## Analysis of Genetic Linkage of HIV from Couples Enrolled in the HPTN 052 Trial

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## **Linkage Analysis**

## 39 transmission events were included in the April 28<sup>th</sup> DSMB report.

Linkage analysis was performed to identify the subset of transmission events where the index participant was the likely source of the partner's HIV infection. The DSMB report included 28 linked transmission events. Those data were used to analyze the impact of immediate ART on HIV prevention.

One additional linked transmission event was identified after the DSMB meeting. This additional event was in the delayed ART arm, further strengthening the association between immediate ART and HIV prevention.

## Phylogenetic analysis of *pol* sequences obtained by population sequencing

Statistical (Bayesian) analysis of genetic distances from *pol* sequence pairs

Phylogenetic analysis of *env* sequences obtained by next generation sequencing

## **Genetic Diversity of HIV**



#### **HIV RNA**



## **HIV Diversity in an Infected Person**

















#### **Population Sequencing**



#### Phylogenetic analysis of pol sequences obtained by population sequencing

Statistical (Bayesian) analysis of genetic distances from *pol* sequence pairs

Phylogenetic analysis of *env* sequences obtained by next generation sequencing

## **Phylogenetic Analysis**



Adapted from Margulies et al., Genome Res 2007;17:760-77 ©2007















Phylogenetic analysis of *pol* sequences obtained by population sequencing

# Statistical (Bayesian) analysis of genetic distances from *pol* sequence pairs

Phylogenetic analysis of *env* sequences obtained by next generation sequencing

Statistical (Bayesian) Analysis Comparison of genetic similarity values from paired sequences (*pol*)

#### **Representative data from one transmission event**

Pair type	Basis	Sample source	# pairs	Similarity values
"Known" linked	Two samples from the same person	Index-index Partner-partner	1 1	99.8 98.2
"Known" unlinked	Unrelated individuals	10 other index participants / site	>50	91.2-95.3
Unknown	Transmission event	Index-partner (2 each)	4	98.4-98.9

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## **Initial Linkage Assessment**

#### 26 events analyzed were clearly linked using both methods – those 26 events were classified as linked

The linkage status of 12 events could not be definitively determined

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## **Population Sequencing**



#### **Consensus sequence** e.g., AACTGATCGGAA...

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## **Next Generation Sequencing**



sequence 1 sequence 2 sequence 3 sequence 4 sequence 5 sequence 6...

sequence 1 sequence 2

sequence 3

sequence 4\*\*

sequence 5

sequence 6...

#### Example of an Unlinked Event (env)





## **Next Generation Sequencing**

One additional event was confirmed to be linked by next generation sequencing after the April 28<sup>th</sup> DSMB meeting.

This event was in the delayed study arm, further strengthening the association between immediate ART and HIV prevention.

This partner seroconverted ~1 month after the index started ART. The partner was most likely infected shortly before the index started ART, or shortly after the index started ART before the index was virally suppressed.

## **Summary of Linkage Analysis**

#### **29 linked events**

- In 2 cases, linkage was only apparent using next generation sequencing
- 1 immediate ART arm; 28 delayed ART arm

#### 7 unlinked events

• 4 immediate ART arm; 3 delayed ART arm

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#### **3 additional events**

- 2 events were not classified based on available data
- 1 late event is still being analyzed
- All 3 were in the delayed arm, not on ART

Linkage was <u>not</u> associated with:

- Geographic region
- Index gender
- Index CD4 cell count at enrollment
- Years between enrollment and seroconversion

	Linked	Unlinked	P value
Index study arm			0.018
Immediate ART	1 (3%)	3 (43%)	
Delayed ART	28 (97%)	4 (57%)	
Index on ART at time of SC			0.0076
Yes	2 (7%)	4 (57%)	
No	27 (93%)	3 (43%)	
# sex partners ≤3 months before SC			<0.0001 <sup>a</sup>
>1	0 (0%)	4 (57%)	
=1	26 (90%)	3 (43%)	
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1 linked transmission in the immediate ART arm identified pre-DSMB 1 linked transmission in the delayed ART arm identified post-DSMB

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#### \*Data was missing for one participant

#### Conclusions

We used a combination of laboratory and statistical methods to determine the linkage status of transmission events in HPTN 052

These data were used to identify the endpoints used in the analysis of HIV prevention in the HPTN 052 trial

A manuscript describing the analysis of HIV linkage in HPTN 052 is In Press in the Journal of Infectious Diseases

## Thanks

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