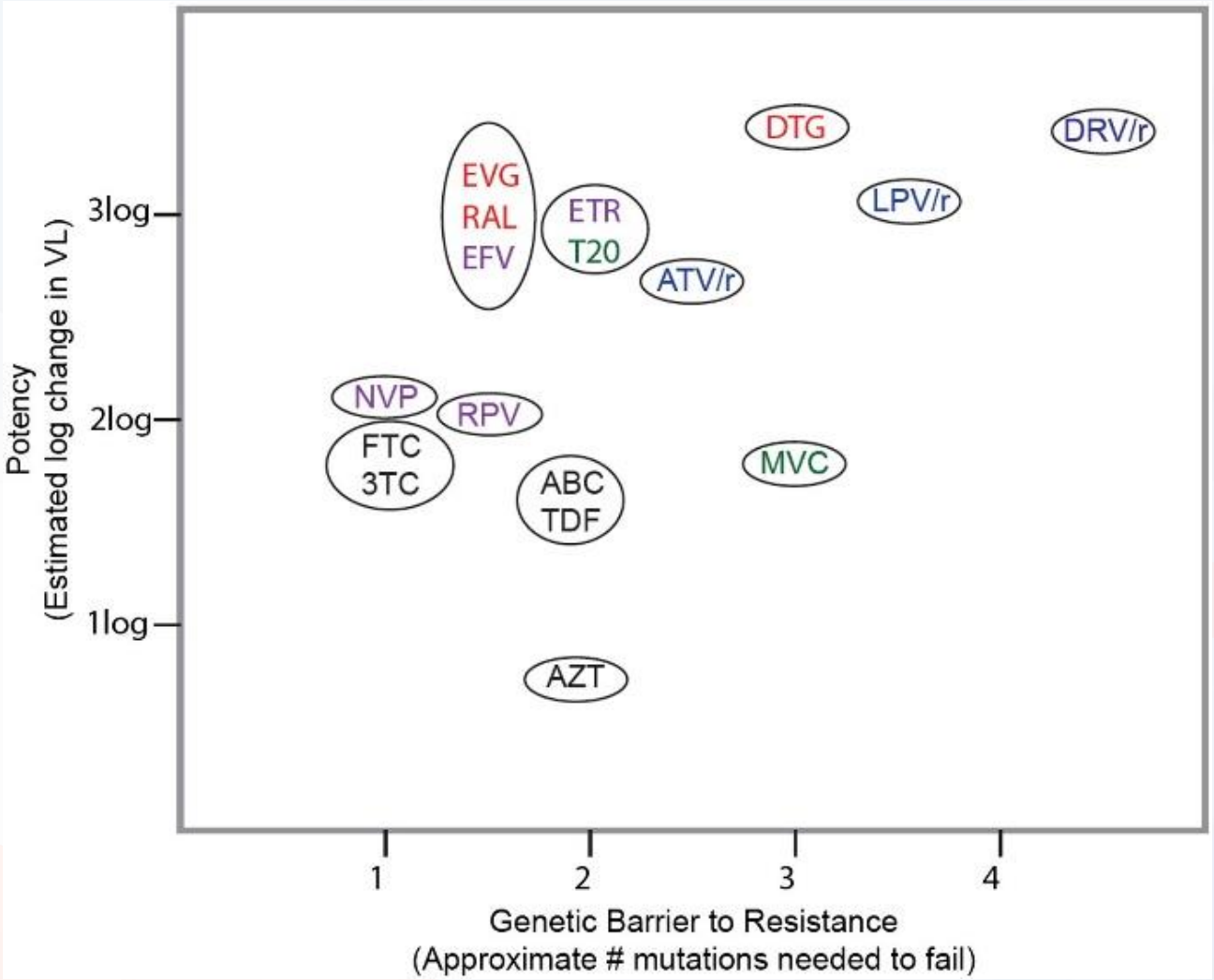
https://www.helpstoptheviruspro.com/en/barrier-to-resistance

Reemergence of "wild-type" HIV



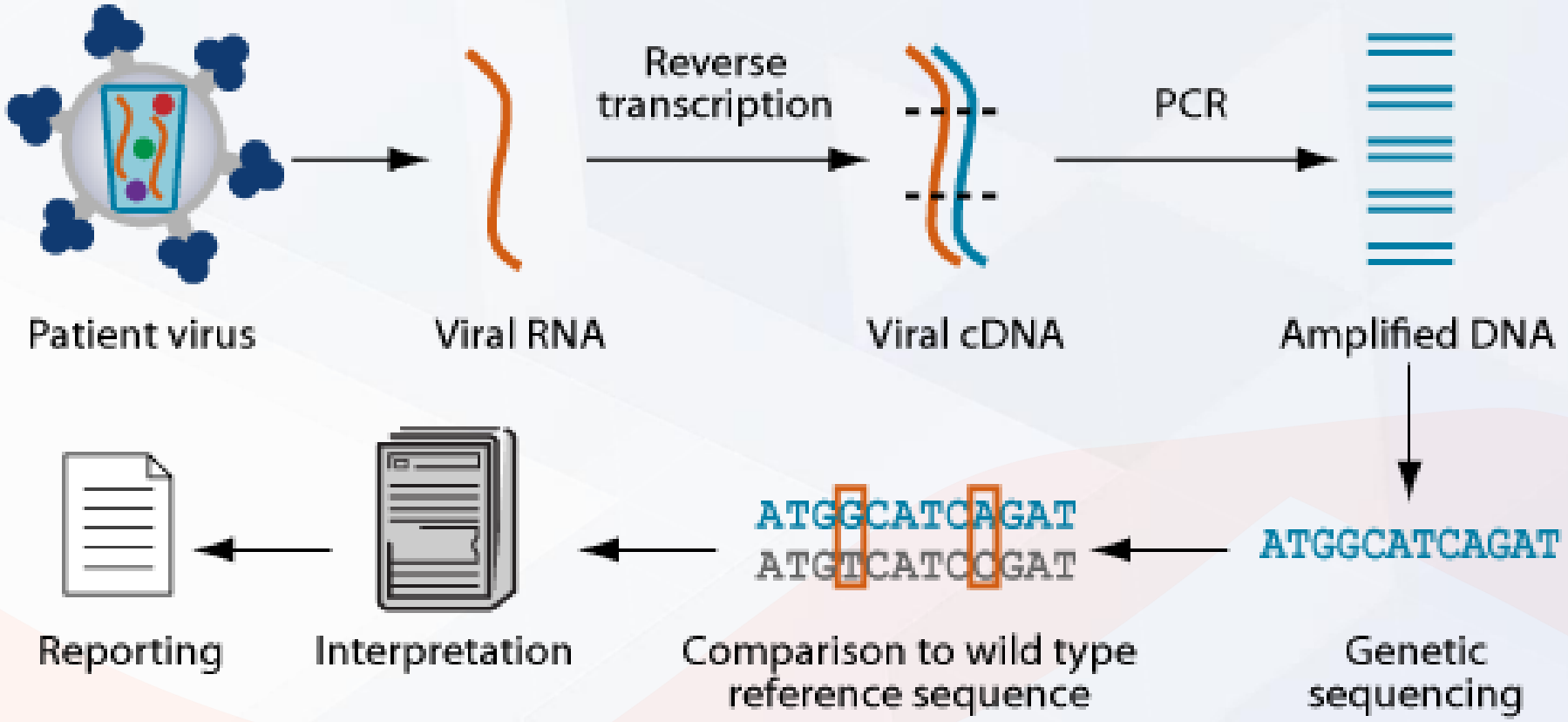
https://www.hiv.uw.edu/go/antiretroviral-therapy/evaluation-management-virologic-failure/core-concept/all

Genetic barrier to resistance



Clutter, et al. *Infect Genetics Evol* 2016; 46: 292-307

Genotype resistance testing



https://monogrambio.labcorp.com/resources/genotyping

Genotype report

	Drug		GenoSure®MG	Assessment*	Comments
	Generic Name	Brand Name	Drug Resistance Associated Mutations Detected	Drug	
NRTI	Abacavir	Ziagen	None	ABC	Sensitive
	Didanosine	Videx	None	ddI	Sensitive
	Emtricitabine	Emtriva	None	FTC	Sensitive
	Lamivudine	Epivir	None	3TC	Sensitive
	Stavudine	Zerit	None	d4T	Sensitive
	Tenofovir	Viread	None	TFV	Sensitive
	Zidovudine	Retrovir	None	ZDV	Sensitive
NNRTI	Efavirenz	Sustiva	K103N	EFV	Resistant
	Etravirine	Intelence	None	ETR	Sensitive
	Nevirapine	Viramune	K103N	NVP	Resistant
	Rilpivirine	Edurant	K103N	RPV	Sensitive
PI	Atazanavir	Reyataz	A71V	ATV	Sensitive
		Reyataz / r‡	A71V	ATV/r	Sensitive
	Darunavir	Prezista / r‡	V11I	DRV/r	Sensitive
	Fosamprenavir	Lexiva / r‡	V11I	AMP/r	Sensitive
	Indinavir	Crixivan / r‡	A71V	IDV/r	Sensitive
	Lopinavir	Kaletra‡	A71V	LPV/r	Sensitive
	Nelfinavir	Viracept	A71V	NFV	Sensitive
	Ritonavir	Norvir	A71V	RTV	Sensitive
	Saquinavir	Invirase / r‡	A71V	SQV/r	Sensitive
	Tipranavir	Aptivus / r‡	A71V	TPV/r	Sensitive

“K103N”

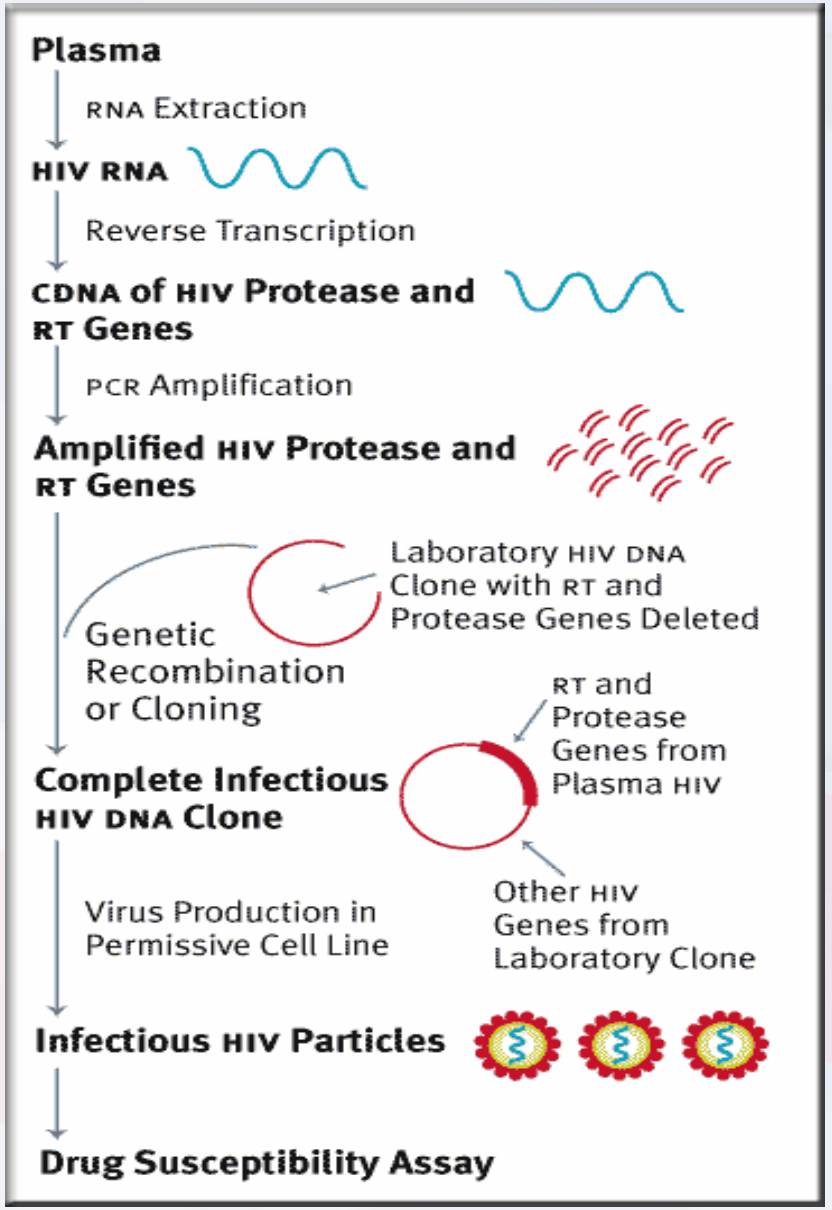
An asparagine (**N**) is substituted for the “wild-type” lysine (**K**) at codon 103 in the reverse transcriptase (RT) gene

This change corresponds to an increase in $IC_{50/90}$ of this viral strain to levels above physiologic drug concentrations

https://monogrambio.labcorp.com/sites/default/files/2019-10/Sample%20Report%20GenoSure%20MG%2004-11-2017.pdf

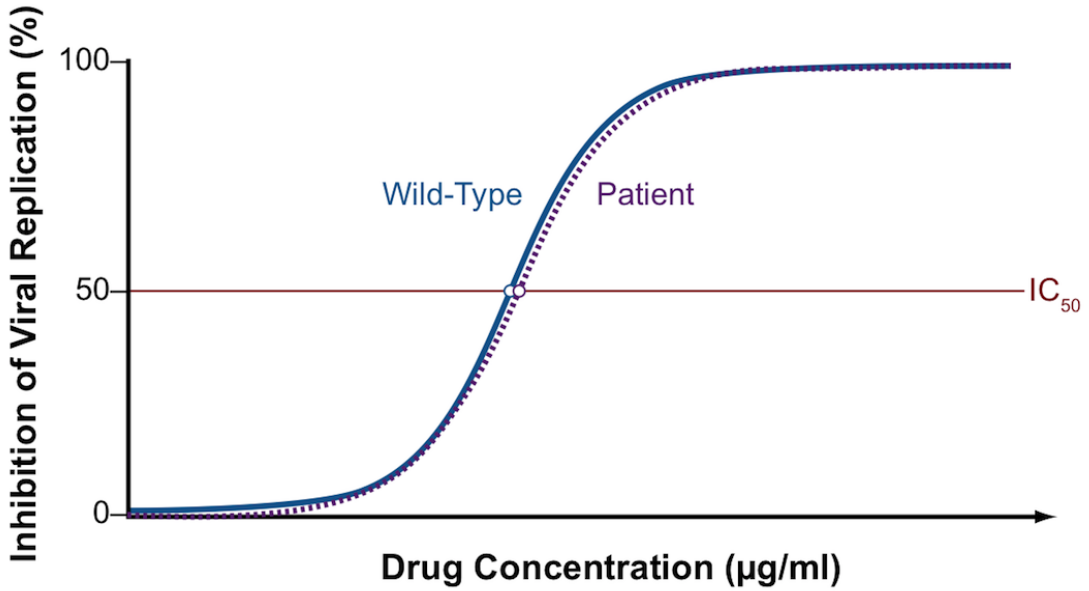
Phenotype resistance testing

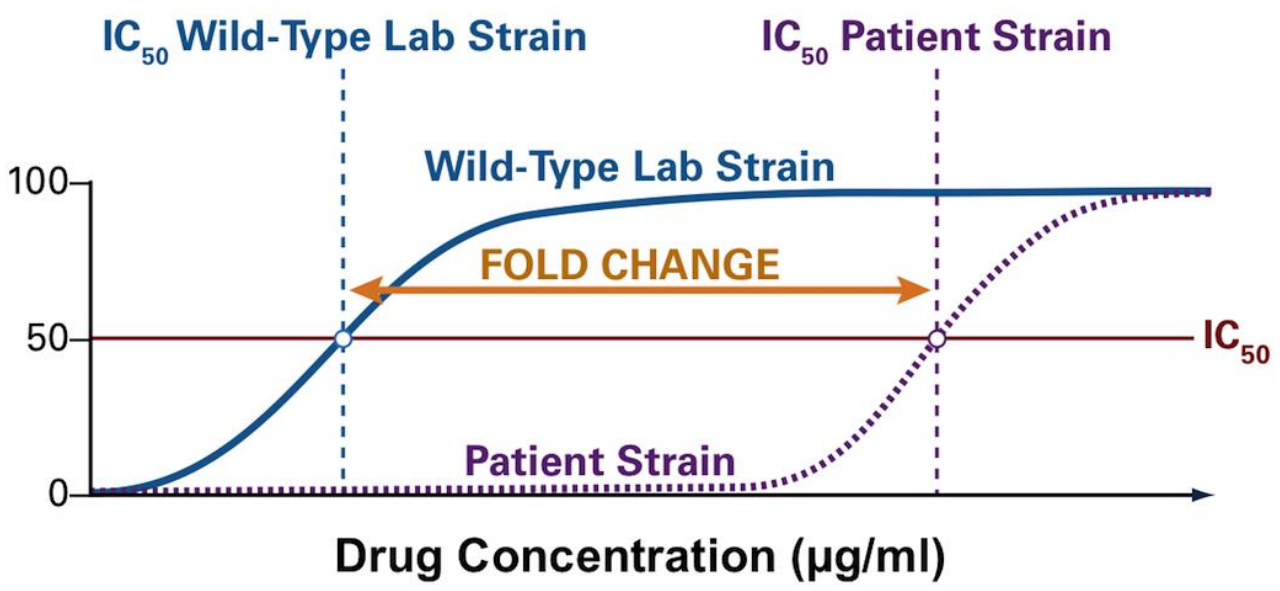
- Tests viability of a “synthetic” version of patient’s HIV in the presence of ART
- Results reported as fold-change in susceptibility to ART



Slide content from: NWAETC

Phenotype resistance testing





Drug-susceptible virus

Drug-resistant virus

https://www.hiv.uw.edu/go/antiretroviral-therapy/evaluation-management-virologic-failure/core-concept/all

Phenotype report

	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		
NRTI	**Abacavir**	Ziagen	*(4.5 - 6.5)*	**3.98**		**Y**	**N**	**Sensitive**	16
	Didanosine	Videx	*(1.3 - 2.2)*	**1.99**		**P**	**N**	**Partially Sensitive**	
	Emtricitabine	Emtriva	*(3.5)*	**>MAX**		**N**	**N**	**Resistant**	
	Lamivudine	Epivir	*(3.5)*	**>MAX**		**N**	**N**	**Resistant**	
	Stavudine	Zerit	*(1.7)*	**1.51**		**Y**	**N**	**Sensitive**	3
	Zidovudine	Retrovir	*(1.9)*	**7.91**		**N**	**N**	**Resistant**	3
	Tenofovir	Viread	*(1.4 - 4)*	**1.16**		**Y**	**N**	**Sensitive**	3
	NRTI Mutations		**M41L, M184V, T215Y**						

https://monogrambio.labcorp.com/sites/default/files/2019-10/PSGT_report_new_Watermark.pdf

Genotypic vs. Phenotypic Resistance Tests

	Genotype	Phenotype
Basis of test	Detects **drug resistance mutations** present in relevant viral genes	Measures the **ability of a virus to grow** in different antiretroviral drug concentrations
Interpretation	Requires knowledge of mutations selected by individual antiretrovirals and potential for cross-resistance conferred by certain mutations	Visual interpretation by bars indicating susceptibility to individual agents
Sensitivity	Enhanced sensitivity for detecting mixtures of wild-type and resistant virus	Results reflect susceptibility of dominant viral species
Availability of results	1-2 wks	2-3 wks
Relative cost	**Lower cost** than phenotypic assays	**Higher cost** than genotypic assays

Other resistance testing platforms

- **GenoSure® Prime** – Includes integrase gene mutations*
- **Trofile® co-receptor tropism assay** – Determines CCR5 co-receptor status of virus
- **GenoSure® Archive** & **Trofile® DNA** – DNA sequencing assays for use in patients with undetectable or low-level HIV RNA in plasma

**Make sure you know whether your assay includes integrase resistance or not!*

Testing for resistance – DHHS Guidelines (last update March 2023)

Antiretroviral Therapy-Naive Persons:

- At entry into care **(AII)**
- Genotypic testing preferred **(AIII)**
- In persons with **acute or recent (early) HIV infection**, in **pregnant people** with HIV, or in people who **will initiate ART on the day of or soon after HIV diagnosis**, ART initiation should not be delayed while awaiting resistance testing results **(AIII)**
- If transmitted INSTI resistance is a concern, providers should ensure that genotypic resistance testing also includes the integrase gene **(AIII)**

Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the Use of Antiretroviral Agents in Adults and Adolescents Living with HIV. Department of Health and Human Services. Available at https://clinicalinfo.hiv.gov/en/guidelines/hiv-clinical-guidelines-adult-and-adolescent-arv/whats-new

Definitions

- **Virologic suppression**:
  - Confirmed HIV RNA below LLOD (<20 copies/mL)
- **Virologic failure**:
  - Inability to achieve or maintain HIV RNA <200 copies/mL
- **Incomplete virologic response**:
  - 2 consecutive HIV RNA ≥200 copies/mL after 24 weeks on ART without documented virologic suppression.
- **Virologic rebound**:
  - Confirmed HIV RNA ≥200 copies/mL after virologic suppression
- **Virologic blip**:
  - An isolated detectable HIV RNA level after virologic suppression followed by a return to virologic suppression
- **Low-level viremia (LLV)**:
  - Confirmed detectable HIV RNA <200 copies/mL (not a blip)

Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the Use of Antiretroviral Agents in Adults and Adolescents Living with HIV. Department of Health and Human Services. Available at https://clinicalinfo.hiv.gov/en/guidelines/hiv-clinical-guidelines-adult-and-adolescent-arv/whats-new

Testing for resistance – DHHS Guidelines (last update March 2023)

Antiretroviral Therapy-Experienced Persons:

- In the following patients:
  - Virologic failure and HIV RNA levels >200 copies/mL
    - **AI** for >1000; **AIII** for 501-1000; **CIII** for 201-500
  - Suboptimal viral load reduction **(AII)**
- While the person is taking prescribed ARV drugs or **within 4 weeks** after discontinuing **(AII)**
  - **After 4 weeks**, resistance testing may still provide useful information…**previously selected resistance mutations can be missed due to lack of drug-selective pressure (CIII)**

Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the Use of Antiretroviral Agents in Adults and Adolescents Living with HIV. Department of Health and Human Services. Available at https://clinicalinfo.hiv.gov/en/guidelines/hiv-clinical-guidelines-adult-and-adolescent-arv/whats-new

Testing for resistance – DHHS Guidelines (last update March 2023)

Antiretroviral Therapy-Experienced Persons (Cont'd):

- Given the long half-lives of the **long-acting injectable ARV drugs**, resistance testing (including testing for resistance to INSTIs) should be performed in **all persons who have experienced virologic failure on a regimen of long-acting CAB and rilpivirine or acquired HIV after receiving CAB-LA as PrEP, regardless of the amount of time since drug discontinuation (AIII)**
- **Genotypic testing is preferred (AII)**
- Addition of phenotypic resistance testing is recommended for persons with known or suspected complex drug-resistance mutation patterns **(BIII)**
- All prior and current drug-resistance test results should be considered **(AIII)**

Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the Use of Antiretroviral Agents in Adults and Adolescents Living with HIV. Department of Health and Human Services. Available at https://clinicalinfo.hiv.gov/en/guidelines/hiv-clinical-guidelines-adult-and-adolescent-arv/whats-new

Volume 30 Issue 4 October/November 2022

Special Contribution

2022 Update of the … in HIV-1

Annemarie M. Wensing, MD, … Silberstein, PhD; Charlotte C… Roger Paredes, MD, PhD; Rob…

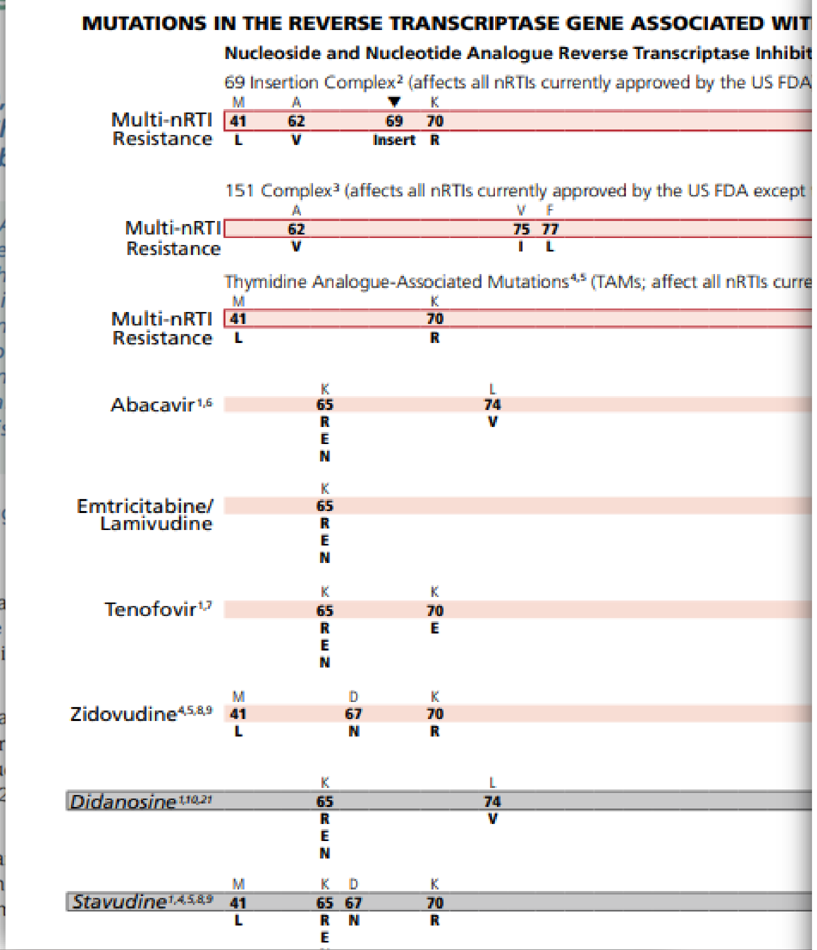
*The 2022 edition of the IAS–USA … tance mutations list updates the … published in September 2019. Th… listed are those that have been … specific criteria for evidence an… scribed. The **Figure** is designed to … tioners to identify key mutation… with resistance to antiretrovira… therefore, in making clinical deci… ing antiretroviral therapy.*

Keywords: HIV, antiretroviral, drug… TAM, therapy, mutations

The 2022 edition of the Internationa… ciety–USA (IAS–USA) drug resistance … updates the **Figure** last published i… 2019.[1] In this update:

- Cabotegravir, fostemsavir, and iba… now been approved by regulator… many countries are all now inclu… sid inhibitor lenacapavir (GS 62… added to the **Figure**.[2]
- A new section on specific drugs a… been added to this update for in… recently approved drugs, that n… added to the **Figure**.

IAS–USA *Topics in Antiviral Medicine*



IAS–USA *Topics in Antiviral Medicine*

User Notes

1. Mutations at the C-terminal reverse transcriptase domains (amino acids 293-560) outside of regions depicted on the **Figure Bar** may contribute to nucleoside (or nucleotide) analogue reverse transcriptase inhibitor (nRTI) and nonnucleoside analogue reverse transcriptase inhibitors (NNRTI) HIV-1 drug resistance. The clinical relevance of these connection domain mutations arises mostly in conjunction with thymidine analogue–associated mutations (TAMs) and M184V and they have not been associated with increased rates of virologic failure of etravirine or rilpivirine in clinical trials.[1-3] K65E/N/R variants are reported in patients experiencing treatment failure of tenofovir (ie, tenofovir disoproxil fumarate [TDF] or tenofovir alafenamide [TAF]), stavudine, or didanosine. The K65R/N variants may be selected by tenofovir, didanosine, abacavir, or stavudine and are associated with decreased viral susceptibility to these drugs.[4-8] The K65R may be more easily selected in subtype C clades.[9] K65E usually occurs in mixtures with wild-type virus. Patient-derived viruses with K65E and site-directed mutations replicate very poorly in vitro; as such, no susceptibility testing can be performed.[10,11] Some nRTI mutations, like T215Y and H208Y,[12] may lead to viral hypersusceptibility to NNRTIs, including etravirine.[13] The presence of these mutations may improve subsequent virologic response to NNRTI-containing regimens (nevirap… 69 without the insertion may be associated with broad nRTI resistance.

3. Since no differences in resistance patterns have been observed between TDF and TAF, both drugs are referred to as "tenofovir" on the **Figure Bar**.[19] Tenofovir retains activity against the Q151M complex of mutations.[4] Q151M is the most important mutation in the complex (ie, the other mutations in the complex [A62V, V75I, F77L, and F116Y] in isolation may not reflect multi-nucleoside resistance).

4. Mutations known to be selected by TAMs (ie, M41L, D67N, K70R, L210W, T215Y/F, and K219Q/E) also confer reduced susceptibility to all currently approved nRTIs[20] except emtricitabine and lamivudine, which in fact reverse the magnitude of resistance and are recommended with tenofovir or zidovudine in the presence of TAMs. The degree to which cross-resistance is observed depends on the specific mutations and number of mutations involved.[21-24]

5. Although reverse transcriptase changes associated with the E44D and V118I mutations may have an accessory role in increased resistance to nRTIs in the presence of TAMs, their clinical relevance is very limited.[25-27]

6. The M184V mutation alone does not appear to be associated with a reduced virologic response to abacavir in vivo. When associated with TAMs, M184V increases abacavir resistance.[5,28]

…strand transfer inhibitors (InSTIs) bictegravir and dolutegravir or a boosted protease inhibitor (PI).[29,30]

A reduced response also occurs in the presence of 3 or more TAMs inclusive of either M41L or L210W.[4] The presence of TAMs or combined treatment with zidovudine prevents the emergence of K65R in the presence of tenofovir.[31-33]

8. The presence of M184V appears to delay or prevent emergence of TAMs.[34] This effect may be overcome by an accumulation of TAMs.

9. The T215A/C/D/E/G/H/I/L/N/S/V substitutions are revertant mutations at codon 215 that confer increased risk of virologic failure of zidovudine or stavudine in antiretroviral-naive patients.[35,36] The T215Y mutant may emerge quickly from one of these mutations in the presence of zidovudine or stavudine.[37]

10. The presence of 3 of the following mutations—M41L, D67N, L210W, T215Y/F, K219Q/E—is associated with resistance to didanosine.[38] The presence of K70R or M184V alone does not decrease virologic response to didanosine.[39] However, the mutations depicted on the **Figure Bar** cannot be considered comprehensive because little relevant research has been reported in recent years to update the resistance and cross-resistance patterns for this drug.

11. There is no evidence for the utility of efavirenz, nevirapine, or rilpivirine in patients with NNRTI resistant virus.[40]

https://www.iasusa.org/resources/hiv-drug-resistance-mutations/
https://www.iasusa.org/wp-content/uploads/2022/10/30-4-559.pdf

Mutations in the Reverse Transcriptase Gene Associated With Resistance to Reverse Transcriptase Inhibitors

Nucleoside and Nucleotide Analogue Reverse Transcriptase Inhibitors (nRTIs)

Multi-nRTI Resistance: 69 Insertion Complex (affects all nRTIs currently approved by the US FDA)

M	A	▼	K	L	T	K
41	**62**	**69**	**70**	**210**	**215**	**219**
L	**V**	**Insert**	**R**	**W**	**Y**	**Q**
					F	**E**

Multi-nRTI Resistance: 151 Complex (affects all nRTIs currently approved by the US FDA except tenofovir)

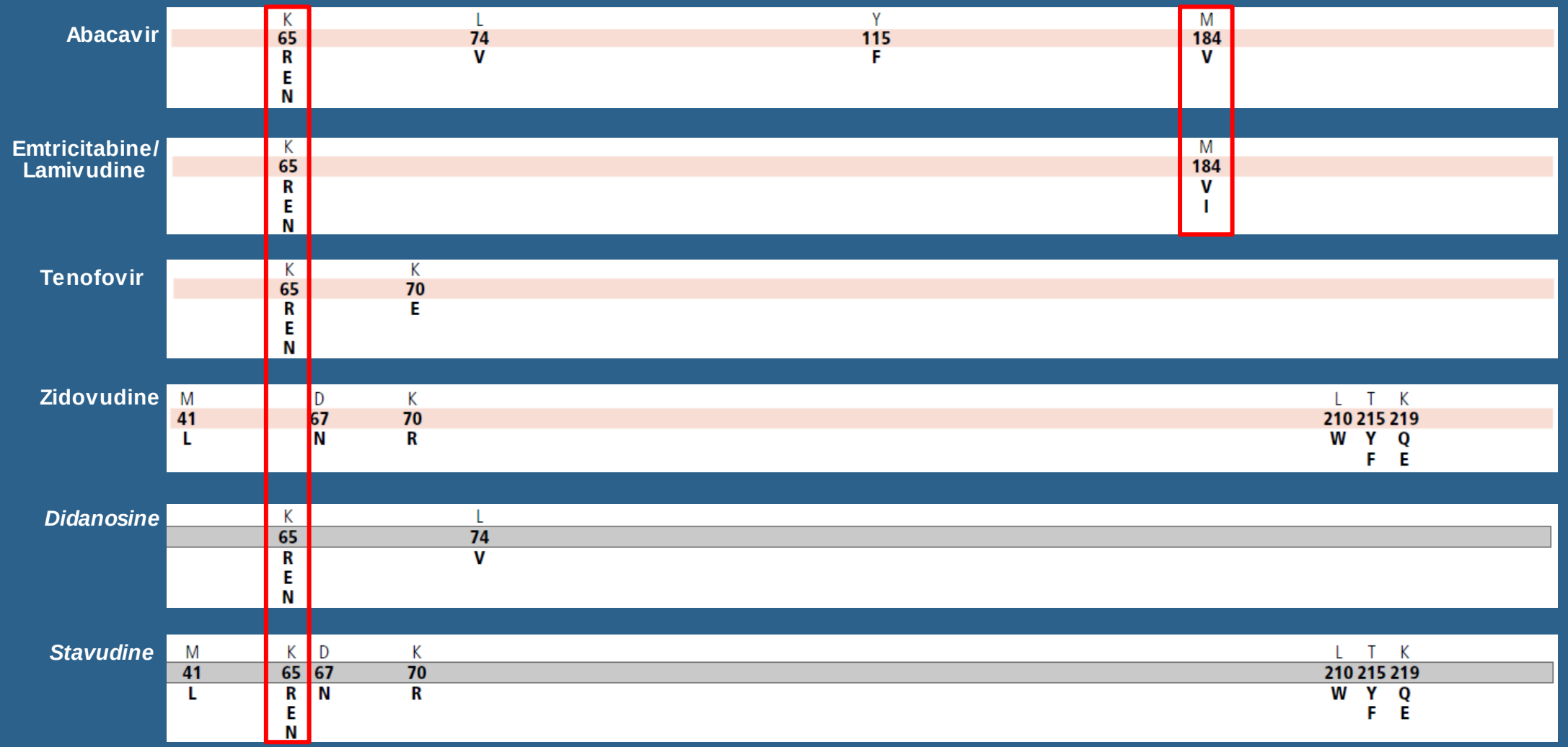
A	V	F	F	Q
62	**75**	**77**	**116**	**151**
V	**I**	**L**	**Y**	**M**

Multi-nRTI Resistance: Thymidine Analogue-Associated Mutations (TAMs; affect all nRTIs currently approved by the US FDA other than emtricitabine and lamivudine)

M	K	L	T	K
41	**70**	**210**	**215**	**219**
L	**R**	**W**	**Y**	**Q**
			F	**E**

Wensing et al. *Top Antivir Med*. 2022;30(4):559-574.
Updates, user notes, and references available at www.iasusa.org.

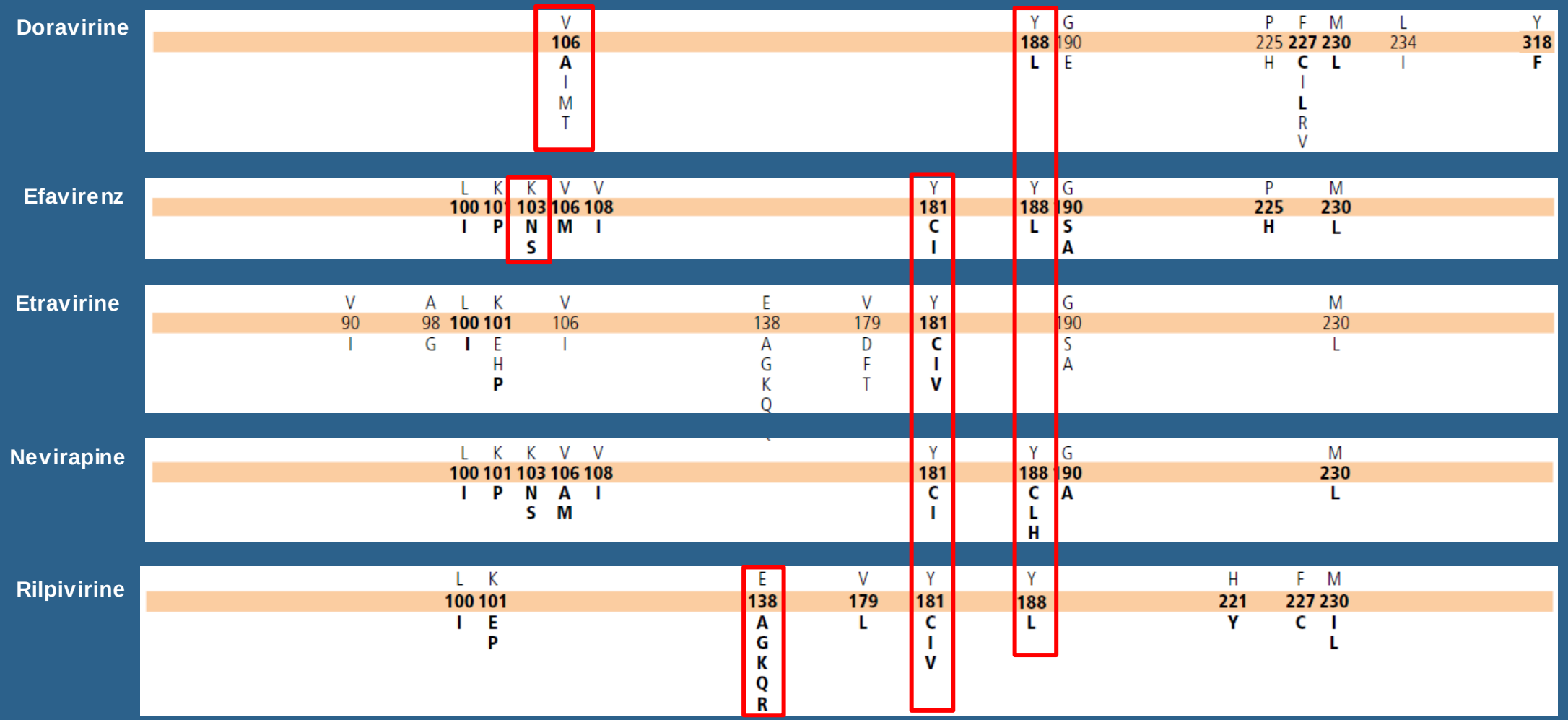
Mutations in the Reverse Transcriptase Gene Associated With Resistance to Reverse Transcriptase Inhibitors (cont'd)



Wensing et al. *Top Antivir Med*. 2022;30(4):559-574.
Updates, user notes, and references available at www.iasusa.org.

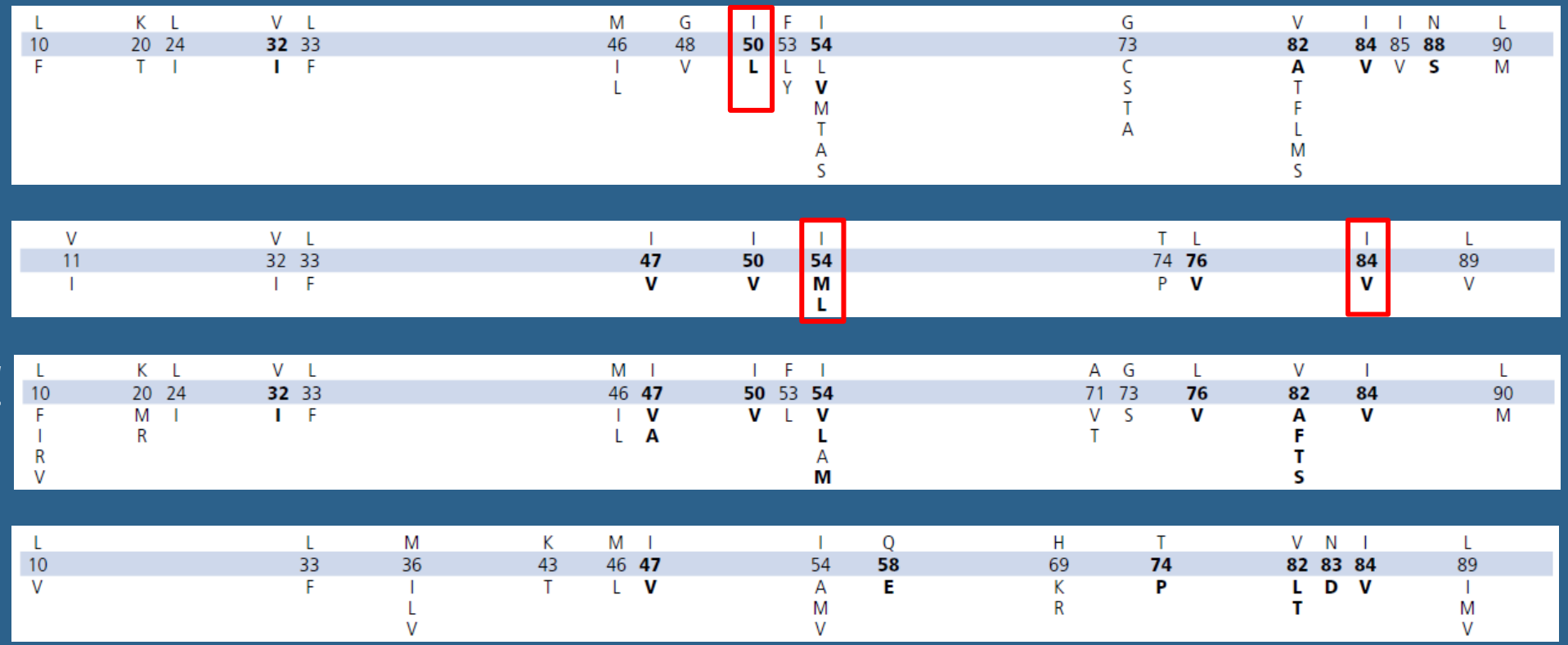
Mutations in the Reverse Transcriptase Gene Associated With Resistance to Reverse Transcriptase Inhibitors (cont'd)

Nonnucleoside Analogue Reverse Transcriptase Inhibitors



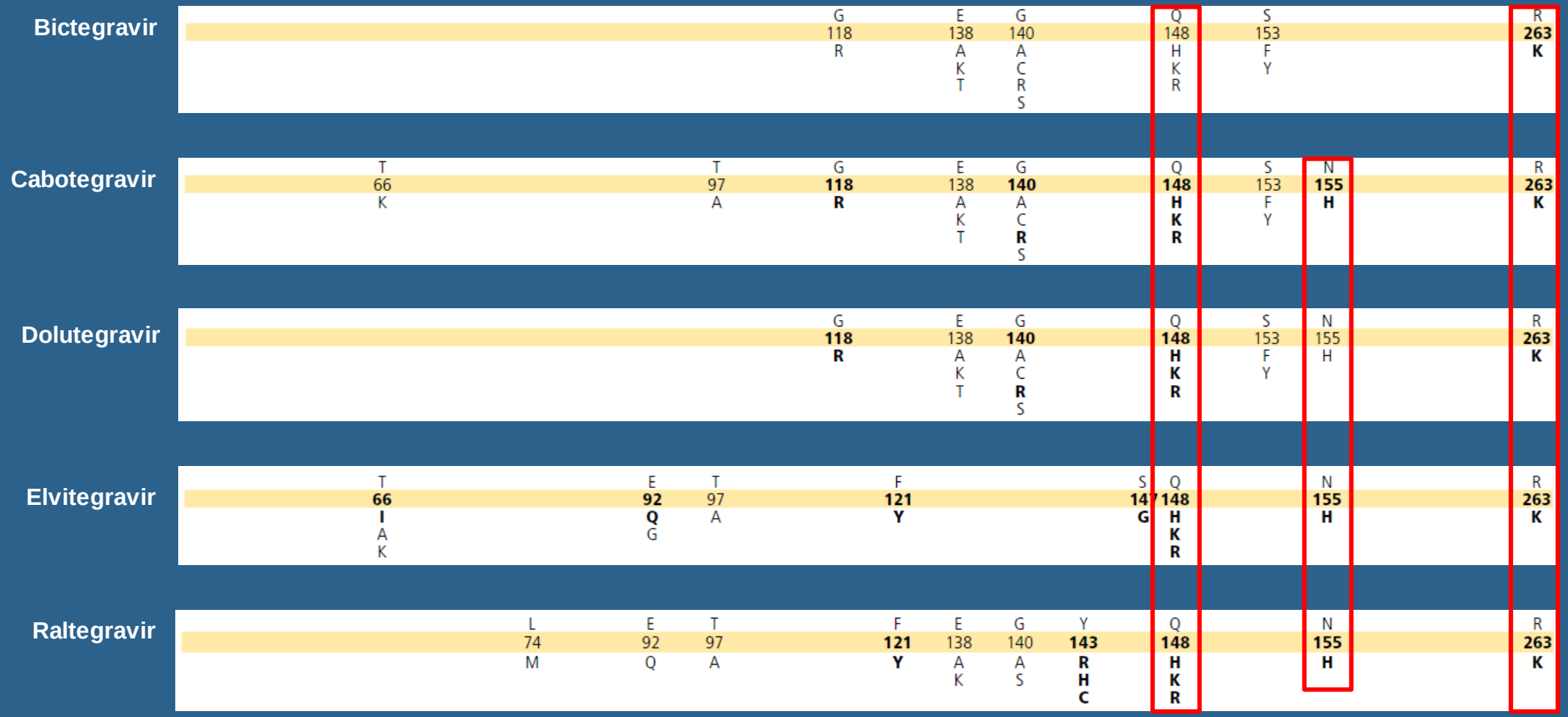
Wensing et al. *Top Antivir Med*. 2022;30(4):559-574.
Updates, user notes, and references available at www.iasusa.org.

Mutations in the Protease Gene Associated With Resistance to Protease Inhibitors



Wensing et al. *Top Antivir Med*. 2022;30(4):559-574.
Updates, user notes, and references available at www.iasusa.org.

Mutations in the Integrase Gene Associated With Resistance to Integrase Strand Transfer Inhibitors

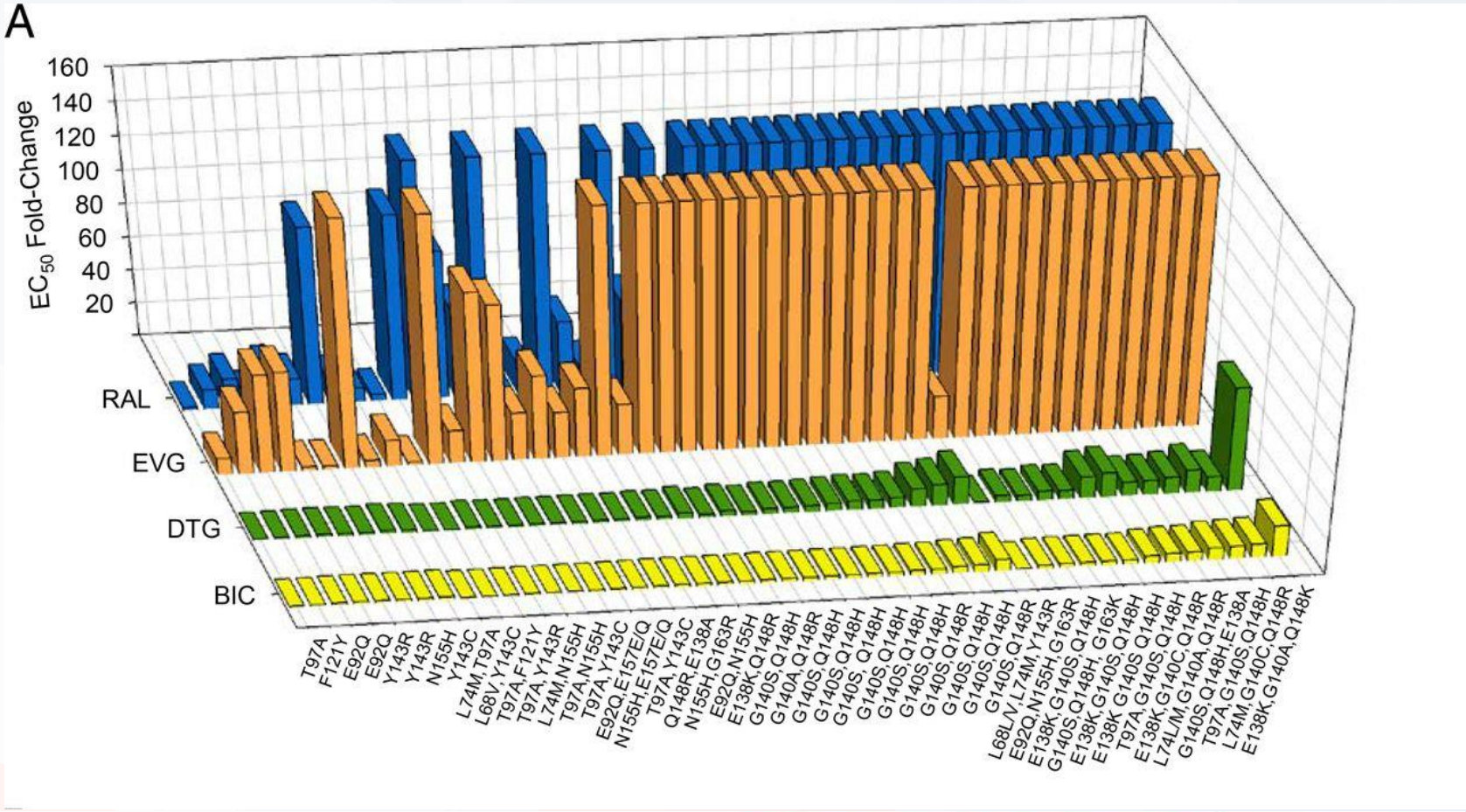


Wensing et al. *Top Antivir Med*. 2022;30(4):559-574.
Updates, user notes, and references available at www.iasusa.org.

Miscellaneous (1): HIV replication "fitness"

- **Fitness** can be measured:
  - in the **lab**
    - "Replicative capacity" – how well/efficiently does the virus replicate?
  - in the **patient**
    - Current viral load in the presence of ART vs. baseline/off ART
- Can explain some phenomena:
  - Meds that shouldn't be active having an impact:
    - 3TC/FTC, other NRTIs
  - Duration of resistance mutations & transmitted resistance
    - M184V vs. K103N

Miscellaneous (2): INSTI resistance



Manuel Tsiang et al. *Antimicrob. Agents Chemother*. 2016;60:7086-7097

Miscellaneous (3):
Resources for ART resistance testing & interpretation

DHHS Treatment Guidelines https://clinicalinfo.hiv.gov/en/guidelines/hiv-clinical-guidelines-adult-and-adolescent-arv/drug-resistance-testing

IAS-USA https://www.iasusa.org/resources/hiv-drug-resistance-mutations/

AIDS Education & Training Center https://aidsetc.org/resource/hhs-adult-art-guidelines-treatment-experienced-patients

Stanford HIV Drug Resistance Database http://hivdb.stanford.edu/

Recap

- **HIV resistance** is a genetic change in the virus that impacts activity of ART drugs
- Resistance can result from **low adherence**, **ART potency**, and/or **drug absorption**
- **Genotype resistance testing is most common**; phenotype testing is available for use in unusual cases
- **Genotype** testing is **recommended in all persons starting ART & in some treatment-experienced persons with detectable viral load**
- The most **common & important mutations differ by ART drug & class**
  - **M184V** is most common NRTI resistance mutation → resistance to 3TC/FTC
- **Online resources & guidelines** are your friends!

Learning Objectives

- By the end of this session, each participant will be able to:
  - ✓ **Define** HIV drug resistance
  - ✓ **List** 2-3 reasons why HIV drug resistance develops
  - ✓ **Describe** the difference between HIV genotype & phenotype testing
  - ✓ **Recognize** 1-2 key resistance mutations
  - ✓ **Identify & access** online HIV resistance resources

Thank you!

Questions or comments?

Todd Hulgan, MD, MPH
Department of Medicine, Division of Infectious Diseases
Vanderbilt University Medical Center
Veterans Affairs – Tennessee Valley Veterans Healthcare System
Nashville, TN
todd.hulgan@vumc.org

AETC Program National Centers and HIV Curriculum

- **National Coordinating Resource Center –** serves as the central web –based repository for AETC Program training and capacity building resources; its website includes a free virtual library with training and technical assistance materials, a program directory, and a calendar of trainings and other events. Learn more: https://aidsetc.org/
- **National Clinical Consultation Center –** provides free, peer-to-peer, expert advice for health professionals on HIV prevention, care, and treatment and related topics. Learn more: https://nccc/ucsf.edu
- **National HIV Curriculum –** provides ongoing, up –to-date HIV training and information for health professionals through a free, web –based curriculum; also provides free CME credits, CNE contact hours, CE contact hours, and maintenance of certification credits. Learn more: www.hiv.uw.edu