Themes

Viral load
Tropism
Drug resistance

HIV Virology & Resistance

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The HIV Virology Timeline

HIV-1 isolated

HIV-1 genome sequenced

HIV replicates at high levels throughout the infection

Virus replication causes immunological compromise

HAART

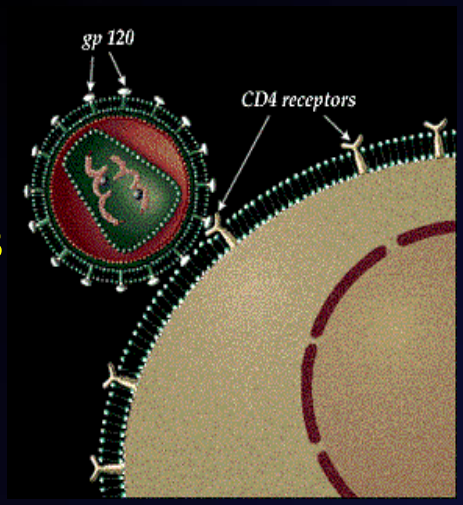
Viral load testing

Virus replication drives disease pathogenesis through Immune activation & inflammation

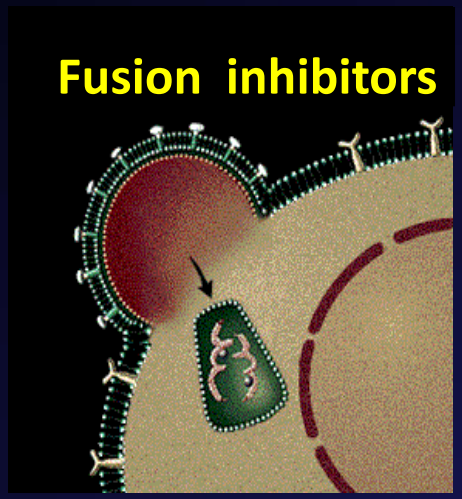
Is HIV eradication possible?

1982 1985 1991 1995 1996 2009 2010 →

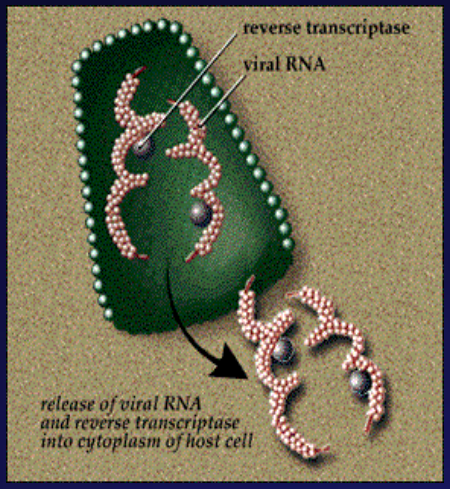
CCR5 antagonists



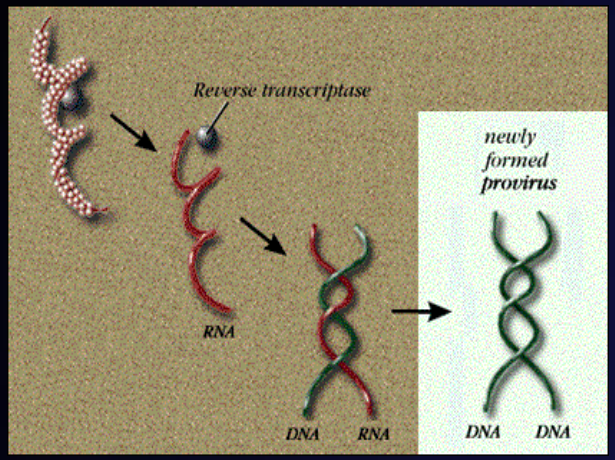
Attachment



Fusion



Release of RNA

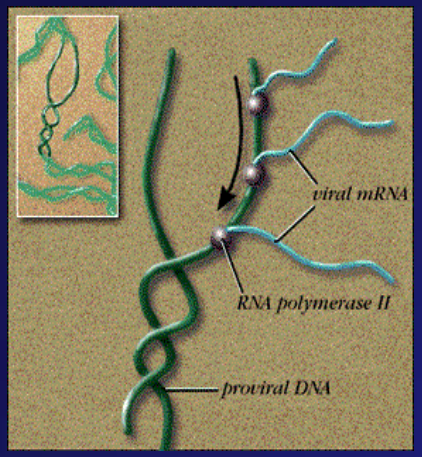


Reverse transcription

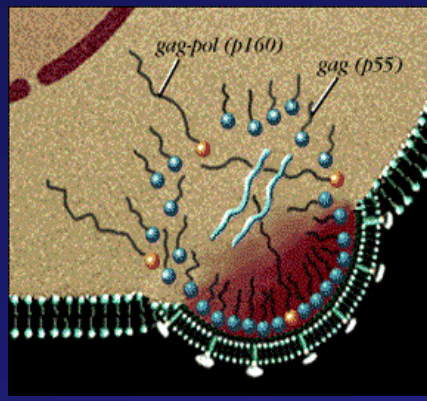
Nucleos(t)ide and Non-nucleoside RT inhibitors

Integration

Integrase inhibitors



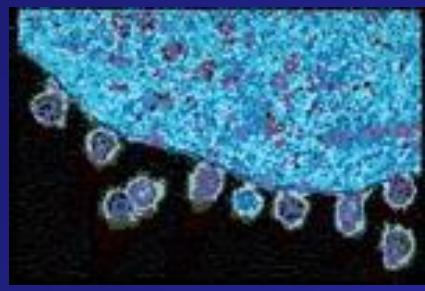
Transcription



Assembly

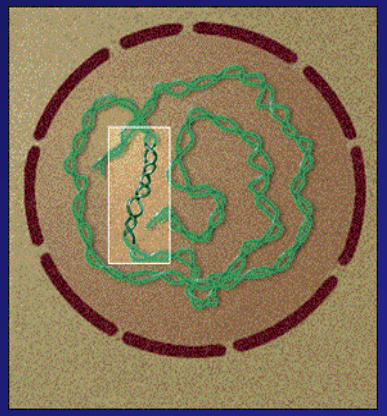
Maturation & budding

Protease inhibitors



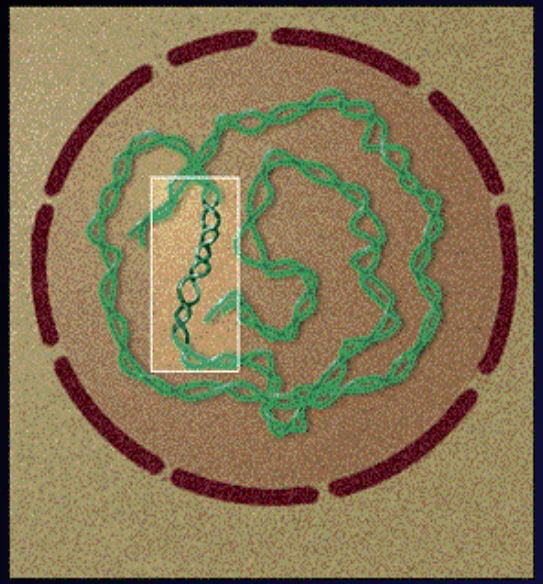
Key virological characteristics of HIV infection

- **High virus replication rate**
  10^9-10^{10} virus particles produced each day
- **Rapid virus clearance**
  $T_{½}$ virus producing cells: <1 day
  $T_{½}$ plasma free: a few hours
- **Genetic evolution**
  All possible point mutations in the viral genome can be generated daily
- **Virus latency – integration into host DNA**
  ~ 1:10^6 resting CD4 T cells



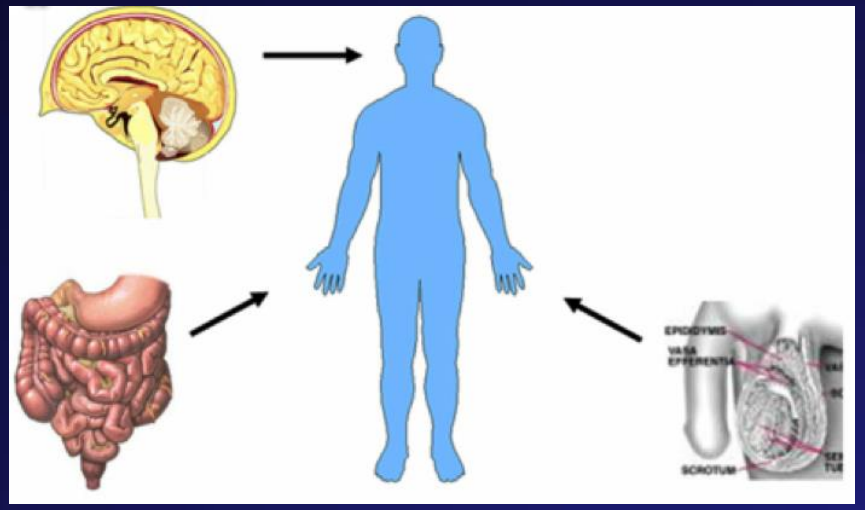
Wong et al. PNAS 1997; Wong et al. Science 1997; Chun et al. Nature 1997; Chun et al. PNAS 1997; Siliciano et al. Nat Med 2003; Strain et al. PNAS 2003

Obstacles to HIV eradication with ART

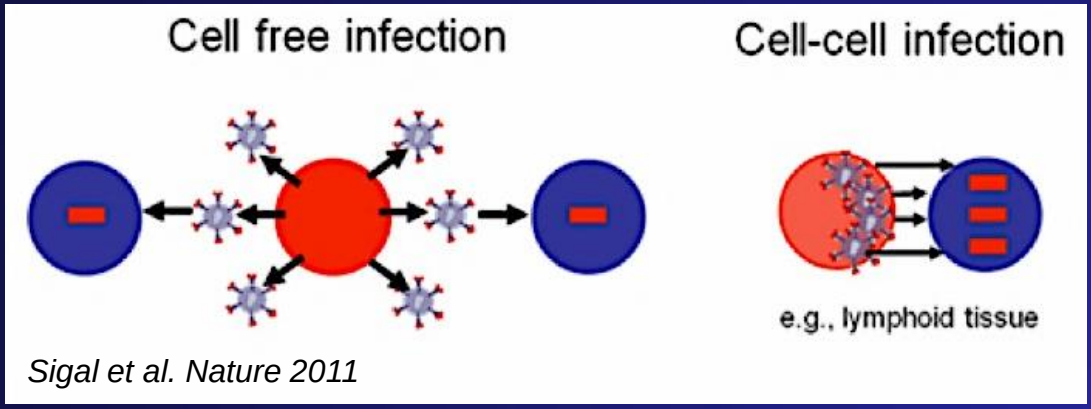


Integration and latency

Sanctuary sites



Cell-to-cell virus spread



Sigal et al. Nature 2011

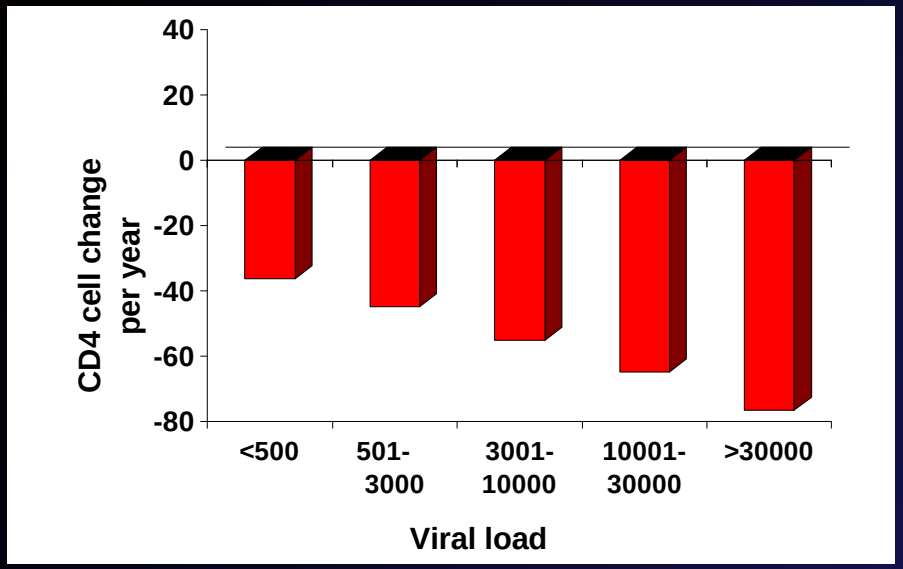
HIV-1 DNA detection during suppressive ART

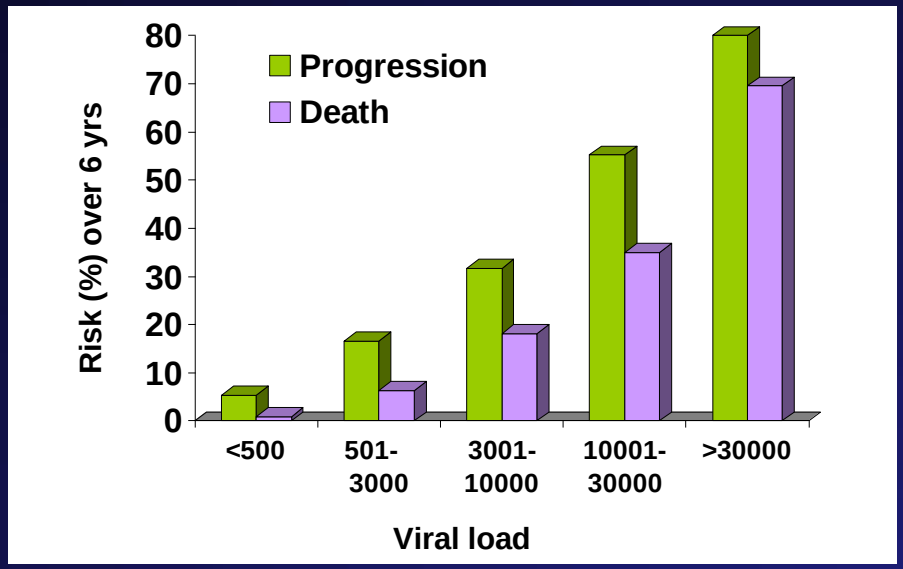
- HIV-1 DNA quantified in PBMC from 104 patients receiving suppressive ART for 1 to 15 years

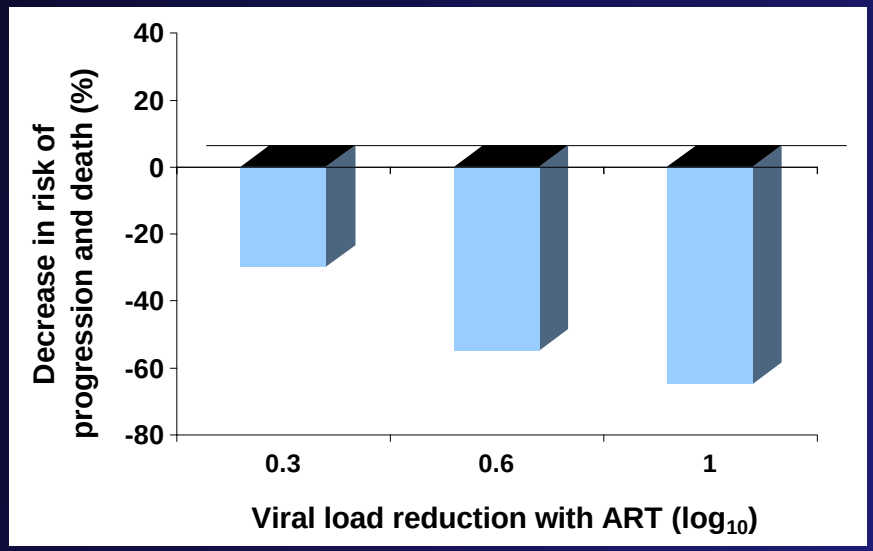
PBMC = Peripheral blood mononuclear cells

Geretti et al. International Workshop on HIV & Hepatitis Viruses Drug Resistance 2013

HIV viral load predicts the rate of CD4 cell loss and disease progression

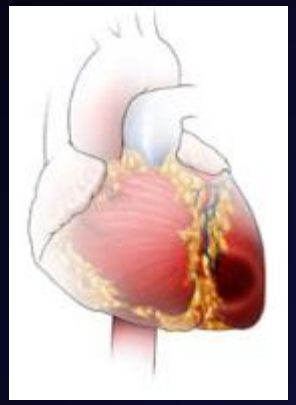






Mellors et al. Science 1996

SMART Study: Stopping ART associated with increased morbidity and mortality



% cumulative clinical events

15
10
5
0

Arm that stopped therapy at CD4 >350 and restarted at CD4 <250 (n= 2495)

p <0.0001

Arm that continued therapy (n=2512)

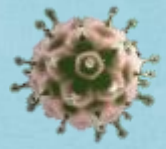
0 4 8 12 16 20 24 28 32 36 40 44

Months after discontinuation

No.												
DC	2495	1839	1441	1112	915	733	569	449	375	297	178	34
VS	2512	1848	1434	1122	931	754	601	490	398	310	184	41

El-Sadr et al. NEJM 2006

Starting first-line ART
DHSS guidelines 2013



	Third agent	NRTIs	Evidence
Recommended	EFV	TDF/FTC	AI
	ATV/r	TDF/FTC	AI
	DRV/r	TDF/FTC	AI
	RAL	TDF/FTC	AI
Alternative	EFV	ABC/3TC	BI
	RPV	TDF/FTC	BI
	RPV	ABC/3TC	BIII
	ATV/r	ABC/3TC	BI
	DRV/r	ABC/3TC	BII
	FPV/r or LPV/r (QD or BID)	ABC/3TC or TDF/FTC	BI
	RAL	ABC/3TC	BIII
	EVG/c	TDF/FTC	BI

EFV = consider avoiding if woman of child-bearing age

TDF = caution if renal insufficiency

ATV = do not use or caution with acid-lowering agents

RPV = not if VL >100,000 cps; caution if CD4 <200; not with PPIs

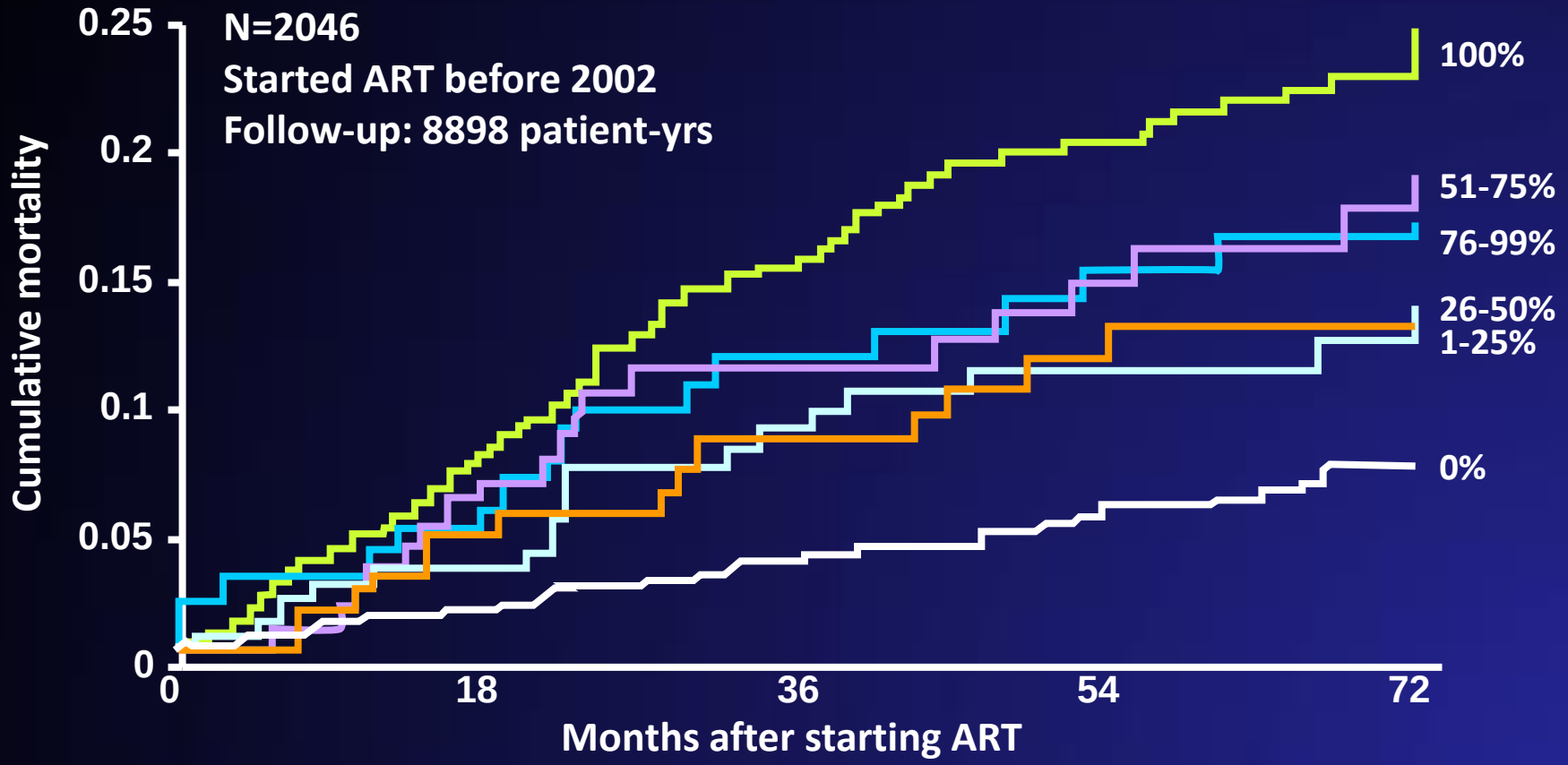
ABC = caution if VL >100,000 cps or high CVD risk; use only if HLA-B57.01 negative

EVG/c = only if eGFR >70 ml/min; potential DDIs with COBI; not with other nephrotoxic drugs

DHSS Guidelines Feb 2013

Mortality according to frequency of viral load measurements >400 cps in first-line ART

Cumulative mortality stratified by % of VL measurements ≥400 cps over first 18 months of ART



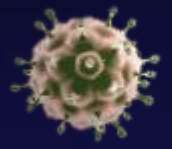
Lohse et al. Clin Infect Dis 2006

The goals of ART

- Restore and preserve immune function
- Reduce HIV-related morbidity and mortality
- Improve quality of life
- Provide maximal and durable VL suppression

- EACS 2012: <50 cps
- BHIVA 2012: <50 cps
- IAS-USA 2012 : <50 cps
- DHHS 2013: <assay detection limits

Defining cut-offs: Viral load assays



- The recommended target for defining ART success dictated by the technical properties of the viral load assay, rather than selected *a priori* based upon clinical significance
  - ***First-generation assays <400 cps***
  - ***Second-generation assays <50 cps***

  (e.g. Roche Amplicor v1.5)

However

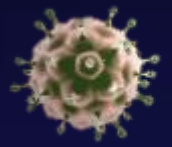
- Patients who achieve and maintain a viral load <50 cps have a small risk of rebound >50 cps during follow-up, and the risk declines further the longer the viral load is <50 cps

Assay discorcordance at the 50 cps cut-off

		>50 cps			
		Amplicor 1.5	RealTime	TaqMan v2	$Artus_{HIV}$ [2]
<50 cps	Amplicor 1.5	-	NA	6%	-
	RealTime	NA	-	13%	5%
	TaqMan v2	5%	7%	-	-
	$Artus_{HIV}$	-	5%	-	-

1. The International Viral Load Assay Collaboration (unpublished);
2. Garcia et al. JCV 2013

Defining virological failure



EACS 2012: Confirmed VL >50 cps 6 months after initiation or modification of ART

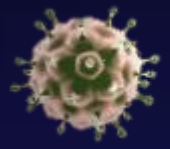
BHIVA 2012: Failure to achieve VL <50 cps 6 months after commencing ART or following suppression <50 cps, confirmed VL rebound >400 cps

DHHS 2013: Inability to achieve or maintain VL <200 cps

IAS-USA 2012: Sustained VL elevation between 50 cps and 200 cps should prompt evaluation of factors leading to failure and consideration of changing ART

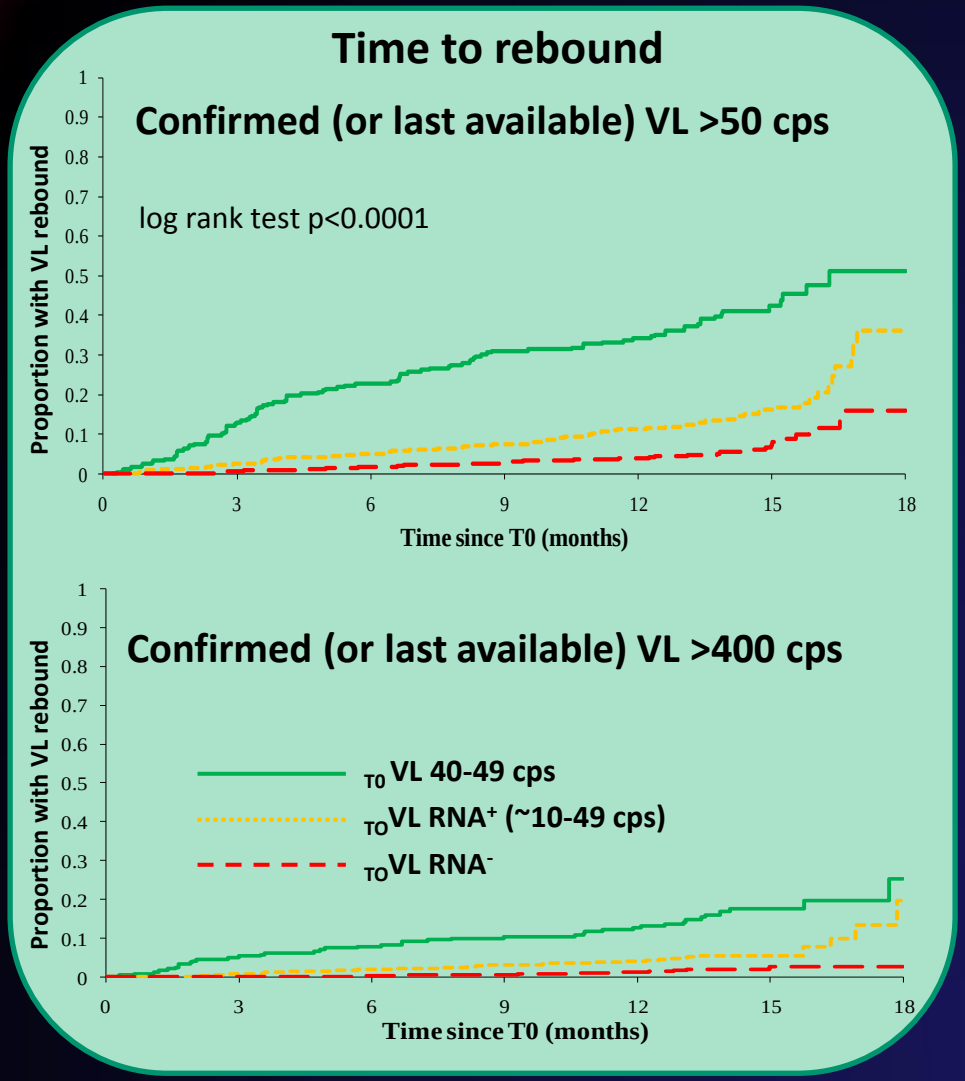
VL = Viral load

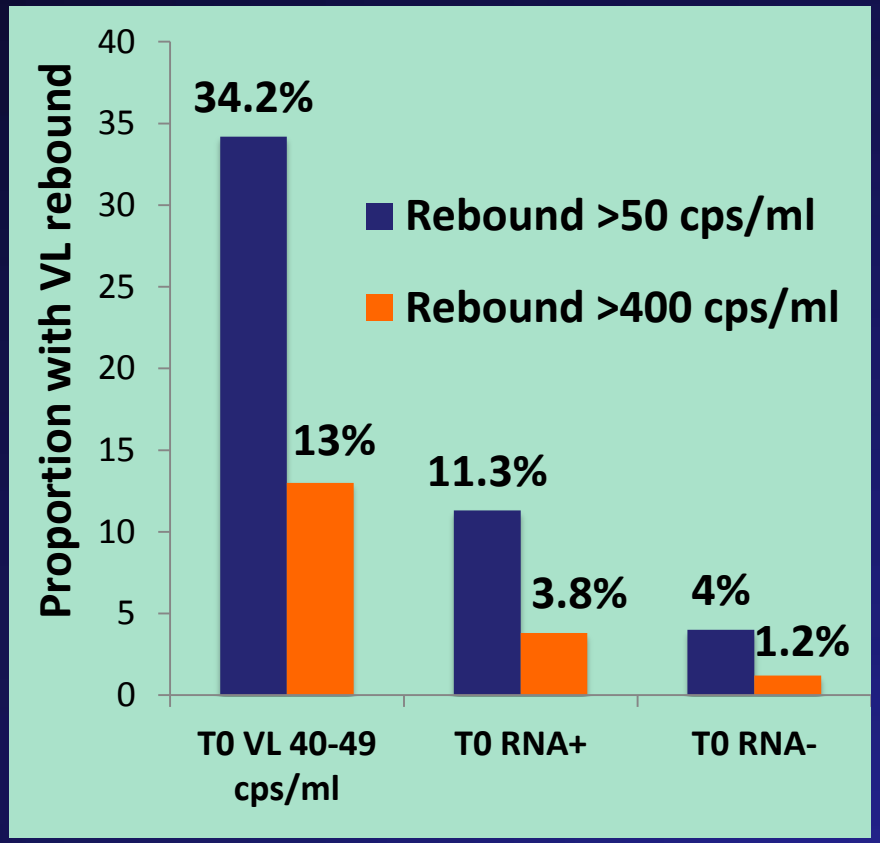
Third-generation viral load assays



- Lower limit of quantification (LLQ) 40 cps (RealTime; Taqman v1) or 20 cps (Taqman v2)
- Report qualitative RNA detection below the LLQ as "target detected"
- Patients on ART can show one of three results:
  - *VL quantified above the LLQ*
  - *RNA detected below the LLQ (RNA^+)*
  - *RNA not detected (RNA^-)*

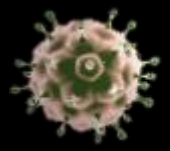
Plasma HIV-1 RNA detection below 50 cps predicts viral load rebound





Doyle et al. Clin Infect Dis 2012

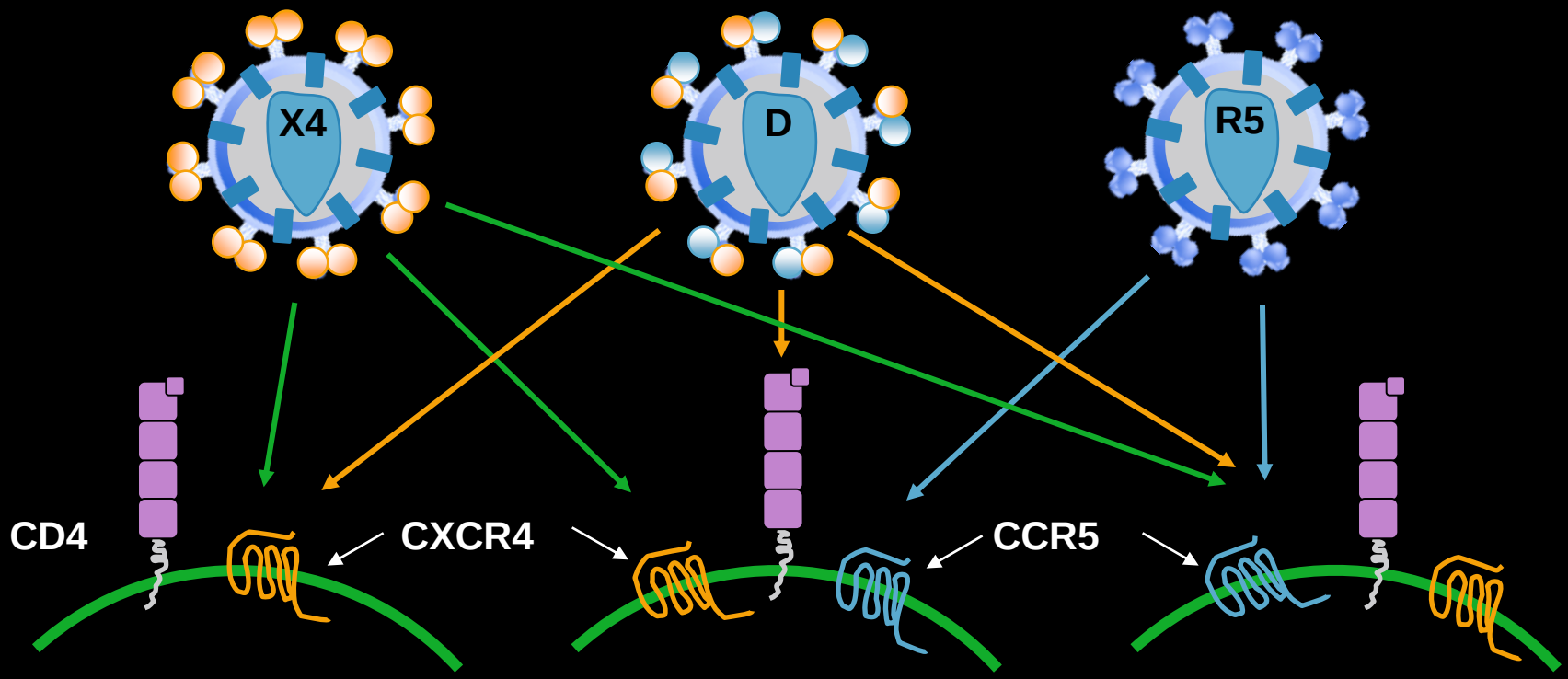
Take-away points



- Viral load informs prognosis, guides ART initiation and is the **key surrogate marke**r of ART efficacy
- The optimal level of viral load **suppression** with ART is <50 cps and probably <10 cps
- There remain uncertainties about the optimal management of **low-level viraemia** leading to discrepancies within guidelines
- Viral load assay **performance** differs
  - *Importance of clinic-lab dialogue*
  - *Importance of using one assay for monitoring*

HIV tropism

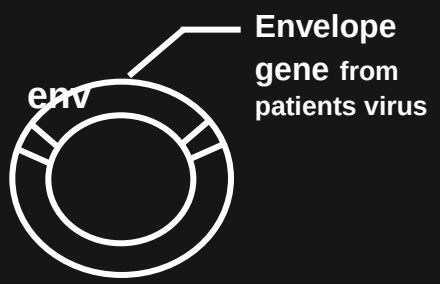
- Defined by the differential use of co-receptors and by the cellular distribution of co-receptors



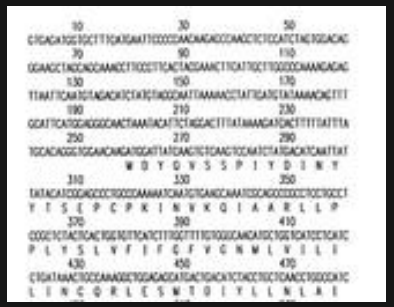
Esté et al. Lancet 2007

Genotypic Tropism Testing

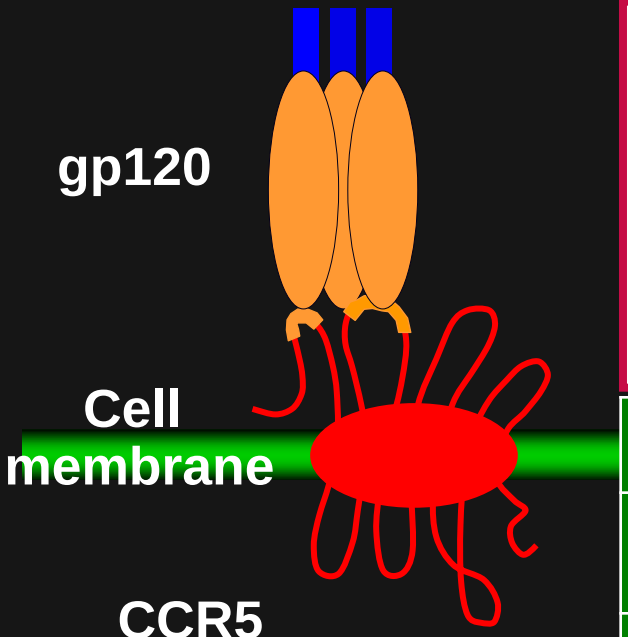
1. Gene sequencing

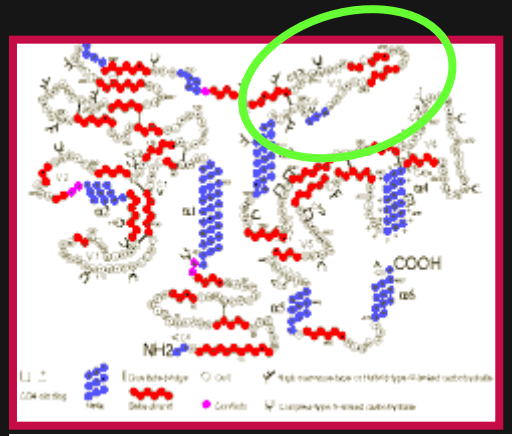


2. Sequence determination



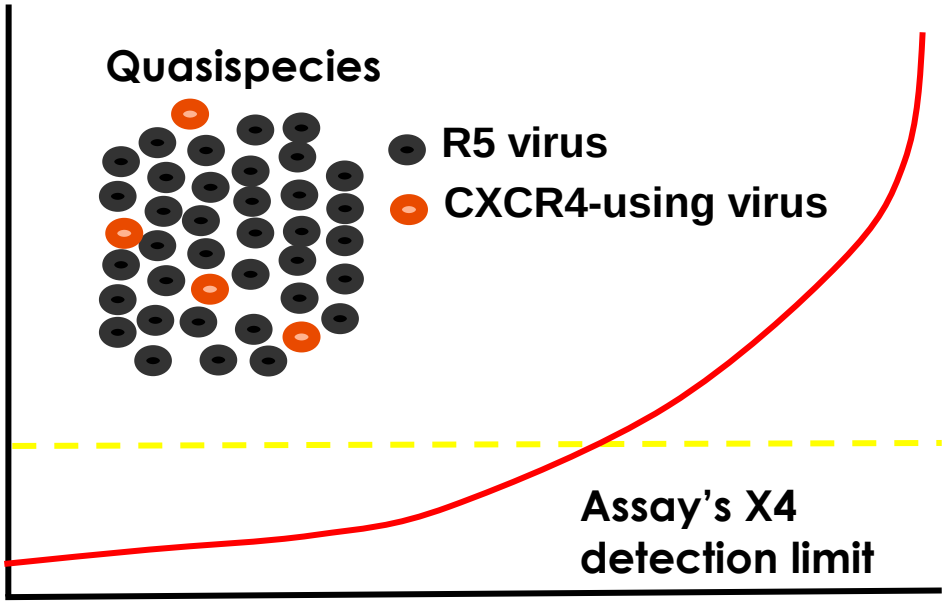
3. Results interpretation via algorithms

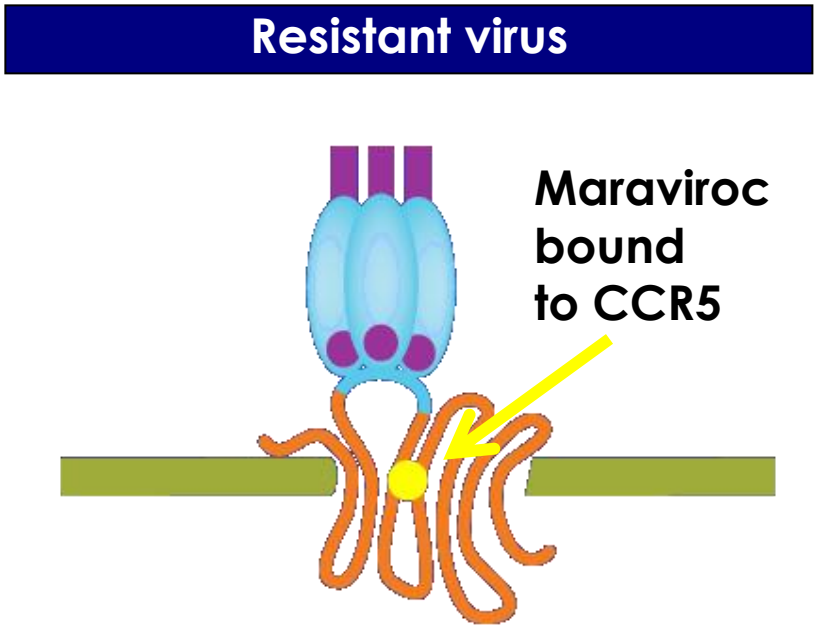




V3 Sequence	
CTRPNNNT-RKSIPMGPG--QAIYATGAIIGDIRQAHC	R5
........R..-.HI..RH..VM...E-...N.....	X4

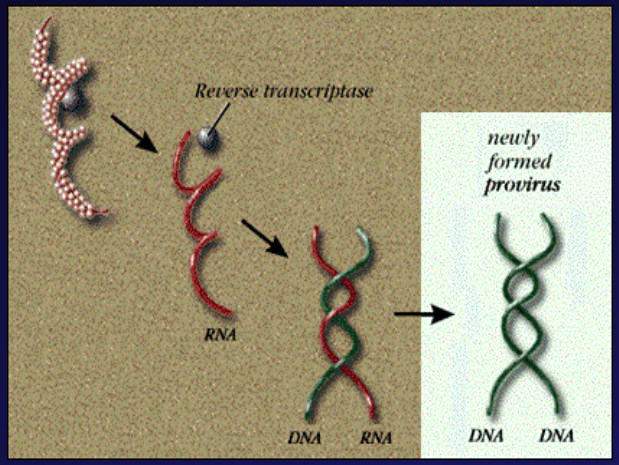
Mechanisms of viral escape during therapy with CCR5 antagonists





Van der Ryst et al. ICAAC 2007

Mechanisms of HIV genetic evolution



1. **Errors by viral reverse transcriptase**

   ~1 mis-incorporation per genome round

2. **Errors by cellular RNA polymerase II**

3. **APOBEC-driven G→A hypermutation**

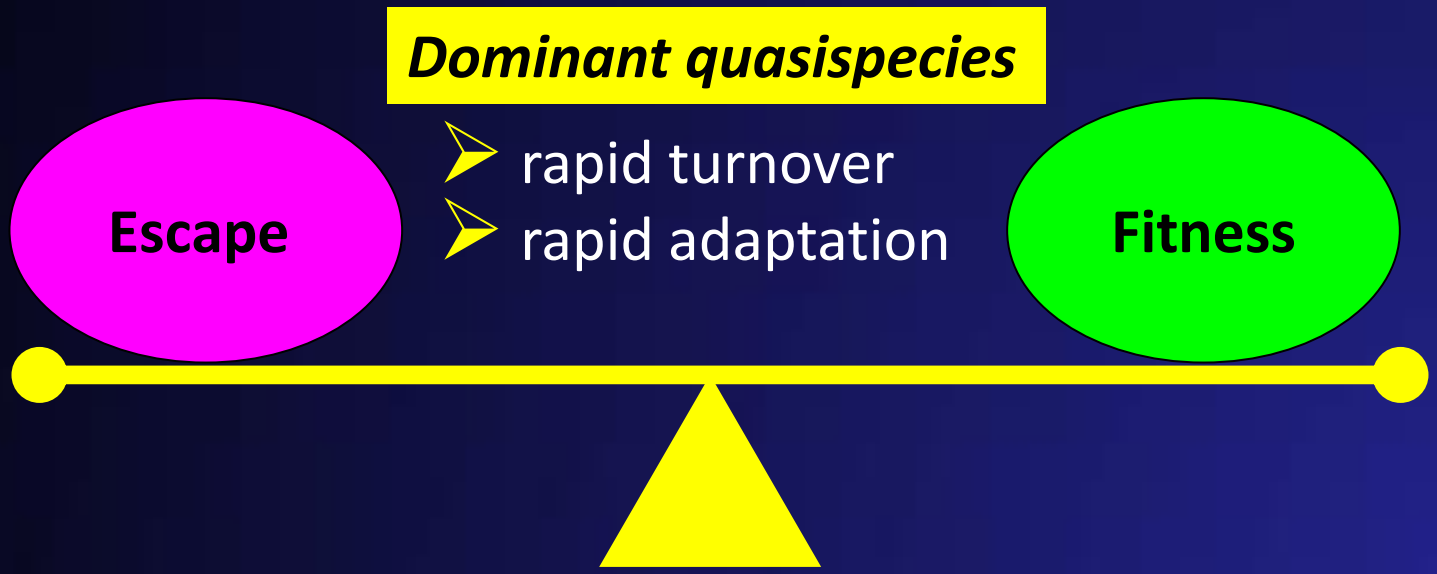
   Deamination of cytosine residues in nascent viral DNA

4. **Recombination between HIV strains**

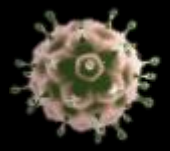
   ~7-30 events per genome round

Consequences of HIV genetic variability

- Challenge for diagnostic assays
- Escape from immune and drug pressure
- Variations in drug susceptibility and resistance pathways
- Viral fitness, tropism and disease pathogenesis

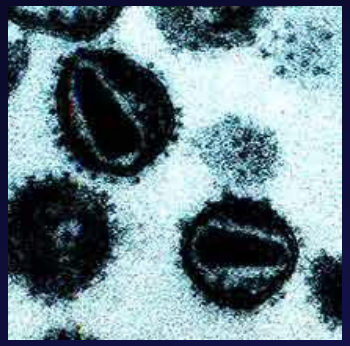


Take-away points



- Drug-resistant mutants emerge **”spontaneously “** during HIV replication
- Due to **impaired fitness**, these “spontaneous” drug-resistant mutants exists only at very low level (in the absence of drug pressure)
- **Single mutants** > double mutants >> triple mutants

PCR



Viral gene (e.g., RT)

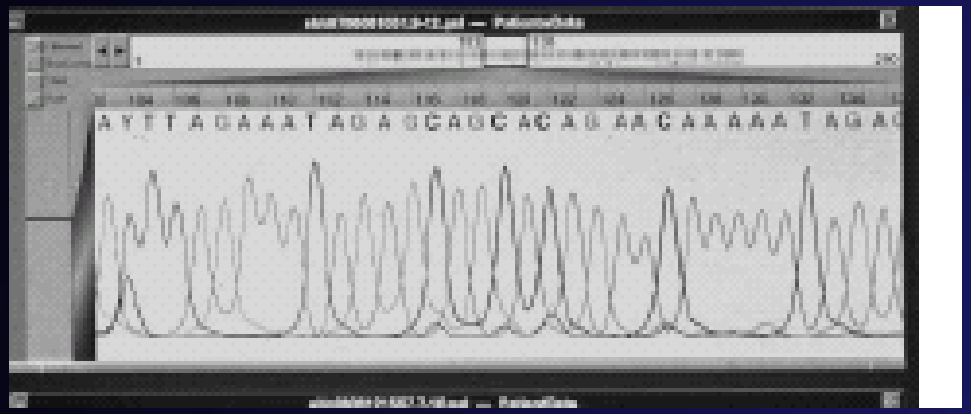
HIV RNA

Plasma

How we detect resistance in routine practice

Sequencing

Mutations



RT M184V

Methionine Valine

@ codon 184 of RT

ATG / AUG GTG / GUG

Recommendations for drug resistance testing[1]

Time	Comment	Method	Evidence
Diagnosis	Recommended	Genotypic	Ia
Starting ART	Recommended if not already carried out*	Genotypic	Ia
After starting ART	Consider if <1log VL drop after 4 wks	Genotypic	IV
	Consider if VL >50 cps/ml after 12-16 wks	Genotypic	III
	Recommended if VL >50 cps/ml at 24 wks	Genotypic	Ia
ART failure	Recommended**	Genotypic	Ia

*Repeat testing to detect superinfection not routinely recommended but may be considered in selected cases (IIb)[2]

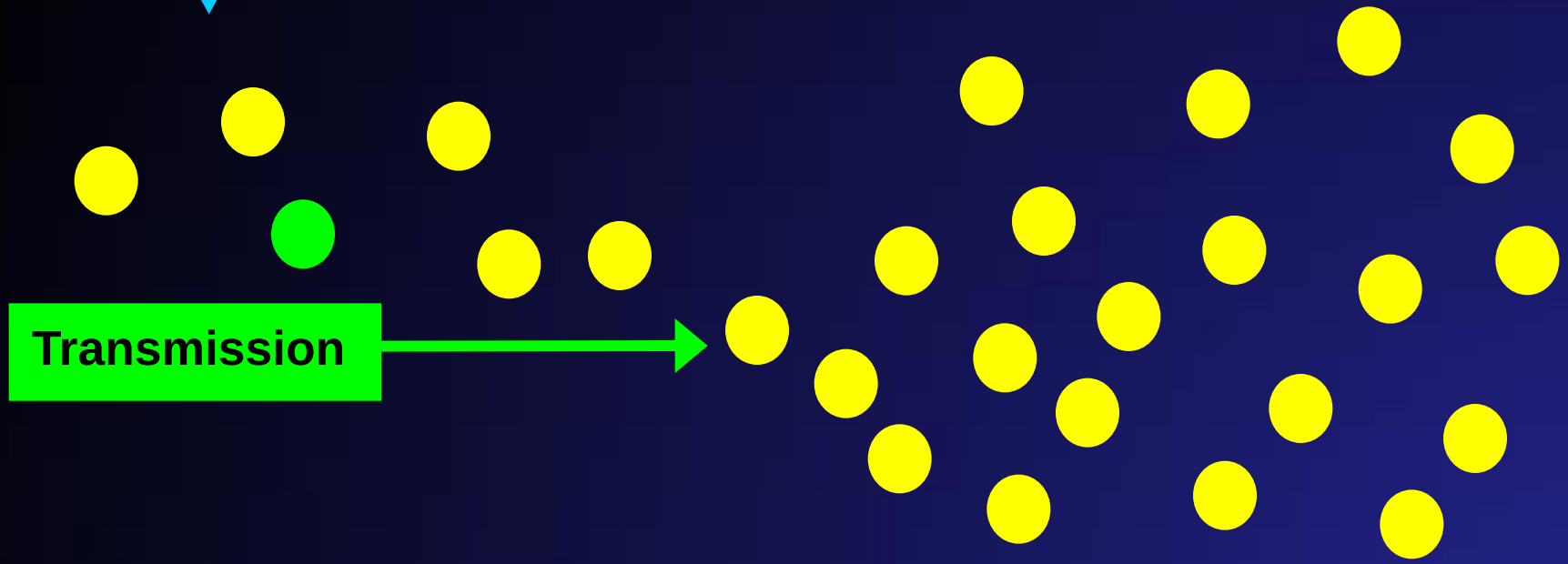
**Consider phenotypic or virtual phenotypic testing if interpretation is uncertain

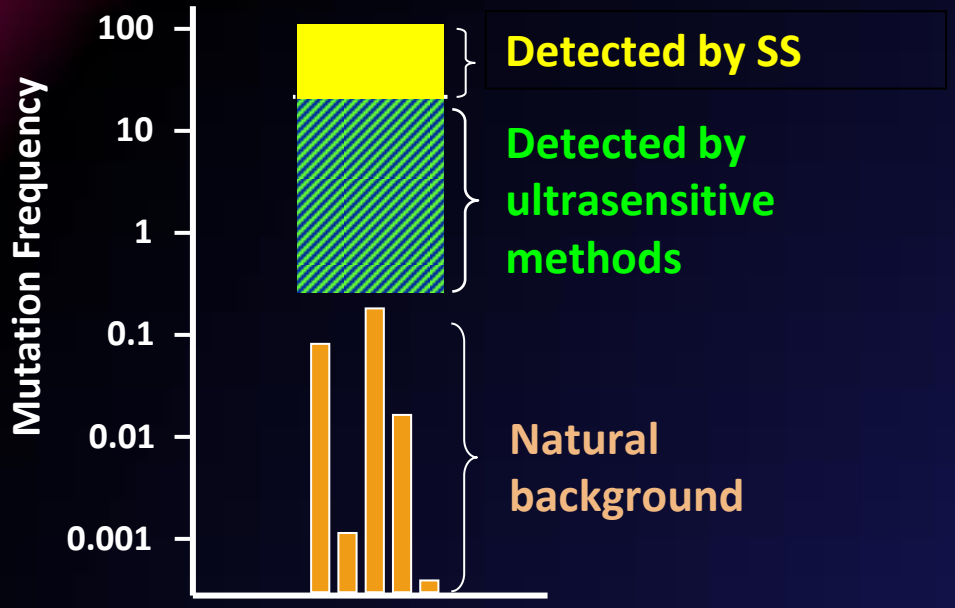
VL = Viral load

1. BHIVA guidelines for the routine investigation & monitoring of adult HIV-1 infected individuals 2011; 2. Doyle, AIDS 2011

Transmitted drug resistance

Stable after transmission
Gradual reversion over time
Persistence at low frequency in plasma
Persistence in latently infected cells





15-30%

Limit of detection of Sanger sequencing (SS)

Impact of transmitted drug resistance

Resistance in ART-naïve patients (FIRST Study, n=258)

	Resistance test	
RAMs	SS	UDS
NNRTI	7%	15%
NRTI	6%	14%
PI	2%	5%
Any	14%	28%

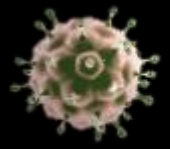
Risk of failure of first-line NNRTI-based ART

- SS RAMs: HR 12.4 [3.4-45.1]
- UDS RAMs: HR 2.5 [1.2-5.4]

SS = Sanger sequencing; UDS = Ultra-deep sequencing; RAMs = Resistance-associated mutations

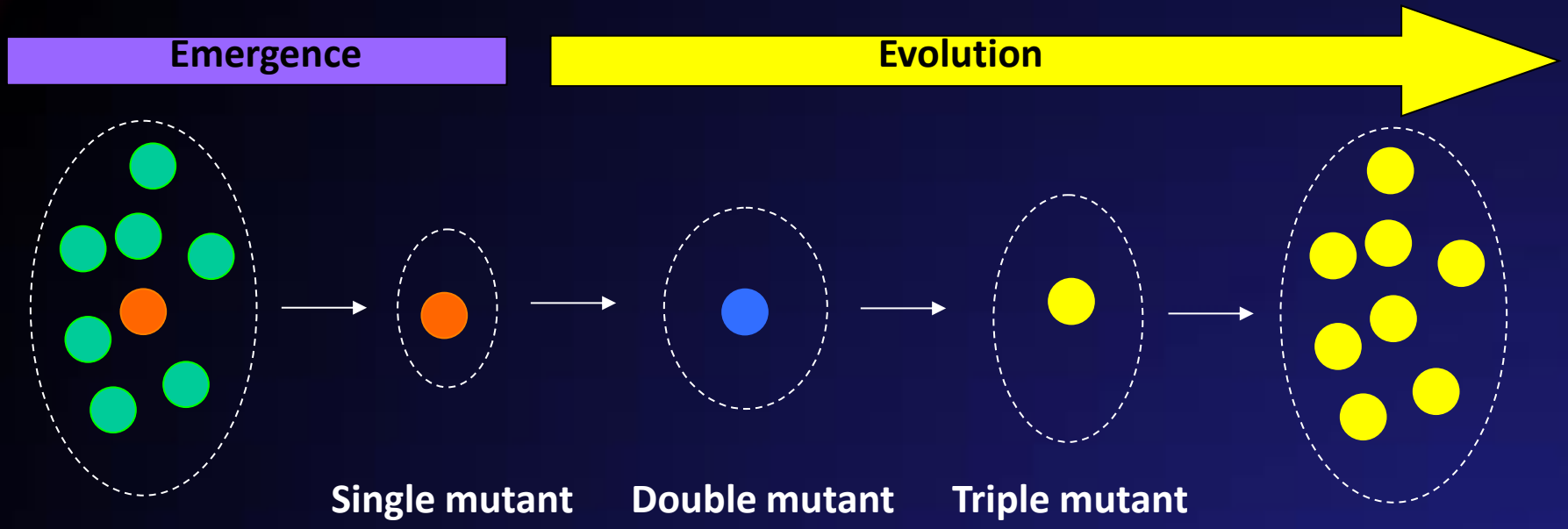
Simen et al. JID 2009

Take away points



- Transmitted drug resistant mutants **persist** as dominant species at variable rates in the absence of drug pressure
- Resistant mutants that have **apparently disappeared** persist at low frequency in plasma and are “archived” in latently infected cells
- Transmitted NNRTI and NRTI mutants reduce responses to NNRTI-based ART with a **dose-dependent effect**

Emergence and evolution of resistance



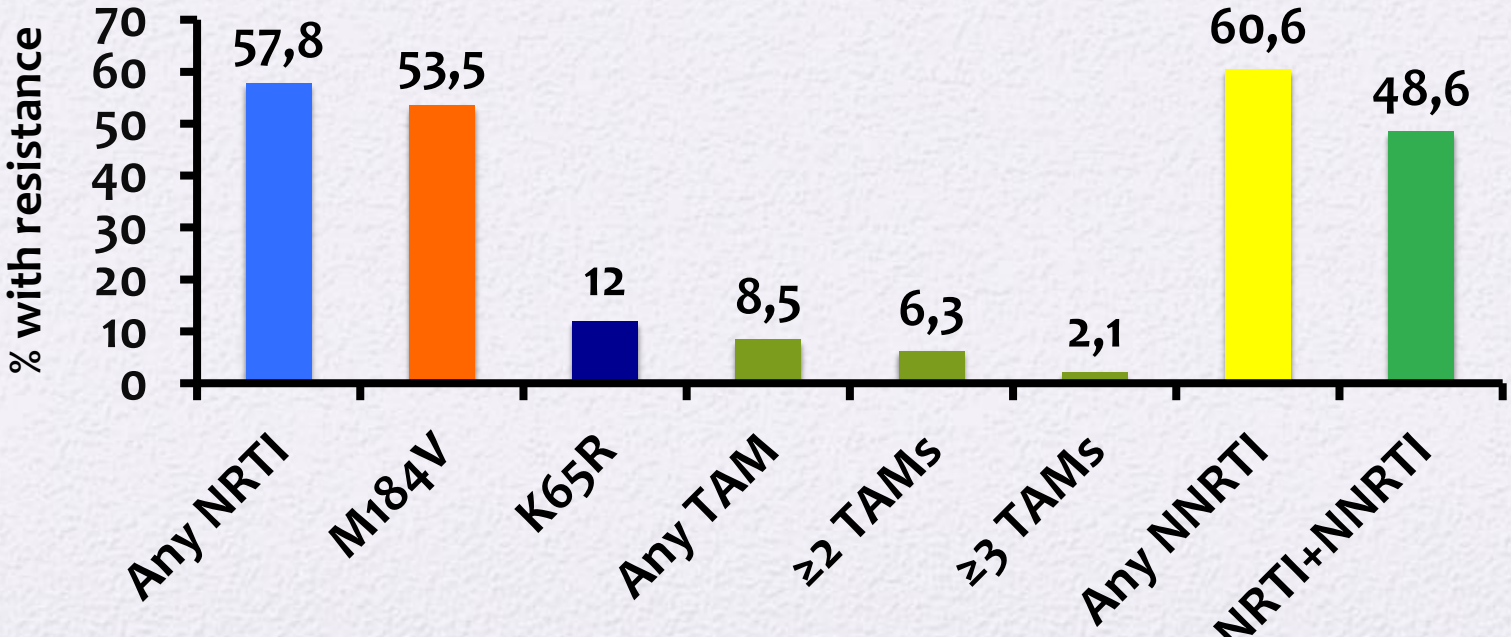
- Increasing number of mutations
- Accumulation of mutations on the same viral genome
- Initially reduced viral fitness
- Compensatory changes restore fitness over time

Genetic barrier & Cross-resistance

Class	ARVs	Genetic barrier	X-resistance
NRTIs	ZDV/3TC, d4T/3TC	+/++	+++
	ABC/3TC, TDF/3TC	+	+++
	TDF/FTC	+/++	+++
NNRTIs	EFV, NVP, RPV	+	+++
	ETV	+/++	+++
PIs	Unboosted	+/++	++/+++
	Boosted	+++/++++	+/++
Fusion inhibitors	T20	+	-
CCR5 antagonists	MVC	+/++	-
Integrase inhibitors	RAL, EVG	+	+++
	DTG	++/+++	++

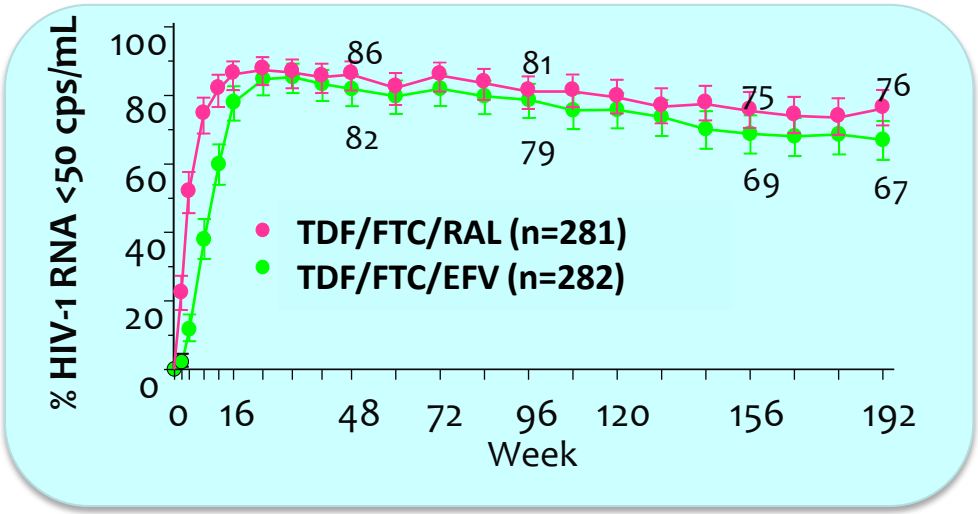
Resistance after failure of first-line NNRTI-based ART

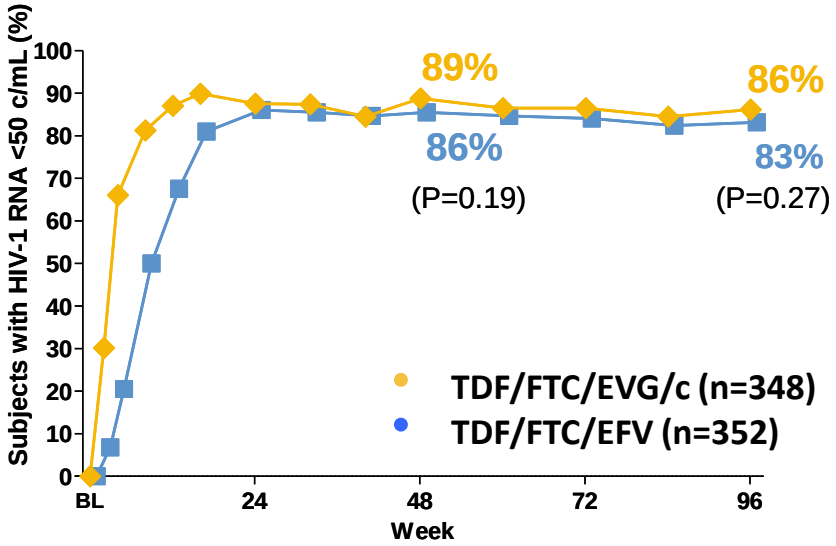
- Cohort from 6 sub-Saharan African countries (Kenya, Nigeria, South Africa, Uganda, Zambia, and Zimbabwe)
- 142 patients with viral load >1000 cps after 12 months of ART assessed for resistance



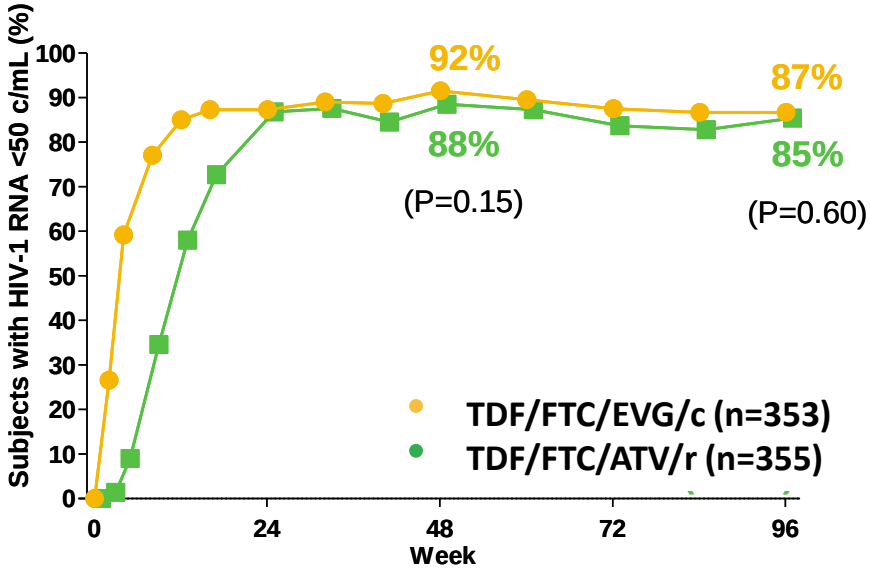
Hamers et al, Lancet Infect Dis 2011

EFV, RAL, EVG/c, or ATV/r in first-line ART





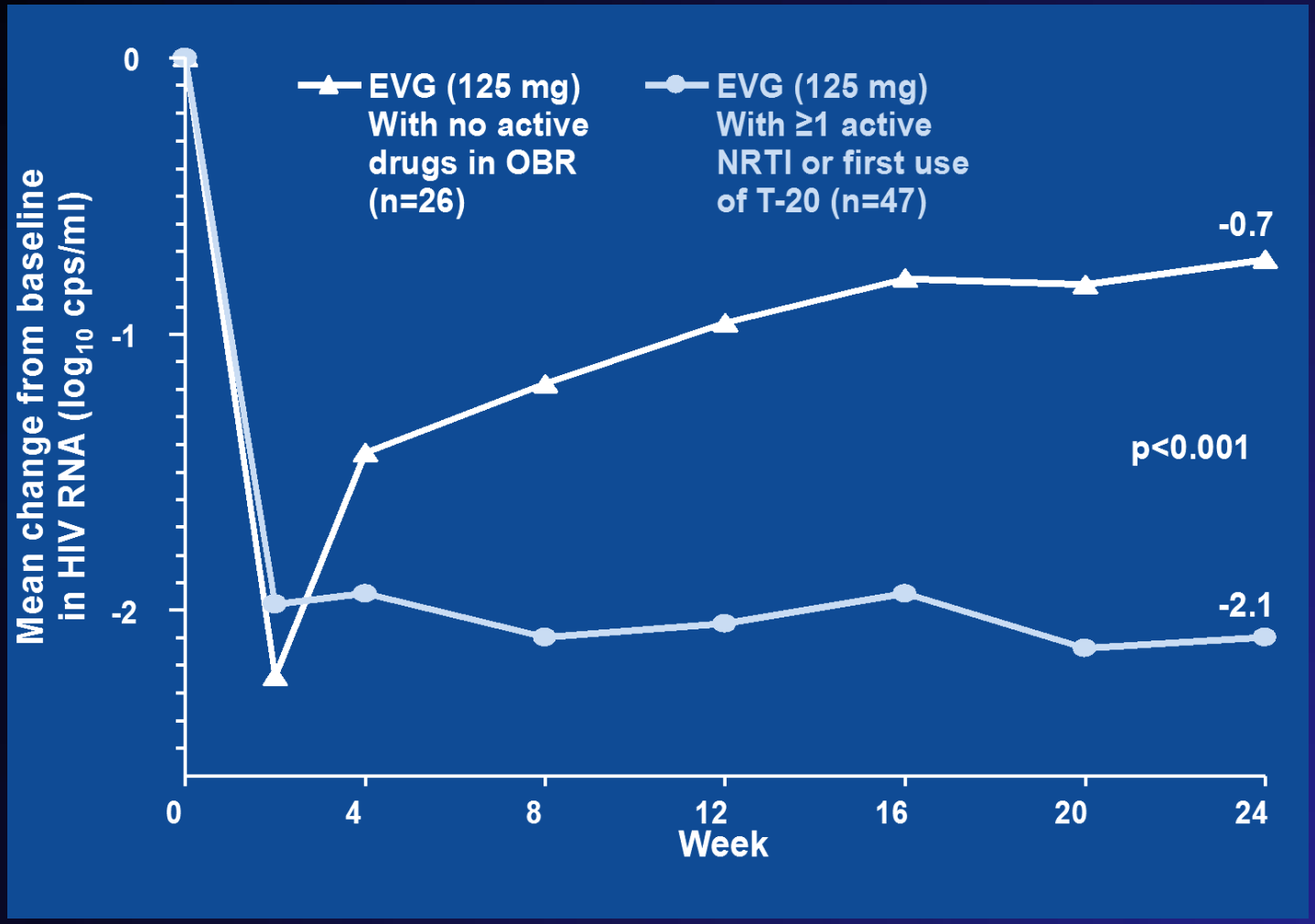
RAMs	STARTMRK wk 192		236-102 wk 96		236-103 wk 96	
	RAL	EFV	EVG	EFV	EVG	ATV
INSTI	4/16	-	9/17	-	5/17	-
EFV	-	7/14	-	10/23	-	0/15
ATV	-	-	-	-	-	0/15
NRTI	6/18	6/14	10/17	3/23	5/17	0/15
M184V/I	6/18	5/14	10/17	3/23	5/17	0/15
K65R	0/18	1/14	4/17	3/23	1/17	0/15



De Jesus et al, HCT 2012; Zolopa et al, ICDTHI 2012; Rockstroh et al, ICDTHI 2012

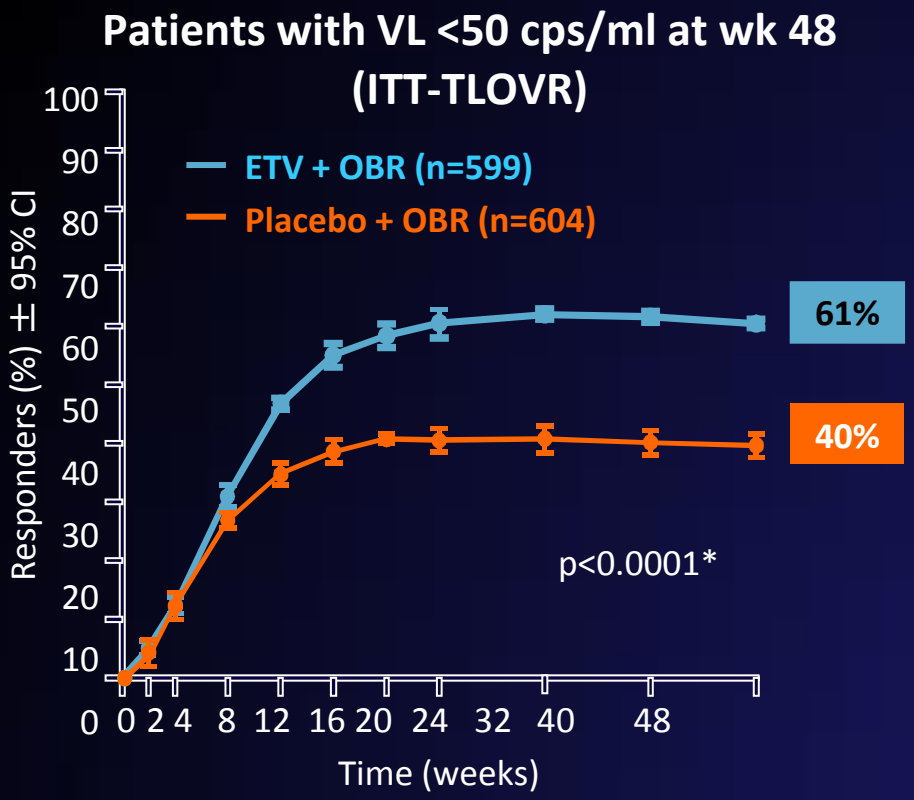
EVG/r with a weak or a strong backbone

Study GS-US-183-105



Zolopa CROI 2007

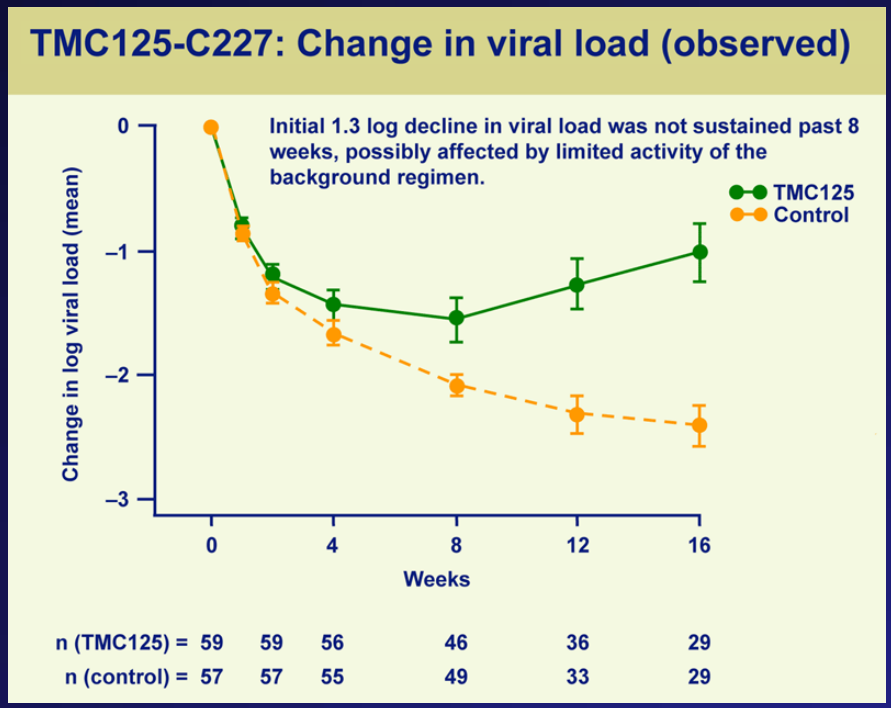
Etravirine with a strong backbone: DUET studies



ART-experienced patients with NNRTI and PI resistance

OBR = Optimized Background Regimen

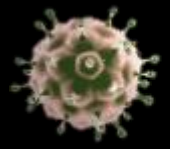
Etravirine with a weak backbone: TMC125-C227



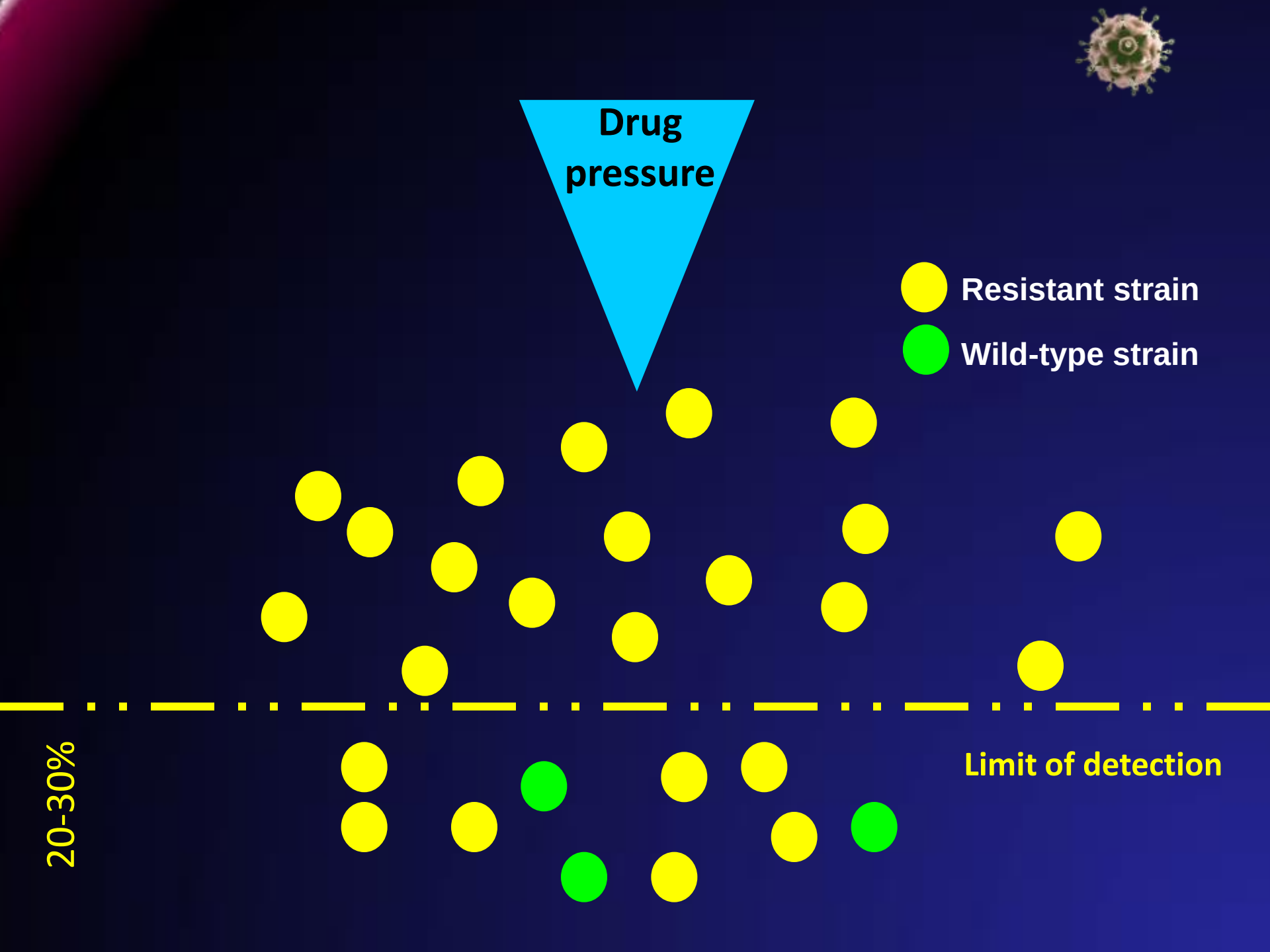
ART-experienced patients with NNRTI resistance

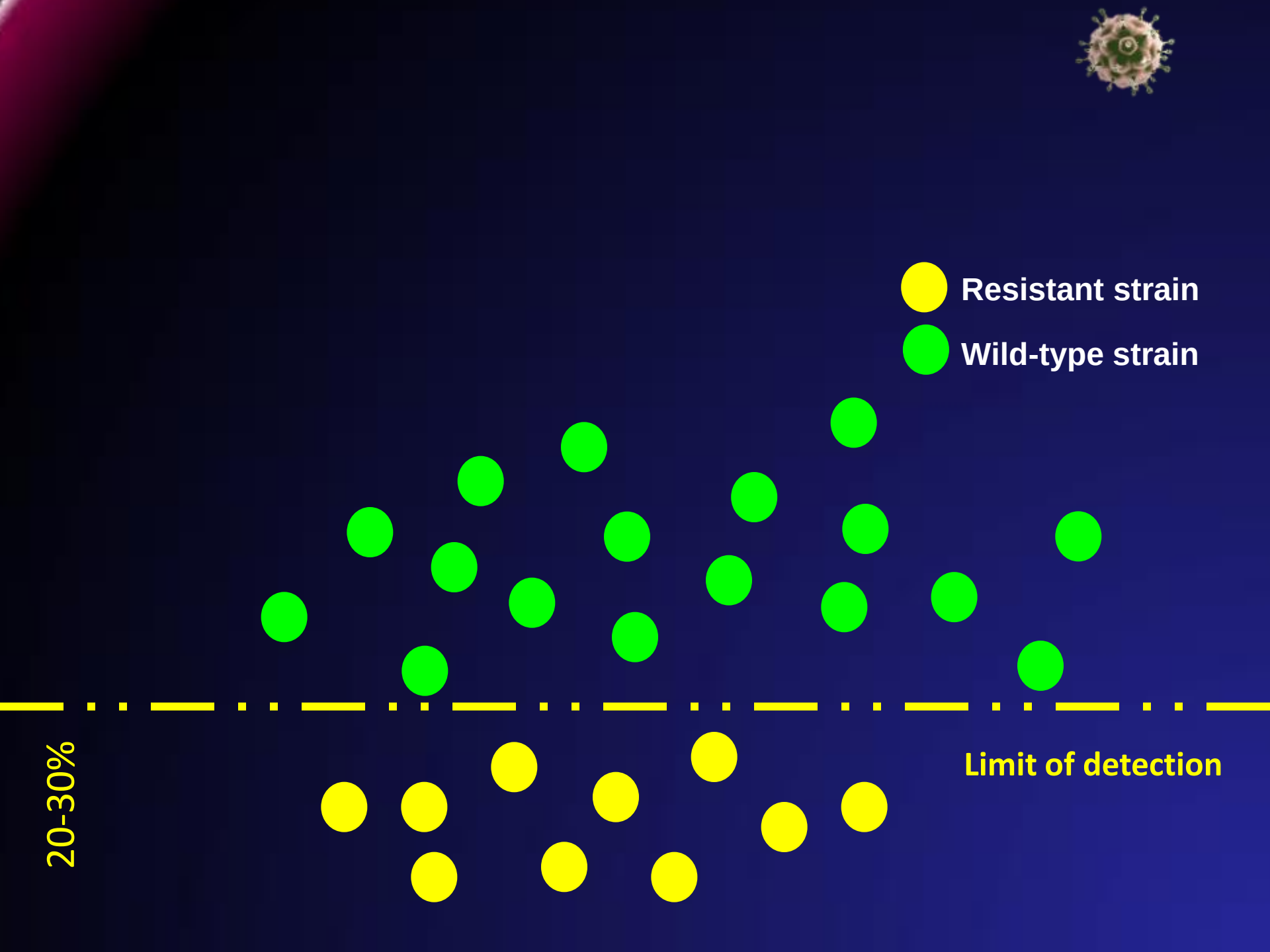
Katlama, AIDS 2009; Ruxrungtham, HIV Med 2008

Take away points



- The **genetic barrier** to resistance is defined by the number of mutations required to reduce susceptibility, fitness cost of mutations, interactions between mutations, drug levels, activity of other drugs in the regimen
- Patients receiving **NRTIs, NNRTIs** or **INSTIs** usually show resistance at the time of virological failure
- PI/r **augment** the genetic barrier of the entire regimen
- PI/r **protect** the residual activity of drugs with partial resistance and low genetic barrier

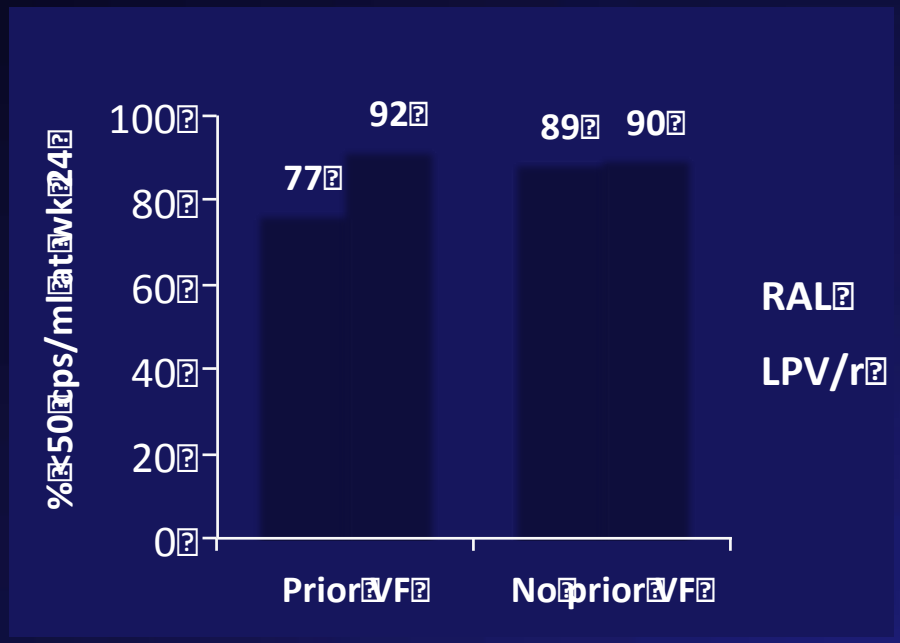

Drug pressure
Resistant strain
Wild-type strain
Limit of detection
20-30%


Resistant strain
Wild-type strain
Limit of detection
20-30%

SWITCHMRK: Replacing LPV/r with RAL

- Patients on ≥2NRTIs + LPV/r with VL <50 cps for ≥3 months randomised to continue LPV/r or switch to RAL

	RAL (n=350)	LPV/r (n=352)
Mean CD4 count	436	454
LPV/r >1 year	83%	82%
Prior ART duration, yrs	3.4	4.1
Previous ARVs, n	5	5
LPV/r as first regimen[a]	37%	37%
Previous VF[b]	32%	35%
% <50 cps at wk 24	81-88%	87-94%



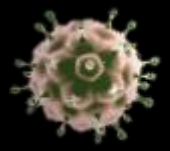
[a]Data obtained retrospectively
[b]Investigator-reported history

VF = Virological Failure

- Non-inferiority of RAL not demonstrated
- Less diarrhoea and better lipids on RAL

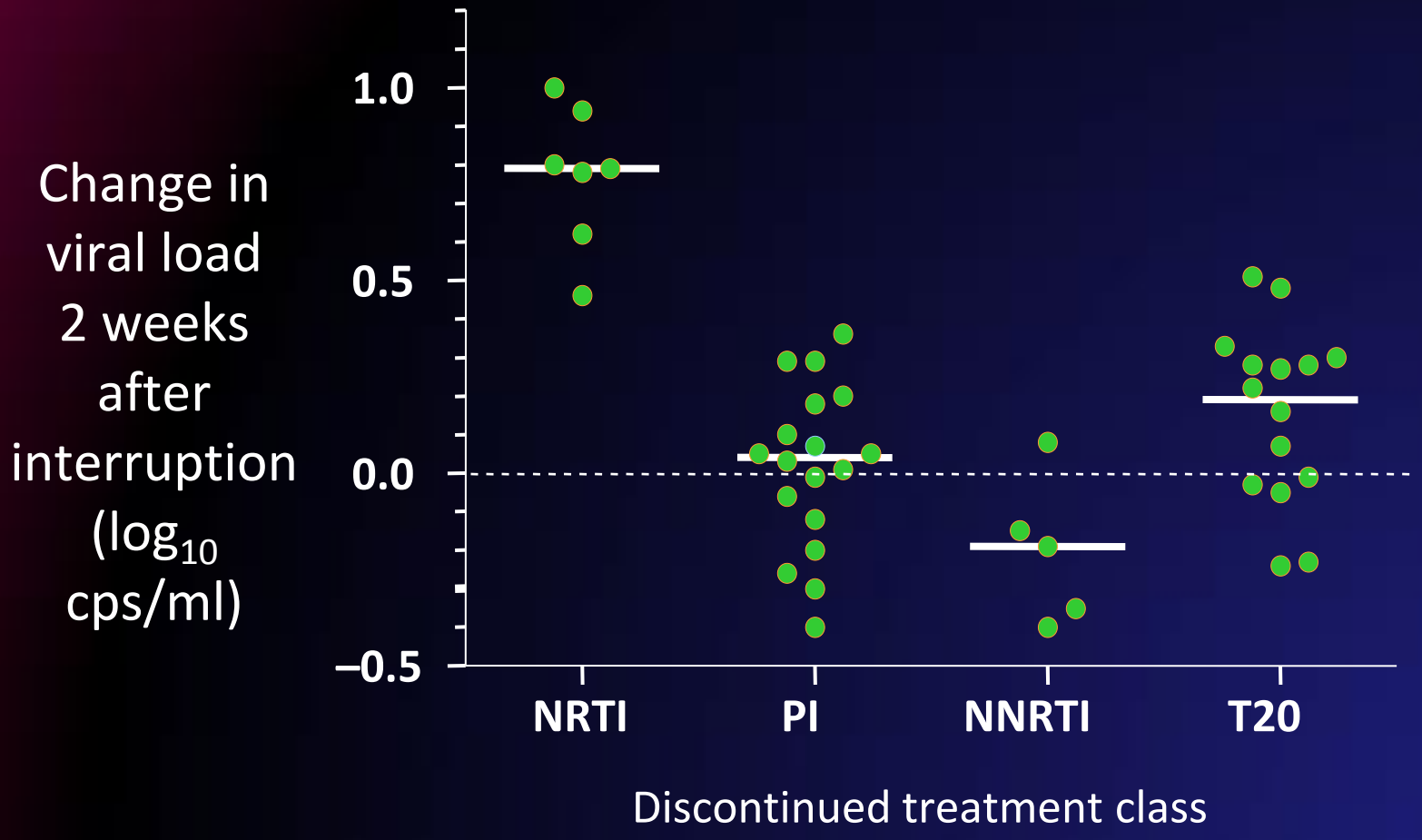
Eron et al, Lancet 2010

Take away points



- Once drug pressure is removed, resistant mutants are **outgrown** by fitter wild-type virus becoming undetectable by routine tests
- Resistant mutants that have **apparently disappeared** persist at low frequency in plasma and are “archived” in latently infected cells
  - *The memory of resistance is long-lived*
  - *Archived resistance can compromise a regimen with a low genetic barrier*
  - *When changing ART, consider the overall ART history and take into account past resistance(known or likely)*

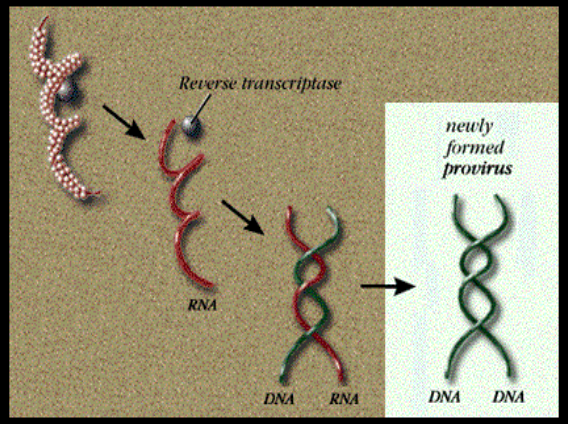
Partial treatment interruption in patients with resistance reveals residual activity

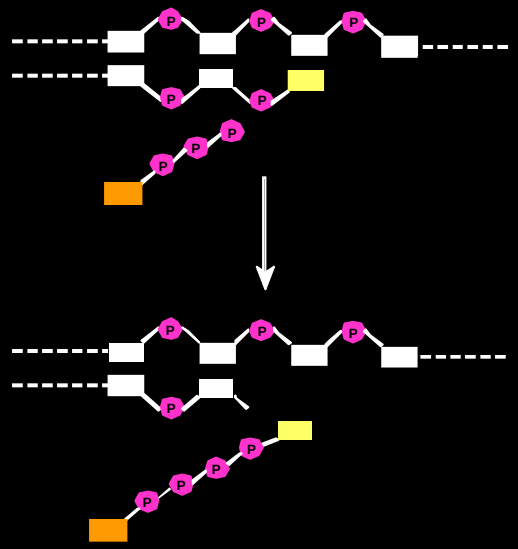


Deeks, CROI 2005

Mechanisms of resistance: Primer unblocking in NRTI resistance

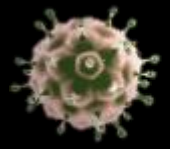
- T215Y-mediated resistance
- Hydrolytic removal of the chain-terminating NRTI enables DNA synthesis to resume
- M184V antagonises the process delaying the emergence of T215Y and increasing susceptibility to ZDV, d4T and TDF





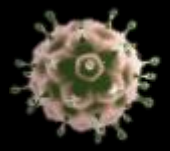
Gotte, J Viol 2000

Take away points

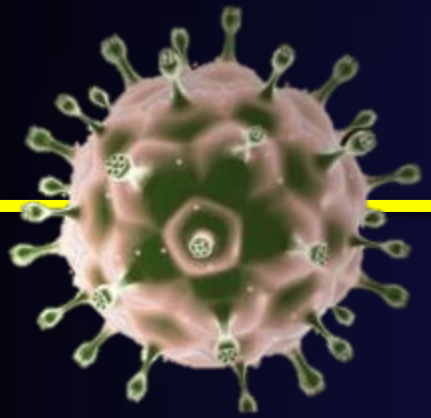


- Resistant mutants often display reduced fitness with a **beneficial effect** on viral load and CD4 counts
- **Compensatory changes** emerge over time that partially restore virus fitness
- **Antagonistic effects** between mutations can also have beneficial effects
- Best evidence of residual activity despite resistance for the **NRTIs**

Clinical implications



- The likelihood of drug-resistance depends upon the individual drug, the overall regimen and the drug levels
- Avoid functional monotherapy with drugs that have a low genetic barrier to resistance
- In the presence of partial resistance ensure optimal activity of the overall regimen to prevent further resistance
- Be mindful of pre-existing resistance when switching patients with suppressed viraemia
- ***Preventing the accumulation of resistance remains a key goal of successfully managed ART***



Thank you