

# A Pilot Study to Analyze HIV-1 Fitness Evolution under a Protease Inhibitor-Based Therapy Shows a Diverse Response Depending of the Basal Genotypic Context of the Virus

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## Abstract

**Background:** In this study we have used HIV-1 isolates from individuals receiving a protease inhibitor (PI) based regimen, to analyze the impact of basal genetic background on viral fitness evolution.

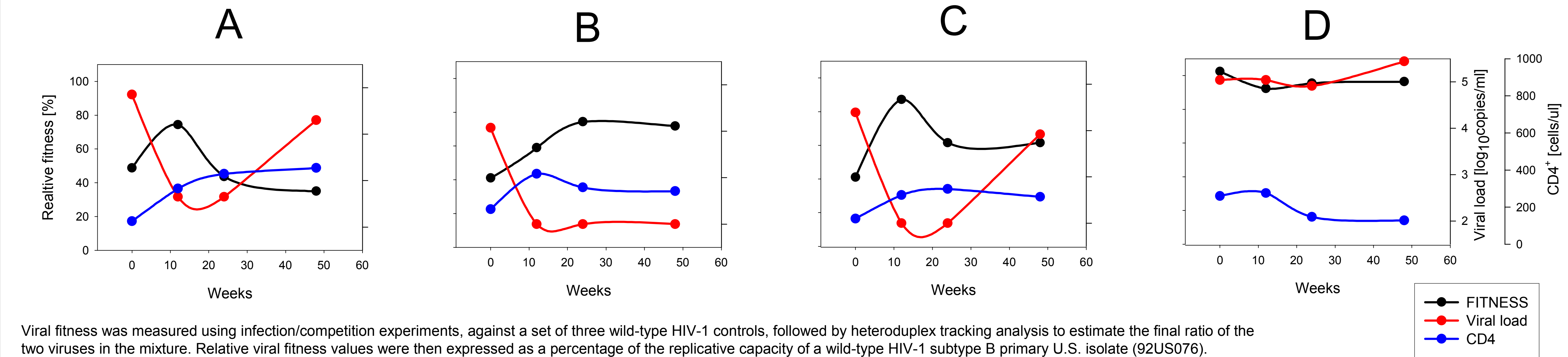
**Methods:** Sixteen paired plasma samples and HIV-1 isolates, corresponding to weeks 0, 12, 24, and 48 post-treatment, were obtained from four randomly selected AZT-experienced, PI naïve patients enrolled in the ACTG 315 study (i.e., zidovudine + lamivudine + ritonavir). Viral fitness was measured using competition experiments, against a set of three wild-type HIV-1 controls, followed by HTA to estimate the final ratio of the two viruses in the mixture. Relative viral fitness values were then expressed as a percentage of the replicative capacity of a wild-type subtype B primary U.S. isolate. Viral RNA was extracted from the plasma samples and genomic regions encoding PR and RT (*pol* gene) were RT-PCR amplified and sequenced.

**Results:** Pre-therapy genotypic analyses of PR and RT revealed two patients harboring wild-type viruses, whereas two had AZT resistant strains. One of the subjects who harbored AZT mutations also had polymorphisms in the PR gene at codons associated with PI resistance (i.e., 36, 77, and 93). After 48 weeks, only the patients with baseline AZT mutations had developed resistant virus to all three antiretroviral drugs (PR, 82; RT, 41-219, 184). Compensatory mutations in PR (10, 36, 54, 77, and 93) were observed only in the virus that contained PR polymorphisms at baseline. Ex vivo viral fitness evolution paralleled these genotypic changes. At week 48, high viral replicative capacity was observed in both wild-type viruses (71.8% and 96.3%), while lower viral fitness was estimated for the drug resistant viruses (34.9% and 61.5%). Interestingly, in those patients failing therapy, two distinct evolution patterns were observed when viral fitness at baseline and post-therapy were compared. An increase in viral fitness (+50% of the initial fitness value) was observed in the drug resistant isolate carrying natural compensatory mutations in the PR gene. In contrast, a reduction in replicative capacity (-28%) was calculated for the virus harboring only primary PR mutations. In addition, despite a similar viral load rebound, an inverse relationship was observed between viral fitness and CD4 counts (61.5%, 267 cells/μl vs. 34.9%, 443 cells/μl) in these two subjects.

**Conclusions:** Selection of drug resistant viruses with impaired fitness is often followed by generation and selection of additional compensatory mutations, which enhance replication capacity. However, in this study, we observed that the evolution of viral fitness depends on the initial HIV-1 genetic background and it correlates well with the virologic response to antiretroviral therapy. Thus, natural polymorphisms associated with resistance to PI- or RT-inhibitors, may lead to a more rapid recovery of viral replication capacity and further HIV pathogenesis.

## Results (I)

Figure 1. Correlation between HIV-1 relative fitness and virological/immunological response.



Viral fitness was measured using infection/competition experiments, against a set of three wild-type HIV-1 controls, followed by heteroduplex tracking analysis to estimate the final ratio of the two viruses in the mixture. Relative viral fitness values were then expressed as a percentage of the replicative capacity of a wild-type HIV-1 subtype B primary U.S. isolate (92US076).

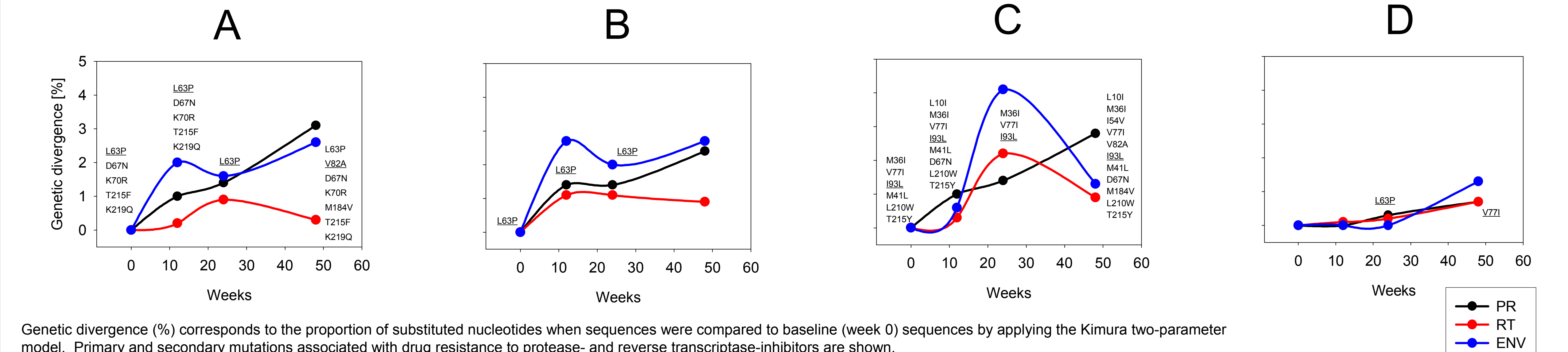
Table 1. Clinical and virological data from HIV-1 infected individuals and isolated viruses

Patient (ACTG code)	sample ID	Date	Week	CD4 (cells/ul)	Viral load (log <sub>10</sub> copies/ml)	TCID <sub>50</sub> (IU/ml)	Protease (PR) mutations <sup>a</sup>	Reverse transcriptase (RT) mutations <sup>a</sup>	Relative fitness <sup>b</sup> [%]	Biological phenotype <sup>c</sup>			
										306	322	V3 net charge	Phenotype
A (610471K)	A0	5/6/96	0	156	4.85	10 <sup>4</sup>	L63P	D67N, K70R, T215F, K219Q	48.7	S	D	+3	NSI
	A12	7/31/96	12	331	2.65	10 <sup>4</sup>	L63P	D67N, K70R, T215F, K219Q	74.4	S	D	+4	NSI
	A24	10/24/96	24	411	2.65	10 <sup>4</sup>	L63P	-	43.6	S	D	+3	NSI
	A48	4/10/97	48	443	4.30	10 <sup>4</sup>	L63P, V82A	D67N, K70R, M184V, T215F, K219Q	34.9	S	D	+3	NSI
B (610363J)	B0	6/19/96	0	205	4.08	10 <sup>5</sup>	L63P	-	41.0	S	E	+4	NSI
	B12	9/11/96	12	395	2	10 <sup>4</sup>	L63P	-	58.9	S	E	+3	NSI
	B24	12/4/96	24	322	2	10 <sup>5</sup>	L63P	-	74.3	S	E	+4	NSI
	B48	5/21/97	48	302	2	10 <sup>3</sup>	-	-	71.8	S	E	+3	NSI
C (610185H)	C0	6/18/96	0	149	4.40	10 <sup>5</sup>	M36I, V77I, I93L	M41L, L210W, T215Y	41.0	S	D	+3	NSI
	C12	9/18/96	12	276	2	10 <sup>4</sup>	L10I, M36I, V77I, I93L	M41L, D67N, L210W, T215Y	87.2	S	D	+2	NSI
	C24	12/11/96	24	309	2	10 <sup>6</sup>	M36I, V77I, I93L	-	61.5	S	D	+3	NSI
	C48	5/28/97	48	267	3.93	10 <sup>4</sup>	L10I, M36I, I54V, V77I, V82A, I93L	M41L, D67N, M184V, L210W, T215Y	61.5	S	D	+2	NSI
D (610500F)	D0	10/1/96	0	260	5.04	10 <sup>4</sup>	-	-	102.5	S	D	+4	NSI
	D12	1/15/97	12	276	5.04	10 <sup>5</sup>	-	-	92.3	S	D	+4	NSI
	D24	4/9/97	24	147	4.92	10 <sup>4</sup>	L63P	-	95.4	S	D	+4	NSI
	D48	9/24/97	48	128	5.45	10 <sup>6</sup>	V77I	-	96.3	S	D	+5	NSI

<sup>a</sup> Only mutations associated with drug resistance are depicted; <sup>b</sup> Relative fitness values are expressed as percentage of the replicative capacity of a wild type HIV-1<sub>92US076</sub> clade B primary U.S. isolate; <sup>c</sup> Viral phenotype was estimated based on amino acids at positions 306 and 322 (numbering based on HIV-1<sub>HXB2</sub> strain) in the V3 loop of the *env* gene and V3 loop net charge.

## Results (II)

Figure 2. Genetic diversity in protease, reverse transcriptase and envelope

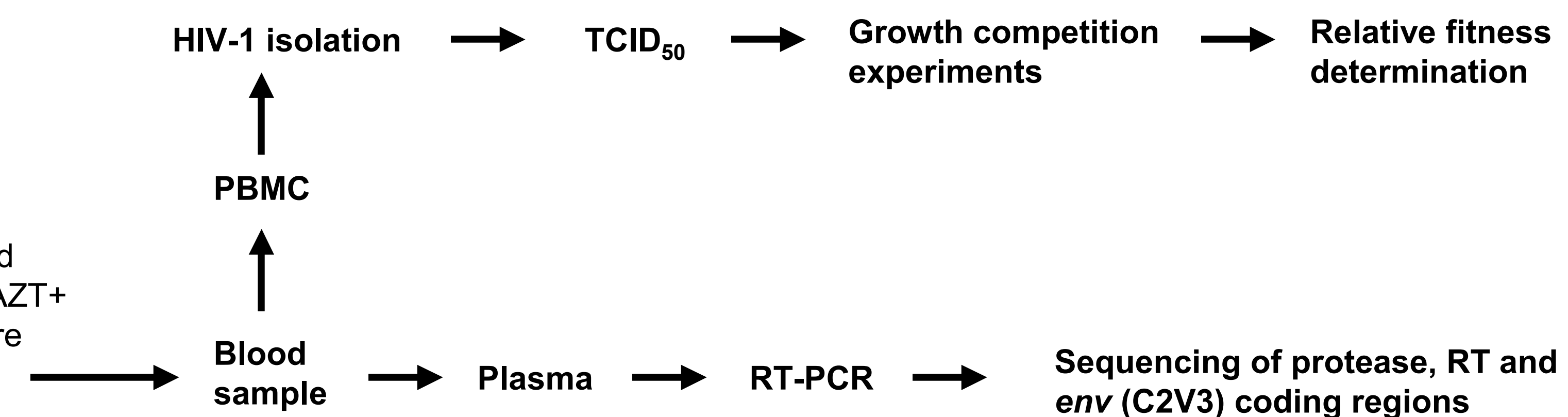


Genetic divergence (%) corresponds to the proportion of substituted nucleotides when sequences were compared to baseline (week 0) sequences by applying the Kimura two-parameter model. Primary and secondary mutations associated with drug resistance to protease- and reverse transcriptase-inhibitors are shown.

## Methods

### ACTG 315 Study

- Nucleoside-experienced patients
- 5 weeks washout period
- Therapy initiated with ritonavir
- After 10 days, AZT and 3TC were added
- HIV-infected individuals, remaining on AZT+ 3TC + RTV despite virologic failure, were selected for this study



## Conclusions

Two phases have been described in the evolution of viral fitness during antiretroviral therapy: (i) selection of drug resistant viruses with impaired fitness, followed by (ii) generation and selection of additional compensatory mutations, which enhance replication capacity. However, in this study, we observed that the evolution of viral fitness depends on the initial HIV-1 genetic background and it correlates well with the virologic response to antiretroviral therapy. Thus, natural polymorphisms associated with resistance to PI- or RT-inhibitors, may lead to a more rapid recovery of viral replication capacity and further HIV pathogenesis. It is clear then that viral fitness studies on HIV-1 isolates from antiretroviral naïve individuals could help to understand the impact of certain intrinsic compensatory/accessory mutations on viral replication.