HIV Drug Resistance

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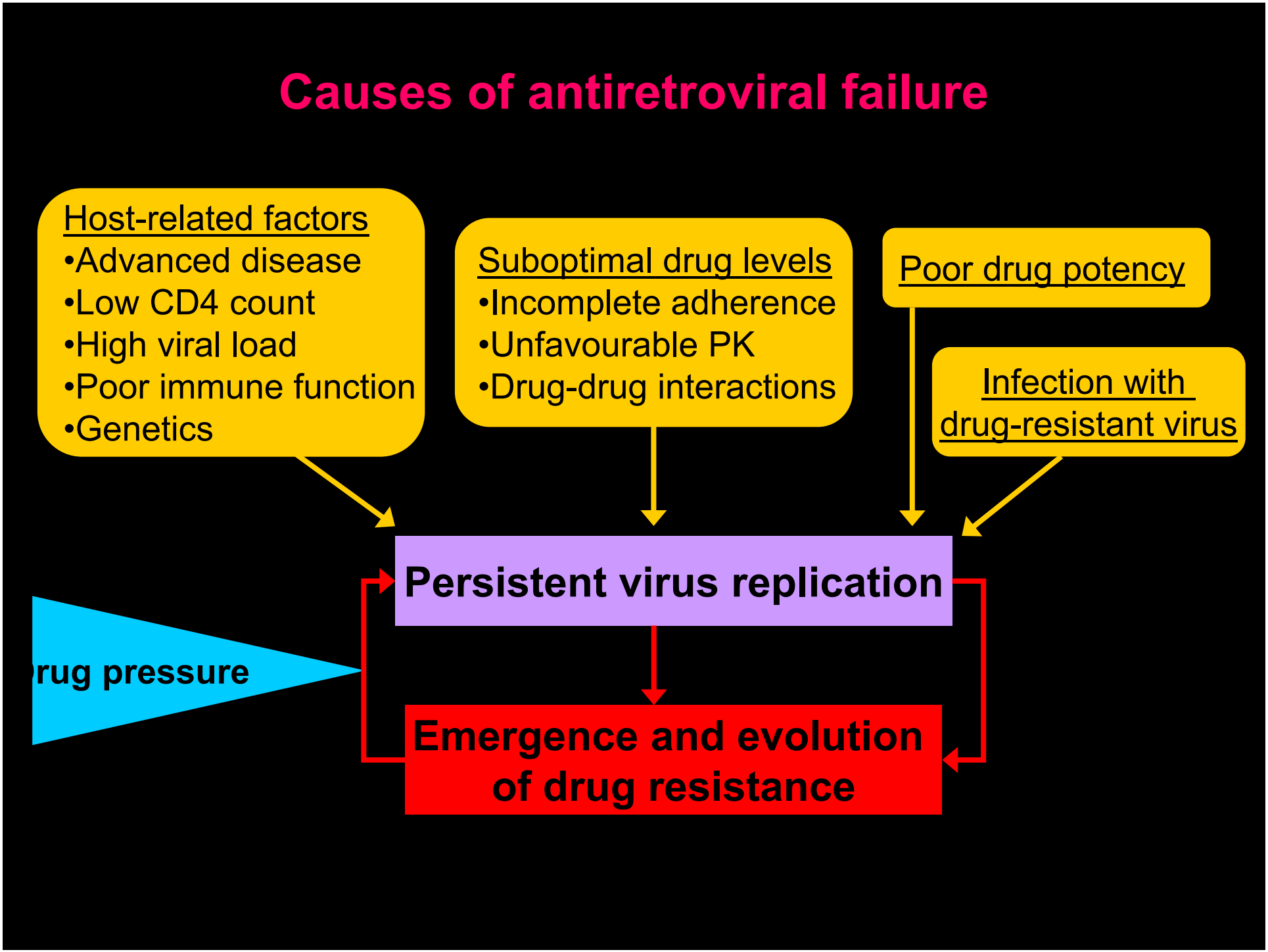

Causes of antiretroviral failure
Host-related factors
•Advanced disease
•Low CD4 count
•High viral load
•Poor immune function
•Genetics
Suboptimal drug levels
•Incomplete adherence
•Unfavourable PK
•Drug-drug interactions
Poor drug potency
Infection with drug-resistant virus
Persistent virus replication
rug pressure
Emergence and evolution of drug resistance

Long-term implications of antiretroviral resistance

- Accumulation of drug resistance reduces effective, well tolerated and less expensive treatment options
  - *Multi-drug resistance linked to increased morbidity and mortality*[1]
- Comprehensive long-term management of HIV infected patients must strive to minimize drug resistance as a key goal

1. Zaccarelli M, AIDS 2005

Antiretroviral resistance: *Themes*

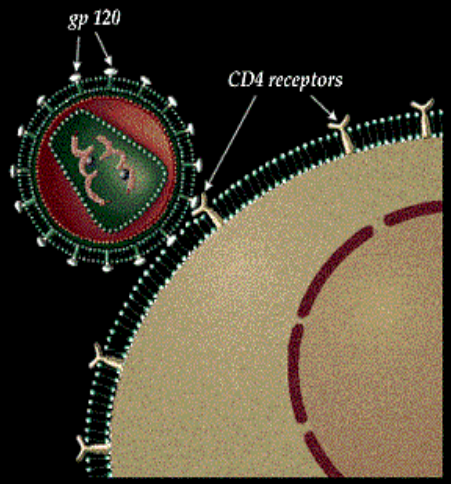
- Mechanisms & Principles
- Clinical implications

Definition of antiretroviral resistance

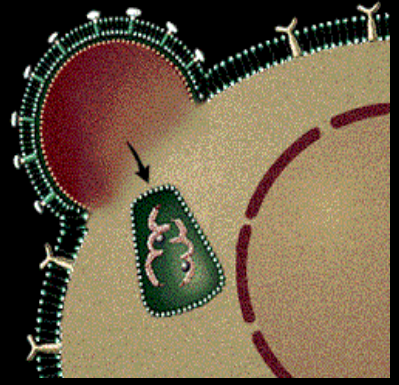
- Changes in the viral genetic sequence (mutations) that confer drug resistance - ***genotypic resistance***
- Commonly reduce drug susceptibility compared with the susceptibility of wild-type viruses - ***phenotypic resistance***

Mediated by:

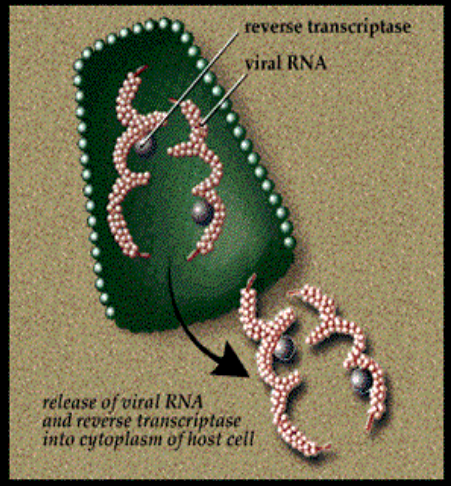
- Changes in the molecular target of therapy (detected in routine tests)
- Changes in other viral proteins (e.g., Gag) that indirectly interfere with drug activity (more difficult to detect)



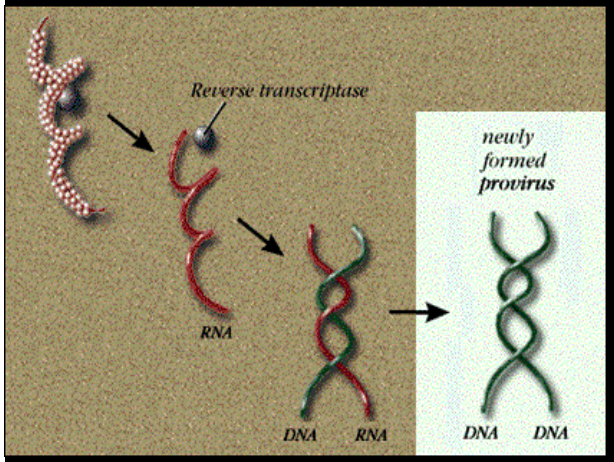
Attachment



Fusion

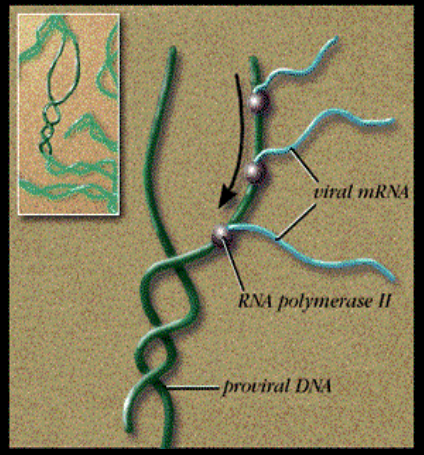


Release of RNA

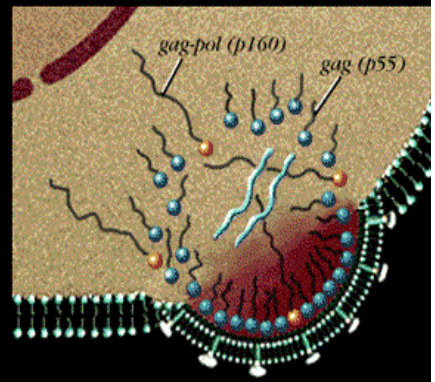


Reverse transcription

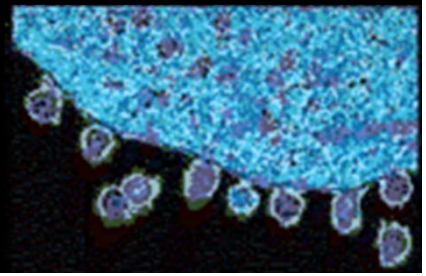
Integration



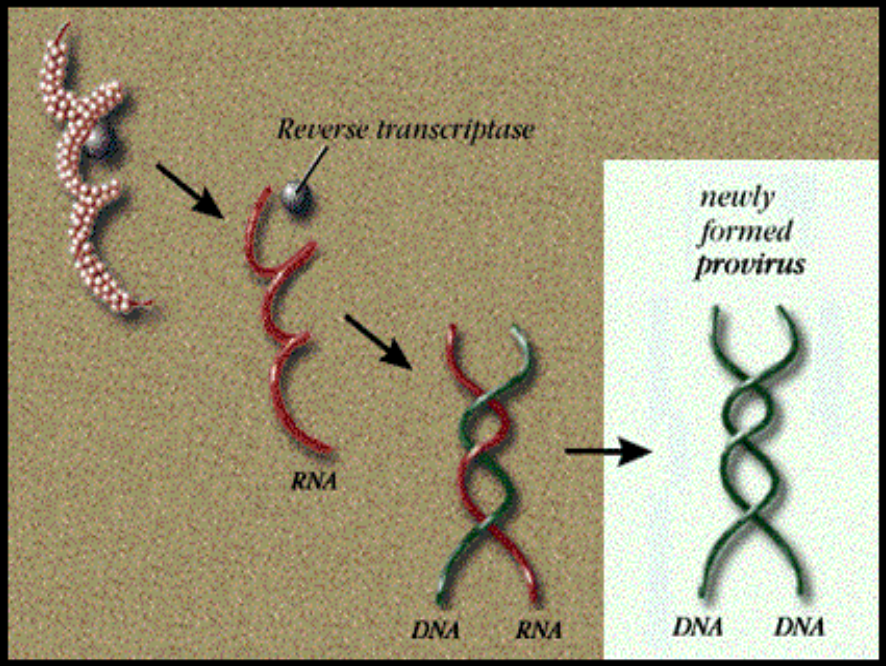
Transcription



Assembly

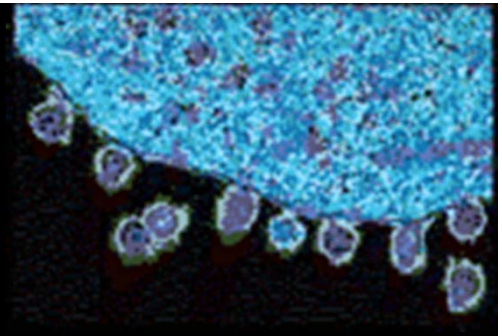


Maturation and budding



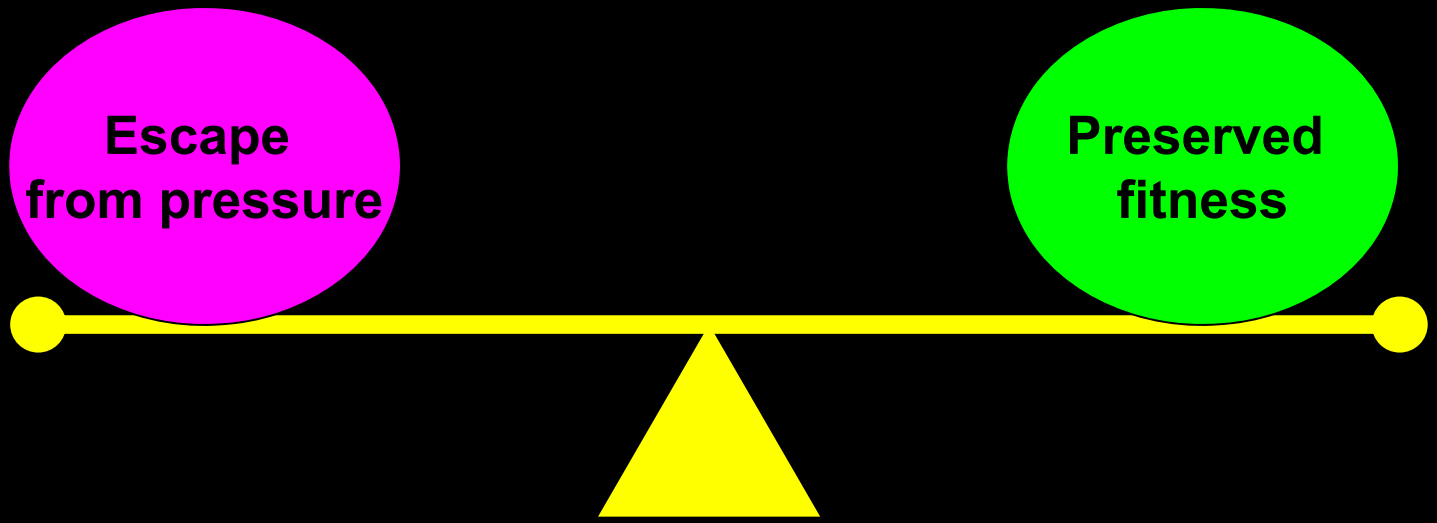
- **Mechanisms of genetic evolution**

1. **Rapid evolution through point mutations**
   - **RT error rate: ~1 per genome round**
   - **Replication rate: 10^9-10^{10} virus particles daily**
   - ***All possible mutations generated daily***
2. **Genetic evolution through recombination**
   - **Recombination rate: 7-30 per genome round**

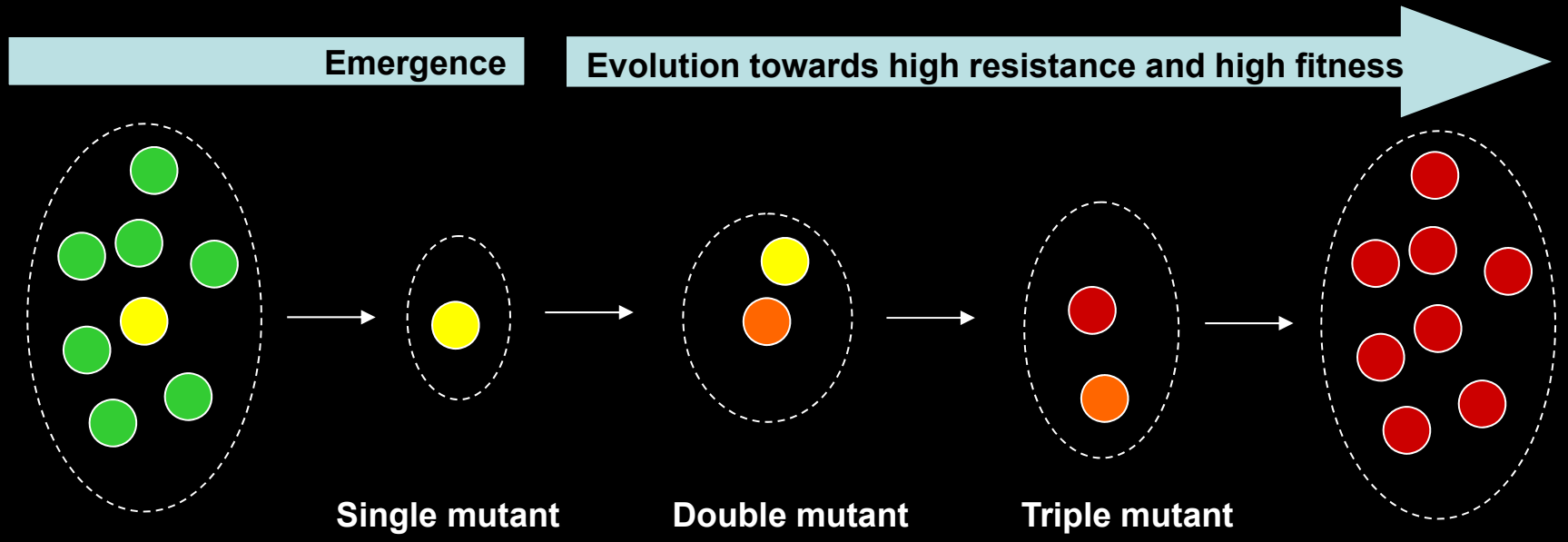


- rapid turnover
- rapid adaptation

Dominant quasispecies

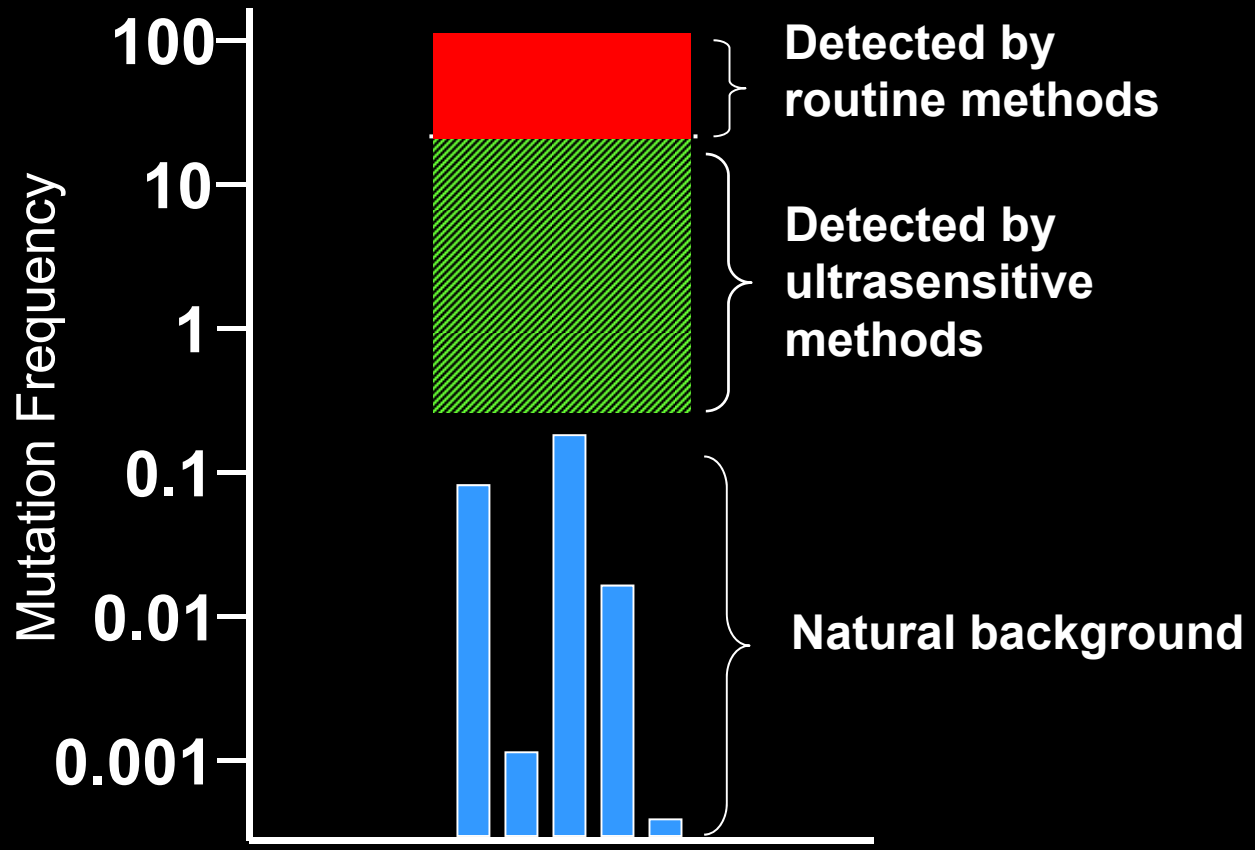


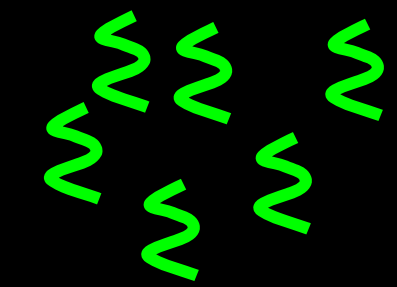
Emergence and evolution of resistance



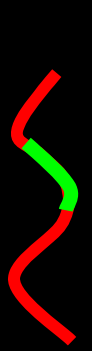
- Increasing resistance and cross-resistance
- Accumulation of mutations on the same viral genome
- Compensatory changes that restore fitness

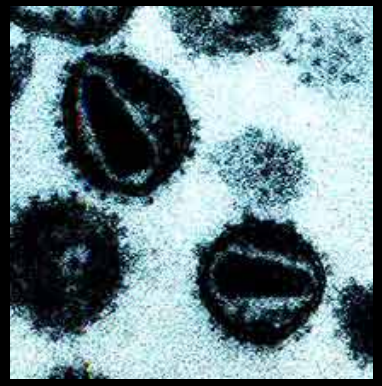
Detection of resistant mutants





PCR





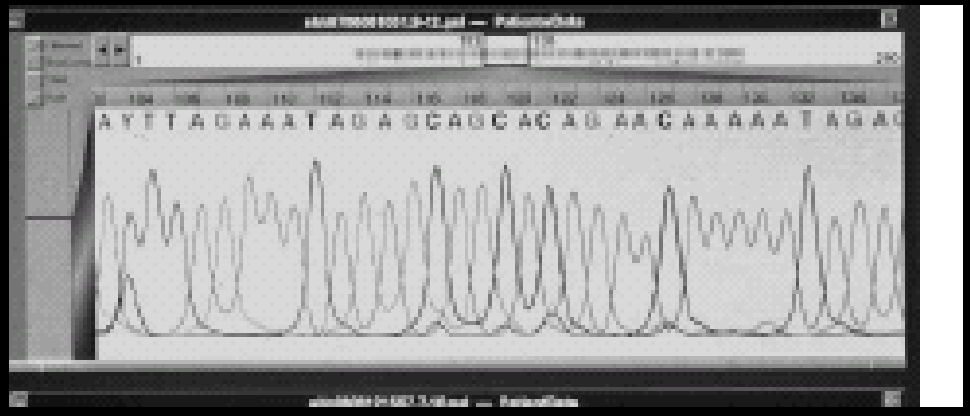
PR and RT

HIV RNA

Plasma

Sequencing

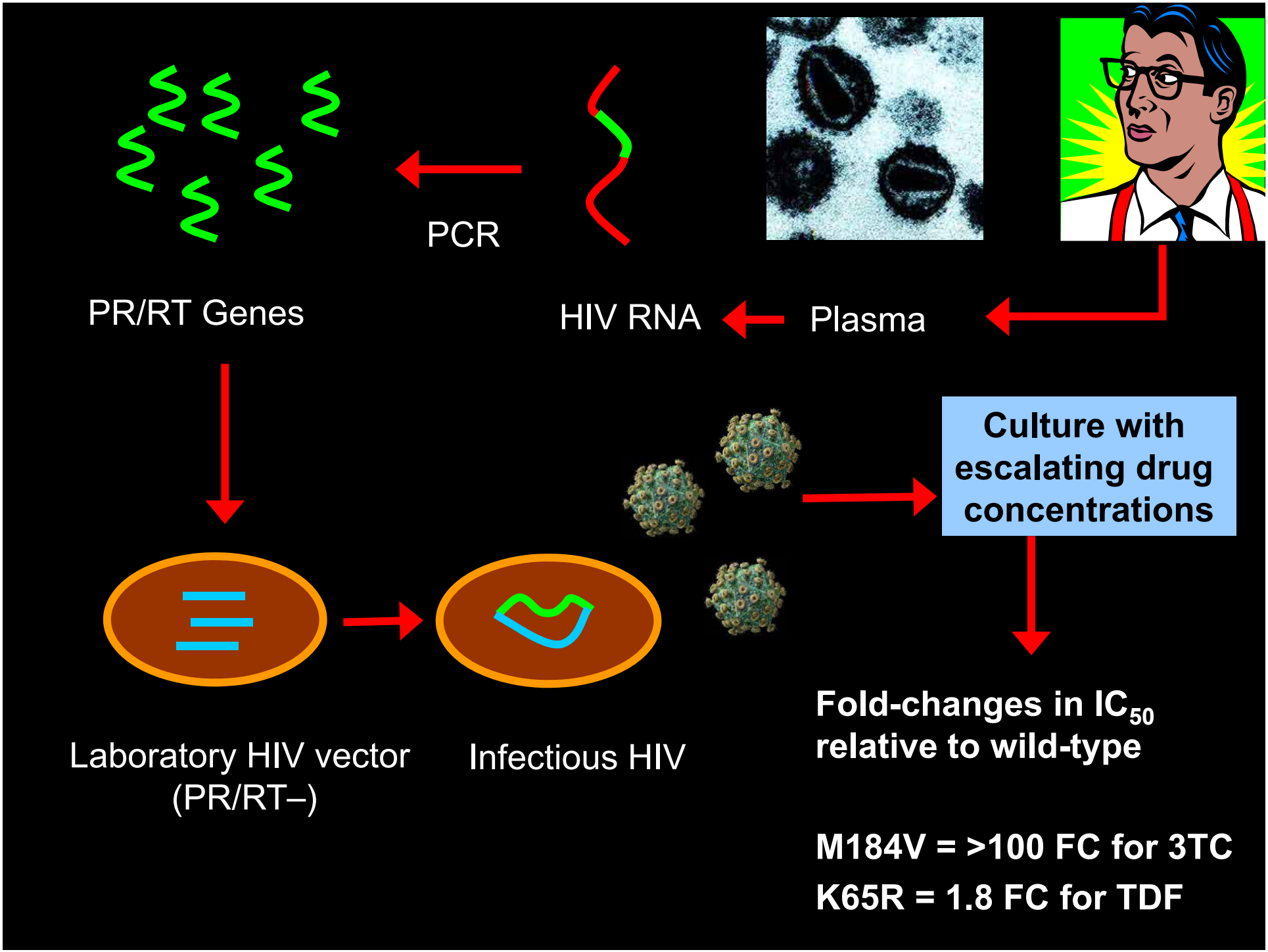
Mutations



RT M184V

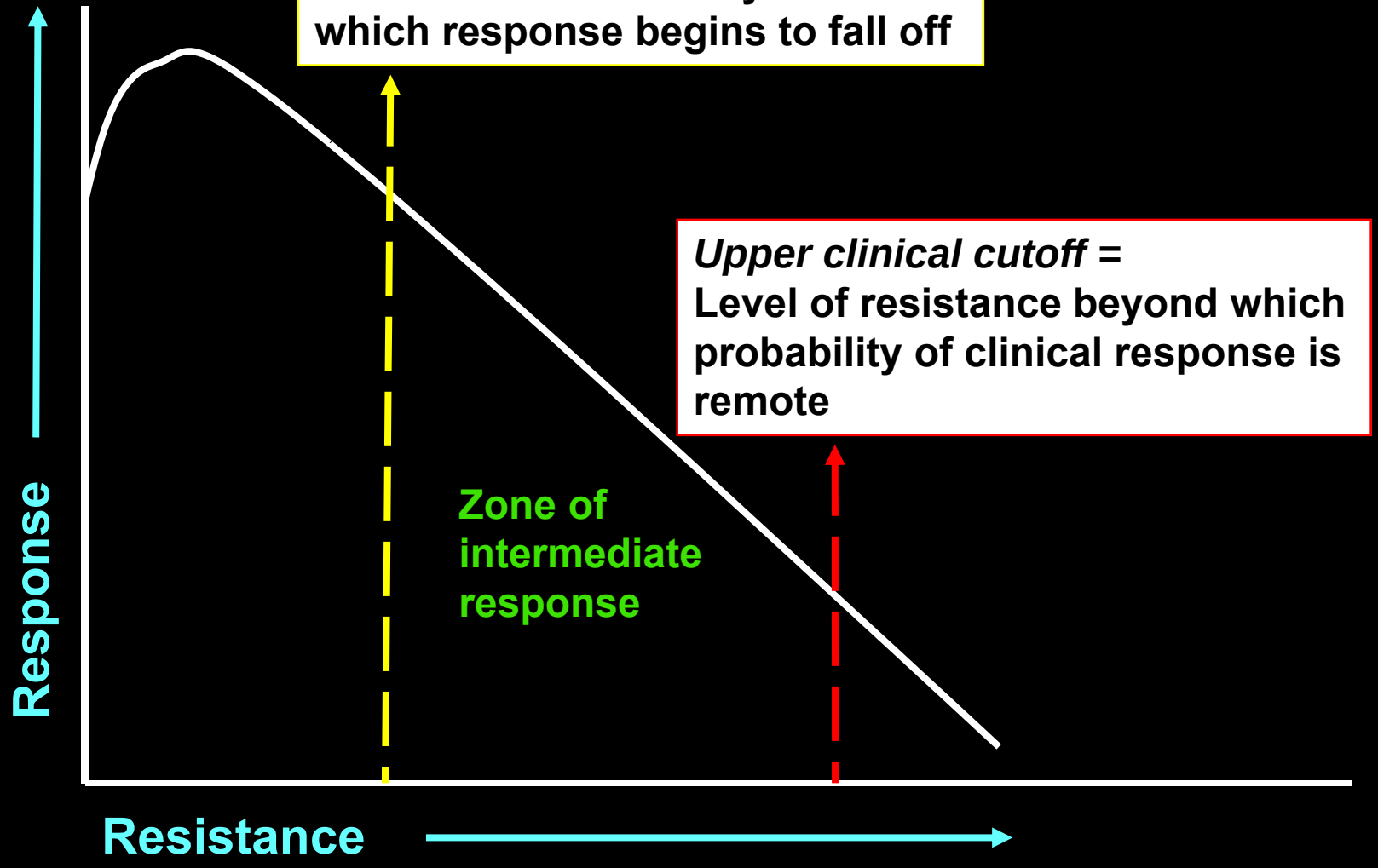
Methionine → Valine

@ codon 184 of RT

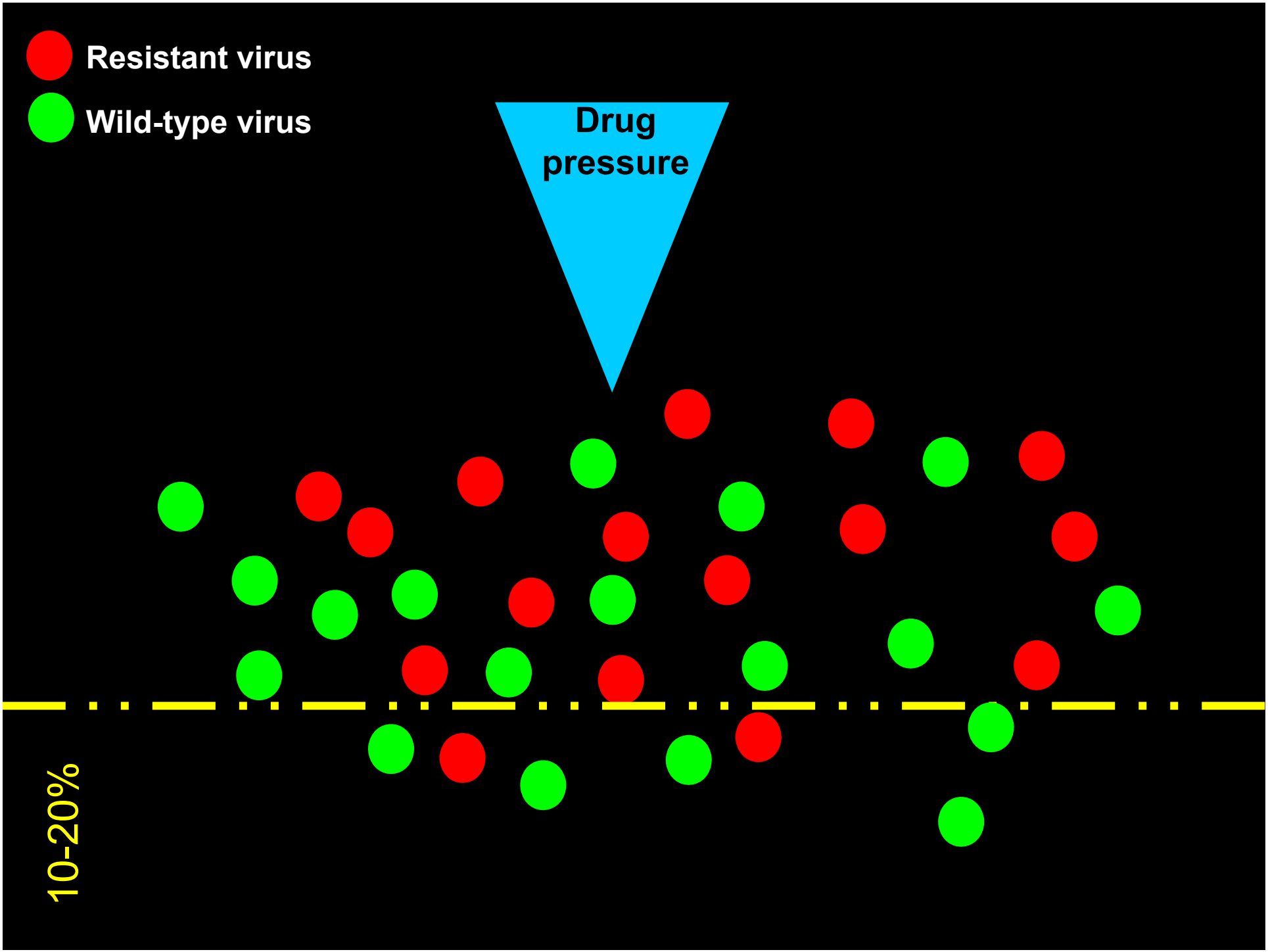

PCR
PR/RT Genes
HIV RNA
Plasma
Culture with escalating drug concentrations
Laboratory HIV vector (PR/RT–)
Infectious HIV
Fold-changes in IC_{50} relative to wild-type
M184V = >100 FC for 3TC
K65R = 1.8 FC for TDF

Clinical cut-offs

Lower clinical cutoff =
Level of resistance beyond which response begins to fall off



Upper clinical cutoff =
Level of resistance beyond which probability of clinical response is remote


Resistant virus
Wild-type virus
Drug pressure
10-20%

Principles of resistance

- Drug-resistant mutants are **selected** (not created) by drug pressure if virological suppression is incomplete
- Ongoing virus replication under drug pressure leads to the further **evolution** of resistance and cross-resistance
- Resistant mutants often display reduced **fitness** but compensatory changes emerge over time that partially restore virus fitness
- If therapy is discontinued resistant mutants disappear from the dominant quasispecies, **persisting** as minority species and archived resistance in latently infected cells

Clinical implications

- Continuing a failing treatment can be deleterious
  - *Use only for patients lacking effective treatment options*
  - *Judicious selection of drugs with expert advice*
  - *Maintain for the shortest period possible pending the availability of new treatment options*
- In treated persons, resistance test results obtained after therapy is discontinued are not reliable
- Resistance is long-lasting, even if undetectable
- Resistance results must be interpreted in the context of the patient's treatment history

Cross-resistance potential

NRTIs	*significant to complete*
NNRTIs	*complete for NVP and EFV*
PIs	*significant with multiple mutations*
Entry Inhibitors	*complete for MRC and VRC*
Integrase Inhibitors	*complete for RAL and ELV*

HAART regimens have a different genetic barrier to the emergence of resistance

Defined by:
Speed of emergence of resistance
No. mutations required to compromise drug activity

2NRTI PI/r

2NRTI PI

3TC ZDV NNRTI
3TC ABC ZDV
3TC ABC EFV
3TC/FTC TDF EFV

TDF 3TC ABC
TDF 3TC ddI
ABC ddI d4T
TDF ddI EFV
TDF 3TC NVP?

Function of:

- *Inhibitor/Target interactions*
  - *Affinity*
  - *Fitness cost*
- *Concentration of the drug*

Kempf D, JID 2004; Phillips A, AIDS 2005; Gulick, 7th ICDTHI 2004; Cahn P, 1st IAS 2001; Feinberg J, 14th IAC 2002; Molina JM, 15th IAC 2004; MacManus S, AIDS 2004; Ananworanich J, 3rd IAS 2005; Malan R, 13th CROI 2006; Miller MD, 6th CDTHI 2002; Arribas JR, 7th CDTHI 2004 ; Molina JM, 15th IAC 2004

Resistance in clinical trials of first-line HAART

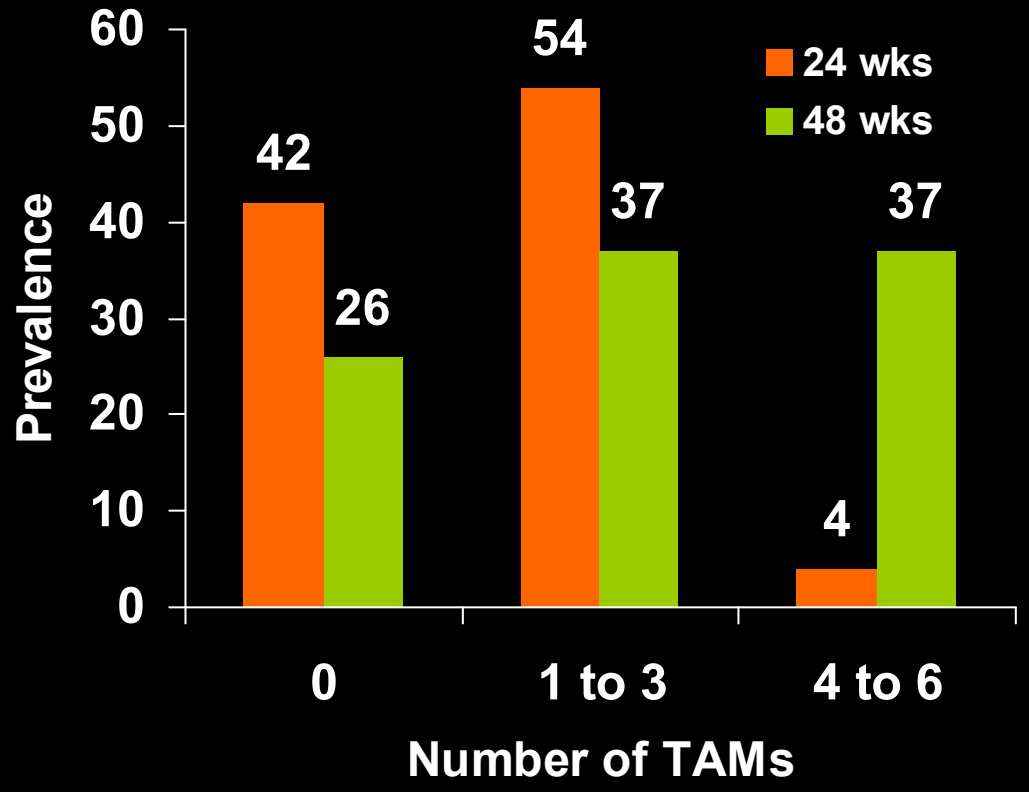
wk 48

Study	Number	Regimen		n	NRTI-R (%)		3rd drug-R (%)
GS934	257	TDF FTC	EFV	12	FTC TDF	17 0	75
GS934	254	ZDV 3TC	EFV	23	3TC ZDV	30 4	70
CNA30021	770	ABC 3TC	EFV	38	3TC ABC	47 21	58
GS903	299	TDF 3TC	EFV	29	3TC TDF	41 24	55
CNA30024	324	ABC 3TC	EFV	13	3TC ABC	31 8	38
ABT418	190	TDF FTC	LPV/r	15	3TC TDF	20 0	0
SOLO	322	ABC 3TC	FPV/r	32	3TC ABC	12 0	0

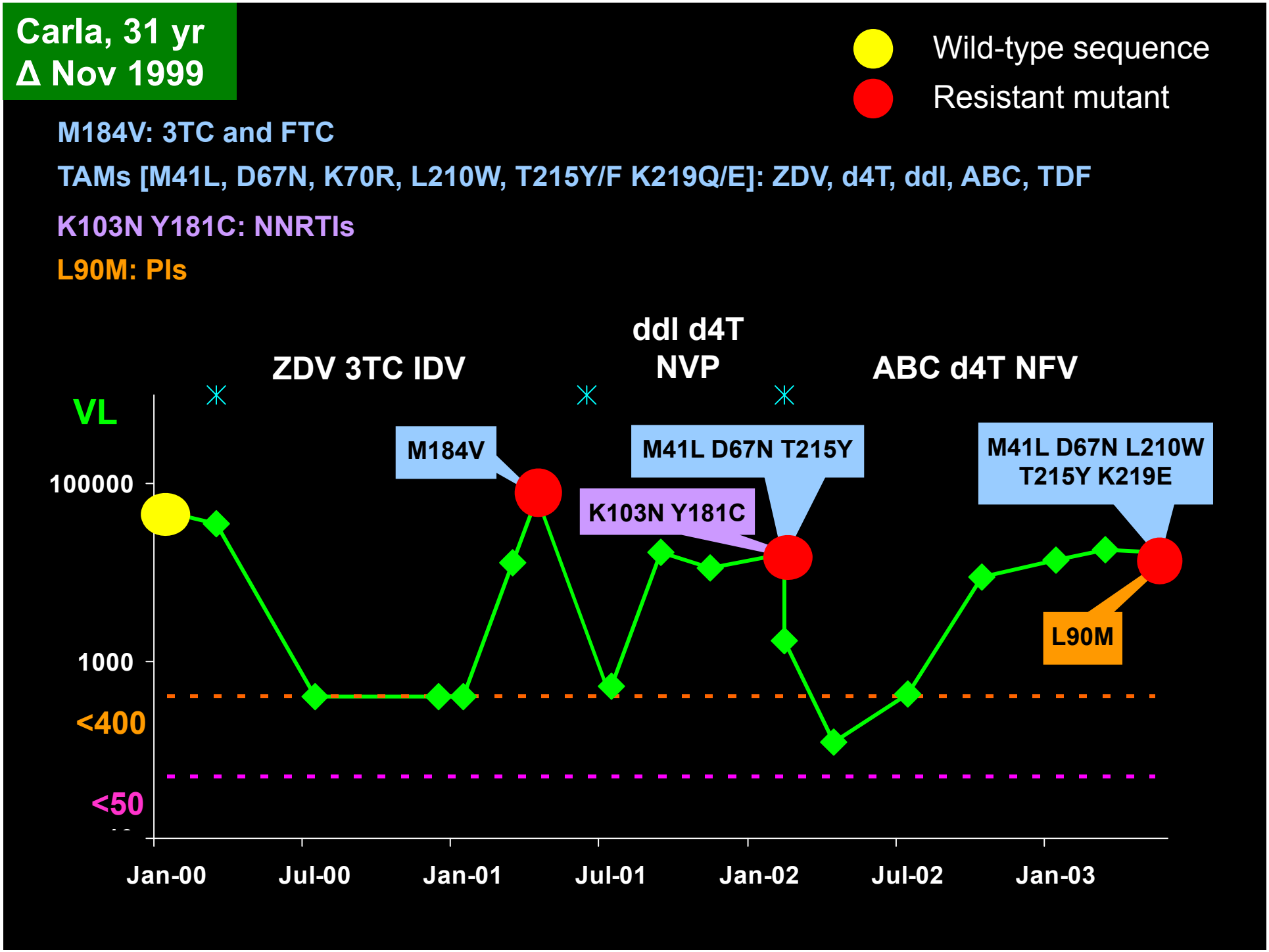
Gallant JE, 16th IAC 2006; Gazzard B, 43rd ICAAC; Miller M, 6th ICDTHI 2002; De Jesus E, 43rd ICAAC 2003; Molina JM, 3rd IAS 2005; MacManus S, AIDS 2004

Prevalence of TAMs in patients receiving ZDV/3TC/TDF in Uganda and Zimbabwe in the absence of viral load monitoring (DART)

- Resistance test results available in subset of patients with viral load >1000 c/ml at wk 24 (n=26/43) and wk 48 (n=35/64)



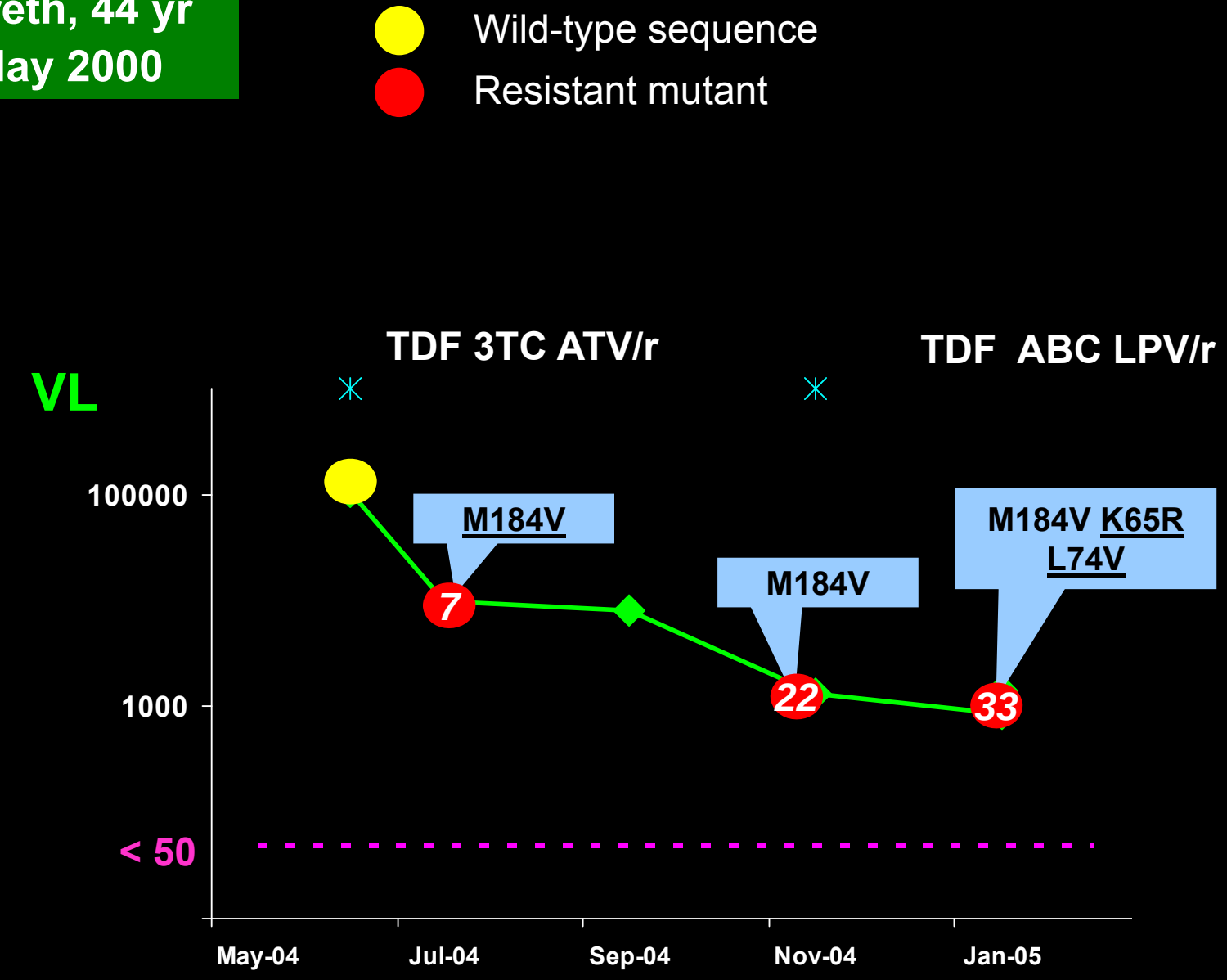
Pillay D, 14th CROI 2007

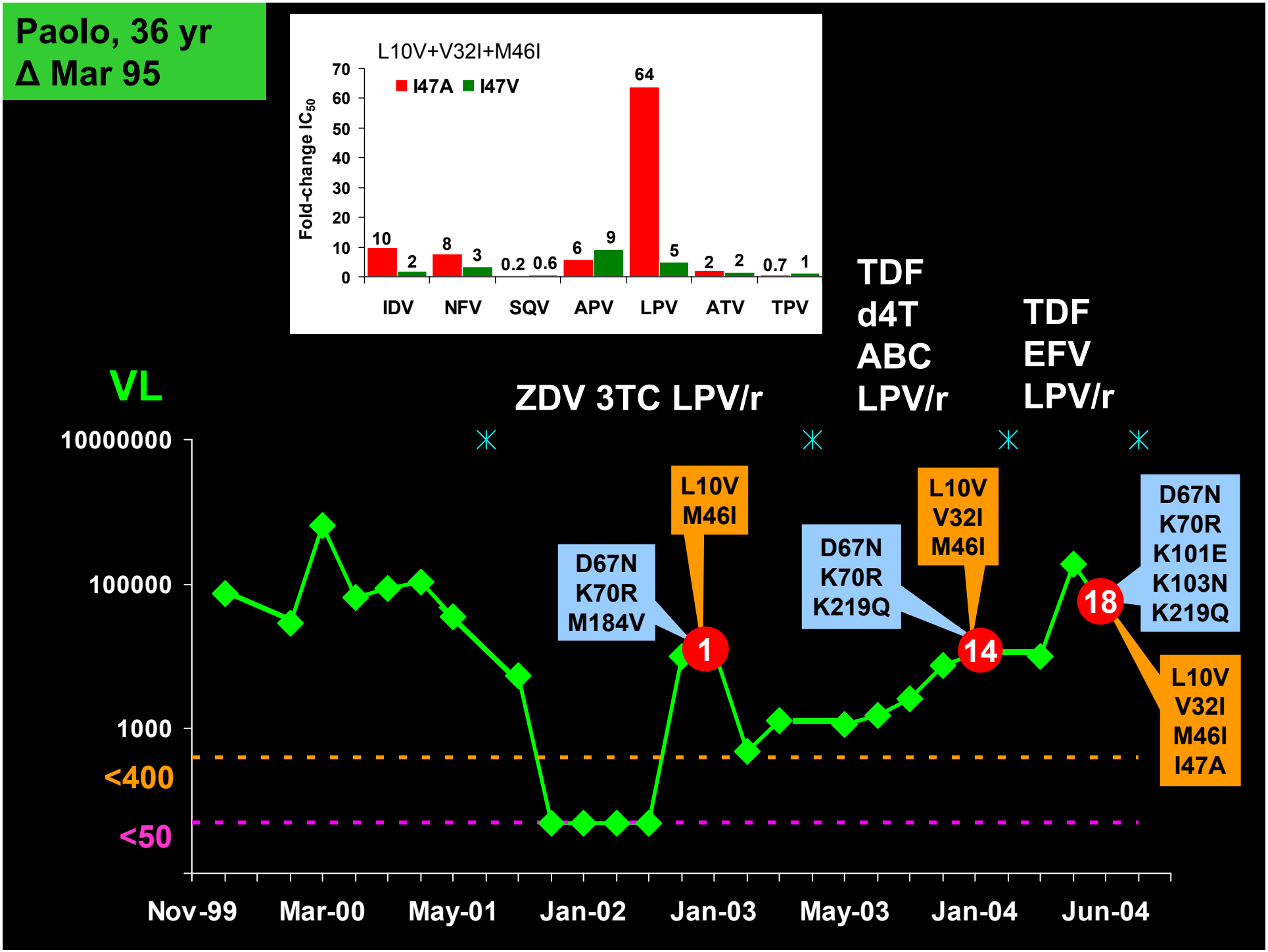

Carla, 31 yr
Δ Nov 1999
Wild-type sequence
Resistant mutant
M184V: 3TC and FTC
TAMs [M41L, D67N, K70R, L210W, T215Y/F K219Q/E]: ZDV, d4T, ddI, ABC, TDF
K103N Y181C: NNRTIs
L90M: PIs
ZDV 3TC IDV
ddI d4T
NVP
ABC d4T NFV
VL
100000
1000
<400
<50
M184V
M41L D67N T215Y
K103N Y181C
M41L D67N L210W
T215Y K219E
L90M
Jan-00
Jul-00
Jan-01
Jul-01
Jan-02
Jul-02
Jan-03

Can we predict emerging resistance during failure of first-line NNRTI-based therapy?

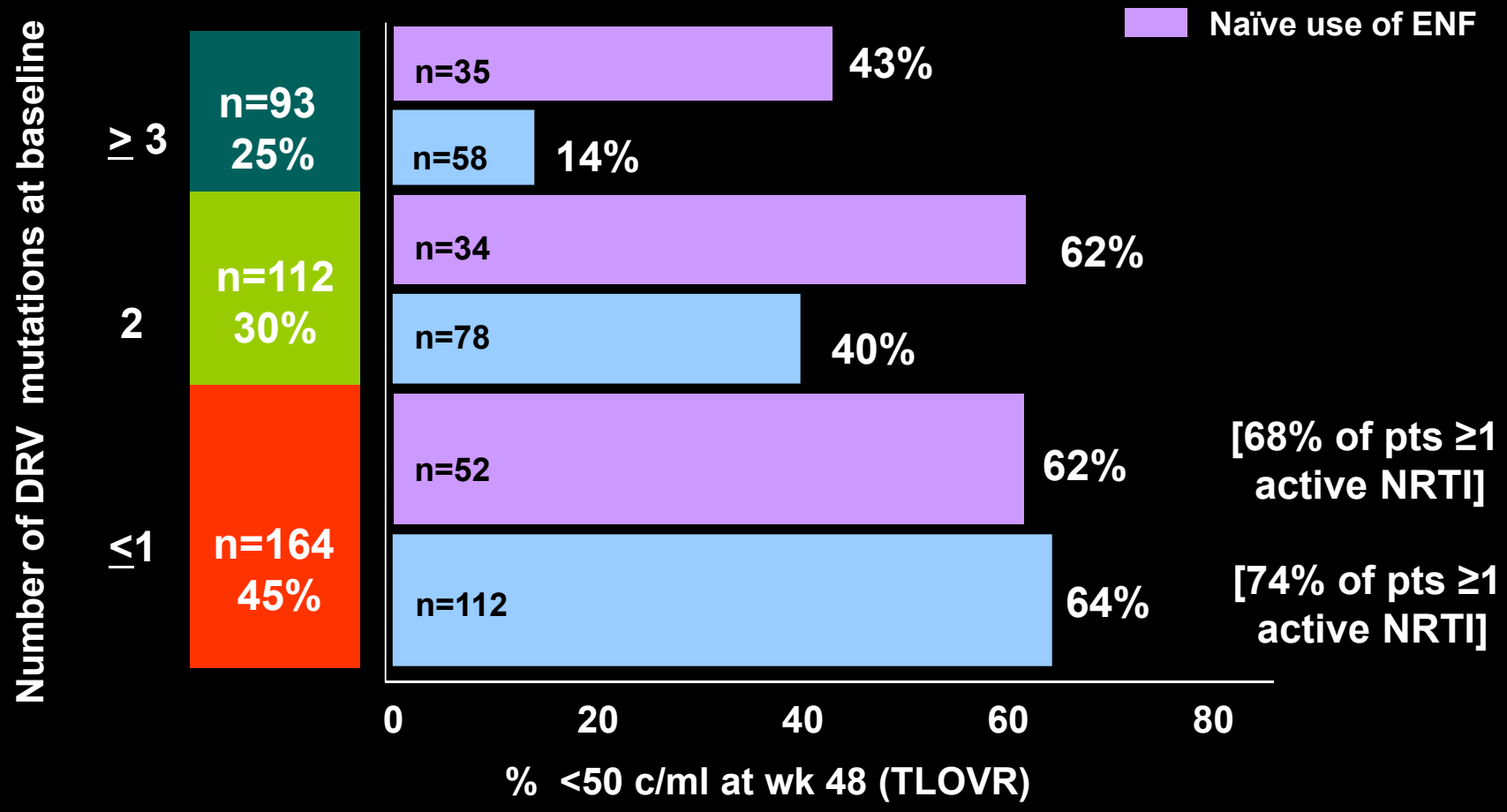
Regimen	Detection	Drugs affected
• ZDV 3TC NNRTI	Early	3TC, NVP, EFV
• ZDV 3TC NNRTI	*Late*	+ ZDV, d4T, ABC > ddI, TDF
• d4T 3TC NNRTI	Early	3TC, NVP, EFV
• d4T 3TC NNRTI	*Late*	+ ZDV, d4T, ABC > ddI, TDF
• ABC 3TC NNRTI	Early	3TC, NVP, EFV
• ABC 3TC NNRTI	Intermediate	+ ABC, ddI
• ABC 3TC NNRTI	*Late*	+ TDF, d4T
• TDF FTC NNRTI	Early	3TC, NVP, EFV
• TDF FTC NNRTI	Intermediate	+ TDF, ABC, ddI, d4T

Gareth, 44 yr
Δ May 2000




Paolo, 36 yr
Δ Mar 95
L10V+V32I+M46I
I47A
I47V
Fold-change IC50
70
60
50
40
30
20
10
0
10
2
8
3
0.2
0.6
6
9
64
5
2
2
0.7
1
IDV
NFV
SQV
APV
LPV
ATV
TPV
TDF
d4T
ABC
LPV/r
TDF
EFV
LPV/r
ZDV 3TC LPV/r
VL
10000000
100000
1000
<400
<50
D67N
K70R
M184V
L10V
M46I
1
D67N
K70R
K219Q
L10V
V32I
M46I
14
18
D67N
K70R
K101E
K103N
K219Q
L10V
V32I
M46I
I47A
Nov-99
Mar-00
May-01
Jan-02
Jan-03
May-03
Jan-04
Jun-04

POWER: Virological response by baseline number of DRV/r mutations*

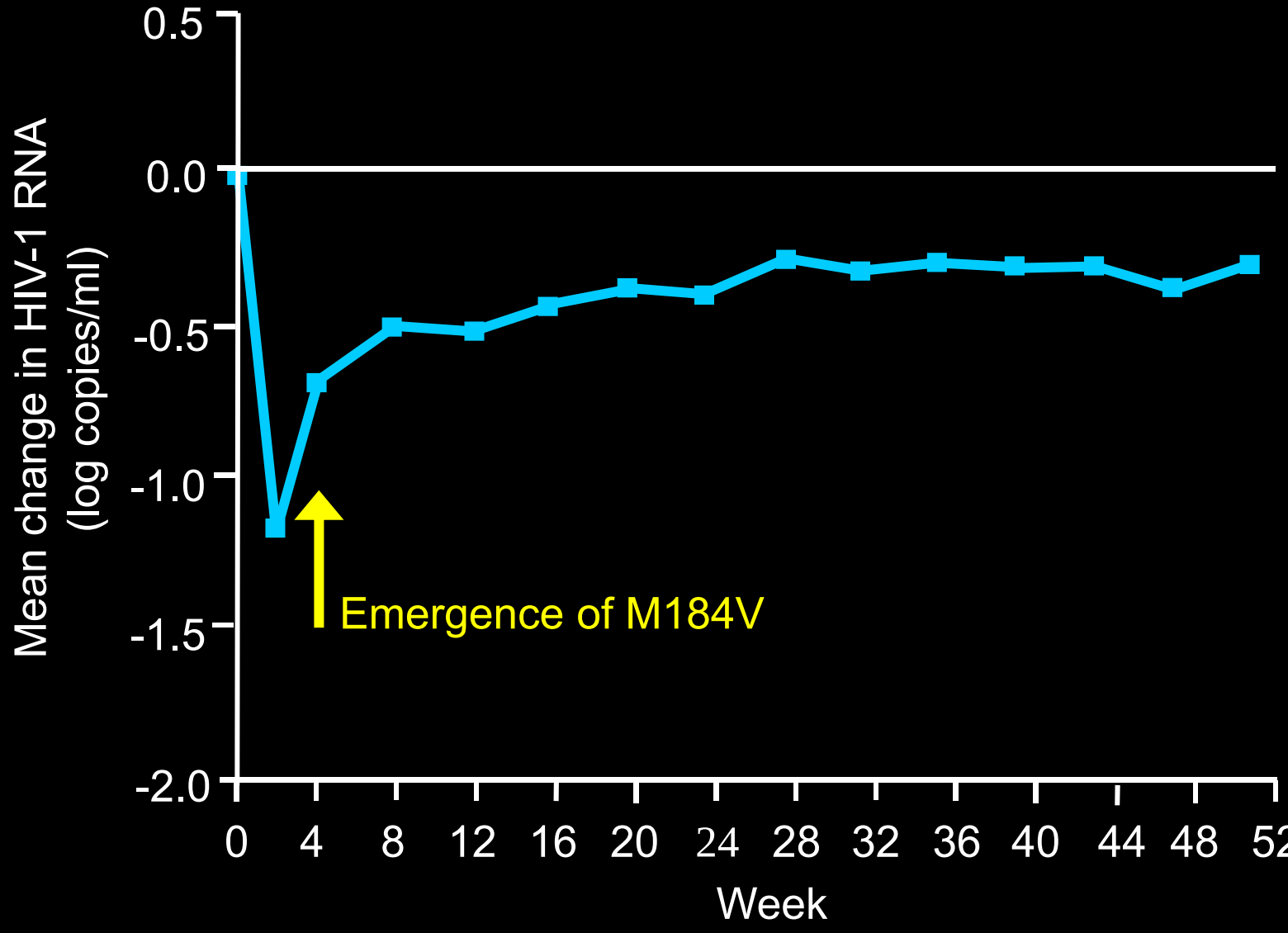


*** V11I V32I L33F I47V I50V I54L/M G73S L76V I84V L89V**

All DRV/r 600/100 mg bd

Adapted from Cohen, 44th IDSA 2006

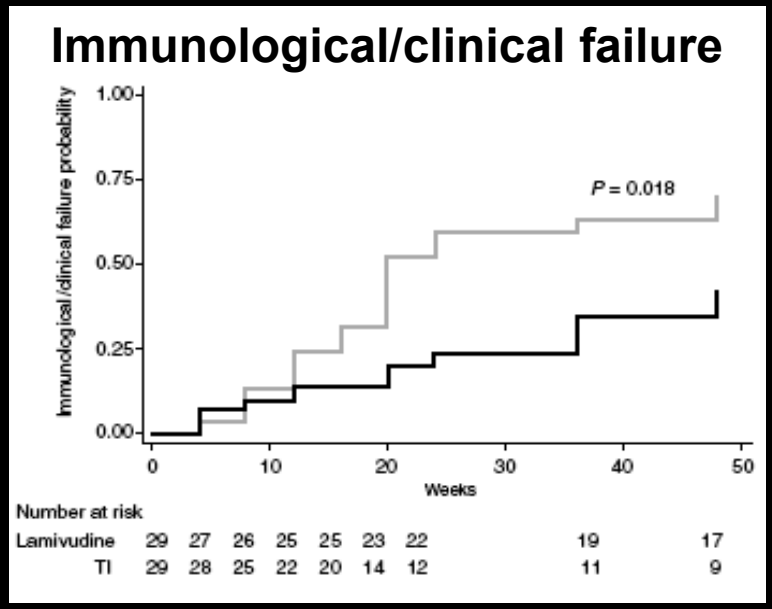
Viral load reduction during 3TC monotherapy (NUCA3001)

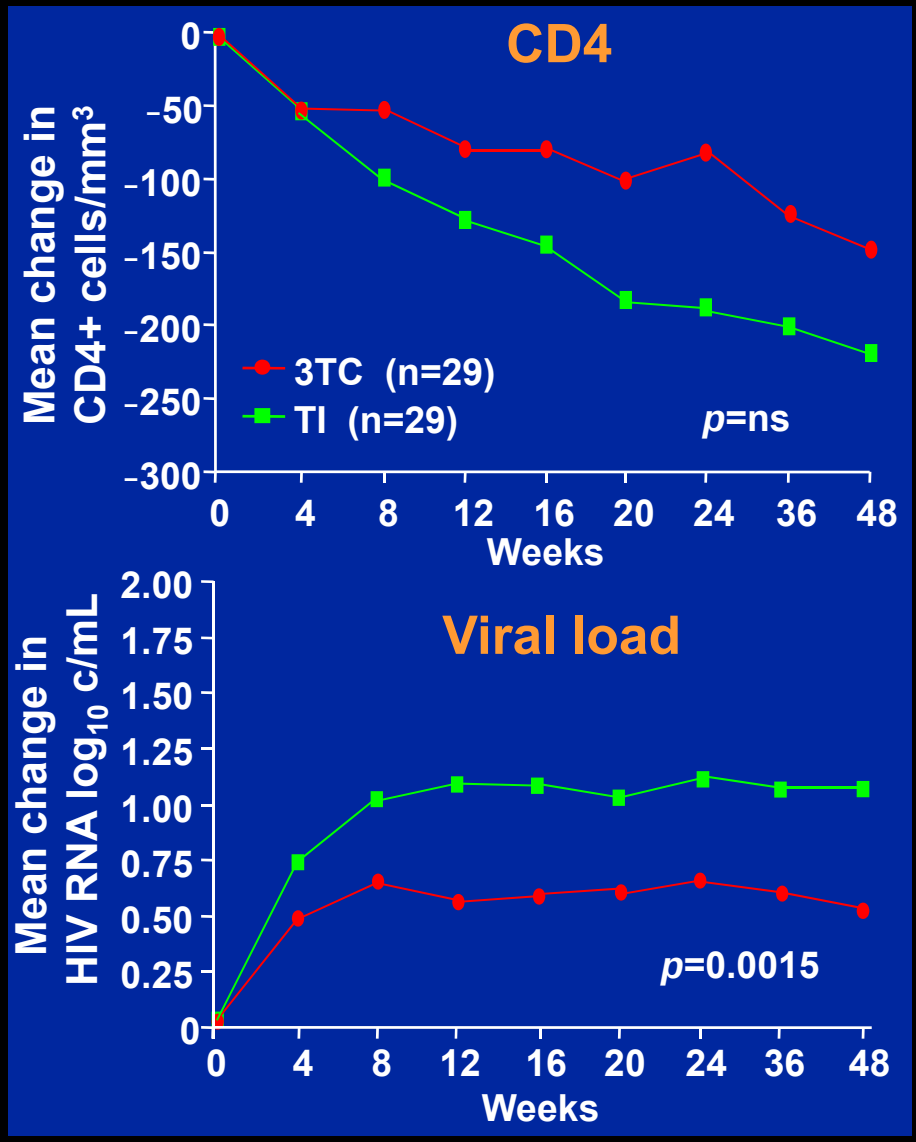


Eron, N Eng J Med 1995

3TC monotherapy *vs* treatment interruption (TI) in patients with resistance [M184V]

- Open-label study
- Patients on 3TC-based therapy with VL>1000, CD4 >500, M184V
- Randomized to 3TC or TI





Castagna A, AIDS 2006

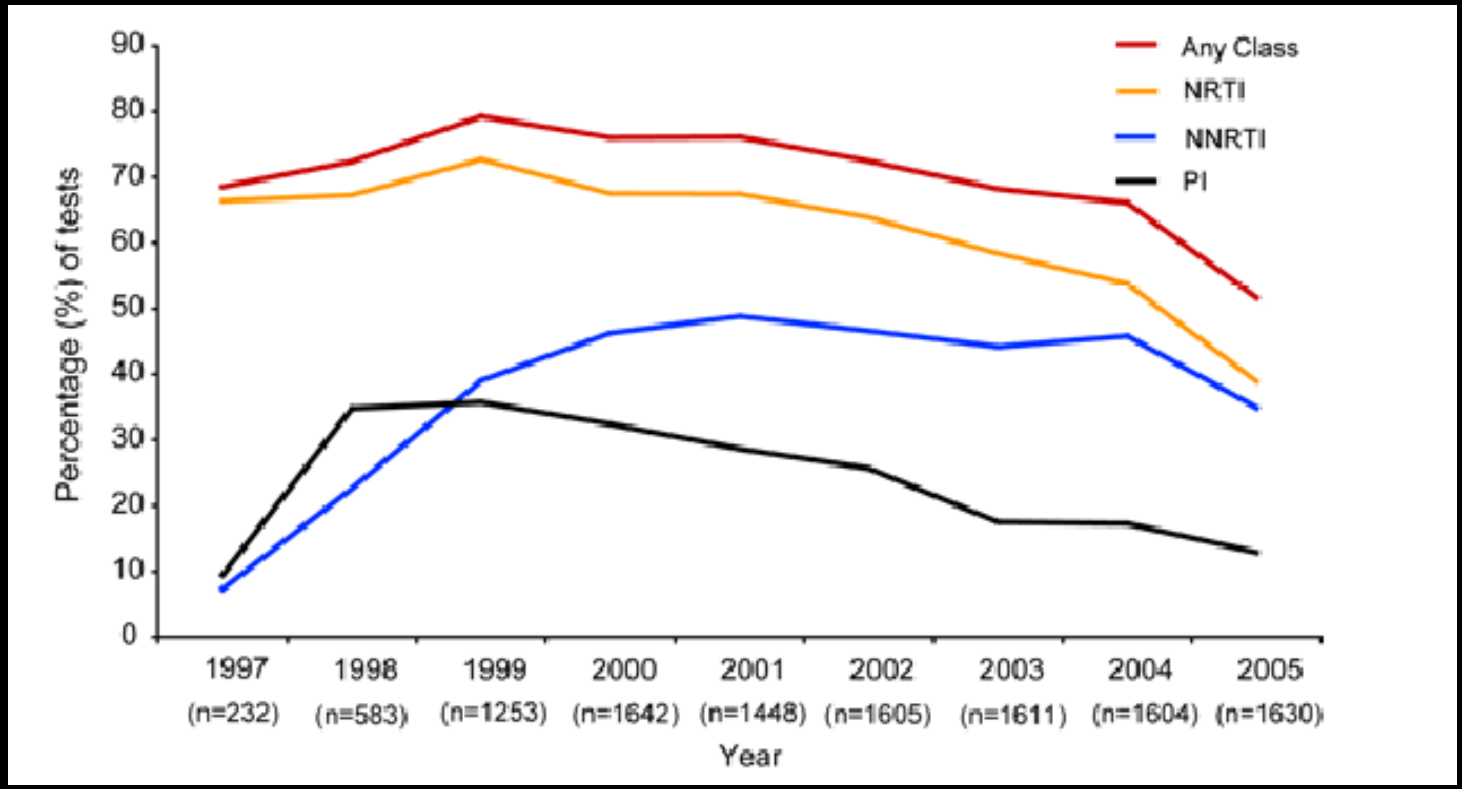
Clinical implications

- Resistance to most NRTIs and PIs is a continuum
- Increasing PI exposure can overcome low to intermediate levels of resistance
- Intermediate levels of drug activity can play an important part in a successful drug regimen
- Resistance can sometimes result in a clinical benefit
  - Mechanisms include:
  - *Residual antiviral activity*
  - *Reduced viral fitness*
  - *Immunological benefit*
  - *Hypersusceptibility effects*

Summary: Determinants of drug resistance in treated patients

- Adherence
- Suboptimal drug levels
- Poor drug potency
- Previous mono or dual therapy
- Genetic barrier of regimen
- Frequency of monitoring
- Management of treatment failure
- Detection method
- Infection with resistant virus

Trends in treatment-associated resistance

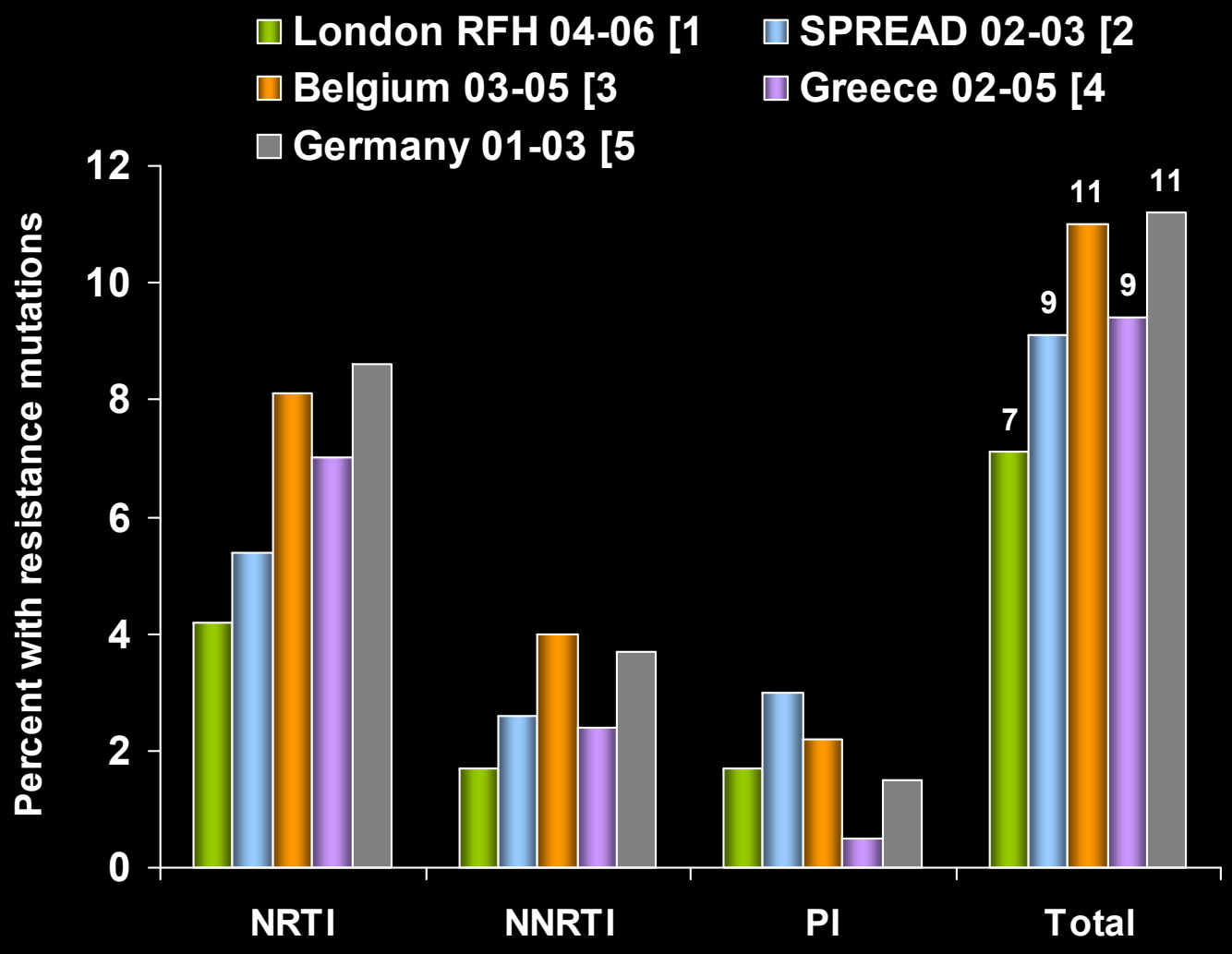


Resistance to at least one drug class: 52%

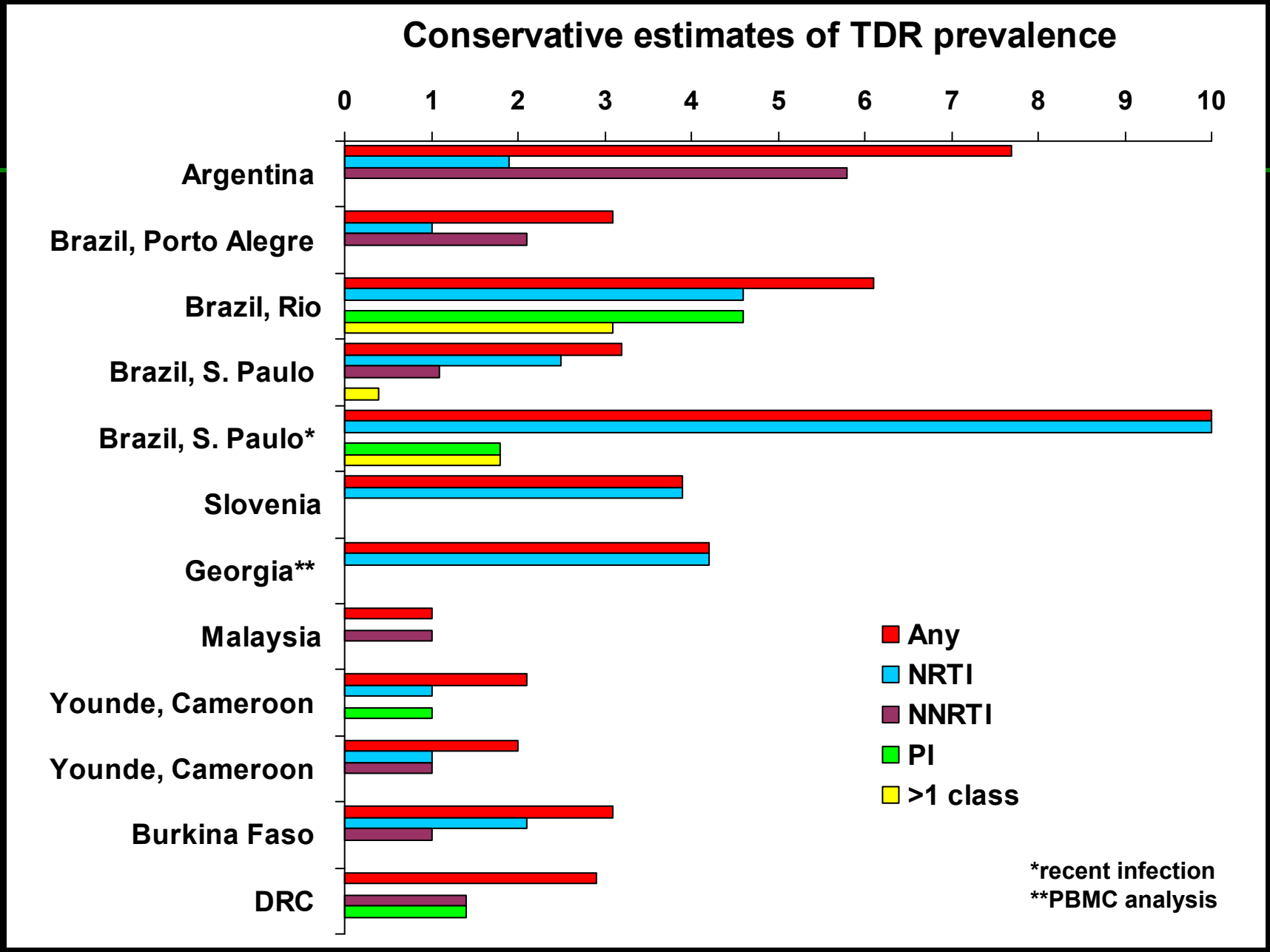
Triple class resistance: 11%

Health Protection Agency, Weekly Report August 2007

European surveys of resistance in newly diagnosed patients



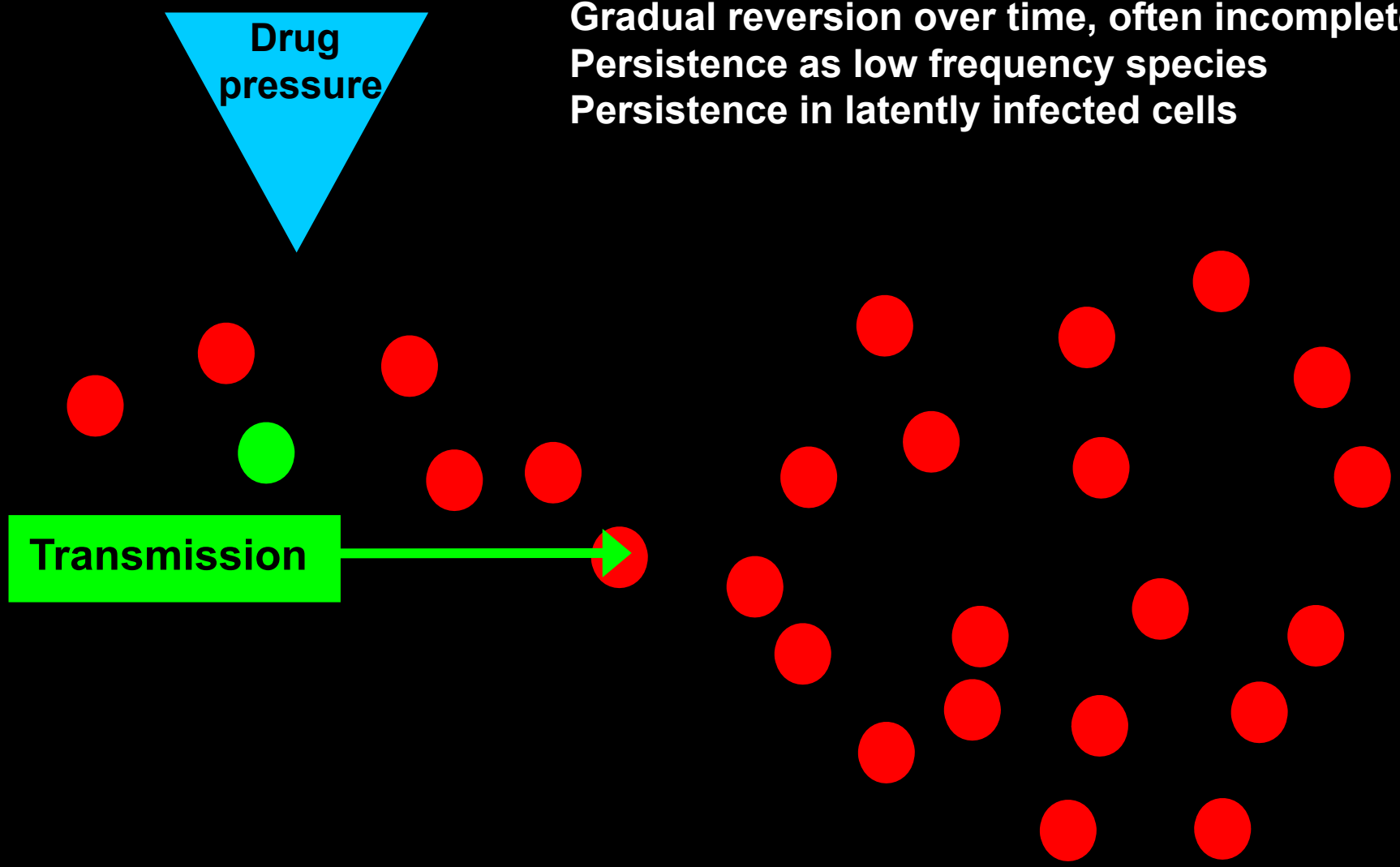
[1]Geretti AM, JAC 2007; [2]Wensing A, 4thERW 2006; [3]Vercauteren, 4th ERW 2006; [4]Paraskevis, 4th ERW 2006; [5]Oette, JAIDS 2006



Geretti AM, Curr Opin Infect Dis 2007

Transmitted drug resistance (TDR)

Stable after transmission
Gradual reversion over time, often incomplete
Persistence as low frequency species
Persistence in latently infected cells



Clinical significance of transmitted drug resistance

- Delayed virological suppression upon starting HAART[1]
- NNRTI TDR increases the risk of treatment failure with NNRTI-based HAART[2]
- NRTI and NNRTI TDR increases the risk of treatment failure in first-line ABC/3TC/EFV or ZDV/3TC/EFV therapy[3]
- TDR reduced the efficacy of NNRTI-based regimens[4]
- Current international guidelines recommend baseline resistance testing
- WHO monitoring TDR in developing countries currently expanding access to antiretroviral therapy

1. Little S, 14th CROI 2007; 2. Kuritzekes D, 14th CROI 2007; 3. Johnson J, 14th CROI 2007; 4. Price H, 4th IAS 2007

- Genotype
  - sequencing of viral genes (reverse transcriptase, protease +/- integrase and envelope and tropism assays)
  - Resistance to specific drugs is *predicted* based on known mutations
  - Requires knowledge of which mutations affect which drugs
- Phenotype
  - Grow virus in culture with various amounts of drugs
  - *Direct measure* of viral resistance
  - Does not explore the underlying mutations, just their affect on the ability of the drug to stop the virus

- Sequence relevant HIV genes and compare result to reference strain

- eg: M 184 V in reverse transcriptase
  - 184 refers to amino acid position 184 in enzyme
  - M (methionine) is the "wild-type" amino acid
  - V (valine) is the "mutant" amino acid

- "Mixtures" are when both WT and mutant amino acids are detected eg M 184 M/V

Relative Advantages

- Wide availability
- Results available in days or weeks*
- Less technically demanding*
- Less expensive*
- FDA approved kits

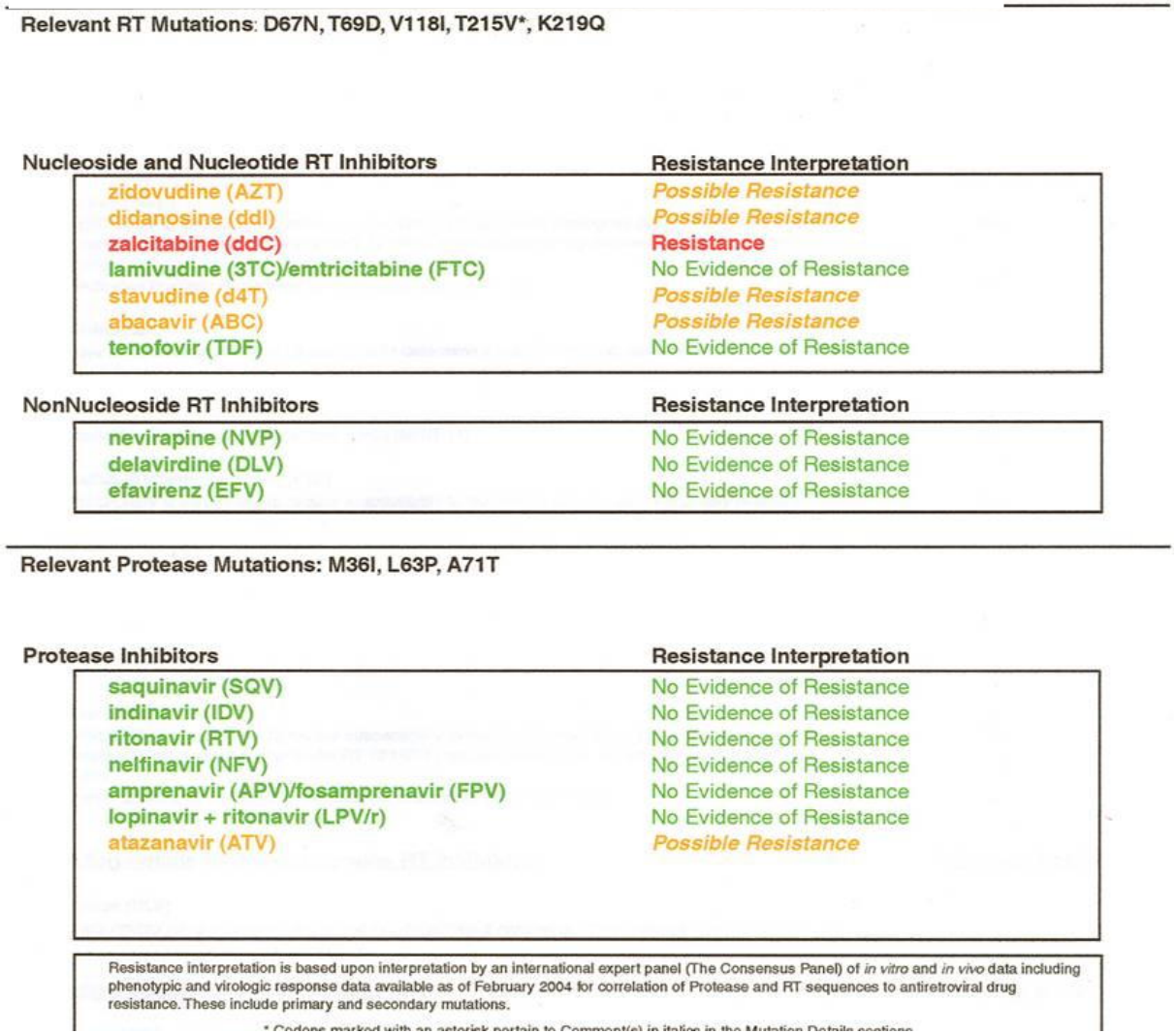
Relative Limitations

- *Indirect* measure of susceptibility
- Expert interpretation required
- Unable to detect overall affect of many mutations
- May miss minor variants

•But even so, still expensive, require technical sophistication,
•turnaround depends on testing volume and
•still uncommonly available for routine clinical care in RCCs

Hirsch. JAMA 1998;279:1984.

Example of Genotype Report



Relevant RT Mutations: D67N, T69D, V118I, T215V*, K219Q

Nucleoside and Nucleotide RT Inhibitors	Resistance Interpretation
zidovudine (AZT)	*Possible Resistance*
didanosine (ddI)	*Possible Resistance*
zalcitabine (ddC)	Resistance
lamivudine (3TC)/emtricitabine (FTC)	No Evidence of Resistance
stavudine (d4T)	*Possible Resistance*
abacavir (ABC)	*Possible Resistance*
tenofovir (TDF)	No Evidence of Resistance

NonNucleoside RT Inhibitors	Resistance Interpretation
nevirapine (NVP)	No Evidence of Resistance
delavirdine (DLV)	No Evidence of Resistance
efavirenz (EFV)	No Evidence of Resistance

Relevant Protease Mutations: M36I, L63P, A71T

Protease Inhibitors	Resistance Interpretation
saquinavir (SQV)	No Evidence of Resistance
indinavir (IDV)	No Evidence of Resistance
ritonavir (RTV)	No Evidence of Resistance
nelfinavir (NFV)	No Evidence of Resistance
amprenavir (APV)/fosamprenavir (FPV)	No Evidence of Resistance
lopinavir + ritonavir (LPV/r)	No Evidence of Resistance
atazanavir (ATV)	*Possible Resistance*

Resistance interpretation is based upon interpretation by an international expert panel (The Consensus Panel) of *in vitro* and *in vivo* data including phenotypic and virologic response data available as of February 2004 for correlation of Protease and RT sequences to antiretroviral drug resistance. These include primary and secondary mutations.

* Codons marked with an asterisk pertain to Comment(s) in italics in the Mutation Details sections

What was missing from that report?

Example of Genotype Report

The current ARV regimen

Relevant RT Mutations: D67N, T69D, V118I, T215V*, K219Q

Nucleoside and Nucleotide RT Inhibitors	Resistance Interpretation
zidovudine (AZT)	*Possible Resistance*
[illegible] (ddI)	*Possible Resistance*
[illegible] (ddC)	Resistance
lamivudine (3TC)/emtricitabine (FTC)	No Evidence of Resistance
stavudine (d4T)	*Possible Resistance*
abacavir (ABC)	*Possible Resistance*
tenofovir (TDF)	No Evidence of Resistance

NonNucleoside RT Inhibitors	Resistance Interpretation
nevirapine (NVP)	No Evidence of Resistance
delavirdine (DLV)	No Evidence of Resistance
efavirenz (EFV)	No Evidence of Resistance

Relevant Protease Mutations: M36I, L63P, A71T

Protease Inhibitors	Resistance Interpretation
saquinavir (SQV)	No Evidence of Resistance
indinavir (IDV)	No Evidence of Resistance
ritonavir (RTV)	No Evidence of Resistance
nelfinavir (NFV)	No Evidence of Resistance
amprenavir (APV)/fosamprenavir (FPV)	No Evidence of Resistance
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* Codons marked with an asterisk pertain to Comment(s) in italics in the Mutation Details sections

General limitations of resistance assays

- Resistance tests are most accurate in assessing the *current* regimen
  - If resistance has ever developed, then archived mutations exist
  - If no drug pressure exists, “wild type” virus will often overgrow the mutant strains
- VL generally at least 1000 copies for most commercial assays
- Mutations are detected only if mutant virus is at least 10-20% of virus population
  - Viral “mixtures” or minor variants can be missed

Resistance Testing in Rx-Naive Patients Should Be Done at Diagnosis

2002
New Dx

	DRUG			PHENOSENSE™ SUSCEPTIBILITY	Evidence of Susceptibility		Comments
	Generic Name	Brand Name	Cutoffs (Lower - Upper)	Fold Change	Pheno Sense	Gene Seq	
NRTI	**Abacavir**	Ziagen	***(4.5)***	**1.0**	Y	Y	
NRTI	**Didanosine**	Videx	***(1.7)***	**0.8**	Y	Y	
NRTI	**Lamivudine**	Epivir	*(2.5)*	**1.2**	Y	Y	
NRTI	**Stavudine**	Zerit	***(1.7)***	**0.9**	Y	Y	
NRTI	**Zalcitabine**	Hivid	*(1.7)*	**0.7**	Y	Y	
NRTI	**Zidovudine**	Retrovir	*(2.5)*	**2.6**	N	N	Mixture(1)
NRTI	**Tenofovir**	Viread	~~*(1.4)*~~	**1.0**	Y	Y	
NRTI	NRTI Mutations		**M41L, T215N/Y**				

Increasing Drug Susceptibility Decreasing (.1 | 1 | 10 | 100)

2005
Still
Rx Naive

	DRUG			PHENOSENSE™ SUSCEPTIBILITY	Evidence of Susceptibility		Net Assessment	
	Generic Name	Brand Name	Cutoffs (Lower - Upper)	Fold Change	Pheno Sense	Gene Seq		
NRTI	**Abacavir**	Ziagen	***(4.5)***	**0.82**	Y	Y	Sensitive	
NRTI	**Didanosine**	Videx	***(1.3)***	**0.95**	Y	Y	Sensitive	
NRTI	**Emtricitabine**	Emtriva	*(3.5)*	**0.73**	Y	Y	Sensitive	
NRTI	**Lamivudine**	Epivir	***(3.5)***	**0.74**	Y	Y	Sensitive	
NRTI	**Stavudine**	Zerit	***(1.7)***	**0.86**	Y	Y	Sensitive	
NRTI	**Zidovudine**	Retrovir	*(1.9)*	**0.77**	Y	Y	Sensitive	14
NRTI	**Tenofovir**	Viread	~~*(1.4)*~~	**0.68**	Y	Y	Sensitive	
NRTI	NRTI Mutations		**T215T/S**					

Increasing Drug Susceptibility Decreasing (.1 | 1 | 10 | 100)

DHHS Guidelines perform genotype

- when managing suboptimal viral load reduction or virologic failure >1000 (? >500 copies/ml)
- in the setting of virologic failure, testing should be done while the patient is on therapy, or within 4 weeks of stopping
- for all pregnant women prior to therapy, or for pregnant women with detectable VL on therapy

Genotype interpretation

- Memorize mutations (not my favourite)
- Pattern recognition
- Most assay reports come with an “expert” interpretation included, but be careful
  - http://hivdb.stanford.edu is a reliable resource
- Limited by complex interactions of multiple mutations, especially for protease inhibitors and TAMs
- Newer drugs come with “prediction rules”

Mutations Selected by nRTIs

Abacavir		K 65 R			L 74 V	Y 115 F	M 184 V			
Didanosine		K 65 R			L 74 V					
Emtricitabine		K 65 R					M 184 V I			
Lamivudine		K 65 R					M 184 V I			
Stavudine	M 41 L	K 65 R	D 67 N	K 70 R				L 210 W	T 215 Y F	K 219 Q E
Tenofovir		K 65 R		K 70 E						
Zidovudine	M 41 L		D 67 N	K 70 R				L 210 W	T 215 Y F	K 219 Q E

Mutations Selected by 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 F	Q 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)

M			D					K											L	T	K
41			**67**					**70**											**210**	**215**	**219**
L			N					R											W	Y F	Q E

- M184V: Resistance to lamivudine and emtricitabine
  - Some resistance to didanosine and abacavir
  - May restore some activity to zidovudine and tenofovir
- K65R: Signature tenofovir mutation
  - Also resistance to abacavir, stavudine, didanosine
- L74V: Resistance to abacavir and didanosine
- Thymidine analogue mutations (TAMS): all NRTIs
- Even with NRTI resistance, most NRTI retain some activity and may help suppress HIV

Thymidine analogue mutations

- Zidovudine and thymidine are thymidine analogues
- Share common resistance patterns (TAMs)
- Common mutations include M41L, L210W, T215Y, D67N, K70R, K219Q/E/N
- TAMs emerge via one of two pathways
  - Pattern 1: 41, 201, 215: confers resistance to ALL NRTIs + tenofovir
  - Pattern 2: 67, 70, 219: confers resistance mainly to ZDV and d4T

Review: Cohen CJ et al *Primer on interpretation of HIV drug resistance testing* UpToDat

- At least 3 TAMs before completely lose virologic activity of ZDV or d4T

- Some NRTI mutations eg T215Y can lead to hypersensitivity of HIV to NNRTIs[1]
  - Nevirapine, efavirenz and etravirine
  - Can improve virologic response to NNRTIs

- M184V appears to delay emergence of TAMs

1. Clark SA et al AIDS 2006 20 981

M184V

- Most prevalent mutation in treated patients
- Often first mutation to appear
- Causes high level resistance to lamivudine and emtracitabine
- Modest reduction in susceptibility to abacavir and ddI
- Confers hypersusceptibility to zidovudine, stavudine and tenofovir
  - Partially reverses resistance to these drugs
- HIV with this mutation is "less fit"
  - Continuing lamivudine with this mutation leads to persistent mean 0.5 log decrease in VL

Johnson V et al. Topics in HIV Medicine 2010 18: 156
Thompson MA et al JAMA 2010 304:321

K65R

- Signature mutation for tenofovir resistance
- Also found in 4-11% of pts with non subtype B HIV failing stavudine and not on tenofovir[1].
- Confers cross resistance to abacavir, stavudine and ddI
  - Particularly with non subtype B
  - However like M184V it is associated with ↓ viral fitness
    - This may be additive with M184V
  - Also K65R induces hypersusceptibility to zidovudine
    - Like M184V
- 3 or more TAMs (including M41L, L210W, T215Y) also confers resistance to tenofovir
  - Presence of TAMs in patients on tenofovir may decrease likelihood of K65R selection[3]
- Emtricitabine may protect against emergence of K65R[2]

1. Wallis C et al JAIDS 2010; 53 480
2. Gallant JE et al NEJM 2006 354 251
3. Von Wyl V et al Clin Infect Dis 200846 1299

Multinucleoside resistance mutations

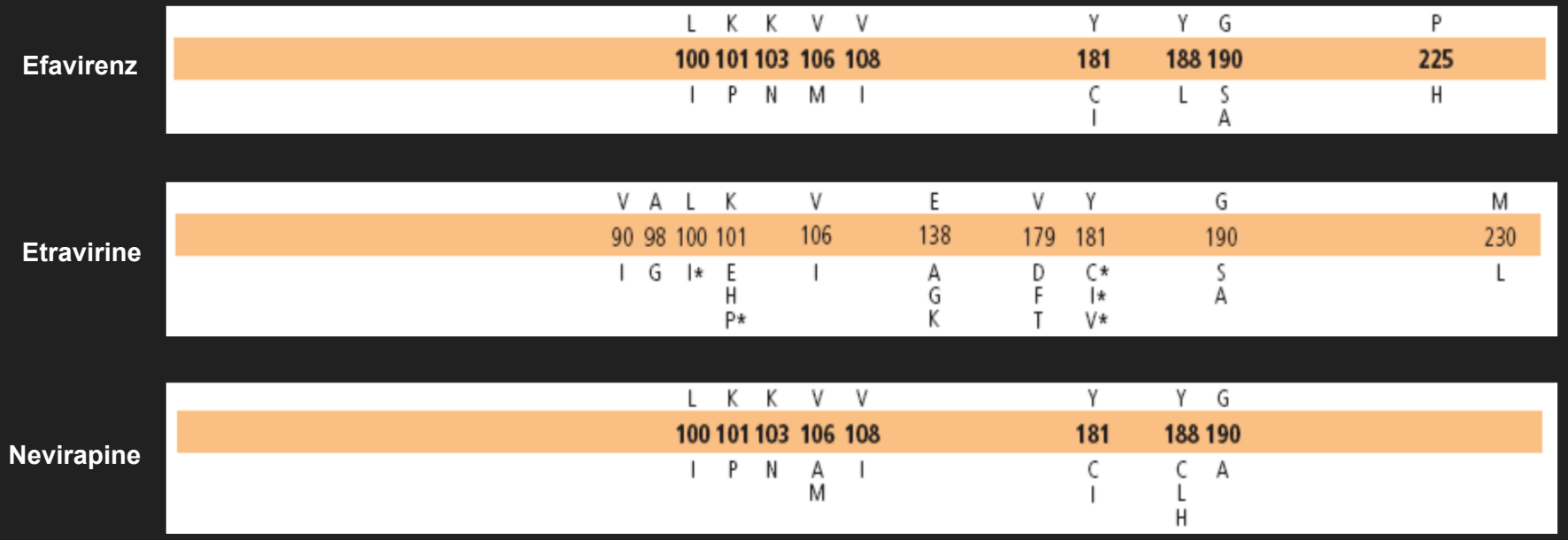
- Q151M complex
  - Resistance to all NRTIs except tenofovir
- T69 insertion mutation (69S)
  - Resistance to all NRTIs including tenofovir when accompanied by TAMs at codons 41, 210 or 215
- These mutations are usually selected by NRTI backbones that include didanosine plus either zidovudine or stavudine
  - Less commonly seen now in USA/Europe/Australia
  - ? prevalent in countries where d-drugs are used

Miller MD et al J Infect Dis 2004 189 937

Mutations associated with loss of protease inhibitor susceptibility

- PIs when given with low dose ritonavir have a high genetic barrier to resistance
- Usually need multiple mutations to accumulate before decrease in drug susceptibility
- Can be classified as primary (major) or secondary (minor) mutations
  - Primary mutations emerge first, reducing antiviral activity
  - Secondary mutations typically emerge later in the presence of continued drug pressure when on a non-suppressive regimen
  - May increase replicative fitness of strains carrying primary mutations
- Most data on PI mutations come from studies of unboosted Pis
- When ritonavir-boosted PIs are used by patients with no pre-existing mutations, treatment failure is rarely associated with PI resistance

Mutations Selected by NNRTIs



Major nnRTI mutations

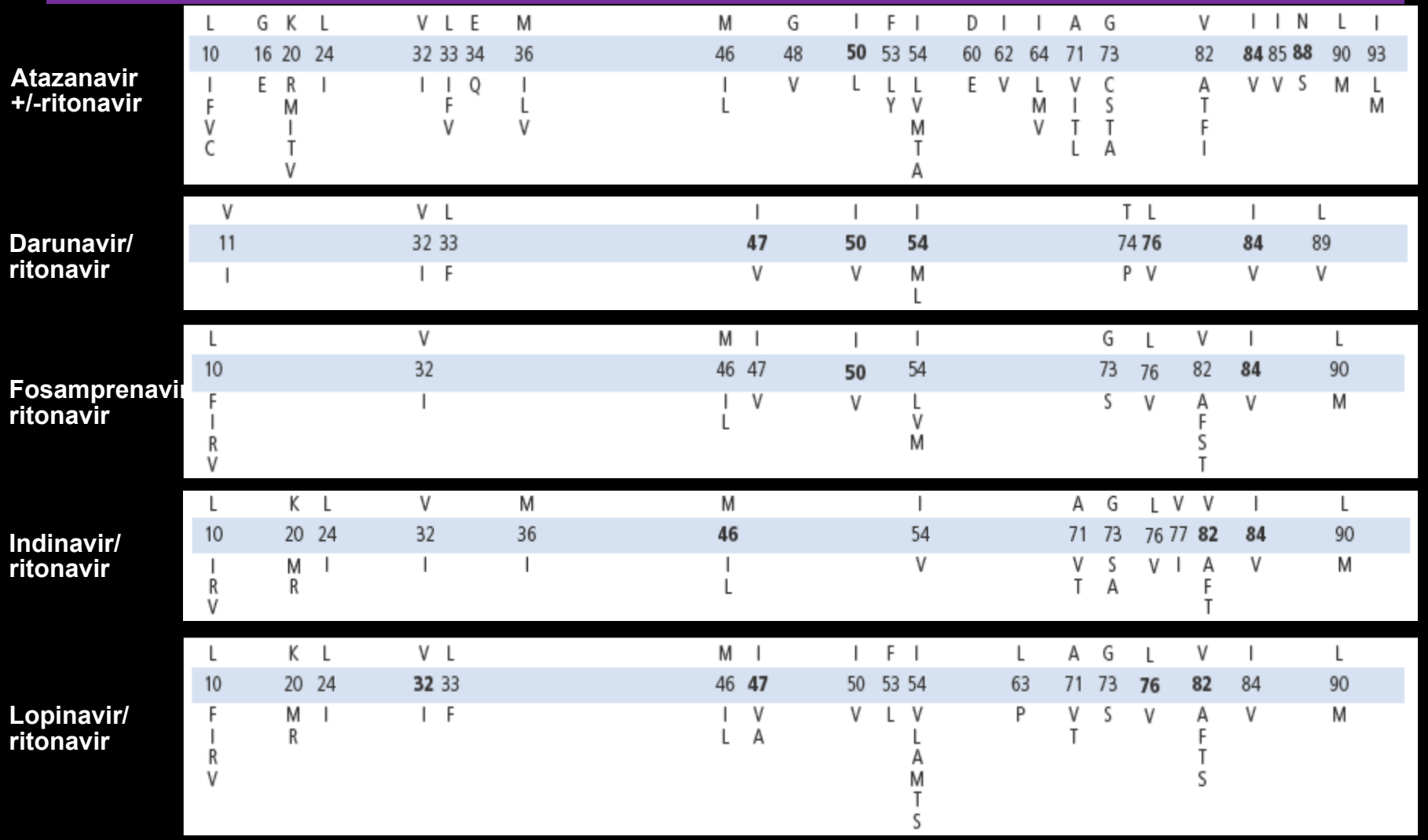
- K103N
  - Most common nnRTI mutation
  - High level resistance to efavirenz / nevirapine
  - Not etravirine
- Y181C
  - High level resistance to nevirapine, intermediate efavirenz
  - Some etravirine resistance

Y181C

- Nevirapine and efavirenz have a low genetic barrier to resistance
  - Significant cross resistance between NVP and EFV
  - A single mutation can confer cross resistance
- Y181C and K103N are the most common mutations seen with NNRTI use
  - Y181C is selected more commonly by nevirapine
  - K103N is selected more commonly by efavirenz
  - Both lead to high level resistance
- Etravirine is a 2nd generation NNRTI: Higher genetic barrier to resistance

Johnson V et al. Topics in HIV Medicine 2010 18: 156
Thompson MA et al JAMA 2010 304:321

Mutations Selected by PIs



Mutations Selected by PIs (cont)

Nelfinavir

L	D	M	M	A	V	V	I	N	L
10	**30**	36	46	71	77	82	84	88	**90**
F I	N	I	I L	V T	I	A F T S	V	D S	M

Saquinavir/ ritonavir

L	L	G	I	I	A	G	V	V	I	L
10	24	**48**	54	62	71	73	77	82	84	**90**
I R V	I	V	V L	V	V T	S	I	A F T S	V	M

Tipranavir/ ritonavir

L	L	M	K	M	I	I	Q	H	T	V	N	I	L
10	33	36	43	46	**47**	54	**58**	69	**74**	**82**	**83**	**84**	89
V	F	I L V	T	L	V	A M V	E	K R	P	L T	D	V	I M V

- "Signature" mutations for non-boosted PI
  - D30N: nelfinavir
  - I50L: atazanavir
  - I50V: fosamprenavir
- Boosted PIs usually do not select for mutations if used as first PI with NRTIs
  - Otherwise may have broad general cross resistance
- Gets very complicated very quickly

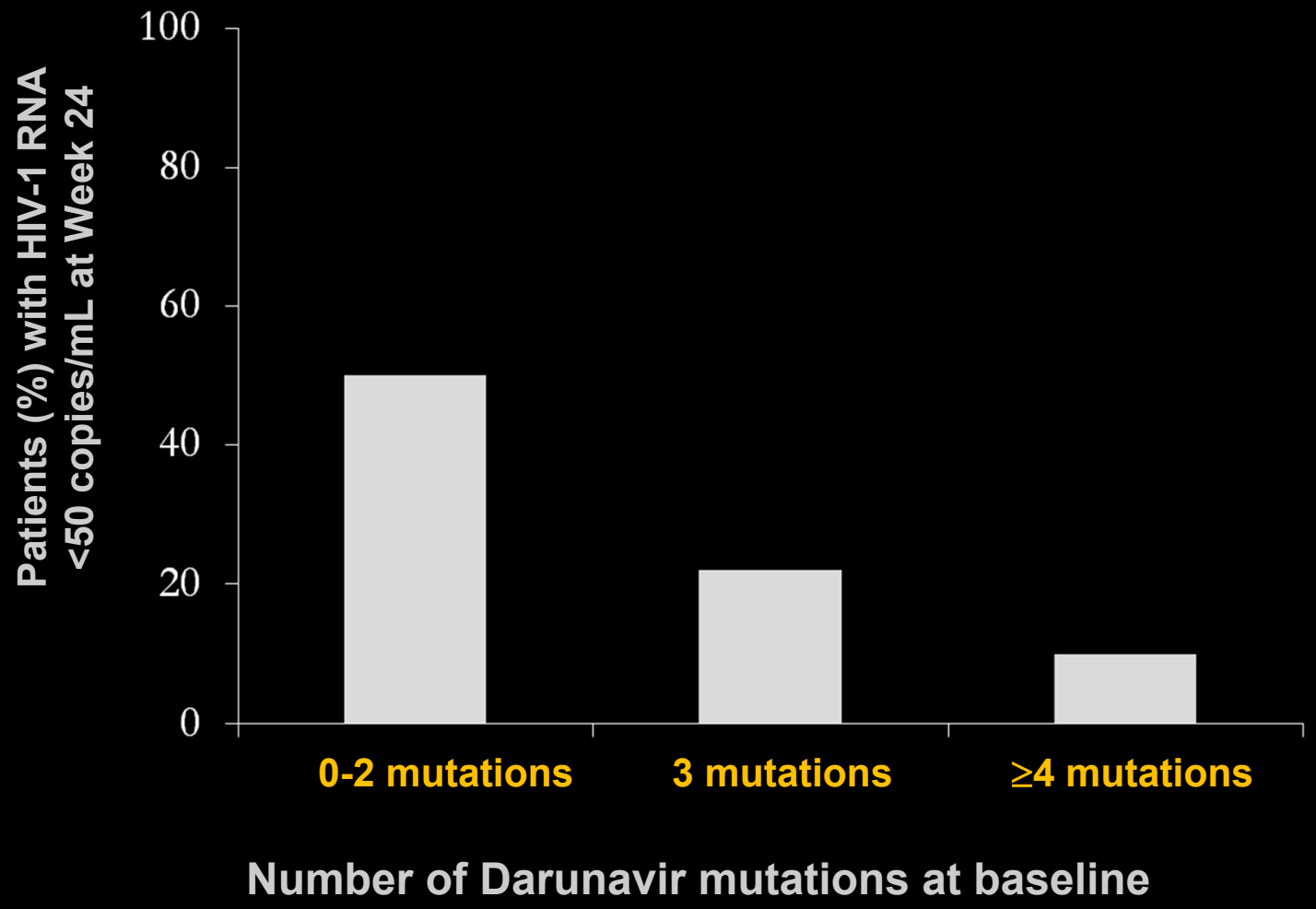
Newer protease "mutation score"

- Darunavir is a newer PI with activity against resistant HIV

- POWER studies showed patients treated with darunavir and optimized background Rx had VL < 50 copies/mL better than comparator PIs
- Response to darunavir was found to be dependent on 11 PI mutations at baseline

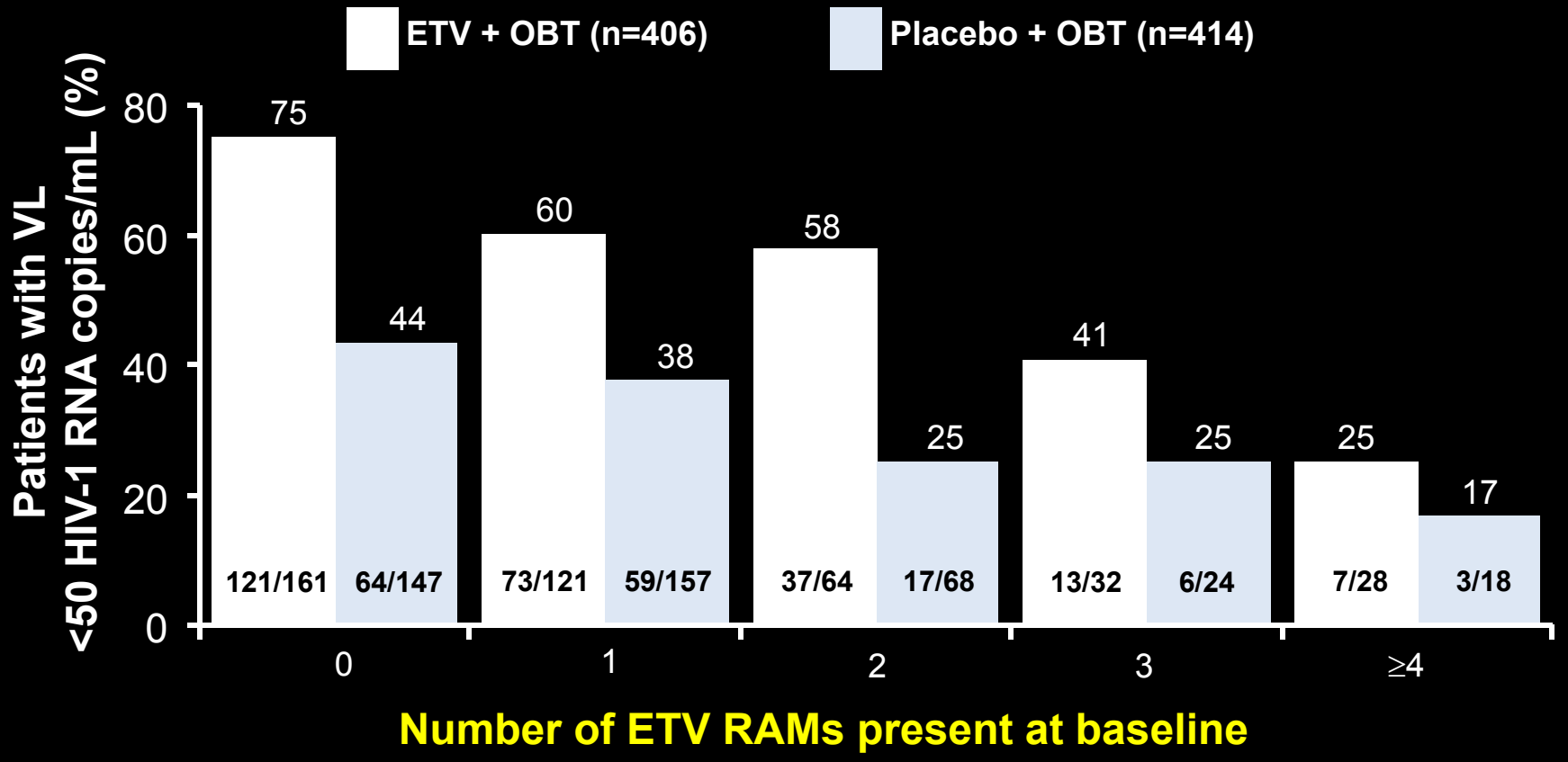
V	V	L	I	I	I	T	L	I	L
11	32	33	**47**	**50**	**54**	74	**76**	**84**	89
I	I	F	V	V	M L	P	V	V	V

Darunavir response by DRV score



Impact of baseline resistance in DUET

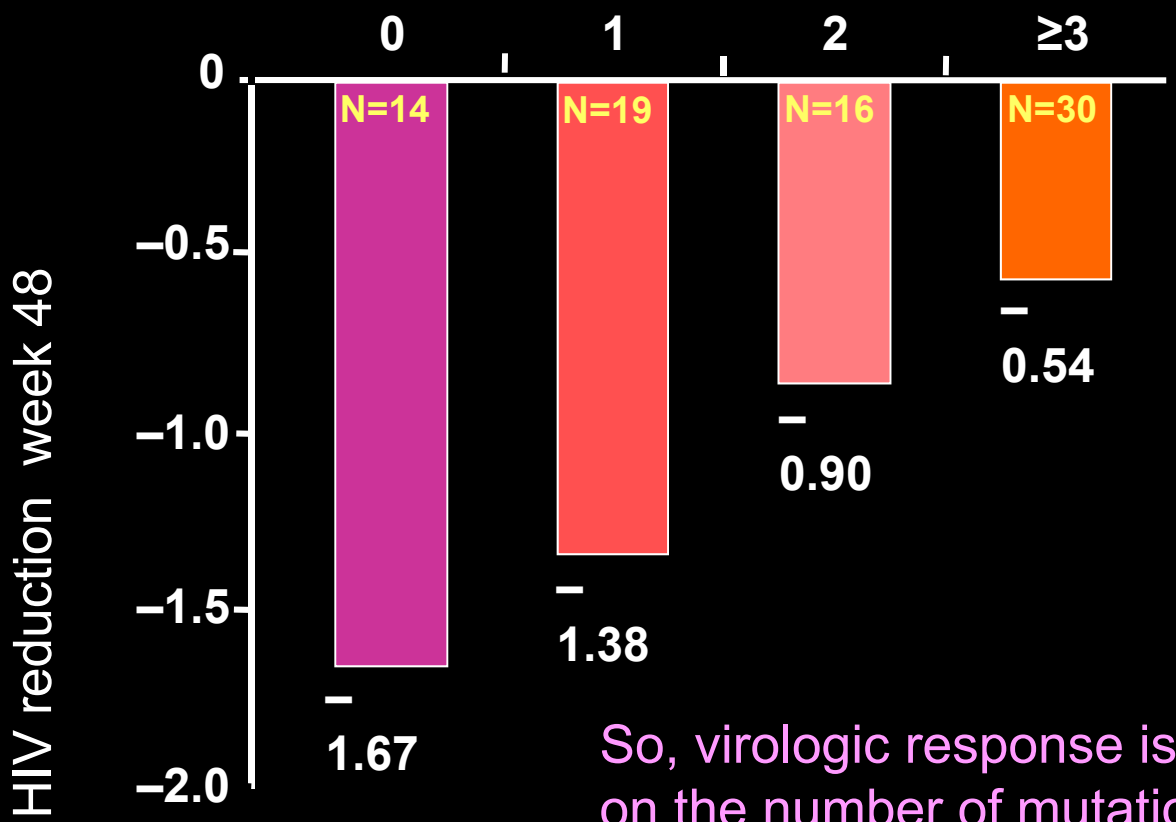
number of baseline ETV RAMs correlated with the virologic response



3 or more ETV resistance associated mutations give a reduced response to ETV

Vingerhoets J, et al. 11th EACS 2007. Abstract P7.3/05

Etravirine (TMC 125): Baseline resistance predicts virological response



So, virologic response is dependent on the number of mutations at baseline

Mutations in the Integrase Gene Associated With Resistance to Integrase Inhibitors

Raltegravir	E	Y	Q	N
	92	**143**	**148**	**155**
	Q	R H C	H K R	H

Raltegravir resistance

- Raltegravir failure is associated with integrase mutations in at least 3 genetic pathways defined by at least 2 mutations
  - Major mutation at 148, 155, or 143
  - One or more additional minor mutations

- Most common and highest degree of resistance is Q148H plus G140S

How adherent do you have to be to prevent resistance?

- Old days: 95% adherence with unboosted Pis necessary for full virologic suppression[1]
- Recently: boosted PIs (+EFV) seem more forgiving[2,3]
  - ? Longer half life, higher plasma levels
- Adherent only 70-89% of time
  - strongly associated with viral rebound and clinically significant resistance[4]
- *NB!* Patient self-reported adherence overestimated by as much as 20%[5]

1. Paterson DL et al Ann Int Med 2000 133 21;
2. Bangsberg DR et al Clin Infect Dis 2006 43 939
3. Raffa et al J Infect Dis 2008 47 397.
4. Sethi AJ et al Clin Infect Dis 2003 37 1112.
5.Arnsten JH et al Clin Infect Dis 2001 33 1417

Conclusions

- Resistance can occur in patients naïve 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

Resistance database initiative: Opportunities for patient care & research

Brendan Larder

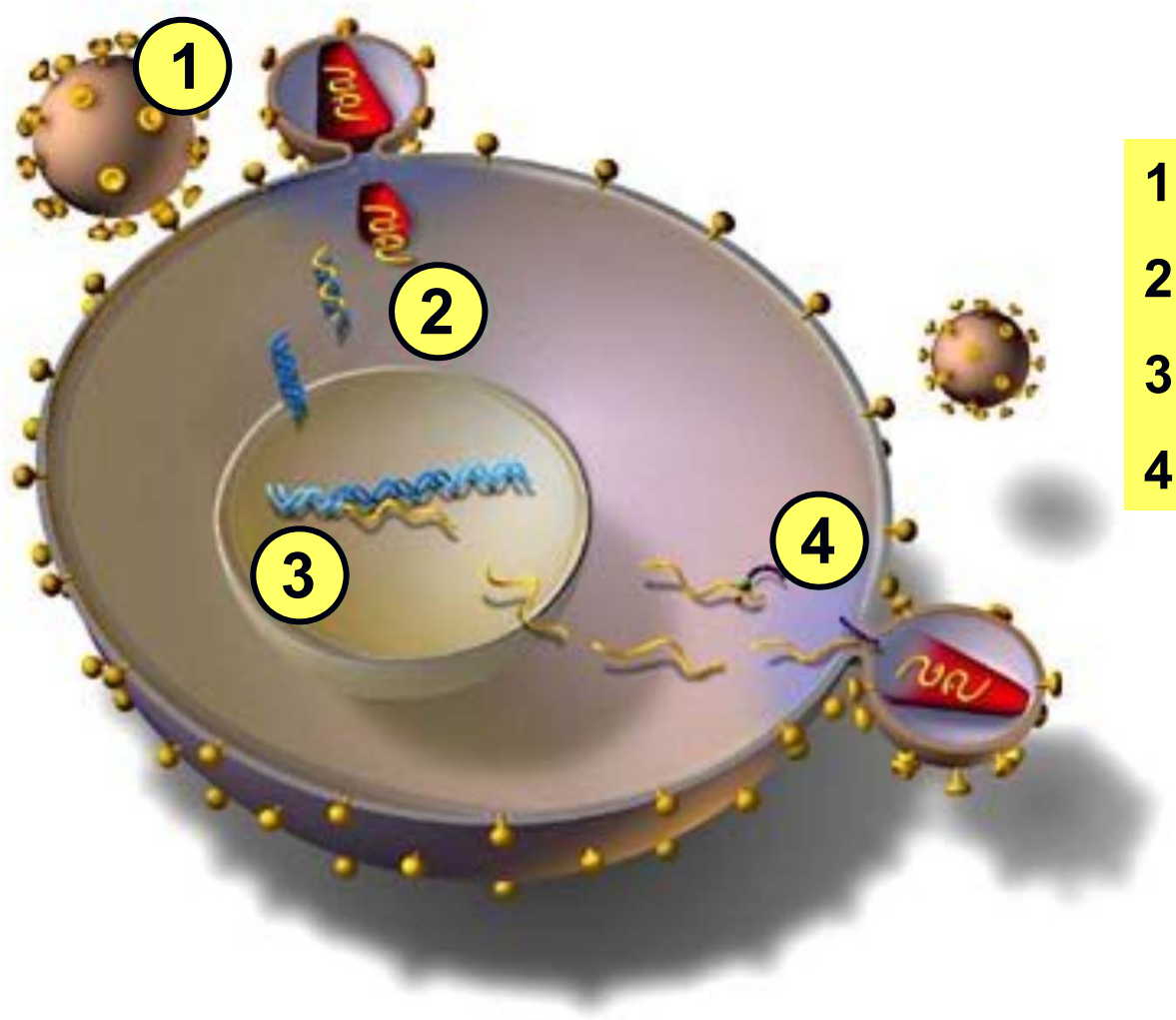
HIV Resistance Response Database Initiative

UK

Content

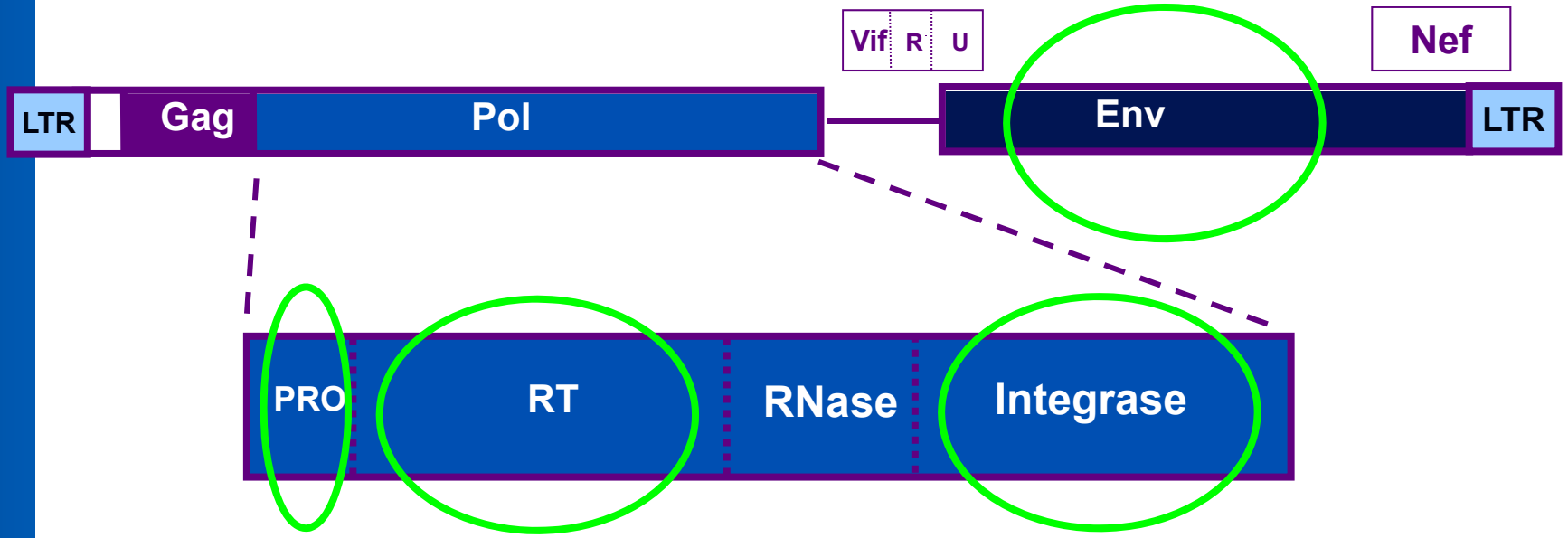
1. Background to HIV drug resistance
2. The RDI and its approach
3. Overview of our work to date
4. Modelling response to treatment in resource-limited settings
5. Future plans

HIV-1 Life Cycle



1. Attachment
2. RT
3. Integration
4. Maturation

Therapeutic Targets



Approved antiretroviral drugs: 1987

Zidovudine - AZT

Approved antiretroviral drugs: 2010

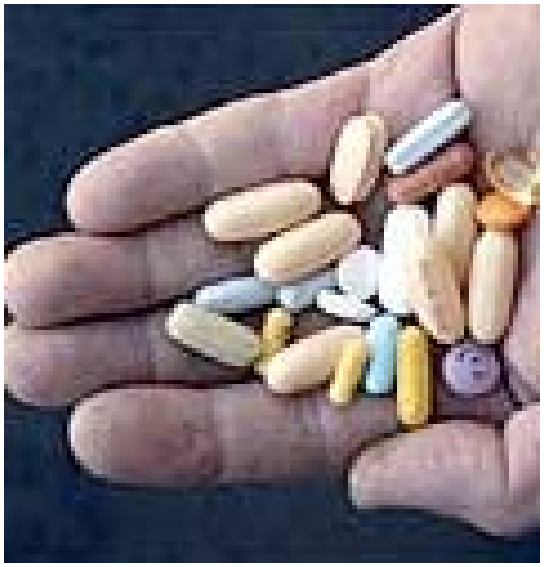
NRTIs		NNRTIs		Protease Inhibitors	
Abacavir	ABC	Delavirdine	DLV	Amprenavir	APV
Didanosine	ddI	Efavirenz	EFV	Atazanavir	ATV
Emtricitabine	FTC	Etravirine	ETR	Darunavir	DRV
Lamivudine	3TC	Nevirapine	NEV	Fosamprenavir	FPV
Stavudine	d4T			Indinavir	IDV
Tenofovir	TDF			Lopinavir/r	LPV
Zidovudine	ZDV			Nelfinavir	NFV
				Ritonavir	RTV
Fixed-Dose Combinations		**Entry Inhibitors**		Saquinavir	SQV
ZDV/3TC		Enfuvirtide	ENF	Tipranavir	TPV
ABC/3TC		Maraviroc	MVC		
TDF/FTC					
ABC/ZDV/3TC		**Integrase Inhibitor**			
EFV/TDF/FTC		Raltegravir	RAL		

Approved antiretroviral drugs - in practice

NRTIs		NNRTIs		Protease Inhibitors	
Abacavir	ABC	~~Delavirdine~~	~~DLV~~	~~Amprenavir~~	~~APV~~
~~Didanosine~~	~~ddI~~	Efavirenz	EFV	Atazanavir	ATV
Emtricitabine	FTC	Etravirine	ETR	Darunavir	DRV
Lamivudine	3TC	Nevirapine	NEV	Fosamprenavir	FPV
~~Stavudine~~	~~d4T~~			~~Indinavir~~	~~IDV~~
Tenofovir	TDF			Lopinavir/r	LPV
Zidovudine	ZDV			~~Nelfinavir~~	~~NFV~~
				Ritonavir	RTV
Fixed-Dose Combinations		**Entry Inhibitors**		~~Saquinavir~~	~~SQV~~
ZDV/3TC		~~Enfuvirtide~~	~~ENF~~	~~Tipranavir~~	~~TPV~~
ABC/3TC		Maraviroc	MVC		
TDF/FTC		**Integrase Inhibitor**			
ABC/ZDV/3TC		Raltegravir	RAL		
EFV/TDF/FTC					

Change in pill burden

1996



3x Daily

2010

Once Daily

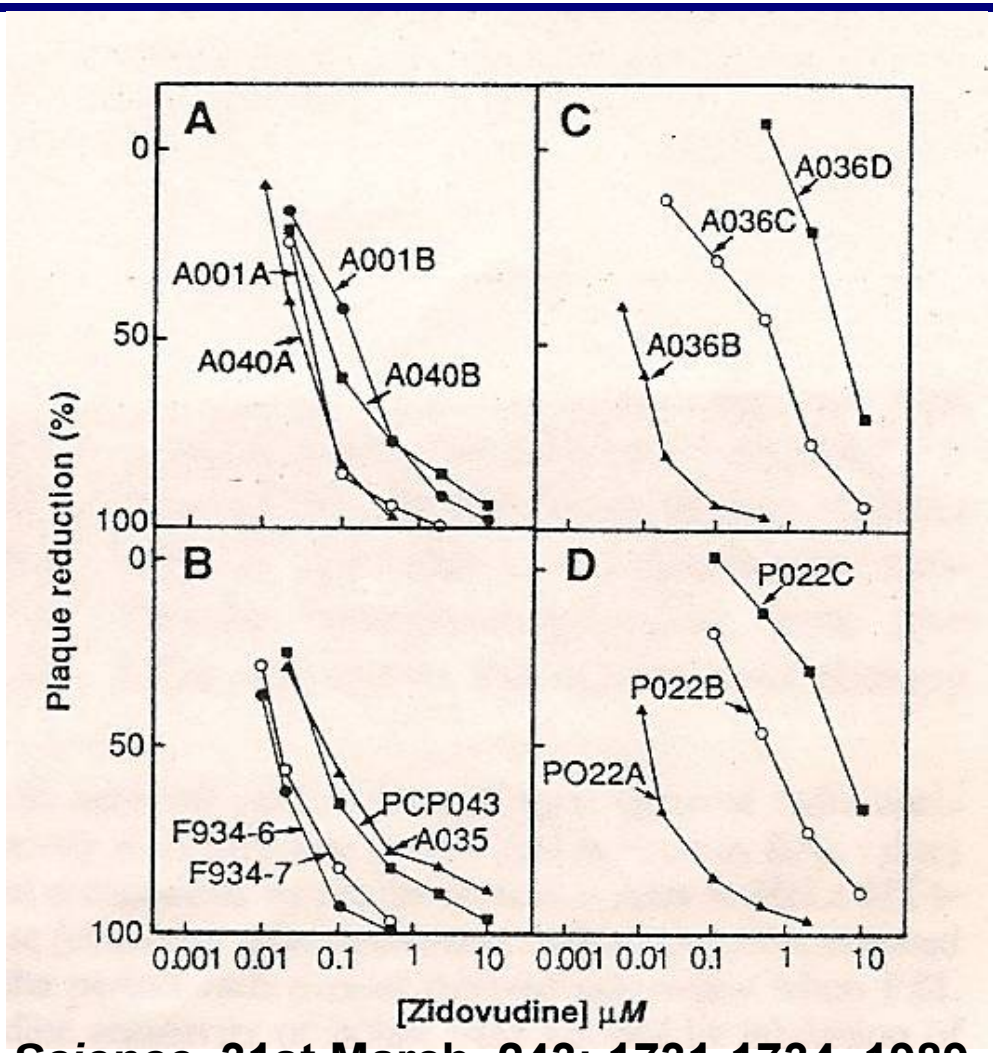
HIV drug resistance

- Resistance is an inevitable consequence of sub-optimal drug therapy
- Partial suppression of HIV promotes resistance (via selection of mutations)
  - Sub-optimal dosing of drugs
  - Patient non-adherence to ARVs, etc.
- Transmission of drug-resistant virus is now fairly common

Measuring Resistance

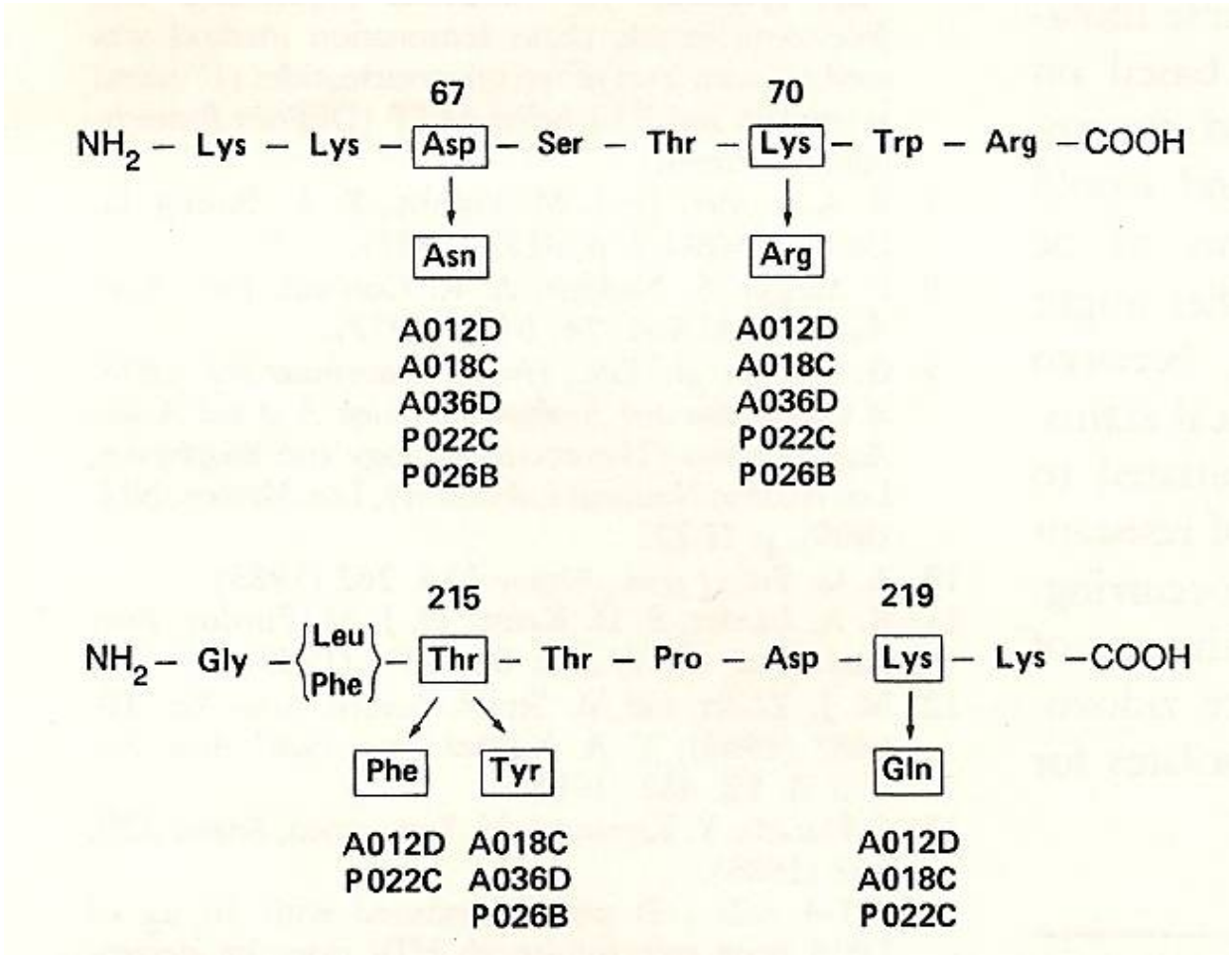
- **Phenotyping**
  - Measured by growing HIV in cells in the presence of different amounts of drug
  - Single or multiple round recombinant assays are used
- **Genotyping**
  - DNA sequencing
  - BUT… viral mutations detected by this test require interpretation

AZT resistance development - progressive & to high-levels



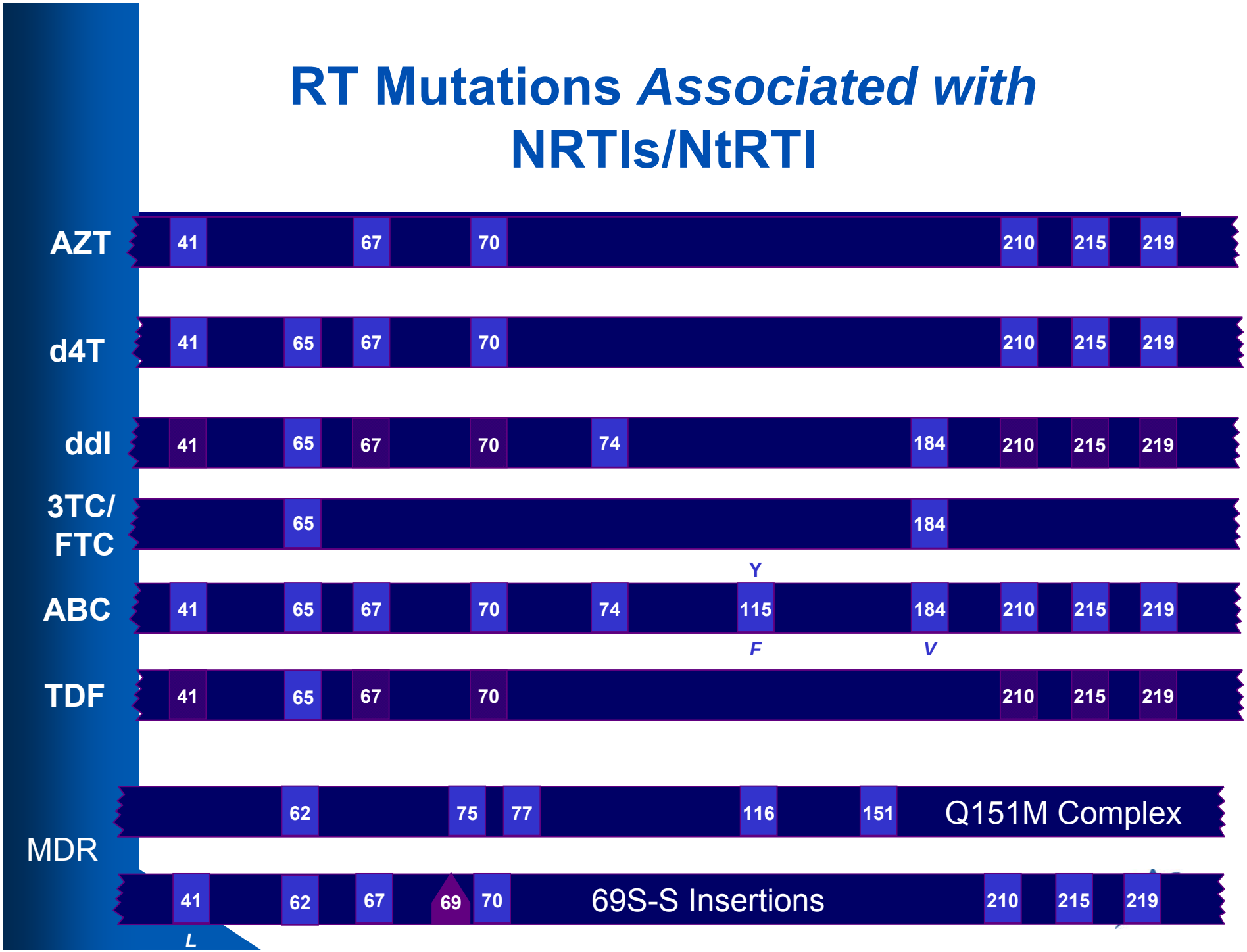
***Science*, 31st March, 243; 1731-1734, 1989**

Specific AZT mutations: the beginning of 'genotyping'

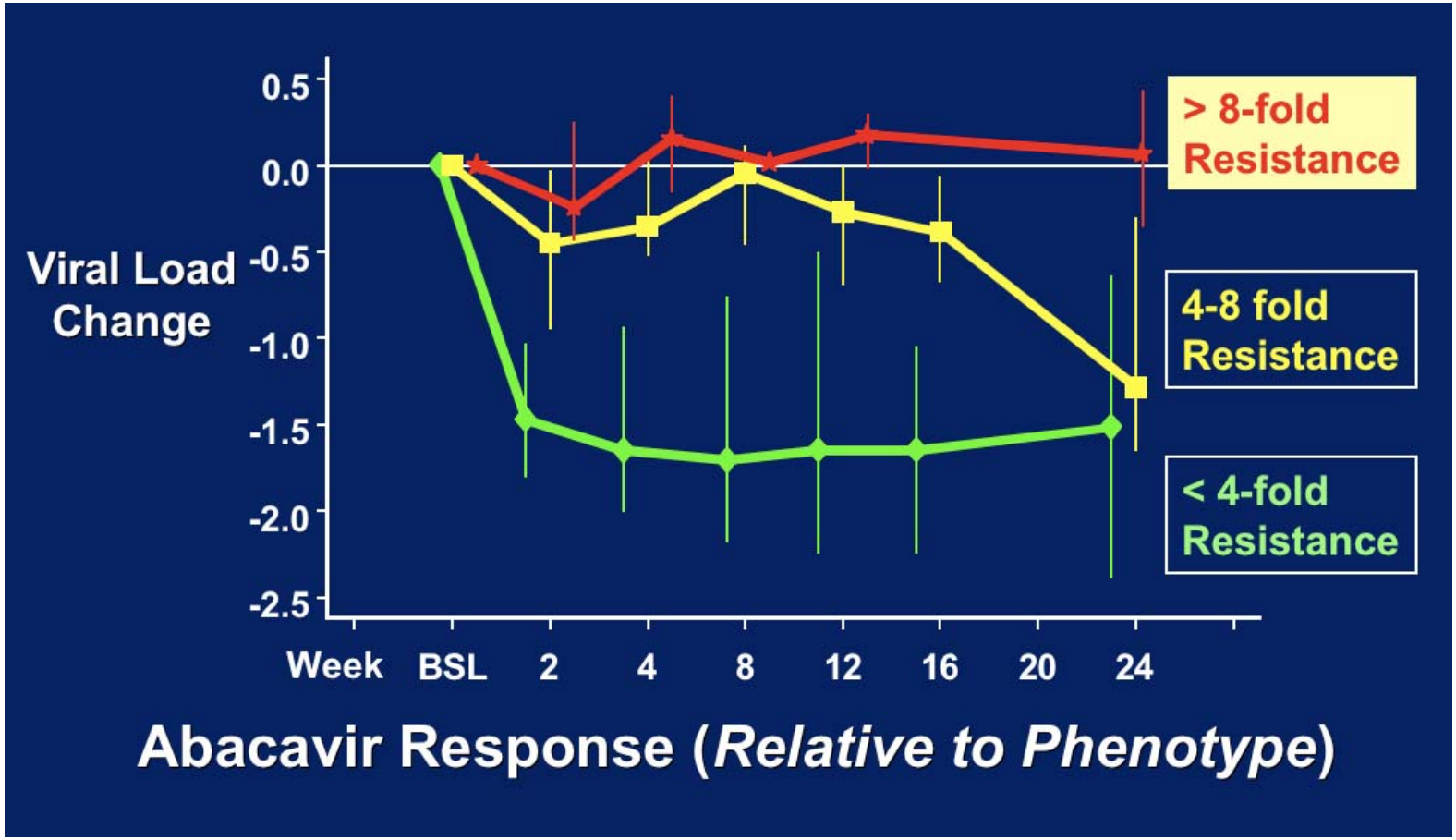


Science, 1st Dec, 246; 1155-1158, 1989

RT Mutations *Associated with* NRTIs/NtRTI



Resistance blunts clinical response



RDI: HIV Resistance Response Database Initiative

Objectives:

- To establish an independent global database of clinical response data for modelling treatment response
- Use computational modelling to predict ***quantitative virological response to combination therapy directly from genotype & other information***
- To harness the models as a free treatment selection support tool on the internet
- To improve treatment decision-making, patient outcomes & save drugs/budgets

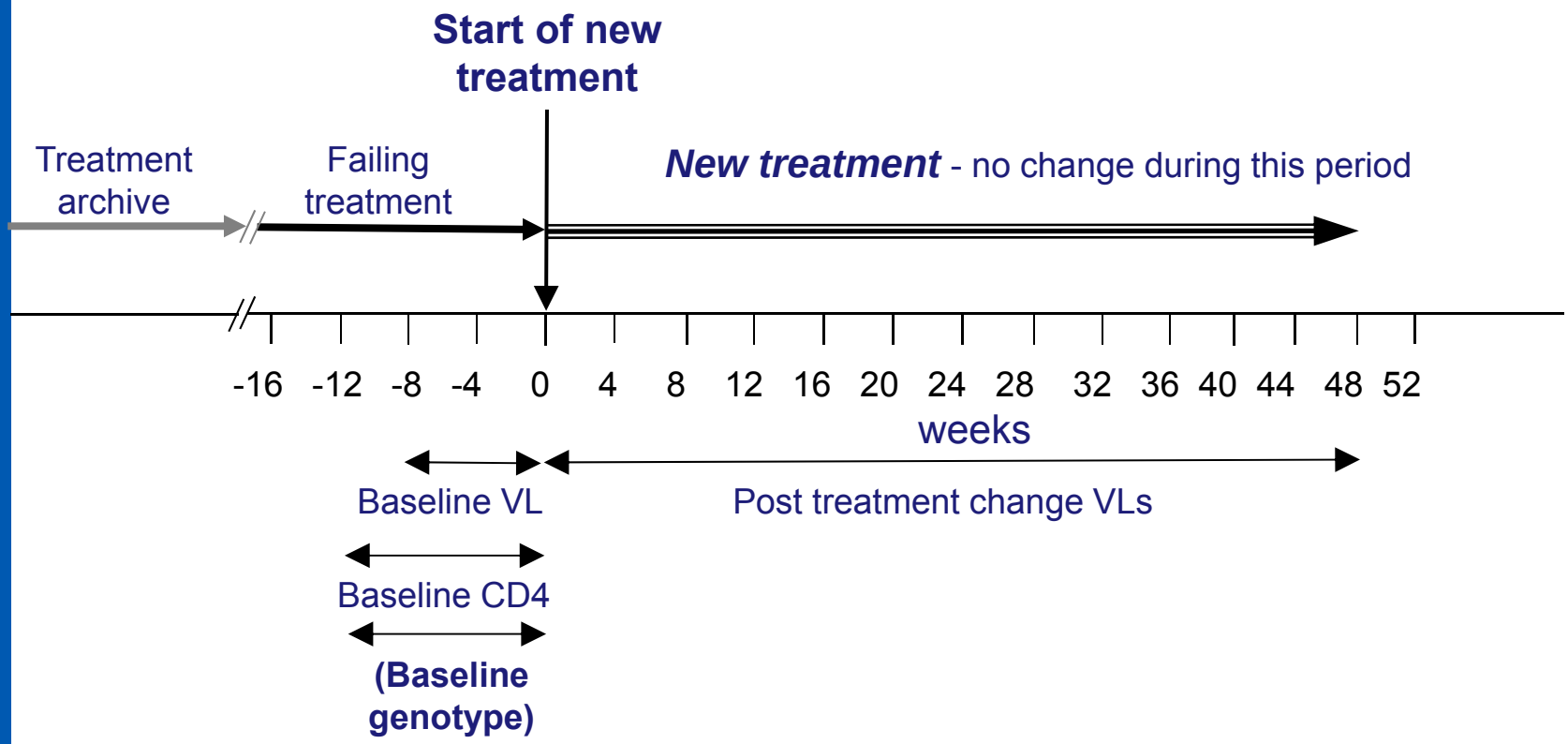
RDI status

- Established as a not-for-profit in 2002
- Data collected from ≈ 60,000 patients from 20 main sources & several hundred clinics in 15 countries
- Dozens of studies over 8 years optimising methods

Why use computational modelling?

- Useful where there are complex, non-linear interactions between multiple variables
- High-level computer models ‘learn’ by example
  - in this case from extensive, real clinical data
- The models can give ***quantitative*** predictions of viral load response to drug ***combinations***
- Main methods investigated:
  - artificial neural networks (**ANN**), random forests (**RF**),

The Treatment Change Episode (TCE)



What do the models predict & how can we assess their accuracy?

- **The absolute viral load after treatment change**
  - Correlation between predicted and actual virological response using an independent test set of different patients (Δ viral load)
- **Whether or not viral load will be above or below 50 copies/ml** (the current 'undetectable' cut-off in many settings) This can be adjusted
  - ROC curves are constructed to determine prediction accuracy

Key results

- Combined models can predict absolute virological responses with correlation ≥ 0.85 (r^2 of ≥0.7)
- Recent models predict probability of viral load going undetectable (<50 copies) with accuracy of ≥80%
- Recent models (trained with >8,000 TCEs) predict virological response *without need for genotype* with similar accuracy

The rationale for RDI in resource-limited setting

- ART continues to be rolled-out in resource-limited settings
- ART failure rates are as expected
- Resistance is a major factor
- Very little access to resistance tests
- Limited (but increasing) treatment options means selections are critical
- Expertise limited in some settings

RF models developed to predict VL<50 copies: modeling without genotype

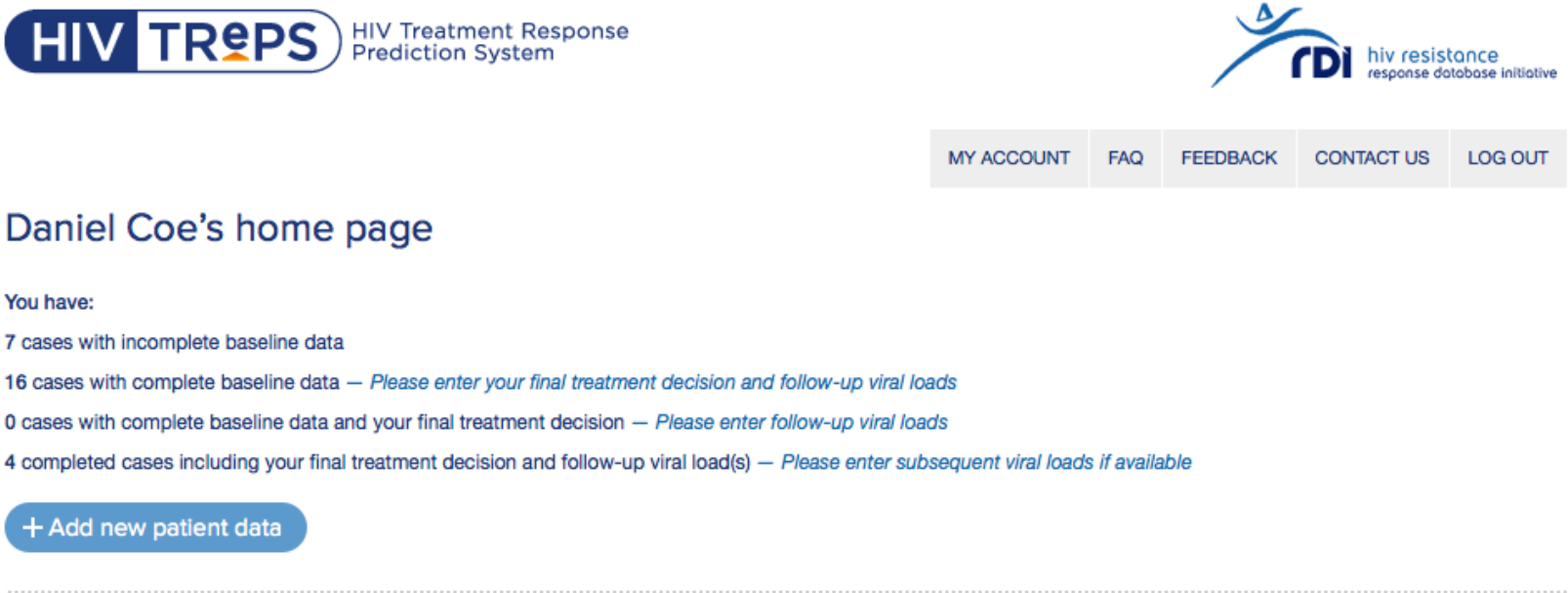
	Training set	Test set	Sens	Spec	Accuracy	AUC
RF binary, including genotype	3,188	100	86%	76%	82%	0.88
RF binary, no genotype	3,188	100	71%	89%	78%	0.86
RF binary, no genotype	8,214	400	86%	77%	82%	0.88

HIV-TRePS

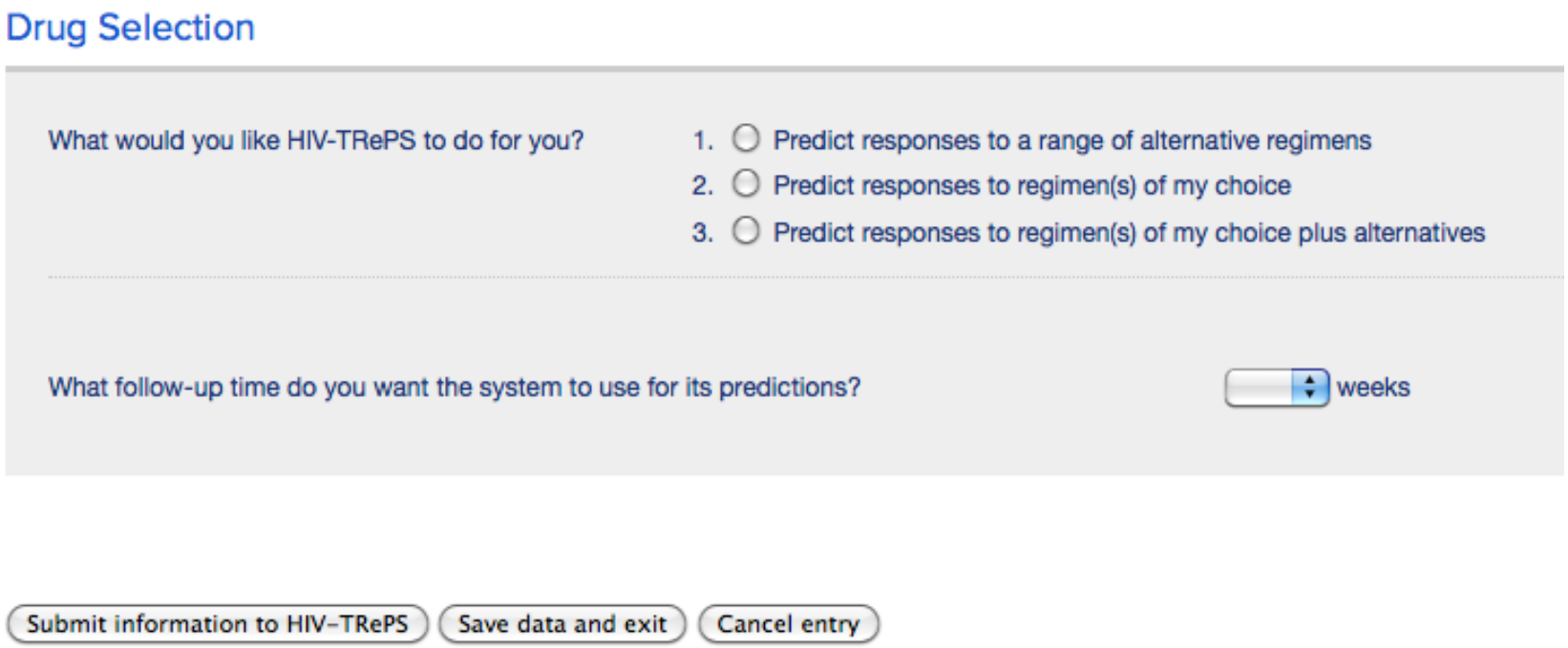
- Free system accessed via RDI web site
- Password-protected user accounts
- Flexible user options e.g.,
  - Model responses to regimens of your choosing
  - Model responses to alternatives, or both
  - Exclude unavailable or unsuitable drugs
- Real time report
- Data saved in user's archive
- Follow-up data collection facility

http://www.hivrdi.org/treps/login.php

HIV-TRePS – home page 1



HIV-TRePS – a baseline screen



HIV-TRePS – a sample report

HIV TRePS HIV Treatment Response Prediction System

rdi hiv resistance response database initiative

10th May 2010

RDI ID	Patient ID	Date of Birth	Sex	User name	Centre
146	bren2		M	Mr Daniel Coe	RDI

Baseline Information

Viral Load	CD4	Protease Mutations	RT Mutations	
26/04/2010	26/04/2010	26/04/2010	NRTI	NNRTI
45,888	300	10F/I/R/V,20M/R,32I,46I/L 54V/L/M,71V/T,77I,82A/F/S,90M	41L,44D,67N,184V 210W,215Y	108I,181C/I/V
Previous Drug Exposure		**Unavailable Drugs**	**Excluded Drugs**	**User Selection**
3TC,D4T,AZT,NVP,IDV,rtv,SQV		ENF	D4T,ENF,IDV	ABC,TDF,ETV,DRV/rtv,RTG ABC,F/3TC,AZT,NVP,LPV/rtv,RTG F/3TC,TDF,ETV,ATZ,RTG

Predictions

HIV-TRePS (v1.0) was instructed to model responses to the user-defined anitretroviral regimens plus alternatives

The regimens are listed in order of predicted virological response with the regimen most likely to reduce the viral load to <50 copies ranked first.

The probability of response (viral load <50 copies) is listed next to each regimen.

Those regimens that the system predicts will produce a response (using the cut-off value for the probability of response that produces optimal accuracy) are highlighted in green and those predicted to fail in red.

A: Top 5 predictions with no more drugs than the user's first selection (5 drugs)

Rank	Regimen	Probability of VL<50	Response Category
1	DRV,F/3TC,RTG,rtv,TDF	76%	Response
2	AZT,DRV,F/3TC,RTG,rtv,TDF	76%	Response
3	AZT,DRV,RTG,rtv,TDF	75%	Response
4	AZT,ETV,F/3TC,RTG,TDF	74%	Response
5	AZT,F/3TC,RTG,TDF	74%	Response
User	ABC,DRV,ETV,RTG,TDF	60%	Response
User	ATZ,ETV,F/3TC,RTG,TDF	42%	Response

rdi

Thank you

HIVTRI slide

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