



The HIV-1 Reverse Transcriptase Connection Domain From Treatment-Experienced Patients Contributes to AZT Resistance

Krista Frankenberry

HIV Drug Resistance Program, USA

C-Terminal HIV-1 RT Domains Obtained From Treatment-Experienced Patients Contributes to AZT Resistance

*Galina N. Nikolenko, Krista A. Frankenberry, Sarah Palmer,
Frank Maldarelli, John W. Mellors, John M. Coffin and
Vinay K. Pathak*



HIV Drug Resistance Program

National Cancer Institute at Frederick



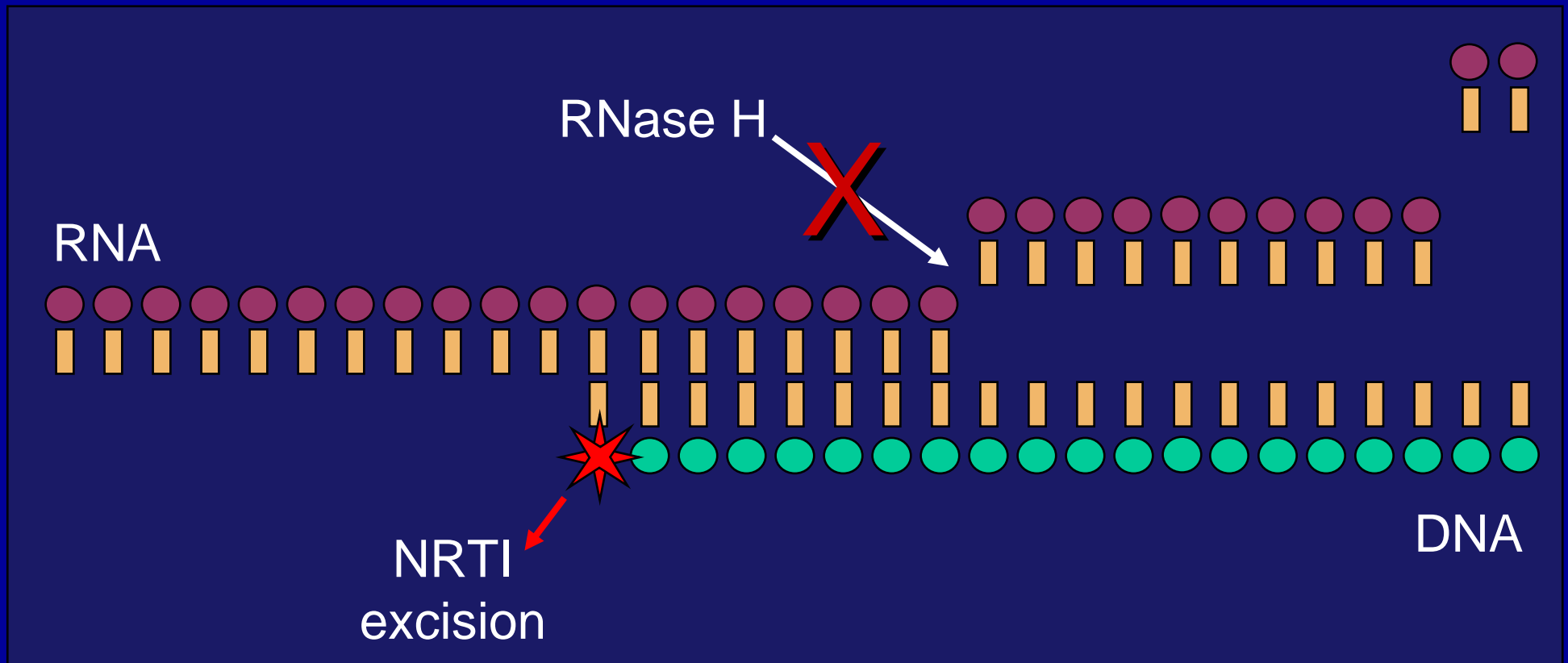
Mechanisms of Resistance to Nucleoside Reverse Transcriptase Inhibitors (NRTIs)

- Nucleotide excision:
 - AZT resistance by thymidine associated mutations (TAMs)
Arion D. et al. Biochemistry 1998; 37: 15908-15917
Meyer P.R. et al. PNAS 1998; 95:13471-13476
- Discrimination between the nucleoside analog and normal dNTP:
 - 3TC resistance by M184V
Sarafianos S.G. et al. PNAS 1999; 96:10027-10032

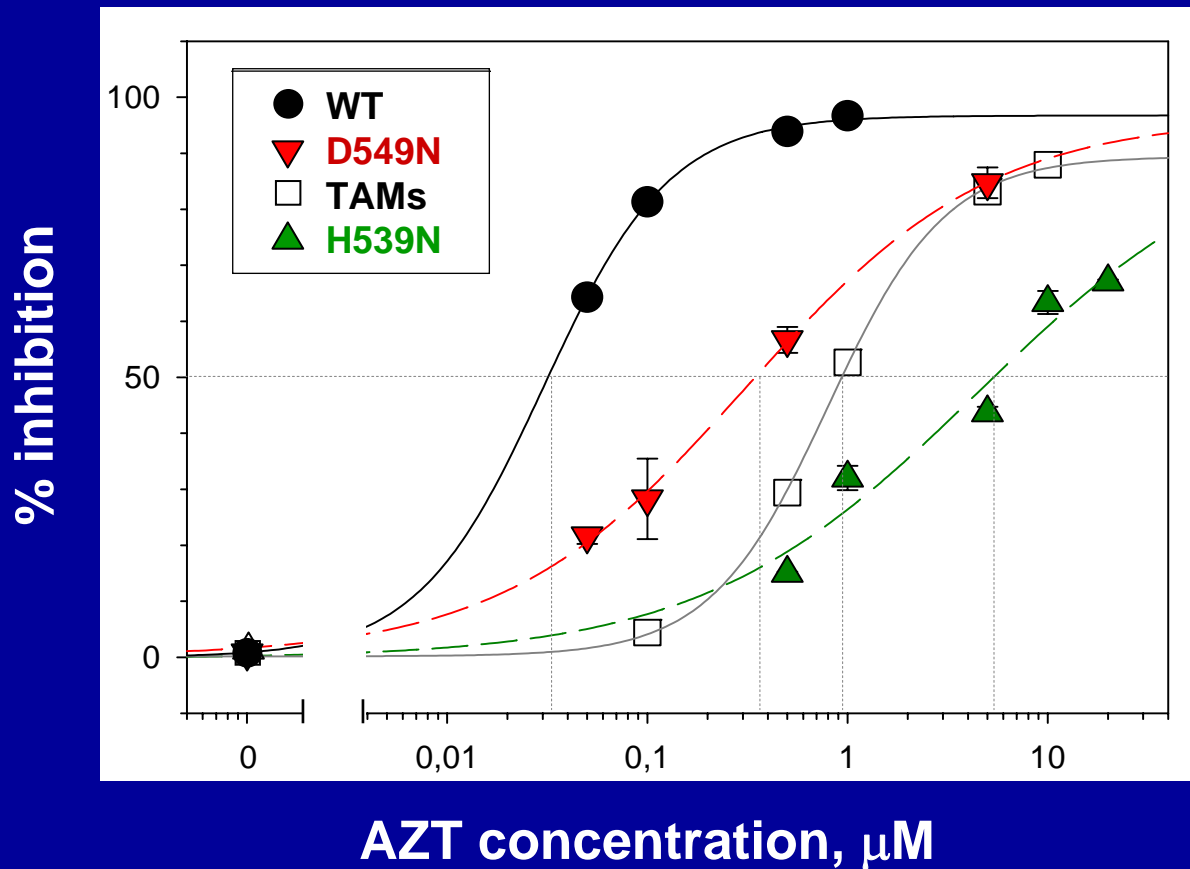
- Balance between RNase H and nucleotide excision

Nikolenko G. N. et al. PNAS 2005;102: 2093-98

Prediction: Reducing RNase H activity will increase resistance to NRTIs by providing more time for nucleotide excision



Mutations in RNase H Increase Resistance to AZT

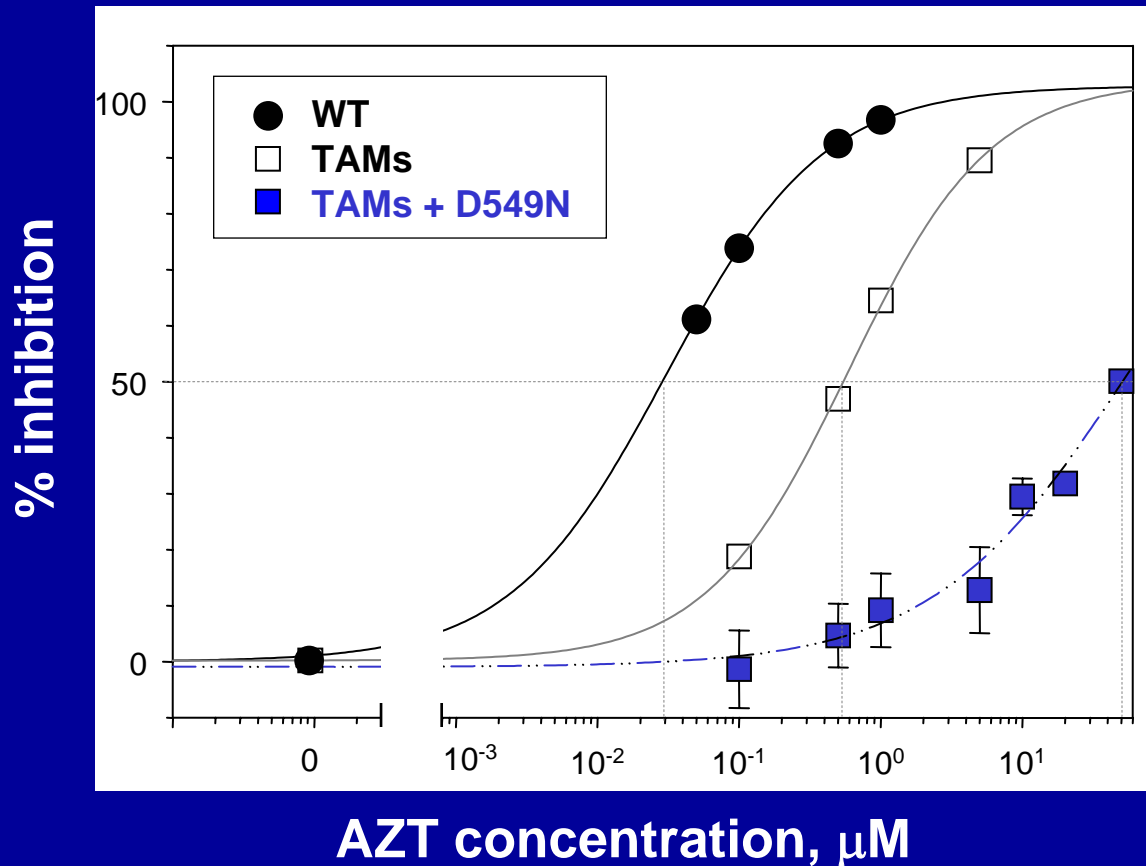


	IC ₅₀ [μM]	fold/WT
WT	0.04	1X
D549N	0.52*	13X
TAMs	0.99*	25X
H539N	7.96*	200X

*P < 0.005

TAMs = D67N, K70R, T215Y, K219Q

RNase H Mutations Increase AZT Resistance Synergistically in Combination with TAMs



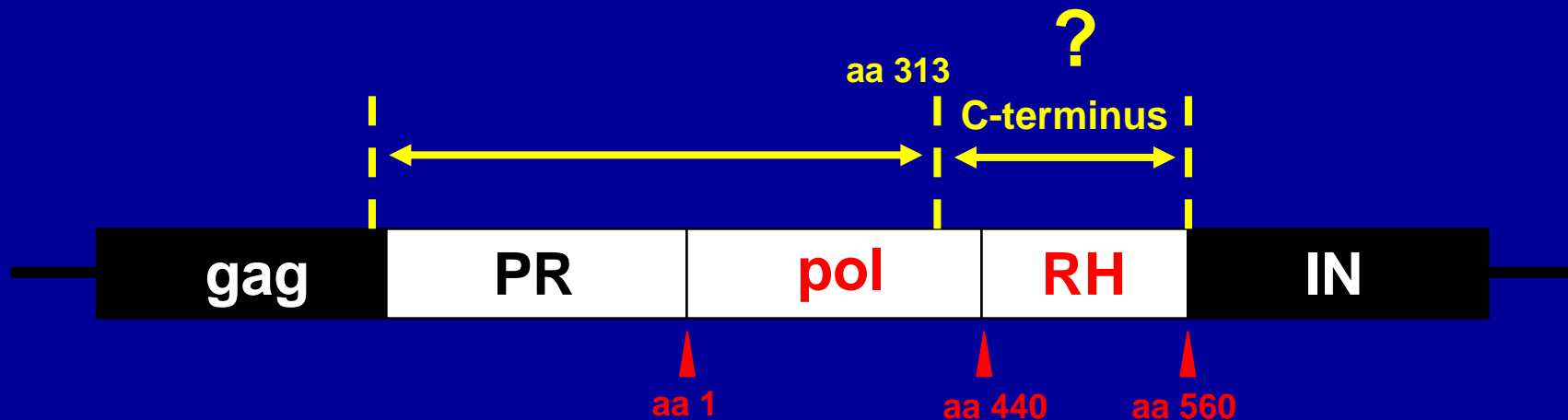
	IC ₅₀ [μM]	fold/WT
WT	0.04	1X
TAMs	0.99*	25X
TAMs + D549N	~50.00	1250X

*P < 0.001

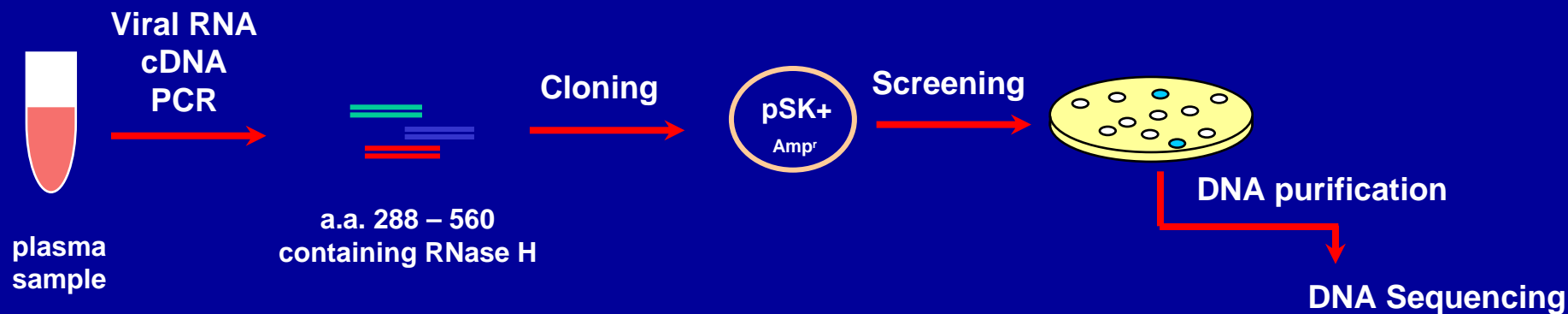
TAMs = D67N, K70R, T215Y, K219Q

What is the Role of RNase H in Clinical NRTI Resistance?

- In standard genotypic/phenotypic assays, only part of RT is analyzed



HIV-1 C-Terminal Domains Derived from Patients: Genotypic Analysis



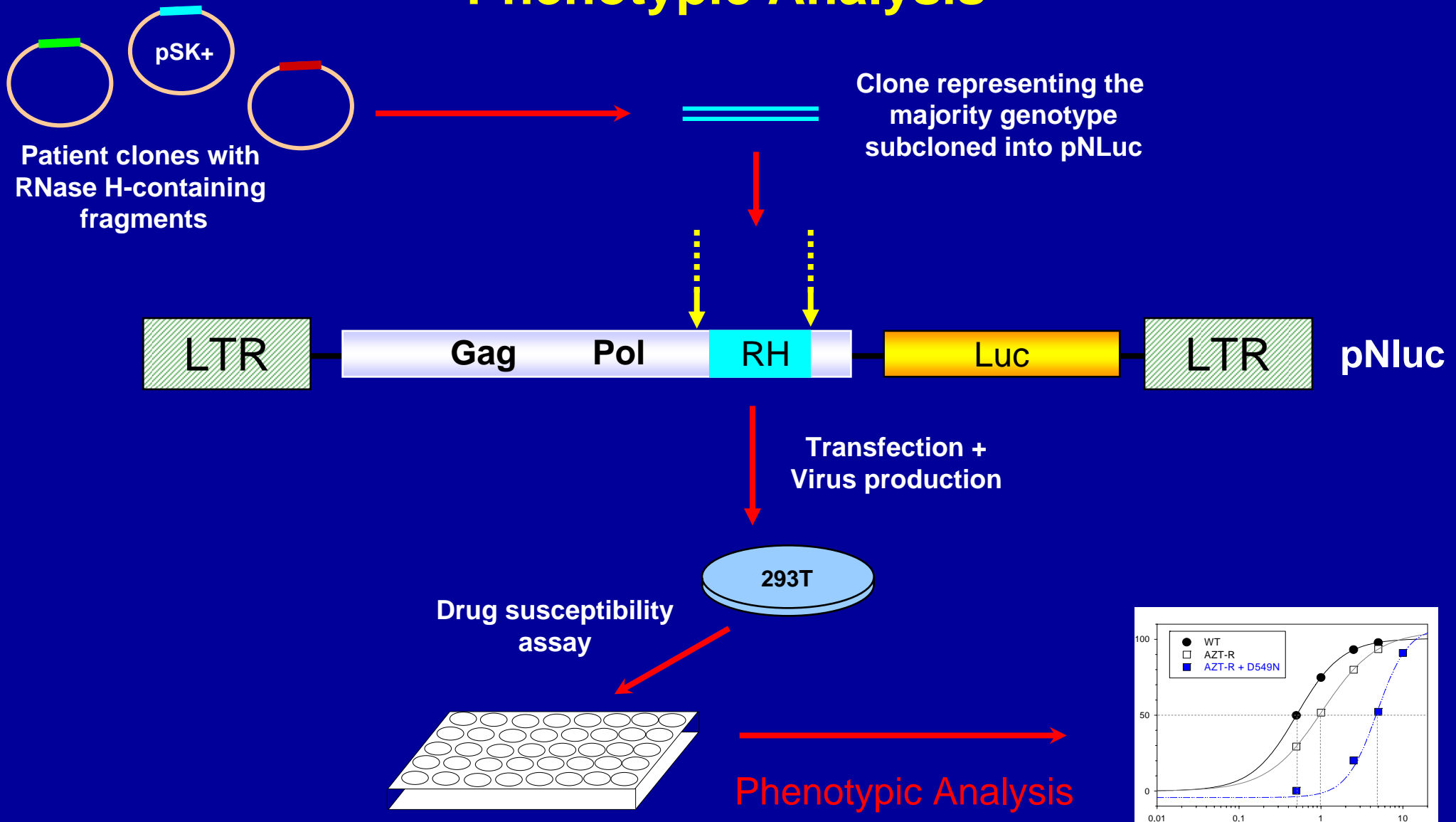
- NRTI-experienced and naïve patients
- Cloned and sequenced 20-40 PCR products
- Clones representing the majority genotype selected for further analysis

```

IVGAETFYVDGAANRETKSGKAGYVTDGRQKVVSLTDITNQ
-----H-----S-----
-----NN-----S-----
-----R-----P-----
-----N-----S-----
-----R-----R-A-I-----
-----Q-----S-----
-----N-----N-----
-----R-----S-----
-----G-----NK-----IT-----
-----S-----
-----N-----S-----
-----N-----R-----NK-----ISI-----
-----S-----T-----
K-----
H-----
    
```

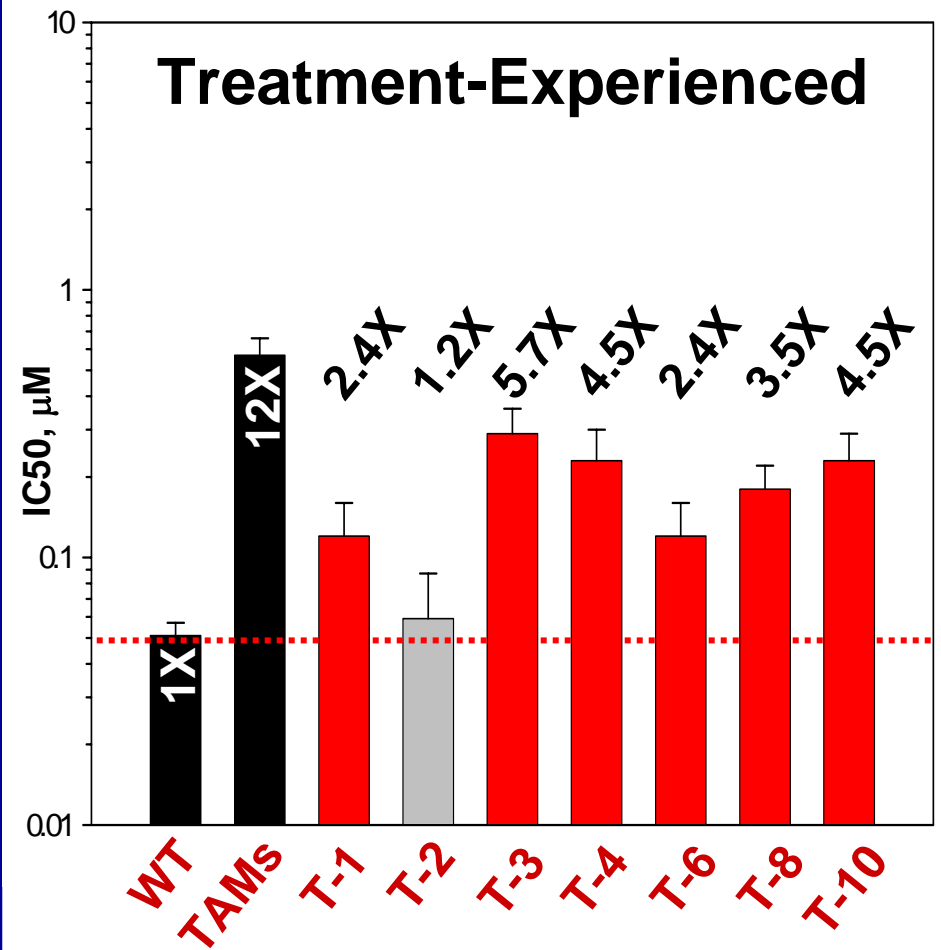
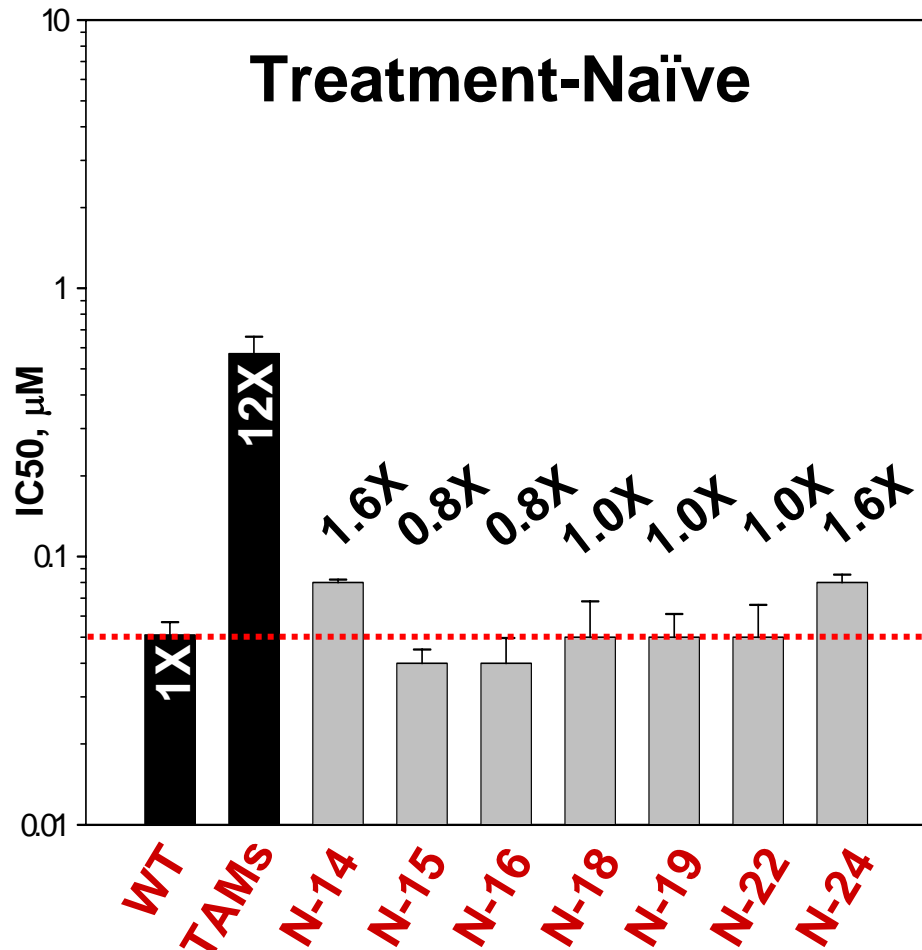
Genotypic Analysis

HIV-1 C-Terminal Domains Derived from Patients: Phenotypic Analysis



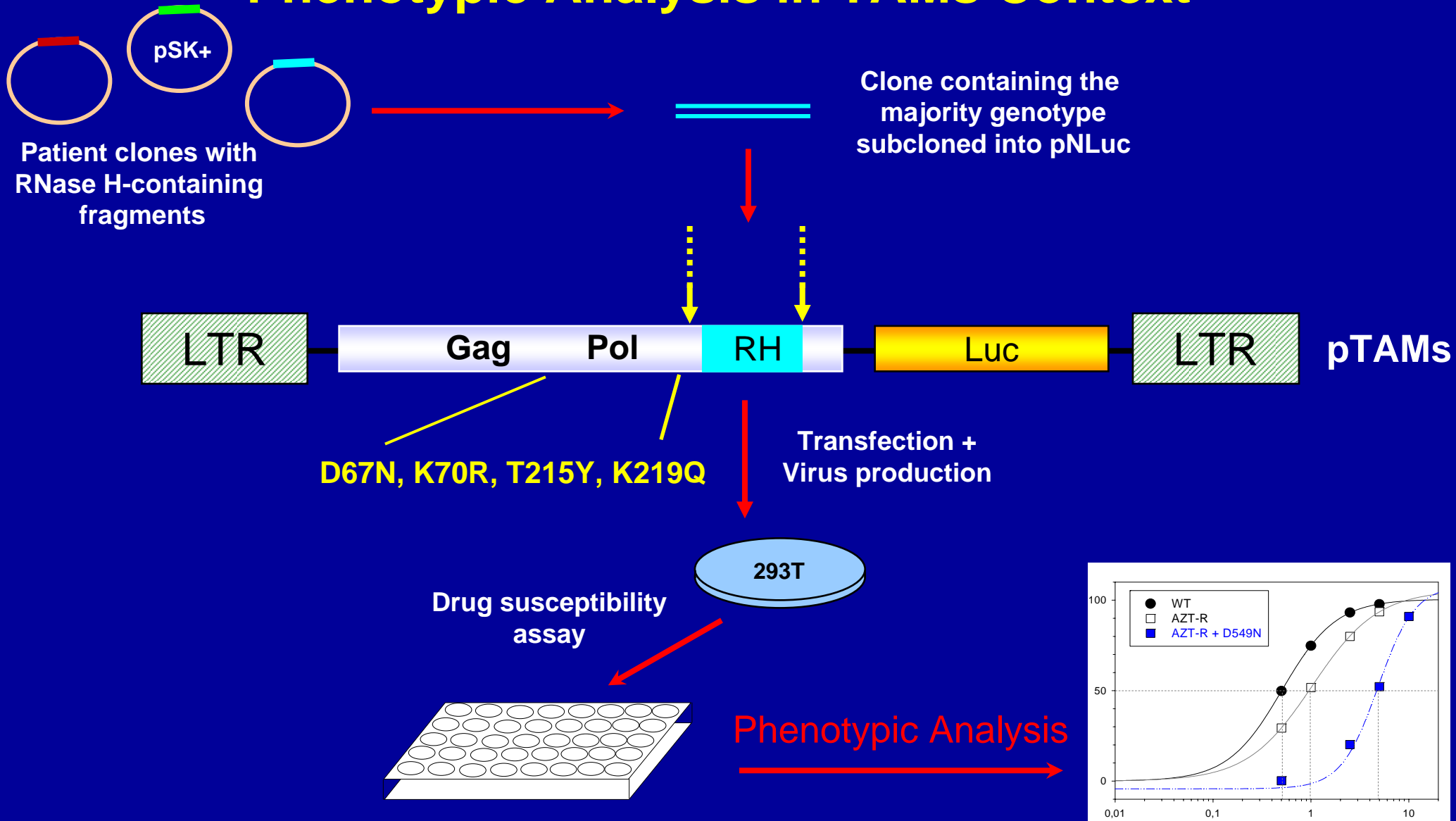
PoI-WT + C-Terminal Domain From Patients

AZT^R



TAMs = D67N, K70R, T215Y, K219Q

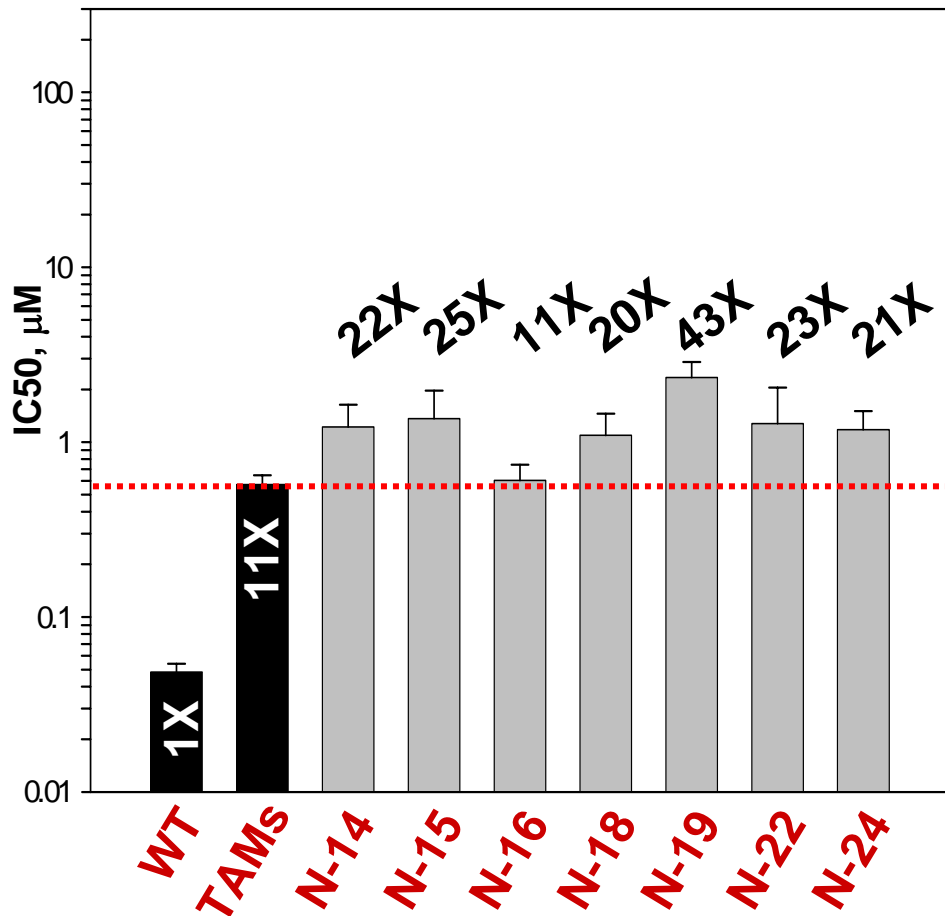
HIV-1 C-Terminal Domains Derived from Patients: Phenotypic Analysis in TAMs Context



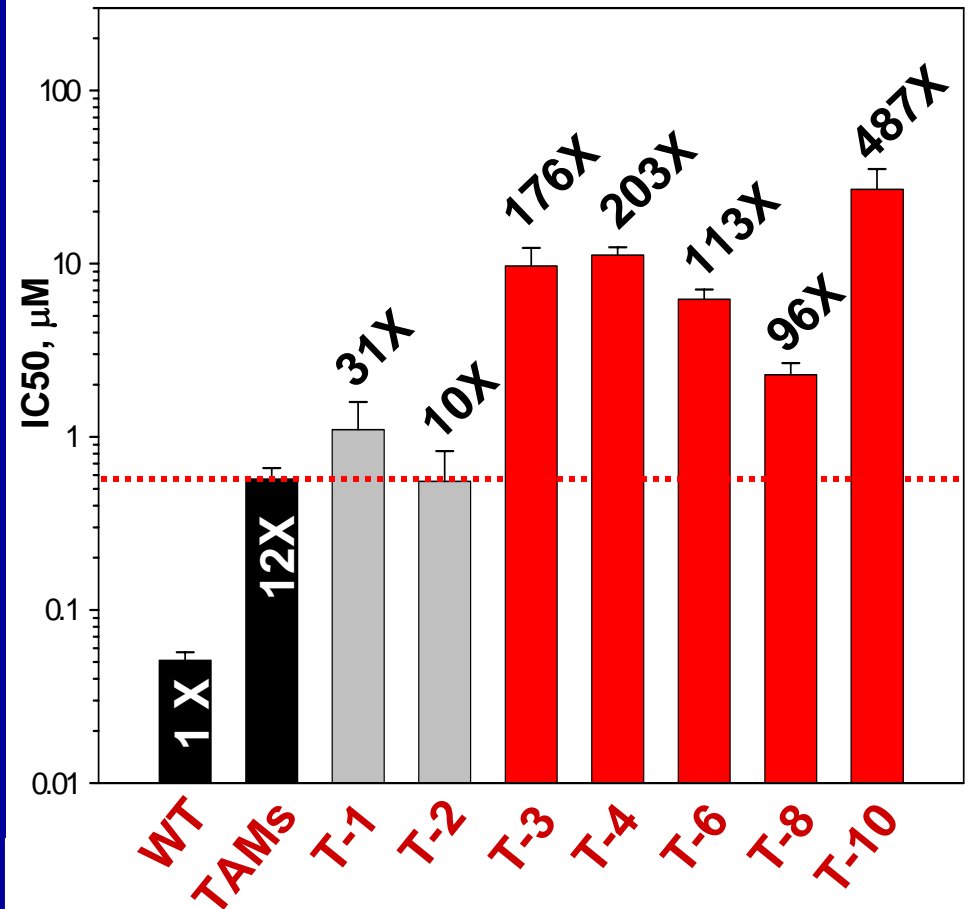
PoI-TAM + C-Terminal Domain From Patients

AZT^R

Treatment-Naïve



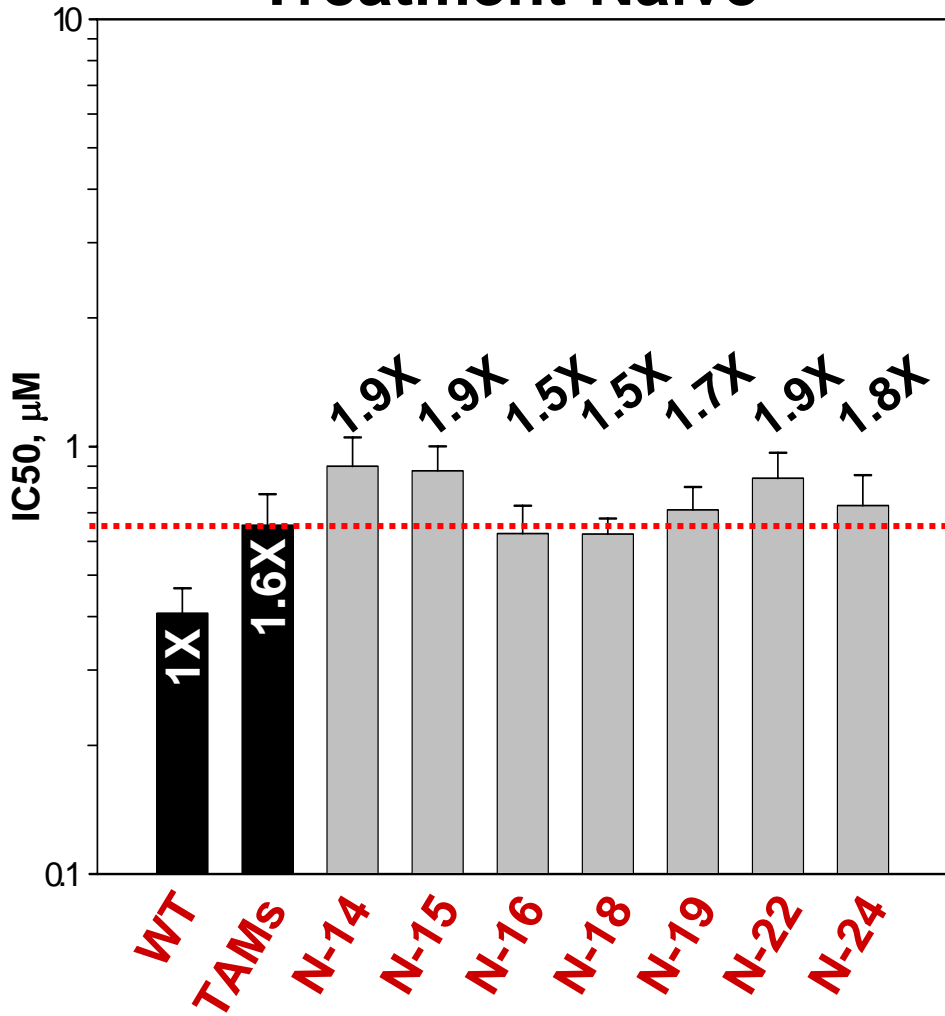
Treatment-Experienced



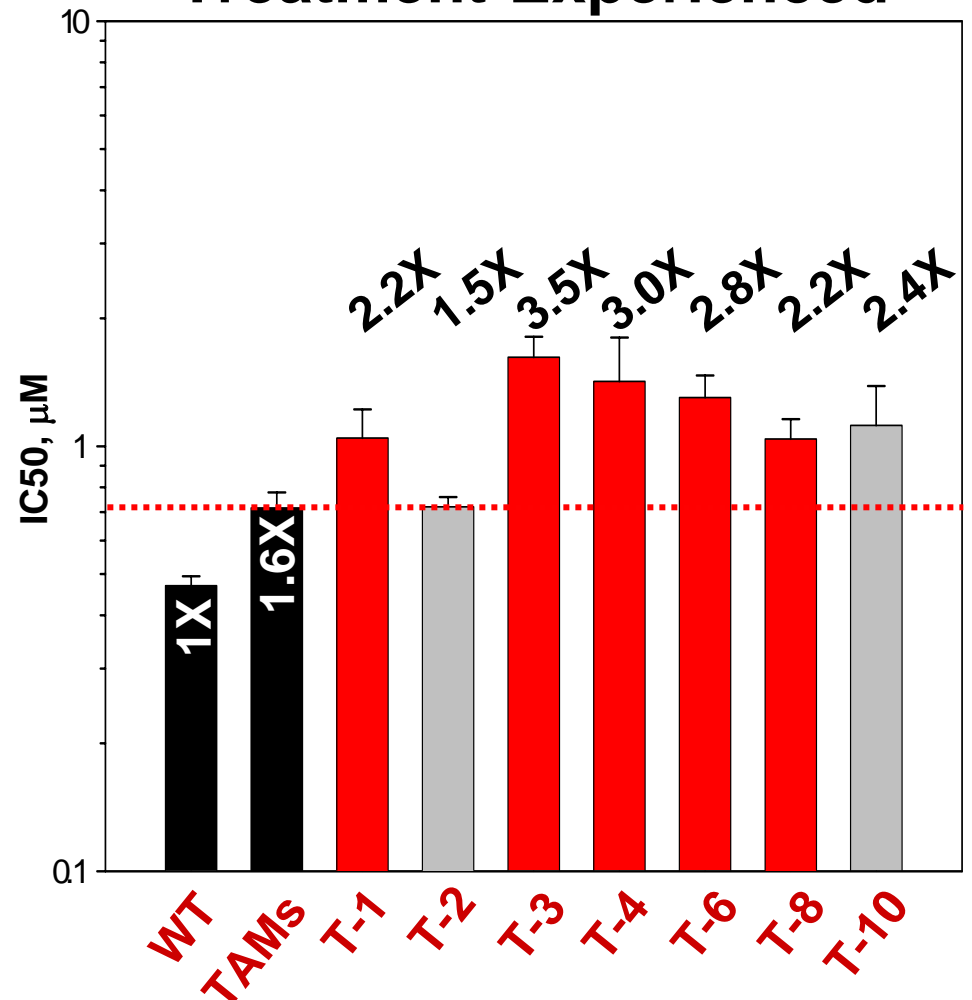
Pol-TAM + C-Terminal Domain From Patients

d4T^R

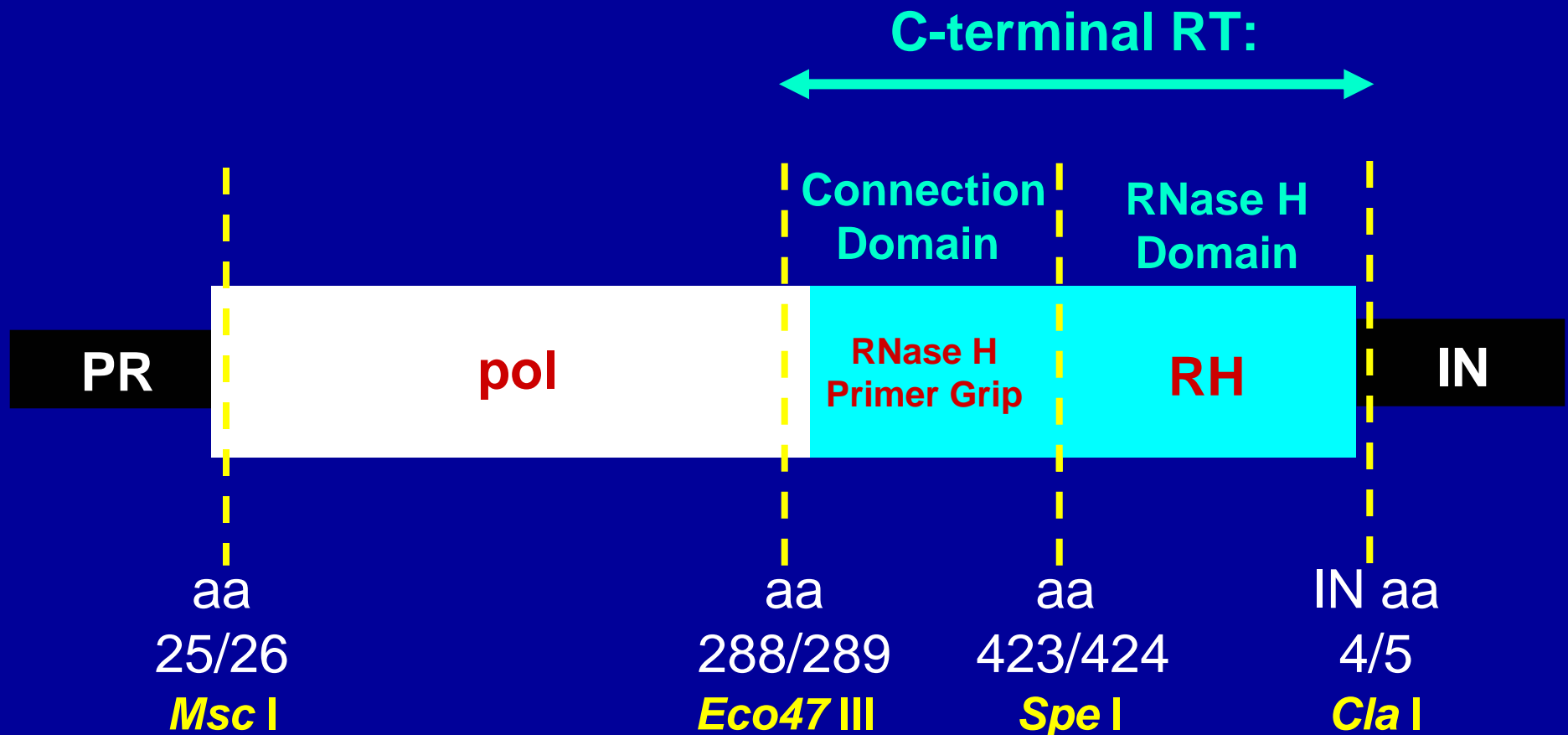
Treatment-Naïve



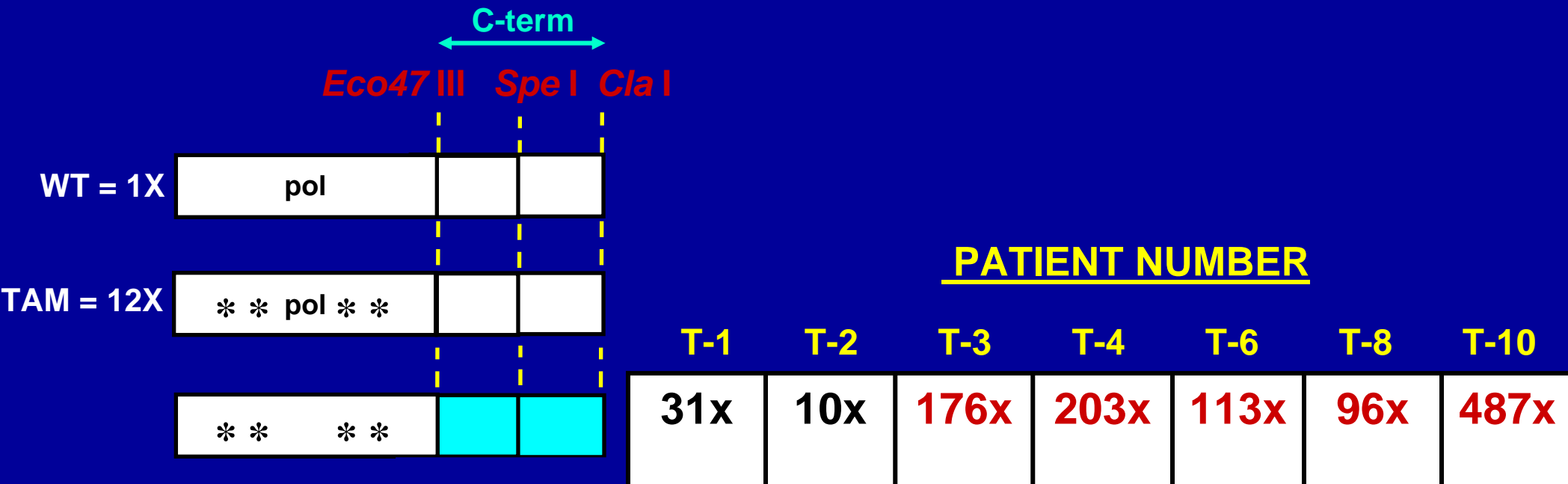
Treatment-Experienced



Where is the NRTI Resistance Located?



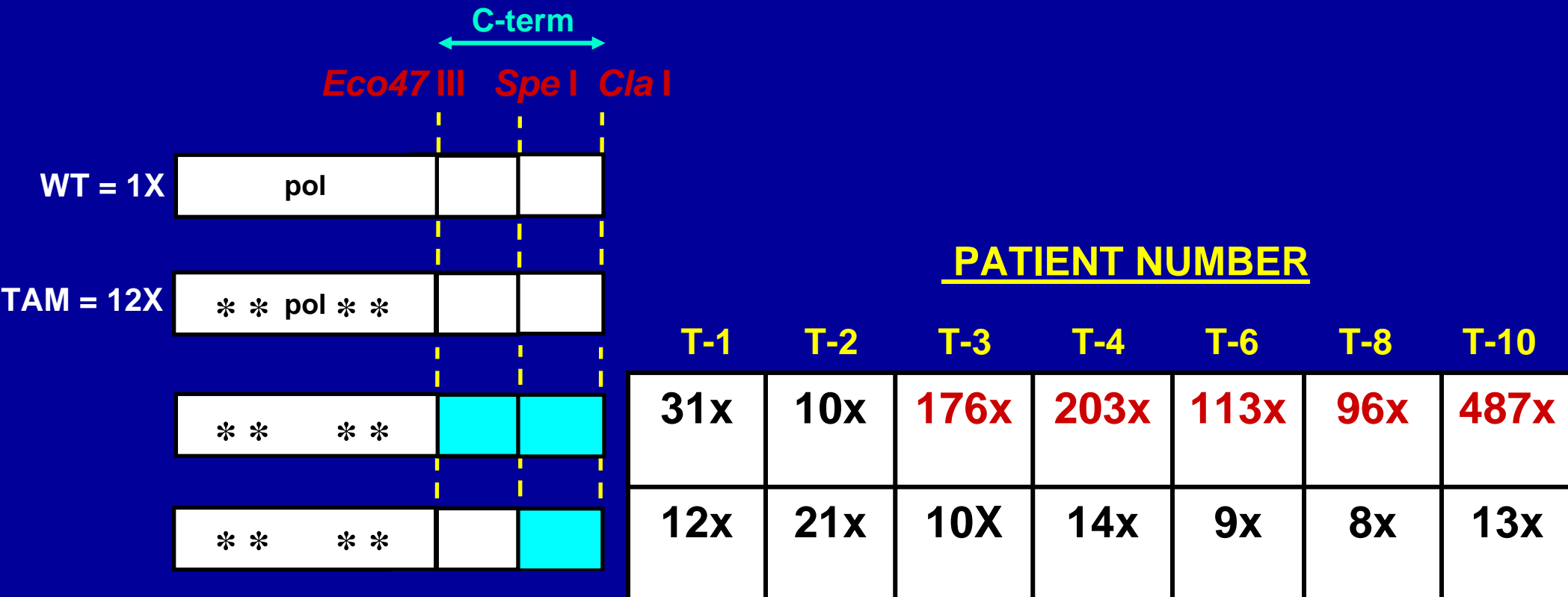
Patient AZT Resistance: Fold Increase Over WildType



■ = patient domain

TAM = 67N, 70R, 215Y, 219Q

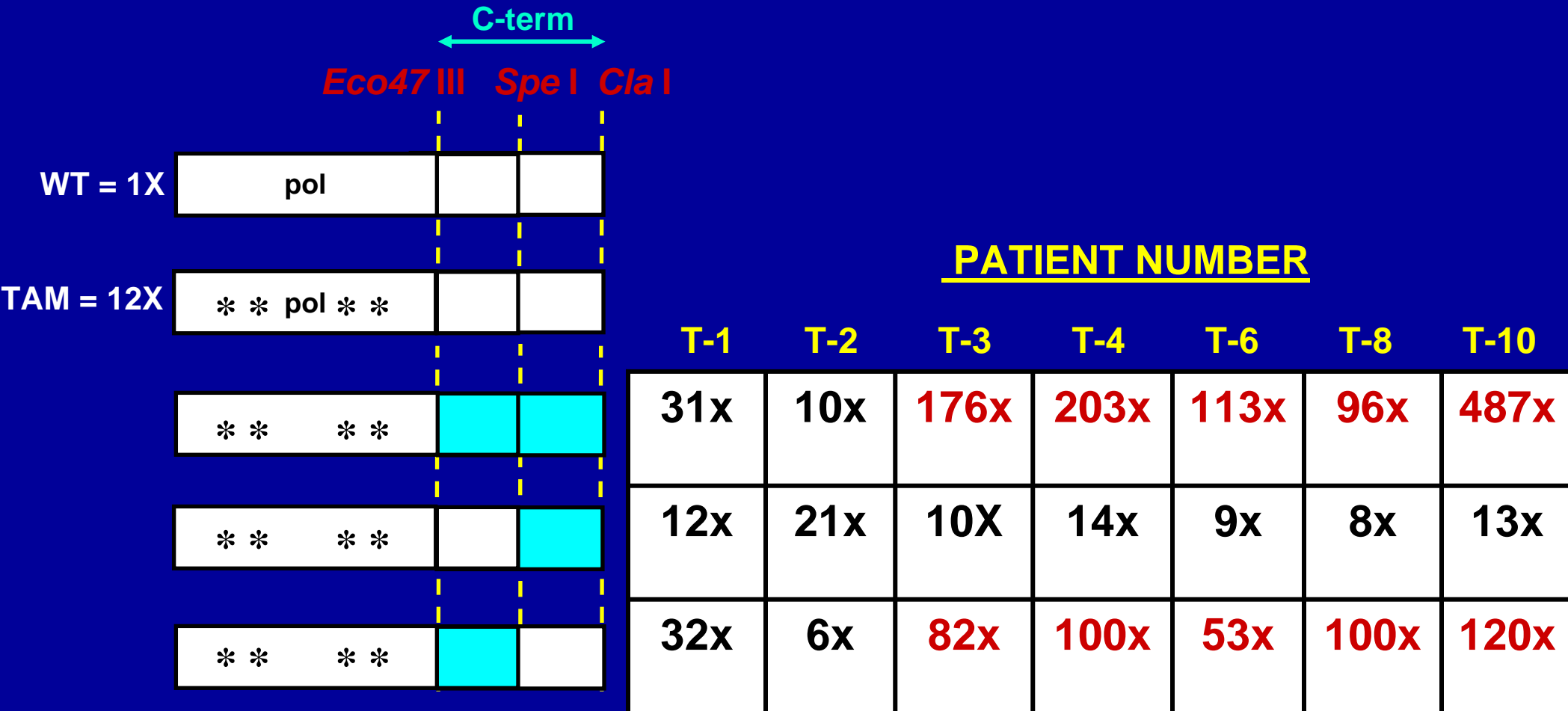
Patient AZT Resistance: Fold Increase Over WildType



■ = patient domain

TAM = 67N, 70R, 215Y, 219Q

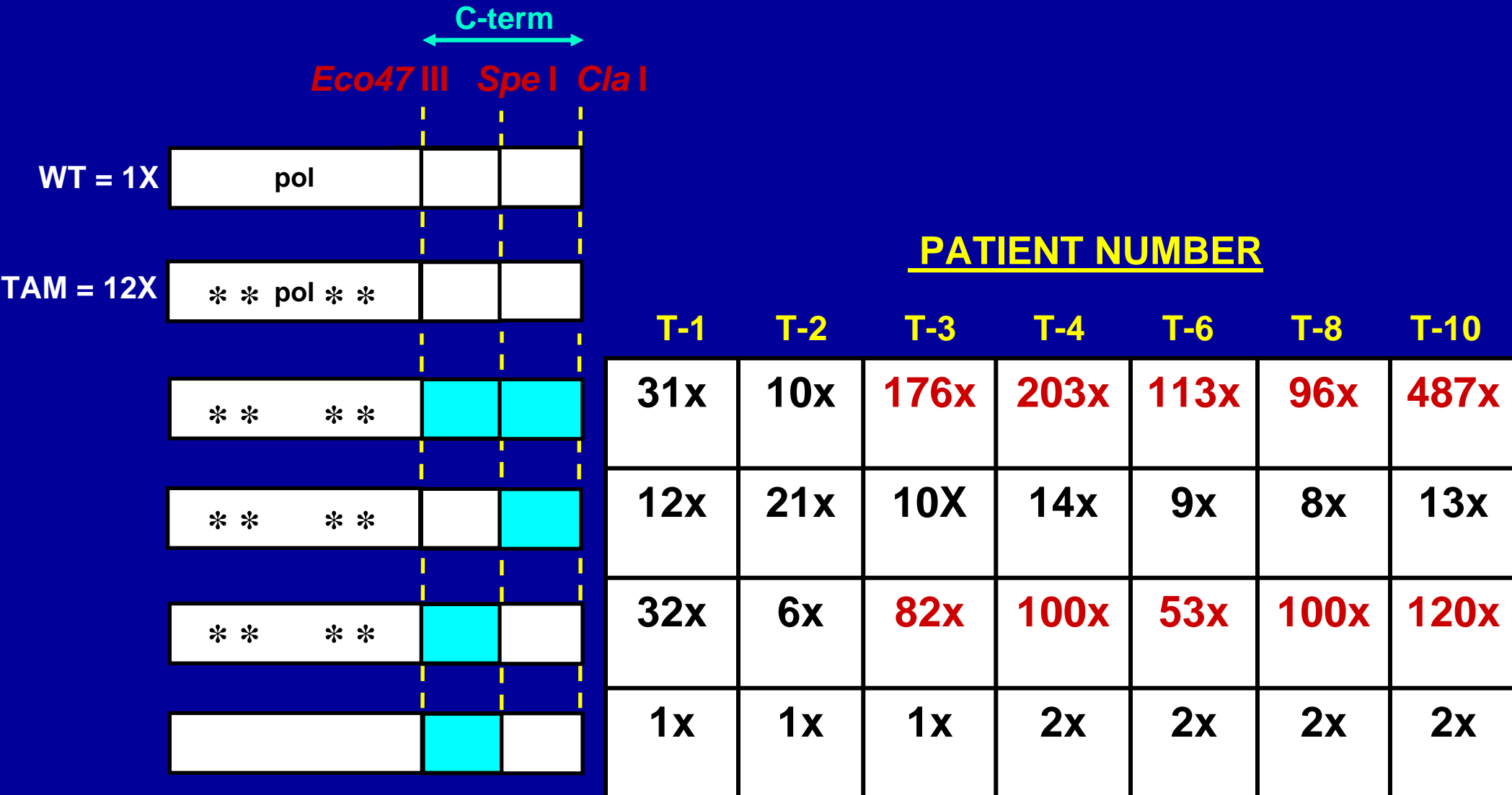
Patient AZT Resistance: Fold Increase Over WildType



■ = patient domain

TAM = 67N, 70R, 215Y, 219Q

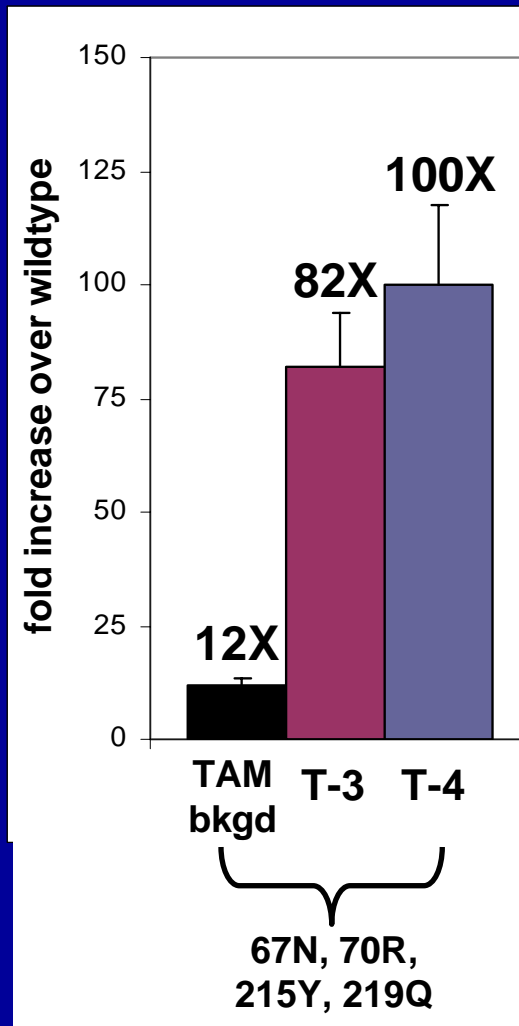
Patient AZT Resistance: Fold Increase Over WildType



= patient domain

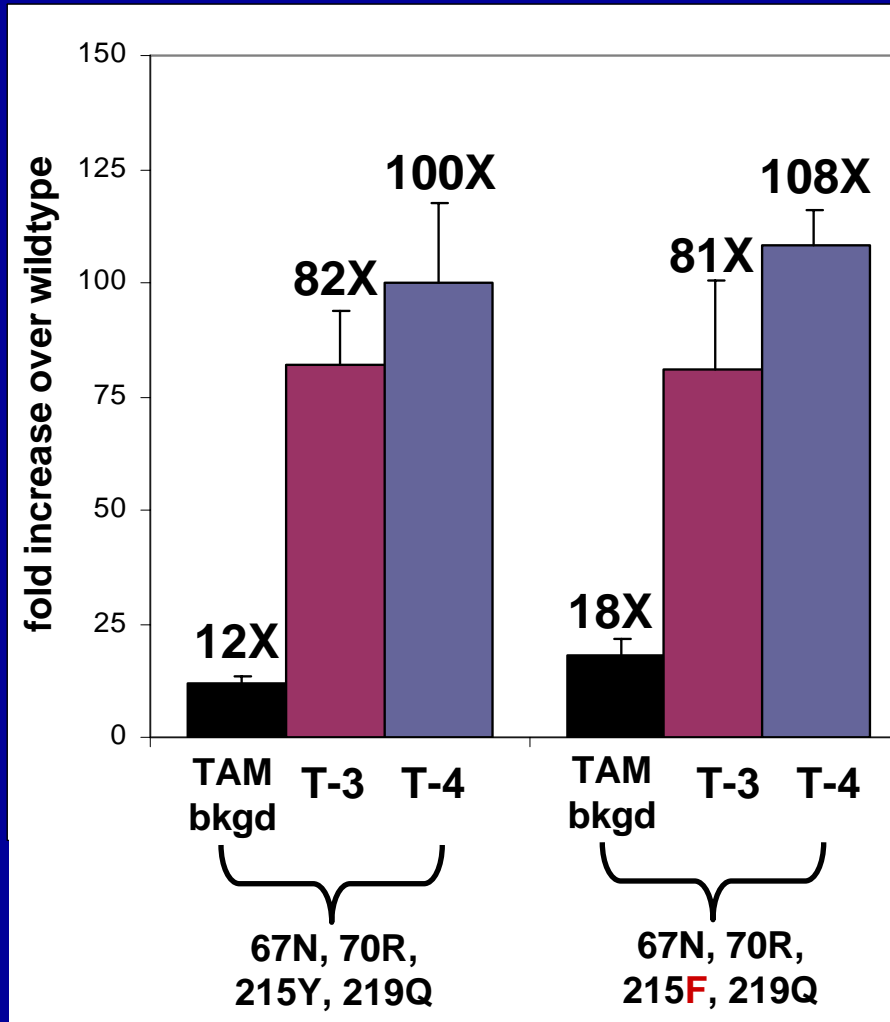
TAM = 67N, 70R, 215Y, 219Q

Connection Domain from Treatment-Experienced Patients Enhances AZT Resistance with Different TAM Combinations



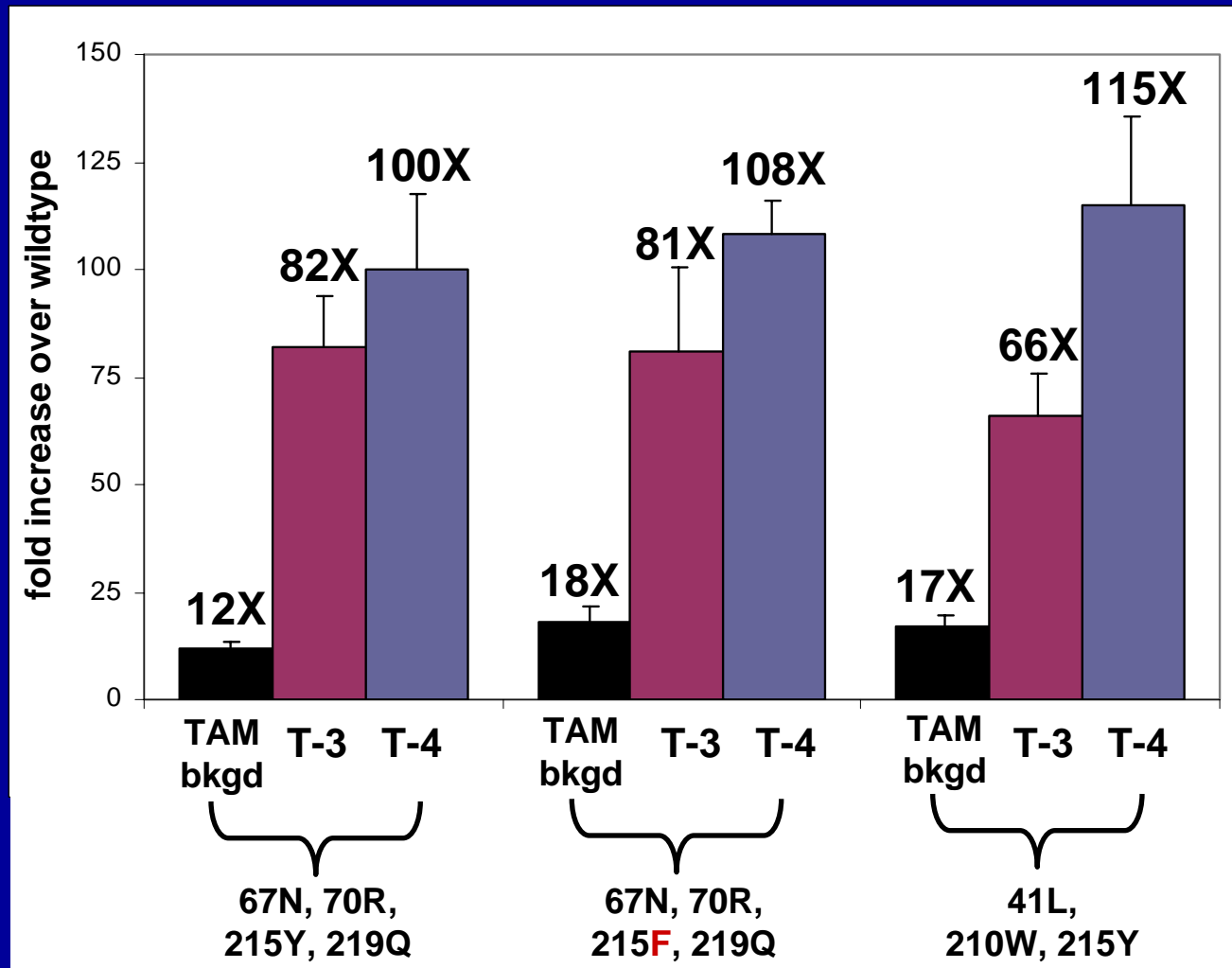
DIFFERENT TAM COMBINATIONS IN RT POL DOMAIN

Connection Domain from Treatment-Experienced Patients Enhances AZT Resistance with Different TAM Combinations



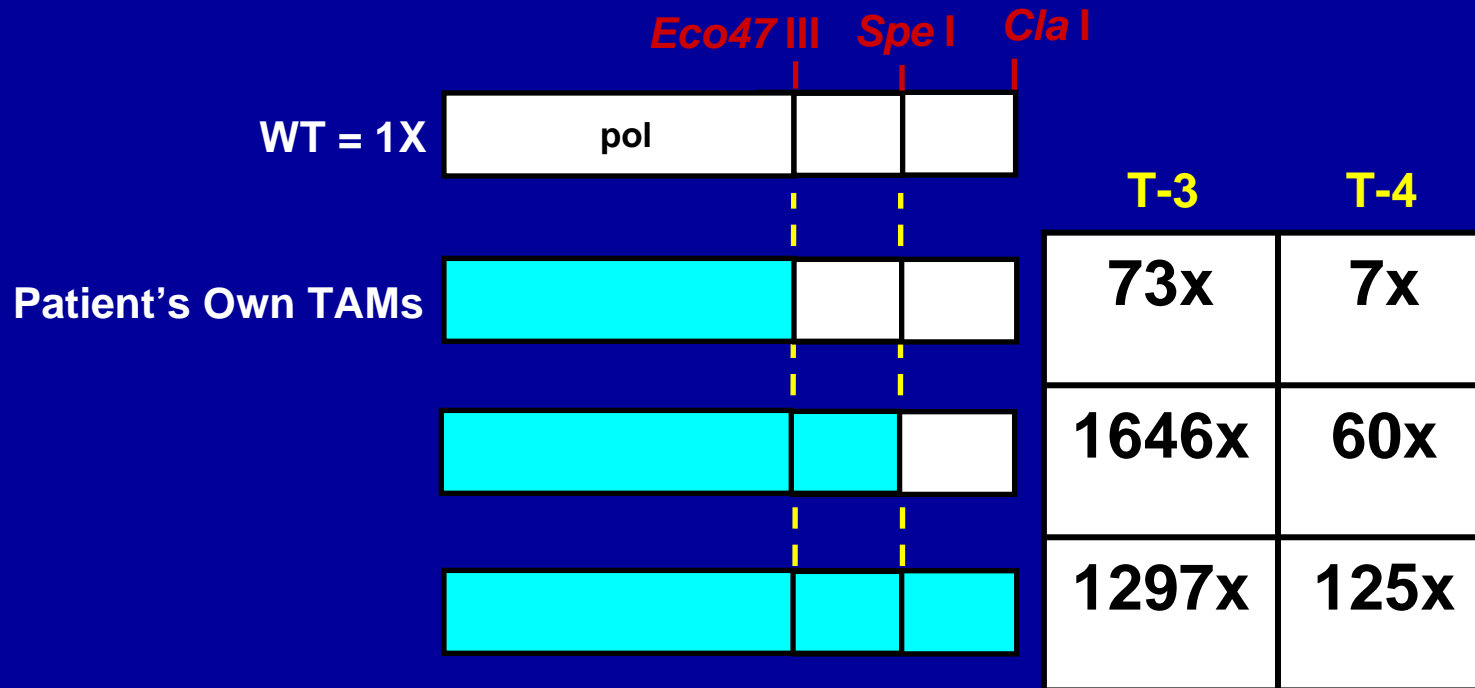
DIFFERENT TAM COMBINATIONS IN RT POL DOMAIN

Connection Domain from Treatment-Experienced Patients Enhances AZT Resistance with Different TAM Combinations



DIFFERENT TAM COMBINATIONS IN RT POL DOMAIN

AZT Resistance is Increased In the Context of the Patient's Own TAMs Combination



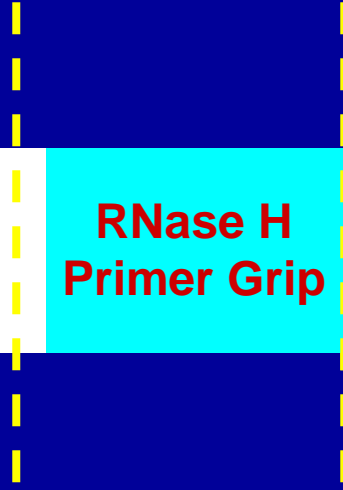
T-3 pol domain = 67N, 70R, 215F, 219Q

T-4 pol domain = 41L, 67N, 184V, 210W, 215Y, 219N

■ = patient domain

Which Mutations in the Connection Domain Contribute to NRTI Resistance?

Connection
Domain



aa

288/289

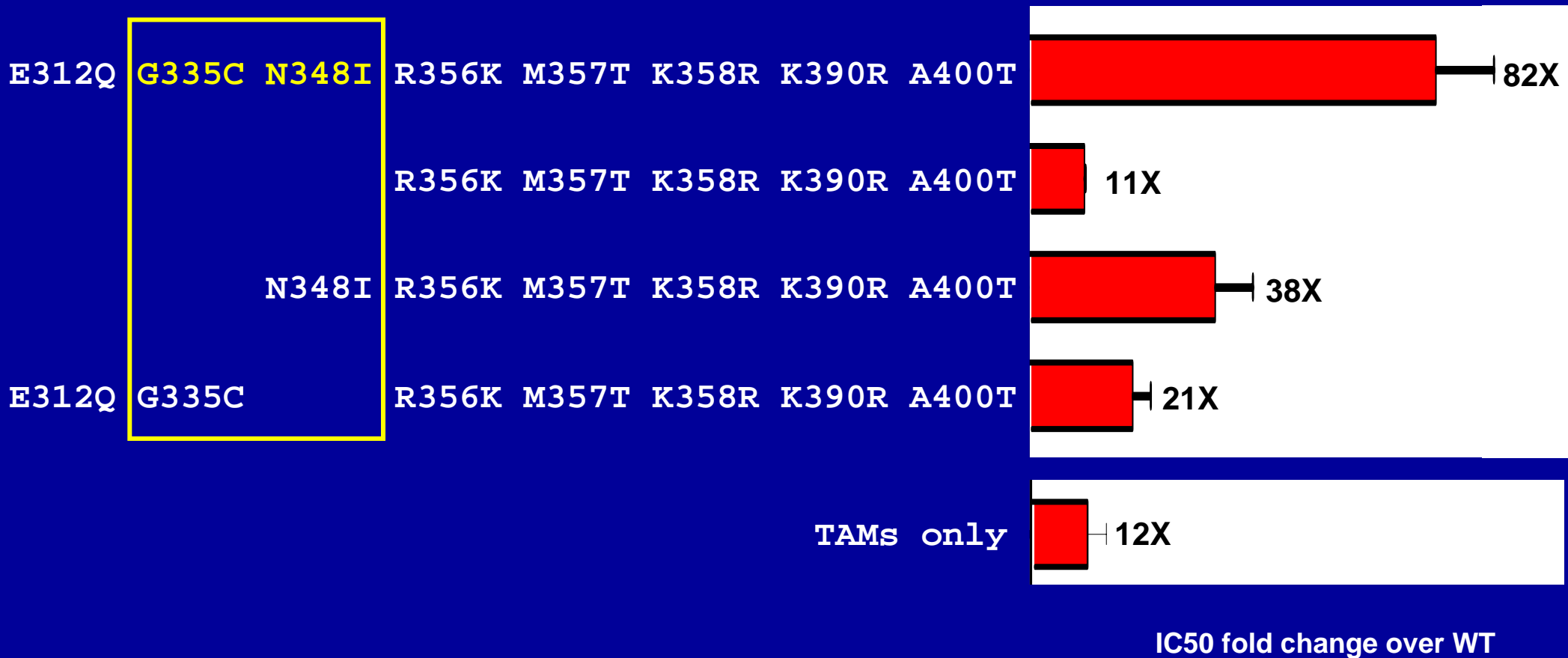
Eco47 III

aa

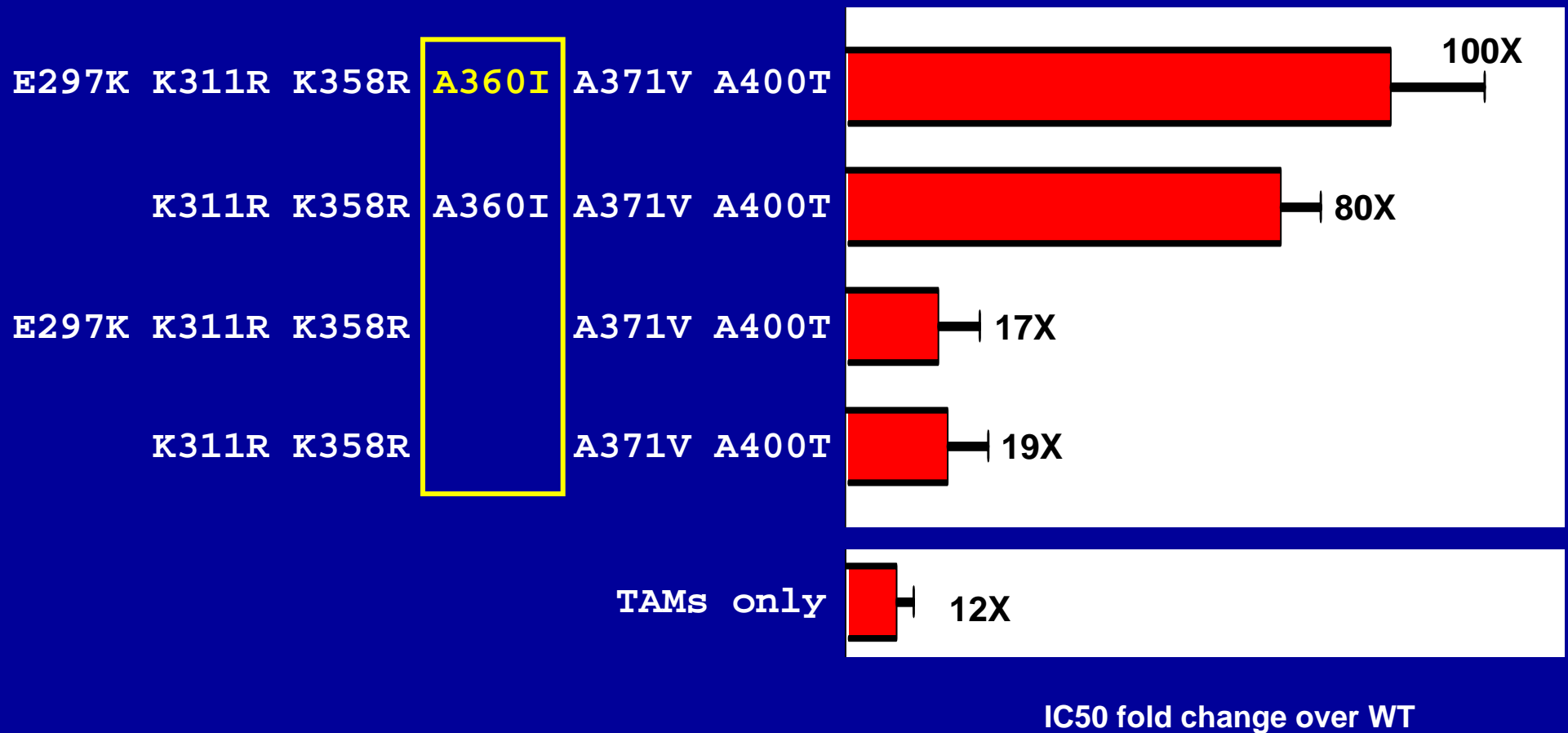
423/424

Spe I

Mutational Analysis of Patient T-3: Increase in AZT Resistance Over WildType



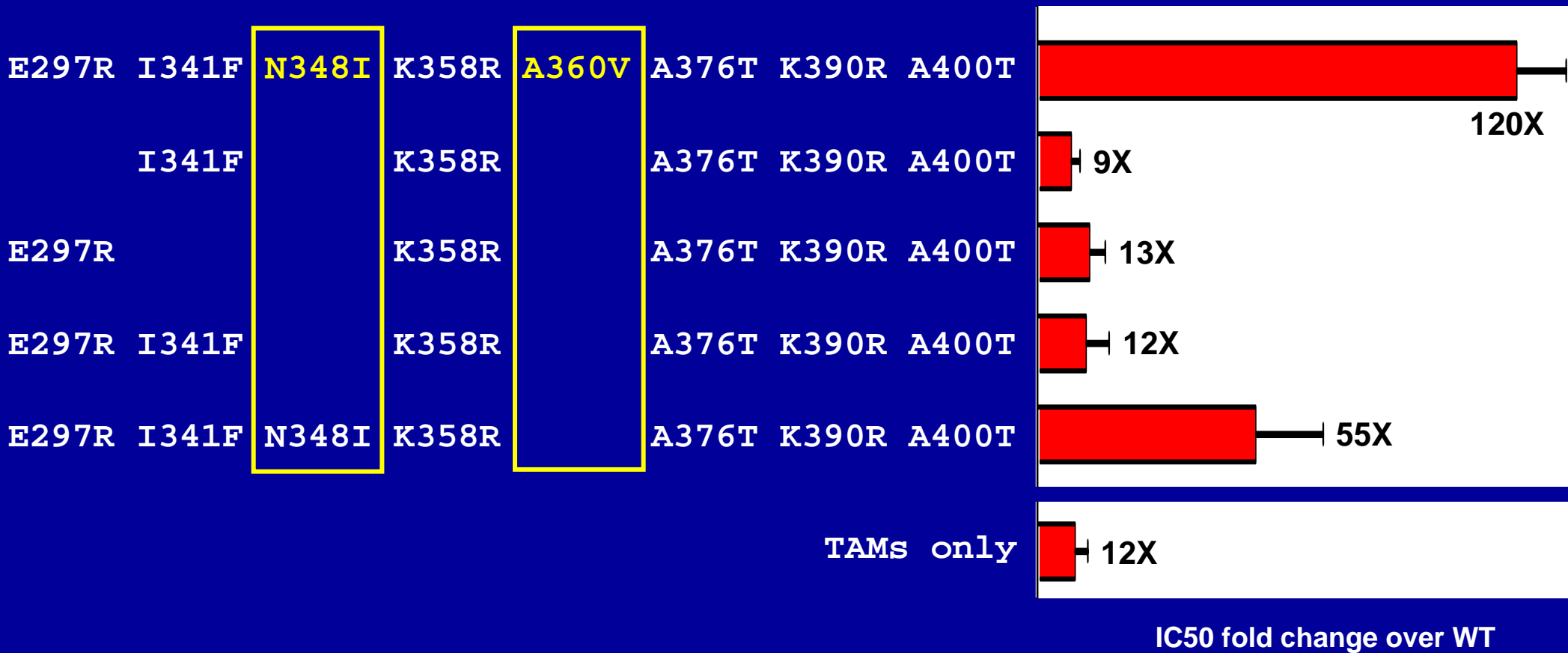
Mutational Analysis of Patient T-4: Increase in AZT Resistance Over WildType



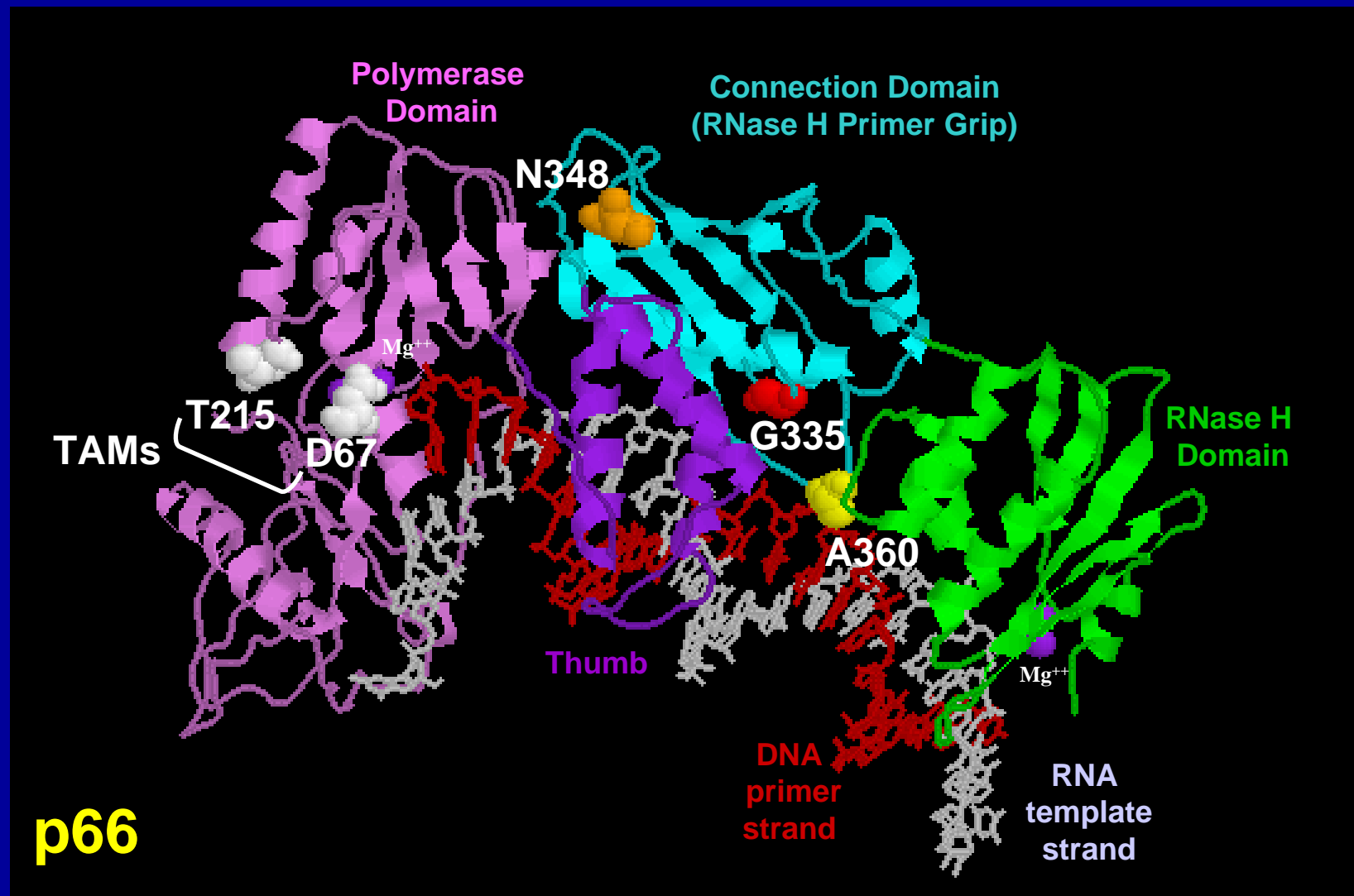
Mutational Analysis of Patient T-6: Increase in AZT Resistance Over WildType



Mutational Analysis of Patient T-10: Increase in AZT Resistance Over WildType



Position of Connection Domain Mutations that Contribute to AZT Resistance



Conclusions

- **The Connection Domain containing the RNase H Primer Grip from NRTI-experienced patients confers resistance to AZT and d4T.**
- **Mutations G335C/D, N348I, A360I/V were found to play a role in AZT resistance in the presence of TAMs.**
- **It is important to carry out genotypic and phenotypic drug resistance assays with the entire RT.**

Acknowledgements

Galina Nikolenko
Vinay Pathak lab
Viral Mutation Section

Vinay Pathak lab
Viral Mutation Section

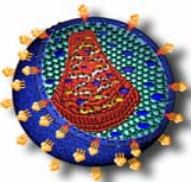
Wei-Shau Hu lab
Viral Recombination Section

John Coffin
HIV Drug Resistance Program

John Mellors
University of Pittsburgh

Frank Maldarelli
Host Virus Interaction Unit

Sarah Palmer
Virology Core Facility



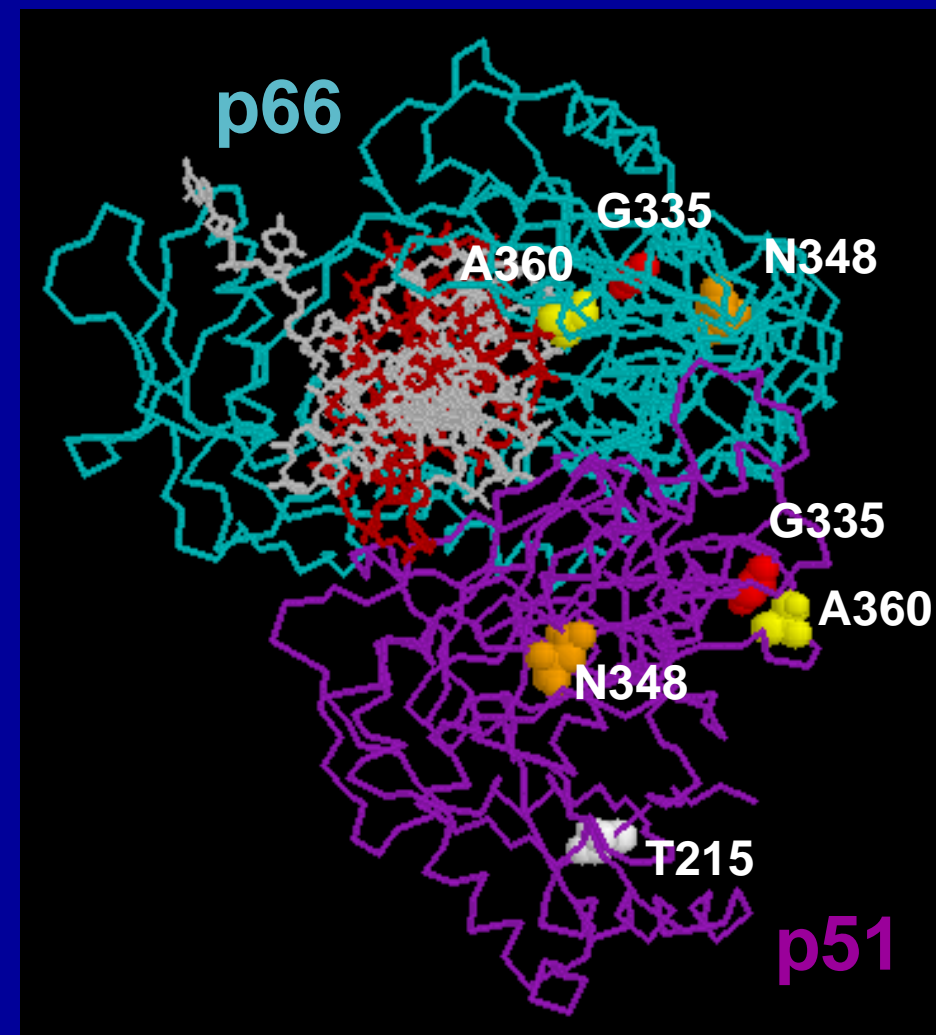
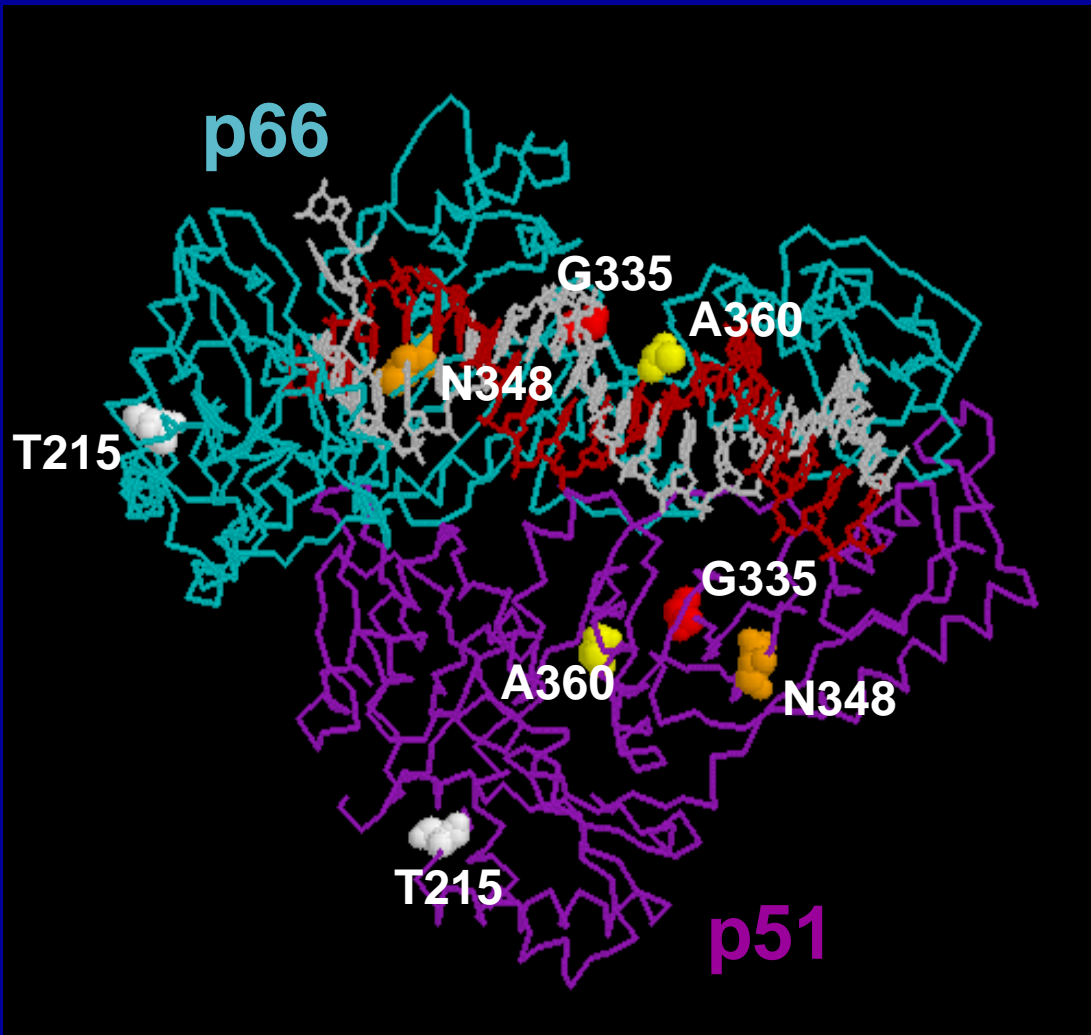
HIV Drug Resistance Program

National Cancer Institute at Frederick

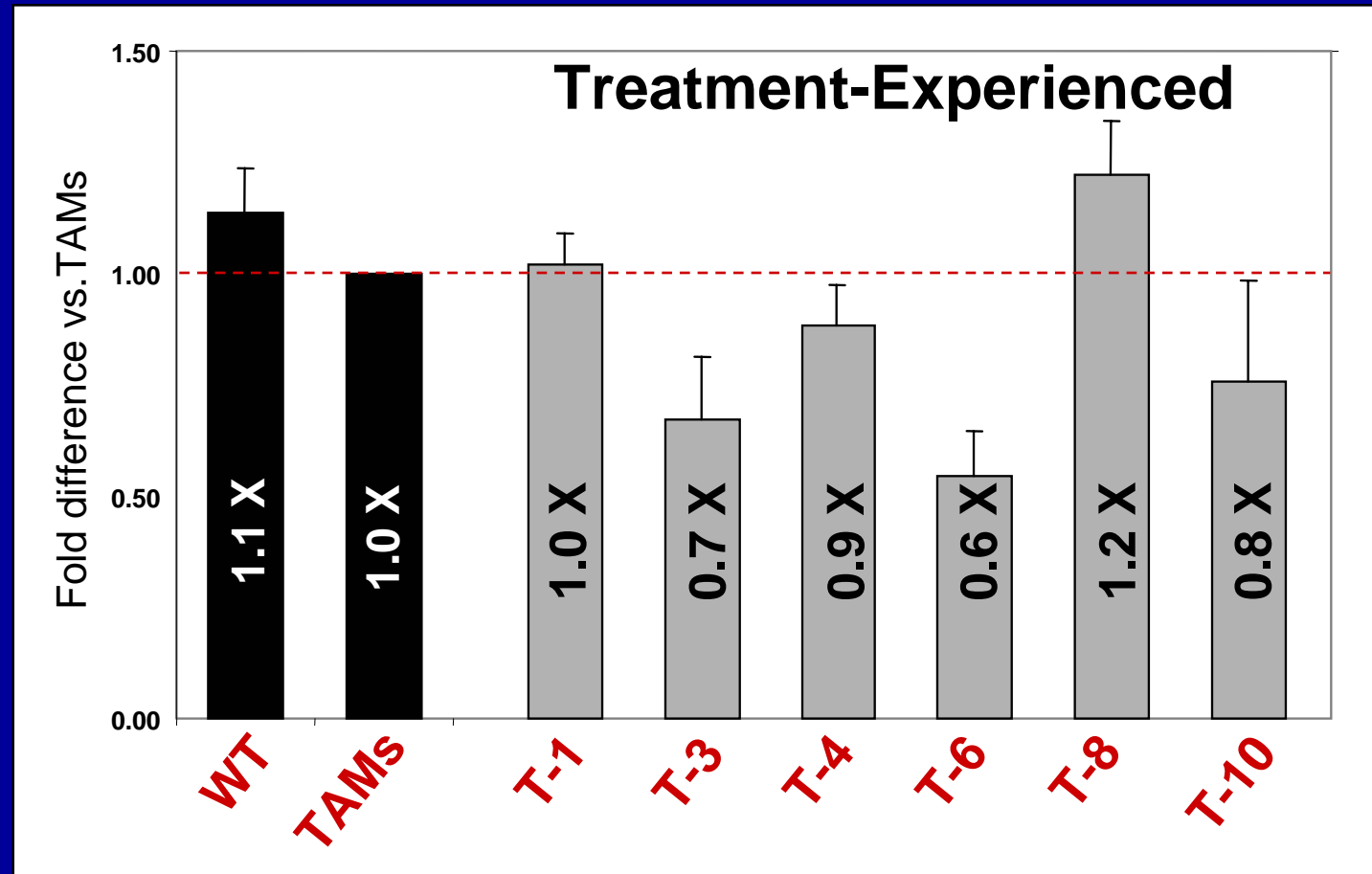
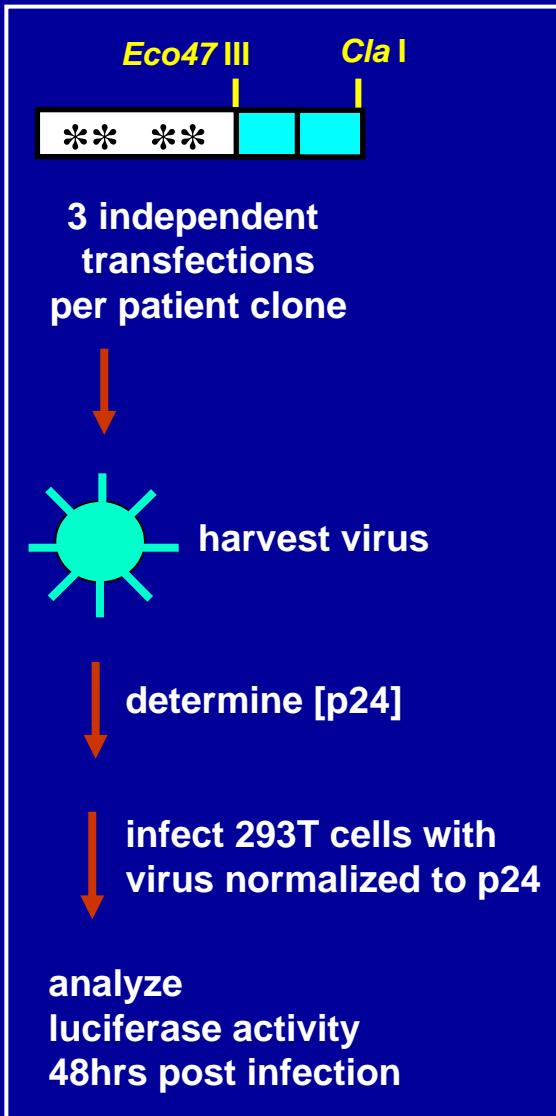




Position of Connection Domain Mutations in p66 vs p51 RT subunits



Replicative Capacity of Treatment-Experienced Patient Viral Clones



N-terminal Additions to the RNase H Domain Increase RNase H Activity *In Vitro*

RNase H Activity

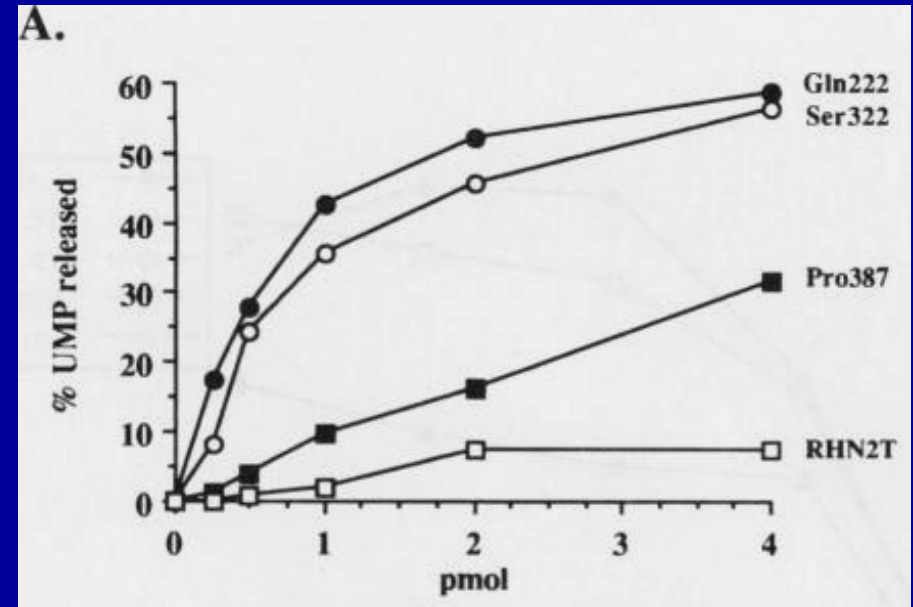
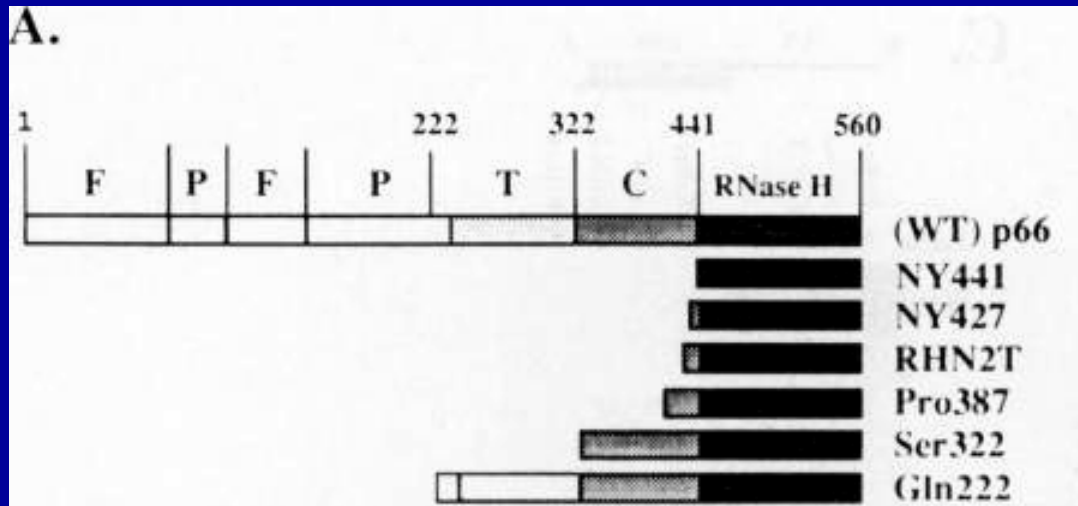
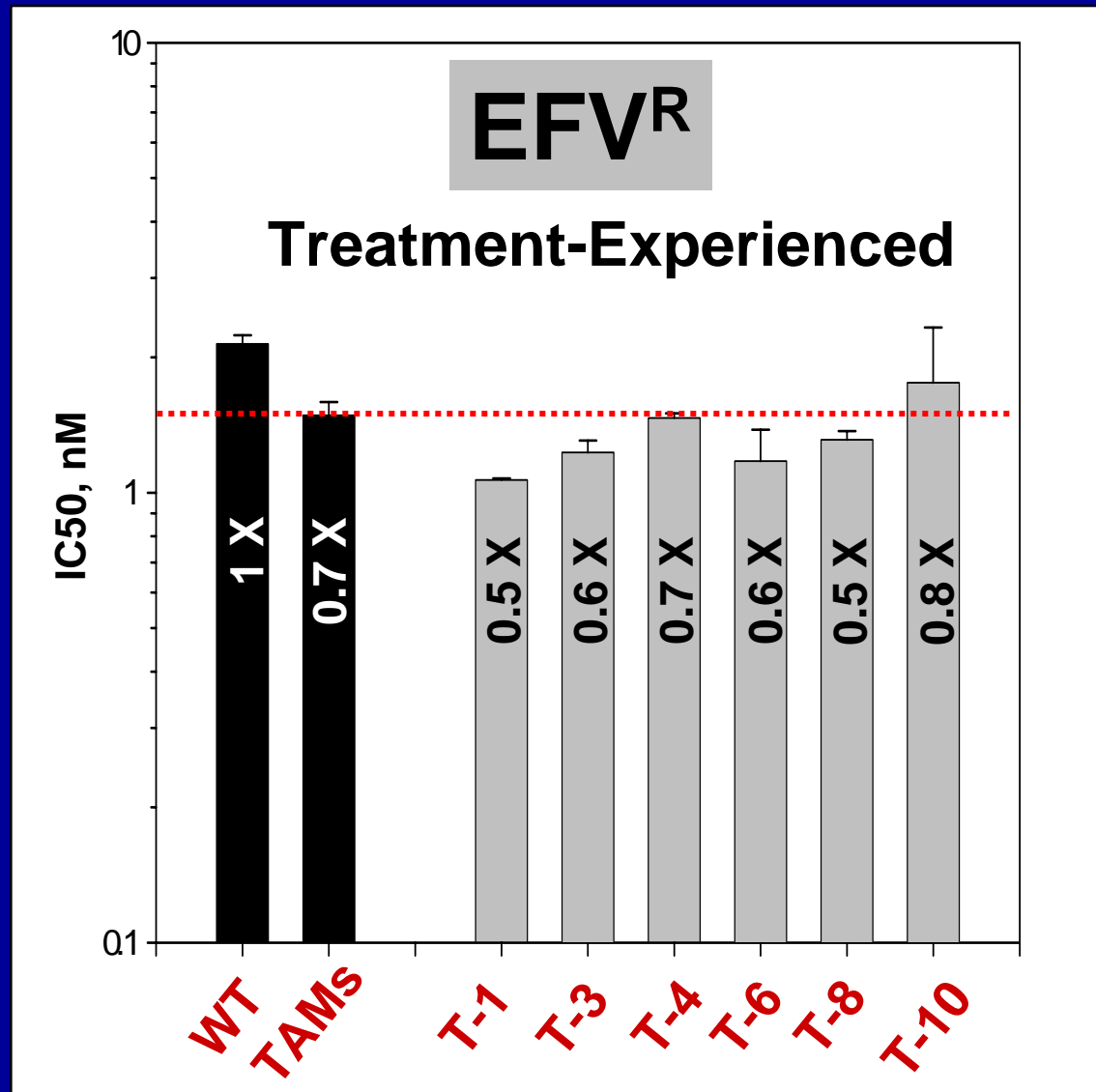
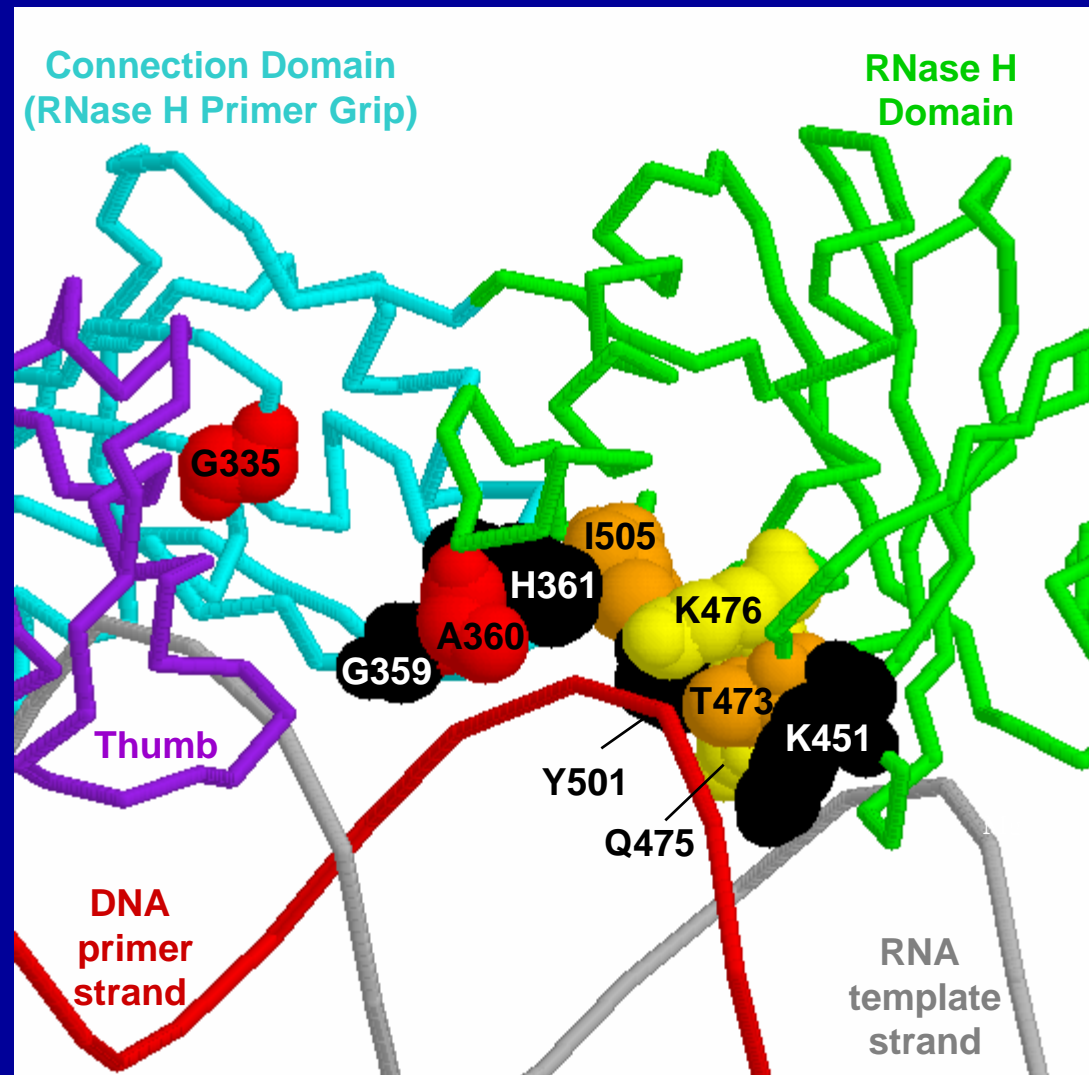


Figure modified from: *Smith, S.S., K. Gritsman and M. Roth. J. Of Virol, 1994: 5721-5729*

C-Terminal Domains From Patients Treated with NNRTIs Do Not Increase EFV Resistance in the Presence of TAMs



Position of RNase H Primer Grip Amino Acid Residues Relative to G335 and A360 in p66



Patient #	<u># yrs on therapy</u>	<u>Prior Therapy</u>			<u>Therapy at sampling</u>
		NRTI	NNRTI	PI	
1	9.9	AZT,3TC, ddI, ABC	NVP	NFV, SQR	d4T, ddI RTV/IDV
2	----	----	----	----	ABC, APV, LPV/RTV
3	6.4	AZT,3TC, d4T, ddI	NVP	----	d4T, ddI, EFV
4	6.5	AZT,3TC, ddI	NVP	RTV, IDV RTV/SQV	d4T, 3TC, ABC,EFV, NFV
6	4.2	AZT,3TC, d4T, ABC	EFV	APV, LPV IDV, NFV	AZT, 3TC, ddI, EFV, IDV, RTV/LPV
8	nd	ABC,ddI	----	APV, LPV/RTV	ddI, TDF, DLV, APV
10	6.5	AZT,3TC, d4T	----	IDV	d4T, 3TC, RTV/SQR

Major RT Mutations in Treatment-Experienced Patients

Patient #	<u>M41L</u>	<u>D67N</u>	<u>T69D</u>	<u>K70R</u>	<u>L74V</u>	<u>M184V</u>	<u>L210W</u>	<u>T215Y</u>	<u>K219Q</u>
1	+	+/-					+	+	
2	+	+	+	+				+ (F)	+(W)
3		+	+ (N)	+				+ (F)	+
4	+	+				+	+	+	+(N)
6	+				+	+		+	
8	+				+(I)		+	+	
10						+			

Pol-WT + C-Terminal Domain From Patients

d4T^R

