

HIV Resistance Testing: Improving Clinical Utility

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OUTLINE

- **Epidemiology**
- **Resistance Assays**
 - Interpretation, cut-off analyses
- **Evolving Issues**
 - Minority variants
 - Data on triple NRTI regimens
- **New Drugs**

EPIDEMIOLOGY

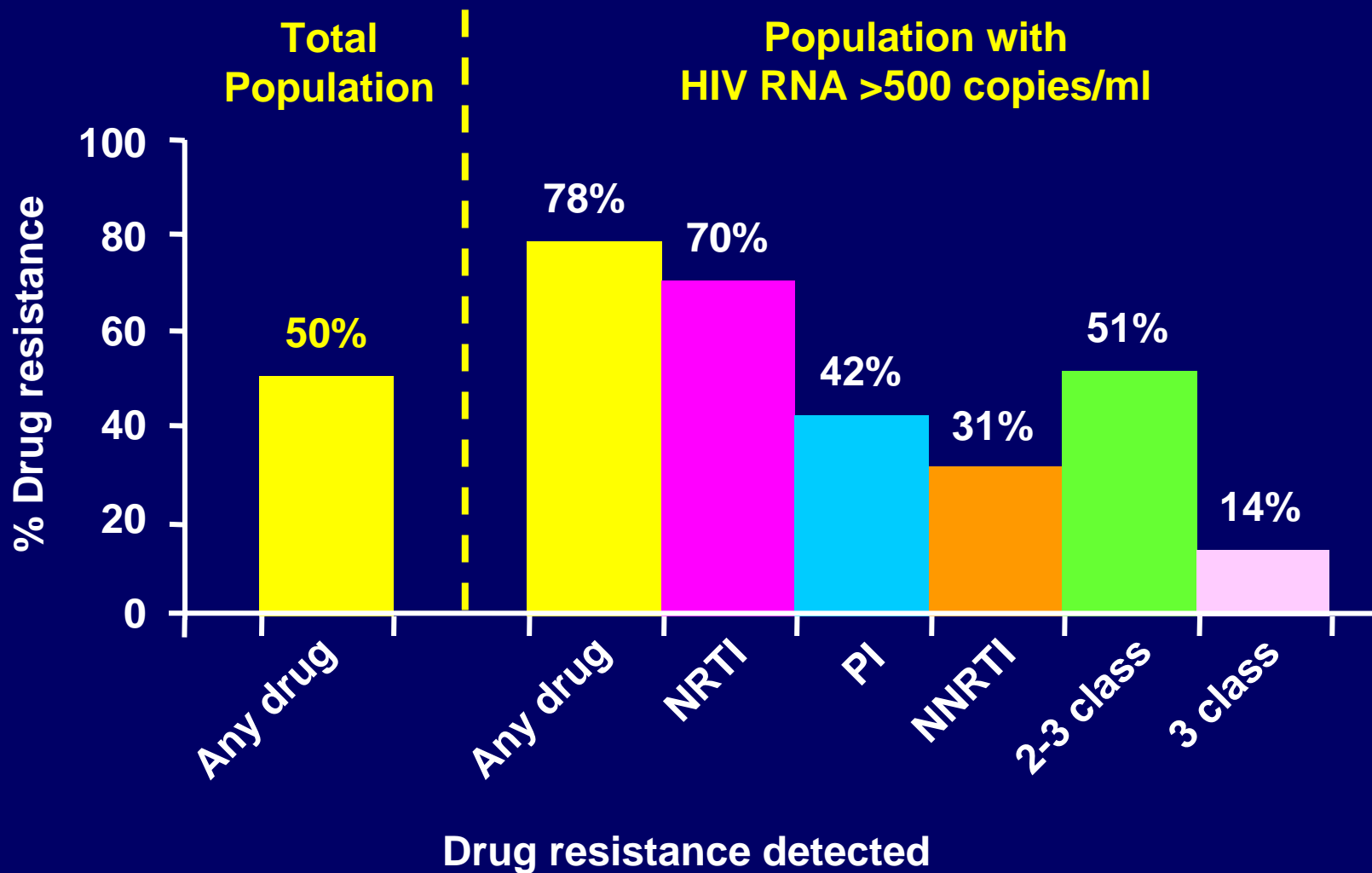
U.S. Surveillance of HIV Drug Resistance at Diagnosis

- 89 diagnostic and clinical sites in 6 U.S. states
 - 85/89 were counseling and testing sites
- 828 newly diagnosed patients
 - 787/828 (95%) genotyped
- Overall HIVDR prevalence was 14.5%

Prevalence of HIVDR among new 787 HIV diagnostic specimens from 89 sites in six states

Categories	Participants with HIVDR
Any drug class: RTI or primary PI	114 (14.5%)
NRTI	56 (7.1%)
NNRTI	66 (8.4%)
PI	22 (2.8%)
Two or more drug classes	24 (3.1%)

Overall Prevalence of HIV Drug Resistance



Case 1

Clinical History

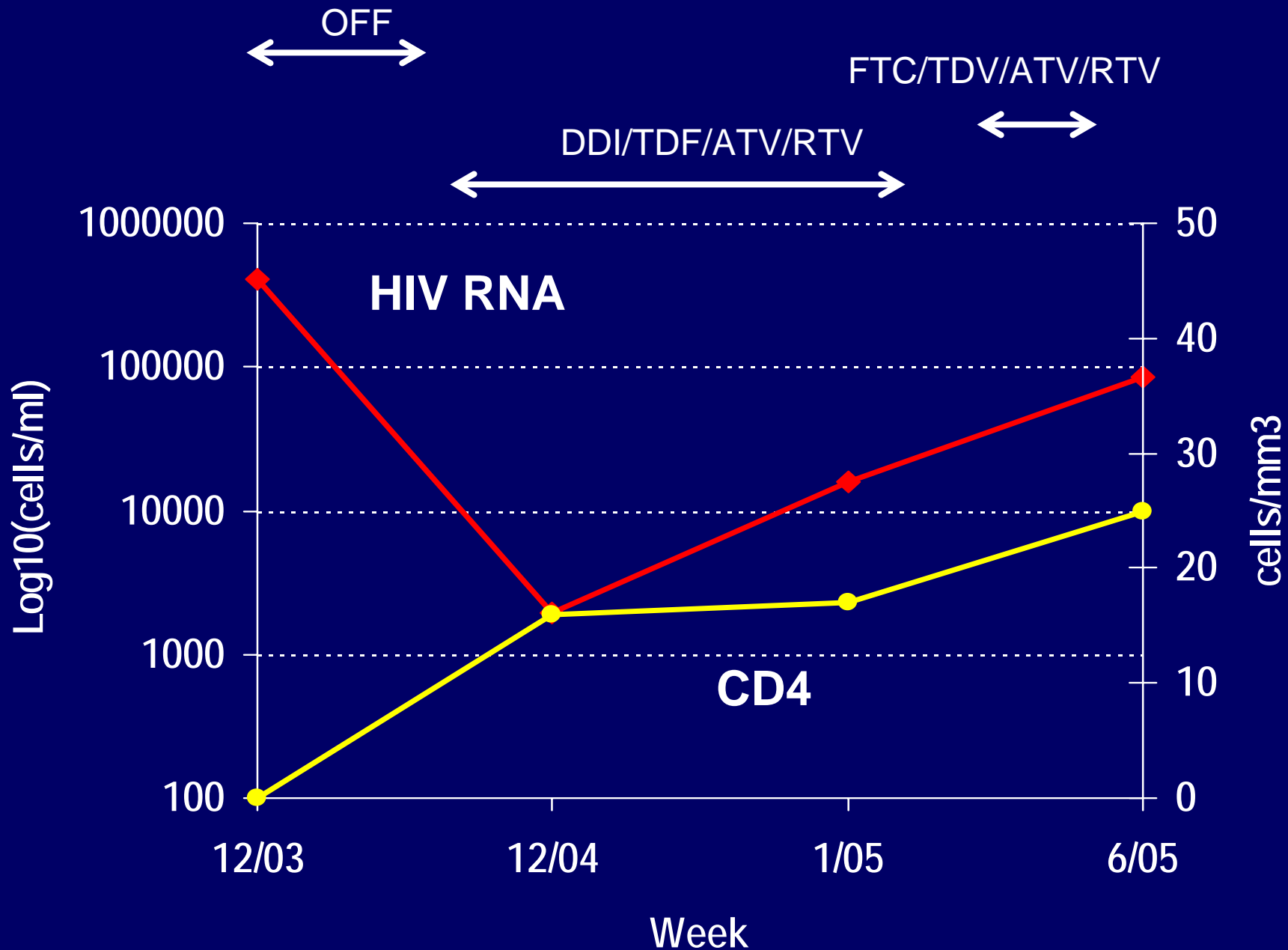
- 40 year old gay male
- CD4 25
- HIV RNA 84,600 copies/ mL
- OI History
 - cryptosporidium
 - KS
 - bacterial pneumonia
- Other Hx
 - Hep C
 - Amphetamine use

Case 1

Past ARV

- 95: ZDV (transient)
- 95-96: d4T
- 96-97: SQV/ RTV
- 97-98: 3TC/DLV/NFV
- 98-00: 3TC/ABC/EFV/FTV/RTV
- 00-04: off treatment
- 04-05: DDI/TDF/ATV/RTV
- Current: FTC/TDF/ATV/ RTV
- Intolerance: ZDV, LPV, NVP
- Prior treatment summary
 - » 3 class exposure
 - » 10 years NRTI
 - » 7 years PI
 - » 15 drugs used in past

HIV RNA and CD4



Case 1

Past Genotype Results

- NRTI: M184V, K219E, K65K/R
- NNRTI: K103N, Y181C
- PI: L10I, K20I, V32I, L33F, M36M/I, K43T, M46I, I54L, L63P, A71V, I84V, L90M

Phenotype Result

DRUG		SUSCEPTIBILITY	
Generic Name	Brand Name	Fold Change	Increasing Drug Susceptibility Decreasing
Abacavir		9.18	
Didanosine		3.31	
Emtricitabine		>MAX	
Lamivudine		>MAX	
Stavudine		1.06	
Zidovudine		0.53	
Tenofovir		1.28	
NRTI Mutations		K65R, M184V	

Delavirdine		0.60	
Efavirenz		0.55	
Nevirapine		0.46	
NNRTI Mutations		none	

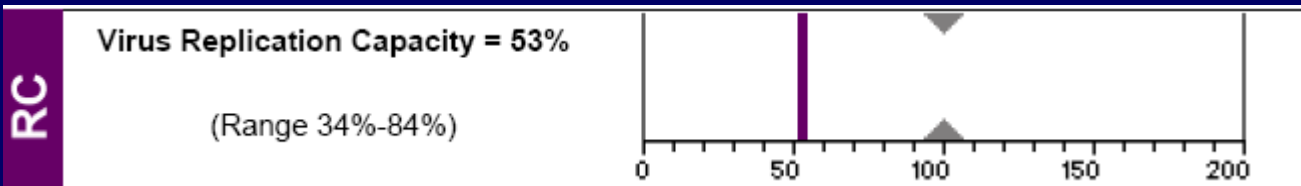
Case 1

Current Genotype Results

- NRTI: K65R, M184V
- NNRTI: None
- PI: L10I, K20I, V32I, L33F, E34Q, K43T, M46I, I54L, L63P, A71V, T74P, V77I, I84V, L90M

Case 1

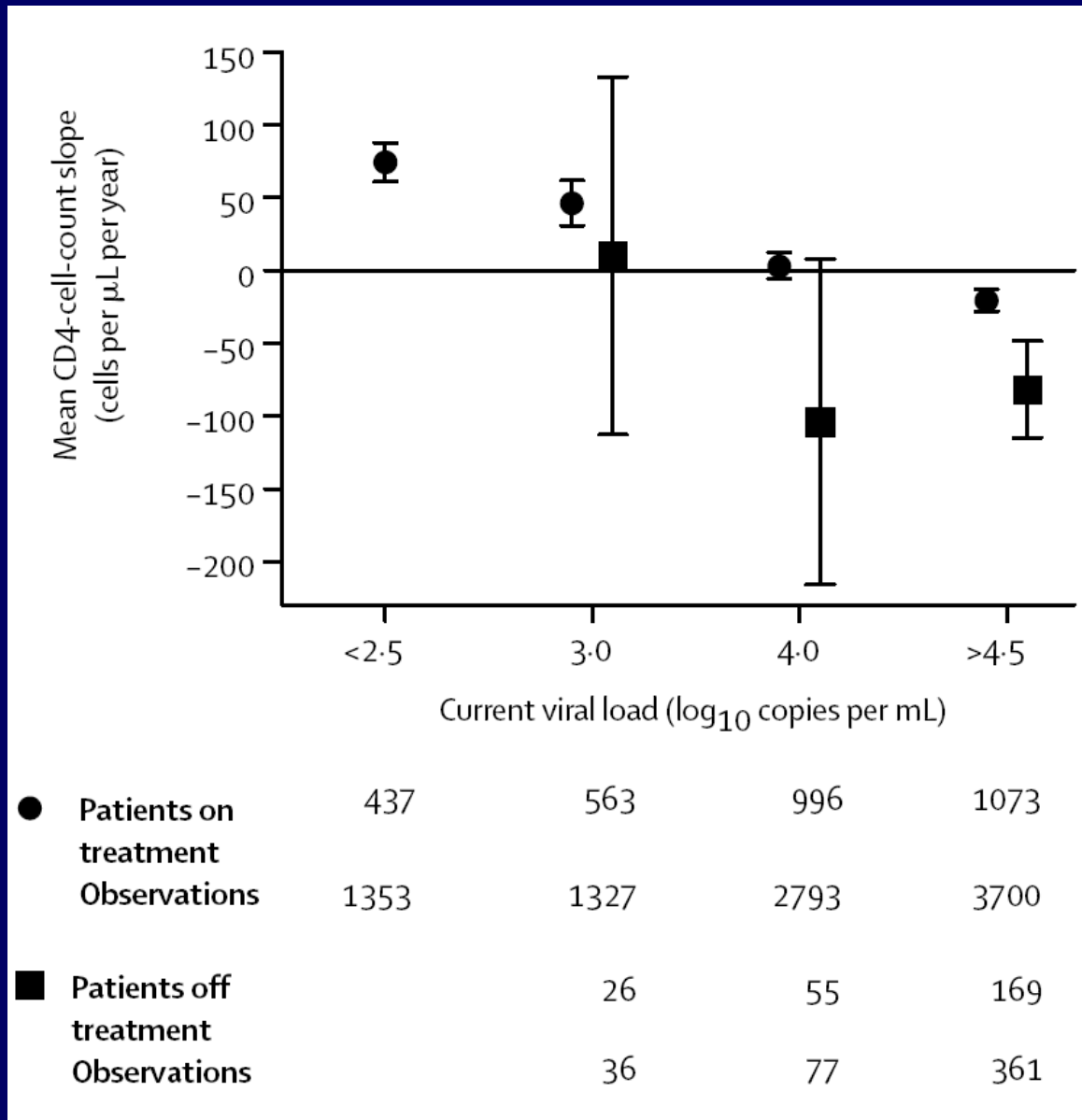
PI	Atazanavir	73		ATV	N	N	Reduced Susc.
				ATV/r	N	N	Reduced Susc.
	Fosamprenavir	61		AMP	N	N	Reduced Susc.
	Indinavir	20		IDV	N	N	Reduced Susc.
				IDV/r	N	N	Reduced Susc.
	Lopinavir	33		LPV/r	N	N	Reduced Susc.
	Nelfinavir	147		NFV	N	N	Reduced Susc.
	Ritonavir	>MAX		RTV	N	N	Reduced Susc.
	Saquinavir	19		SQV	N	N	Reduced Susc.
Tipranavir	4.88		TPV/r	N	Y	Reduced Susc.	
PI Mutations		L10I, K20I, V32I, L33F, E34Q, K43T, M46I, I54L, L63P, A71V, T74P, V77I, I84V, L90M					



Switch Decisions in Patients with Three Class Resistance

- Stop all ARV
- Maintain current regimen and wait for better options
- Switch now- to what?

CD4 Decline in Patients on or off Treatment



RESISTANCE TEST INTERPRETATION

Havana Trial Results: HIV RNA <400 at Week 24 (Per Protocol Analysis)

	Genotype	No genotype
Expert	69%	49%
No expert	46%	36%

$P < 0.05$ for 2 main factors

**RESISTANCE TEST
INTERPRETATION- Genotype**

www.hivdb.stanford.edu

The screenshot shows a Netscape browser window titled "Stanford HIV Drug Resistance Database - Netscape". The address bar contains "http://hivdb.stanford.edu/". The browser's toolbar includes icons for Back, Forward, Reload, Home, Search, Netscape, Print, Security, Shop, and Stop. The main content area features the title "Stanford HIV Drug Resistance Database" and a descriptive paragraph: "A curated database containing nearly all published HIV RT and protease sequences; a resource for researchers studying evolutionary and drug-related variation in the molecular targets of anti-HIV therapy." Below this is a navigation menu with links for Home, Seq Analysis, Database Queries, Resistance Notes, User Guide, and Contact Us. The "Database Query Pages" section lists several options: "Protease inhibitors, RT inhibitors" (Retrieve sequences of isolates from persons receiving a selected antiretroviral therapy), "Protease mutations, RT mutations" (Retrieve sequences of isolates containing selected mutations), "Protease inhibitor susceptibilities, RT inhibitor susceptibilities" (Retrieve published drug susceptibility data for isolates with selected mutations), and "Mutation profiles: Protease, RT, Position Summary" (Retrieve summary mutation data according to treatment and subtype). It also lists "Other pages: References, Advanced query pages, GenBank, ...". The "Sequence Analysis Programs" section includes "HIVseq" (Compare new RT and protease sequences to published sequences with the same mutations), "HIVdb" (Infer drug resistance to 16 available drugs using rules hyperlinked to data within the database), and "Release notes" for the above programs, for creating algorithms using the "Algorithm Specification Interface (ASI)", and for comparing algorithms (HIValg). The "Drug Resistance Notes" section is partially visible at the bottom. The browser's status bar at the bottom indicates "Go to your personal start page".

Stanford HIV Drug Resistance Database

A curated database containing nearly all published HIV RT and protease sequences; a resource for researchers studying evolutionary and drug-related variation in the molecular targets of anti-HIV therapy.

Home Seq Analysis Database Queries Resistance Notes User Guide Contact Us

Database Query Pages

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Retrieve sequences of isolates from persons receiving a selected antiretroviral therapy

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Sequence Analysis Programs

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Drug Resistance Notes

Go to your personal start page

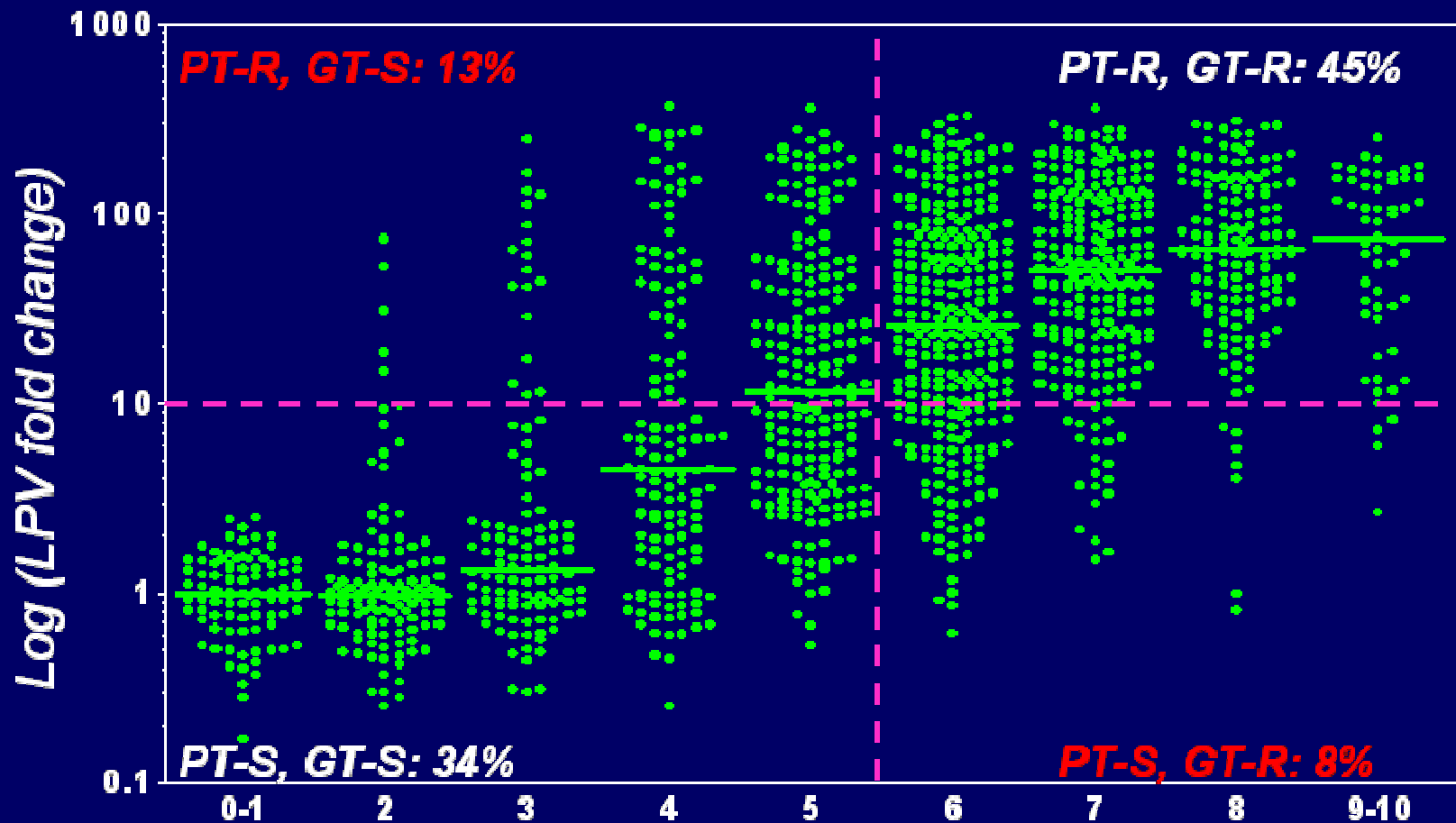
Lopinavir Resistance – Original Algorithm

- Mutations associated with LPV phenotypic resistance from Abbott clinical trial samples¹
 - L10F, I, R, or V, K20M or R, L24I, M46I or L, F53L, I54L, T, or V, L63P, A71I, L, T, or V, V82A, F, or T, I84V, L90M
- Clinical response rates decrease when LPV FC > 10 (“PT-R”) or LPV mutation score > 5 (“GT-R”)²

¹ Kempf et al., *J. Virol.* 75:7462, 2001

² Kempf et al., *Antivir. Ther.* 7:165, 2002

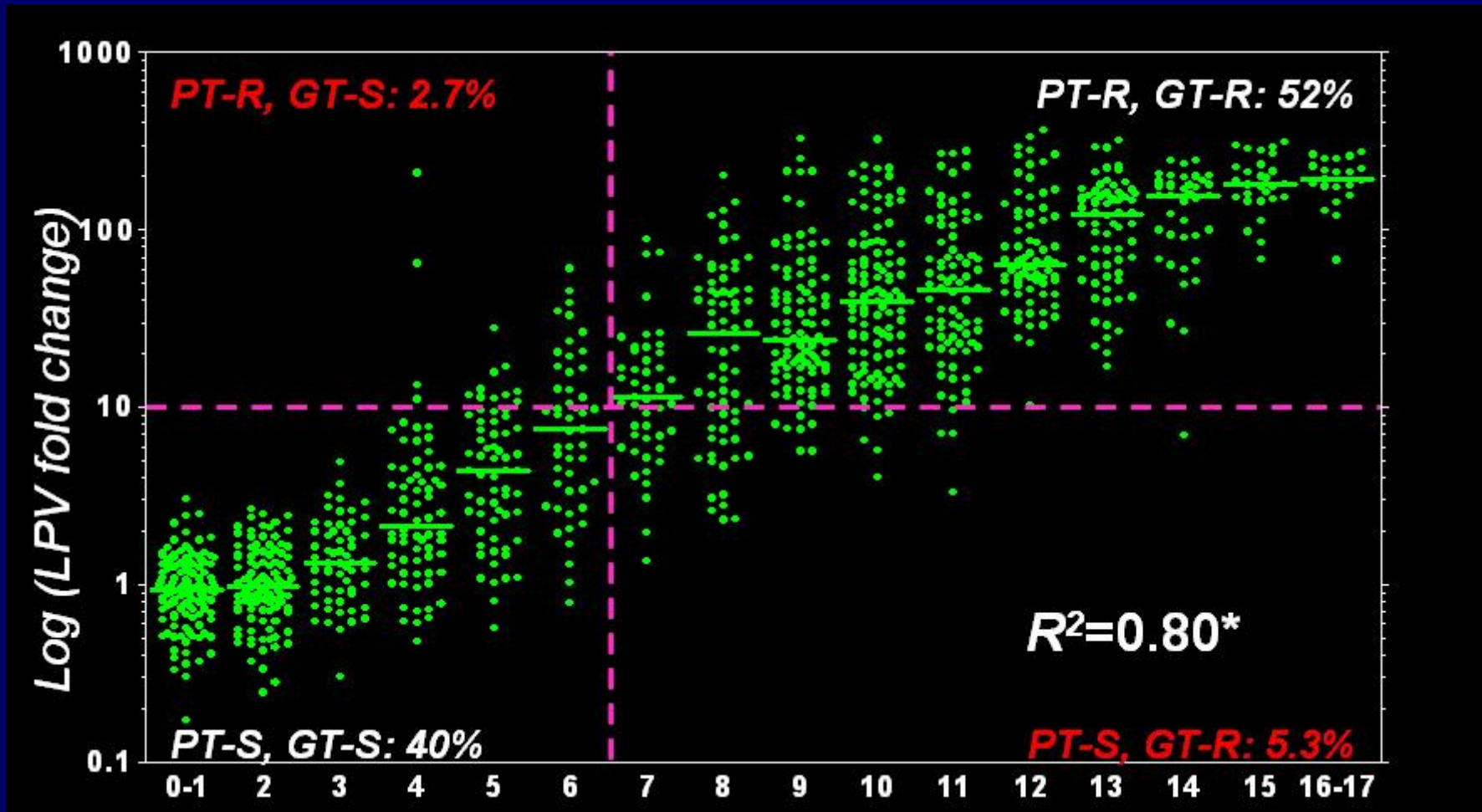
LPV Phenotype and Mutation Score



Mutations Associated with LPV FC >10 in samples with < 6 LPV Mutations

- **Known LPV mutations**
 - I54V, V82A
- **New variants at known positions**
 - I54A, S, M, V82S
- **New positions**
 - V32I, L33F, G48V, I47V, I50V
 - G16E, E34Q, K43T, L76V, L89IM

Accuracy of New LPV Genotype Algorithm



Testing the LPV Rules for Prediction of Virologic Outcomes in Clinical Cohorts

- **CBIG Cohort¹: 105 PI-experienced pts received LPV/r**
 - Prior PIs: NFV (63%), IDV (50%), RTV (40%), SQV (31%), APV (9%)
 - Compared ability of rules to predict change in VL: RCG, ATU, ABT and Monogram rules
 - Parkin Score was preferred in multivariate models
- **Abbott study 8882: 148 PI-experienced pts; tx LPV/r**
 - Prior PIs: NFV (43%), IDV (42%)
 - Week 48 response (% with VL < 400 copies/ml) vs. mutation score according to: ABT, VL, Virco, ATU, CBIG, RCG
 - Only Monogram algorithm significant in multivariate model

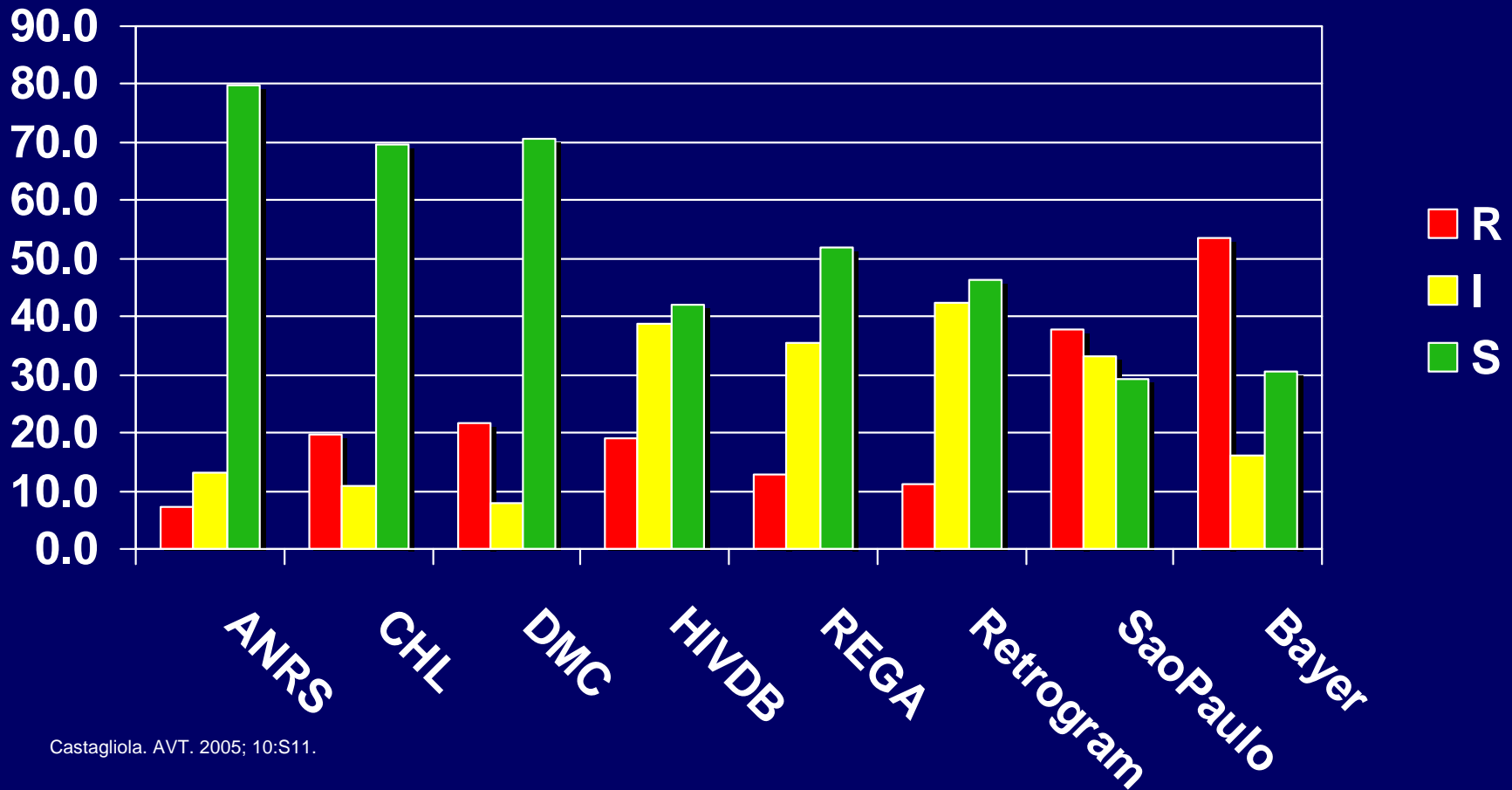
1. Rice et al. 2nd IAS Conf., 2003 Poster #3225

2. Norton et al. XIII IDRW 2004, poster #117

Level of resistance to ABC: Eight Genotype Interpretation Algorithms

N=583

VL 4.4 (3.8-5.0), mean change in VL: -1.6 log₁₀ copies/ml



Evaluation of interpretation systems for abacavir

	R	I Mean change in VL relative to R (95% CI)	S Mean change in VL relative to R (95% CI)	P value I/R and S/R
ANRS	0.00	+0.64	+0.66	0.007/0.001
CHL	0.00	+0.6	+0.22	<0.001/0.10
DMC	0.00	+0.47	+0.17	0.03/0.19
HIVDB	0.00	+0.48	+0.07	<0.001/0.62
REGA	0.00	+0.58	+0.21	<0.001/0.20
RetroGram	0.00	+0.14	-0.06	0.42/0.71
SaoPaulo	0.00	+0.10	-0.20	0.45/0.15
Bayer	0.00	+0.37	-0.02	0.02/0.88

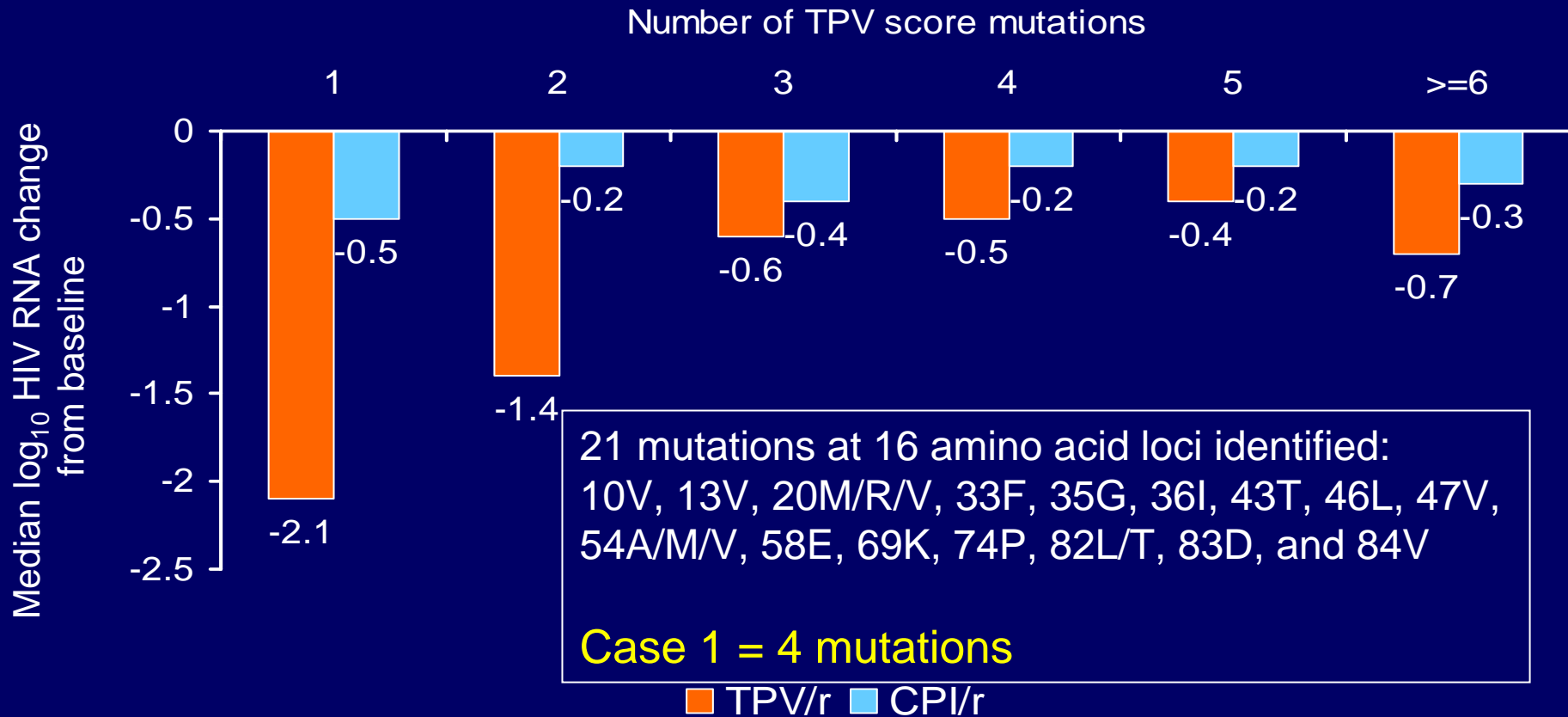
adjusted on VL and number of active drugs using ANRS rules

Positive estimates from the model indicate a larger difference in reduction in log viral load

TPV RESIST Studies

Change in VL at Week 24

According to TPV Score Mutations



Virtual phenotype (Virco): genotype interpretation system

- **Matching a patient's viral genotype to a large genotype/ phenotype dataset based on key mutations affecting drug susceptibility and not the entire amino acid sequence.**
- **Each drug has a distinct list of key mutations summarized as a series of logical expressions**
 - 8-14 groups of mutations
 - Overall 30-54 mutations/drug are considered

SUMMARIZING THE PATIENT VIRAL GENOTYPE AS A MUTATIONAL PROFILE

Example: 44D, 41L, 210W, 67N plus multiple others
Calculate 3TC mutational profile:



- 44D | 44A
- 151M
- 65R
- 215Y | 215F
- 210W
- 184V | 184I | 184T
- 41L | 70R
- 62V | 75I | 77L | 116Y
- (69S | 69A) & ([69S] | [69A] | [69C] | [69V] | [69T])
- 67N | 219Q | 219E | 219N
- 118I

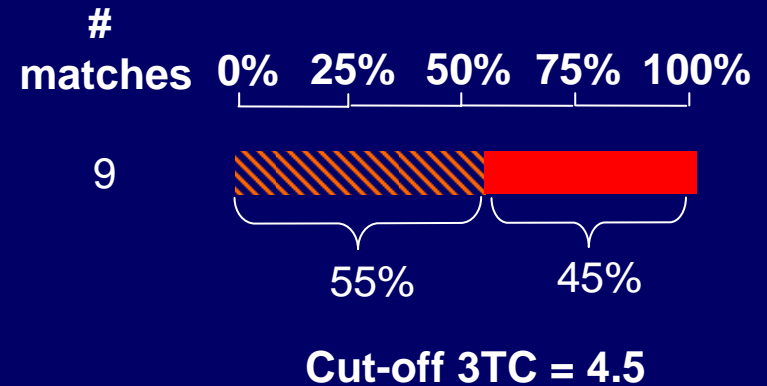
● Profile '10001010010'

Using the mutational profile to search for matches in 3TC database

Phenotypic Information

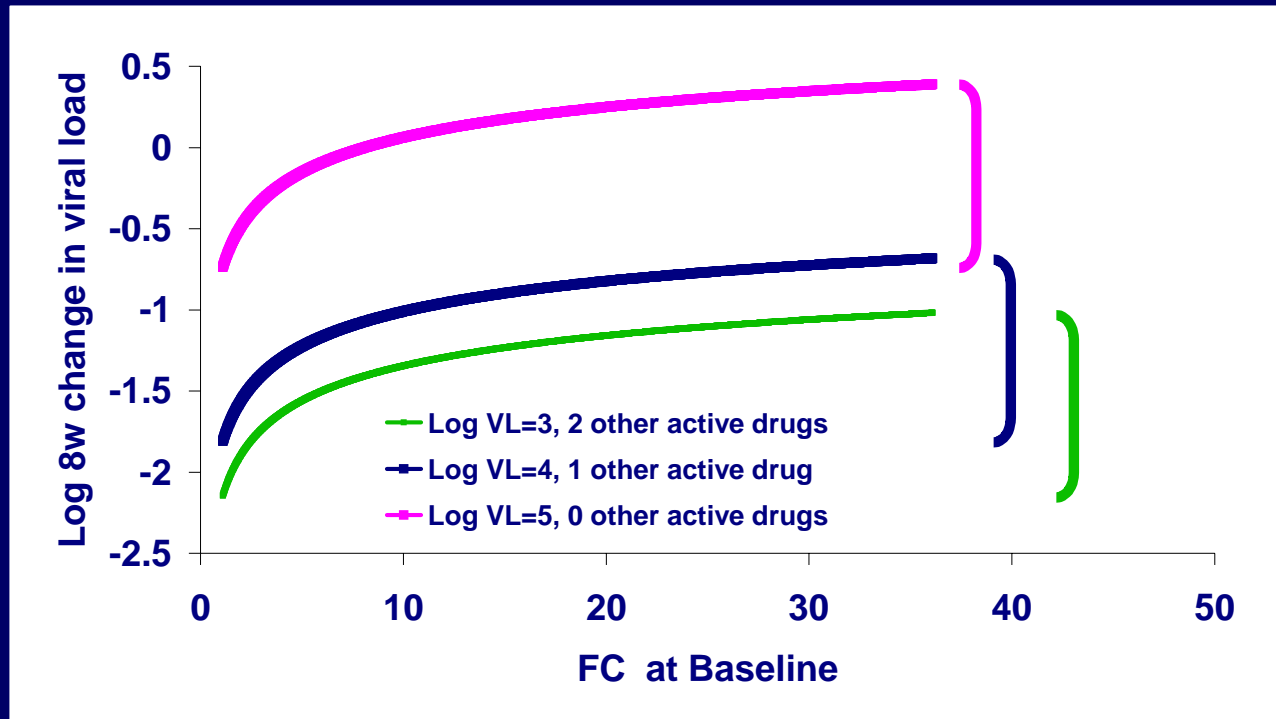
10001010010	→	3.0
10101011010		
10001010010	→	3.3
10111010010		
10001010010	→	4.6
10001010010	→	4.7
10101010010		
10001011010		
10001010010	→	4.8
11001010010		
10001010010	→	3.1
10001010011		
10001010010	→	2.9
10001010010	→	4.7
10001010010	→	2.8
10001010010		
11101010010		

3.3



Mean fold-change

Identifying the Effect of a Single Drug in a Combination Regimen

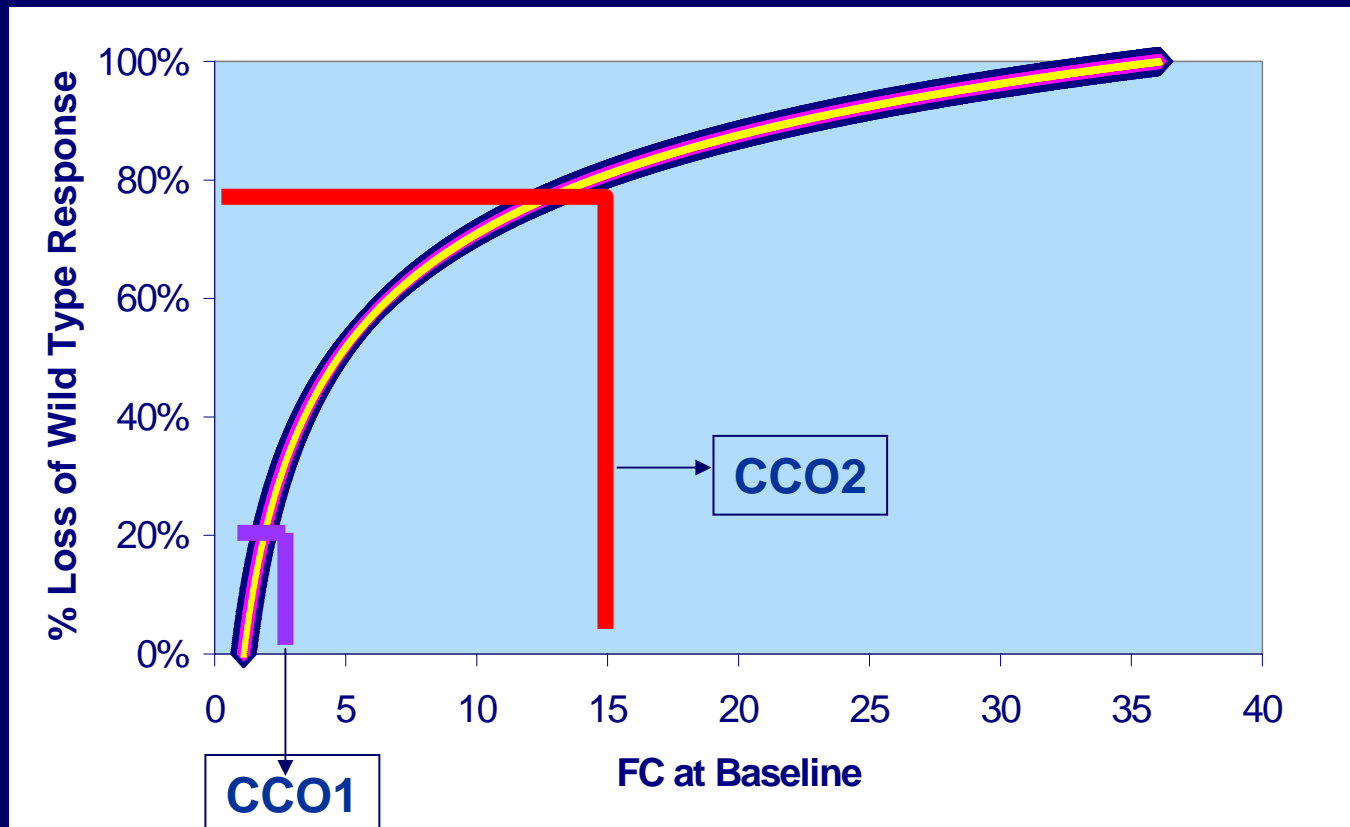


✓ Model predicts response to entire combination regimen; depends on multiple variables

✓ Single Drug Effect = $\Delta VL_{wt} - \Delta VL_{Max FC}$

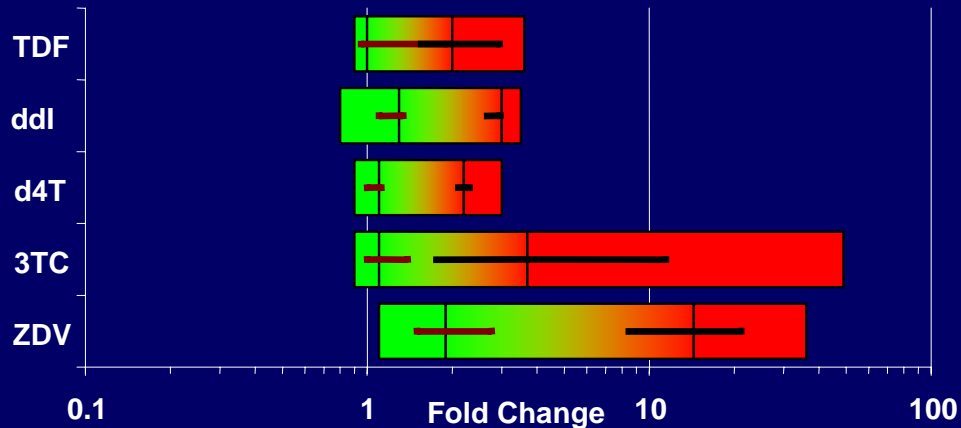
Defining Clinical Cutoffs Based on Loss of Drug Effect

- ✓ Identify two cutoffs/drug as the predicted virtual phenotype Fold Change associated with:
 - 80% loss of the response demonstrated by a wild type virus
 - 20% loss of the response demonstrated by a wild type virus

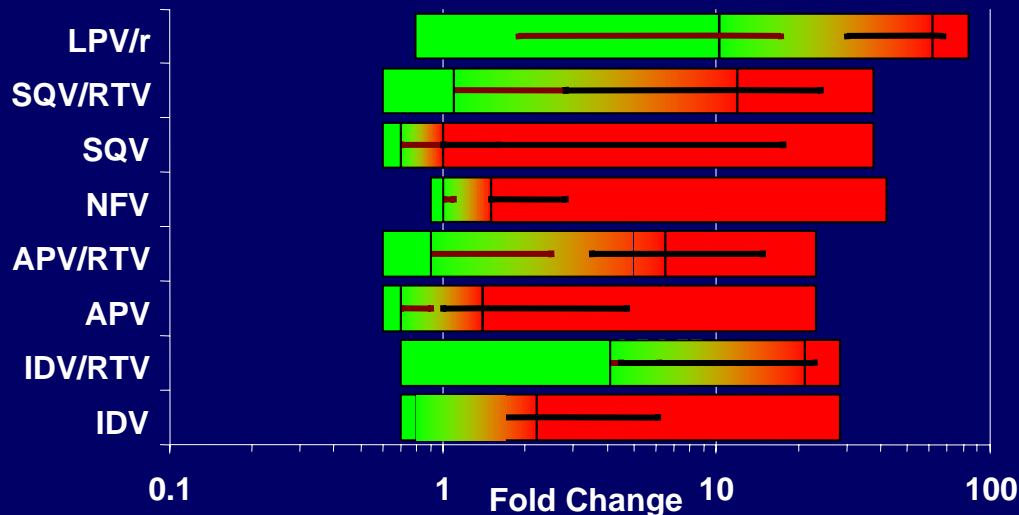


Estimation of phenotypic clinical cutoffs (CCO) for virtual phenotype

Assessment of variability of CCOs for NRTIs

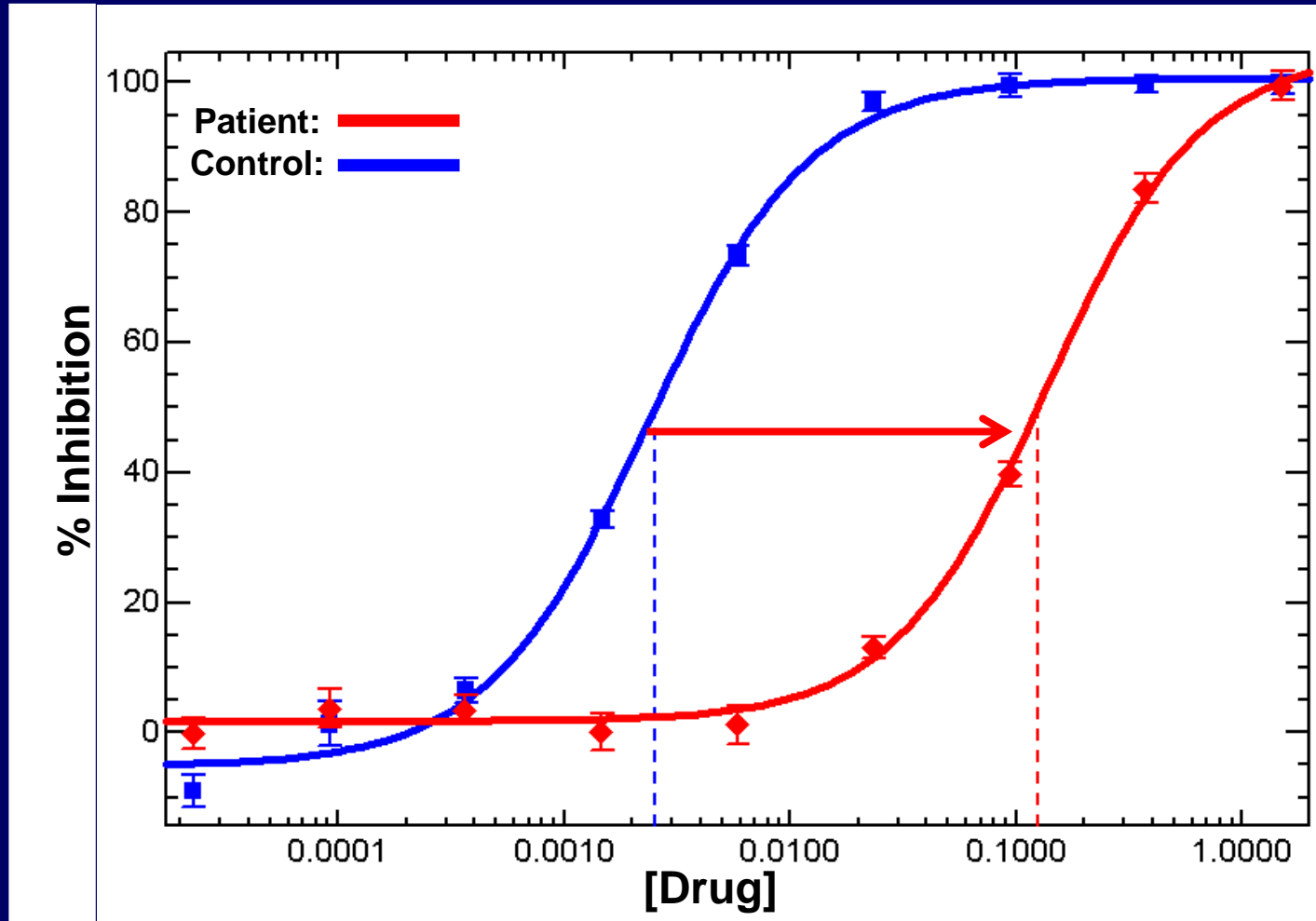


Assessment of variability of CCOs for PIs

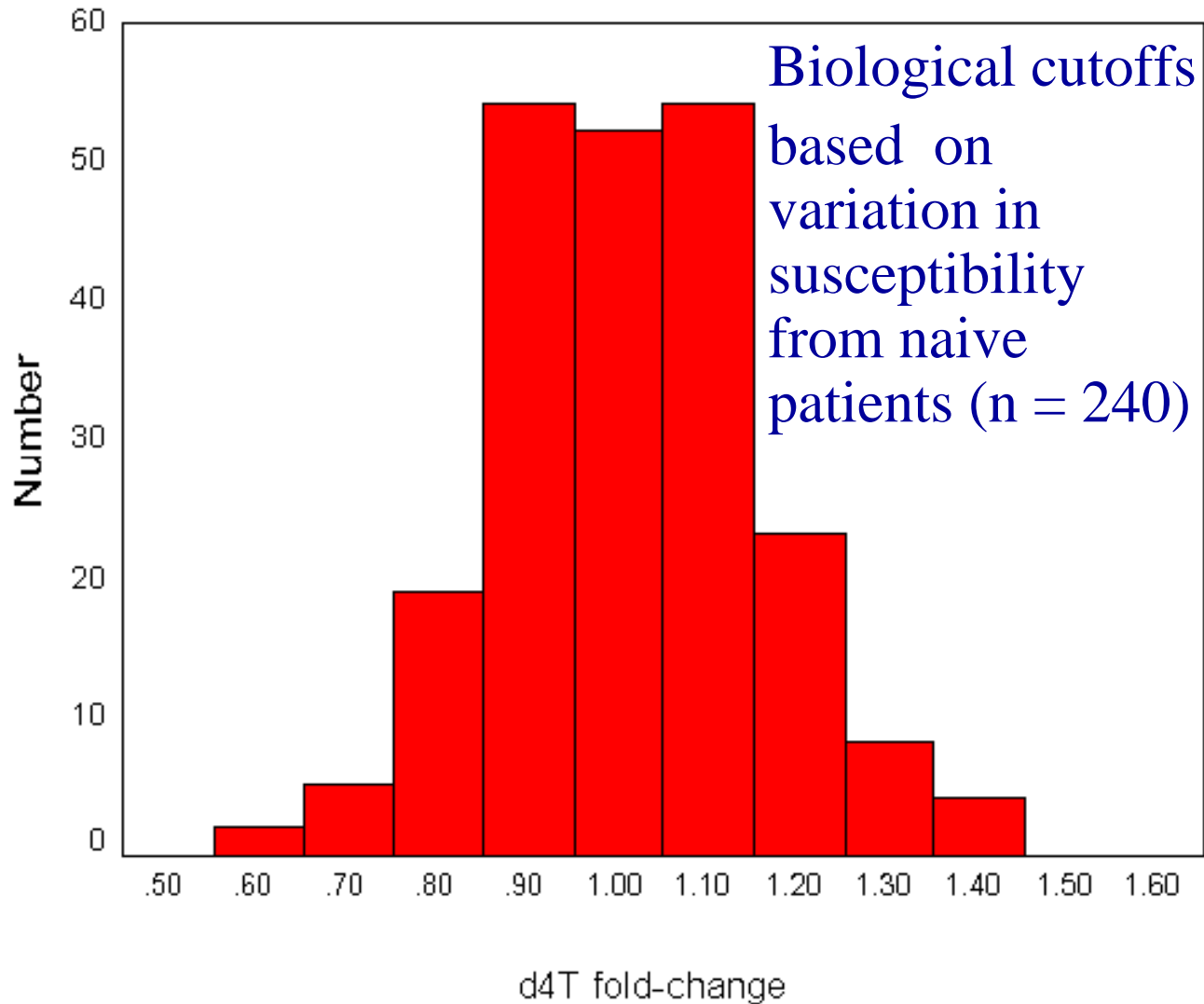


**RESISTANCE TEST
INTERPRETATION- Phenotype**

Measurement of Phenotypic Drug Resistance



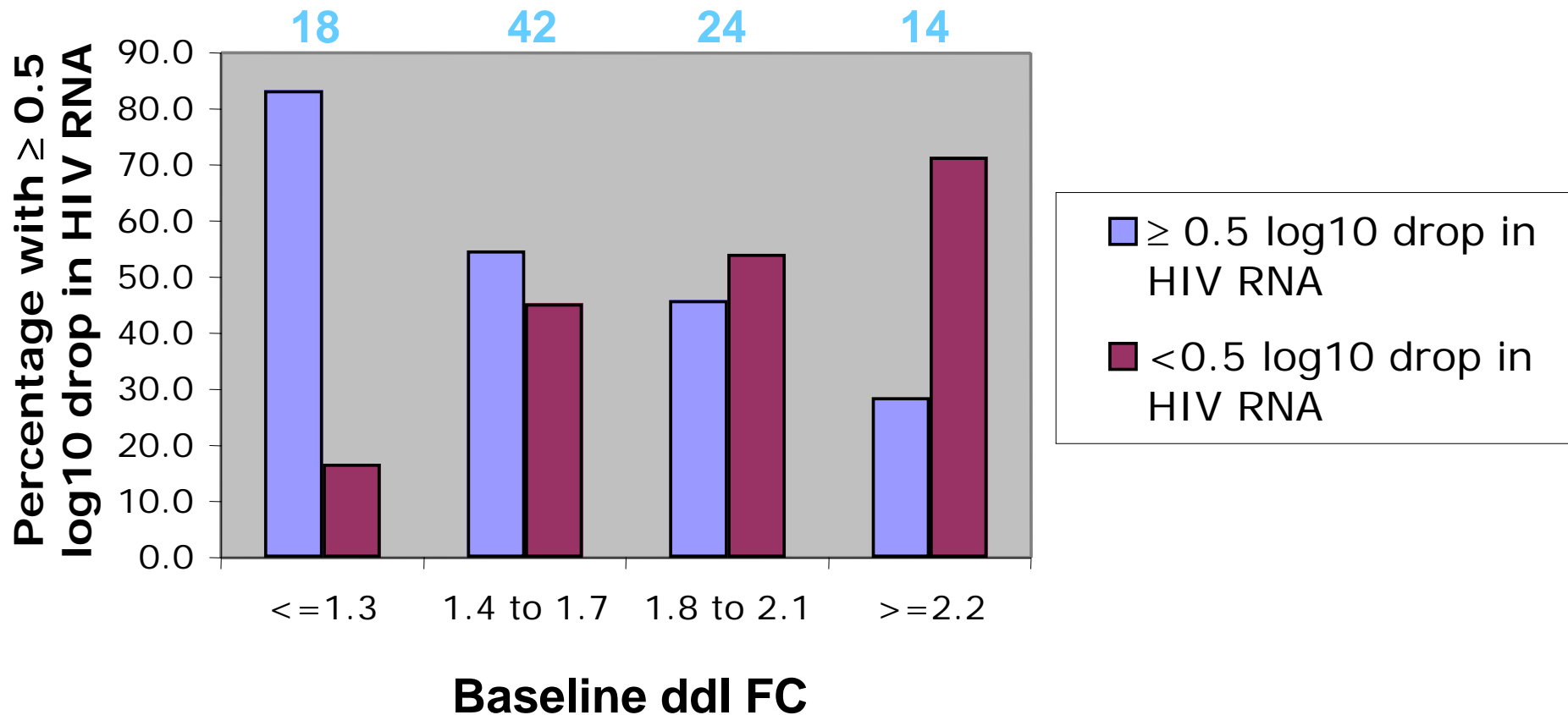
Biologic Cutoffs for ViroLogic Assay: D4T



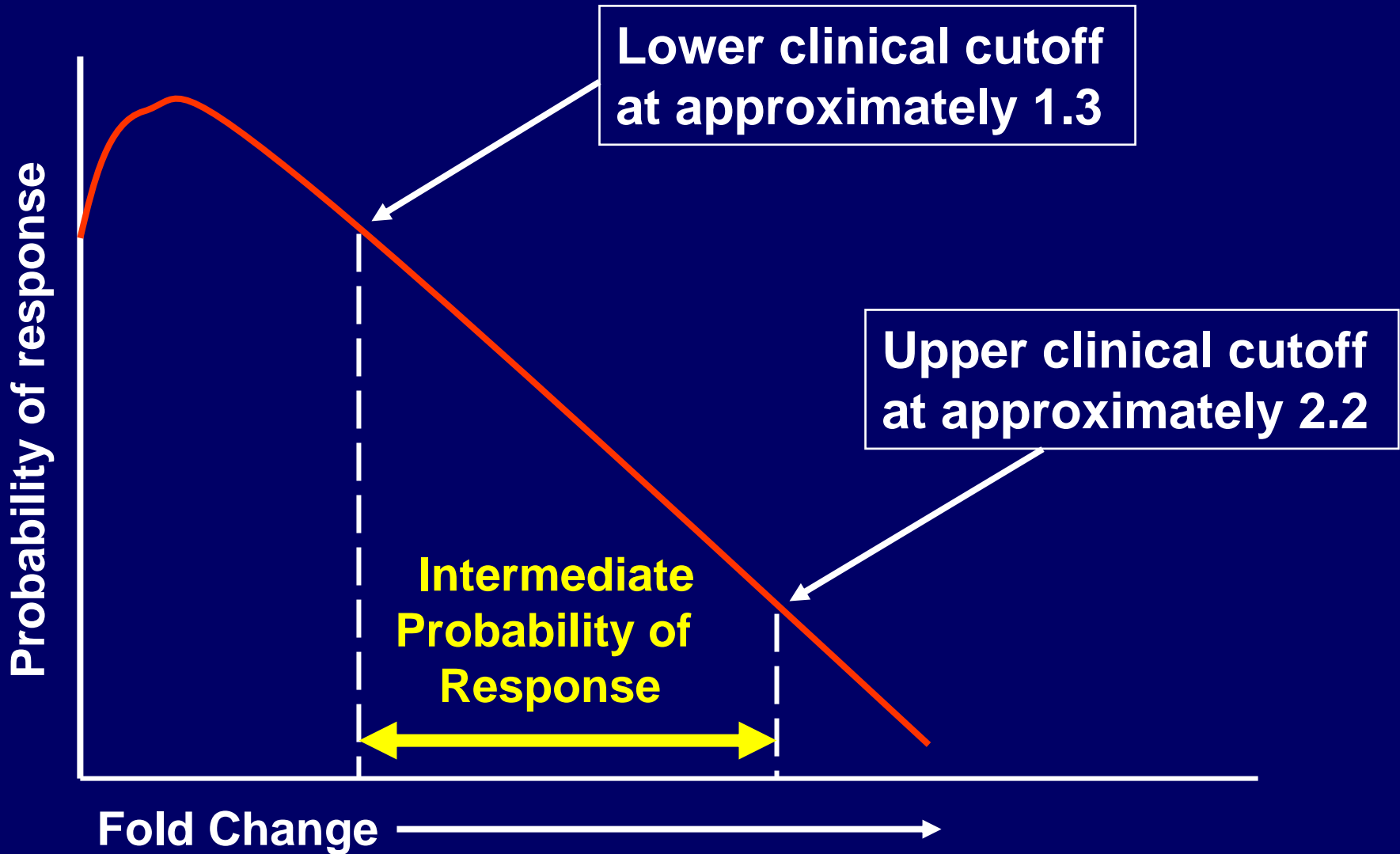
JAGUAR

Baseline ddl Fold Change and Virologic Response (discrete virologic outcome)

Virologic Response to ddl @ 4 weeks



Proposed Clinical Cutoffs for ddl



CASE 2

CASE 2

Clinical History

- **41 year old gay male**
- **CD4 428**
- **HIV RNA 9663 copies/ mL**
- **OI History**
 - **Cryptococcal meningitis (5 yr prior)**
 - **Recurrent Zoster**
- **Other Hx**
 - **Hep B**
 - **GERD**

CASE 2

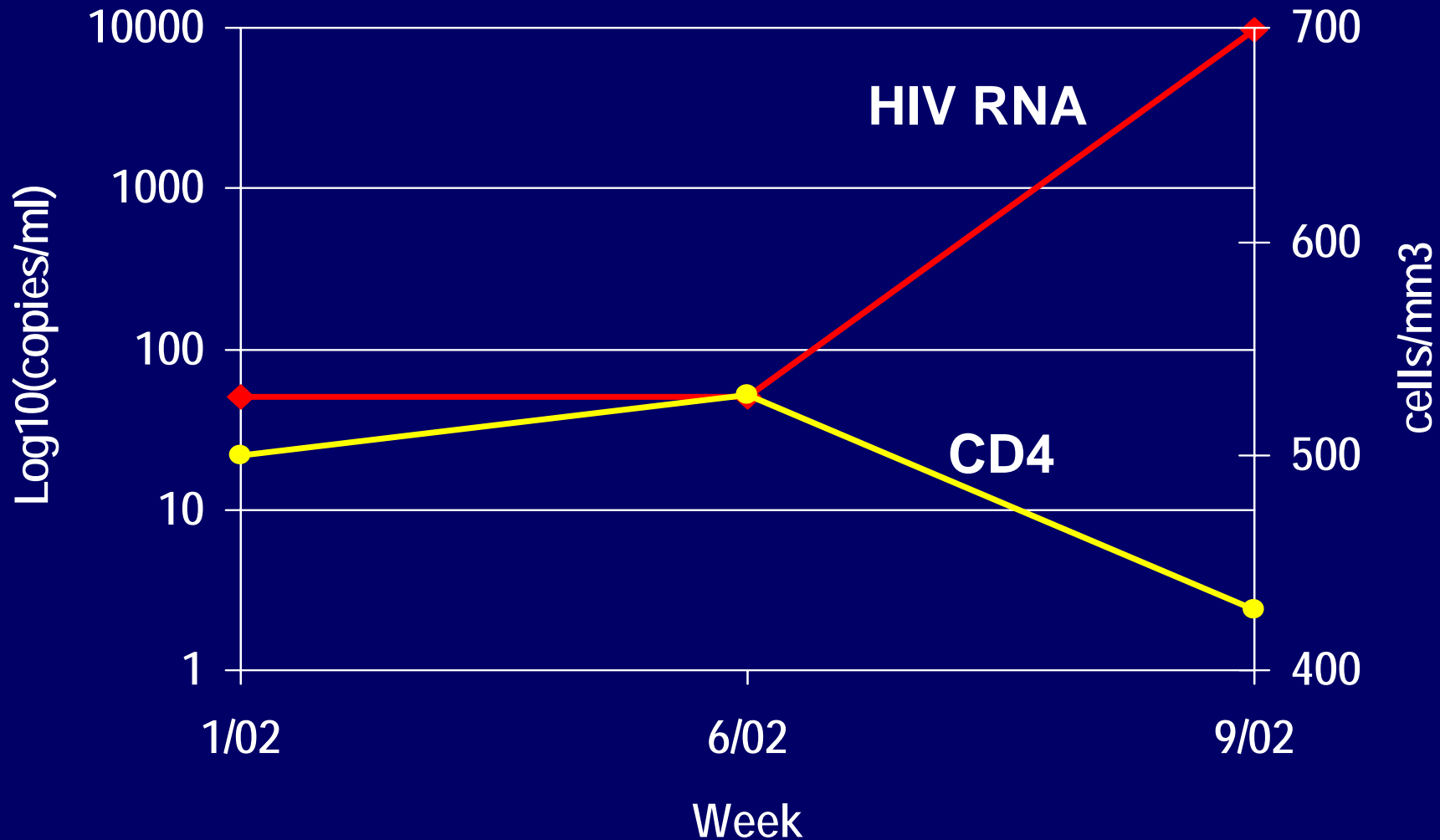
Past ARV

- 97-99: d4T/ 3TC/ IDV (stopped due to increased lactate)
- 99-02: ABC/ 3TC/ IDV (HIV RNA < 50; switched to simplify)
- 5/02- 9/02 (current): TDF/ 3TC/ ABC
- Prior treatment summary
 - 2 class exposure
 - 5 years on triple drug ARV regimen
 - Virologic suppression on PI based regimen
 - 5 drugs exposed in past

HIV RNA and CD4

ABC/ 3TC/ IDV

3TC/TDF/ABC



Phenotype Result CASE 2

DRUG		SUSCEPTIBILITY	
Generic Name	Brand Name	Fold Change	Increasing Drug Susceptibility Decreasing
NRTI	Abacavir	3.9	
	Didanosine	1.5	
	Lamivudine	>MAX	
	Stavudine	0.9	
	Zalcitabine	2.2	
	Zidovudine	0.4	
	Tenofovir	0.5	
NRTI Mutations		K65K/R, M184V	
NNRTI	Delavirdine	1.0	
	Efavirenz	0.5	
	Nevirapine	0.6	
	NNRTI Mutations		none

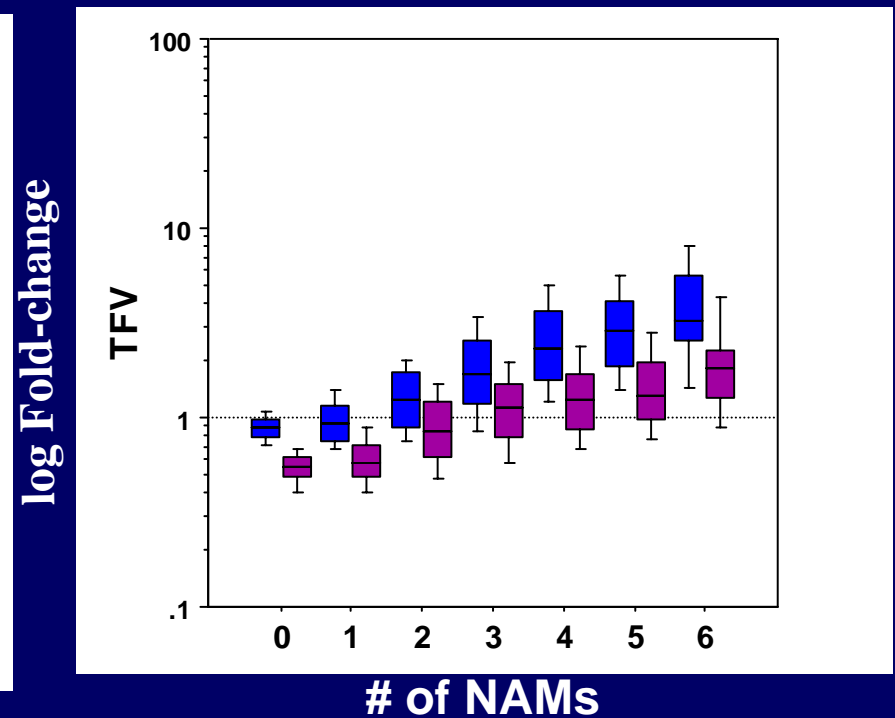
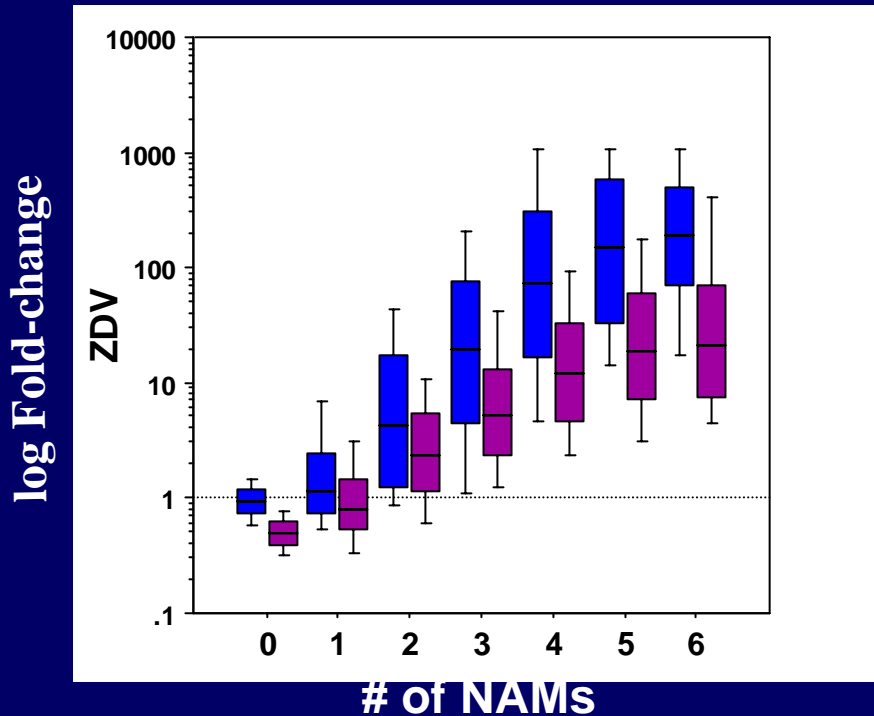
Phenotype Result CASE 2

DRUG		SUSCEPTIBILITY				
Generic Name	Fold Change	← Increasing	Drug Susceptibility	Decreasing →		
		.1	1	10	100	1000
PI	Amprenavir	0.5				
	Indinavir	1.0				
	Lopinavir	0.7				
	Nelfinavir	1.7				
	Ritonavir	0.8				
	Saquinavir	0.9				
	PI Mutations	L63P, V77I				

NAMs Decrease while M184 V Increases Susceptibility to the Group 1 NRTI

Group 1: AZT, TDF, d4T

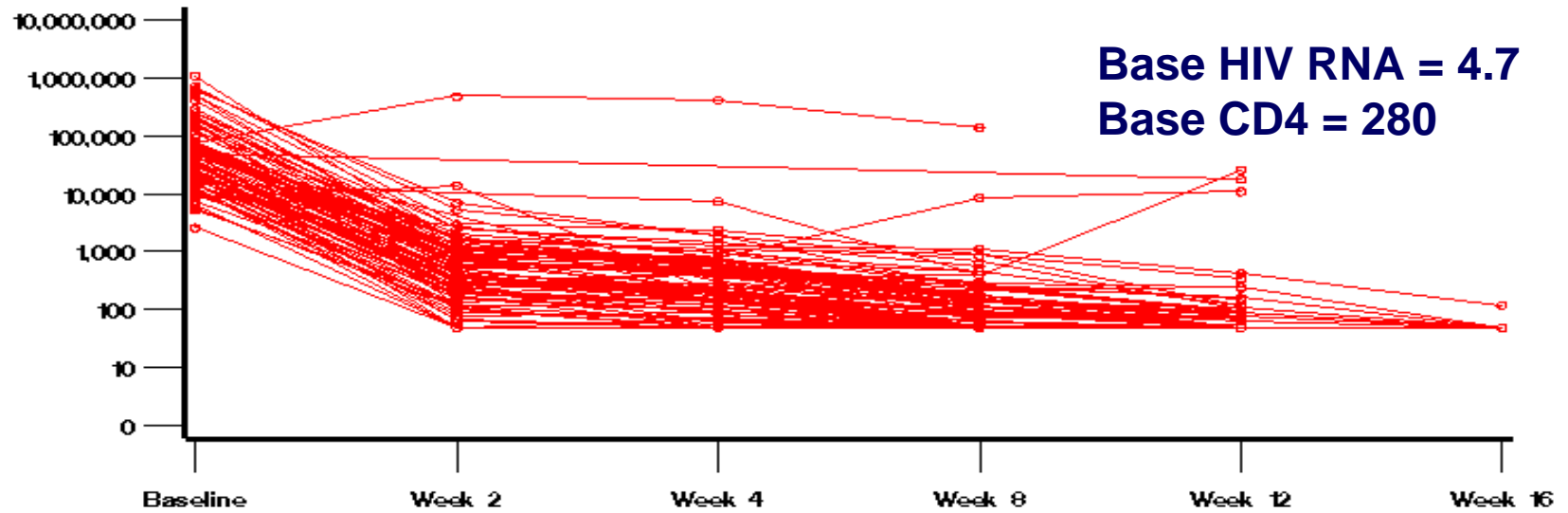
M184V mutation increases susceptibility to these drugs



NAMs: M41L, D67N, K70R, L210W, T215Y/F, and K219Q/E/N
Group 2: ddI, ddC, ABC, 3TC

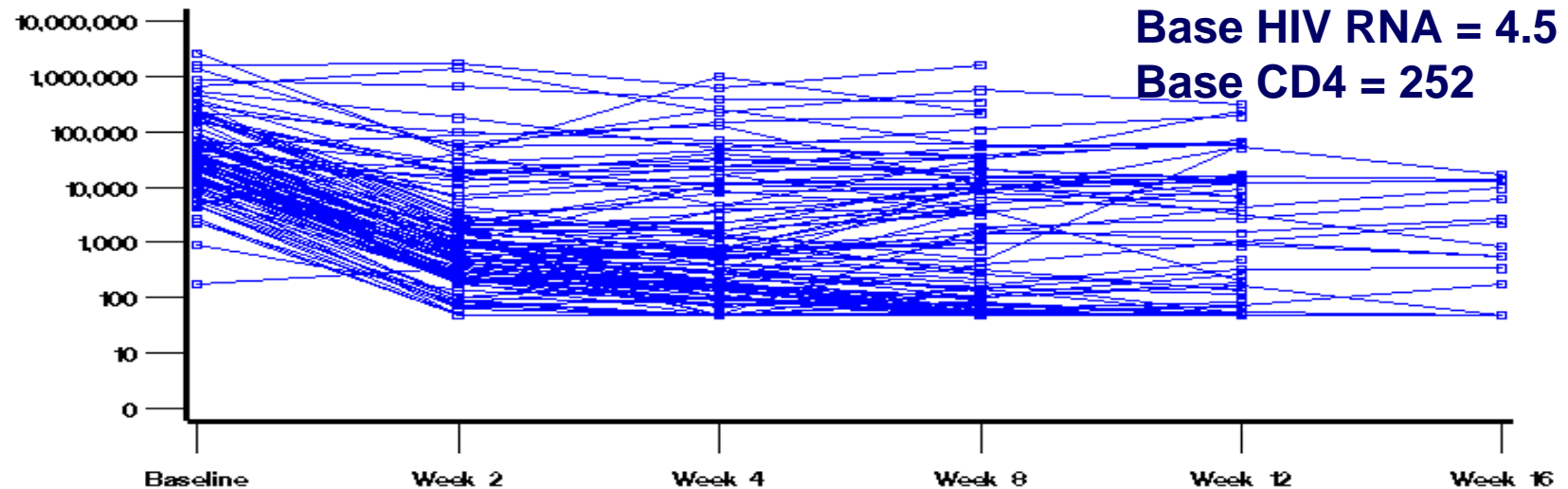
EFV + ABC/3TC (n=92)

HIV RNA, c/mL



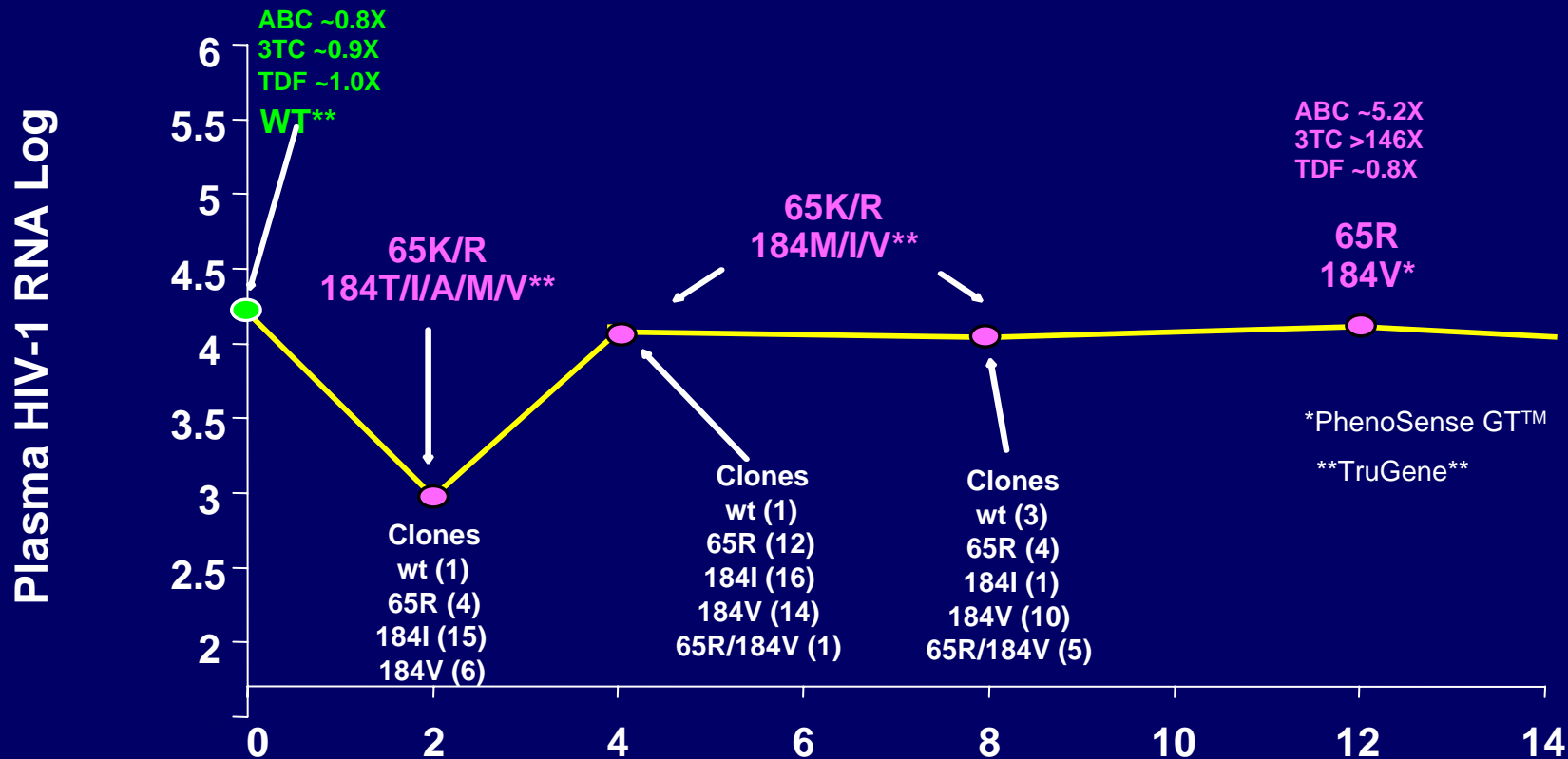
TDF + ABC/3TC (n=102)

HIV RNA, c/mL



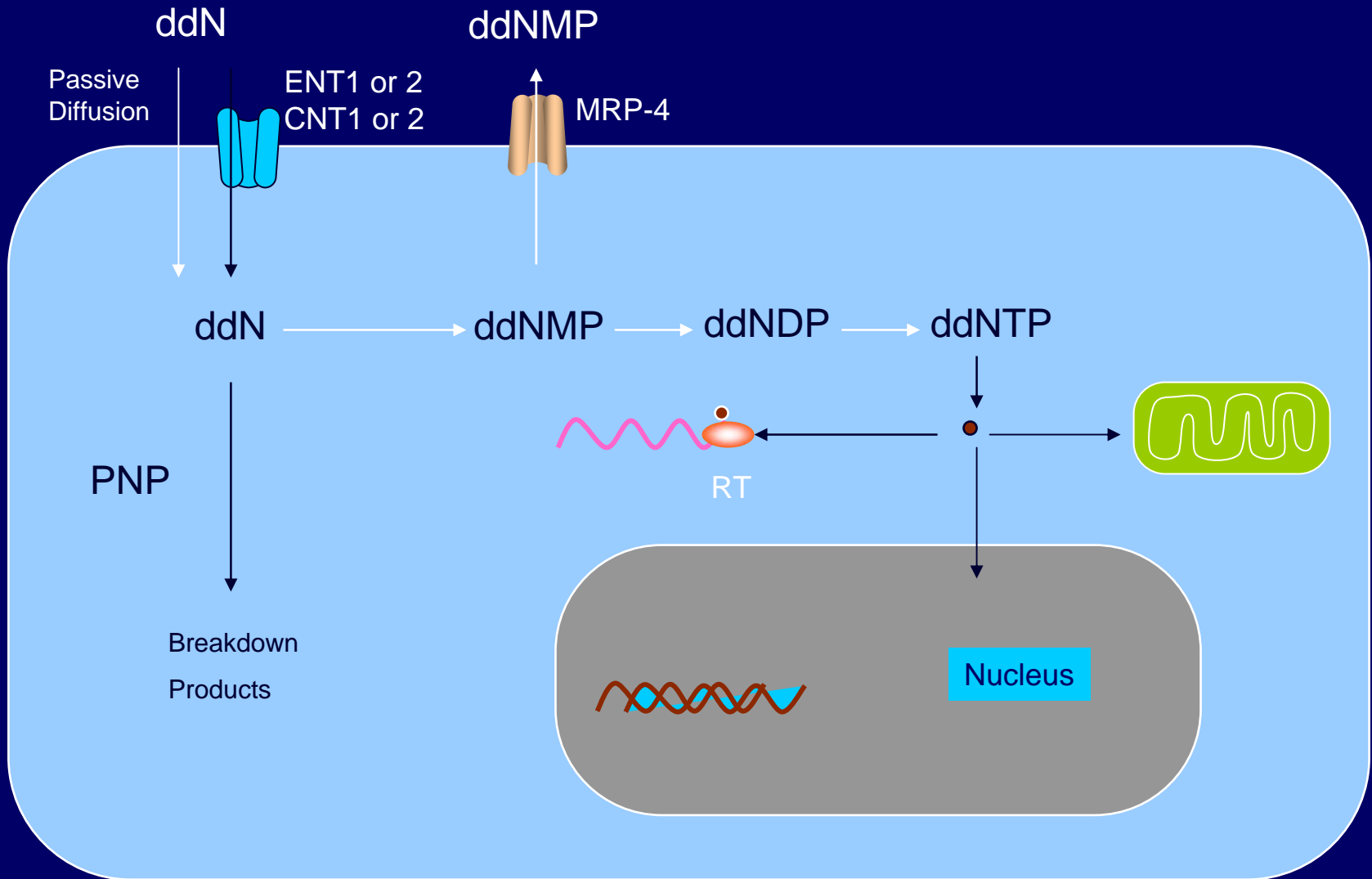
Subject 1

ESS30009



M184V/I mutations in 40 of 41 (98%) and K65R in 22 of 41 (54%) K65R. Clonal analysis in 4 with only M184V/I: K65R was observed in 5 of 22 (23%), 1 of 27 (3.7%), 1 of 8 (13%), and 1 of 119 (0.8%)

Intracellular Transport of Nucleosides



MINORITY VARIANTS

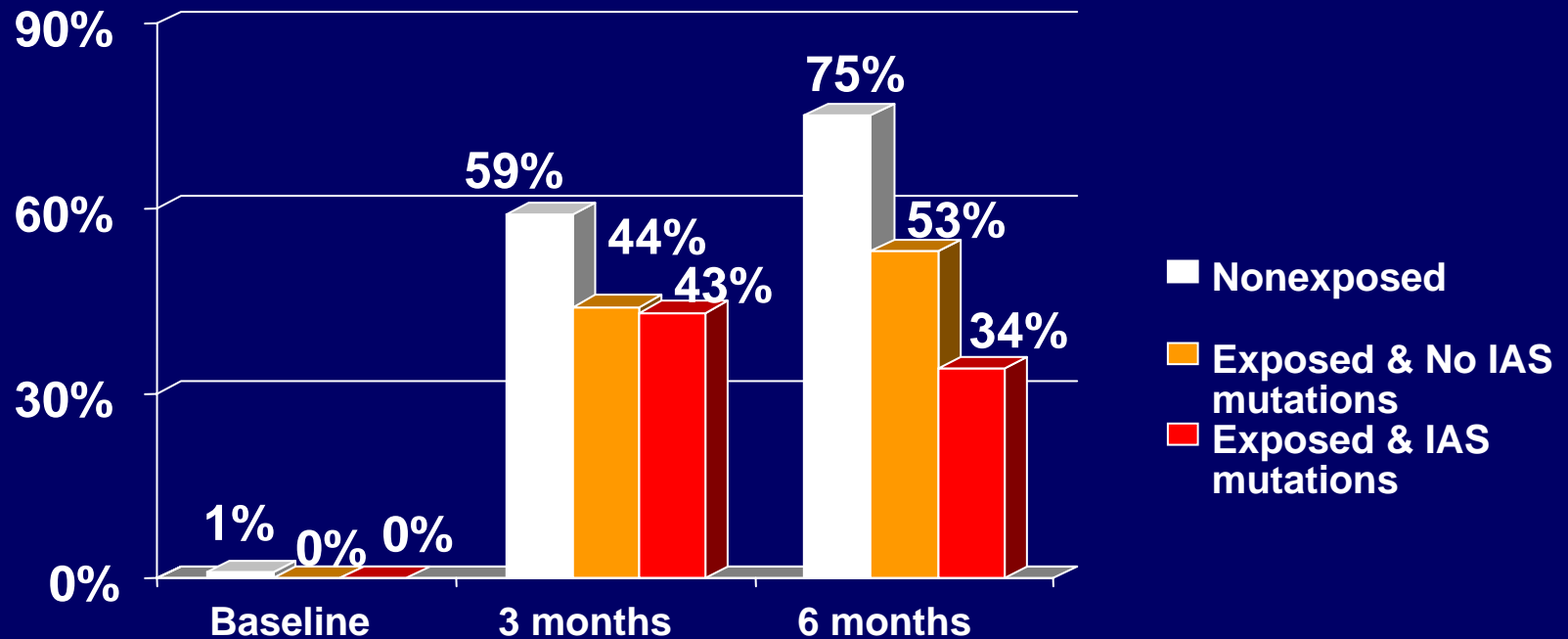
Allele Specific PCR to Detect Persistence of NVP Resistance

- **Twenty-seven women enrolled in a South African MTCT trial**
- **All were given single-dose nevirapine to be taken at the onset of labor**
- **Plasma samples were collected at four time points:**
 - » **baseline (pretreatment)**
 - » **1-4 months**
 - » **5-9 months**
 - » **11-14 months**

Conclusions

- ❖ **Using allele specific PCR, the frequency of NNRTI-resistant variants in women after sdNVP was 69% -- about twice that previously reported with standard genotyping**
- ❖ **NNRTI-resistant variants selected by single-dose nevirapine were detected in the majority of women 6 months after the standard genotype became negative**
- ❖ **The frequency of NNRTI-resistant variants declined with time but could persist above pretreatment levels for at least 14 months**

3 and 6 month responders (50 copies/mL)

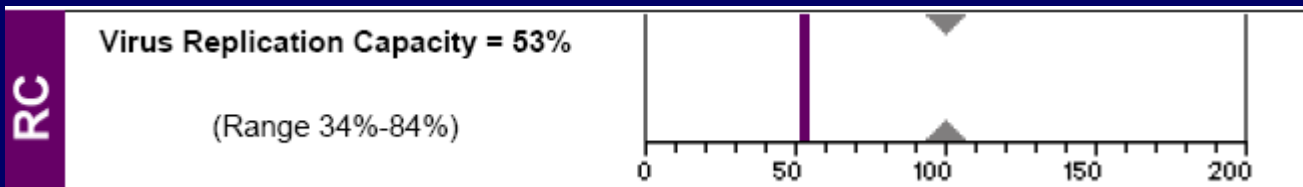


No NVP	42	37	28
NVP no MUT	139	112	110
NVP+MUT	61	58	50



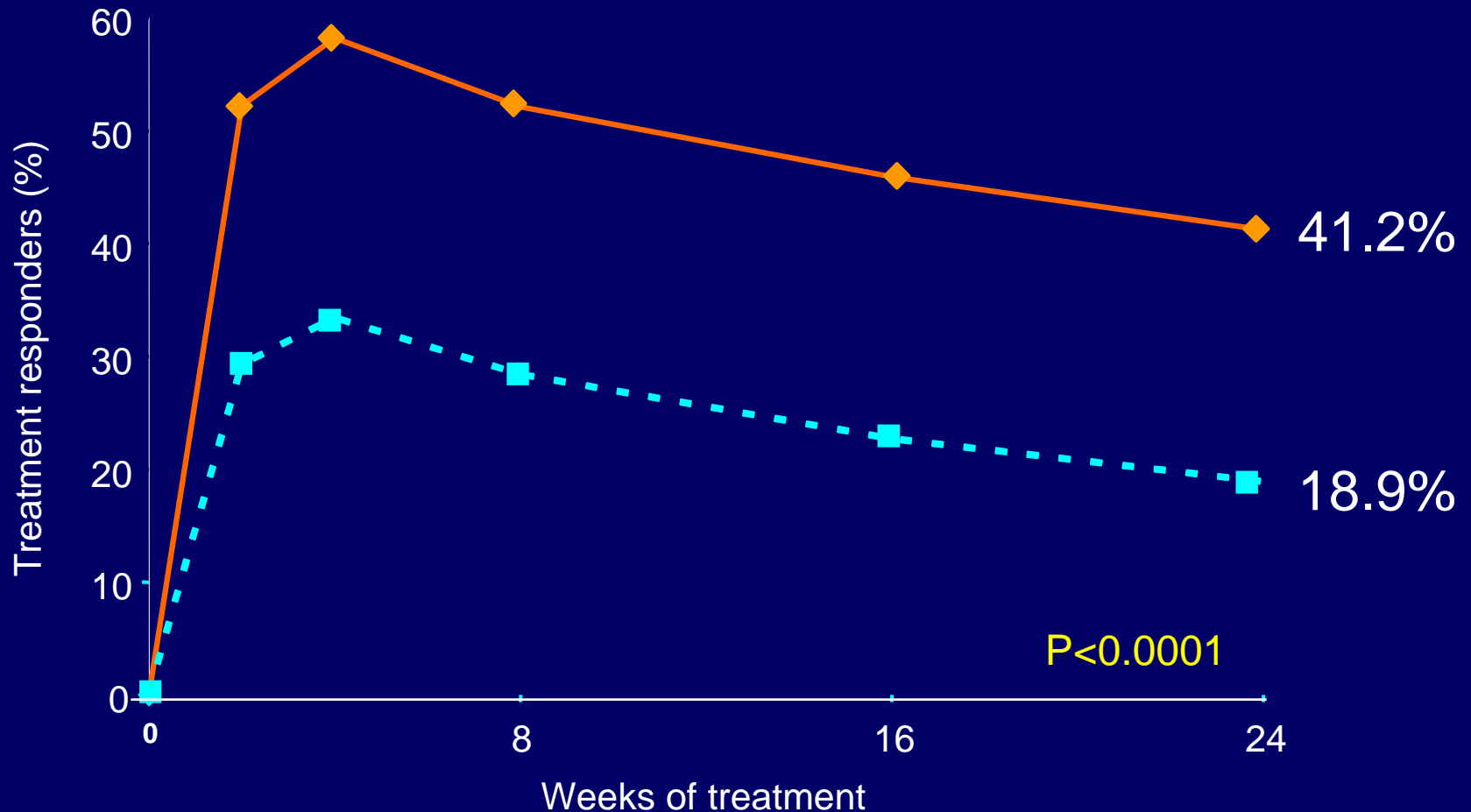
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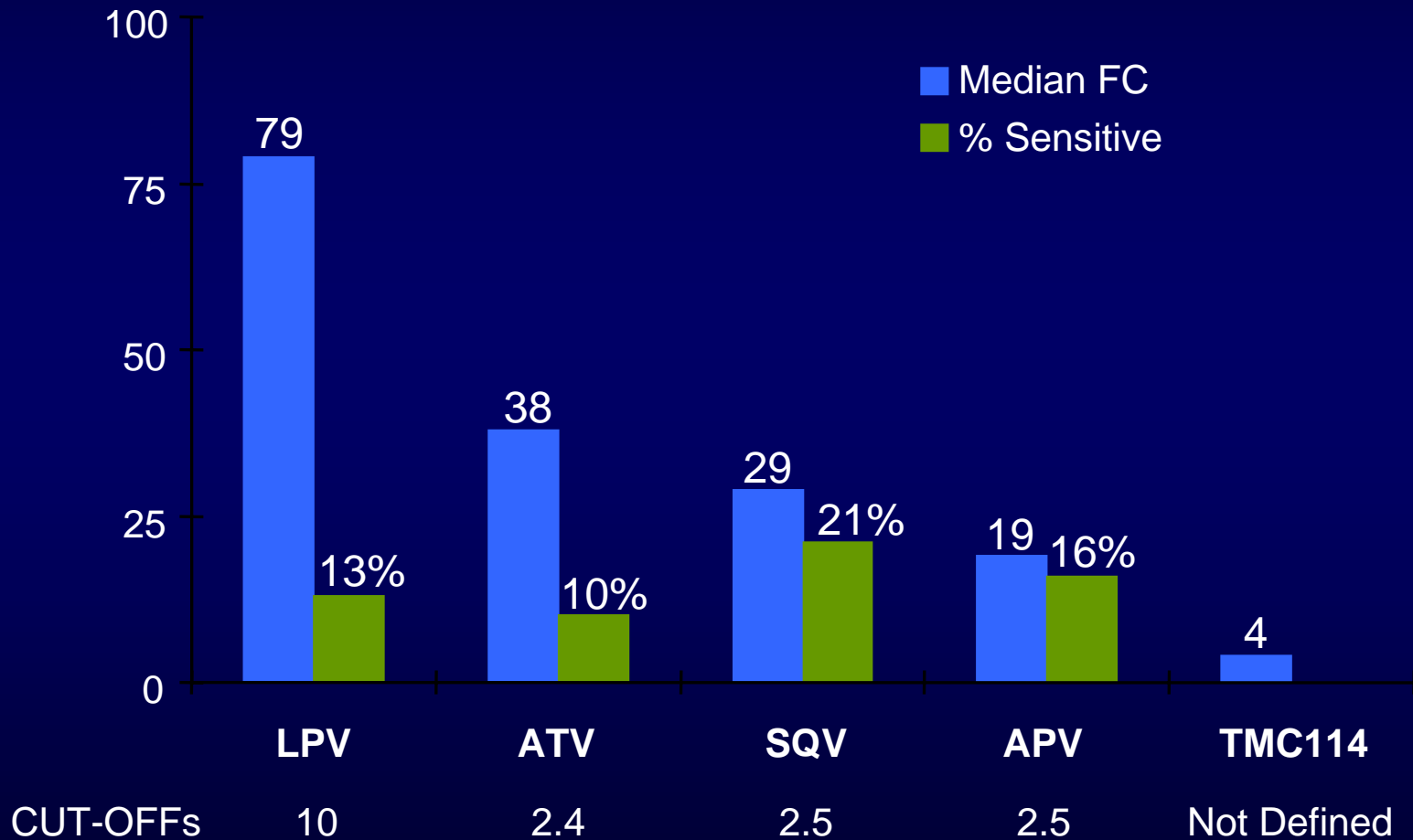


TPV RESIST Studies

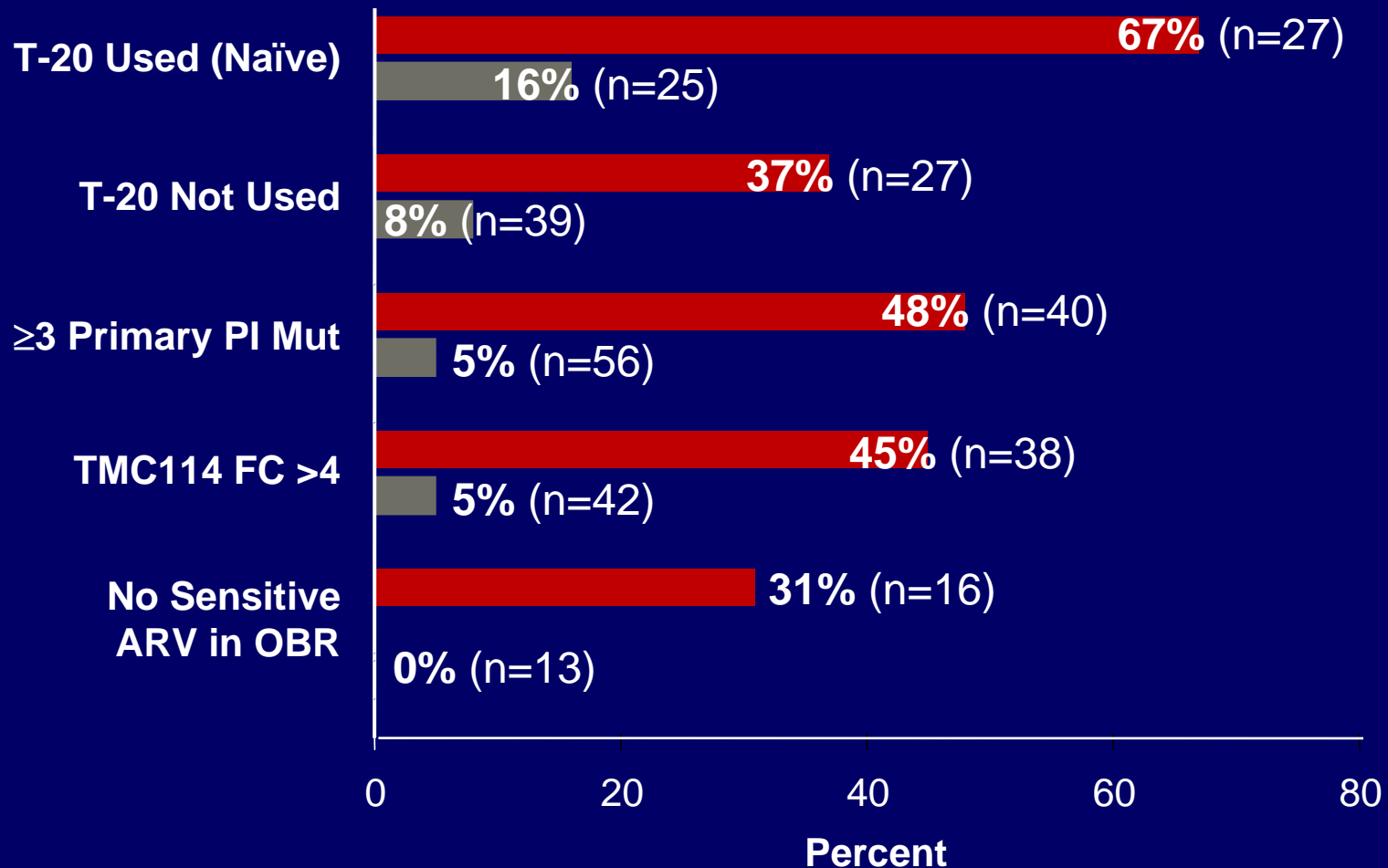
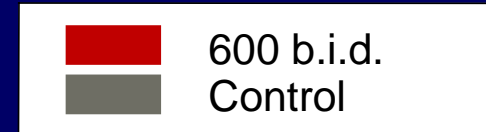
Proportion With Treatment Response ($\geq 1 \log_{10}$ Viral Load Reduction)



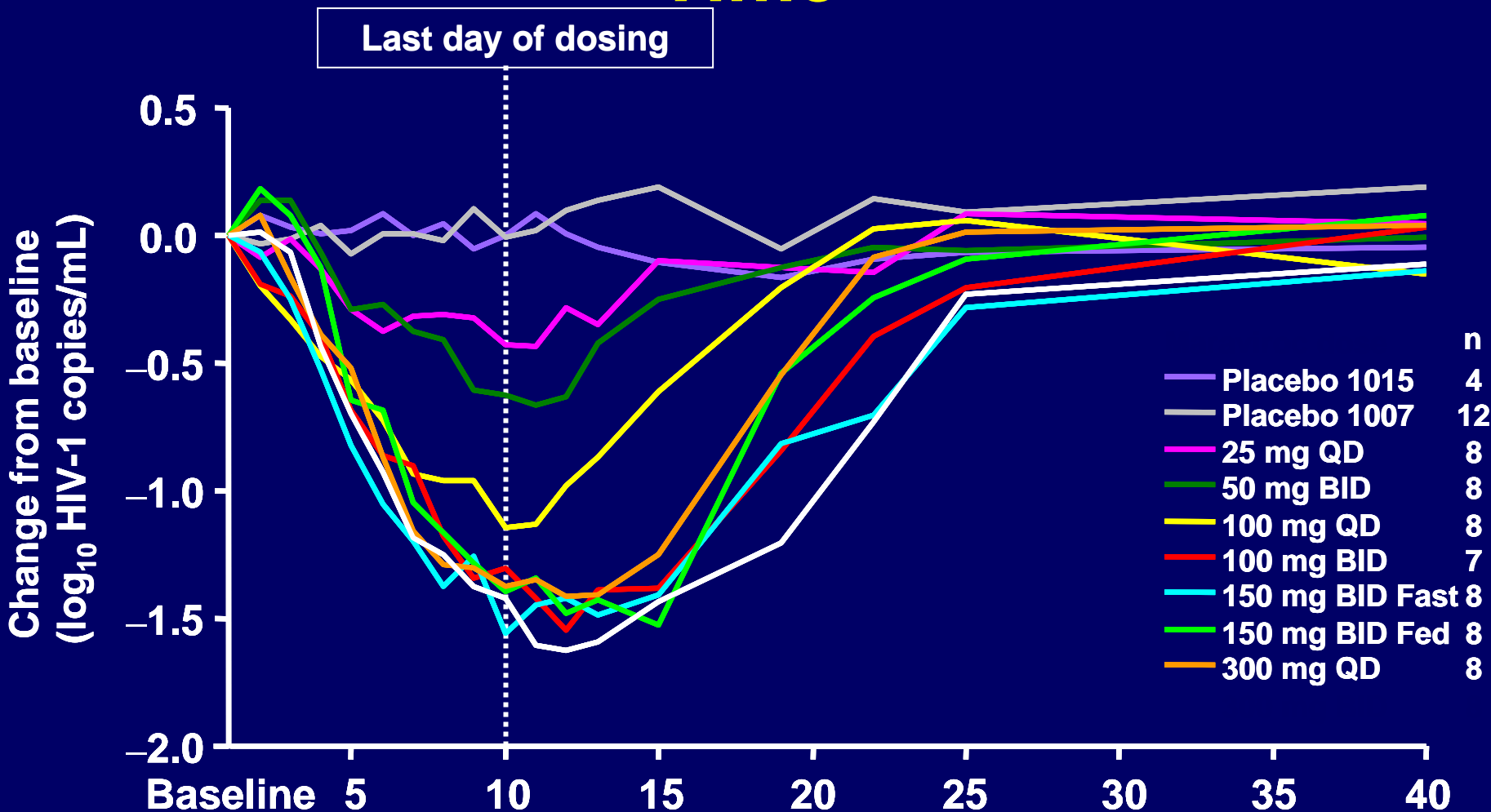
Baseline PI Susceptibility (Antivirogram[®])



HIV RNA <50 at Week 24: ITT NC=F (Cont'd)



MVC Efficacy Results: Mean Reduction in Viral Load over Time

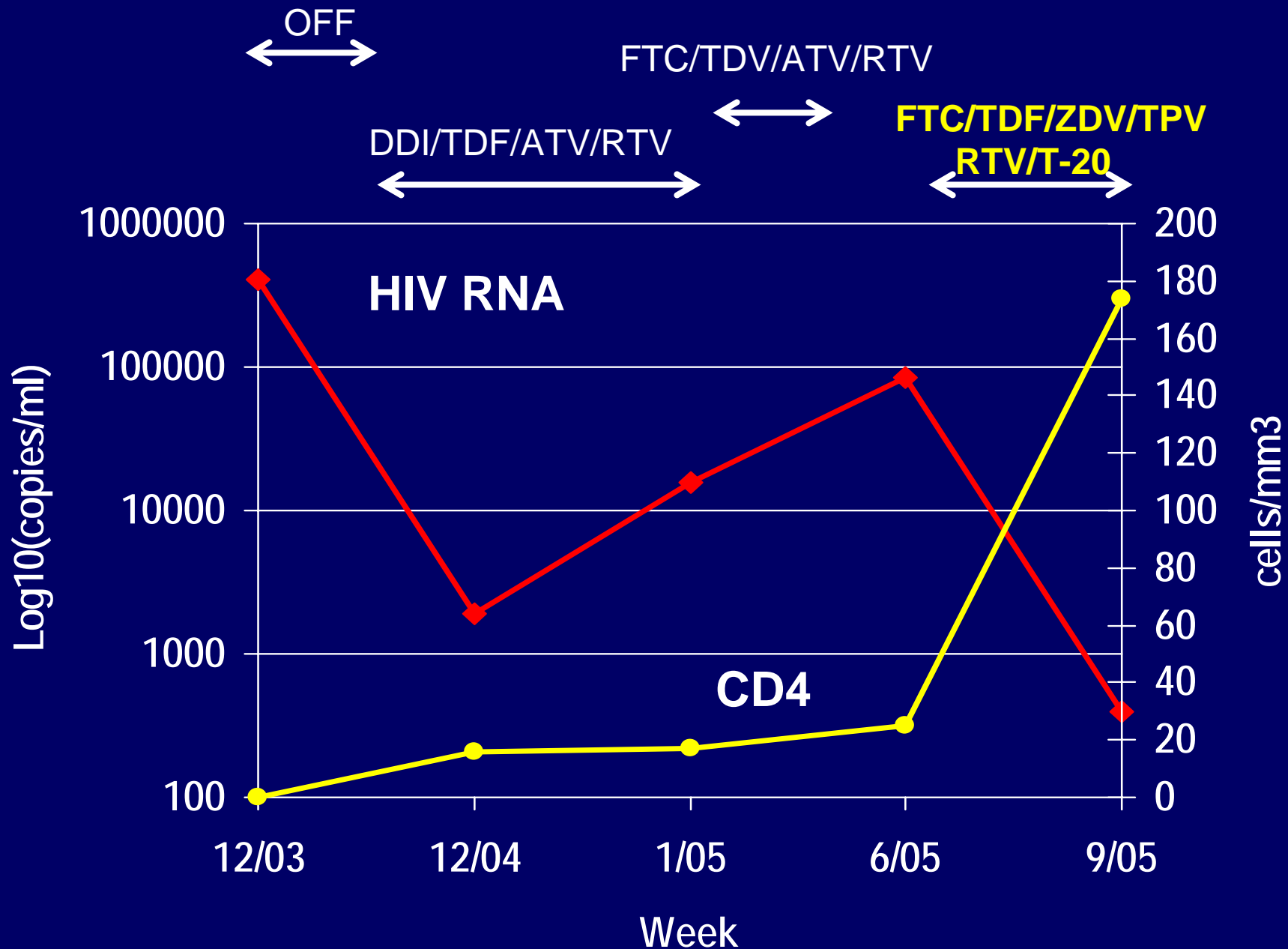


Case 1

Regimen Selected

- FTC
- TDF
- ZDV
- TPV
- RTV
- T-20
- Rationale
 - » extensive prior treatment
 - » broad 3 class cross resistance
 - » limitations from prior toxicity
 - » choice to recycle agents based on lowest (relative) phenotype fold change-accounting for clinical cut-points

HIV RNA and CD4



Case 1 Summary

- Larger than expected HIV RNA response
- Attained undetectable viral load, ? only transiently
- large increase in CD4
- CD4 and viral load changes of this magnitude associated with improved survival
- Realistic goal in highly experienced, 3 class resistant patients to maintain CD4; long term viral suppression may not be currently possible