

# Resistance testing at low viral load: a case story

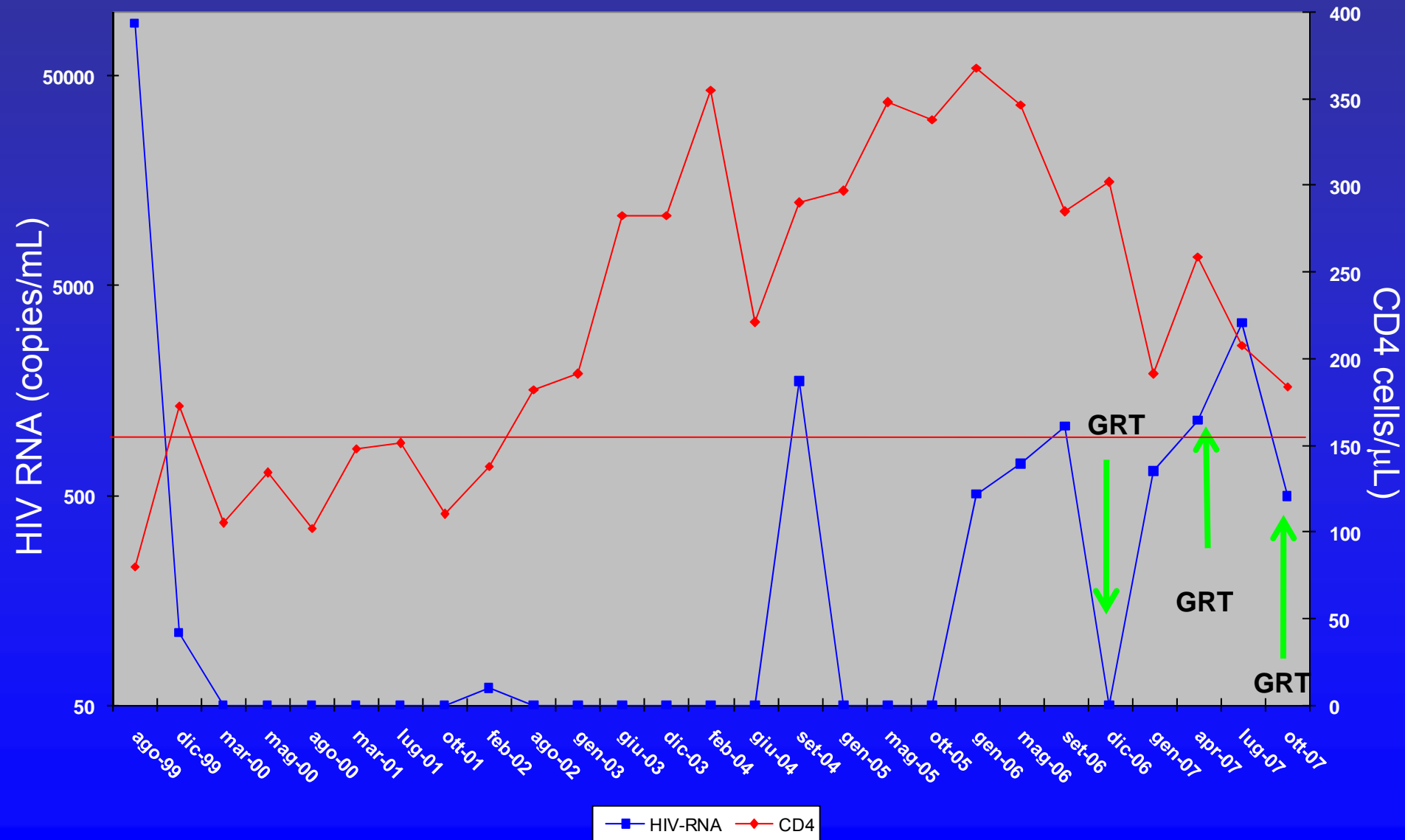
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- Man, 50 years, IDU
- First positive anti-HIV test in Jan '85
- HBsAg, syphilis negative, anti-HCV positive
- Loss to follow-up for 14 years (refuses medical treatment)
- AIDS-related event (oesophageal candidiasis) in Aug '99
- No suboptimal therapies, on cART since 1999.....

# Treatment regimens

- Sept '99- Nov '99: AZT+3TC+NFV (epigastric pain, loose stool)
- Nov '99- May '04: d4T+3TC+NVP (hepatotoxicity)
- May '04- Oct'07: TDF+3TC+D4T (several episodes of non-adherence)

# Viro-immunological follow-up



# Changing Treatment Goals for Treatment-Experienced Patients

## Goals of Therapy 2003

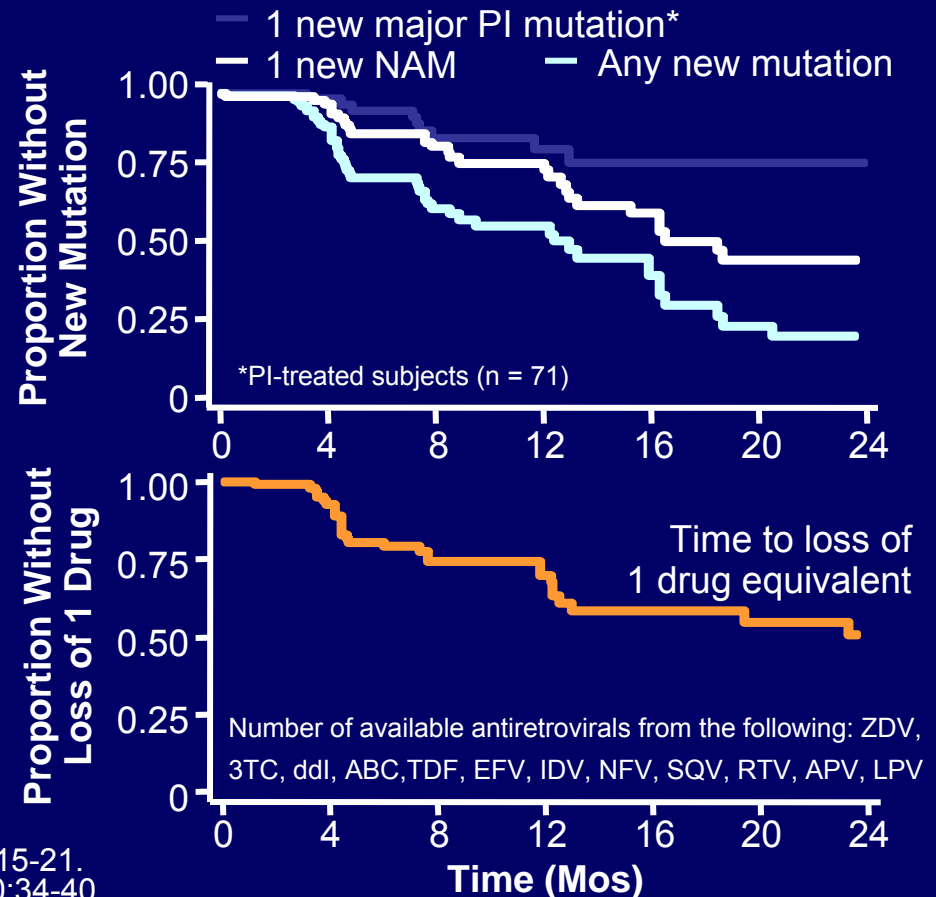
- Preserve immune function
- Maximize reduction in plasma HIV RNA levels
- Minimize toxicity

## Goals of Therapy 2008

- Full viral suppression is an achievable goal
  - Newer drugs
  - Drugs in newer classes
- Use **multiple active drugs to achieve full suppression**
- Minimize toxicity (NRTI)

# Risk of Delayed Switch on Stable HAART

- SCOPE cohort of ART-experienced subjects (n = 106)<sup>[1]</sup>
  - Stable HAART for ≥ 120 days
  - HIV-1 RNA > 1000 c/mL
  - ≥ 1 resistance mutation
  - Resistance testing every 4 mos until HAART modification
- Emergence of new mutations at 1 yr
  - Any: 44% (95% CI: 33% to 56%)
  - NAMs: 23% (95% CI: 15% to 34%)
  - PI: 18% (95% CI: 9% to 34%)
- Persistent viremia on HAART runs risk of limiting future treatment options
- Other studies show similar results<sup>[2-4]</sup>



1. Hatano H, et al. CROI 2006. Abstract 615.
2. Lafeuillade A, et al. IAC 2004. Abstract WeOrB1293.
3. Margot NA, et al. J Acquir Immune Defic Syndr. 2003;33:15-21.
4. Napravnik S, et al. J Acquir Immune Defic Syndr. 2005;40:34-40.

# Genotypic Resistance Tests

- **11/12/06 (VL<50 c/mL): not amplifiable**
- **11/04/07 (VL: 1136 c/mL):**

- ✓ RT: K65R, T69AV, V118I, M184V, K219KR
- ✓ PR: I62V, I64M

**Resistance: 3TC/ FTC, ABC (all), ddI (REGA), TDF (ANRS)**

**Intermediate: d4t , TDF (Stdb , REGA), ddI (ANRS, Stdb), AZT (REGA)**

**Susceptible: all NNRTI and PI, d4T(ANRS), AZT (ANRS; Stdb)**

- **19/10/07 (VL: 436 c/mL):**

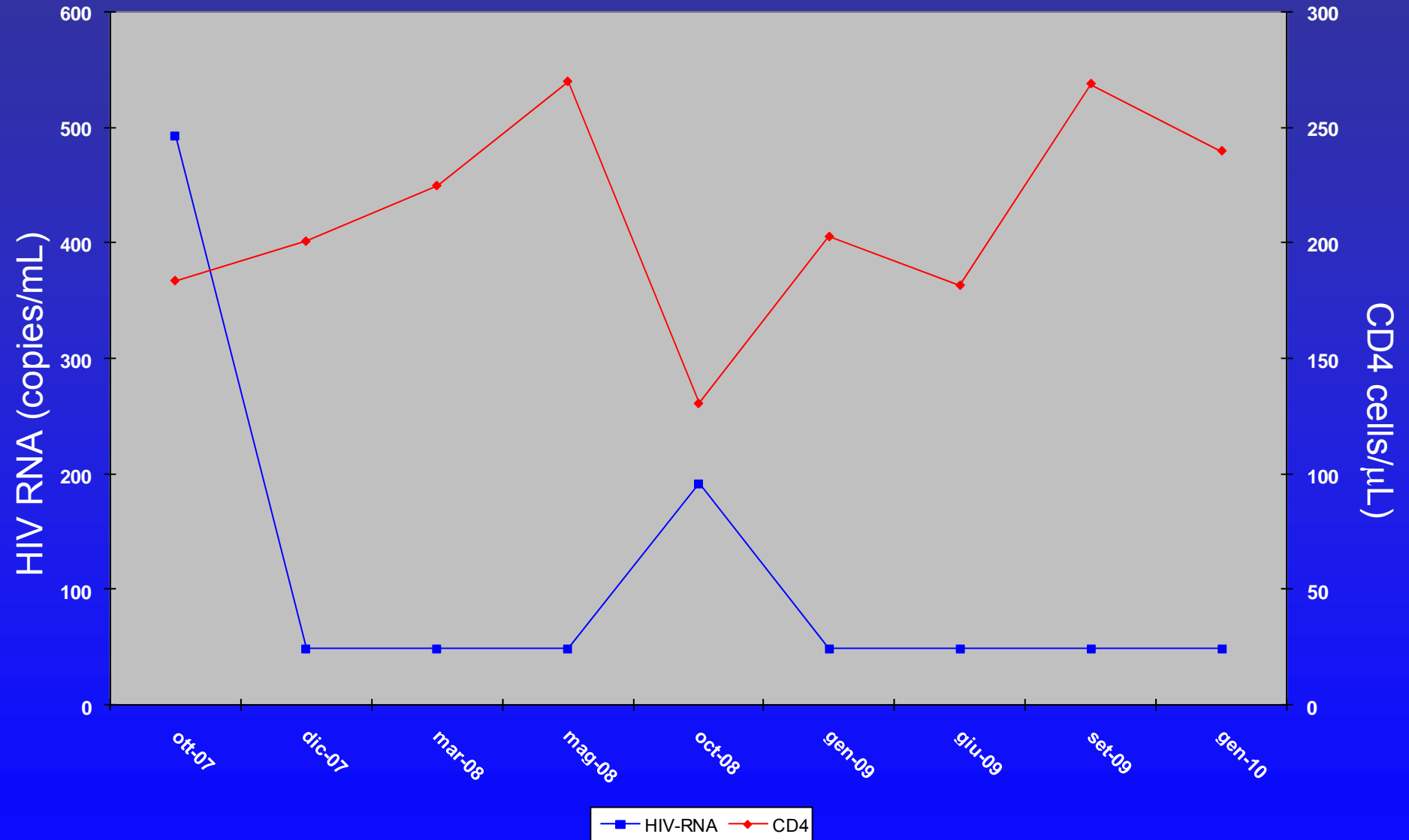
- ✓ RT: K65R, T69AV, V118I, M184V, K219KR
- ✓ PR: I62V, I64M

**Resistance: 3TC/ FTC, ABC (all), ddI (REGA), TDF (ANRS)**

**Intermedediate: d4T, TDF (Stdb , REGA), ddI (ANRS, Stdb), AZT (REGA)**

**Susceptible: all NNRTI and PI, d4tT(ANRS), AZT (ANRS; Stdb)**

# Oct-07: TDF+3TC+ATV/r



# New extraction/amplification methods

RNA estratto dal plasma con  
l'estrattore automatico  
Nuclisens Easy MAG  
(Biomerieux)

DNA estratto dal sangue  
intero con l'estrattore  
automatico Bio Robot EZ1  
(Qiagen)

Amplificazione genica mediante una "home  
made" Nested-PCR utilizzando il sistema  
Expand High Fidelity PCR della Roche

Sequenziamento genico con il sistema  
ViroSeq HIV-1 (Abbott) e il sequenziatore  
automatico ABI 3100 (Applied Biosystem)

Analisi delle sequenze mediante i programmi  
ViroSeq HIV-1 e Stanford hivbd

# Retrospective testing of low VL sample

- **11/12/06 (VL: <50 c/mL):**

- ✓ RT: K65R, T69AV, V118I, M184V

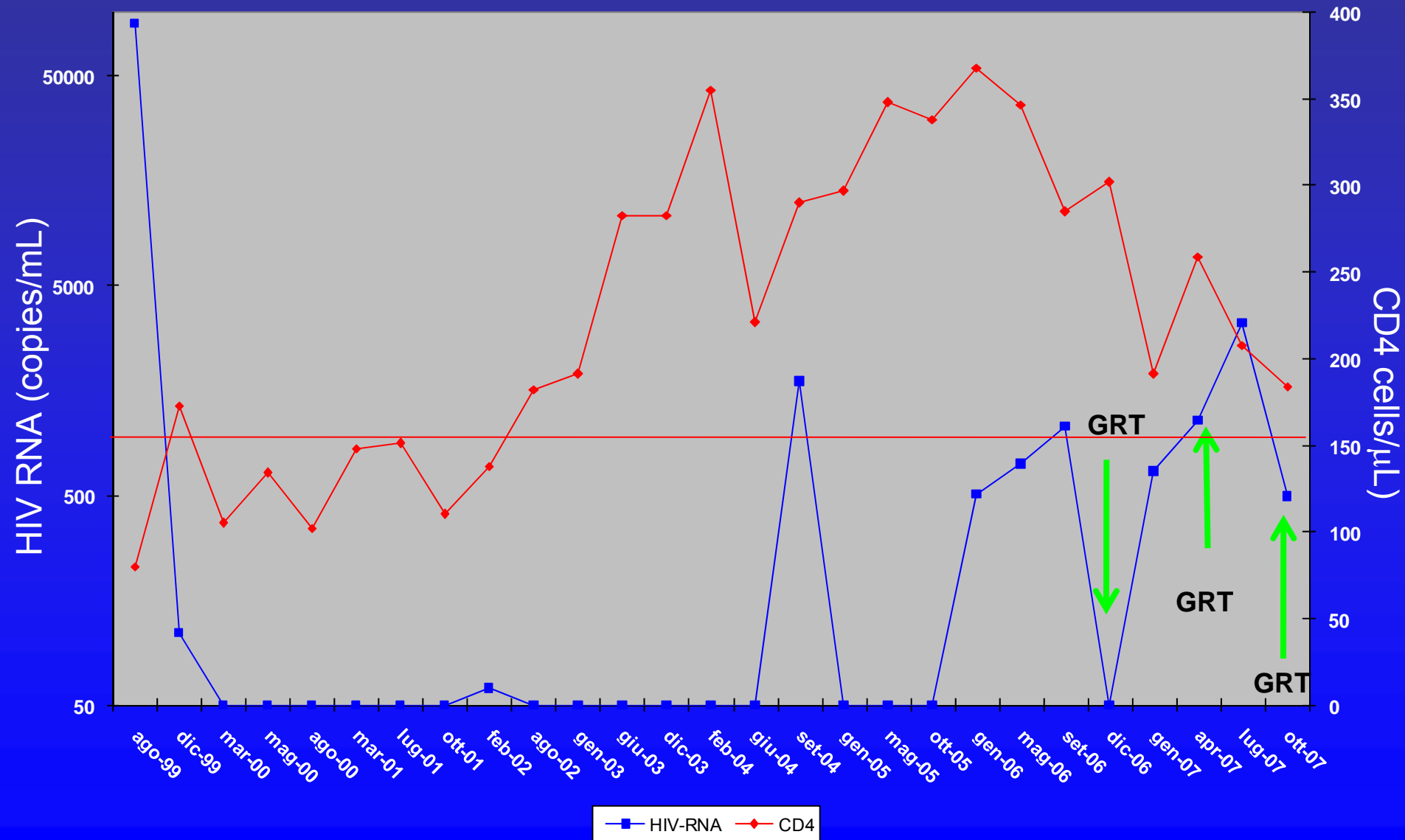
- ✓ PR: I62V, I64M

**Resistance: 3TC/ FTC, ABC (all), ddi (REGA),  
TDF (ANRS)**

**Intermediate resistance: TDF (Stdb , REGA),  
ddi (ANRS, Stdb),**

**Sensitivity: all NNRTI and PI, d4T (all), AZT  
(all)**

# Viro-immunological follow-up



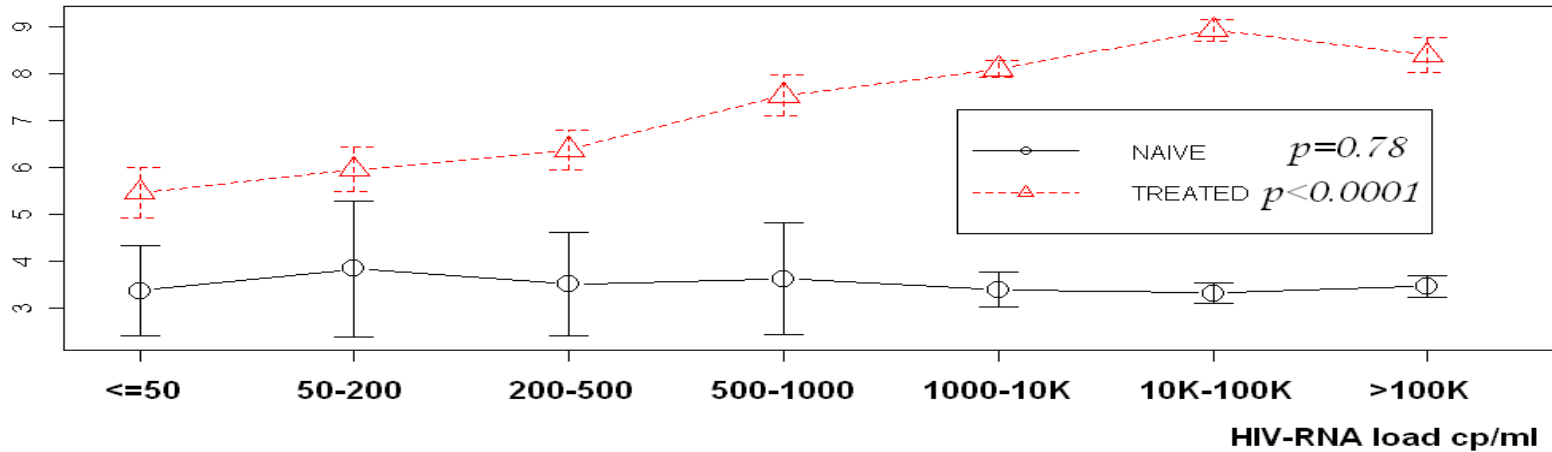
# Question

- DRM at low VL are generally fewer:
  - less viral replication ?
  - failure to detect them?

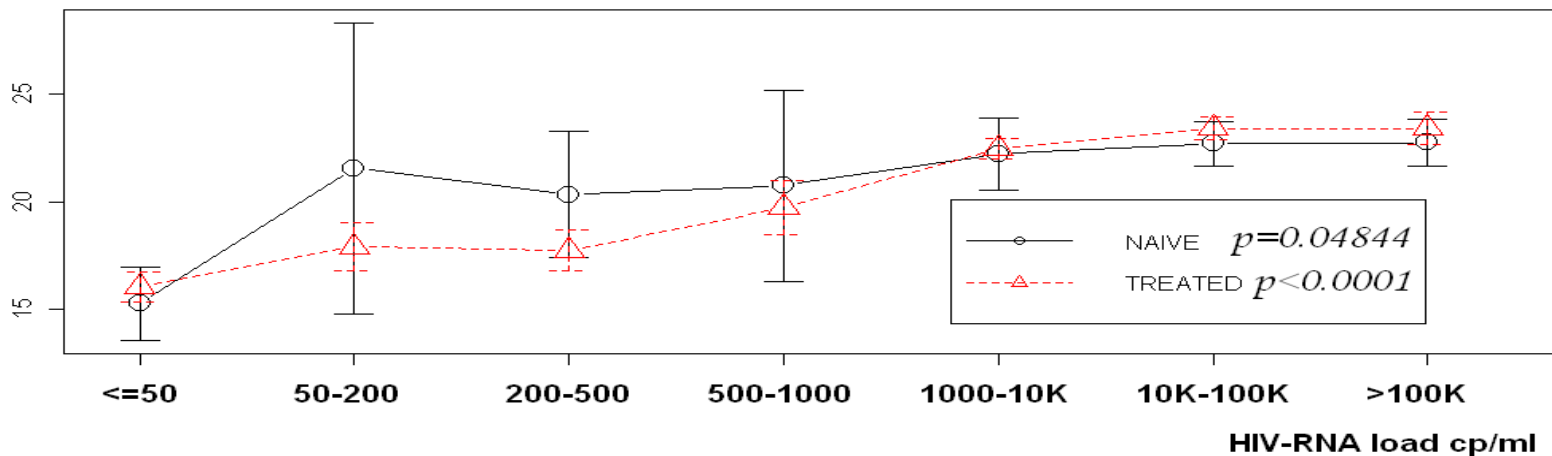
# No. of IAS DRM and polymorphisms according to VL (Sehere)



no. of IAS DRM



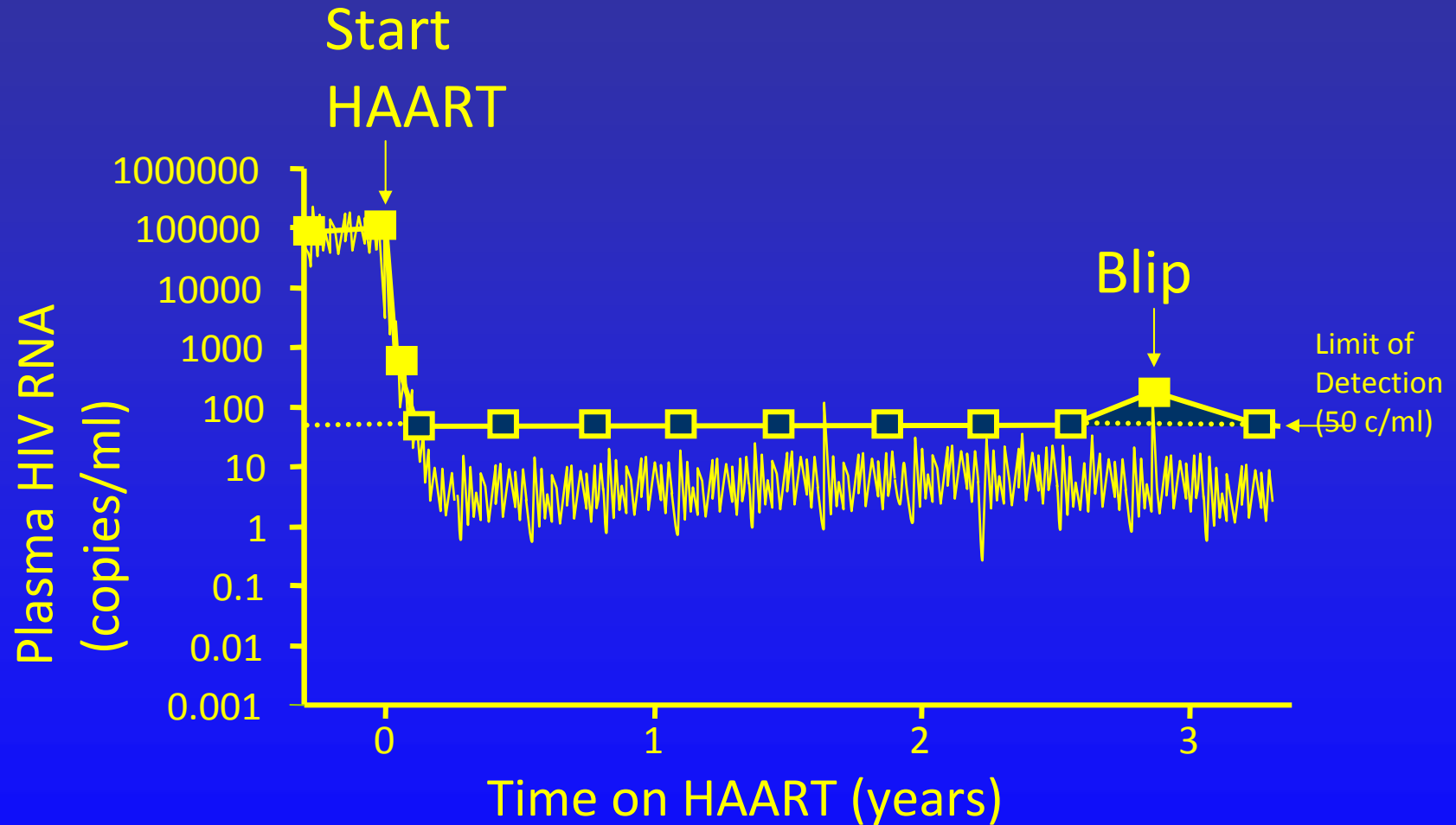
no. of polymorphisms



# Discussion

- Resistance accumulates at low viral load
- The accumulation of resistance mutations can be prevented by early switching based on genotype information
- Even at  $VL < 500$  genotyping resistance testing can, in some instances, be performed and give useful clinical information

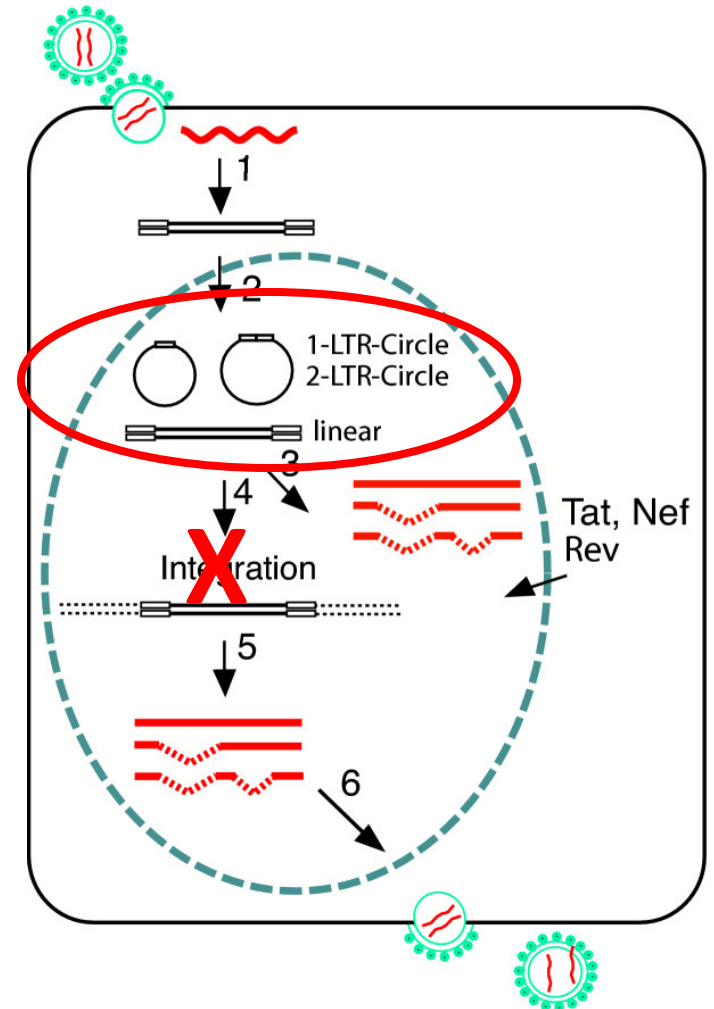
# No new resistance during blips



Nettles et al, JAMA, 2005

# Some Evidence for Productive Replication in ART-suppressed Patients

- 2LTR episomal HIV DNA is produced after nucleus import and pre-integration.
- Can be detected with episome-specific PCR
- 13/44 (30%) RGV intensified patients had a transient increase in 2LTR circles
- Only those with increase in 2LTR had decline in CD8 activation during RGV intensification.



Sharkey, Nat Med, 2000; Wu, Retrovirology, 2004; Martinez-Picado and Massanella, CROI 2009

# Acknowledgements

- Simona Di Giambenedetto
- Manuela Colafigli
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