ARV resistance and why it matters (especially for tenofovir)

Dr Edmnund Wilkins

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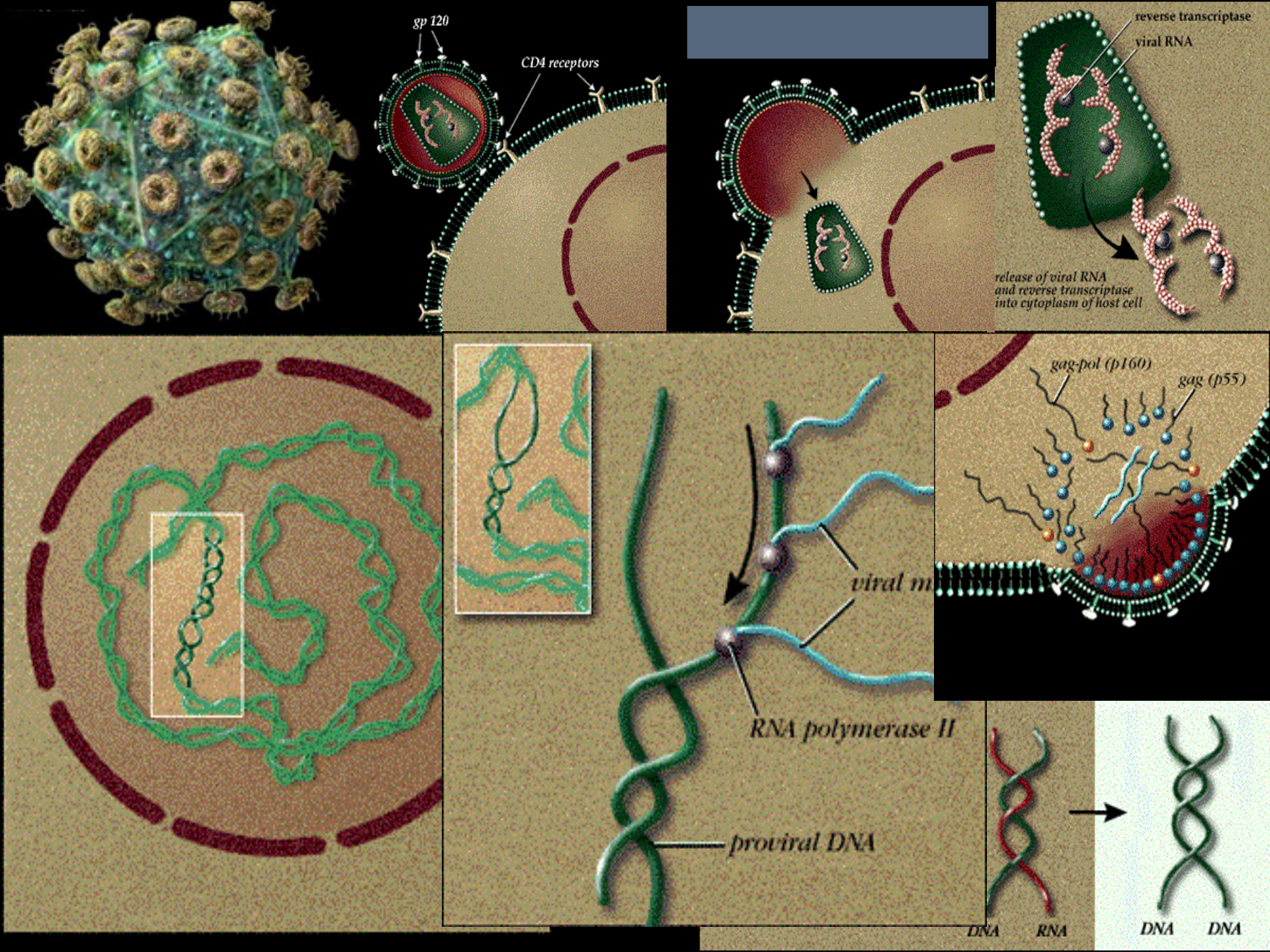
gp 120
CD4 receptors
reverse transcriptase
viral RNA
release of viral RNA
and reverse transcriptase
into cytoplasm of host cell
gag-pol (p160)
gag (p55)
viral m
RNA polymerase II
provirial DNA
DNA
RNA
DNA
DNA

The aim of this session

- What is resistance?
- Why does it occur?
- Why are we so bothered?
- The resistance test – when, why and how?
- The virological benefits and concerns of using TDF 1st line
- How do we minimise the risk of resistance, particularly to tenofovir?

What is resistance?

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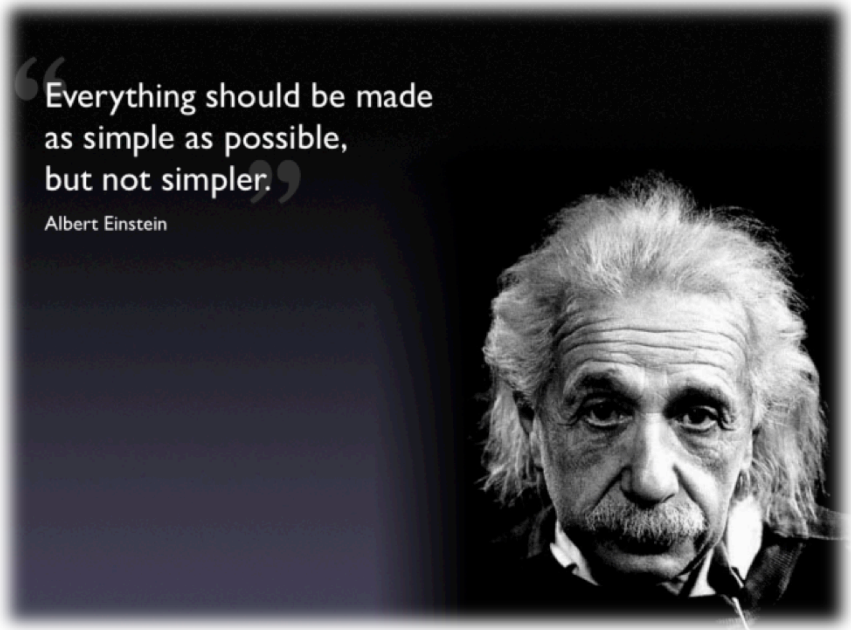
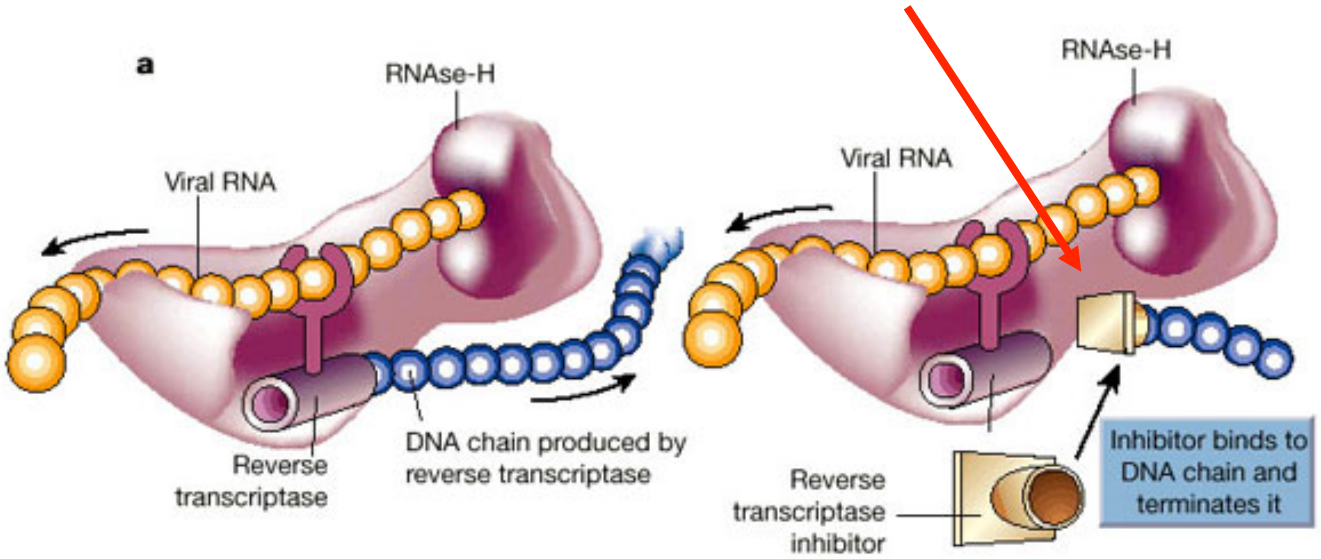
Basic Theory – what is resistance

- Mutations of the viral genetic material that result in the drugs no longer being able to block viral replication

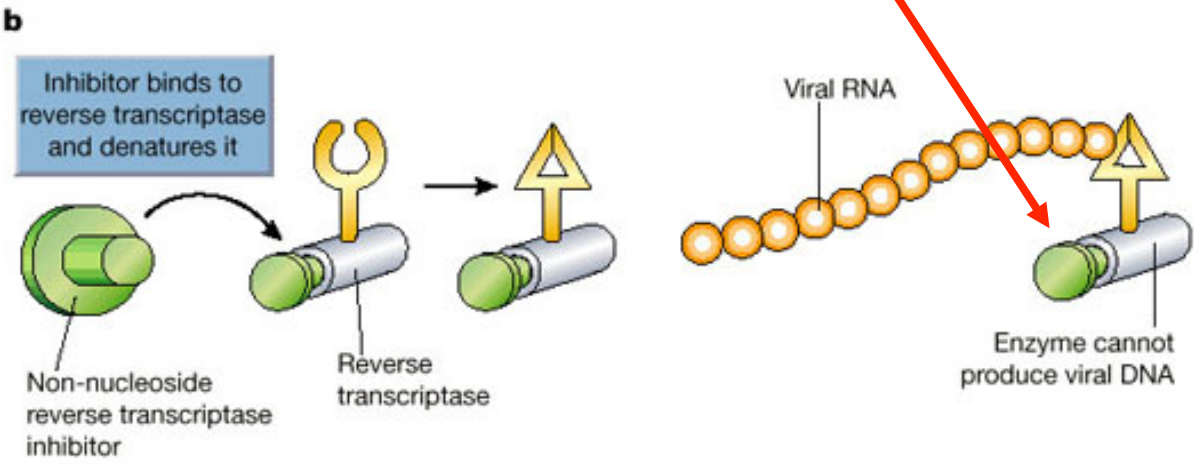


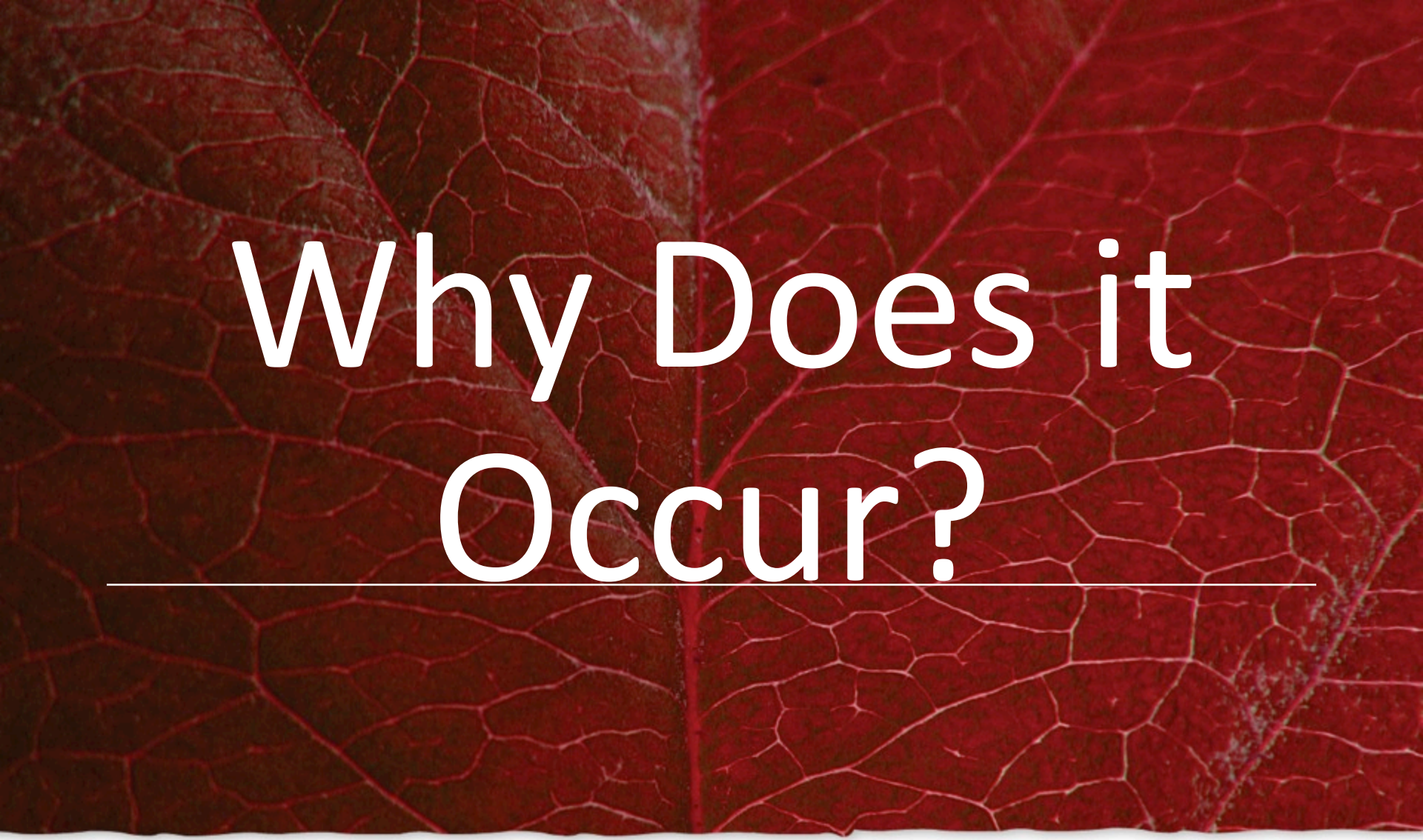
What is happening at the molecular level?

NRTI



NNRTI





Why Does it Occur?

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Because of the virus…

- HIV has a high mutation rate
  - Makes mistakes when replicating itself
  - Therefore lots of potential to develop resistance
    - >1 billion viral particles made/day
    - → 1-10^6 mutations/day
    - Every single mutation is possible every single day…

Because of the virus…

- Low barrier to resistance
  - It doesn't take many resistance mutations to knock out a drug
  - These mutations not 'lethal'
  - Limited effect on virulence

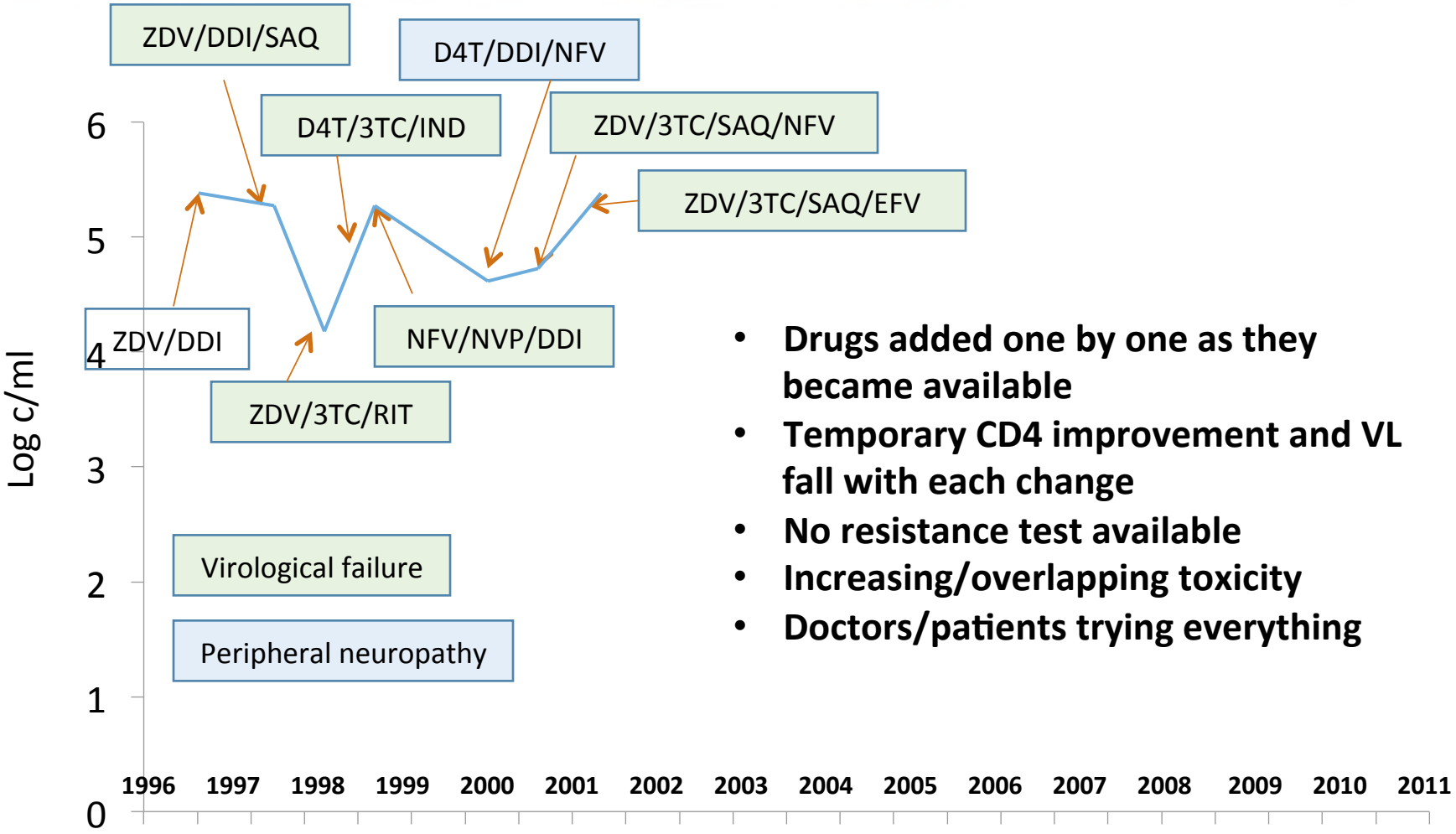
Because of the drugs..

- Viral replication in presence of detectable drug(s) because they are not potent enough

Because of the drugs..

- Viral replication in presence of detectable drug(s) when there is pre-existing/emergent resistance

Often because of past mistakes

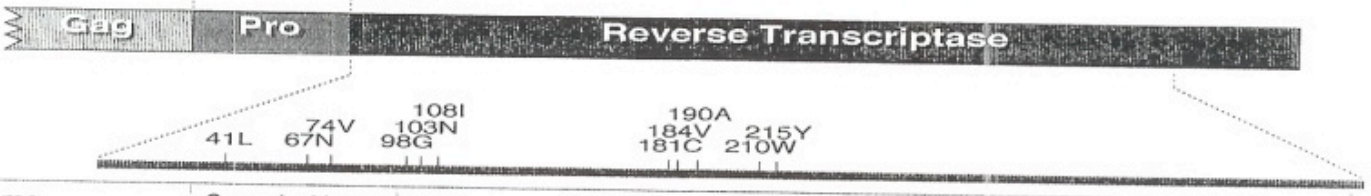


- **Drugs added one by one as they became available**
- **Temporary CD4 improvement and VL fall with each change**
- **No resistance test available**
- **Increasing/overlapping toxicity**
- **Doctors/patients trying everything**

The consequence!

ZDV/DDI/SAQ

D4T/DDI/[illegible]P/r



Trade Name	Generic Name	Muta-tions	Interpretation	Additional Information
Retrovir®	Zidovudine	√	[illegible]	AZT resistance may be reversed
Epivir®	Lamivudine	√	Evidence of Resistance	184: characteristic mutation
Videx®	Didanosine	√	Evidence of Resistance	
Hivid®	Zalcitabine	√	Evidence of Resistance	
Zerit®	Stavudine		No Evidence of Resistance	
Ziagen®	Abacavir	√	Evidence of Resistance	
	Adefovir		No Evidence of Resistance	
Viramune®	Nevirapine	√	Evidence of Resistance	
Rescriptor®	Delavirdine	√	Evidence of Resistance	
Sustiva®	Efavirenz	√	Evidence of Resistance	
Crixivan®	Indinavir	√	Evidence of Resistance	
Norvir®	Ritonavir	√	Evidence of Resistance	
Viracept®	Nelfinavir	√	Evidence of Resistance	
Invirase®/Fortovase®	Saquinavir	√	Evidence of Resistance	
Agenerase®	Amprenavir	√	Evidence of Resistance	

Log c/ml

6
5
4
3
0

2009

Because of the patient..

- Viral replication in presence of detectable drug(s) where poor adherence or drug-drug interactions

Why Are We So Bothered?

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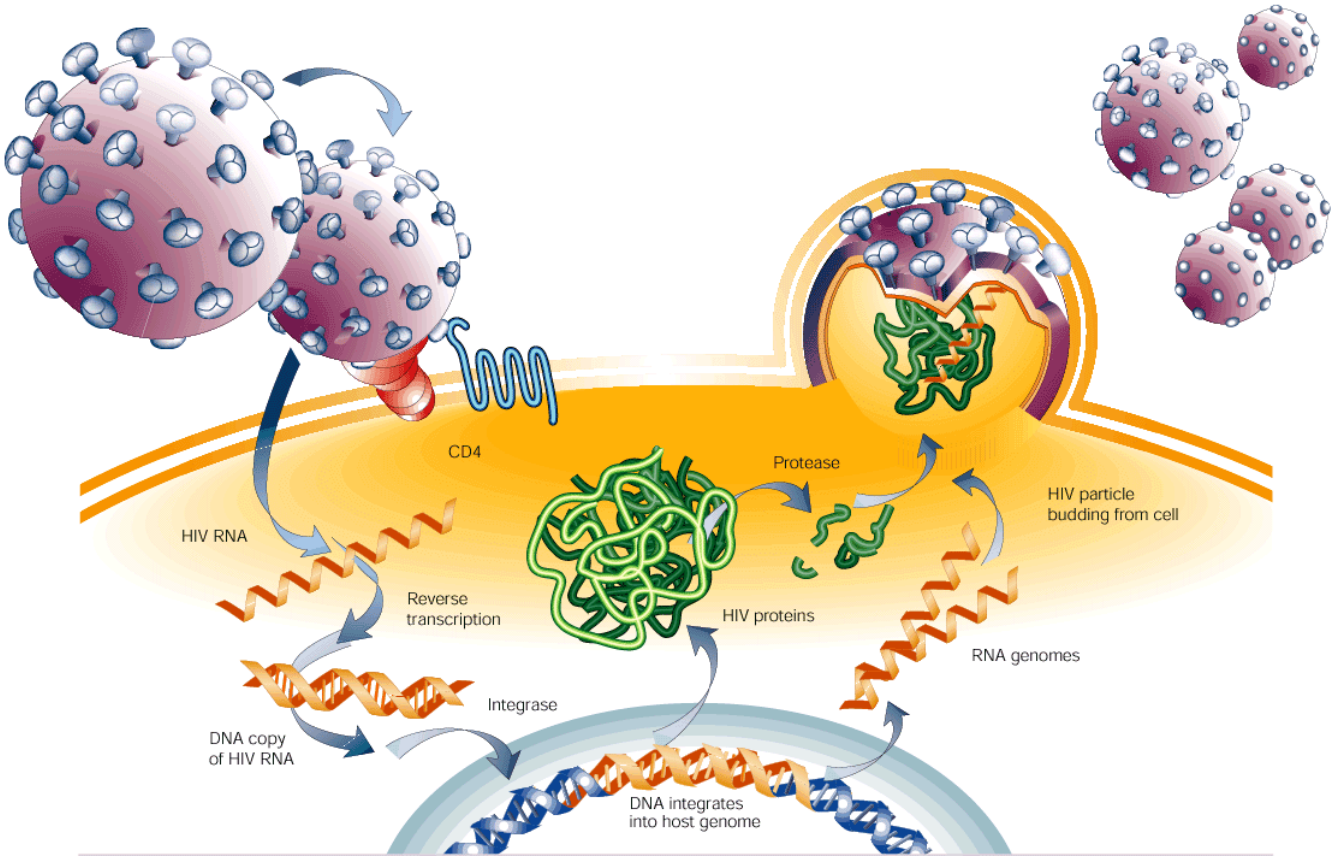
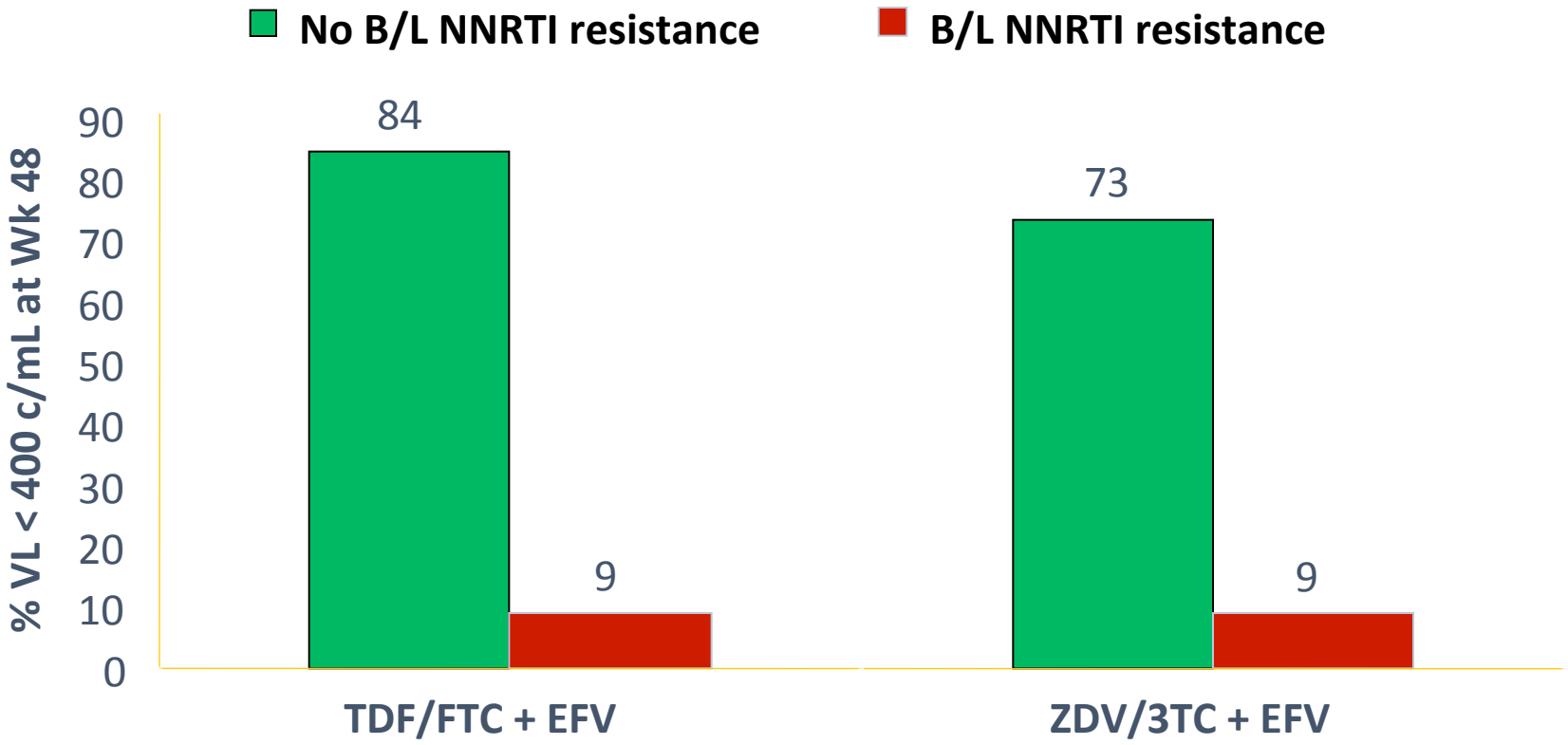
Once resistance develops it is always there – archived



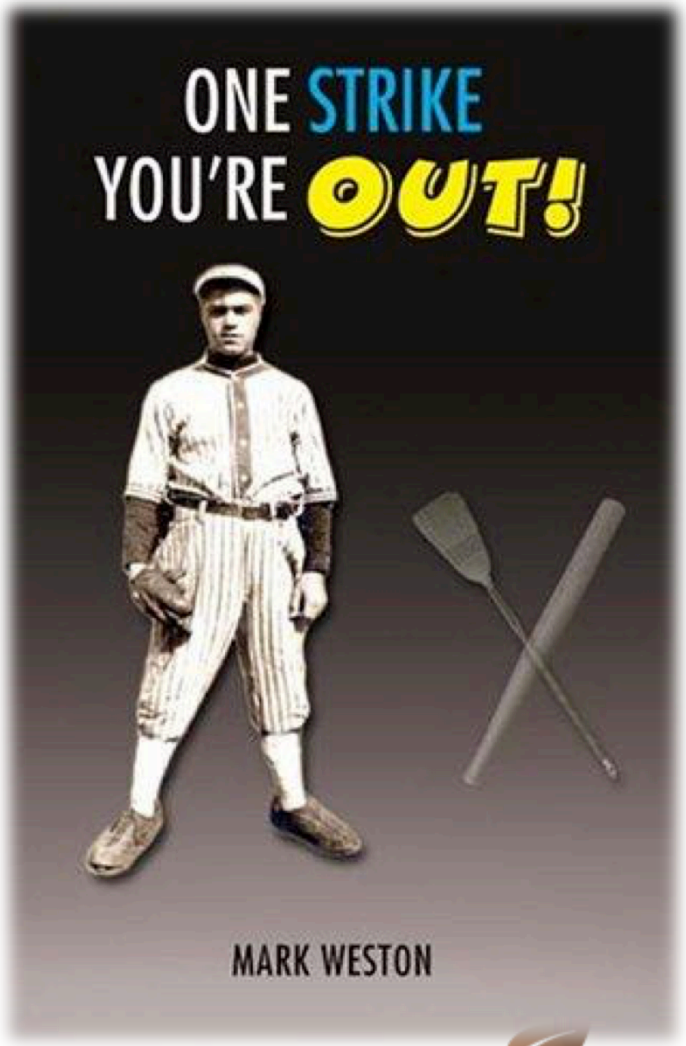
Archived NNRTI Resistance Markedly Reduces Treatment Response



Gallant JE, et al. N Engl J Med. 2006;354:251-260.

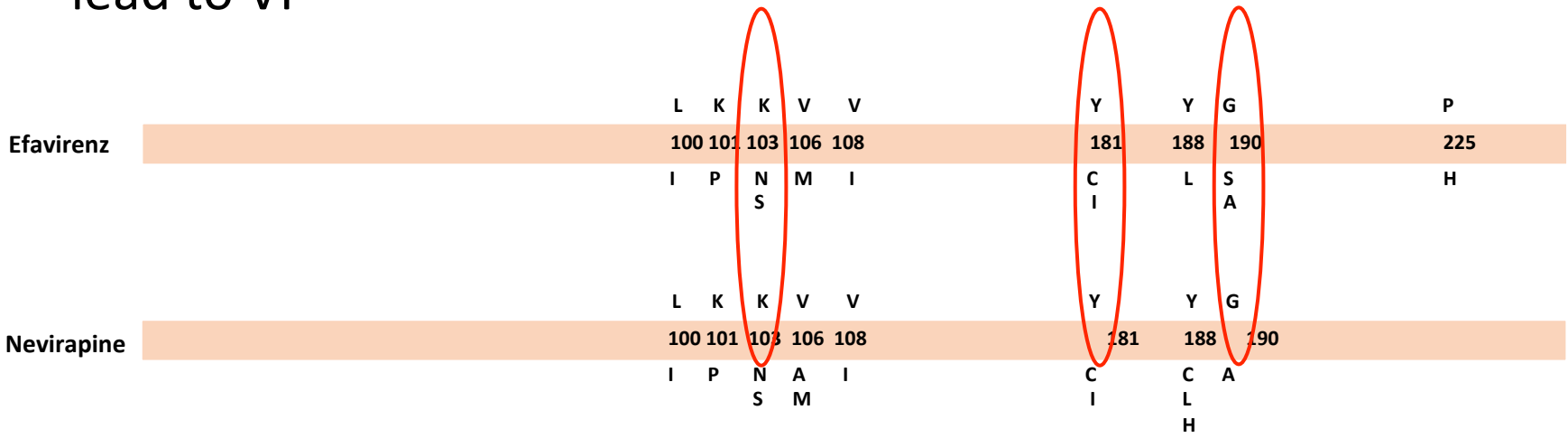
One mutation may be all that is needed

- A single mutation may wipe out activity…..
  - M184V - lamivudine or emtricitabine
  - K103N – nevirapine



One mutation may mean that other drugs have no/reduced activity

Single point mutations in the NNRTI binding pocket (e.g. K103N) lead to VF



- As EFV and NVP share similar binding sites, mutations often lead to cross resistance to the other agent[2]
- NNRTI resistance accumulation can compromise the efficacy of second-generation NNRTIs[3]

1. Johnson VA, et al. Top Antivir Med 2011;19:156–54
2. Delaugerre C, et al. J Med Virol 2001;65:445–48
3. Ghosn J, et al. AIDS Rev 2009;11:165–73

The Resistance Test – when?

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Case 1

- 33 year old heterosexual male
- Presents with oropharyngeal and oesophageal candidiasis
- CD4 142 cells/mL
- Viral load 37,567 copies/ml
- Started on Septrin

ZDV *or* **TDF**

and

3TC or FTC

and

EFV *or* **NVP**

He receives EFV/AZT/3TC

- 33 year old heterosexual male
- Presents with oropharyngeal and oesophageal candidiasis
- CD4 142 cells/mL
- Viral load 37,567 copies/ml
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ZDV *or* **TDF**

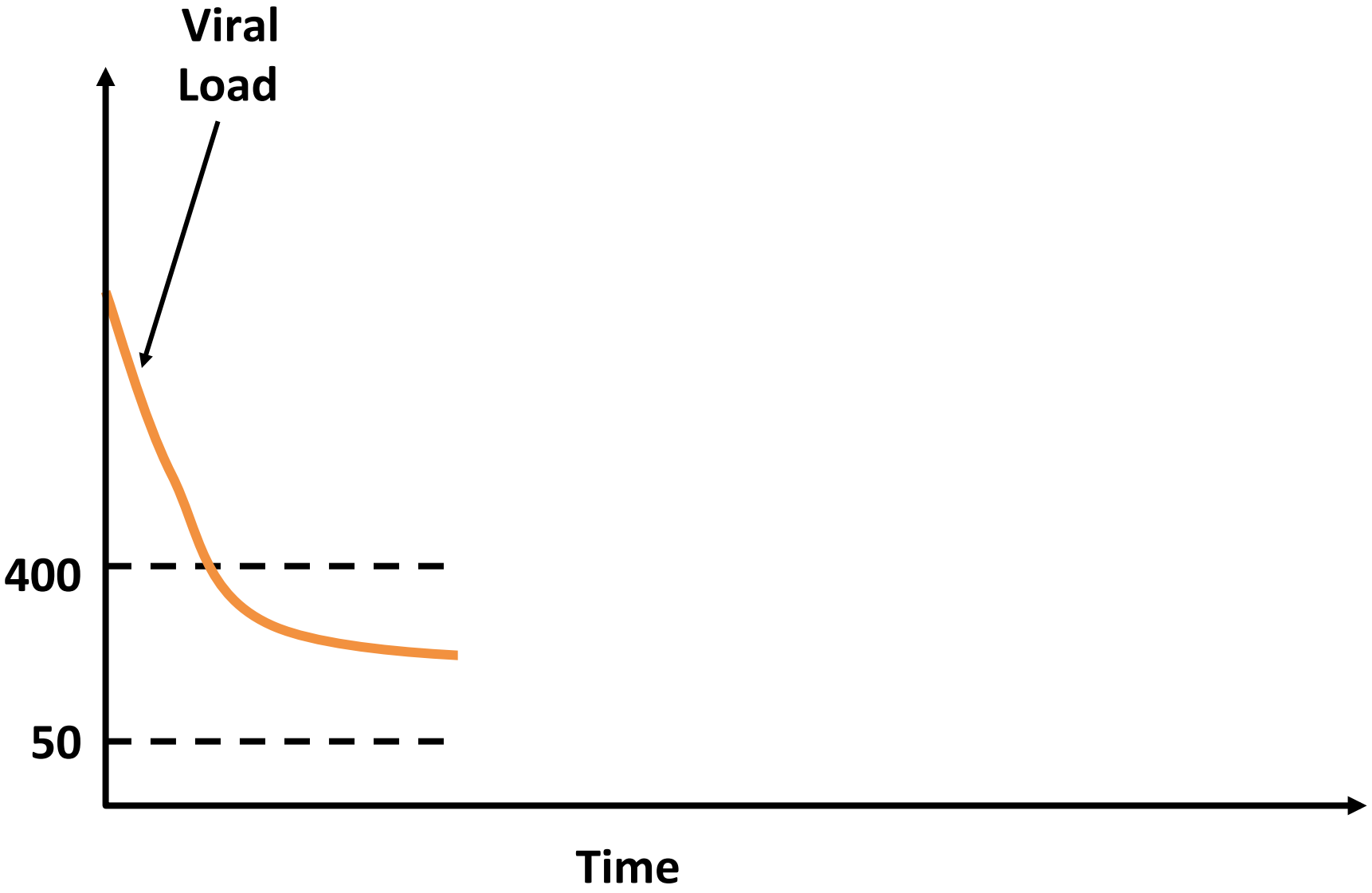
and

3TC

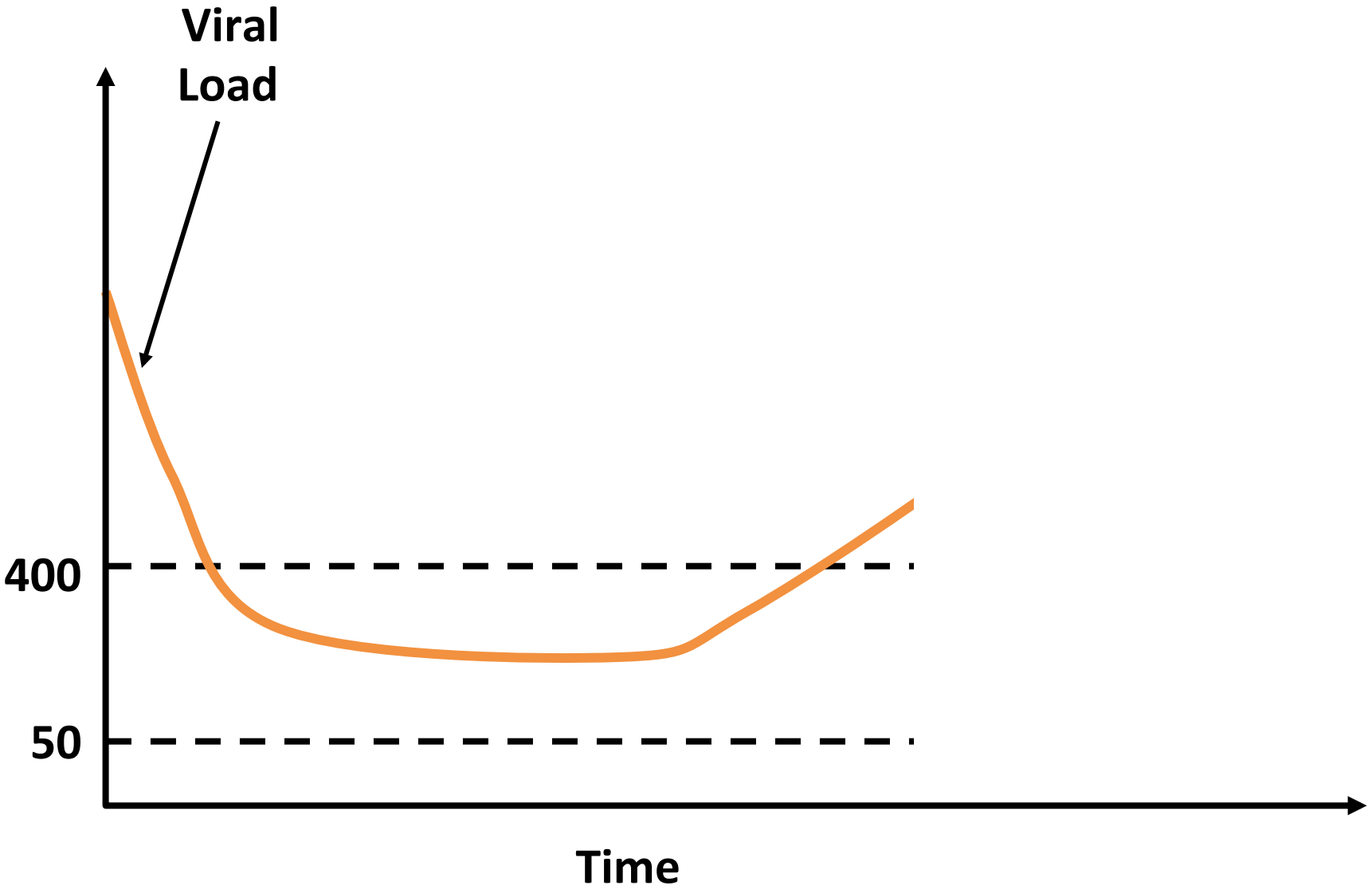
and

EFV *or* **NVP**

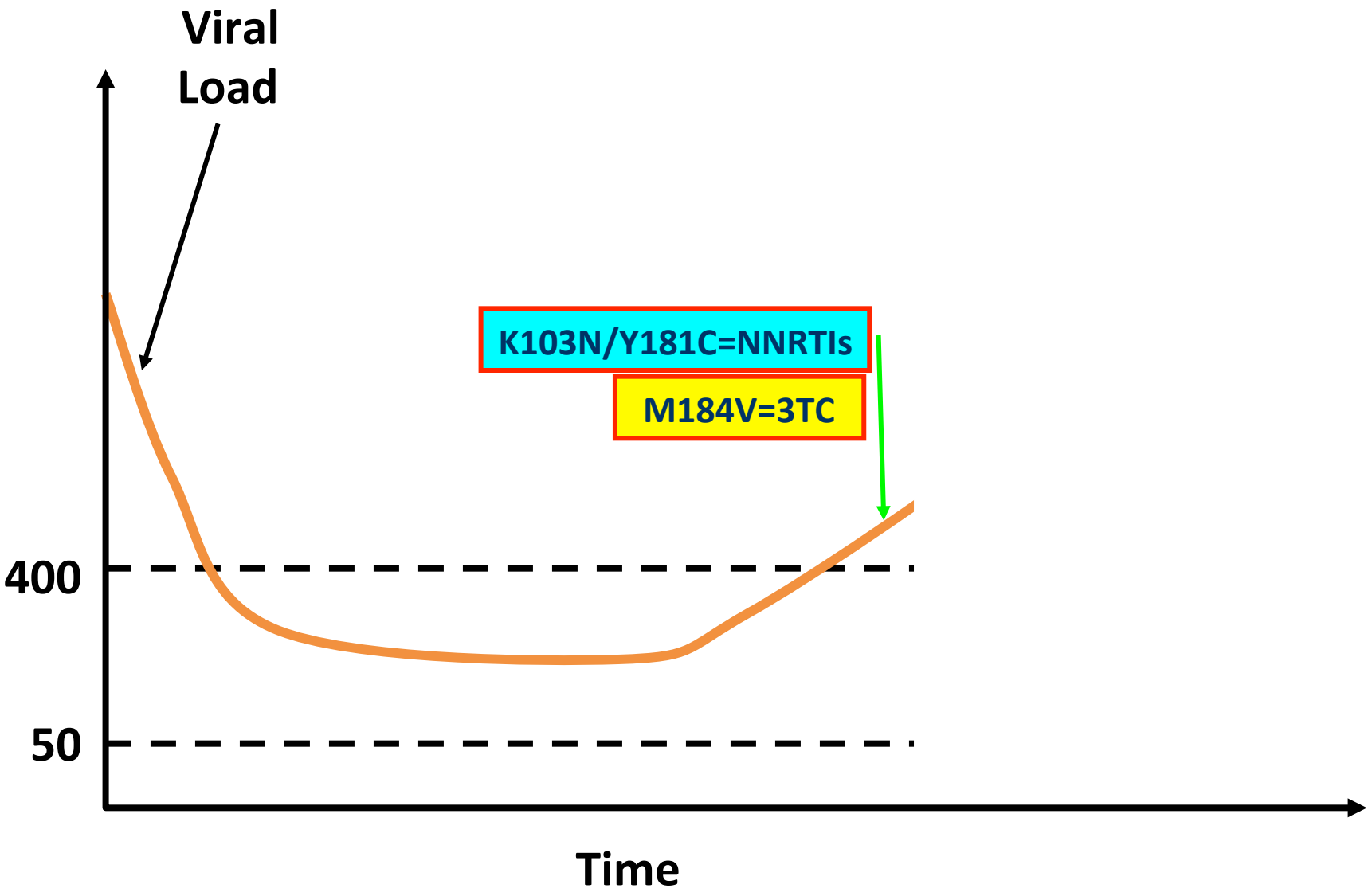
Response to therapy EFV/AZT/3TC



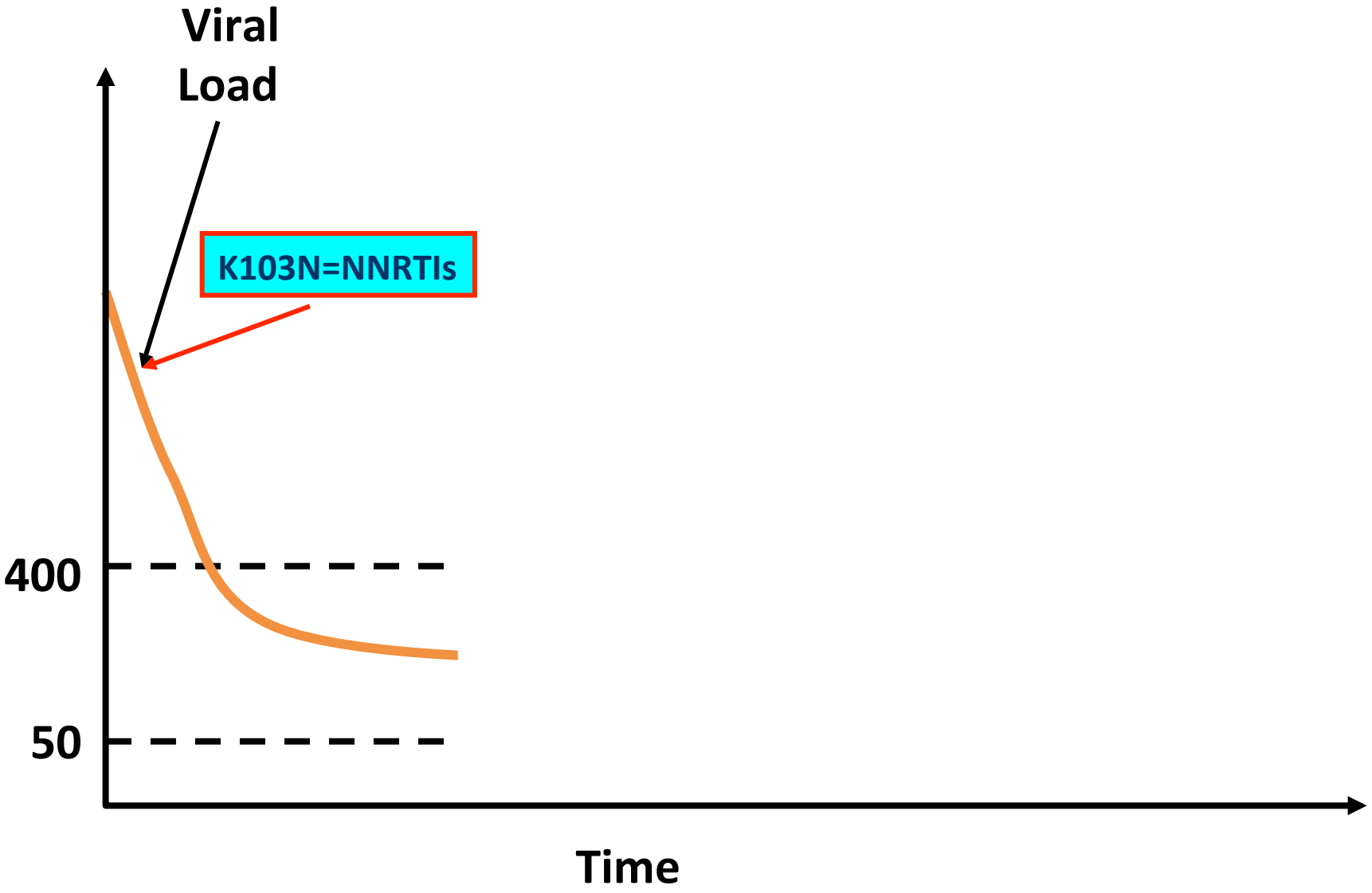
Response to therapy – 6m later



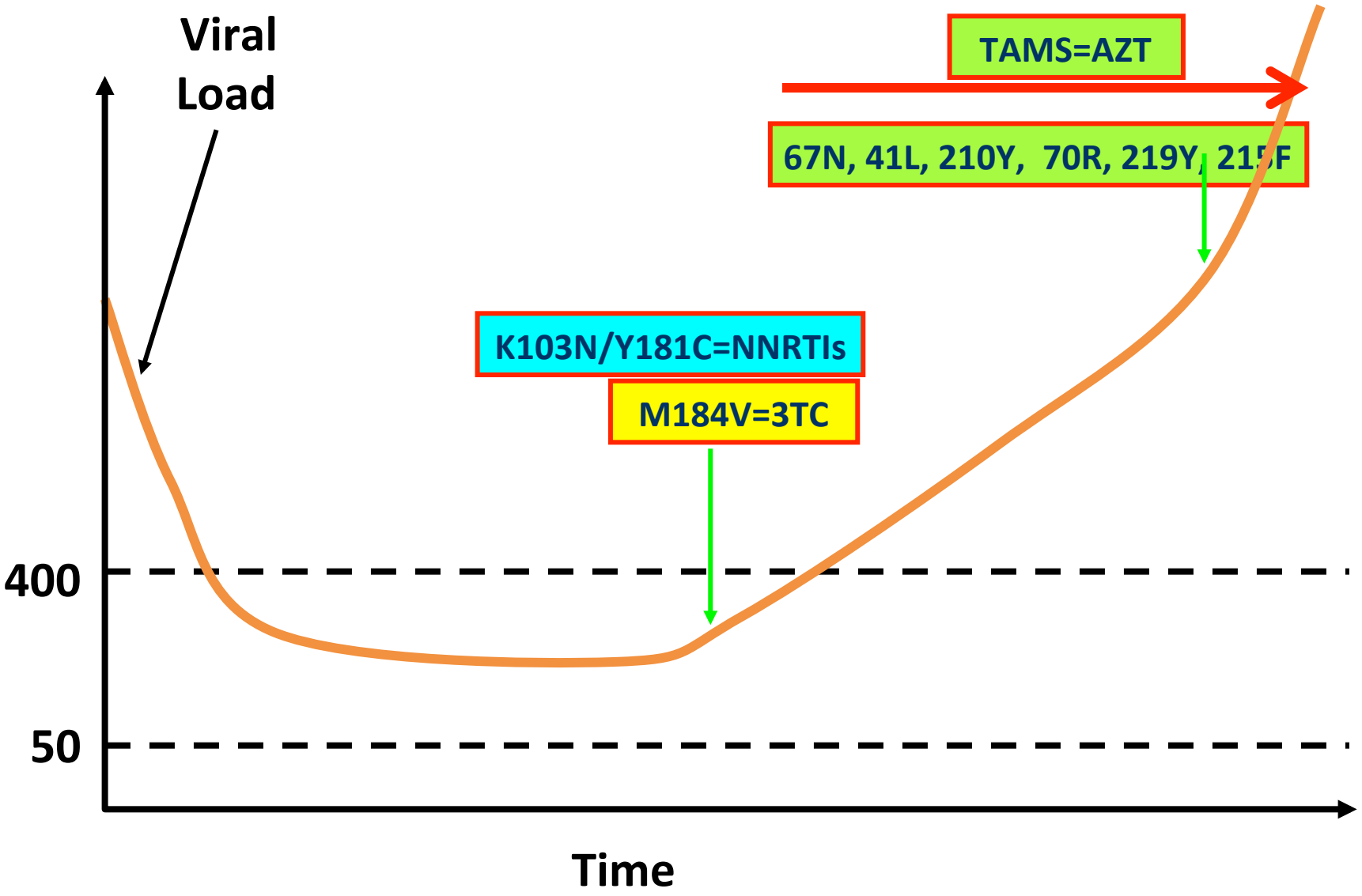
Resistance test at failure: VL 800



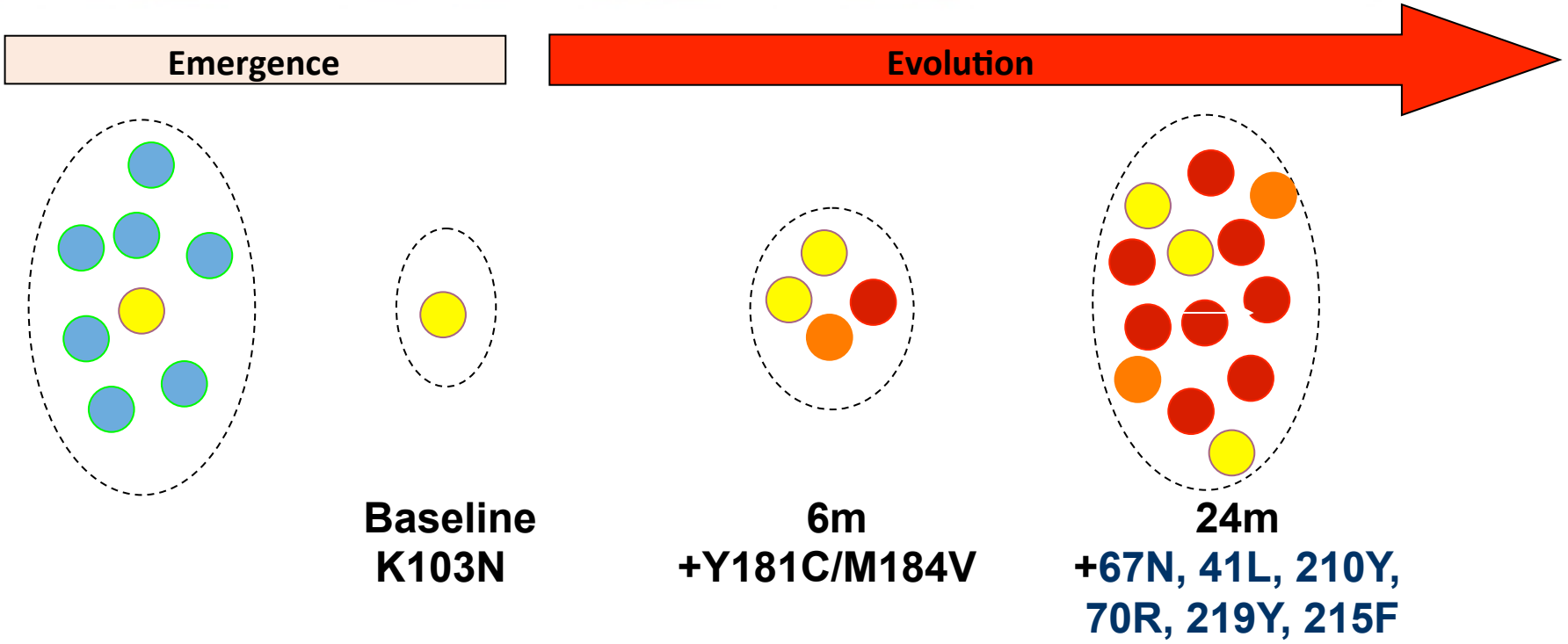
Resistance test of baseline sample



Response to therapy – 12m on



Evolution of resistance



- Increasing number of mutations
- Accumulation of mutations on the same viral genome
- INCREASING RESISTANCE

The resistance test – why?

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For many drugs the more mutations the more resistance…

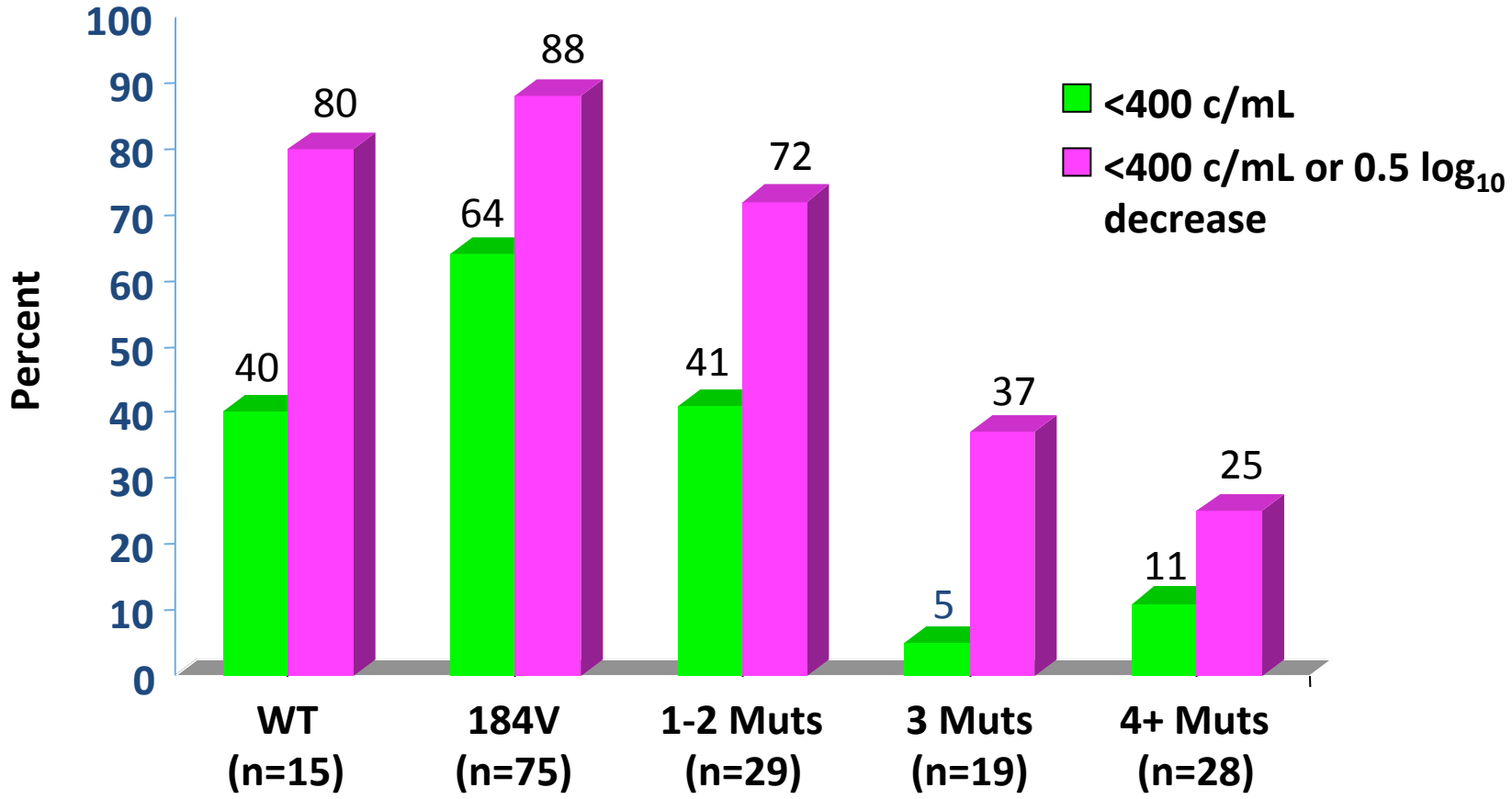
Accumulation of TAMs:

M41L, D67N, K70R, L210W, T215Y/F, K219Q/E

Susceptible Partial Resistance Resistance

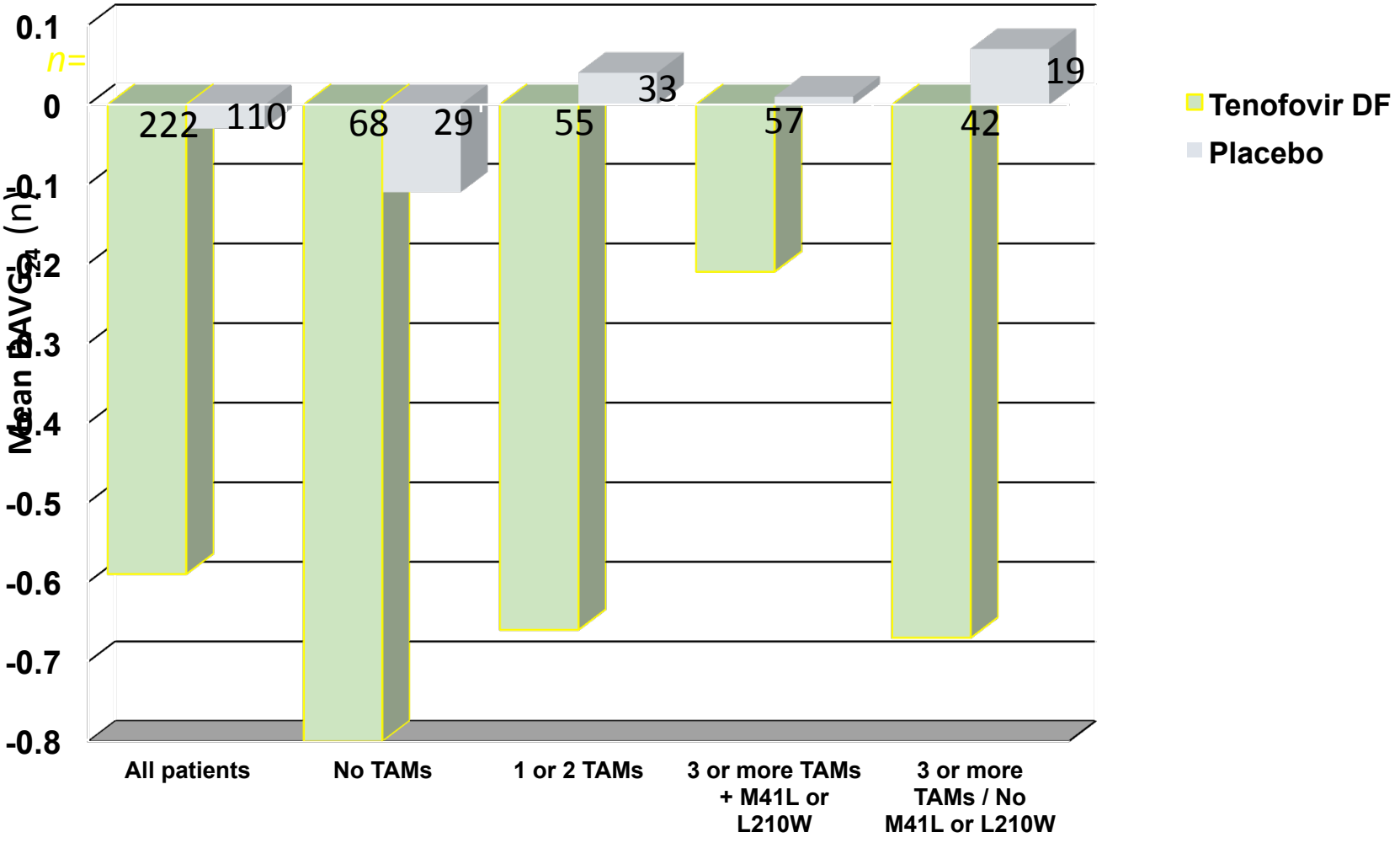
Number of TAMs present

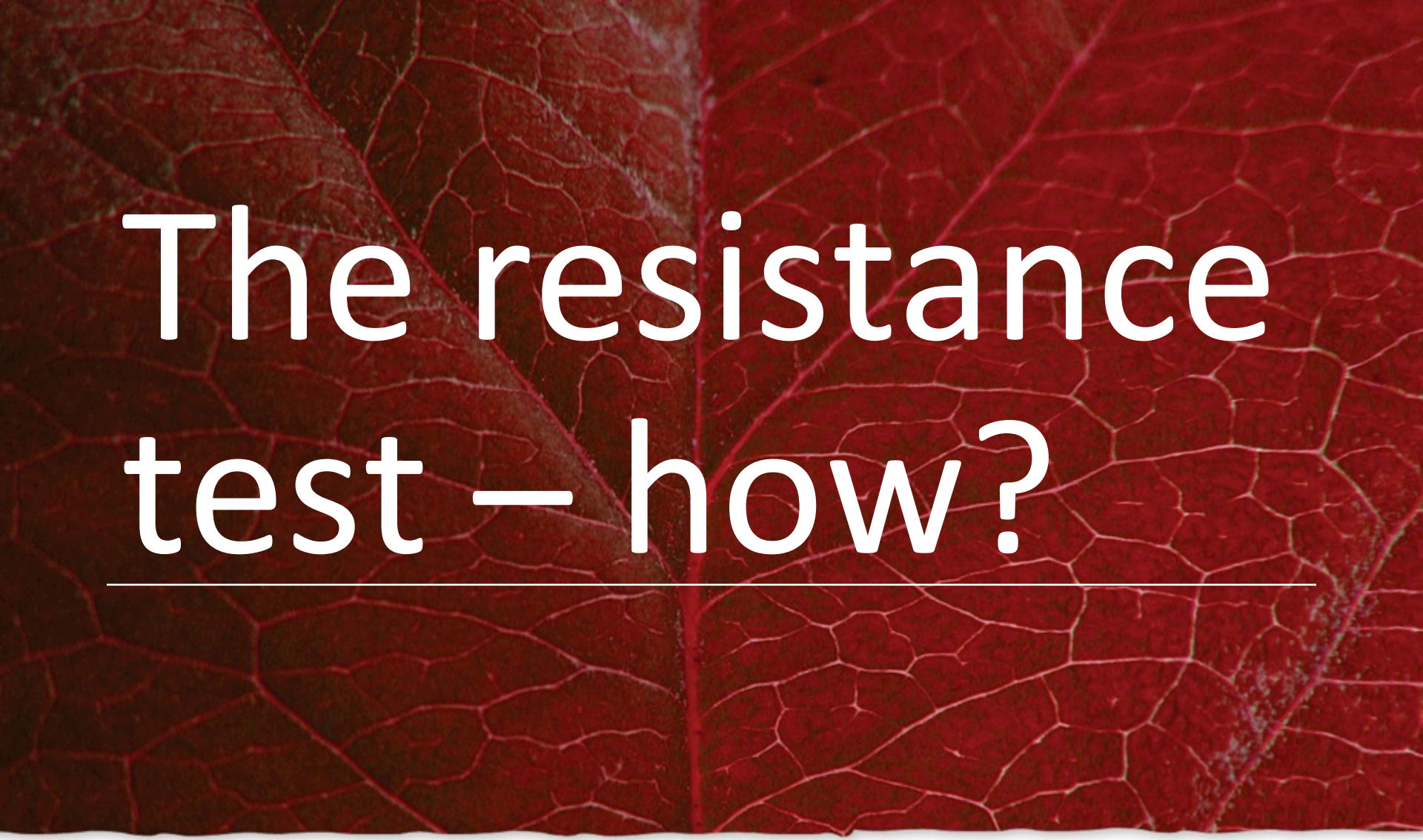
The more AZT resistance, the more abacavir resistance



Data on file, GlaxoSmithKline

The more AZT resistance the more tenofovir resistance





The resistance test – how?

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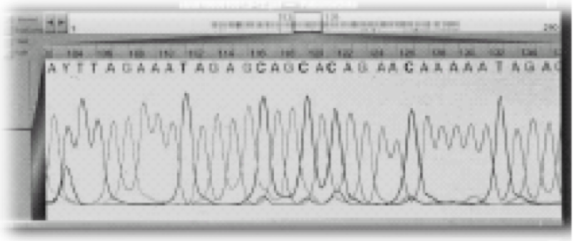
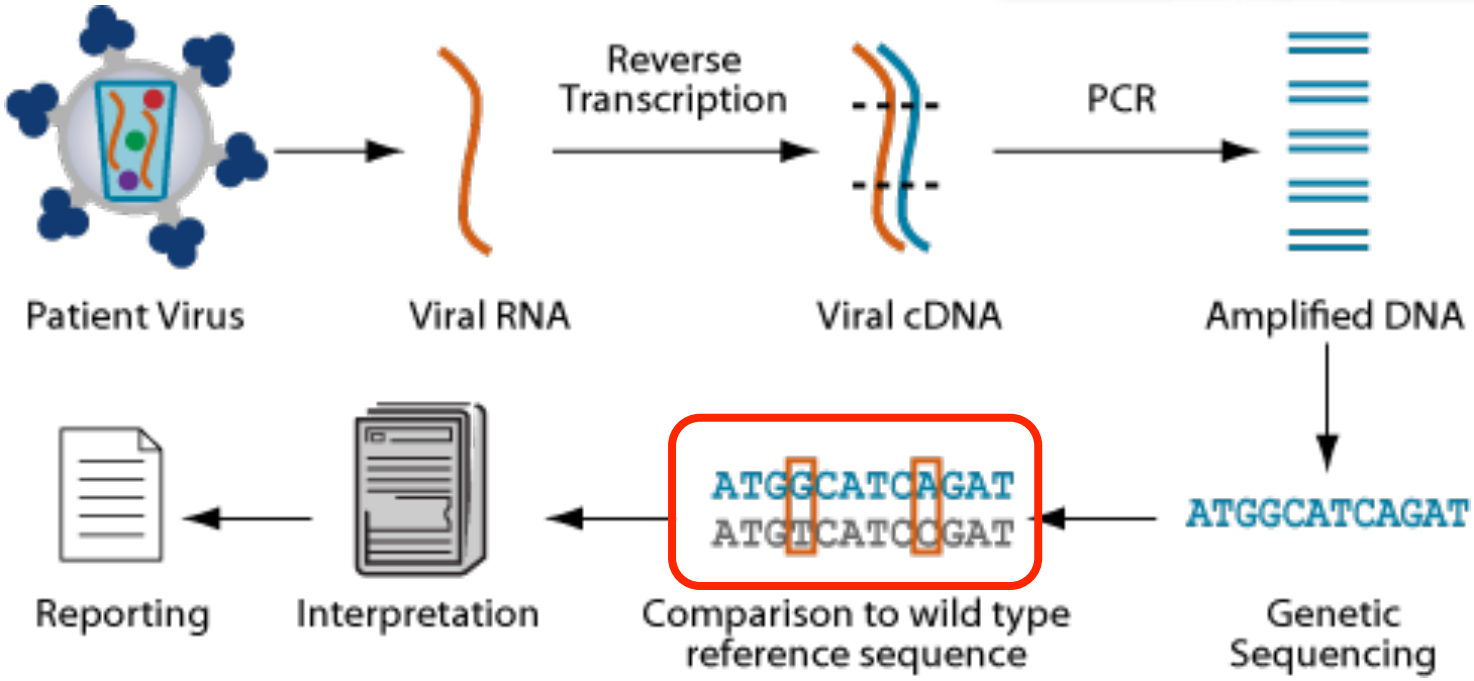
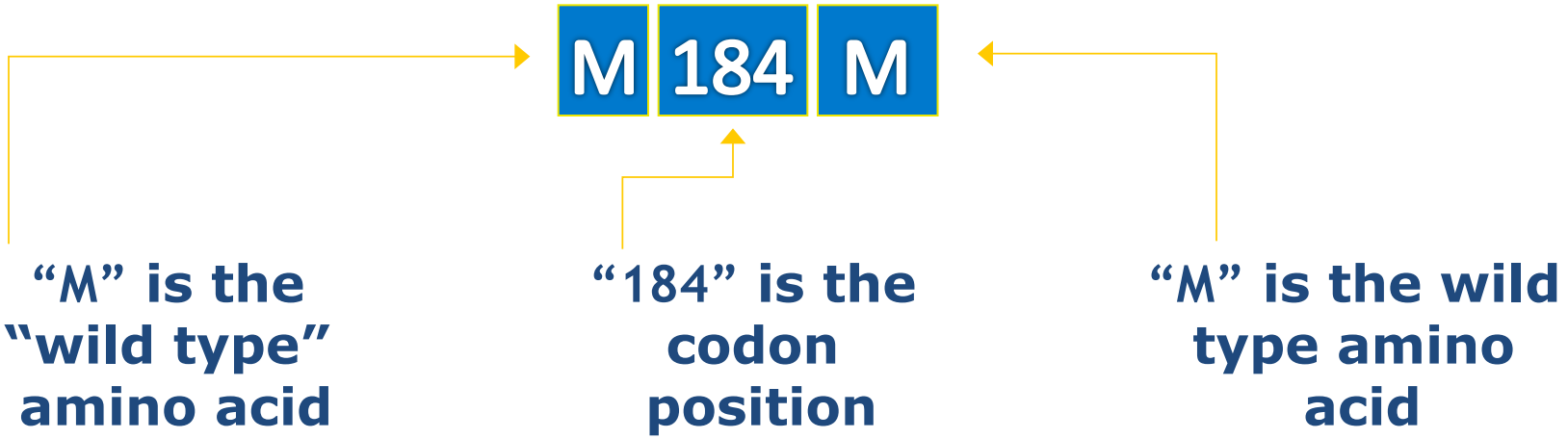
How does the resistance work?



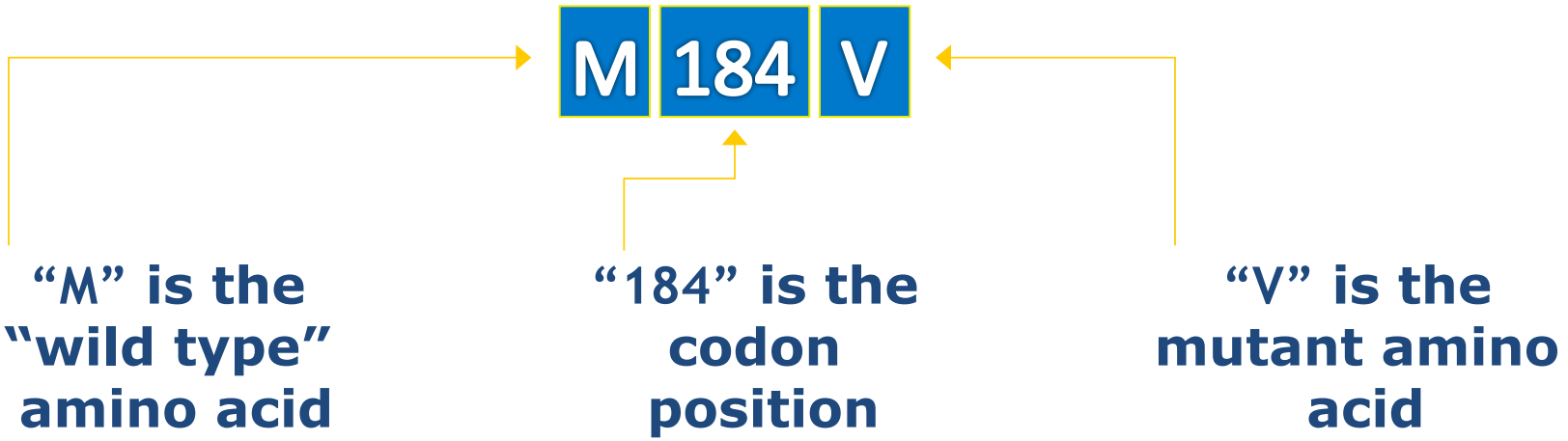


What do these mean…..
Before 3TC

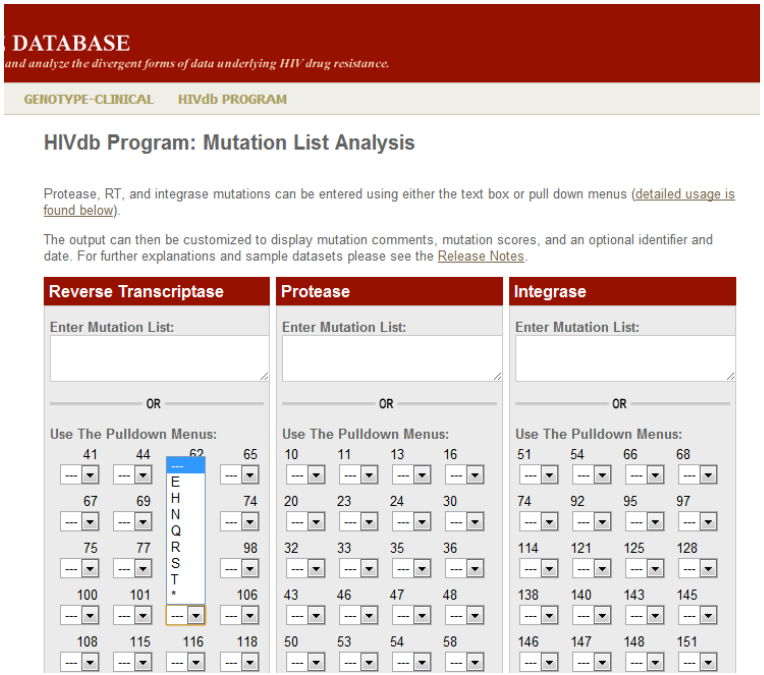


After 3TC and resistance…

- **How do we identify a resistance mutation?**



A prediction of phenotype is then used to give the report



Resistance associated RT Mutations: L100I, K103N, T215S*/Y

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

NonNucleoside RT Inhibitors	Resistance Interpretation
efavirenz (EFV)	Resistance
nevirapine (NVP)	Resistance

Resistance associated PR Mutations: L19I, M46L*, L63P, A71T

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

** Protease Inhibitors administered with low-dose ritonavir for pharmacological boosting.

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 June 2007 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

Limitations of resistance testing

- Population sequencing
  - Standard resistance testing will only detect mutations that are in **>20%** of the circulating virus
- Archived resistance
  - May be so low they cannot be detected…. But they are still there…. and will rapidly re-emerge under drug pressure
  - So you need to look at all ART when VL failure
  - And maybe make a guess

The virological benefits of using TDF 1st line?

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What if with case 1..

- 33 year old heterosexual male
- Presents with oropharyngeal and oesophageal candidiasis
- CD4 142 cells/mL
- Viral load 37,567 copies/ml
- Started on Septrin

ZDV *or* **TDF**

and

3TC/FTC

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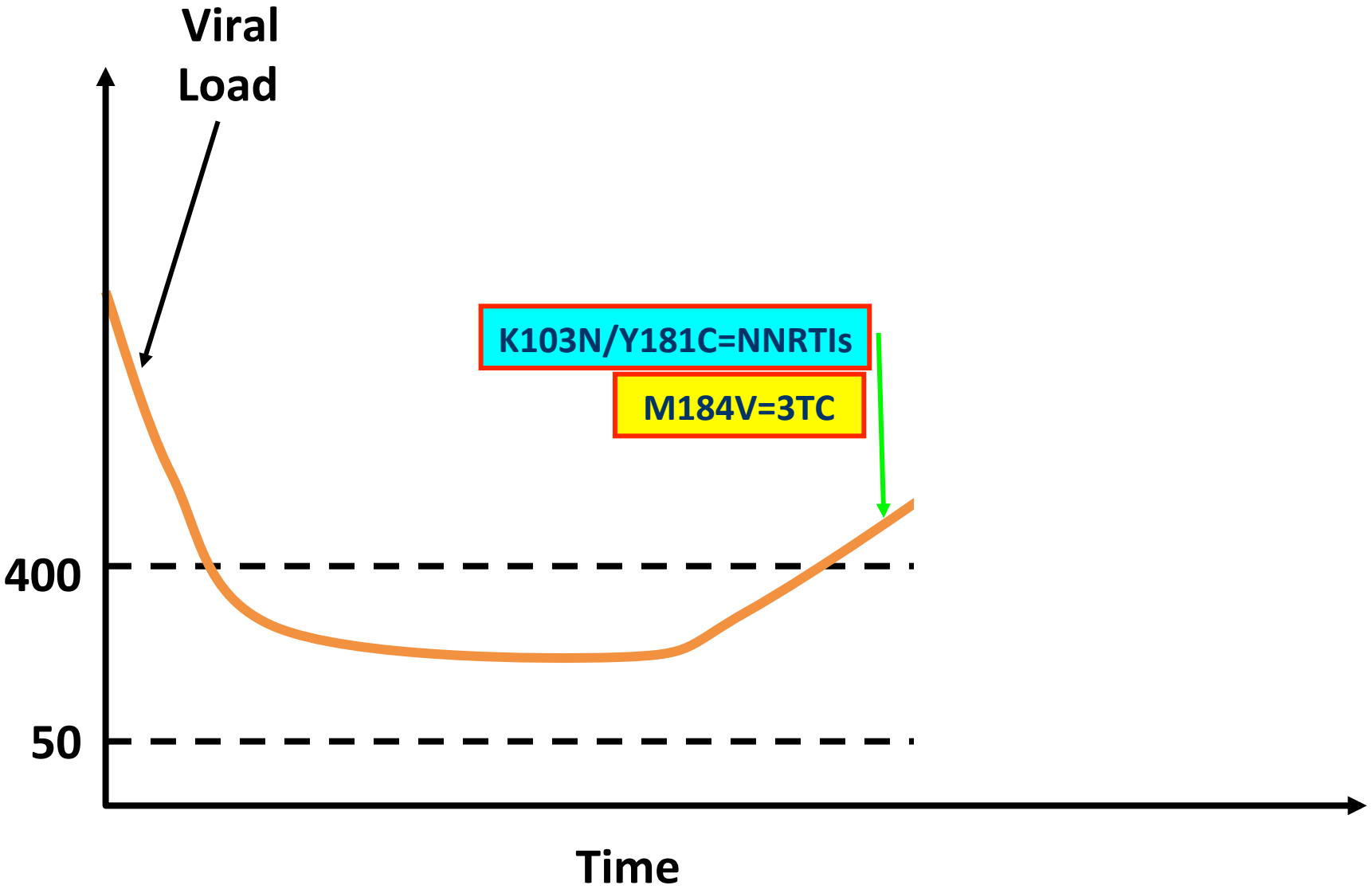
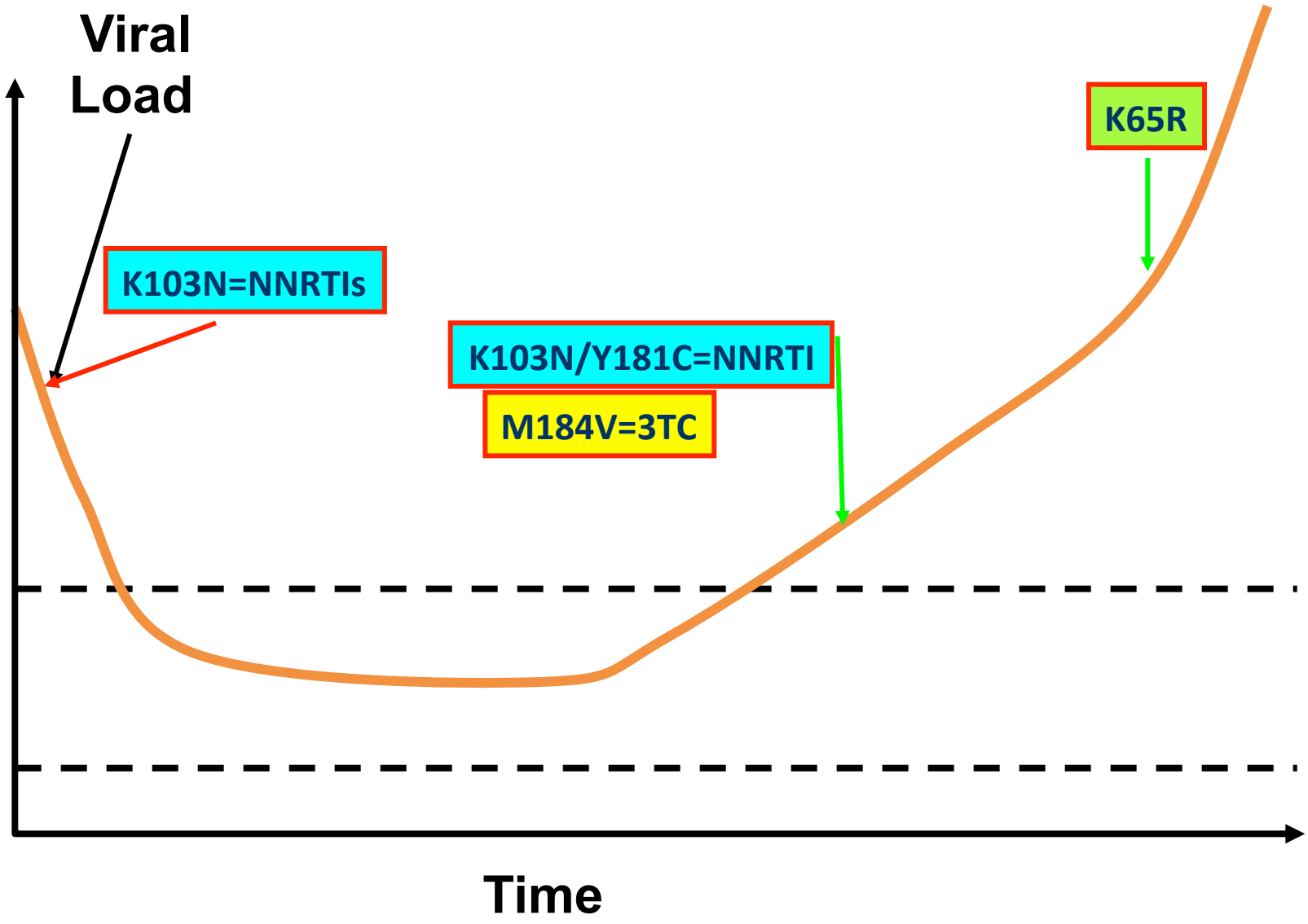
EFV *or* **NVP**

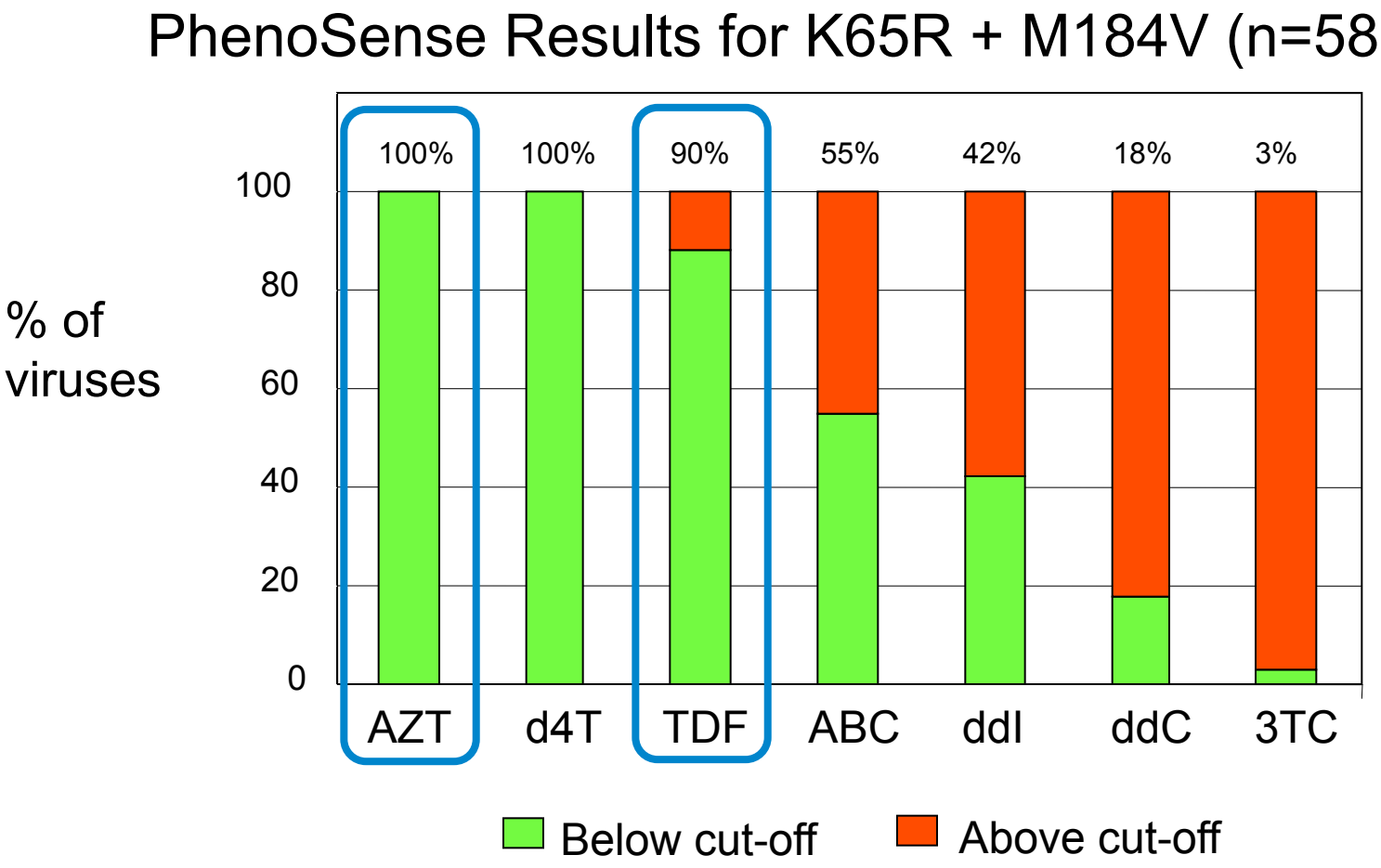
Case 1 with EFV/TDF/3TC: VL 800



Case 1 with EFV/TDF/3TC: VL 1000

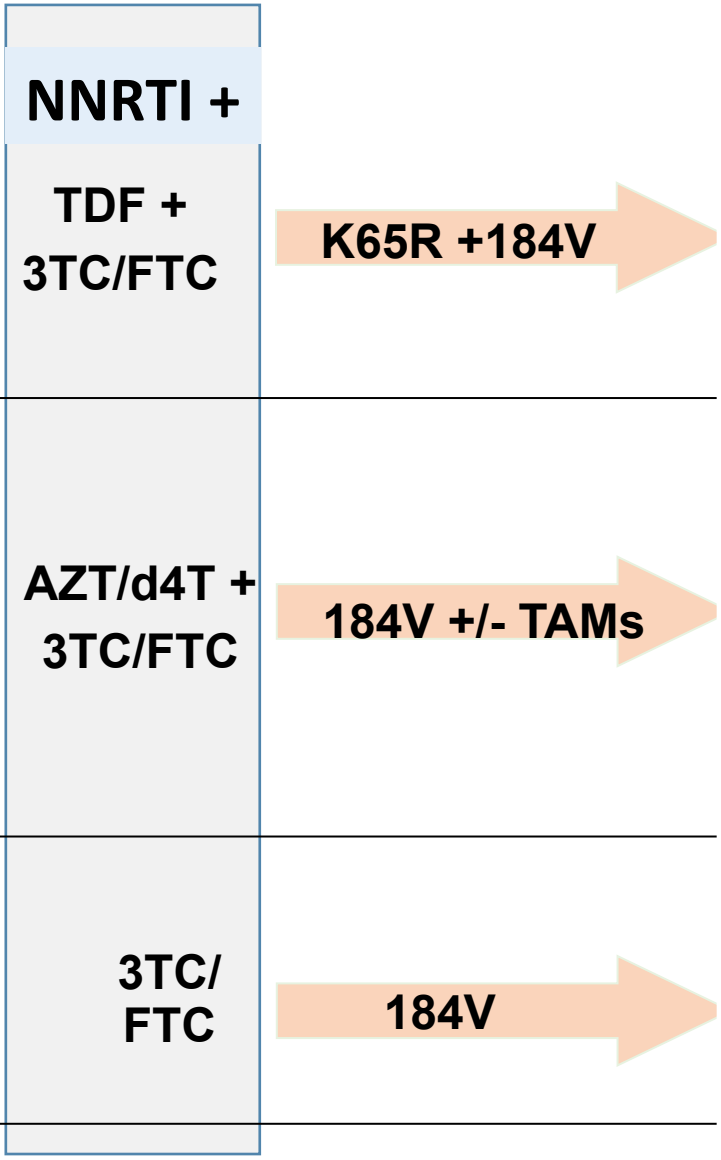


Susceptibility to NRTIs if K65R and M184V develop

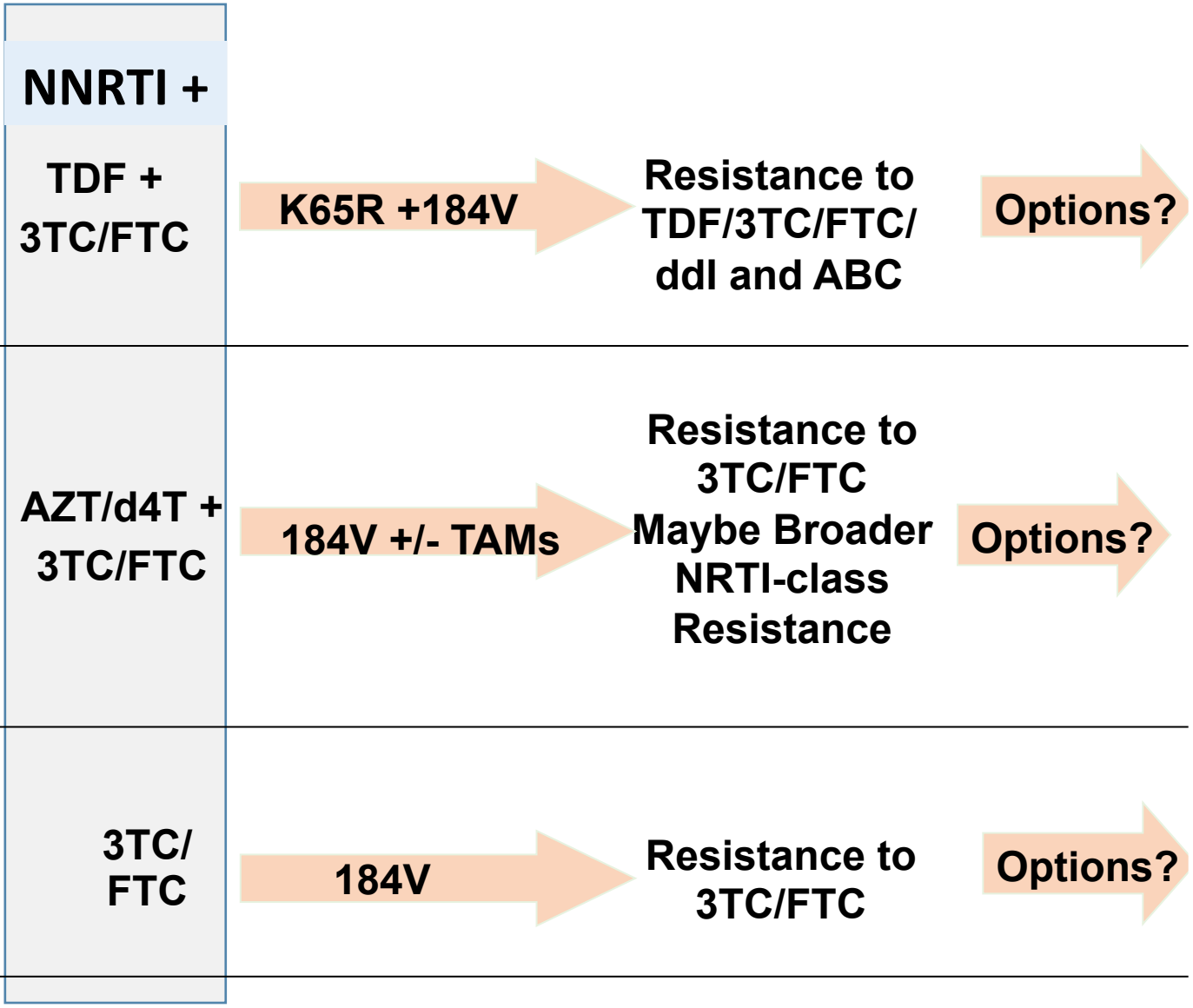


Miller et al (2003) 43rd ICAAC #H-904 and presentation
www.iasusa.org/resistance-mutations/mutations-figures.pdf. Vol 14 issue 3 Aug 06

Hence sequencing Options: PI AND….



Hence sequencing Options: PI AND….



Hence sequencing Options: PI AND….

NNRTI +				Boosted PI +
TDF + 3TC/FTC	K65R +184V	Resistance to TDF/3TC/FTC/ ddI and ABC	Options?	AZT (yes, ↑ activity) d4T (yes) TDF (maybe)
AZT/d4T + 3TC/FTC	184V +/- TAMs	Resistance to 3TC/FTC Maybe Broader NRTI-class Resistance	Options?	TDF, ABC (if <3-4 TAMs) and depending on pattern
3TC/ FTC	184V	Resistance to 3TC/FTC	Options?	AZT (yes, ↑ activity) ABC, d4T, ddI (yes) TDF (yes, ↑ activity)

The virological concerns of using TDF 1st line?

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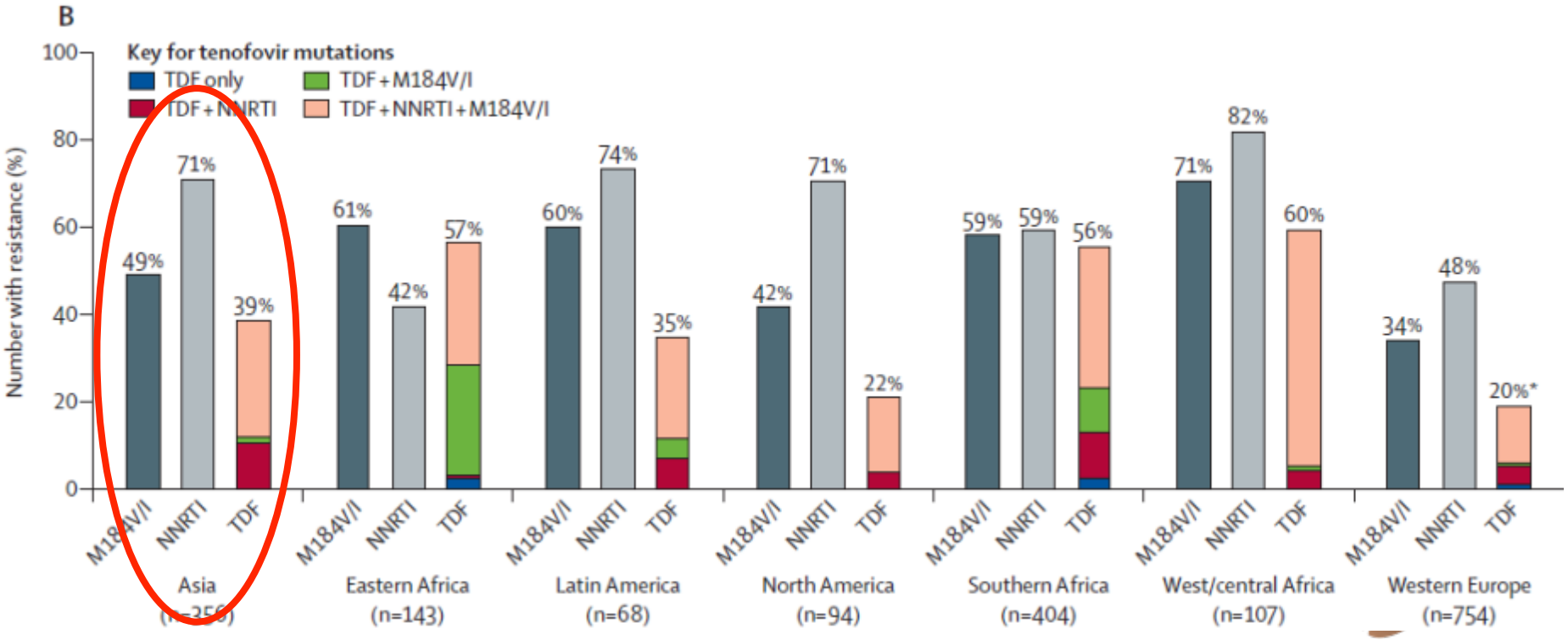
What about tenofovir?

- Recommended with FTC/3TC as 1st line NRTI backbone in all guidelines
- Over 15 million globally receiving ART
- Increasingly used around the world in treatment and prophylaxis
  - AZT and NVP being phased out
  - EFV preferred NNRTI

Resistance rate to TDF

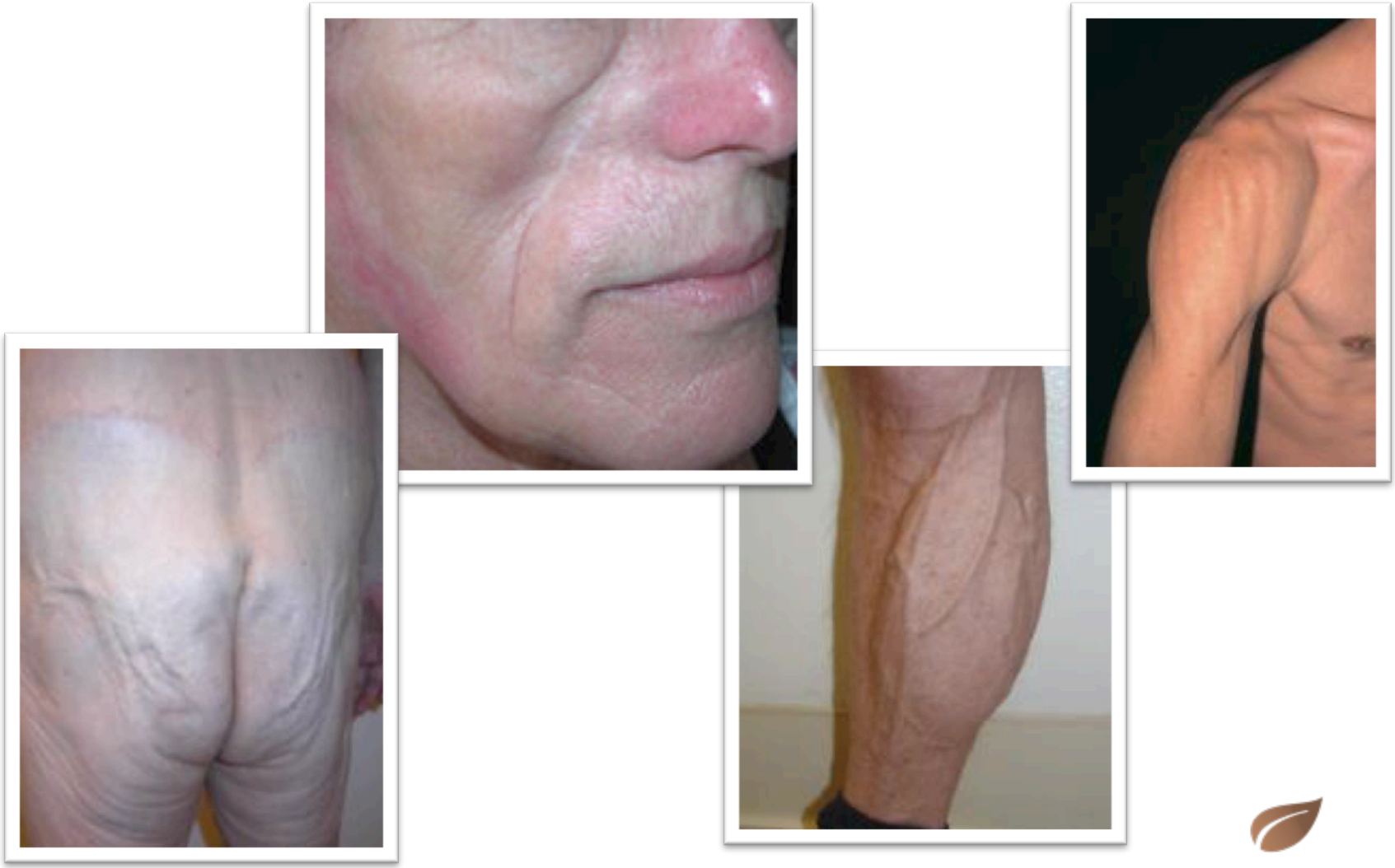
- Signature mutation is K65R
- Resistance rare in high income countries but much commoner in low/middle



TDF resistance

- 20% in Europe to >50% of isolates in sub-Saharan Africa - 39% in Asia
- Estimated that 15-35% of patients on ART in SSA have ARV resistant virus by 12 months
    - Likely that **5-10%** will develop TDF resistance WITHOUT VL monitoring
- Commoner with subtype C (also D4T may generate)
- Associated with:
    - Lower CD4 <100
    - 3TC use vs. FTC
    - NVP use vs. EFV

But TDF is well tolerated with minimal long-term toxicity



So how do we minimise risk of resistance particularly tenofovir?

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Follow national Guidelines

Recommended

ZDV **TDF**

3TC/FTC

EFV **NVP**

70%

Alternative

ZDV *or* **TDF**

and

Dolutegravir
ABC/3TC??

3TC

and

EFV *or* **NVP**

25%

Viral load measurements and resistance

- More frequent routine VL testing will detect virological failure earlier:
  - Identifies patients before resistance has developed
  - Allows earlier switch
  - New regimen more likely to be active
  - More valuable than basing solely on clinical, CD4 markers

Viral load measurements and resistance

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- Viral load levels at which resistance likely to develop
  - **300 – 500**: 3TC, EFV and NVP
  - **500 – 1000**: AZT and ABC
  - **1000**: TDF
  - Rare irrespective of VL: boosted PIs

Your reality!

- Financial constraint and human resources
- Technical expertise
- Transportation of sample from remote areas to the PCR sites
- Infrastructure (electricity etc.)
- Coordination between national lab and township level , within laboratory , and within service provider
- Quality control and trouble shooting

Naïve patient

- Follow National ART guidelines = potent
- TDF with FTC/3TC preferable for sequencing options
- ? Any national resistance surveillance data
- ?? Baseline resistance test
- Check on adherence/tolerability etc.
- If VL fails to fall OR rebounds further resistance test
- Switch early <1000 c/ml

Experienced patient

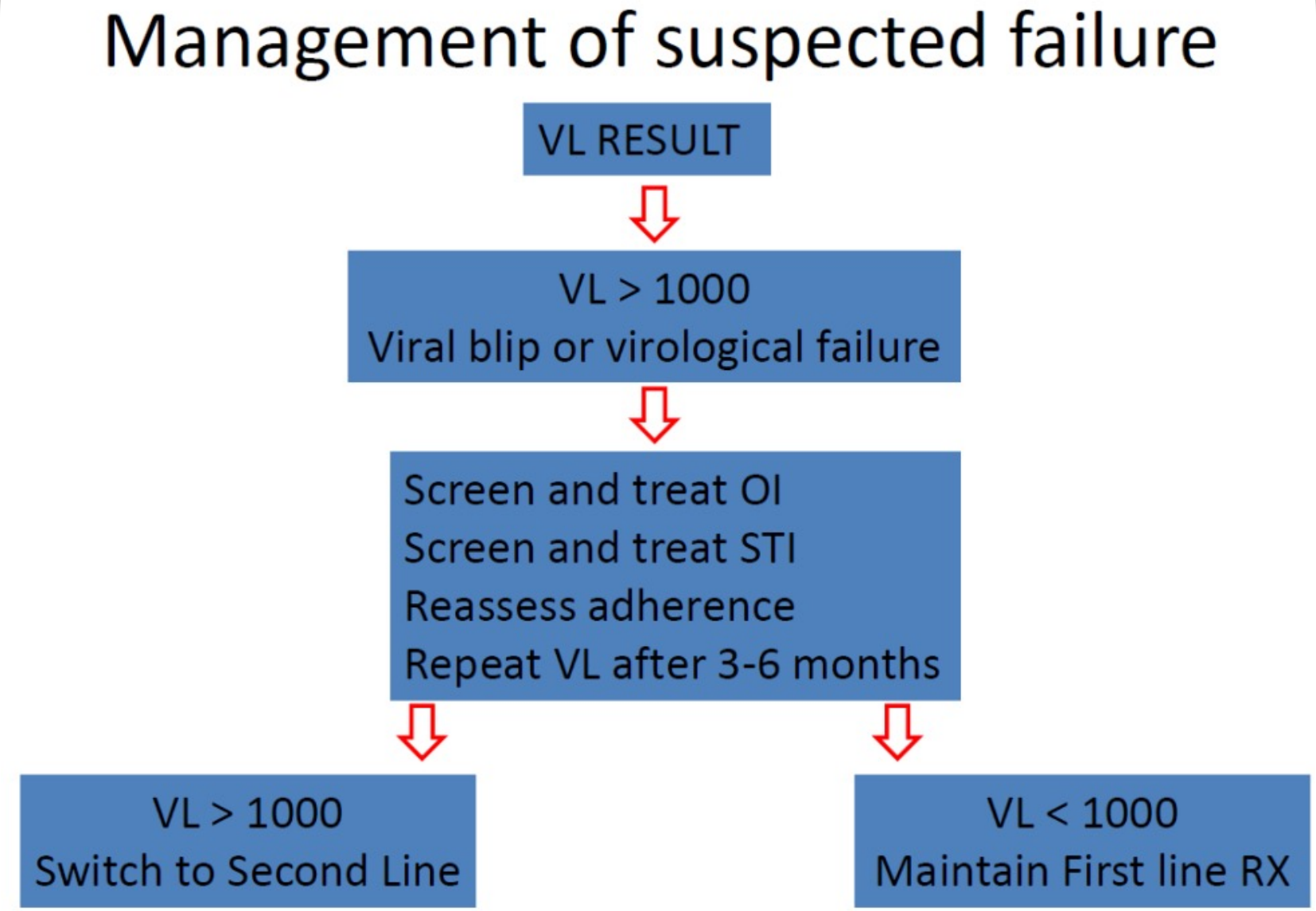
- Know your patient's ART history and:
  - What he was on when his VL increased OR his CD4 fell
  - What he wasn't taking properly/was suboptimal treatment
- Resistance test before new regimen
- If none
  - Assume NNRTI/3TC resistance
  - Boosted PI based new combination
  - NRTI choice dependent on 1st line treatment

Targeted resistance testing

- Baseline resistance testing:
  - Prevents use of partially resistant combinations which may lead to selection of resistance
  - Early virological failure
  - Restricted options

- Resistance testing at first VL >400
  - By definition NOT a blip
  - Early detection of NNRTI and 3TC resistance
  - Preservation of TDF sensitivity
  - Allows continuing use of TDF

Discussion point…
(your workshop 2016)



The aim of this session

- What is resistance?
- Why does it occur?
- Why are we so bothered?
- The resistance test – when, why and how?
- The virological benefits and concerns of using TDF 1st line
- How do we minimise the risk of resistance, particularly to tenofovir?

Discussion and questions?

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