

# HIV The Basics

## Resistance 101: Interpreting and Using the Data

Sources: IAS - <http://www.iasusa.org>

Stanford resistance database - <http://hivdb.stanford.edu>

DHHS - <http://www.aidsinfo.nih.gov/guidelines/>

Update of Drug Resistance Mutations in HIV:2009 *Topics HIV Med.*17:138-145

Elizabeth Race, MD, MPH

Acknowledgements:

Joel E. Gallant, MD, MPH

Johns Hopkins University School of Medicine

Ben J. Barnett, MD

Univ. of Texas Health Science Center, Houston



# Objectives

- At the conclusion of this presentation, listeners should be better able to:
- Identify the most clinically significant antiretroviral resistance mutations associated with various classes of HIV drugs
- Select appropriate types of antiretroviral resistance tests and implement these tests into their clinical practice to maximize successful patient outcomes

## **Off-Label Disclosure:**

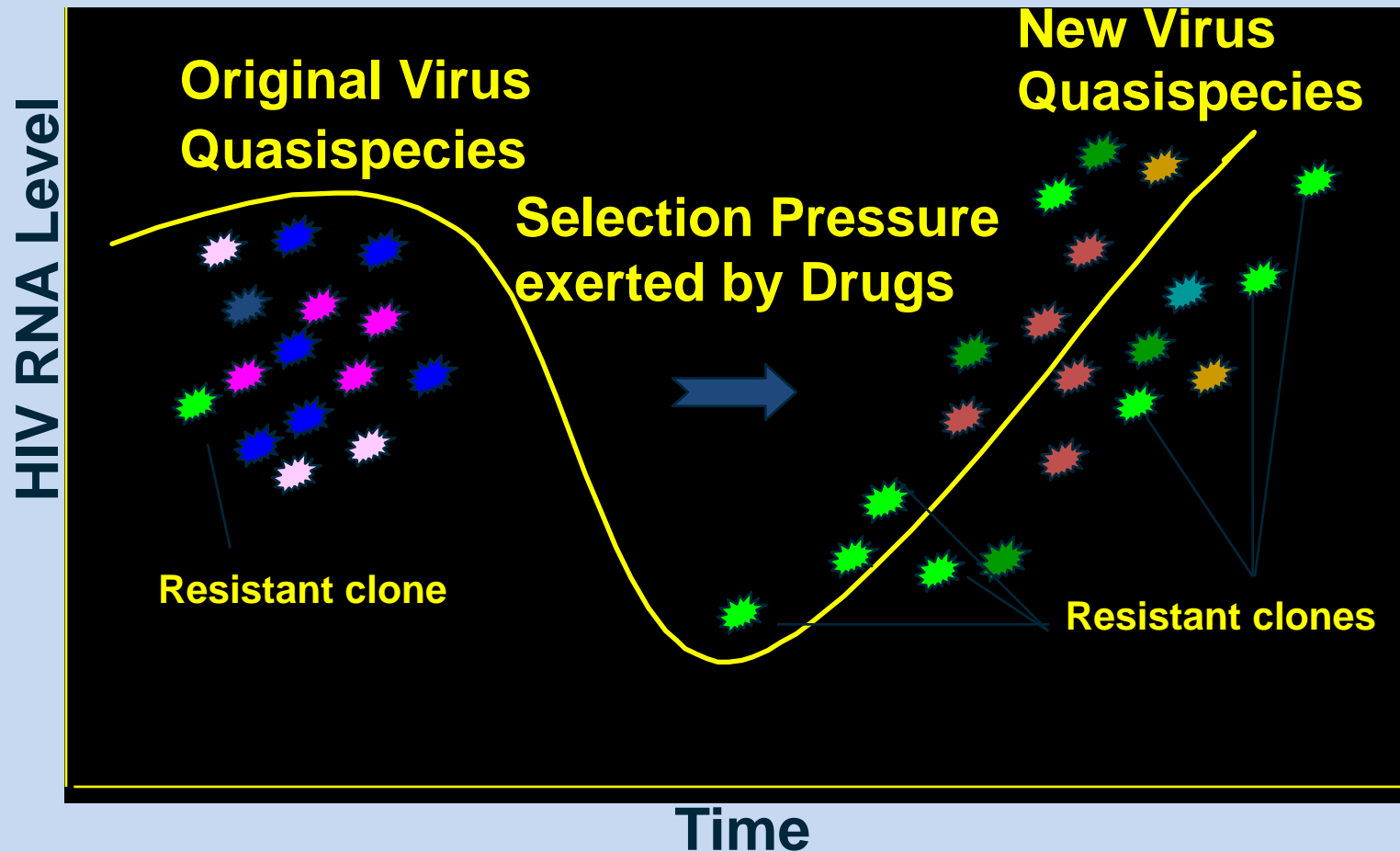
**There will be no off-label/investigational uses discussed in this presentation.**



# What is Resistance & How Does It Occur?

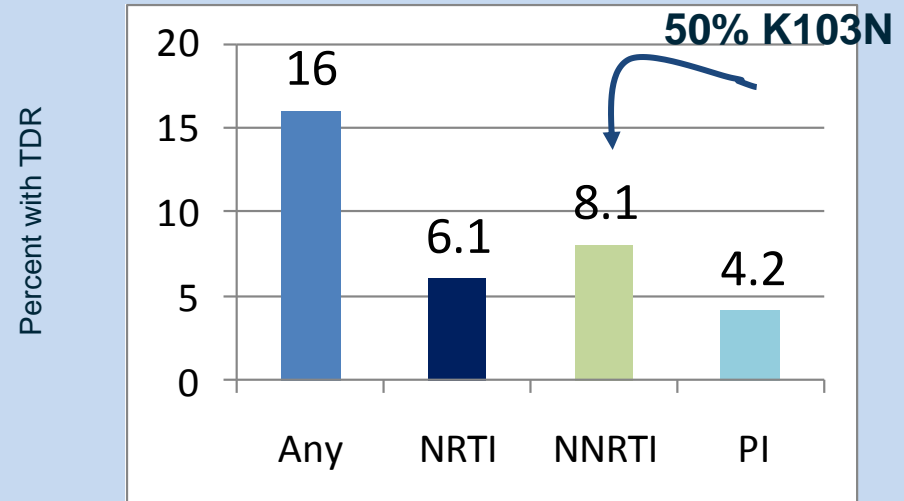
- The ability of HIV to multiply in the presence of suppressive level of antiretroviral drugs; Does **not** make HIV more pathogenic
  - Perhaps less pathogenic? Mutations may impair viral fitness.
- Can occur as primary transmission of resistant virus (TDR)
- Can occur with sub-suppressive levels of anti-viral drugs & then natural selection of mutant strains
  - Partial adherence to regimen
  - Sub-optimal dosing of drugs
  - Drug interactions
  - Incomplete absorption in intestinal tract

# Viral Resistance is the Outcome of Viral Replication, Mutations & Selection Pressure



# US Transmitted Drug Resistance: Newly Diagnosed

- 2007 CDC surveillance for TDR detected 16% of pts with new HIV diagnosis & mutations
  - Most common: NNRTI
  - 83% had single mutation



**Primary Resistance in Young Pts: 55 recently infected pts (16-24 yo) from 15 US cities; approx. 50% AA; 25% Hisp.**

Resistance	By Genotype	By Phenotype
<b>Overall</b>	<b>18%</b>	<b>22%</b>
<b>NNRTI</b>	<b>15%</b>	<b>18%</b>
<b>PI</b>	<b>3.6%</b>	<b>5.5%</b>
<b>NRTI</b>	<b>4%</b>	<b>4%</b>

# Resistance Testing: DHHS Guidelines

- Recommended in acute HIV infection
- Should be done for all pregnant women prior to therapy, or for those entering pregnancy with a detectable HIV VL on therapy
- In chronic infection, recommended for all patients on entry into care, regardless of treatment plan
- Perform when managing suboptimal VL decrease
- In the setting of viral failure, testing should be done while the patient is on therapy, or within 4 wks of stop
- Recommended to assist in selecting active drugs for pts with viral failure & **VL > 1000; Consider in VL > 500**

# Methods & Limitations of Resistance Testing

- Genotype:
  - Direct sequencing of viral genes **reverse transcriptase** and **protease**, less commonly **integrase** and **envelope**
  - Resistance to specific drugs is **predicted** based on known mutations
  - Mutations are detected only if mutant virus is at least 10-20% of virus population; **minor variants can be missed**
- Phenotype
  - Grow virus in culture with various amounts of drugs added
  - Direct measure of viral resistance
  - Does not explore the underlying mutations, just their affect on the ability of the drug to stop the virus
- Resistance tests are most accurate in assessing the *current* regimen; **if resistance has ever been detected, then archived mutations exist**
  - If no drug pressure exists, “wild type” virus will often overgrow the mutant strains

# Genotypes

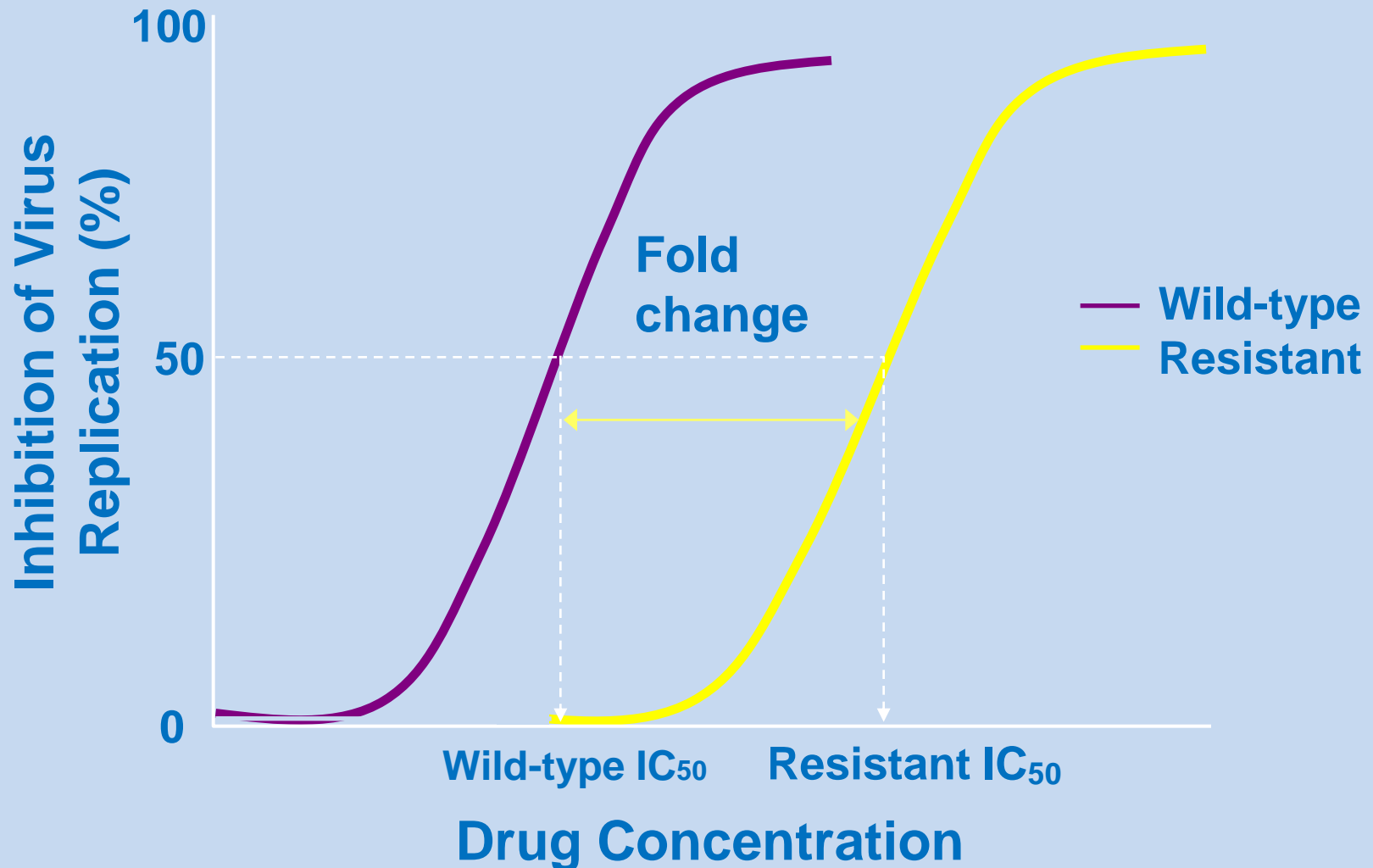
- Involve sequencing of various HIV genes and comparing results to reference wild type (wt) strain
- **Primary mutations** decrease drug susceptibility
  - Example: **M 184 V** in Reverse Transcriptase (RT)
  - **184** refers to amino acid (AA) position **184** in RT
  - **M** (methionine )is the wt AA;**V** (valine) is the mutant
  - “Mixtures” are when both wt and mutant AA’s are detected:  
**M 184 M/V**
- **Secondary mutations** are selected after 1<sup>o</sup> mutations and may have a limited or cumulative effect
- **Multi-Drug Resistance (MDR) mutations** can decrease susc. to many or all drugs in a single class

# HIV Phenotype

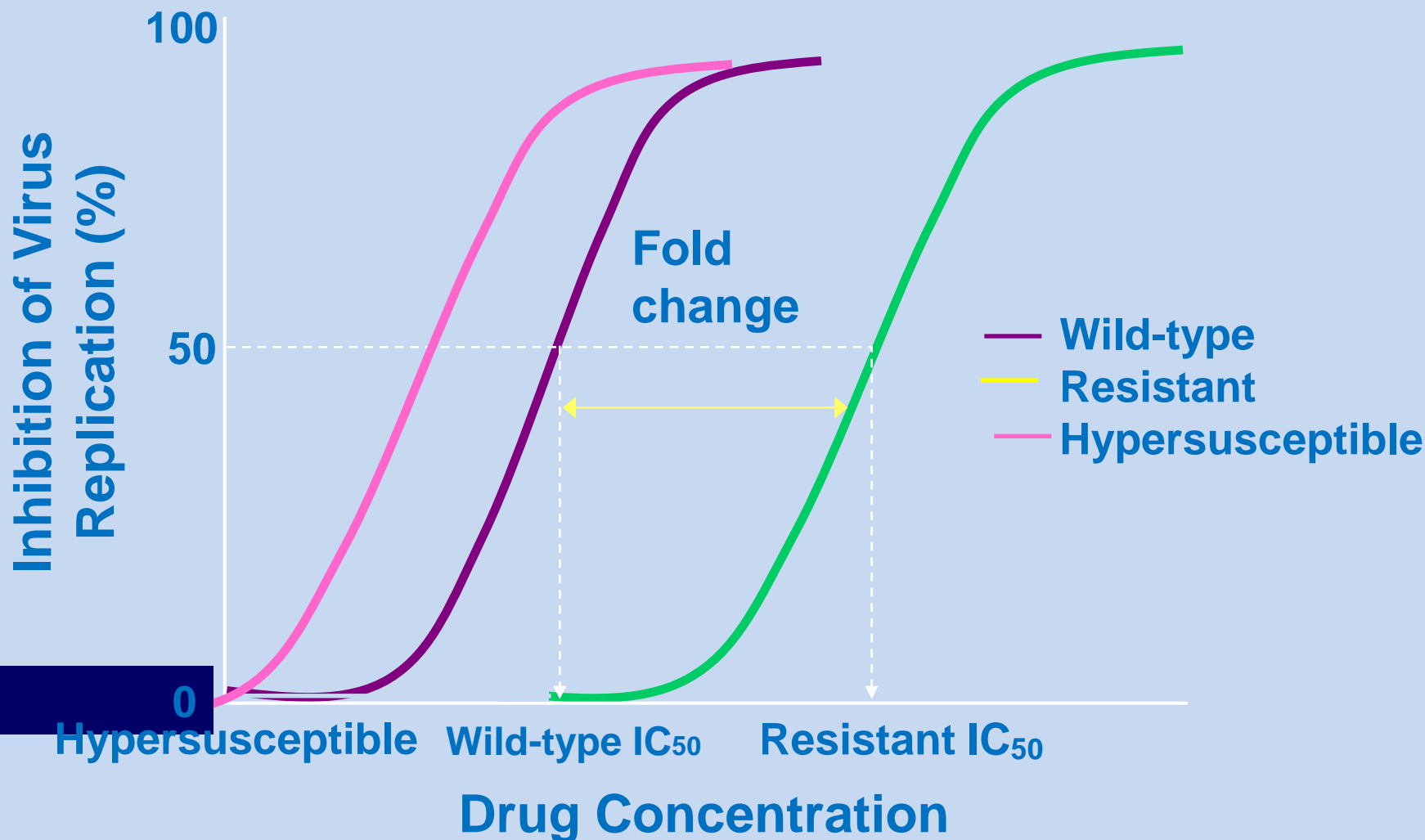
- Phenotype refers to the growth characteristics virus in vitro; most useful for **etravirine** or **PI's**; also **non-subtype B HIV**
- Standard phenotypic testing
  - Results usually expressed as fold-change in susceptibility compared to a laboratory control isolate
  - Interpretation of drug activity dependent on methodology used to define cutoffs (clinical, biological, technical)
- Virtual phenotype testing
  - Matches genotypic data against database of virus samples with paired GT and PT data
  - Confidence level based on number of matching genotypes within the database

✓ Clinical cutoffs : based on pt virological response in clinical trials  
Biologic cutoffs : based on natural variability of wt viruses from naïve pts  
Technical cutoffs: based on assay variability w/ repeated testing of pt samples

# Phenotypic Susceptibility: Relationship Between Drug Concentration and Viral Inhibition

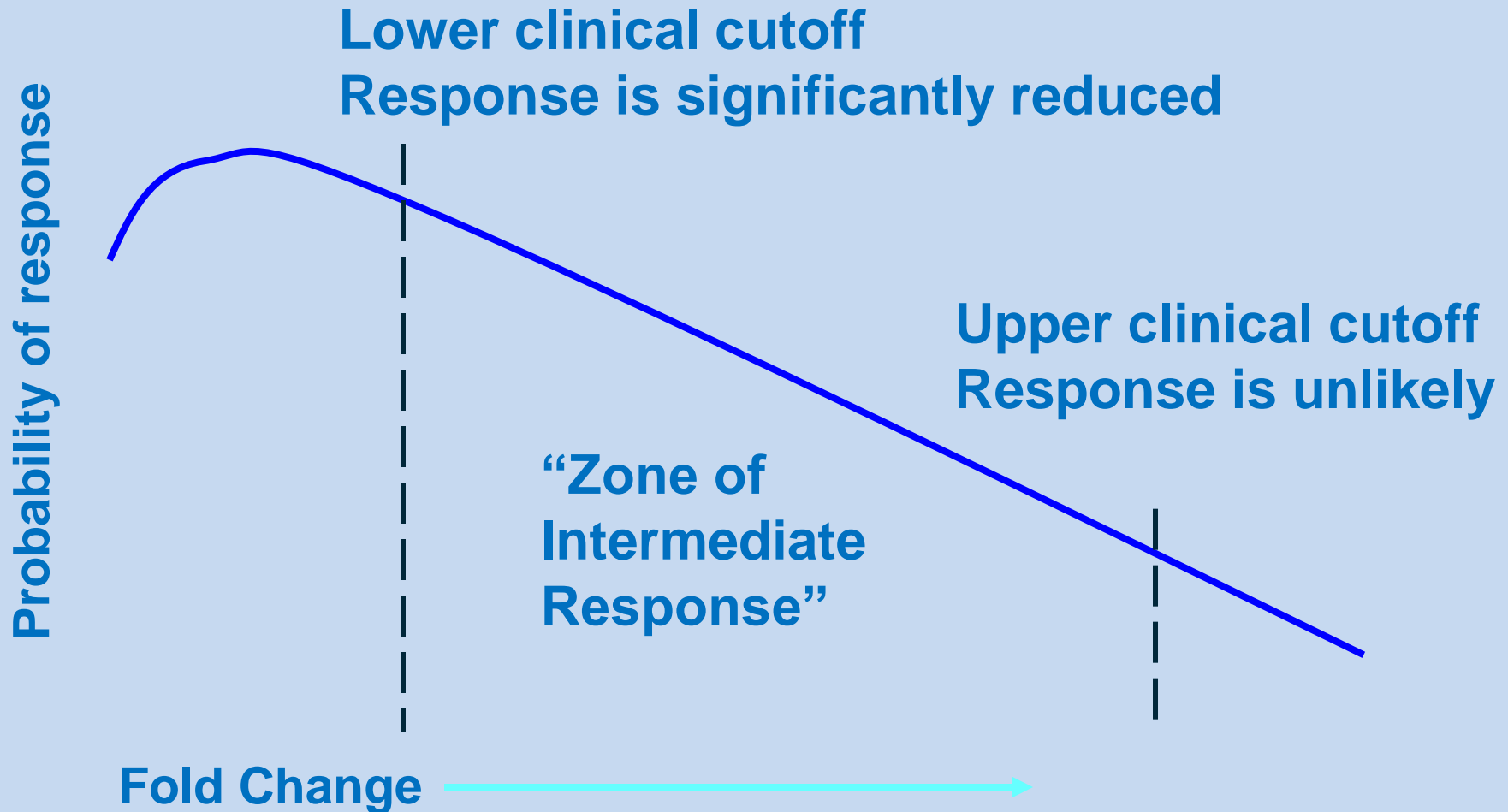


# Phenotypic Susceptibility: Relationship Between Drug Concentration and Viral Inhibition



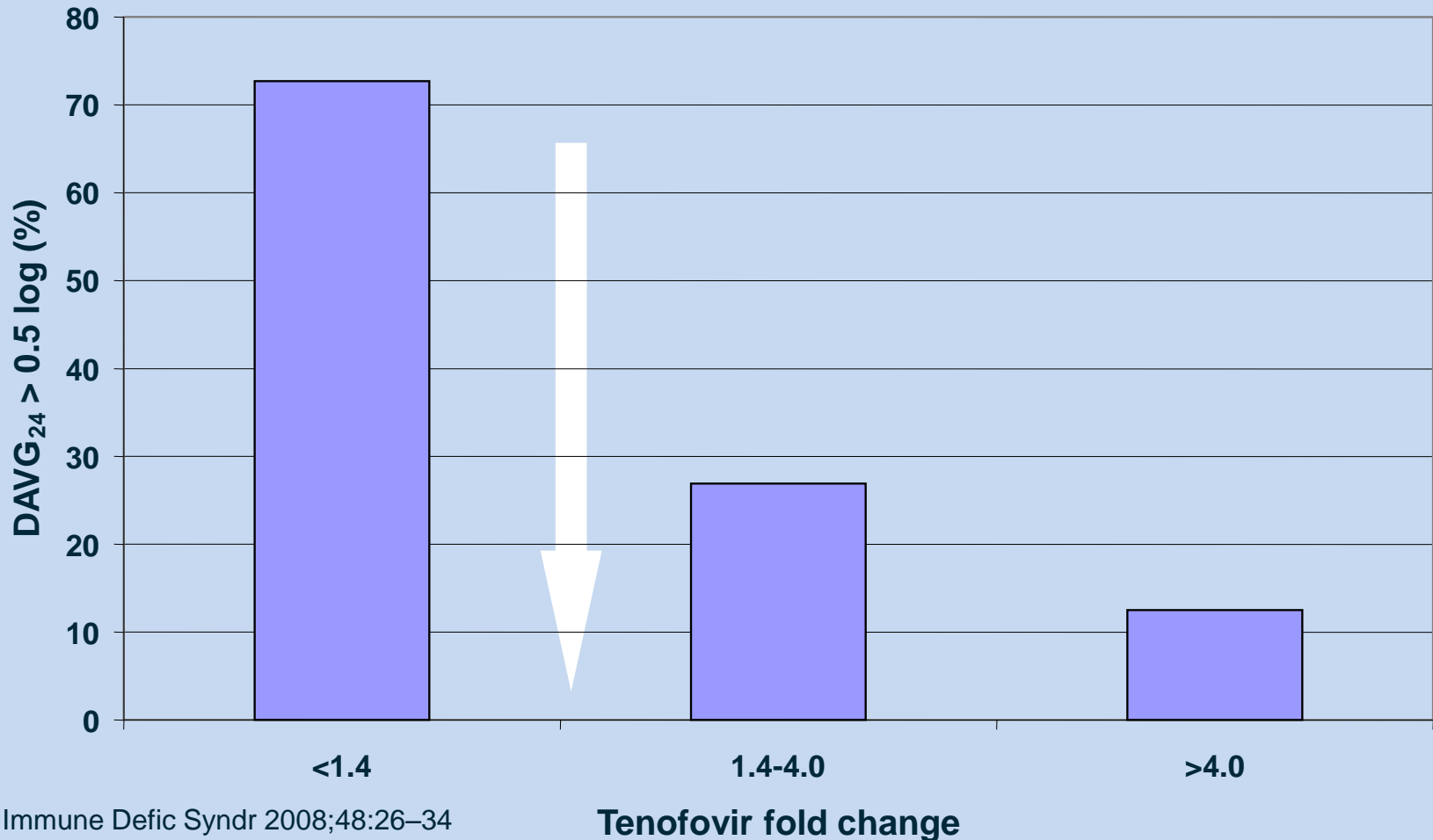
# Interpreting Phenotypes

## Clinical Cutoffs differ for each drug



# TFV cutoff analysis obtained from clinical trial data

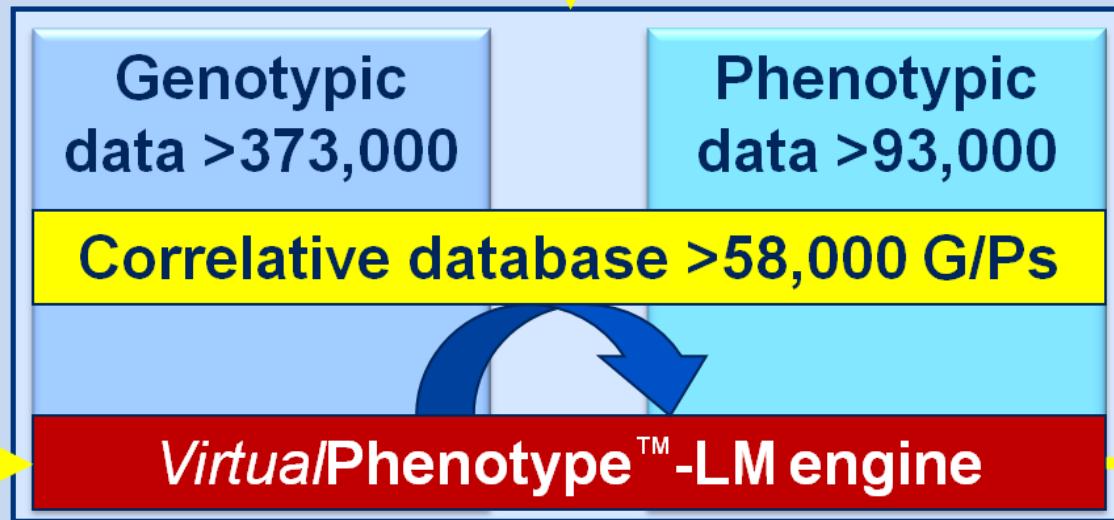
Average change in viral load over 24 weeks



J Acquir Immune Defic Syndr 2008;48:26–34  
B. Lu, et al 11th Resistance Workshop 2001, Seville  
Miller, et al 5th Resistance Workshop 2001, Scottsdale

# Correlative and Clinical Outcomes Databases\* **Virtual Phenotype**

- Routine clinical testing
- Clinical trials
- Research collaborations



Vermeiren H Van  
Craenenbroeck E, Alen P,  
et al. J Virol Methods  
2007; 145(1): 47-55  
Winters B. Van Craenen-  
broeck, J Virol Methods  
162(2009) 101-108

Nucleotide  
sequence  
(...AAGTC  
TCCGCAT  
GCATA...)

Clinical Outcomes Database  
>21,000 patients or  
>8,800 Treatment Change Episodes

Calculated  
Fold-Change  
values in  
IC<sub>50</sub>

Clinical  
Cut-Offs

\*Status Dec 08

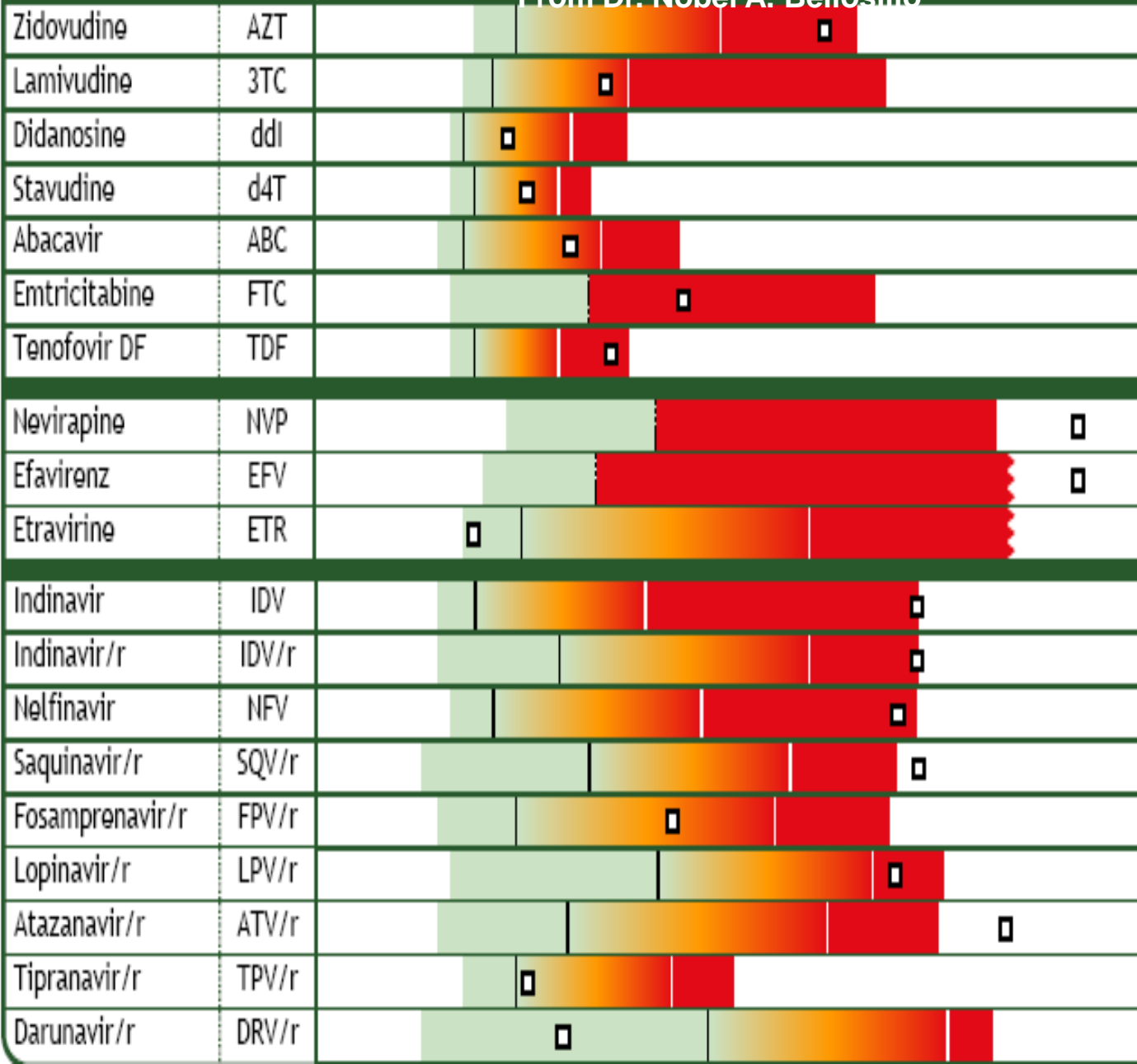
**DRUGS**

# Example of Virtual Phenotype

FC	(95% confidence limits)	CCO 1	CCO 2	BCO
32.2	(30.4-34.1)	1.5	11.4	
3.7	(3.4-4.0)	1.2	4.6	
1.4	(1.4-1.5)	0.9	2.6	
1.7	(1.6-1.8)	1.0	2.3	
2.6	(2.4-2.7)	0.9	3.5	
8.0	(7.5-8.6)			3.1
3.9	(3.7-4.2)	1.0	2.3	
364.8	(302.2-440.5)			6.0
733.0	(613.4-876.0)			3.3
1.0	(0.9-1.1)	1.6	27.6	
80.0	(71.6-89.3)	1.0	5.4	
80.0	(71.6-89.3)	2.3	27.2	
65.9	(60.4-72.0)	1.2	9.4	
81.5	(59.7-111.3)	3.1	22.6	
7.1	(6.8-7.5)	1.5	19.5	
64.2	(60.1-68.7)	6.1	51.2	
191.2	(159.1-229.9)	2.5	32.5	
1.7	(1.6-1.8)	1.5	7.0	
2.4	(2.2-2.7)	10.0	106.9	

From Dr. Nobel A. Bellosillo

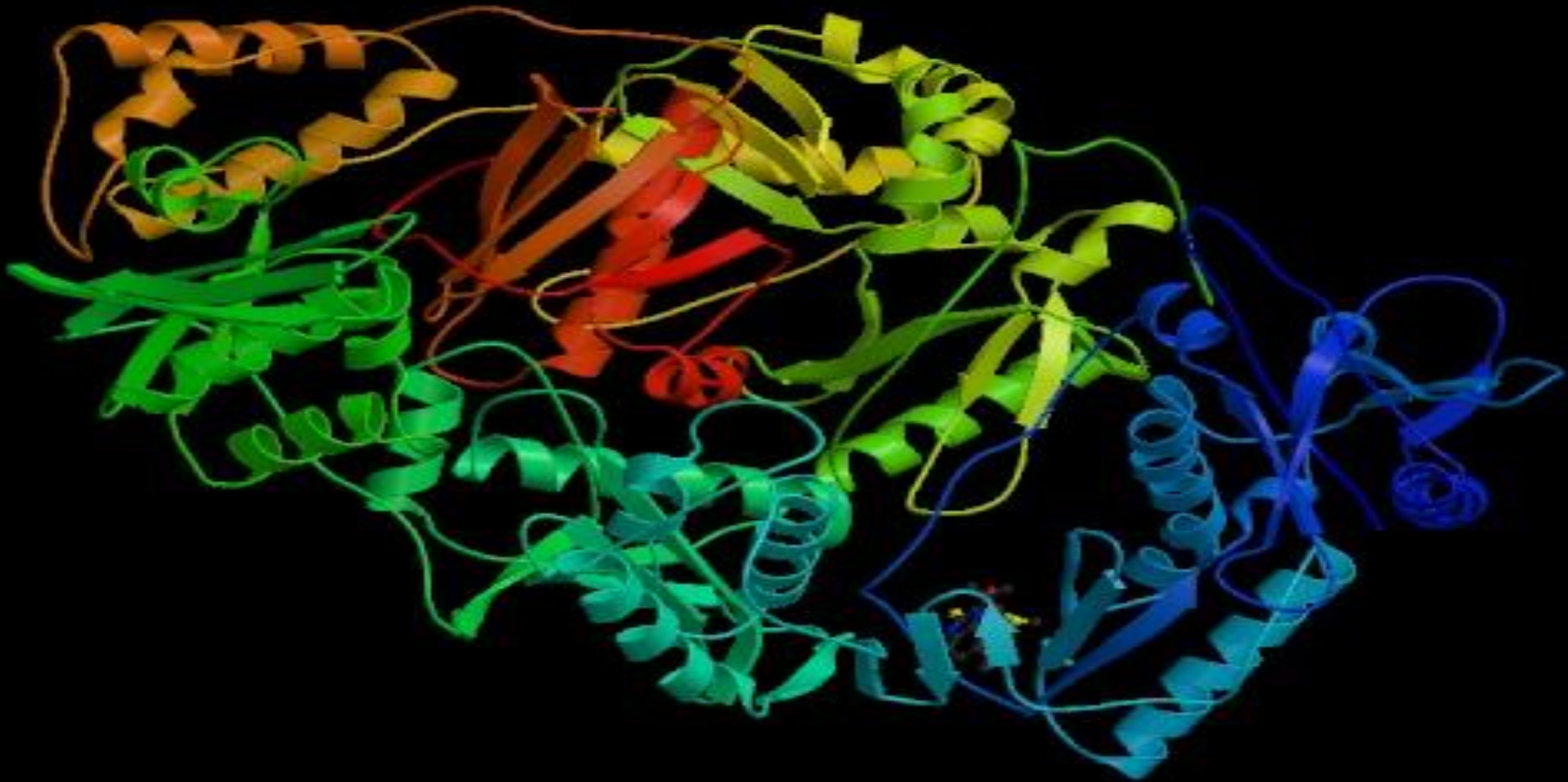
(>200)



# Which Resistance Test When? (DHHS)

- Genotype preferred due to faster result, lower cost and enhances sensitivity for detecting “mixtures”
  - In anti-retroviral naïve patients
  - In patients with sub-optimal viral response on therapy
  - Virologic failure on a first or second regimen
- Phenotype
  - “Addition of phenotypic testing to genotypic testing is generally preferred for persons with known or suspected complex drug resistance mutation patterns, particularly to protease inhibitors”
- Virtual Phenotype (not specifically stated in DHHS)
  - As a substitute when actual phenotype not available

# HIV-1 Reverse Transcriptase



# Major nRTI Mutations

- **M184V** – diminishes viral fitness by approx. 50%\*
  - Resistance to lamivudine and emtricitabine
  - Some resistance to didanosine and abacavir
  - **Restores** some activity to zidovudine/d4t, tenofovir
- **K65R**
  - Broad resistance to all nRTI; but ↑ susc. to AZT
- **L74V**
  - Resistant to abacavir & DDI; ↑ susc.to AZT,TDF
- **TAMs – 215, 41, 210, 67, 70, 219:** ↓ susc. to all nRTI
  - Selected by a prior tx history of AZT, D4T
  - More resistance w/ 41/210/215 than 67/70/219 path
  - **44D, 118I:** ↑ nRTI resistance with 41/210/215 path

# Multinucleoside & Nucleotide Resistance

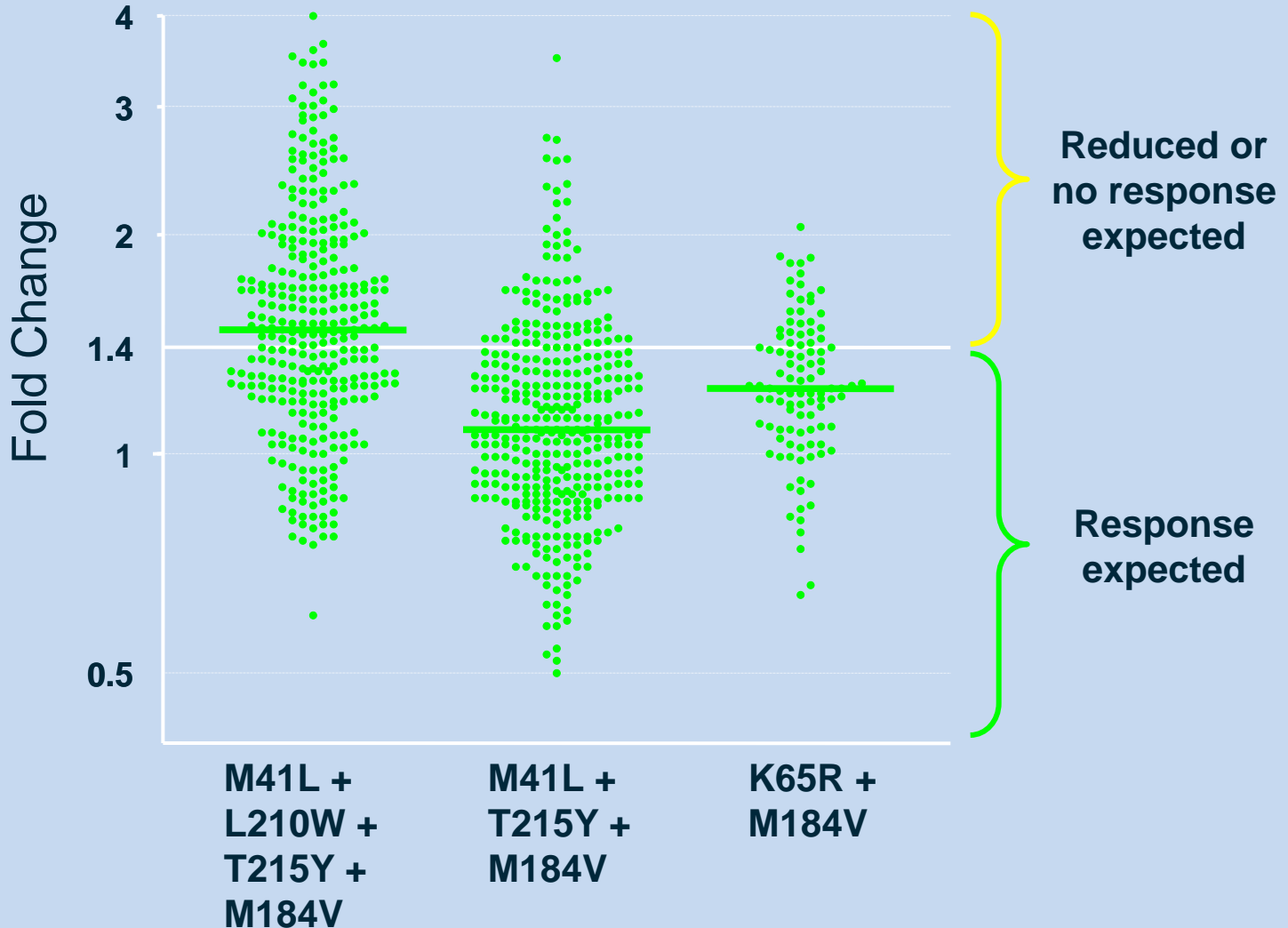
- Multinucleoside resistance is typically associated with high level resistance to most nucleosides:
  - **Q151M** complex; selected for by AZT/DDI use; **TDF susceptibility preserved**
  - serine insertions - **69S(S,S)**; selected for by DDI/D4T; assoc. with **TDF resistance**
  - multiple NAMS, especially with M184V
  - **K65R – only ZDV reliably active**; some d4T/TDF/ABC activity possible, but reduced
- Tenofovir resistance: K65R, **41/210/215Y**, *but may retain phenotypic (and clinical) activity*

# Tenofovir Susceptibility Ranges

Green dots = patient viruses from Monogram database

Grouped by shared mutational pattern

Current genotype algorithms would assess all viruses as **Resistant**



# ARS Question #1

When a treatment-experienced pt's genotype shows K65R, the most likely interpretation is:

1. The pt is and will always be always highly resistant to tenofovir, so it should be stopped
2. The patient has developed the primary tenofovir resistance mutation, but tenofovir may still retain some activity in this pt
3. The pt has transmitted drug resistance from another individual
4. AZT is ***not*** likely to be effective in this pt

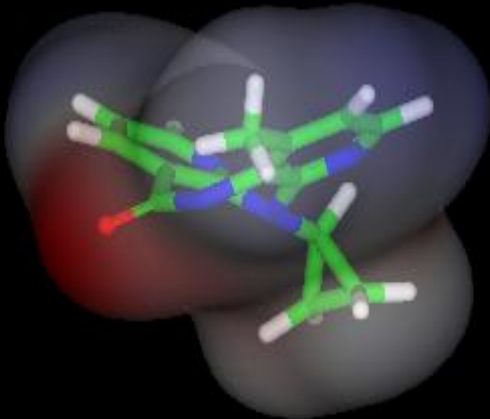
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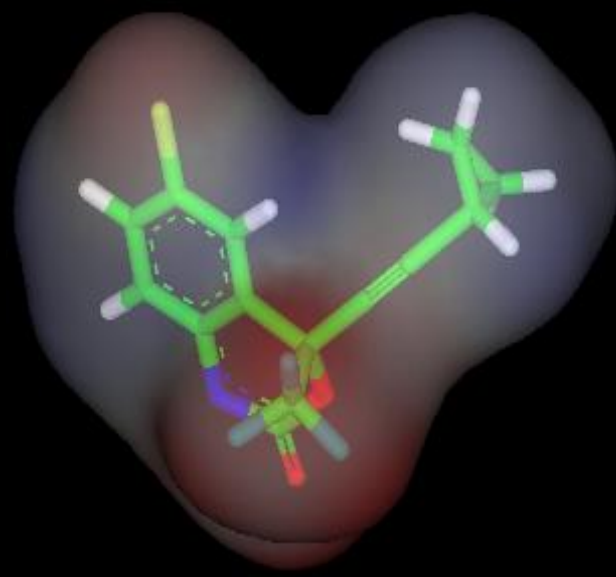
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# Major NNRTI mutations

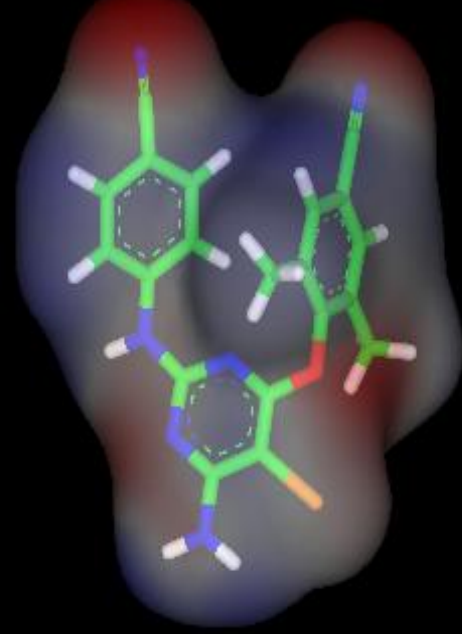
- **K103N**
  - Most common NNRTI mutation
  - High level resistance to efavirenz & nevirapine **but not etravirine**
- **Y181C**
  - High level resistance to nevirapine & intermediate to efavirenz
  - Some etravirine resistance, but provides a mutational foundation for development of higher levels of resistance
- **98/101/106/108/188/G190A** also important; **in non-clade B, 106**
- There is broad cross resistance between nevirapine and efavirenz due to low genetic barriers
  - A single mutation can eliminate activity of EFV or NVP
  - *No impact of NNRTI mutations on viral fitness*, so continued use of NNRTI in the face of resistance adds nothing



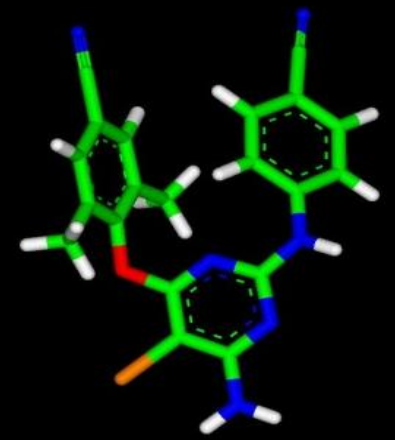
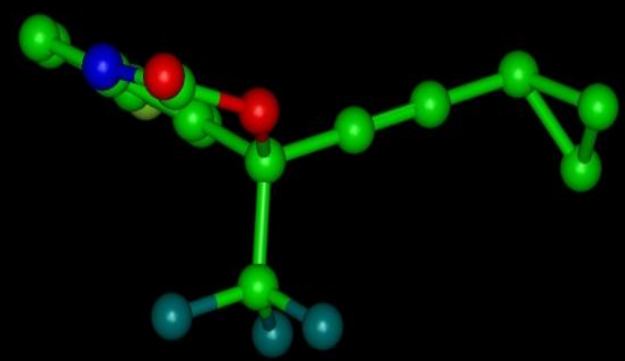
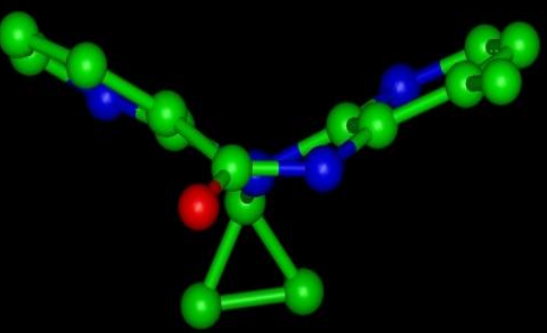
NVP(Nevirapine)



EFV (Efavirenz)



ETR (Etravirine)



# First and Second-Generation NNRTI's

Clotet B, et al. AIDS Review 2004; 6:123-130; K Das, et al. J Med Chem 2004:47:2550-2560; JOA De Kerpel, et al. Presented at 224<sup>th</sup> ACS; Aug 18-22, 2002, Boston MA; Poster 570269.

# Etravirine: Second generation NNRTI

- May retain activity against HIV with NNRTI resistance from NVP or EFV

***K103N alone does not affect etravirine***

- Has a higher genetic barrier than other NNRTI, therefore a mutation score has been developed

**Y181C** yields a “resistance weight factor” of 2.5  
(intermediate)

**G190A** yields a “resistance weight factor” of 1 (low)

- In a study of 14,940 samples submitted for resistance testing: 5,482 (36.7%) had resistance to EFV or NVP, but **67.2% remained sensitive to ETR by genotype and 76.4% by phenotype**

# Etravirine Resistance Score

Weighted mutation score corresponded to response rates as follows :

- 0-2: 74% (highest response)
- 2.5-3.5: 52% (intermediate response)
- $\geq 4$ : 38% (reduced response)

Weighted Mutation Score	1	1.5	2.5	3
Mutation in RT	90I, 179D, 101E, 101H, 98G, 179T, 190A	138A, 106I, 190S, 179F	101P, 100I, 181C, 230L	181I/V

# ARS Question #2

Pick the most correct statement regarding the potential for “sequencing” NNRTI’s:

1. After a pt develops a K103N mutation on efavirenz, *nevirapine* is often effective
2. A Y181C mutation *completely eradicates* the clinical effect of etravirine
3. A provider need not worry about a genotype with “transmitted” K103N in a newly diagnosed pt, since TDF/FTC/EFV is a potent triple ARV
4. Etravirine, combined with additional ARV’s, may be effective for individuals who have failed TDF/FTC/EFV starting regimens

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# Protease Inhibitors

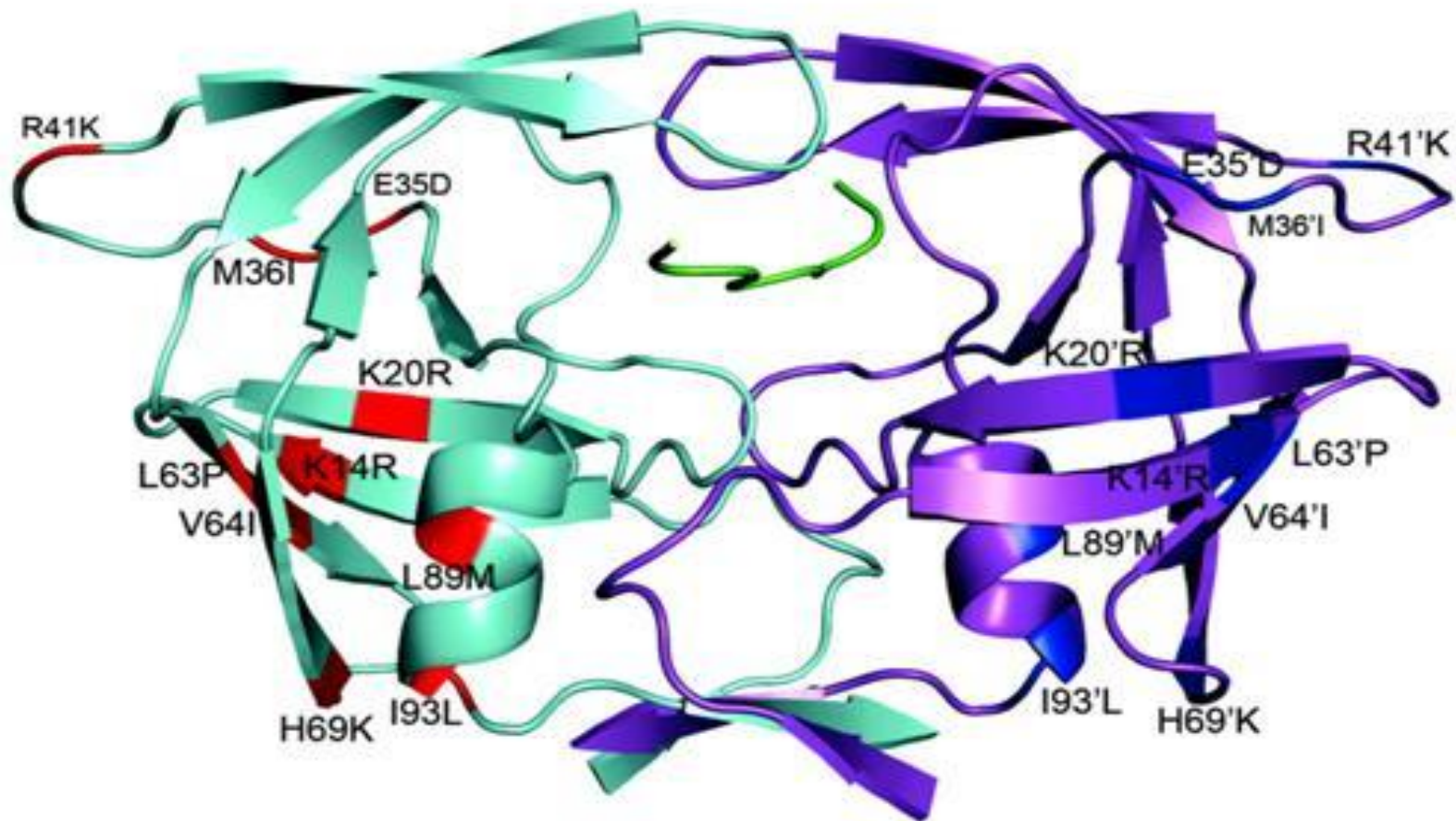


Figure 2. CRF01\_AE protease in complex with p1-p6 (green). Amino acid changes in monomer A (cyan) are indicated in red, and changes in monomer B (magenta) are indicated in blue. (Bandaranayake et al. JV. 2008)

# Major protease mutations

- “Signature” mutations for *non-boosted* PI
  - **D30N**: nelfinavir; no cross-resistance
  - **I50L**: *unboosted* ATV (RTV boosting alters mutations)
  - **I50V**: fosamprenavir; some cross-resistance to lopinavir
  - **G48V**: saquinavir; no cross-resistance
  - **L90M**: often follows unboosted PI's; causes cross-res.
- Boosted PI's (**LPV/r, FPV/r, SQV/r, ATV/r, DRV/r**) usually do not select for resistance if used as **1st PI**
- However, if 1<sup>st</sup>-line boosted PI failure is not addressed promptly, **secondary resistance mutations can accumulate**; ideally obtain phenotype to evaluate

# Resistance to Atazanavir:

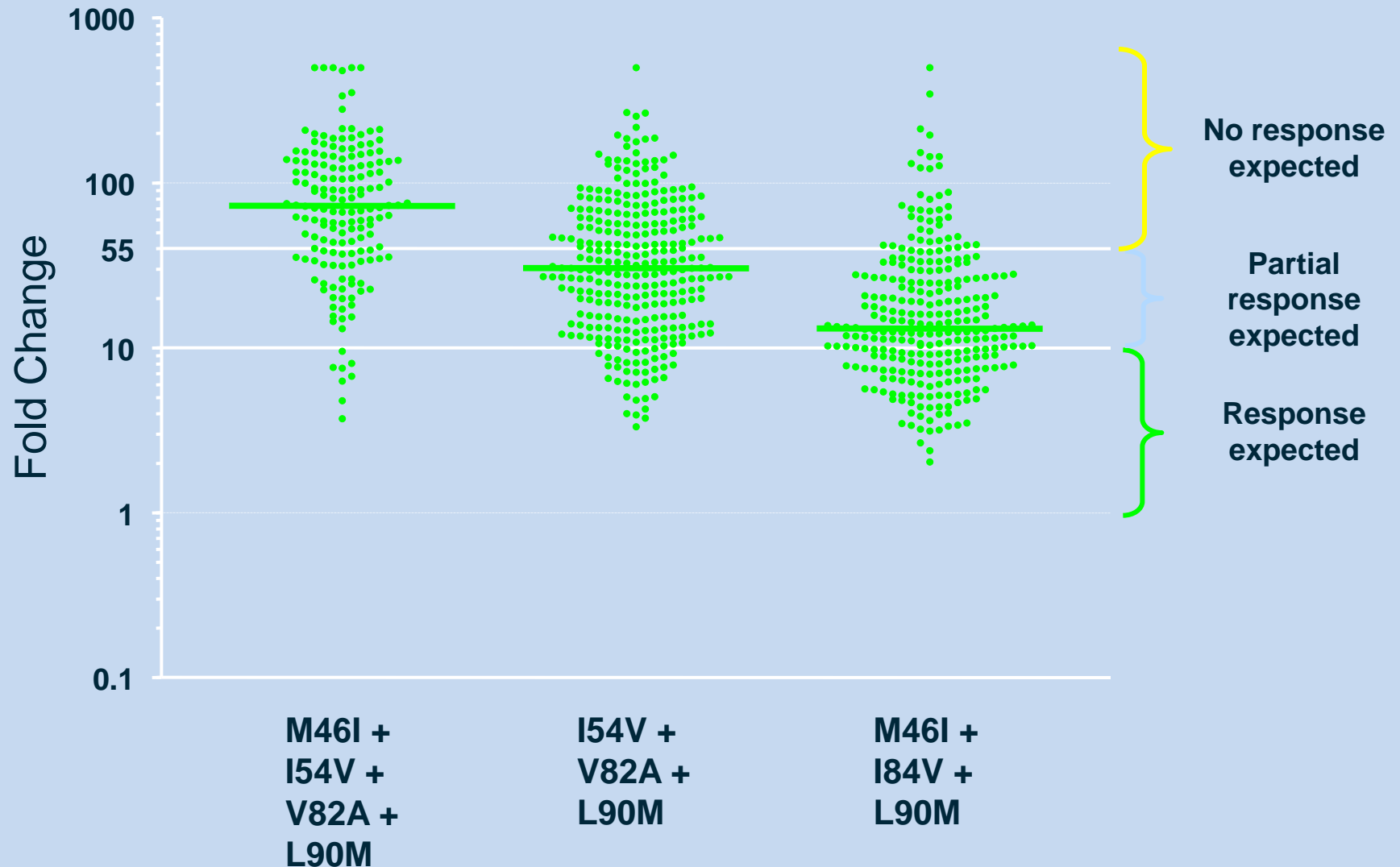
V32I, M46I, I50L, I54L, A71V, V82A, I84V, N88S, L90M

- When given as an initial unboosted PI; selects for **I50L & A71V**
- If used as a subsequent PI; selects for I54L, I84V
- Case report of boosted ATV resistance in a PI-naïve pt: K20T, M36I/V, L63P, A71T, N88S
- Cross-resistance to ATV induced by common PI mutations: V82A & L90M
- In vitro, ATV selects for V32I, M46I, I84V, N88S

# Lopinavir Mutation Score

- **8, 30, 32, 46, 47, 48, 50, 54, 82, 84, 90**
- Clinical response rates decrease when:
  - **LPV mutation score > 5 (“GT-R”)**
  - **LPV IC<sub>50</sub> fold-change > 10 (“PT-R”)**
- Report suggesting that as few as 4 mutations can be associated with high-level resistance (Prado,AIDS, 2002)
- **I50V** confers 48X odds ratio of a FC >10 to LPV/r, even if there are fewer than 6 mutations (Parkin,Antivir Ther 7:S23,2002)
- Add. mutations not described above associated with LPV phenotypic resistance from clinical trial samples:
  - **10, 20, 24, 53, 63, 71**

# Lopinavir Susceptibility Ranges



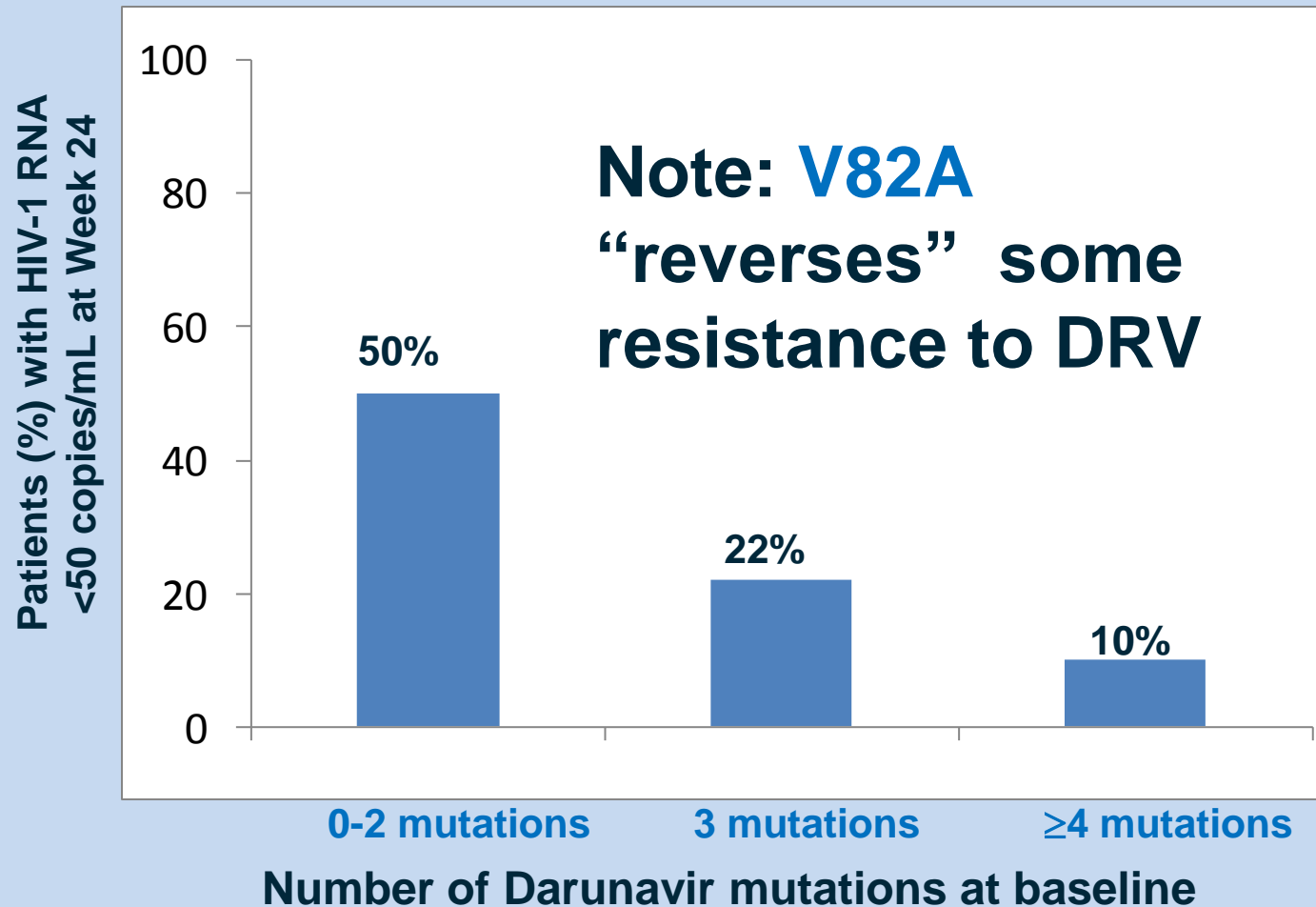
# Darunavir Mutation Score:

V11I, V32I, I33F, I47V, I50V, I54L/M, T74P, L76V, I84V, L89V

- Approved in 1st-line & treatment-experienced pts
- May be used QD in treatment-experienced pts if there are zero DRV resistance mutations
- POWER studies showed patients treated with darunavir and optimized background meds had VL < 50 c/mL greater than for comparator PIs
- Response to darunavir was found to be dependent on 11 PI mutations at baseline

V	V L	I	I	I	T L	I	L
11	32 33	<b>47</b>	<b>50</b>	<b>54</b>	74 76	<b>84</b>	89
I	I F	V	V	M	P V	V	V
				L			

# Darunavir response by DRV score



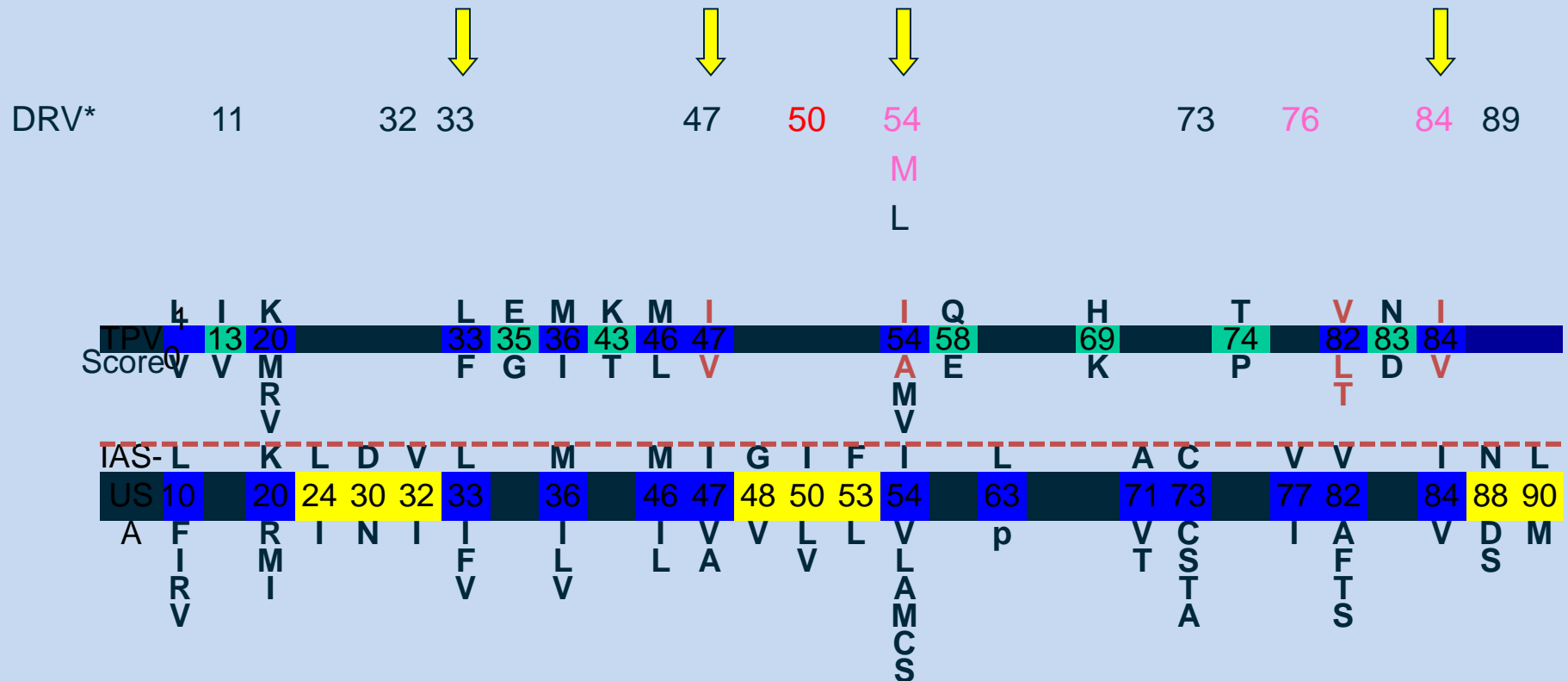
The presence of the **V82A** mutation in patients with three DRV RAMs was assoc. with a virological response comparable to that observed in pts with 2 DRV RAMs.

# Tipranavir: Resistance

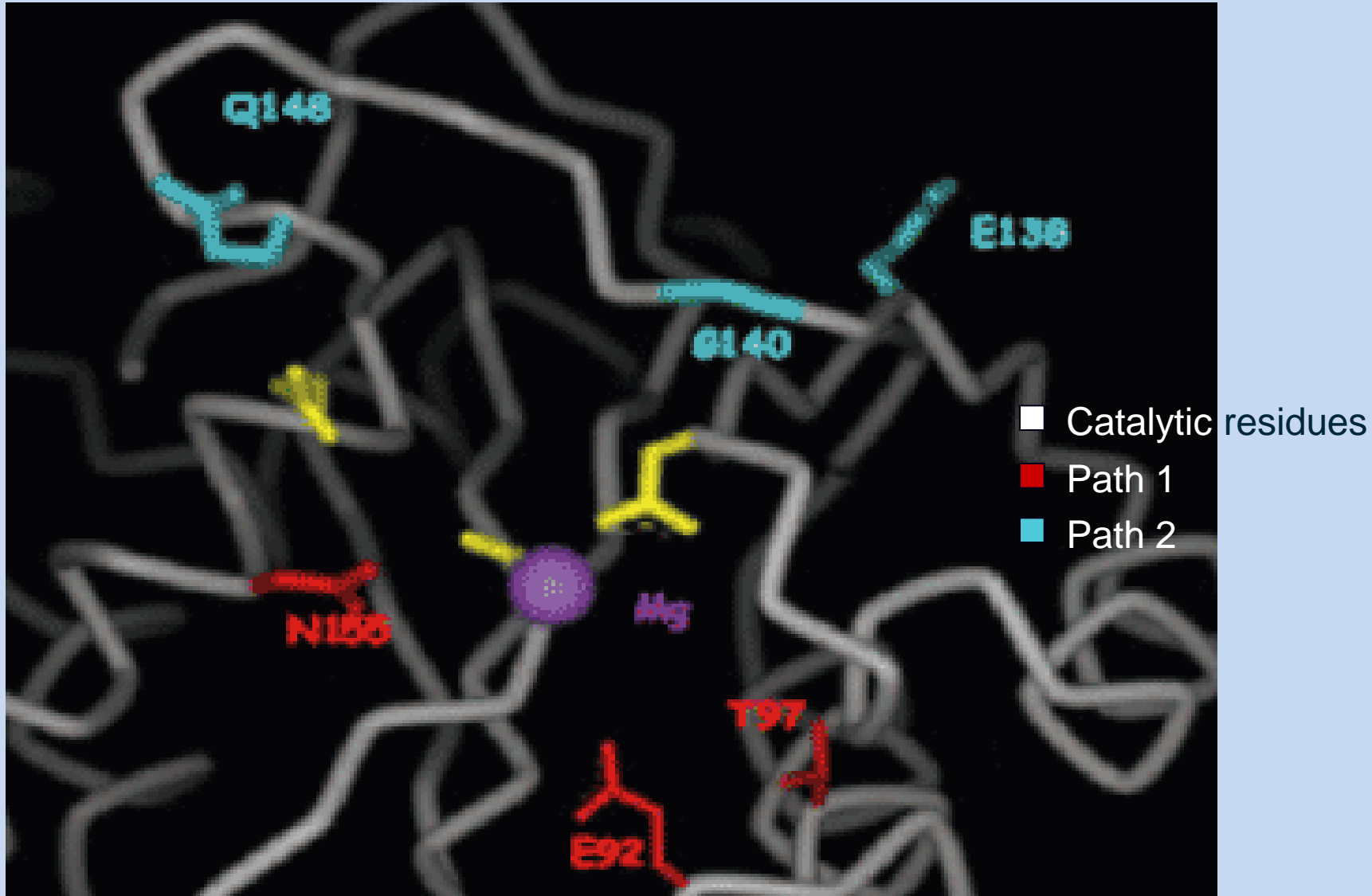
- The most common mutations that developed on tipranavir therapy: **L33V//F, V82T, and I84V**
- Reduced virologic responses assoc. with PI mutations:
  - **L10V, I13V, K20M/R/V, L33F, E35G, M36I, K43T, M46L, I47V, I54A/M/V, Q58E, H69K, T74P, V82L/T, N83D, I84V**
- Note: in very treatment-experienced patients requiring sequencing of protease inhibitors:
  - **70% of pts with reduced susceptibility to tipranavir were susceptible to darunavir**
  - **53% of pts with reduced susceptibility to darunavir were susceptible to tipranavir**

# Darunavir and Tipranavir Have Unique Resistance Profiles:

## DRV and TPV Mutations vs IAS-USA Protease Gene Resistance Mutations



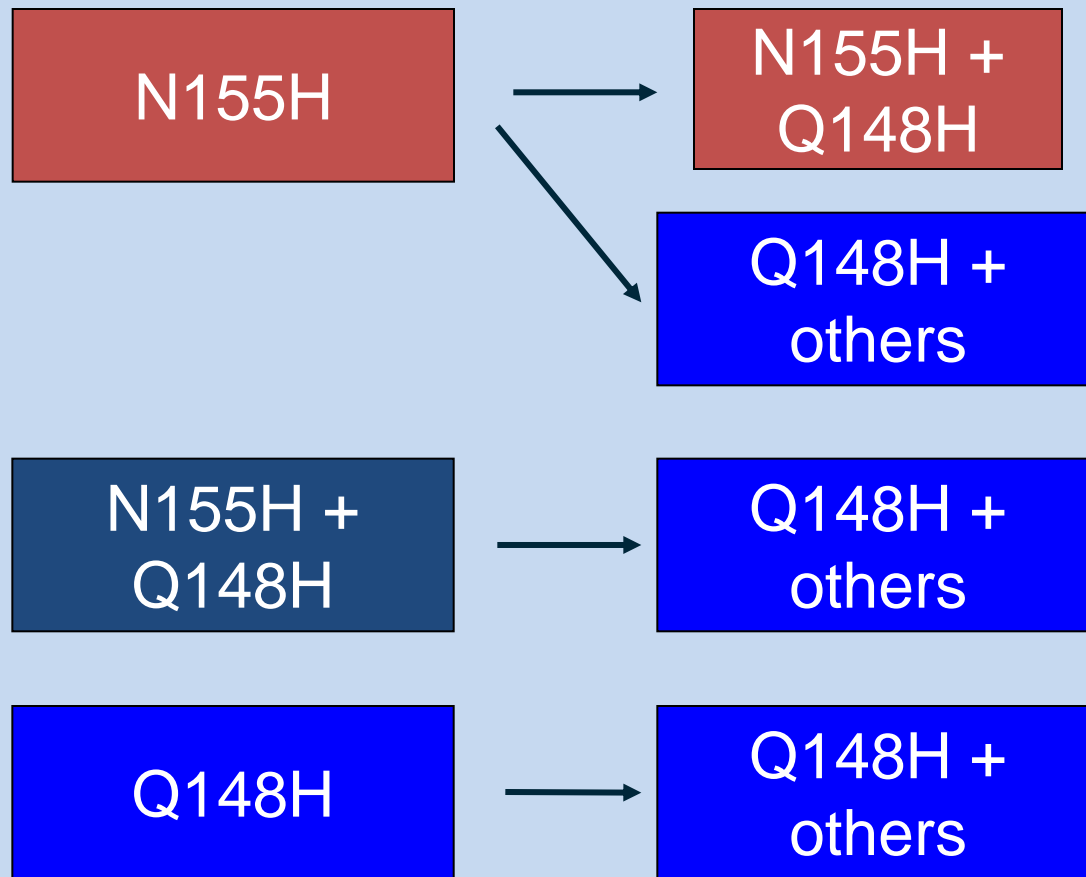
# Raltegravir: Mutation Pathways Leading to Resistance Identified



# Raltegravir: First HIV Integrase Inhibitor

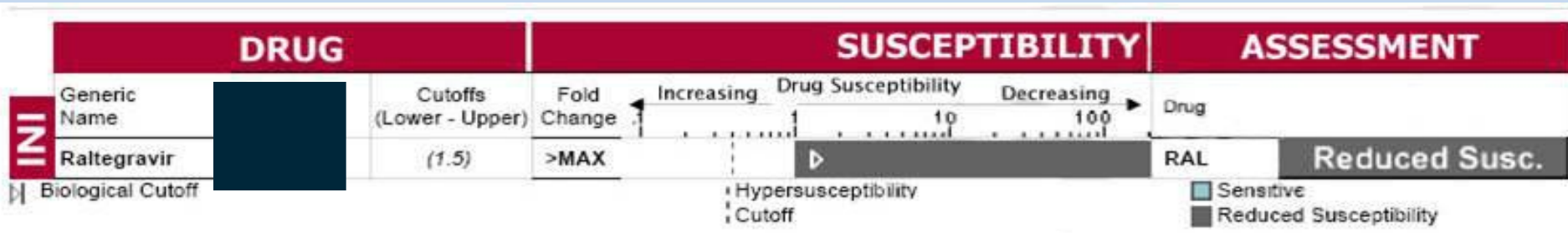
- Raltegravir failure is assoc. with integrase mutations in at least 3 genetic paths defined by at least 2 mutations:
  - Major mutations: **148,155, or 143 +/- minor changes**
- Most common/most resistant = **Q148H plus G140S**
- **Q148H/K/R or Y143R/H/C** associated with high-level phenotypic resistance (> 100-fold change in IC<sub>50</sub>)
- **N155H** associated with low-level phenotypic resistance (< 50-fold change in IC<sub>50</sub>)
- Continued raltegravir in the presence of viral failure & resistance is not recommended
- Cross-resistance from RAL-assoc. mutations may confer reduced susceptibility to investigational integrase inhibitors

# Raltegravir Resistance Evolution



# Integrase Assay Determines RAL Susceptibility

- Phenotypic integrase resistance assay now available
  - Amplification threshold: VL > 500
  - Biological cutoff for RAL is FC > 1.5
  - Report does not detail genotypic mutations



- Integrase genotype assays may be available; useful for determining susceptibility to 2<sup>nd</sup> generation agents
- Transmitted RAL resistance (N155H +2 minors\*) now reported; consider baseline RAL resistance testing prn

\* E157Q& G163R; Boyd SD, et al. Antiviral Therapy 2011;16:257-61; Fransen S, et al. 48<sup>th</sup> Annual CAAC/IDSA 46<sup>th</sup> Annual Meeting. Abstract 1214; From JE Gallant, MD, MPH, at Washington, DC: June 17, 2010, IAS–USA.

# ARS Question #3

Select the most correct statement about what is *currently* known about raltegravir resistance

1. Virologic rebound to raltegravir leads to a single major resistance pathway
2. Transmitted RAL resistance has now been reported, so baseline RAL resistance testing may be considered in high-use areas
3. Once raltegravir resistance has been identified, the drug should still be continued
4. Today, a RAL resistance **genotype** is the most easily available predictive test to measure resistance

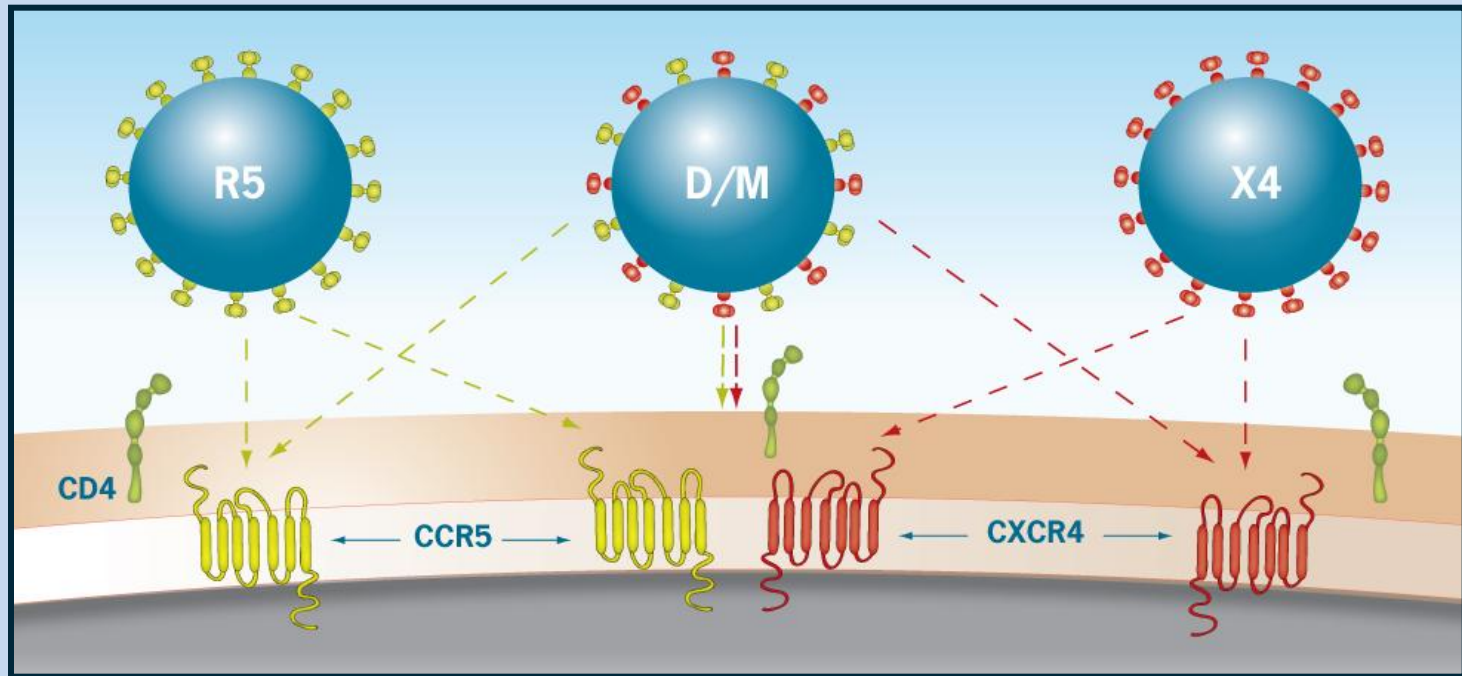
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# Defining Co-Receptor Tropism

- CCR5 and CXCR4 are the primary chemokine co-receptors used by HIV to enter CD4<sup>+</sup> T cells



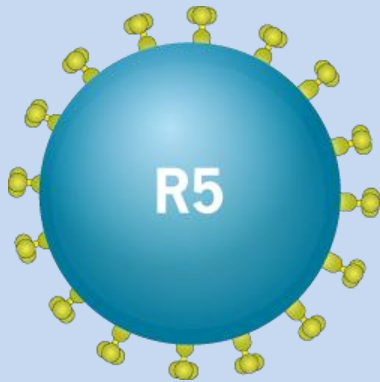
CCR5-tropic (R5) virus enters CD4<sup>+</sup> T cells via CCR5

HIV that can use either CCR5 or CXCR4 is called dual tropic

CXCR4-tropic (X4) virus enters CD4<sup>+</sup> T cells via CXCR4

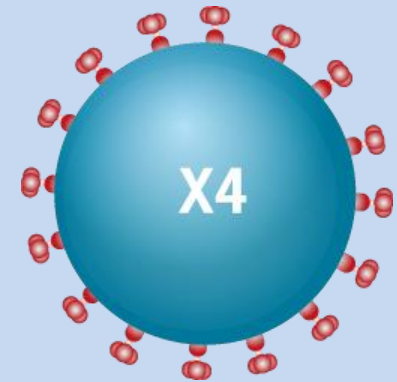
Virus populations containing a mixture of R5-tropic, X4-tropic, and/or dual-tropic HIV are called mixed tropic

# Typical HIV Tropism Patterns

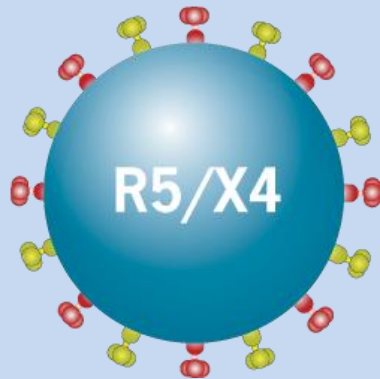


CCR5-  
tropic (R5)

CXCR4-  
tropic (X4)

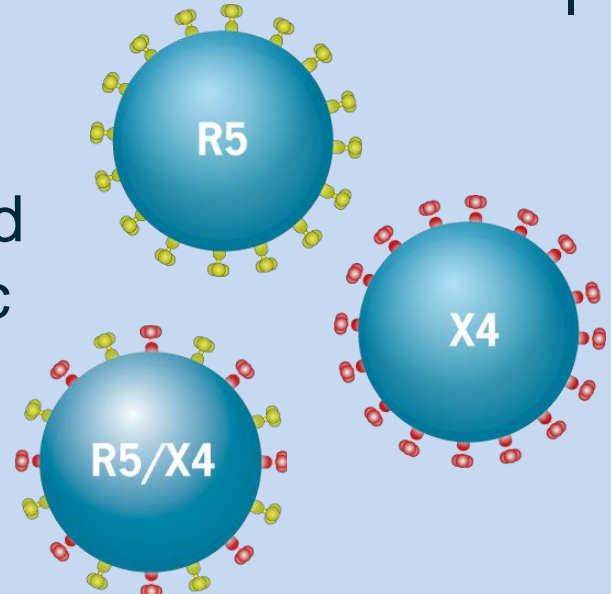


*Dual/mixed-tropic  
(D/M)*



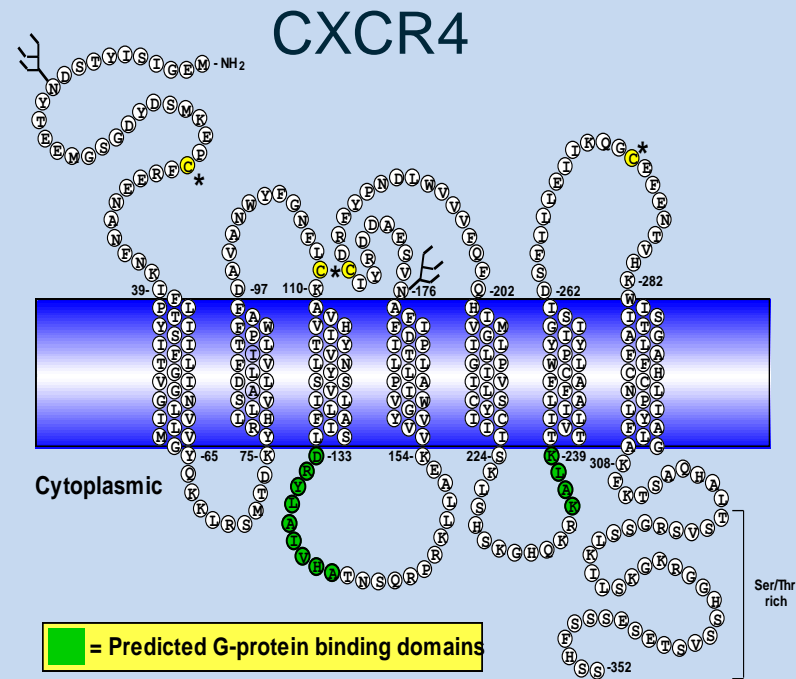
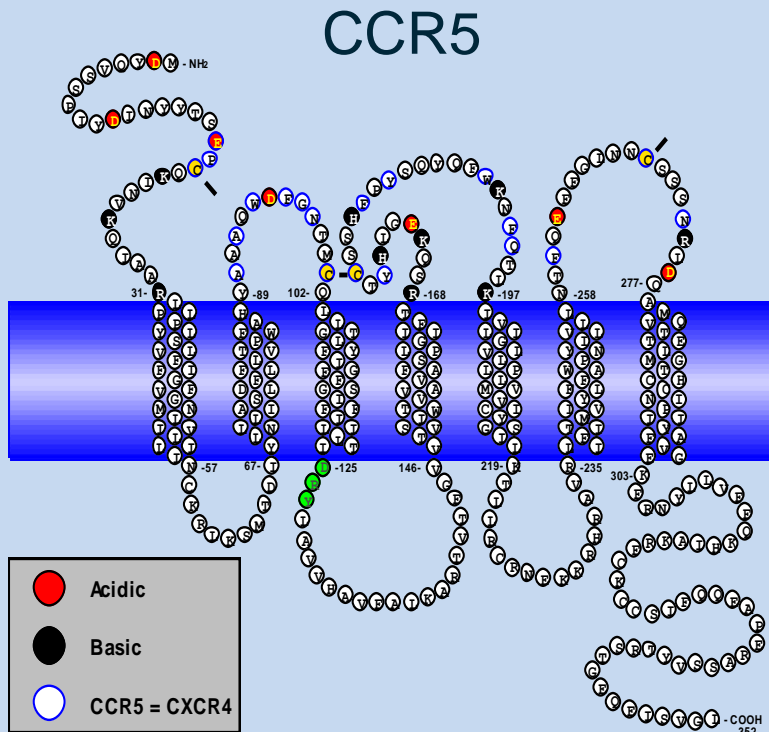
Dual  
tropic

Mixed  
tropic



# Coreceptor Is Required for Viral Entry

- There are 2 HIV coreceptors: **CCR5 & CXCR4**; both are also chemokine receptors
- Most viruses can use only CCR5: **R5 viruses**
- R5 viruses binding CCR5 is associated w/ **virus transmission**
- Some viruses can use both coreceptors: **called Dual-Tropic or Mixed-Tropic HIV (D/M)**; a few use only CXCR4: **X4 viruses**



# Maraviroc

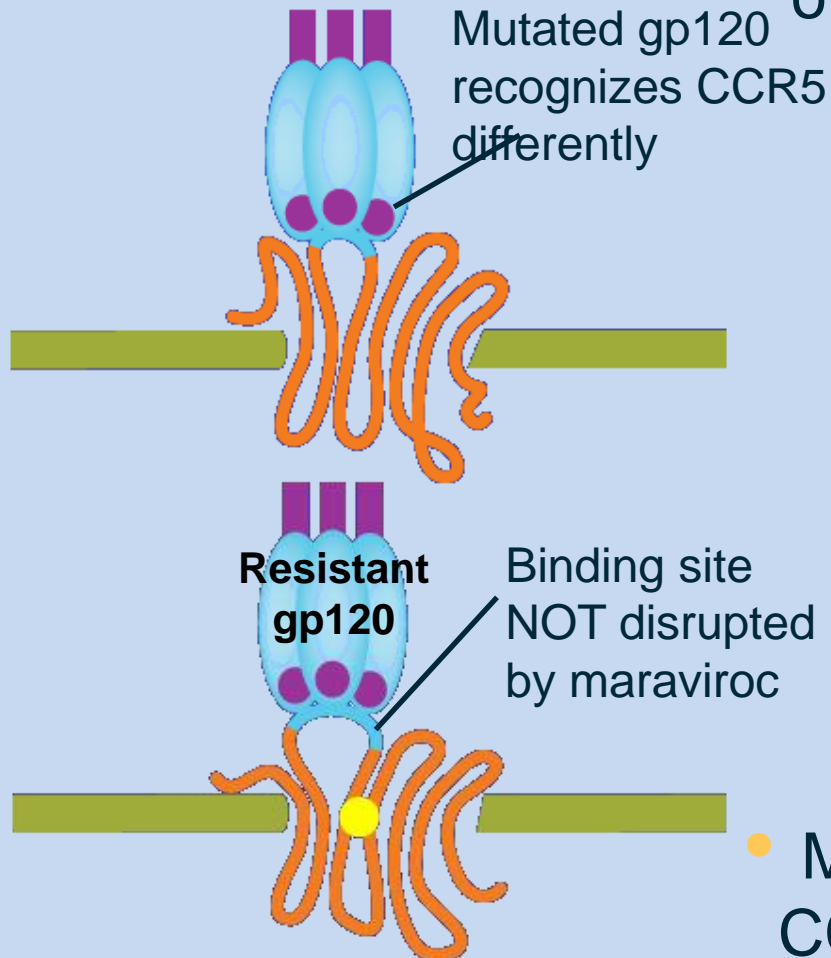
- First HIV CCR5 inhibitor / CCR5 chemokine antagonist
- Effective against strains that use the CCR5 co-receptor for cell entry
  - Little effect on viruses which use CXCR4 co-receptor, or which use both
- Activity of maraviroc is tested by co-receptor “tropism” test, which is not a resistance test
- CCR5 tropism (R5 virus) in ~85% of naïve patients, ~50% of experienced patients

# Mechanisms of Maraviroc Resistance

- **Major: Outgrowth of Dual/Mixed or X4 virus from pre-existing minority population present at levels too low to be detected by current tropism assays**
- Minor: Virus remains R5, with true resistance
  - Mutations in HIV gp120 that allow HIV to bind to CCR5 *even though maraviroc is also bound*
  - Most mutations are variable changes in the V3 loop
  - No consistent signature mutations for MRV resistance
- Because a unique viral drug binding site is not mutated
  - Genotyping cannot be used to predict CCR5 inhibitor efficacy; phenotype test must be used

# Maraviroc-Resistant Virus Can Use Compound-Bound Receptors to Enter Cells

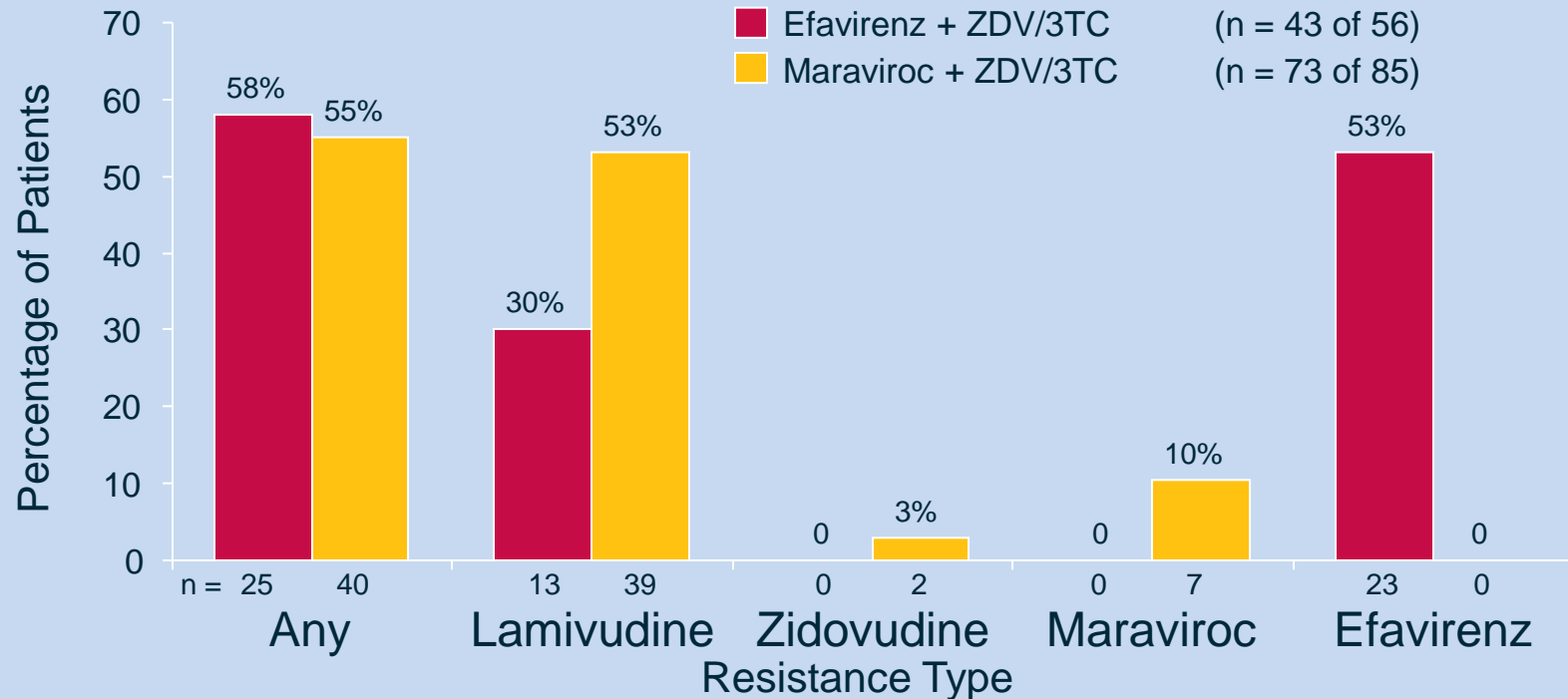
## Maraviroc-resistant virus



- Different resistance characteristics vs. other ARVs due to host cell target
- Maraviroc-resistant HIV-1:
  - Associated with mutations in the pattern of amino acids in the V3 loop of gp120
  - Substitutions outside the V3 loop of gp120 may also contribute to reduced susc. to maraviroc
- MRV-resistant HIV can enter cells via CCR5 even when MRV is bound

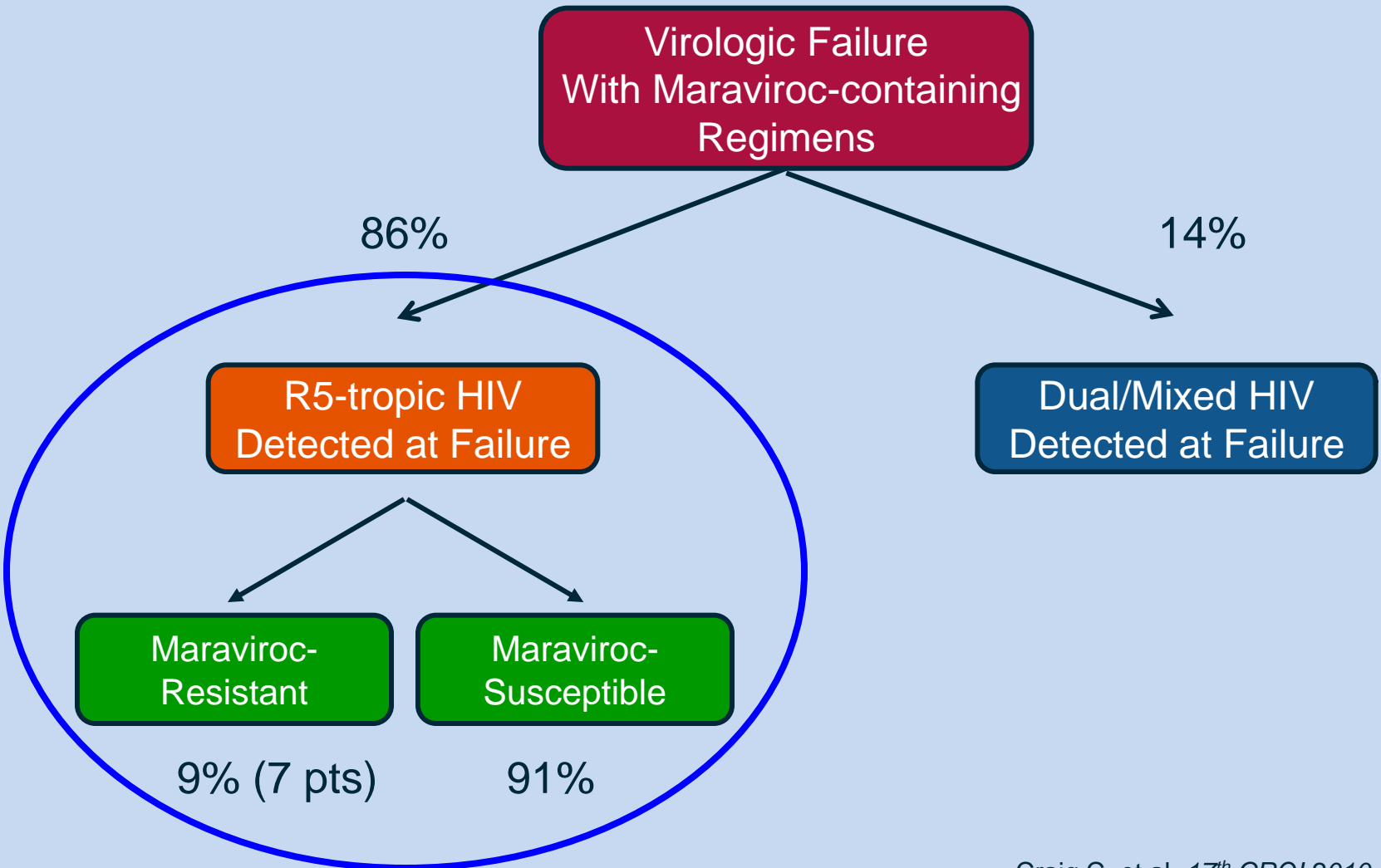
# Virologic Failure With Maraviroc Was Most Frequently Associated With Lamivudine Resistance Mutations

Includes all virologic failures with evaluable post-baseline genotypic and phenotypic data



- Most maraviroc recipients with virologic failure (n=85) had lamivudine resistance
- Most efavirenz recipients with virologic failure (n=56) had efavirenz resistance
- Resistance to lamivudine, zidovudine and efavirenz was determined genotypically; resistance to maraviroc was defined as concentration response curves that did not reach 95% inhibition
- 14% (12/85) of recipients who failed on maraviroc had CXCR4-using virus at the time of treatment failure

# Mechanisms of Virologic Failure in Naive Patients Treated with Maraviroc in the Merit Trial



# Conclusions

- Resistance can occur in patients new to ARV
- Resistance testing can be used to optimize an antiretroviral regimen
  - Must use in context of treatment history and results of all prior resistance tests
  - Goal for all HIV infected patients is HIV RNA < 50
- Factors other than resistance may cause regimen failure
- Resistance testing is reliable and cost-effective but must be interpreted in context and may require expert advice
- Cannot detect “minority” populations (<10-20%?)
- Cannot detect archived resistant virus in reservoirs