HIV DRUG RESISTANCE IN AFRICA

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BARC
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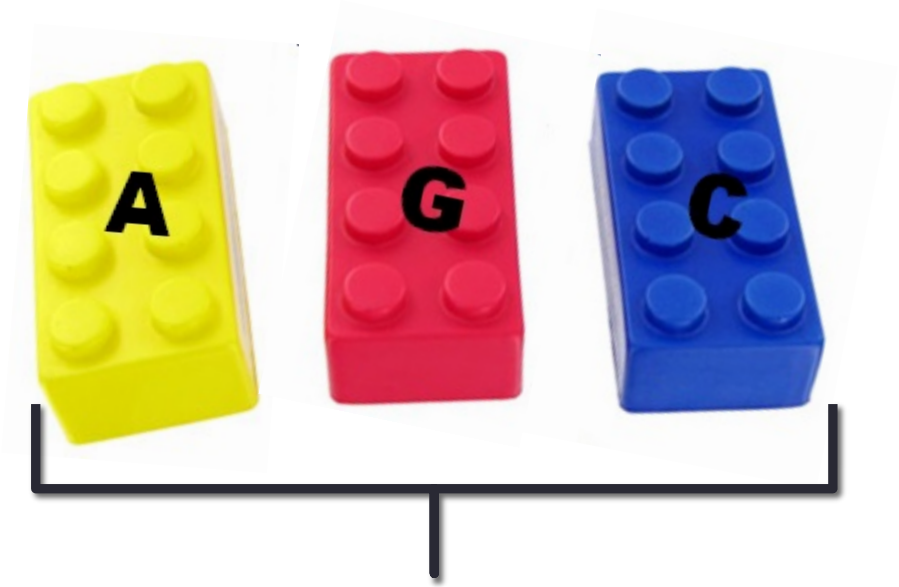
Key to Diagnostic Excellence

What makes up the HIV virus

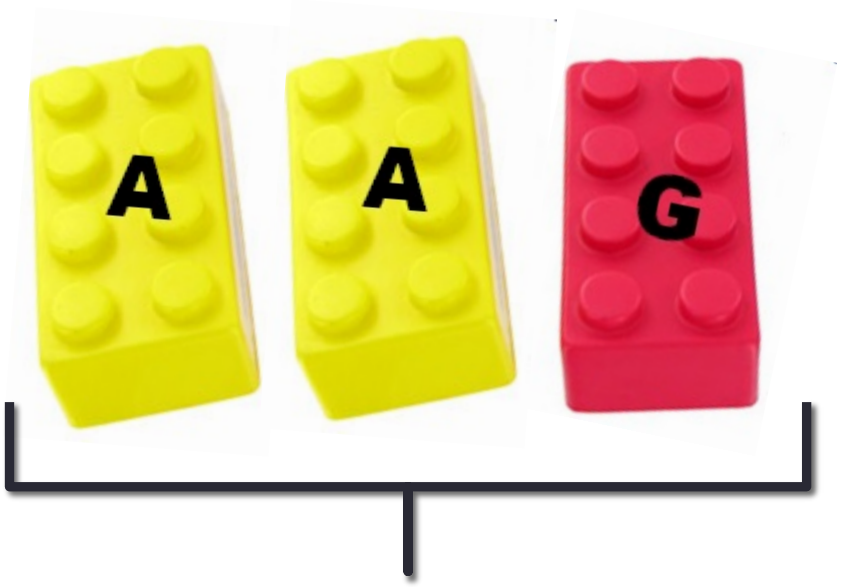
- HIV genome is made up of building blocks known as amino acids, each amino acid consists of three nucleotides.

Amino Acids

- Different Combinations of the nucleotides make up different amino acids



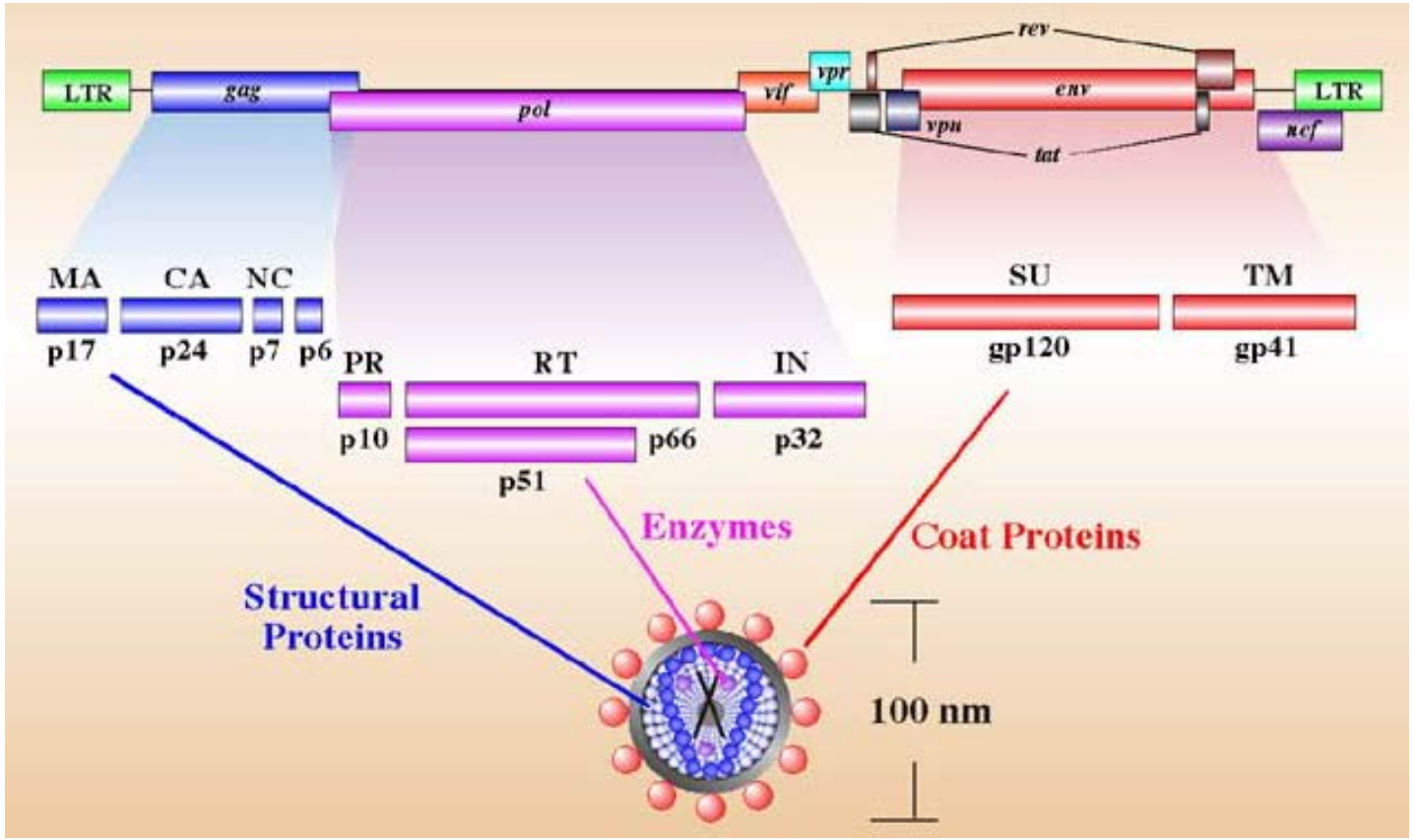
Serine
(S)



Lysine
(K)

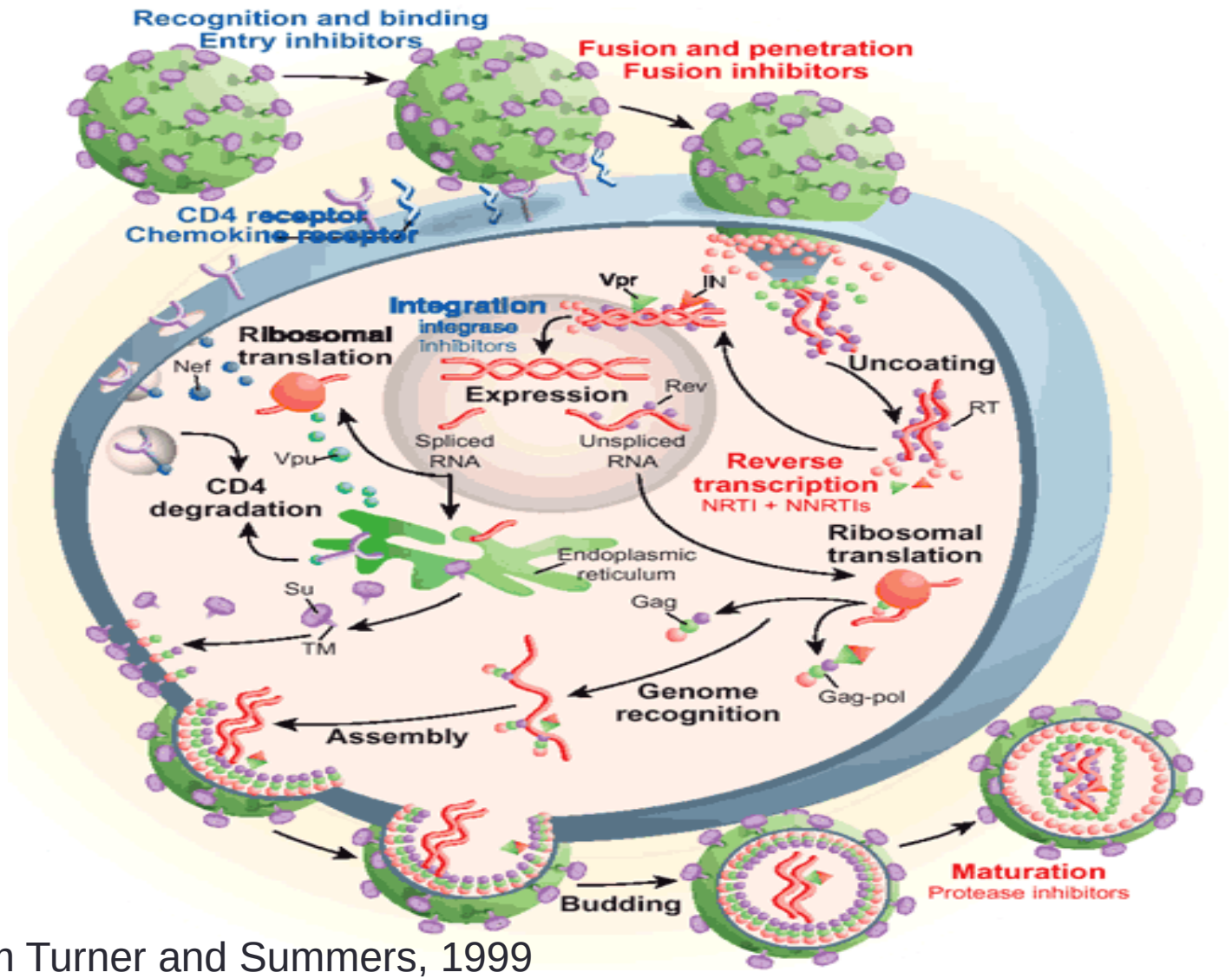
HIV Genome

- Amino Acids make up the HIV genes



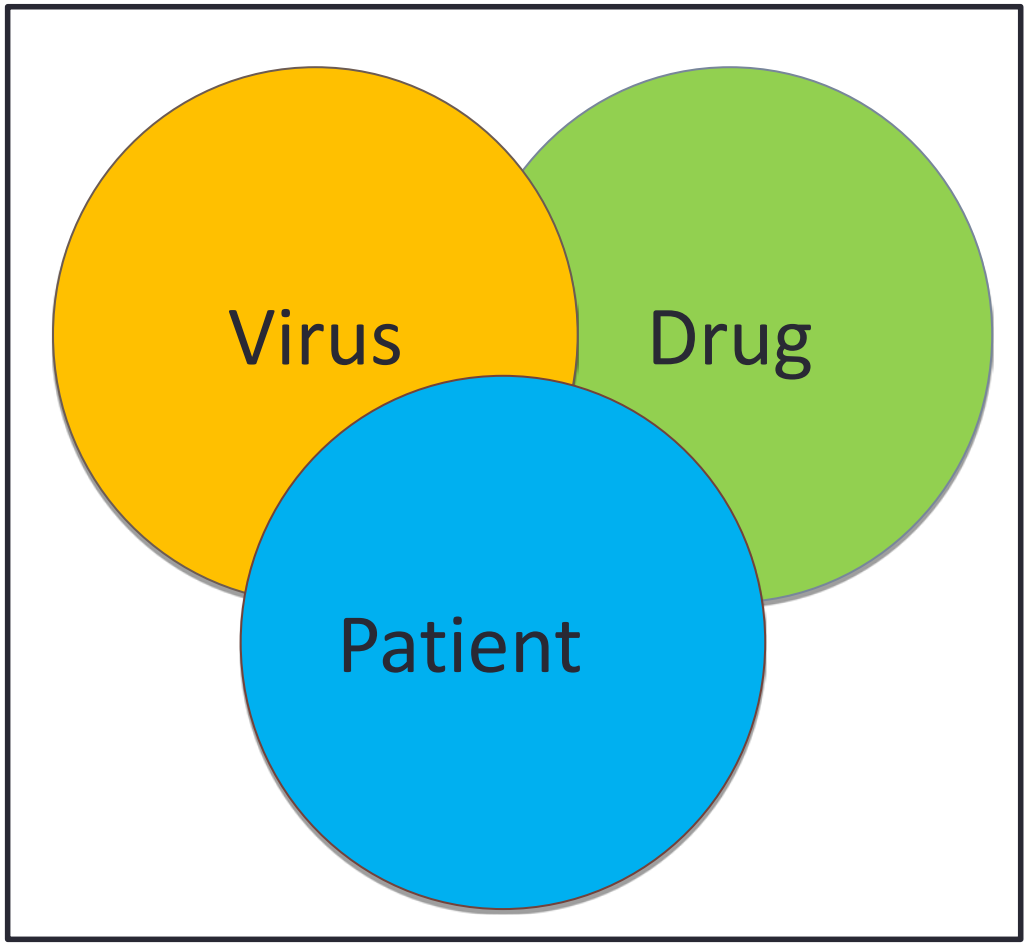
http://www.stanford.edu/group/virus/retro/2005gongishmail/HIV-1b.jpg

HIV Life Cycle & Drug Targets



Modified from Turner and Summers, 1999

Treatment outcome is dependent on several factors



How does resistance occur?

- When HIV replicates it makes mistakes.
- Why does the virus make mistakes:
  - It doesn't check what it is doing (high error rate of the reverse transcriptase [RT] enzyme);
  - Replicates very fast (high HIV replication rate).
- Mistake=Mutation
- Resistance normally occurs at the location of the ARV gene target.

Principles of Resistance

- Specific to one ARV or Cross-Resistance to Multiple ARVs.
- Remove the drug, resistance will decay (fade).
- Mutations can make the virus weak (fade fast); other make the virus as fit as wildtype (slow to fade)
- How easy to get resistance
  - Often dependent on the ARV;
  - Resistance can get worse overtime because mutations keep accumulating.

Example of what a mutation does

THE CAT SAT ON THE MAT

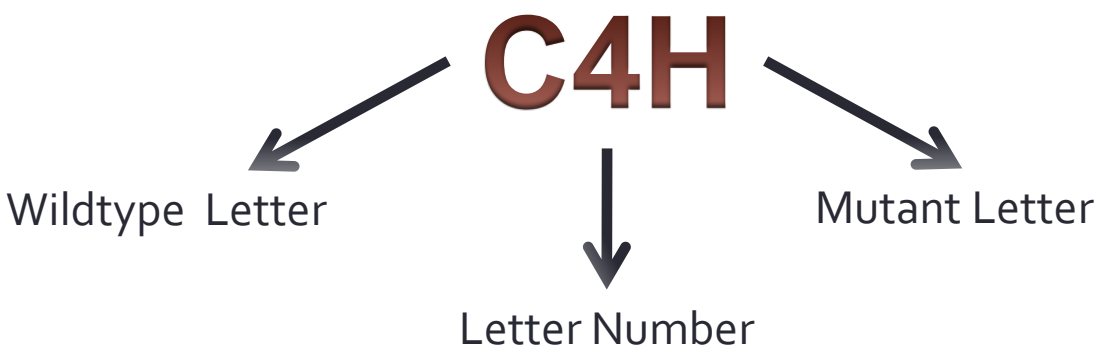
THE **H**AT SAT ON THE MAT

It changes the sentence (gene) so it still makes sense; but says something different.

Naming a Mutation

THE CAT SAT ON THE MAT

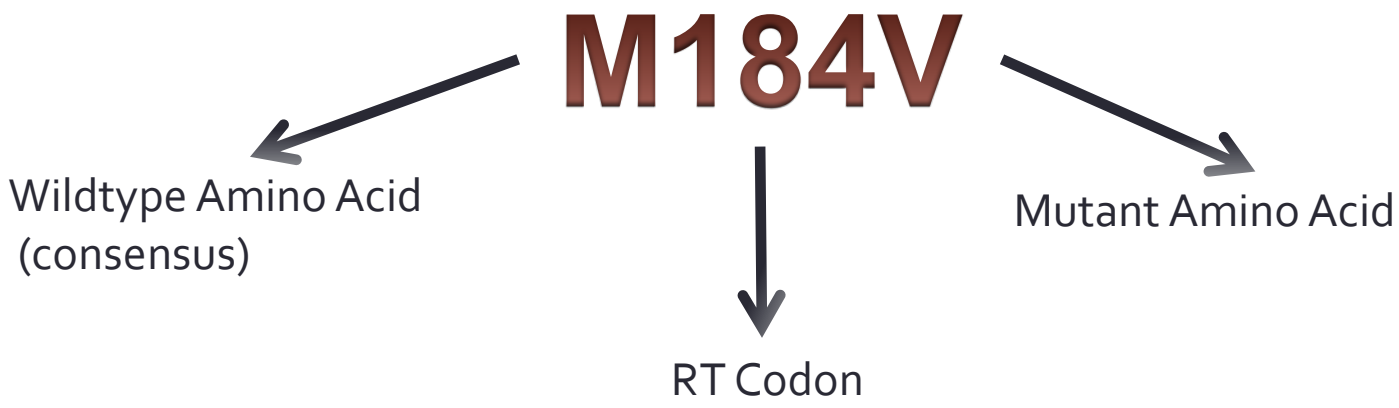
THE **H**AT SAT ON THE MAT



Naming of an HIV Mutation

The length of the gene:

- Protease Region of Polymerase Gene is from Amino Acid 1 to Amino Acid 99
- Reverse Transcriptase Region of Polymerase Gene is from Amino Acid 1 to Amino Acid 540
- Anywhere along these amino acids you can get a change in the sentence and a mutation.



Mixture

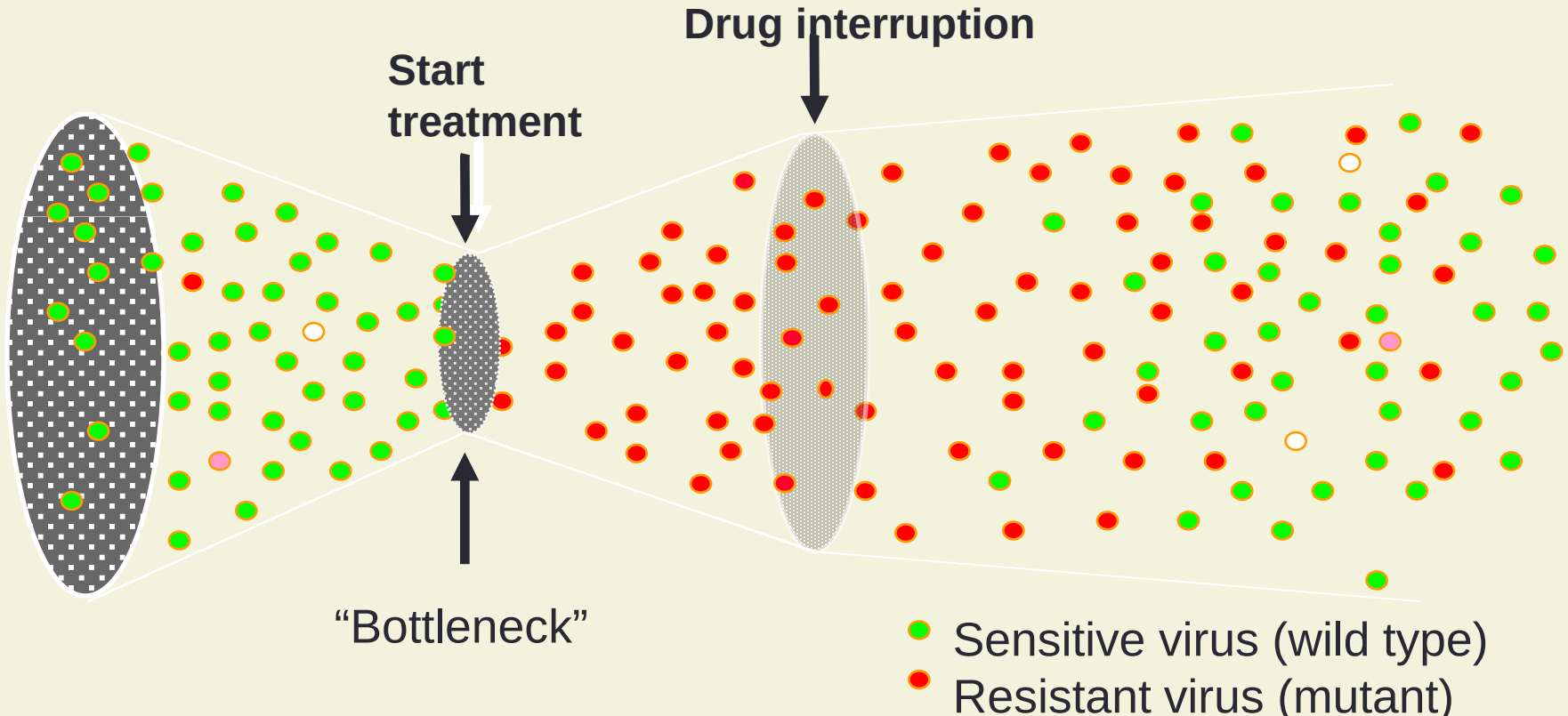
M184M/V

- Means there is both wild-type and mutant viruses present
- Treat as if it were a mutation.

Viral dynamics and resistance

What happens when the virus makes changes to its genes that the antiretroviral are targeting?

- The antiretroviral no longer ‘understands’ the sentence
- This allows the HIV virus to grow
- So you see an increase in HIV Viral Load



How to test for HIV Resistance

Phenotypic Assay

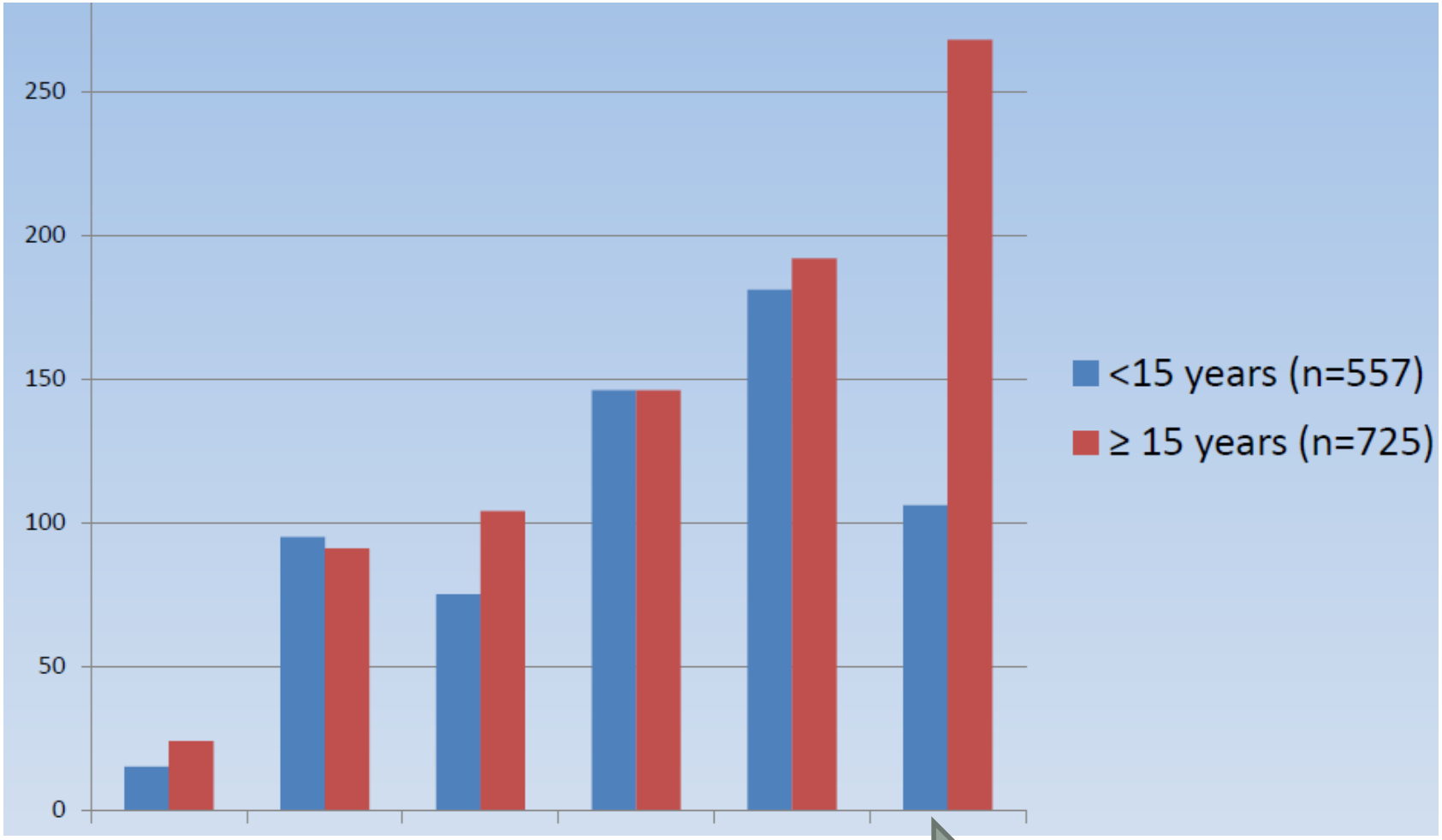
Test what effect the mutation will have on the virus in the laboratory

Genotypic Assay

Determines what mutations are present

- Population Based
  (>25% of a population)
  - Commercial Assays
  - In-house assays
- Minority Variant Assays
  (>1% of a population)
  - Research Assays
- 2x EDTA tubes sent for testing

Treatment Failure and Resistance Testing in South Africa



Van Zyl et al., IAS 2012

Resources for Resistance Testing in Africa?

- Testing Currently Centralised;
- Occurs in research centres and pathology services;
  - Lancet/BARC-SA covers:
    - Kenya, Uganda, Tanzania; Botswana; Zambia; Zimbabwe; Mozambique; Swaziland; Namibia via NIP; South Africa.
  - Uganda JCRC
  - CDC KEMRI in Kenya
  - NHLS in SA (currently centralised in three centres) but increasing capacity through a global fund grant (Stellenbosch; KZN; JHB- expanding to Bloemfontain).
  - AfricaCentre
  - All South African Resistance Data may be uploaded to a central database and one such option is Saturn (originally maintained by NICD).

RESISTANCE
&
MICROBICIDES

Infected Post-Enrollment

Study	# Sequenced		# Resistant to TDF or FTC
	Placebo	Active	
Bangkok Tenofovir	35	15	0
CAPRISA-004	0	35	0
Fem-PrEP	35	33	1 Placebo (M184V) 4 TDF-FTC (M184V/I)
iPrEX	64	36	0
Partners in PrEP	51	27	0
TDF2	24	9	1 Placebo (K65R <1%)
VOICE/MTN-003	128	173	1 TDF/FTC (M184V)

Enrolled during Acute Seroconversion

Study	# Infected at Enrollment	# Resistant to TDF or FTC
Bangkok Tenofovir	2	0
Fem-PrEP	5	0
iPrEX	10	3 (M184I/V)
Partners in PrEP	8	2 (1 K65R + 1 M184V)
TDF2	1	1 (K65R/M184V)
VOICE	9	2 (M184I/V)
TOTAL	**35**	**8 (23%)**

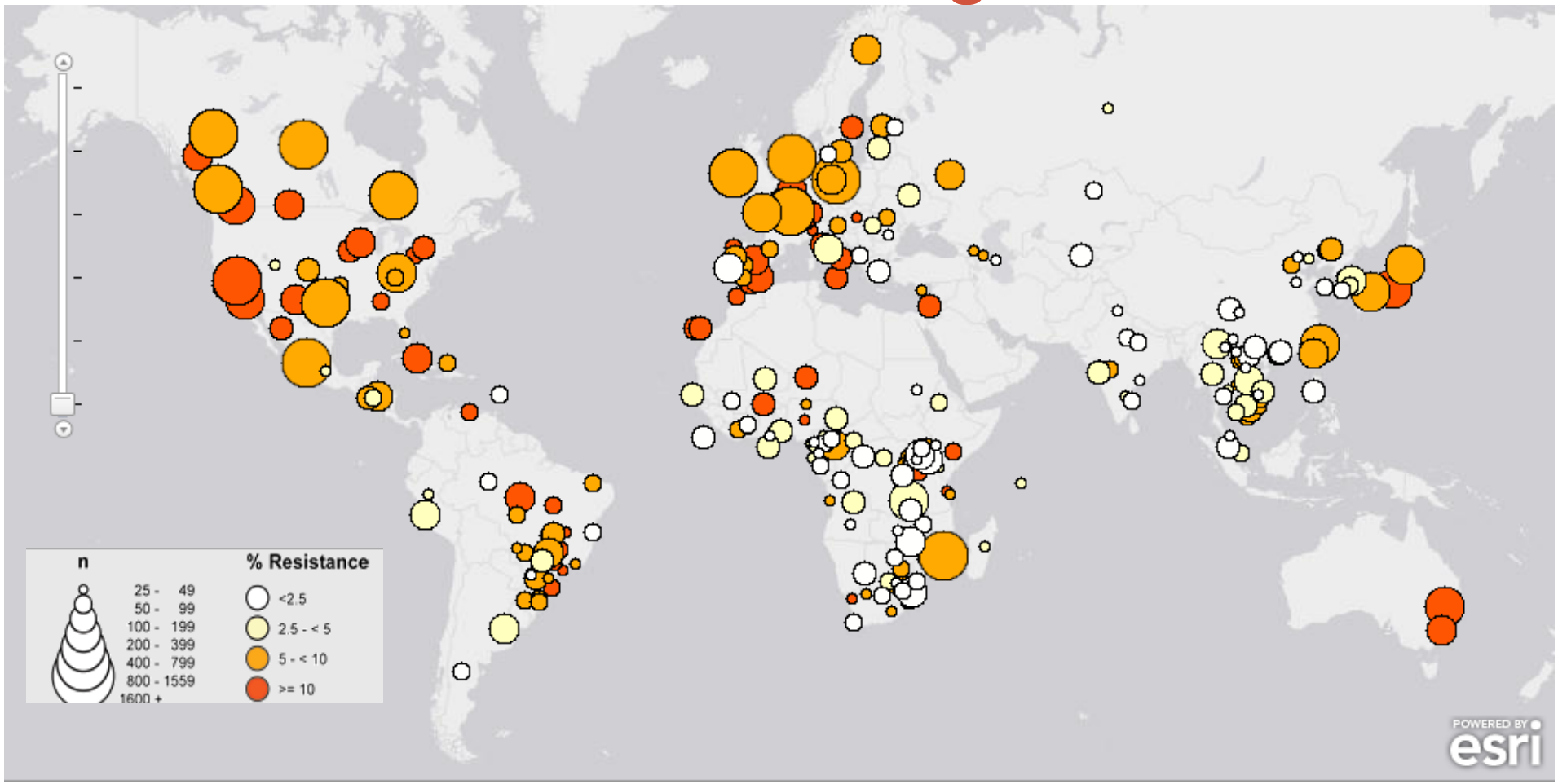
Mellors et al., 2013
MTN regional meeting

VOICE Standard Sequencing

No resistance to TFV	• **TFV oral or gel arms (K65R or K70E)** • 0/173 infected after enrollment • 0/18 acutely infected at enrollment
3 cases of FTC Resistance	• **Oral Truvada arm (M184V/I)** • 1/55 infected after 309 days on product • 2/9 acutely infected at enrollment; on product 26 & 29 d
8 cases of NNRTI resistance (transmitted)	• **All arms (K103N/V106M and/or Y181C)** • 8/355 (all seroconverters) • 2009 WHO TDR mutations (n=34)

Mellors et al., 2013 MTN regional meeting

Surveillance of HIV Drug Resistance



- **Stanford Resistance Database HIV-1 Drug Resistance in ARV-naive Populations**
- Compendium of published virus sequences from 46,765 persons, 264 studies

Transmitted Drug Resistance

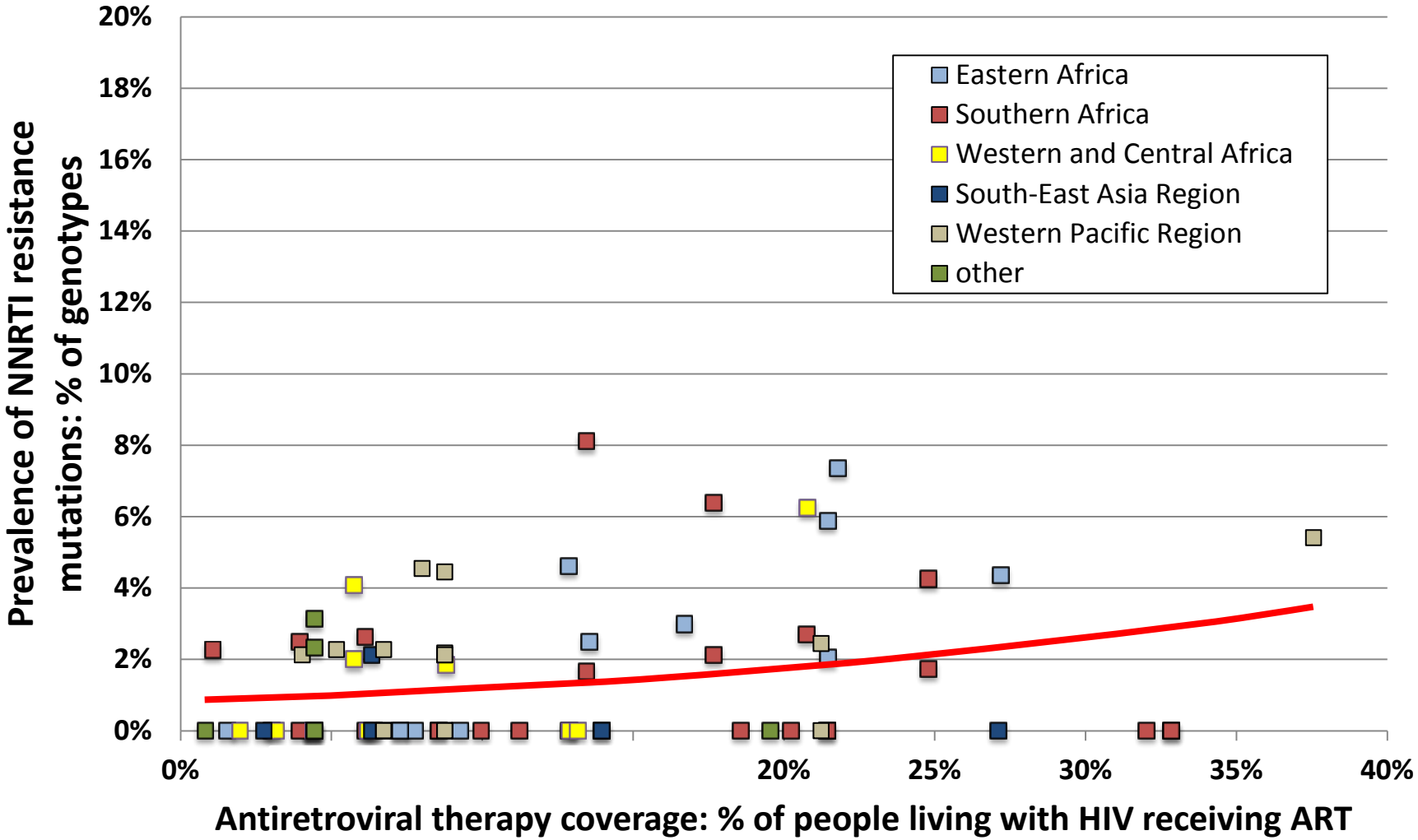
- Longer country has an ARV roll-out program so the prevalence of HIV drug resistance increases.

	Timing of ARV rollout in country/region	Year of HIV infection															P-value
		2005			2006			2007			2008			2009			
		N	DRM	%	N	DRM	%	N	DRM	%	N	DRM	%	N	DRM	%	
Total		45	0	0	118	5	4.2	115	5	4.3	81	5	6.2	49	4	8.2	0.056
Rwanda	Early 2004	17	0	0	18	0	0	20	2	10.0	20	3	15.0	3	0	0	0.081
Kenya	Late 2003	1	0	0	18	1	5.6	16	0	0	19	0	0	10	1	10.0	0.796
Masaka, Uganda	Mid-2004	6	0	0	13	1	7.7	20	0	0	13	0	0	14	0	0	0.327
Entebbe, Uganda	Mid-2004	0	-	-	6	3	50.0	6	1	16.7	11	2	18.2	0	-	-	0.088
Zambia	Late 2002	21	0	0	61	0	0	50	1	2.0	18	0	0	19	3	15.8	0.002
Cape Town	Early 2004	0	-	-	2	0	0	3	1	33.3	0	-	-	0	-	-	0.546

Price, Wallis et al., ARHR 2010

Transmitted HIVDR (WHO surveys)

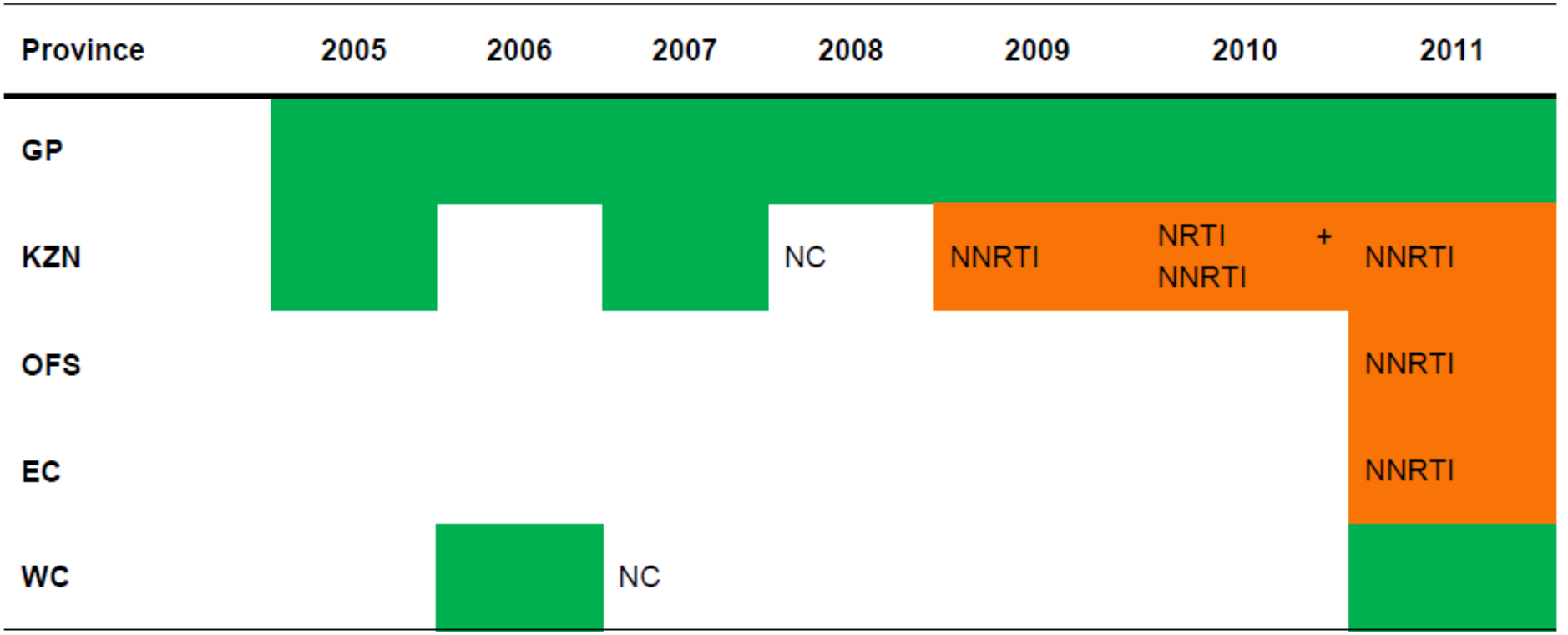
Relationship between transmitted resistance to NNRTI and ART coverage



Notes: P-value adjusted for region: 0.039. Odds-ratio = 1.49 (95% CI 1.07–2.08)

Transmitted Drug Resistance in SA

Table 1: Prevalence classification of transmitted drug resistance (TDR) in selected provinces of South Africa as per the WHO recommended method of using annual antenatal survey (ANSUR) specimens, 2005 – 2011.



Green: Low (<5%) prevalence classification of HIV TDR; Orange: moderate (5-15%) prevalence classification of TDR. NC: not classifiable. GP = Gauteng, KZN = KwaZulu-Natal, OFS = Orange Free State, EC = Eastern Cape, WC = Western Cape. NRTI = nucleoside reverse transcriptase inhibitor, NNRTI = non-nucleoside reverse transcriptase inhibitor.

Hunt et al., 2013 CommDis Bull 11(4) Nov 2013

2012: Transmitted Drug Resistance in SA

Table 1: Distribution of remnant serum specimens obtained by province for estimates of transmitted HIV drug resistance (TDR), South Africa, 2012.

Province	Total number of specimens collected in ANSUR 2012	Provincial contribution to ANSUR	Provincial HIV prevalence estimate	Number of eligible specimens	Number of specimens not available for testing	Number removed - unresolved phylogenetic linkage	Number removed - DLT positive	Number of specimens included in TDR analysis
Eastern Cape	4625	13.5%	29.1%	127	10	3	2	112
Free State	2325	6.8%	32.0%	75	3	0	1	71
Gauteng	6862	20.0%	29.9%	121	25	0	2	94
KwaZulu-Natal	7011	20.5%	37.4%	327	15	0	8	304
Limpopo	3579	10.4%	22.3%	56	14	0	0	42
Mpumalanga	2201	6.4%	35.6%	70	8	0	3	59
North West	2457	7.2%	29.7%	61	14	0	0	47
Northern Cape	1190	3.5%	17.8%	14	7	0	0	7
Western Cape	4010	11.7%	16.9%	35	1	0	0	34
National	34260	100.0%	29.5%	886	97	3	16	770

11% samples collected had ARVs detected in them.

Hunt et al., 2015 CommDis Bull 13(1) April 2015

Transmitted drug Resistance, per province

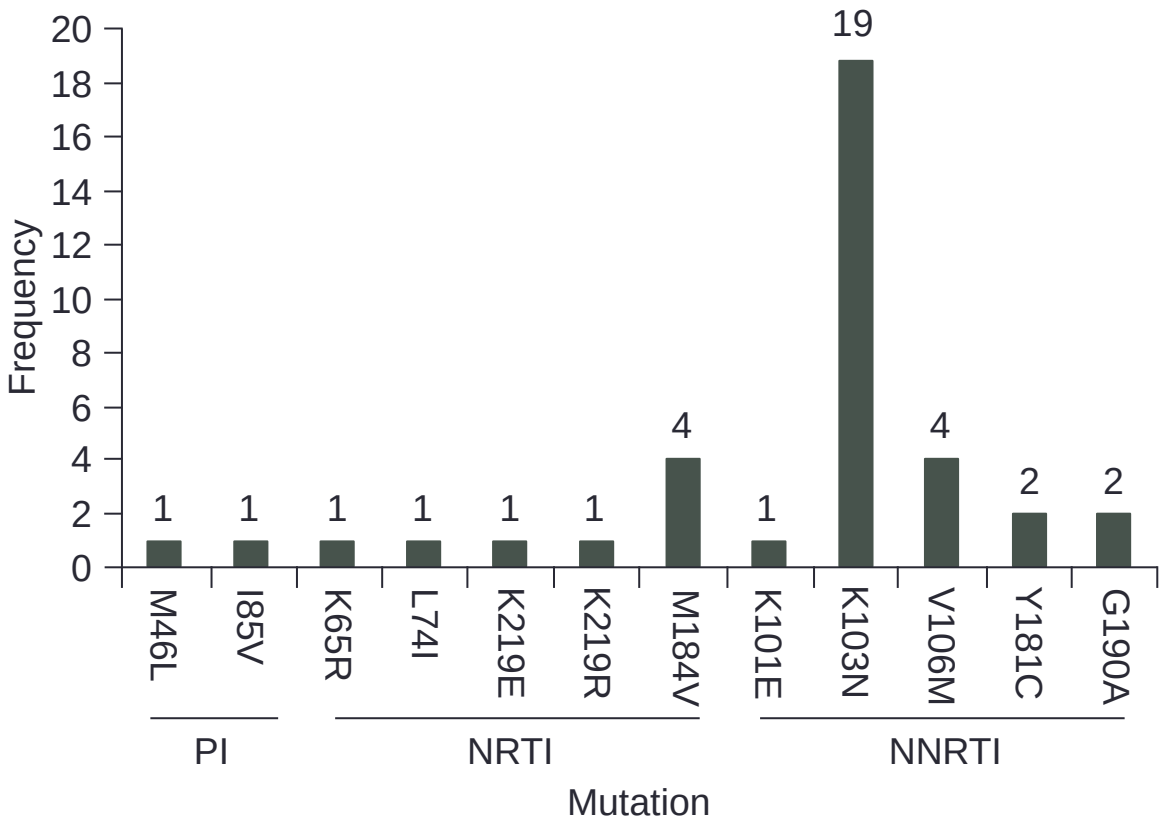
Province	Number of specimens amplifiable by genotyping PCR	Genotyping amplification rate	Number of sequences with PI mutations	PI Point Prevalence (95% CI)	Number of sequences with NRTI mutations	NRTI Point Prevalence (95% CI)	Number of sequences with NNRTI mutations	NNRTI Point Prevalence
Eastern Cape	99	88.4%	0	0% (0 - 3.7)	0	0% (0 - 3.7)	3	3% (1.0 - 8.5)
Free State	54	76.1%	0	0% (0 - 6.6)	1	1.9% (0.3 - 9.8)	4	7.4% (2.9 - 17.6)
Gauteng	65	69.1%	1	1.5% (0.3 - 8.2)	0	0% (0 - 5.6)	6	9.2% (4.3 - 18.7)
KwaZulu-Natal	196	64.5%	0	0% (0 - 1.9)	4	2% (0.8 - 5.1)	8	4.1% (2.1 - 7.8)
Limpopo	20	47.6%	0	0% (0 - 16.1)	0	0% (0 - 16.1)	2	10% (2.8 - 30.1)
Mpumalanga	45	76.3%	0	0% (0 - 7.9)	1	2.2% (0.4 - 11.6)	2	4.4% (1.2 - 14.8)
North West	21	44.7%	2	9.5% (2.7 - 28.9)	0	0% (0 - 15.5)	1	4.8% (0.8 - 22.7)
Northern Cape	4	57.1%	0	0% (0 - 49.0)	0	0% (0 - 49.0)	0	0% (0 - 49.0)
Western Cape	28	82.4%	0	0% (0 - 12.1)	0	0% (0 - 12.1)	2	7.1% (2.0 - 22.6)
National	532	69.1%	3	0.6% (0.1 - 1.6)	6	1.1% (0.5 - 2.4)	28	5.3% (3.7 - 7.5)

NRTI = nucleoside reverse transcriptase inhibitors; NNRTI = non-nucleoside reverse transcriptase inhibitors; PI = protease inhibitors

Hunt et al., 2015 CommDis Bull 13(1) April 2015

Transmitted Drug Resistance: KwaZulu Natal

- MTN009 resistance patterns in worked screened for a PrEP study in 2010-2011



Participants (n)	Resistance profile		
	NRTI	NNRTI	PI
1	K219R	-	-
1	K219E	-	-
14	-	K103N	-
1	-	V106M	-
1	-	-	M46L
1	-	K103N, Y181C	-
1	-	V106M, G190A	-
1	K65R	Y181C	-
1	M184V	K103N	-
1	M184V	K101E, K103N	-
1	M184V	K103N, V106M	-
1	L741, M184V	K103N	-
1	-	K101E, V106M, G190A	185V

Parikh et al., PLOSone 2013

Acknowledgements

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