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HIV-1 superinfection with a drug resistant strain in a patient successfully controlled with antiretroviral treatment

Castro E¹, Zhao H², Cavassini M³, Mullins JI², Pantaleo G¹ and Bart P-A¹

Erika Castro, MD, PhD
Laboratory of AIDS Immunopathogenesis
Division of Immunology and Allergy
BT 06 / CHUV
1011 Lausanne
Switzerland

erika.castro-bataenjer@chuv.ch
Tel +41 21 314 11 60
Fax +41 21 314 11 61

¹Laboratory of AIDS Immunopathogenesis, Service of Immunology and Allergy, University Hospital, Lausanne, Switzerland. ² Department of Microbiology, School of Medicine, University of Washington, Seattle, Washington, United States of America ³ Service of Infectious Diseases, University Hospital, Lausanne, Switzerland.

1. ABSTRACT

Background:

HIV-1 superinfection (HSI) has been mostly reported among untreated patients, during treatment interruption, and more recently in seroconcordant couples with poor viral suppression. Here we report the emergence of HSI in a man successfully controlled with ART following unprotected sex with a seroconcordant partner.

Patients and Methods:

Briefly, blood samples were obtained from 2 men (M1 and M2) chronically infected with HIV-1 and sexual partners since 2006. M1 was diagnosed in 2000 with primary HIV infection, initiated ART in 2000, and has remained on ART with undetectable viremia, normal CD4+ counts (mean=883cells/mm³) and no drug resistance mutations through the end of 2007. In contrast, M2 had not controlled viremia (range: 3 to 4 log) despite 5 years of ART and harbored a triple class resistant virus. In February 2008 M1 presented a plasma viral load of 280 copies/mL that kept increasing and rebounding over the following year. A new viral genotype was detected with triple class resistance in M1. Assessment of both patients' viruses was performed in addition to a phylogenetic analysis of whole-genome (N=28), *env* (N=28) and *gag* (N=25) sequences obtained from viral RNA collected in 2000 and 2008. Sequence alignments were generated with clustal W or MUSCLE and phylogenies inferred with neighbor-joining plus bootstrap resampling or maximum-likelihood methods.

Results:

All 86 viral sequences were assigned to clade B. The genotypic analysis from 2008 revealed 25 new drug resistance mutations to NRTI/NtRTI (6/25), NNRTI (5/25) and PI (14/25) in M1's profile, of which 23 were also present in M2 from the same period. Additionally, M1-2008 sequences clustered within the M2-2008 branches and distinct from M1-2000 sequence clusters in all trees. No recombination between the original M1 and M2 strains was observed, rather, substantial replacement of M1 by M2 sequences was detected upon superinfection.

2. STUDY DESIGN

Patients: Subjects M1 and M2 are HIV-1 chronically infected patients followed at the Immunology and Infectious Diseases Outpatient consultations from the Centre Hospitalier Universitaire Vaudois, respectively. The two men were together from 2006 onward and have unprotected sex. In 2008 an informed consent was obtained from each man, in order to investigate the HIV transmission in the couple.

Resistance Analysis: Viral RNA resistance genotype assessments were generated with Virco® PCR algorithms on a routine basis. The resulting 1.4kb *pol* nucleotide sequences were further analysed with the Stanford University Drug Resistance online tool.

Subtype assignment: Genotype resistance and clade prediction was verified by phylogenetic analysis of HIV alignments comprising also reference sequences from subtypes A to J and CRF01 to CRF15 (data not shown).

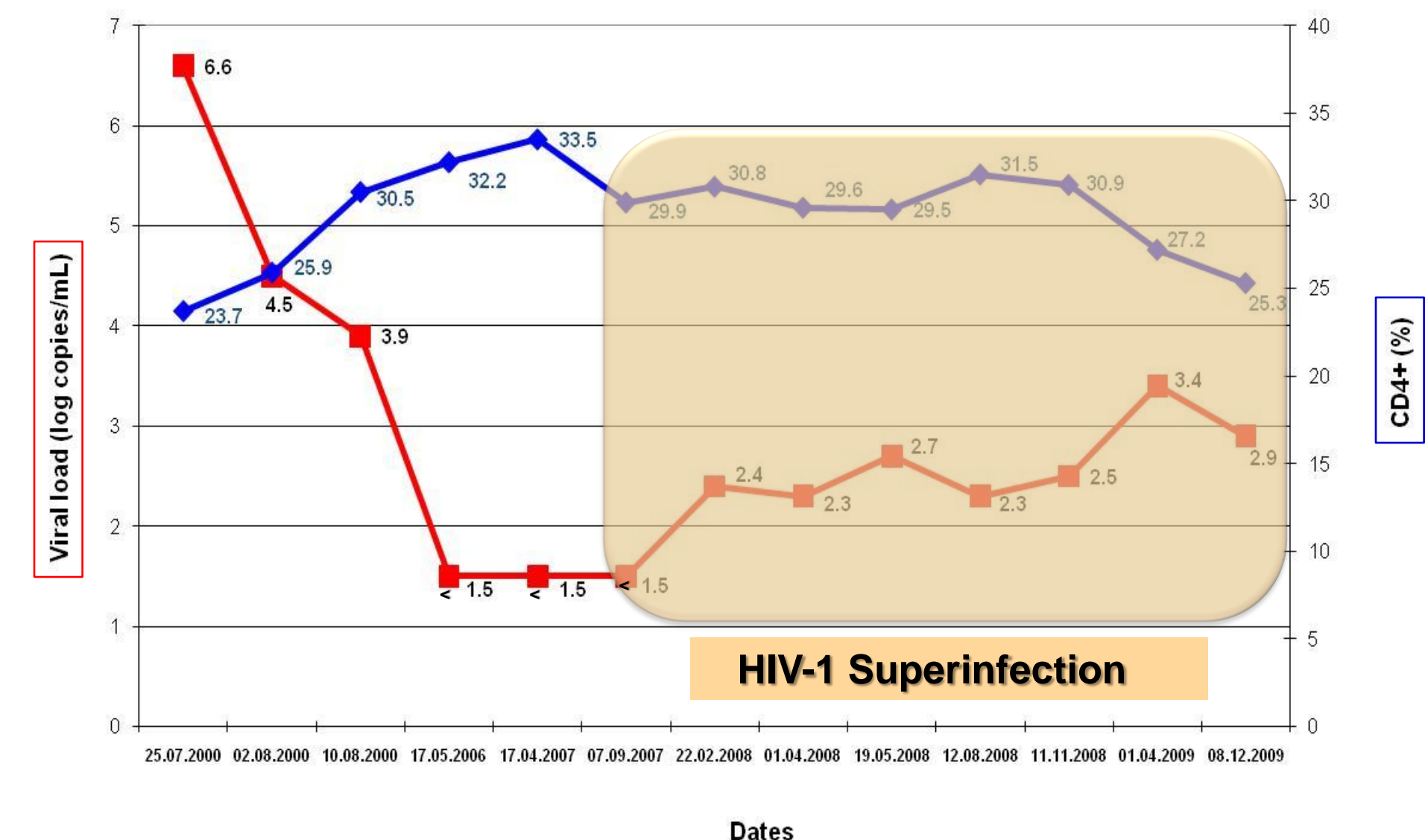
Clone analysis: Viral whole genome sequences analysis was performed at the Molecular Retrovirology Laboratory from the Department of Microbiology at the University of Washington. For this purpose, stored plasma from M1 collected in 2000 and 2008 plus plasma from M2 collected in 2008 were evaluated.

3. PATIENTS INFECTION OUTCOME

Blood specimens were obtained from 2 men (M1 and M2) chronically infected with HIV-1 and sexual partners since 2006.

Patient	Date	Antiretroviral treatment	CD4+ T-cell cells/mm ³ (%)	Virological assessment
M1	Baseline 2000	naïve	405 (23.7)	• Incomplete seroconversion • Viral load = 4x10 ⁶ RNA-copies/mL
	September 2007	ABC + 3TC + AZT	872 (29.9)	• Undetectable viremia after starting ART in 2000. • No emergence of drug resistance mutation.
	February 2008		1025 (30.8)	• Triple class-resistant virus • Viral load = 280 RNA-copies/mL
	April 2009		776 (27.2)	• Same resistant mutation pattern • Viral load = 2460 RNA-copies/mL
December 2009		822 (25.3)	• Viral load = 795 RNA-copies/mL	
M2	March 2008	ABC + 3TC + LPV/r	273 (27.0)	• Persistent triple class-resistant virus • Viral load = 17300 RNA-copies/mL
	June 2008	RAL + MVC + ETV + DRV /r	410 (26.9)	• Viral load <40 RNA-copies/mL

4. VIRAL LOADS AND CD4+ COUNTS FROM M1



Upon HIV-1 superinfection in M1, plasma viral load rebounded by at least a 1.5 log increase. Whereas CD4+ counts (%) have remained within a small range of variation over the same period.

5. RESISTENCE MUTATIONS TO ART

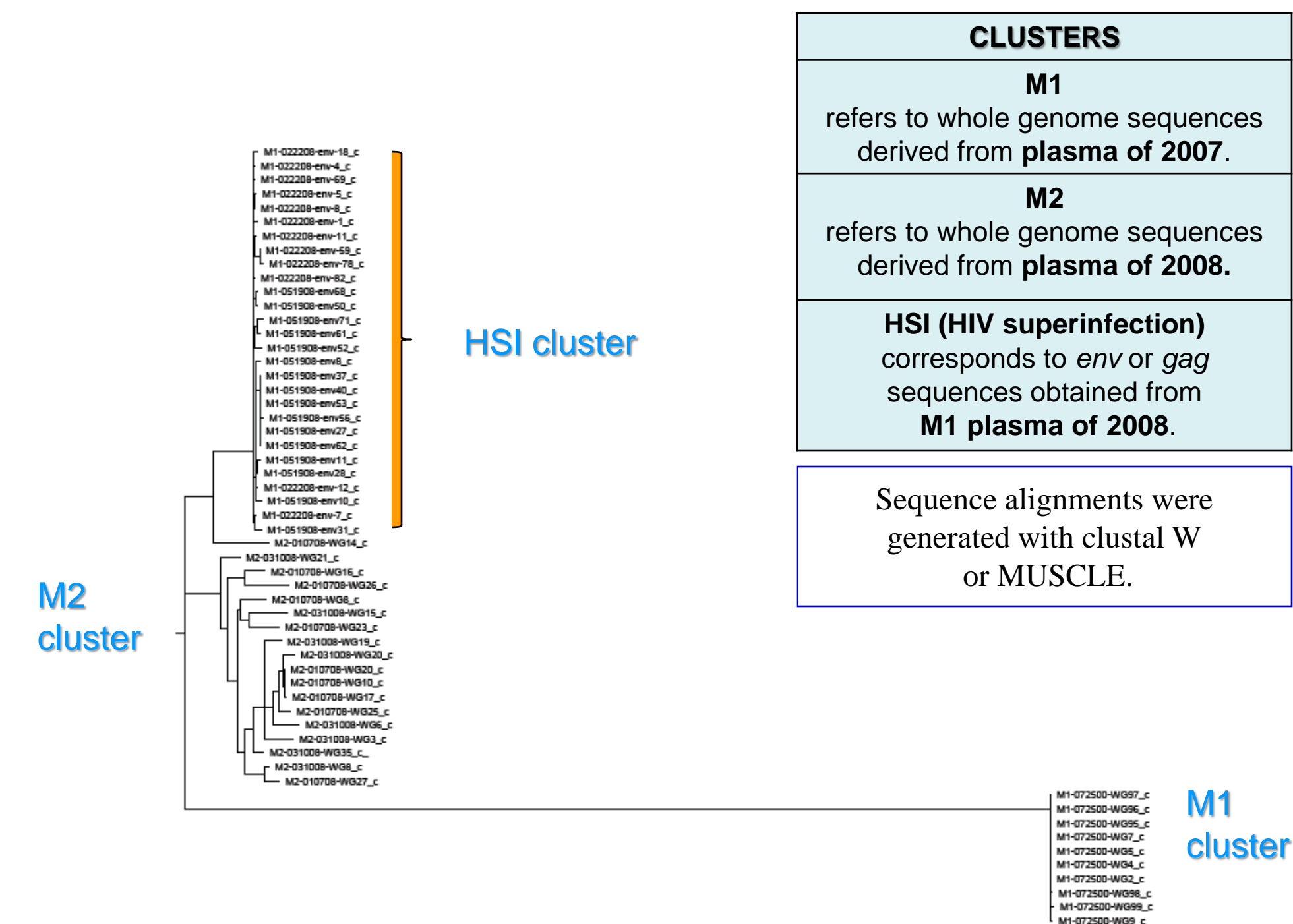
Resistance mutations to ART present in M1 (2008-2009) and M2 (2008) HIV-1 genotypic analyses.

RTI mutations	41L	74I	75M	75T	98G	103N	108I	115FY	184V	215Y	318F	399D		
M1	X	X		X	X	X	X	N	X	X	X	X		
M2	X	X	X		X	X	X		X	X	X	X		
PIs mutations	10V	13V	20R	32I	33I	46I	47V	50V	71I	77I	82A	89V	90M	93L
M1	X	X	X	X	X	X	X	X	X	X	N	X	X	X
M2	X	X	X	X	X	X	X	X	X	X		X	X	X

Overall, all NRTI/NtRTI, NNRTI and PI resistance mutations found in M2 emerged in M1. Mutation 75T has a high frequency in CRF01_AE clade infection and is associated with reduced susceptibility to d4T/ddI. Interestingly, 75T has remained traceable in M1 despite the absence of drug pressure. Additionally, two other new resistance mutations emerged in M1, PI mutation 82A and NRTI mutation 115F. Perhaps these 2 new mutations were selected by previous ART combinations used in patient M1. In fact, M1 received LPV/r (Kaletra) and AZT+3TC (Combivir) prior to Trizivir started at 2002.

6a. Env TREE

Maximum Likelihood tree of *env* 1.6kb alignment of HIV-1 M1 and M2 clone sequences



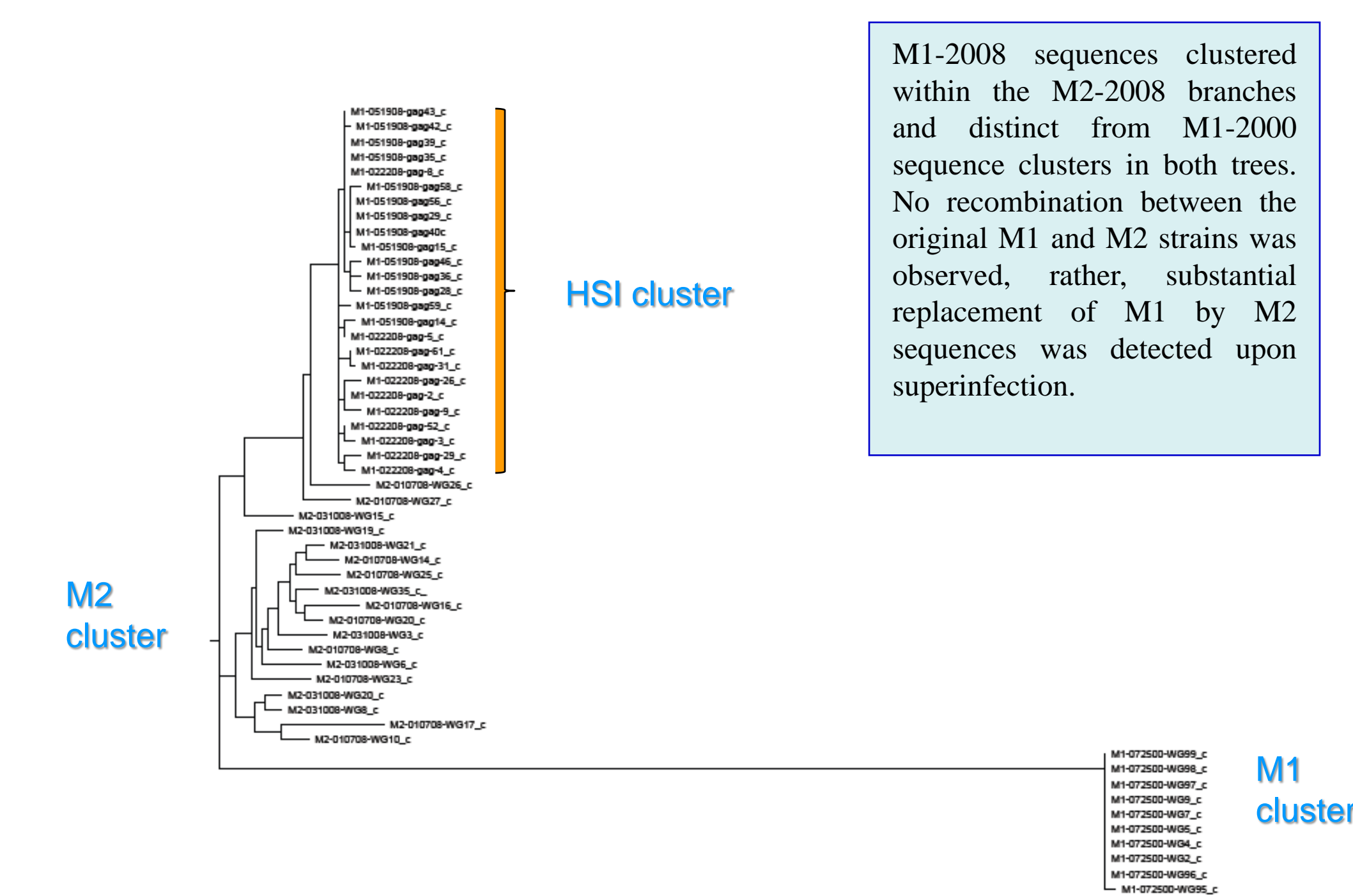
7. CONCLUSIONS

- We detected HIV-1 clade B superinfection in a patient with continuous, efficient long-term ART controlled infection, initially indicators being a detectable and increasing viral load and acquisition of drug resistance mutations derived from the superinfecting strain.
- Upon the onset of HIV-1 superinfection in patient M1 other resistance mutations not present in the superinfecting strain also emerged – probably, selected at early ART regimens and present as minority populations prior to superinfection.
- In contrast to recent reports of superinfection among long-term known seroconcordant couples undergoing ART, this report underscores the fragile barrier exerted by HAART.
- This evidence has important implications for ongoing HIV therapeutic and preventive vaccine strategies as well as pre-exposure prophylaxis.

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6b. Gag TREE

Maximum Likelihood tree of *gag* 1.5kb alignment of HIV-1 M1 and M2 clone sequences



M1-2008 sequences clustered within the M2-2008 branches and distinct from M1-2000 sequence clusters in both trees. No recombination between the original M1 and M2 strains was observed, rather, substantial replacement of M1 by M2 sequences was detected upon superinfection.