Supplemental Digital Content 1. Methods used for linkage analysis.

The genetic linkage status was determined for index-partner pairs using methods described in a previous report (Eshleman, et al. J Infect Dis. 2011; 204:1918-1926). The classification of "linked" indicates that the HIV sequences from an index participant and the corresponding partner are closely related; in these cases, the index was the likely source of the partner's infection. The methods used to assess linkage status are summarized below.

First, *pol* region sequences (HIV protease and reverse transcriptase) were obtained using the ViroSeq HIV-1 Genotyping System (Celera, Alameda, CA). Phylogenetic analysis was performed using sequences from index-partner pairs, unrelated index participants (local controls), and reference sequences. Whenever possible, the analysis was performed using two samples from each individual (index and partner), collected on different dates. Sequences were aligned using MegAlign v5.07 (Clustal W method). PHYLIP (Neighbor-Joining and Consense) was used to generate phylogenetic trees and bootstrap values. Index-partner pairs were provisionally classified as linked if all of the sequences from the two individuals clustered together on a single branch of the tree with a high bootstrap value.

Next, genetic similarity values were calculated for the *pol* region sequences from index-partner pairs and local controls. These data were analyzed using Bayesian methods to determine the probability of linkage between different individuals. Index-partner pairs were provisionally characterized as linked by Bayesian analysis if the linkage probability was ≥0.5 for all pairs of sequences from the two individuals. Index-partner pairs were classified as linked (final status) if they were characterized as linked by both of these methods (phylogenetic and Bayesian analysis of *pol* region sequences); no further analysis was performed for these cases.

For the remaining cases, next generation sequencing was performed for a region of gp41 (HXB2 coordinates: 7691-8374) using a Roche 454 instrument (Roche, Branford, CT). Index-partner pairs were classified as linked (final status) if multiple consensus sequences from the index and the partner clustered together on a branch with a high bootstrap value.

Additional information describing the algorithm used for linkage analysis and the results obtained at each phase of testing for partner infections in HPTN 052 is presented elsewhere (Cohen, et al. New Eng J Med. 2016, In Press).

Supplemental Digital Content 2. Characterization of partner infections.

A. SUBTYPE SUMMARY

HIV subtype was determined by phylogenetic analysis of HIV *pol* sequences generated using the ViroSeq HIV-1 Genotyping System (1,302 nucleotide consensus sequences encoding HIV protease and 335 pairs; sequencing analysis failed in six cases. In all 72 cases, the HIV subtypes of the index and partner were consistent with the prevalent subtype(s) in each country. In 70 of the 72 cases, the subtypes of the index and partner were the same. The partner infections were unlinked in the two cases where the subtypes of the index and partner samples were different.

Country	# index-partner pairs with subtyping results	Subtype(s)
US	1	B
Brazil	4	B (N=2), F (N=1) C/BC recombinant (N=1)
Thailand	2	Both CRF02_AE
India	3	All C
Malawi	41	All C
Zimbabwe	9	All C
Botswana	4	All C
Kenya	5	A1 (N=2), D (N=1), A1 index with D partner (N=1), A1 index with C partner (N=1)
South Africa	3	All C
TOTAL	72	

B. RESISTANCE SUMMARY

Linked infections:
Resistance was detected in 3/46 linked cases (all in the delayed ART arm; in these three cases, the index participant was not on ART at the time of partner diagnosis):

- Two with transmitted resistance
  - In one case, the index and partner both had K103N
  - In one case, the index and partner both had V179D and/or V179E
- In one case, the index had K101E in one of two samples; the partner did not have resistance mutations detected

Unlinked infections:
Resistance was detected in 6/26 unlinked cases (all in the early ART arm; in these six cases, the index participant was on ART at the time of partner seroconversion):

- In four cases, resistance was detected in the index only:
  - K103N, M184V, and Y181C
  - K103N, V106M, M184V
  - M184V
  - K103N and T215S
- In two cases, resistance was detected in the partner only:
  - K103N
  - M184V, T215Y, V108I, Y181C

Supplemental Digital Content 4. Analysis of factors associated with linked partner infection.

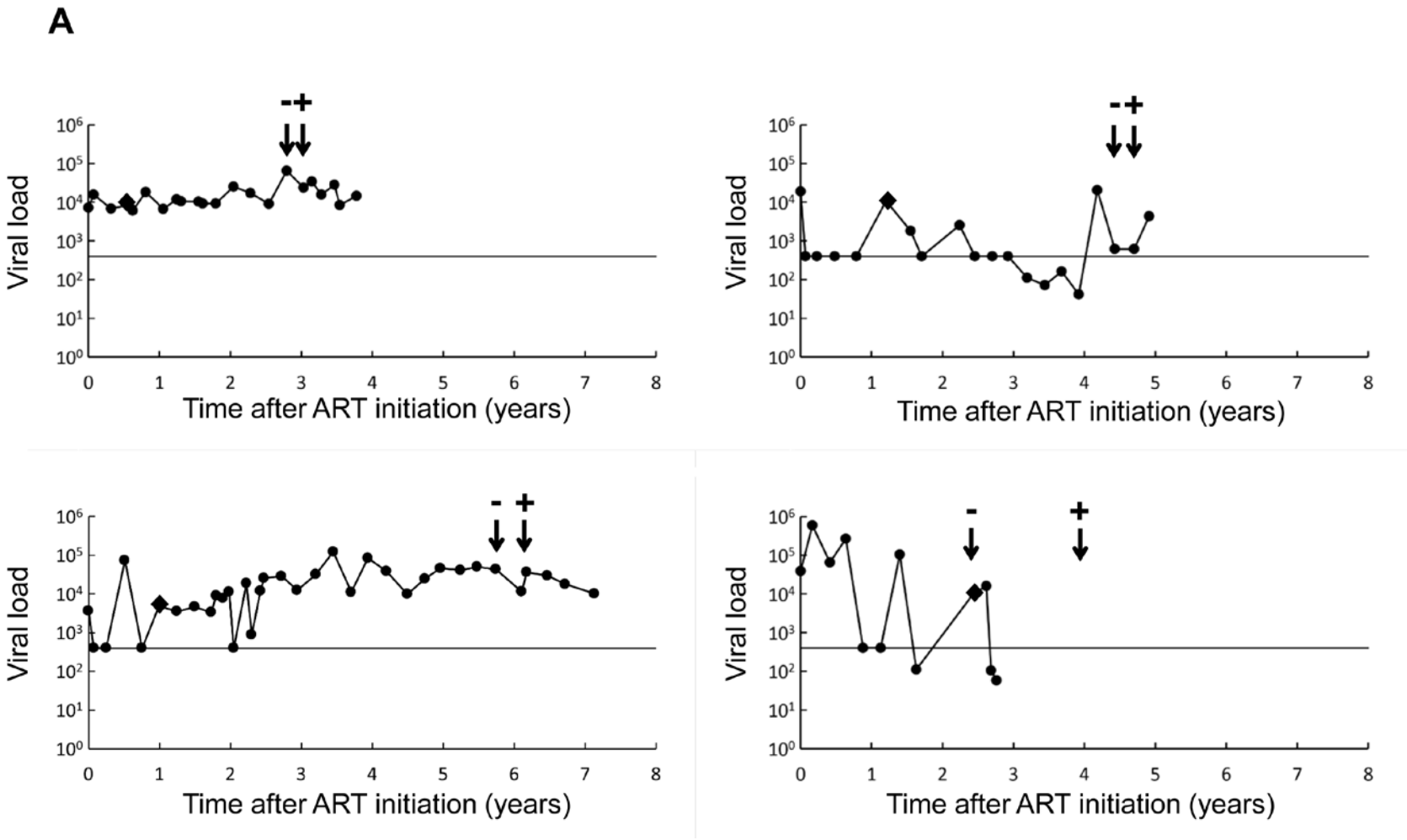
		Linked N=46	Unlinked N=26	P value	Multivariate Model 1 OR (95% CI)	Multivariate Model 1 P value	Multivariate Model 2 OR (95% CI)	Multivariate Model 2 P value
Region	Africa	39 (85%)	23 (88%)	0.74[a]				
	Asia/America	7 (15%)	3 (12%)					
Index sex	Male	21 (46%)	11 (42%)	0.81[a]				
	Female	25 (54%)	15 (58%)					
Couple type	Male-Male	0 (0%)	2 (8%)	0.13[a]				
	Others	46 (100%)	24 (92%)					
Study arm	Early ART	3 (7%)	14 (54%)	**<0.0001[a]**	0.07 (0.01, 0.94)	**0.045**		
	Delayed ART	43 (93%)	12 (46%)		Ref			
Index on ART at SC	Yes	8 (17%)	21 (81%)	**<0.0001[a]**				
	No	38 (83%)	5[c] (19%)					
Index VL >400 at SC	No	4 (9%)	21 (88%)	**<0.0001[a]**	Ref	**0.0006**	Not included	
	Yes	39 (91%)	3 (13%)		**157 (8.8, >999)**			
Index log_{10}VL at SC[d]	Median (IQR)	4.93 (4.11, 5.25)	2.60 (2.60, 2.60)	**<0.0001[b]**	Not included		**12.85 (3.76, 43.99)**	**<0.0001**
Index CD4 at SC	Median (IQR)	379 (308, 476)	540 (484, 683)	**0.0002[b]**	0.31 (0.10, 0.91)	**0.033**		
Yrs enrollment to SC	Median (IQR)	1.5 (0.8, 2.2)	3.3 (2.0, 4.7)	**0.0003[b]**				
Sex partners ≤3 months before SC[e]	>1	0 (0%)	5 (19%)	**0.007[a]**				
	1	41 (89%)	19 (73%)					
	0	3 (7%)	2 (8%)					

Legend for Supplementary Table:

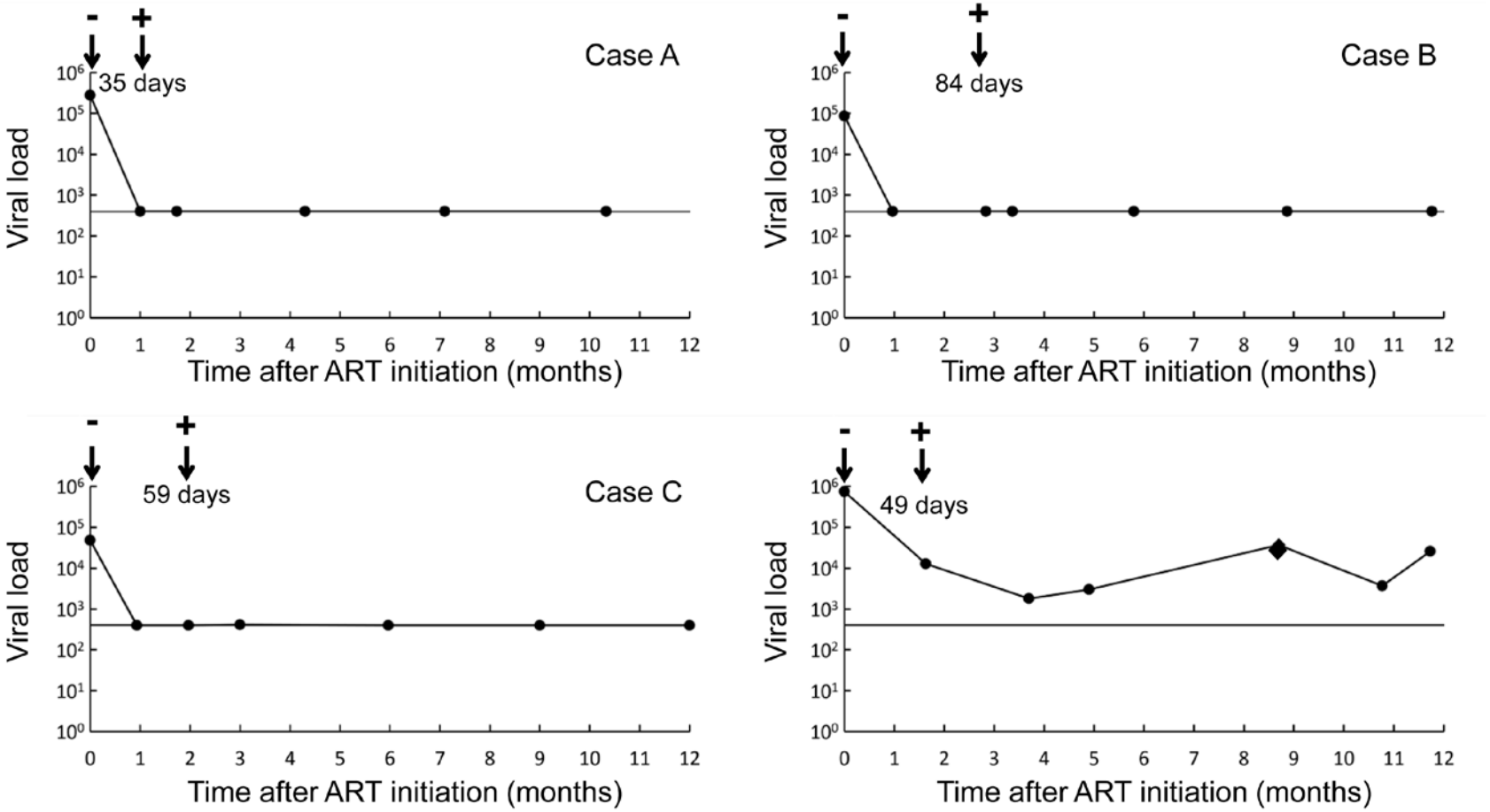
Results are shown for univariate analyses and two multivariate models performed using backward stepwise regression. Multivariate model 1 used the binary variable for viral load (<400 or ≥400 HIV RNA copies/mL); multivariate model 2 used the continuous variable for viral load (log_{10} HIV RNA copies/mL). Significance was defined as $p < 0.05$ (bold text). CD4 cell count data were analyzed per 100 cells/mm^3 increment; continuous viral load data were analyzed per log_{10} increment. Seven participants were excluded from the multivariate analysis because of missing index viral load and/or CD4 cell count data at seroconversion visit (five were missing viral load data; six were missing CD4 cell count data). Factors that remained associated with linked infection in the multivariate models are shown. ART: antiretroviral therapy; SC: seroconversion; IQR: interquartile range; VL: HIV viral load, copies/mL; CD4 cell count: cells/mm^3; Yrs: years; OR: odds ratio; CI: confidence intervals.

[a] p-value from Fisher's exact test.
[b] p-value from Wilcoxon Rank-Sum test.
[c] Two index participants were on ART due to pregnancy. They were considered to be not on ART at the time of seroconversion.
[d] Viral load values <400 copies/mL were assigned a value of 399 copies/mL (equivalent to 2.60 log_{10} copies/mL); 25 index viral load results obtained at the time of seroconversion were <400 copies/mL, including 4/43 results for couples with linked infection; 21/24 results for couples with unlinked infection.
[e] Two partners with linked infection did not provide information about the number of sex partners in the 3 months before seroconversion.

Supplemental Digital Content 3. Relationship of linked partner infections to index viral load.



B


-
+
Case A
35 days
Viral load
Time after ART initiation (months)
Case B
84 days
Case C
59 days
49 days

Legend for Supplemental Digital Content 3:

Viral load data from index participants are plotted as a function of time after initiation of antiretroviral therapy (ART). Viral load values <400 copies/mL were assigned a value of 399 copies/mL. Arrows with negative signs (-) indicate the last visit where the partner tested negative for HIV infection; arrows with positive signs (+) indicate the first visit where the partner tested positive for HIV infection. Black diamonds indicate the date of virologic failure, defined as the first of two consecutive dates more than 24 weeks after ART initiation where the index participant's viral load was >1,000 copies/mL. Supplemental Figure (A) shows data from the four linked partner infections that were diagnosed after index ART failure. The initial ART regimens in these cases were atazanavir (ATV)/lamivudine (3TC)/zidovudine (ZDV) (one case) and efavirenz (EFV)/3TC/ZDV (three cases). In one case, EFV/3TC/ZDV was switched to a second regimen (ritonavir-boosted ATV/3TC/ZDV); in the other three cases, the initial ART regimen was continued until the end of follow-up. Supplemental Figure (B) shows data from the four linked partner infections that were diagnosed shortly after index ART initiation. In all four cases, the initial ART regimen was EFV/3TC/ZDV. The number of days between the index's ART initiation and the partner's HIV diagnosis is shown below the arrow. In three of these cases, the index participant was virally suppressed at the time of the partner's HIV diagnosis (Cases A-C); additional data for those three cases is shown in Figure 2.