

# Drug-resistant HIV-1 Variants Selected in Children with Intermittent Low Level Plasma HIV-1 RNA (Blips) during HAART

N. H. Tobin, Y. Wang, A.J. Melvin, S. DeVange, J. McKernan, G.M. Ellis, K.M. Mohan, G. Pepper, L. Heath, W.E. Naugler, I. Beck, P.Lewis^, G.H. Learn, J.I. Mullins and L.M. Frenkel.  
 University of Washington, Seattle, WA and ^Oregon Health Sciences University, Portland, OR, USA

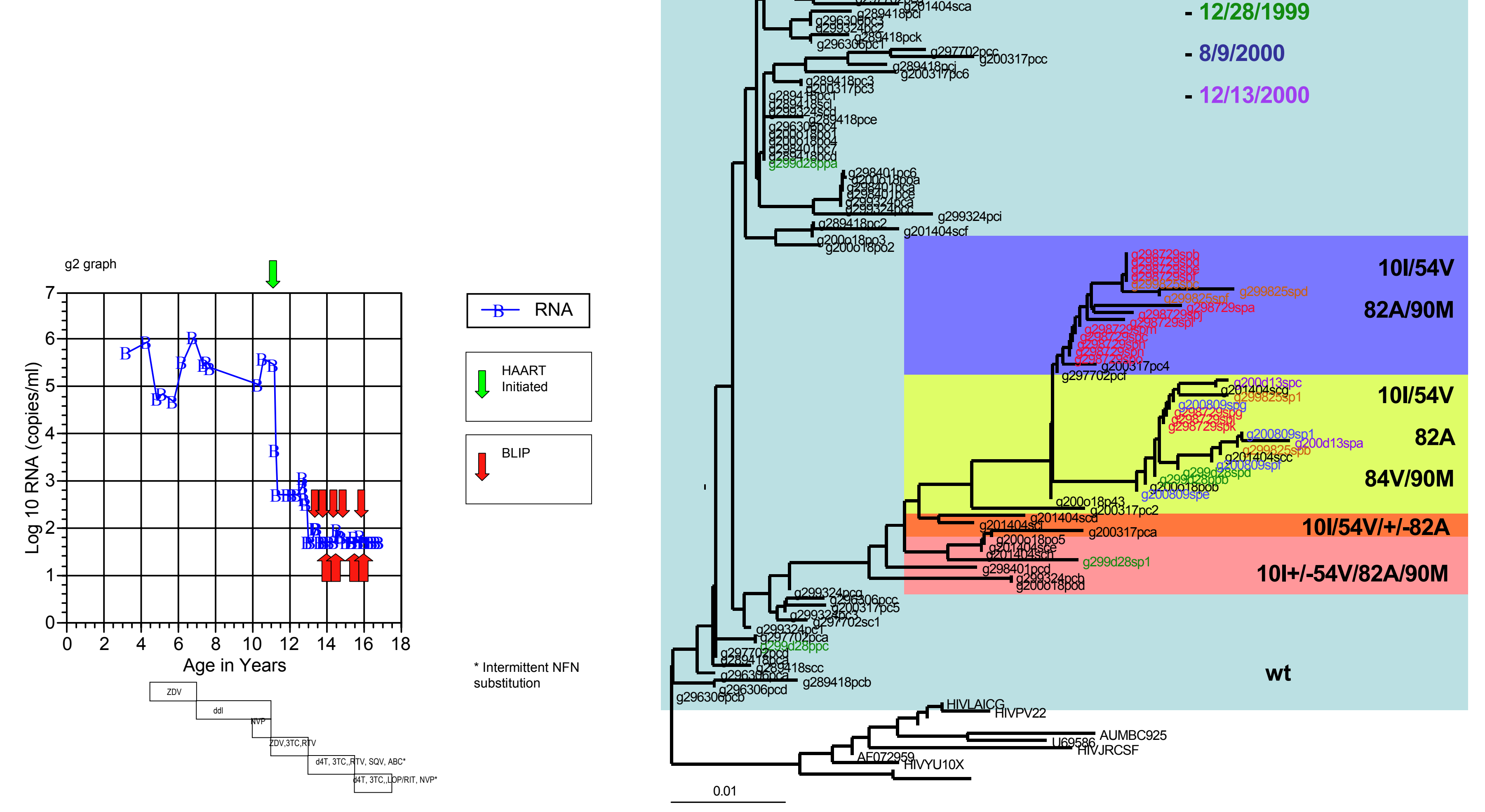
**BACKGROUND:** The significance of intermittent low-level viremia (50-500 copies/mL) in HIV-infected patients on therapy is unknown. Published data suggests the transcription of archival virus only, however, our studies demonstrate the selection of drug-resistant HIV-1 variants during effective HAART.

**METHODS:** Plasma HIV-1 RNA was measured every two-three months after initiation of HAART using the Amplicor Monitor 1.0 through 1997 and the Ultrasensitive Amplicor assay (Roche) from 1998-2001. To evaluate the replicative activity of HIV-1 when plasma RNA levels were detected between 50 and 250 copies/ml, the regions of *pol* encoding PRO and RT and *env* were amplified from the plasma. Phylogenetic analyses were conducted to reveal relationships of plasma virus to PBMC viral populations from over the course of infection. Ten or more amplicons were sequenced directly from end-point dilutions of specimens from multiple time points prior to and during 3-5 years of HAART.

**RESULTS:** Eight children, for whom viral evolution had been characterized since near the time of primary infection, had 19 episodes of intermittent viremia between 50 and 236 copies/ml (median 68). Four children had only one “blip” and four children had two or more “blips”. In phylogenetic analysis plasma virus grouped with PBMC virus from early in infection as well as recently evolved, increasingly drug-resistant virus. For example, a patient with one blip of 104 copies/ml during 5 years of effective HAART had plasma virus characteristic of early infection. In contrast another patient with 10 blips between 50-101 copies/ml and 12 RNA determinations <50 copies/ml during 5.5 years of HAART selected new mutations with genotypic resistance to all 3 classes of antiretrovirals. In addition, an increasing proportion of his PBMCs became infected with highly drug-resistant virus even though his plasma RNA was suppressed and continues to be <50 after multiple treatment intensifications.

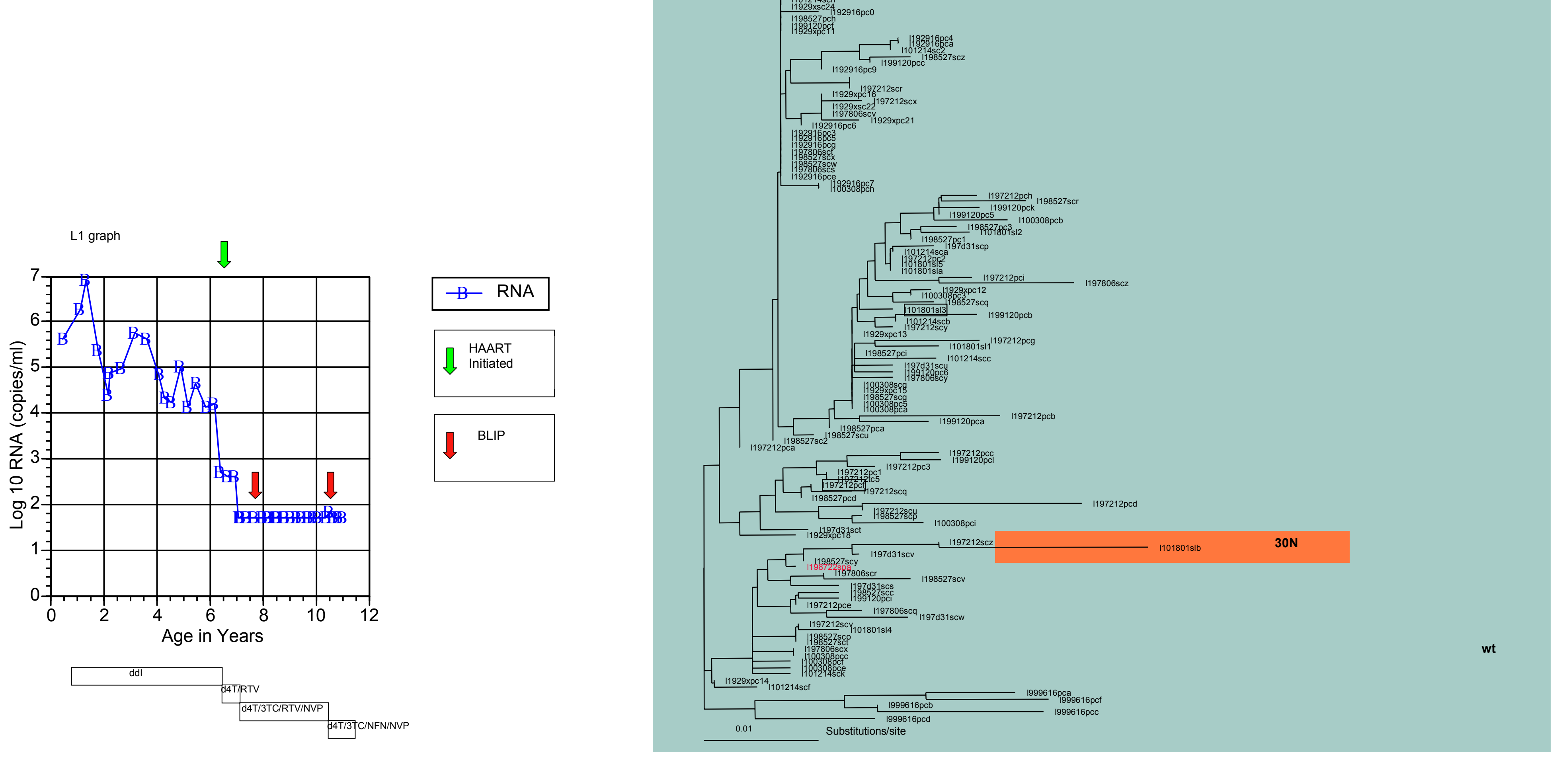
**Figure 1:**

*Patient 1*



**Figure 2:**

*Patient 2a*



**CONCLUSIONS:** Single low-level blips did not result in measurable new mutations and this RNA appeared to reflect transcription of latent virus, suggesting insignificant infection of new cells and minimal evidence of selective pressure. Drug-resistant virus was selected in association with more frequent blips. The clinical significance of plasma blips appears to depend not only their frequency but on the selective pressure of the HAART regimen, with potent therapy limiting outgrowth of fit virus.

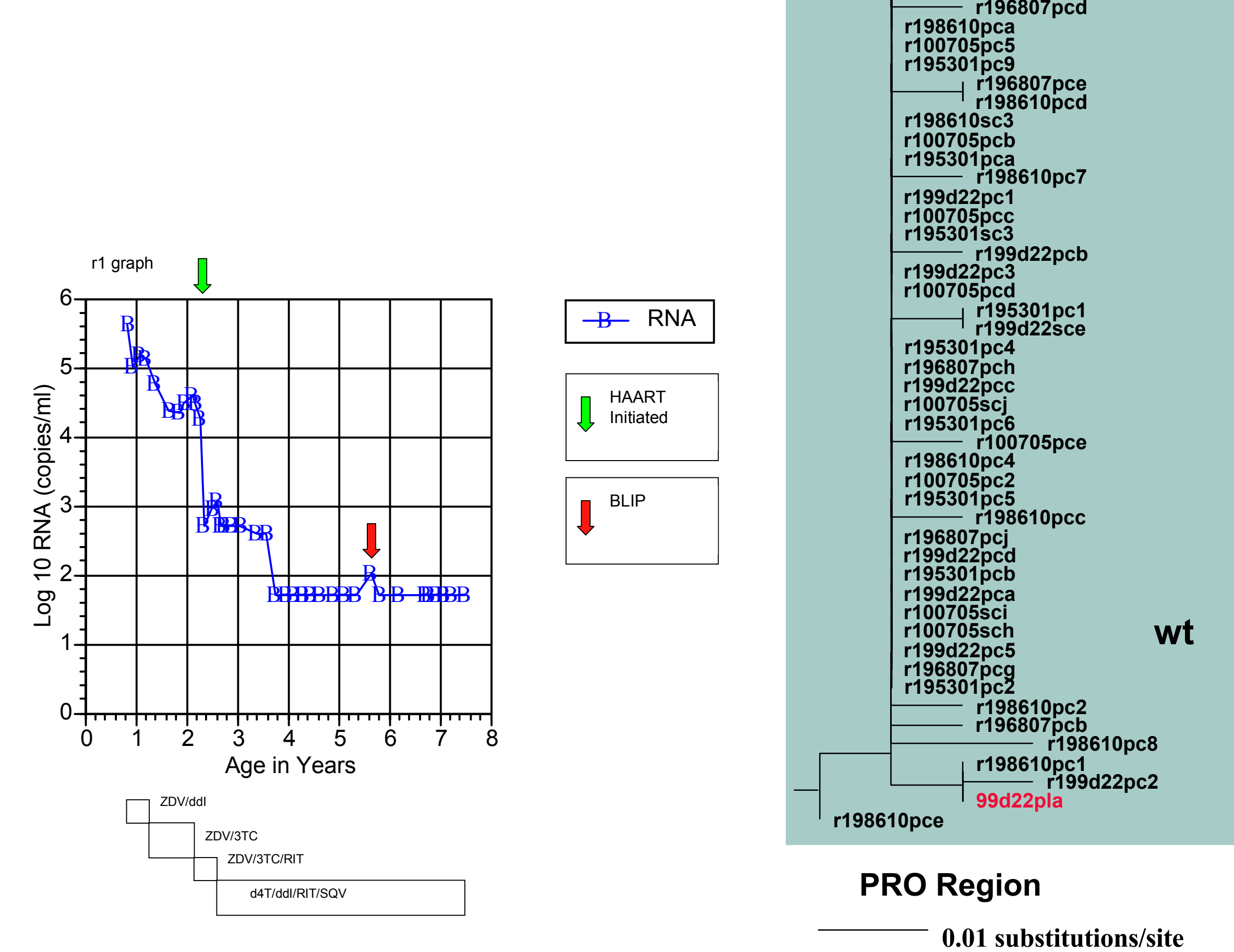
**Figure 1 (above):** HIV RNA graph, antiretroviral history, and phylogram of the protease region for 1 patient demonstrating selection of highly drug-resistant virus during “effective” HAART. *Patient 1* (g2) had 10 (50-101 HIV-1 RNAcopies/ml) blips in 22 measurements during 5.5 years of HAART prior to intensification with lopinavir/ritonavir. He has subsequently had no blips in 7 measurements spanning 1 year. His blip virus selected 10I, 54V, 82A, 84V, and 90M. Sequences from plasma blips are highlighted in color.

**Figure 2 (above and below):** HIV RNA graphs, antiretroviral histories, and phylograms of 6 patients with either no evidence of evolution, *patients 2a* (11), *2c* (h2) and *2f* (v1), or insufficient evidence to determine, patient *2b* (r1), *2d* (m2), and *2e* (s1). Sequences obtained from viral blips are highlighted in color.

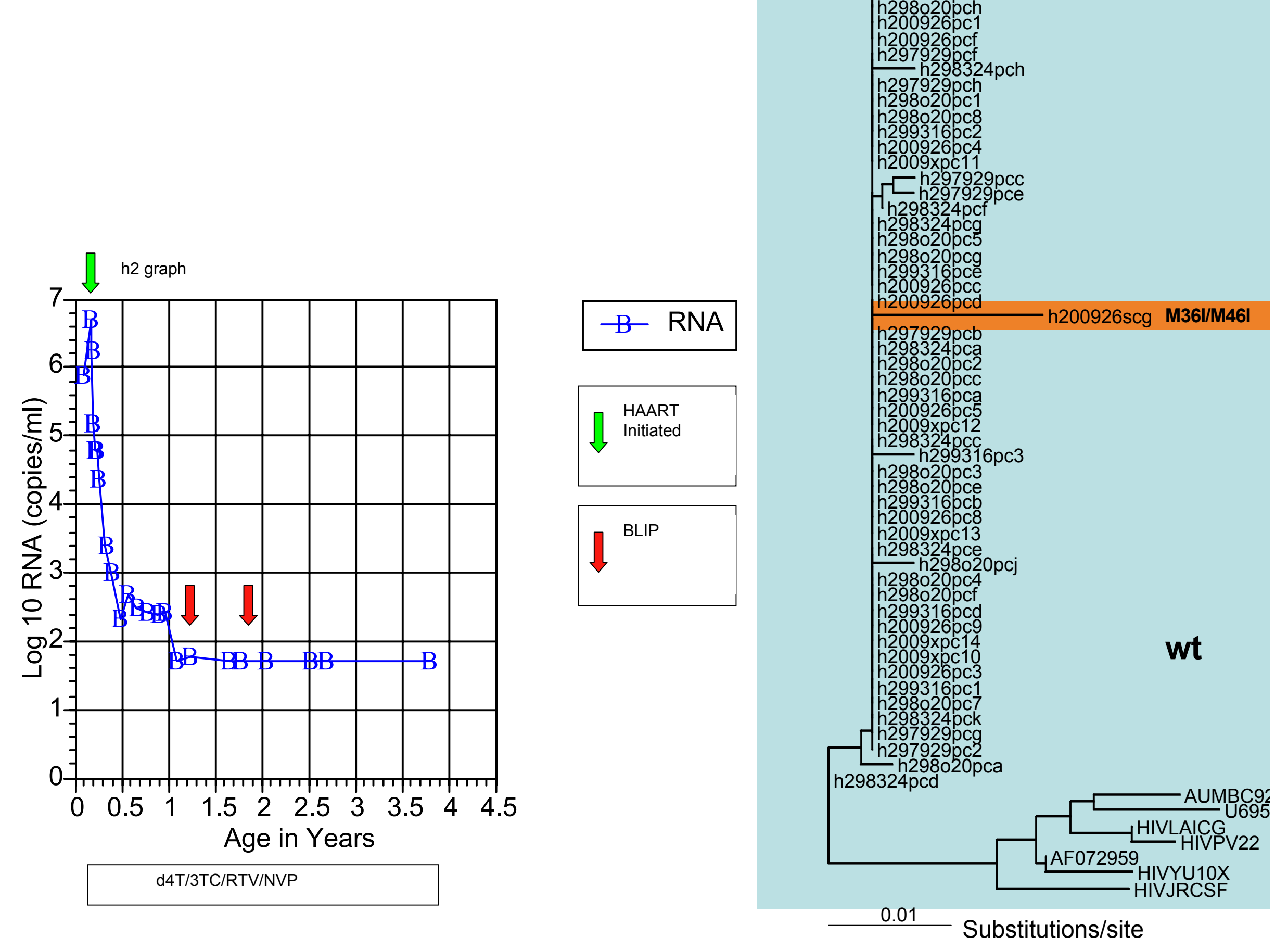
Patient	Number of Blips	Evidence of Evolution
1a	9	Yes
2a	2	No
2b	1	Indeterminate
2c	2	No
2d	2	Indeterminate
2e	1	Indeterminate
2f	1	No

**Figure 2 cont:**

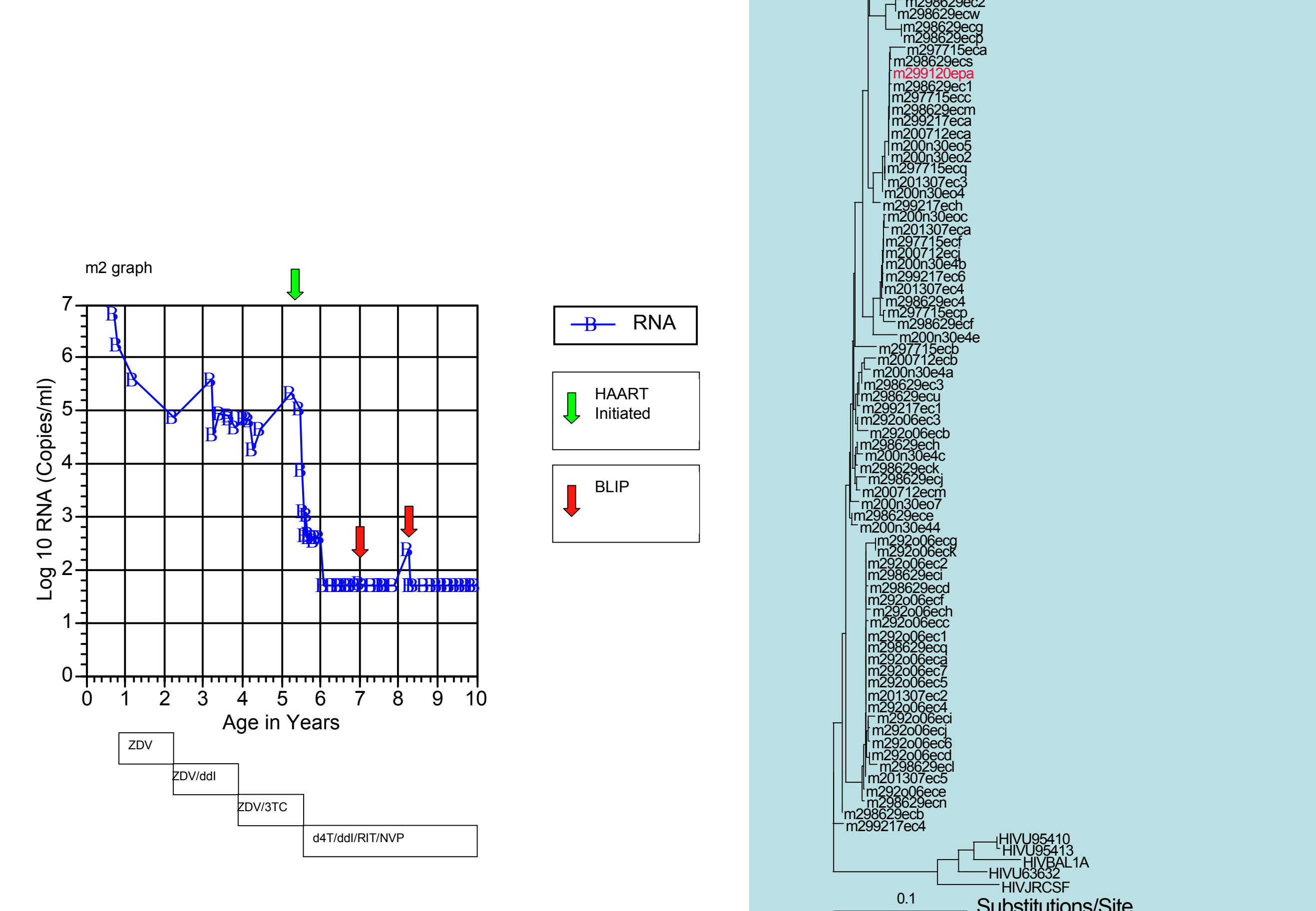
*Patient 2b*



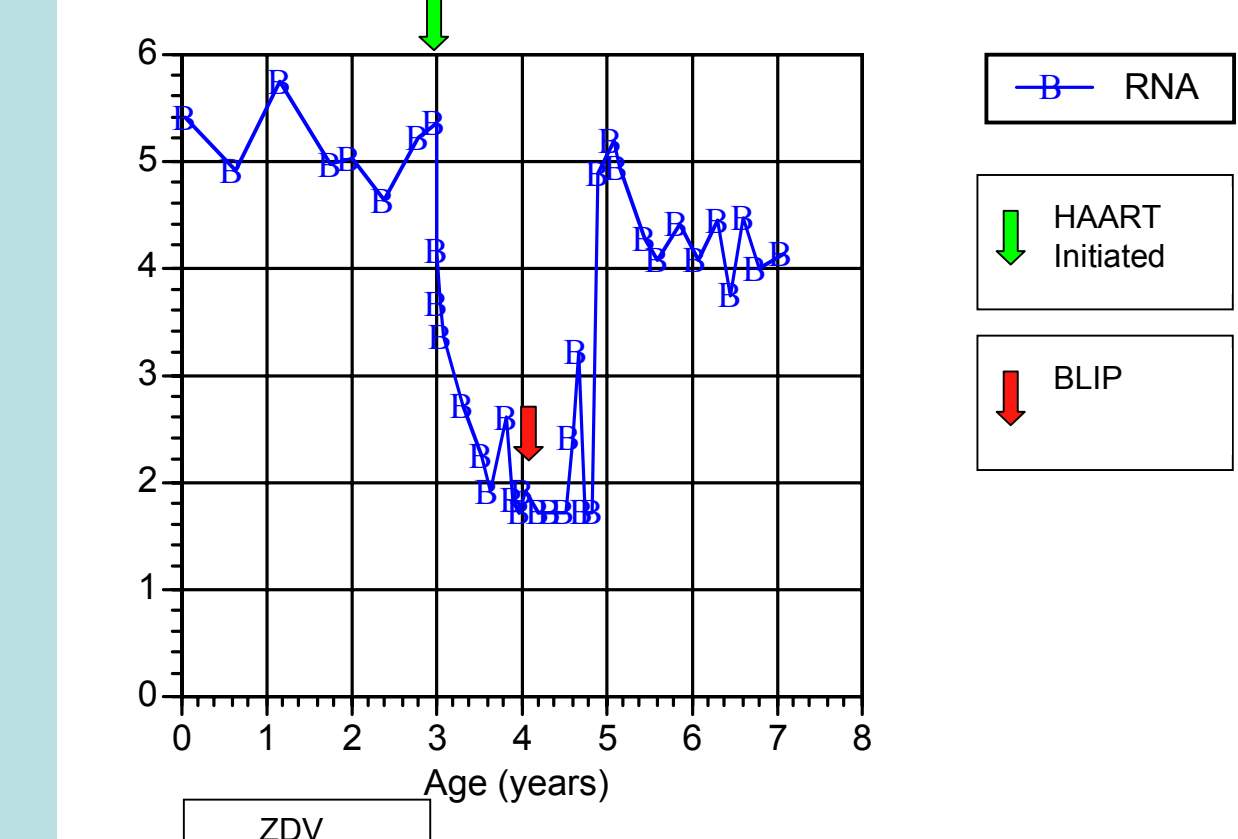
*Patient 2c*



*Patient 2d*



*Patient 2e*



*Patient 2f*

