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Prediction of X4-tropic Human Immunodeficiency Virus from Proviral Envelope Sequence in Patients with Suppressed Viral Load on Antiretroviral Therapy (ART).

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I. Abstract

Background: The presence of X4-tropic HIV negates the antiviral response to R5 antagonists. However, the identification of X4 tropic HIV is currently possible only in patients with HIV viremia. We evaluated the prevalence of X4-tropic HIV among ACTG A5102 patients who had plasma HIV RNA levels < level of detection for > 1 year comparing tropism predicted from the HIV proviral DNA sequence before treatment interruption to the plasma HIV tropism after interrupting antiretroviral therapy (ART).
Methods: Envelope sequence (C2-C3) was amplified from proviral DNA isolated from PBMCs before treatment interruption; co-receptor usage was predicted using the PSSM program (http://indra.mullins.microbiol.washington.edu/pssm/) for the population sequence and 10-20 cloned *env* amplicons were also examined. After antiretroviral therapy was stopped, tropism was evaluated with the Trofile assay from Monogram Biosciences in the matched samples when the plasma HIV RNA level was > 1000 copies/ml.
Results: Among 18 evaluable patients treated for > 1 year, median CD4 was 888/mm3 which decreased to 649 /mm3 2-8 weeks (mean 2.8 weeks) after interruption. The median first detectable RNA > 1,000 copies/ml after stopping therapy was 4.27 log10 c/ml. Proviral *env* sequences predicted X4-tropic virus for 5/18 (28%); in 3 by consensus proviral sequence and in two samples by cloning. For the two cloned samples, 5/20 and 2/12 clones from proviral V3 loop DNA were predicted to be X4. Only the 3 samples with X4 in consensus proviral sequence had dual/mixed (D/M) virus by the Trofile assay from the corresponding plasma samples. These 3 D/M patients exhibited trends towards a lower nadir CD4 count (p=0.076, Mann-Whitney) and less reduction in CD4 count after interruption (p=.085, Mann-Whitney) compared to the patients with only R5-tropic HIV.
Conclusions: Currently, R5 inhibitors are recommended only for those with a measurable plasma HIV level and demonstration of exclusive R5 tropism. Here we show that X4-tropic HIV can be identified and predicted from proviral *env* sequences among suppressed patients with high CD4 cell counts, providing a means to identify potential situations in fully suppressed HIV-infected patients when R5 inhibitors could be effectively used in drug substitution or simplification studies. Surprisingly, there was no evidence of increased CD4 decline among those with X4 tropism.

II. Background and Objectives

ACTG A5102 was a pilot study in which 47 subjects who had been virologically suppressed for more than 1 year on potent ART were randomly assigned (1:1) to receive IL-2 treatments or placebo over three months before treatment interruption (TI) (JAIDS (2006) 42: 140-8). The study enrolled 47 patients. The objective of the study was to determine whether IL2 increased the time that patients maintained a CD4 count > 350 per cu mm off therapy relative to those who did not receive IL-2.

Drugs which inhibit CCR5 co-receptor binding and infection (R5 inhibitors) are currently recommended for patients only when their virus is demonstrated to use exclusively CCR5 as a co-receptor. For patients who are virologically suppressed, it is not possible to determine the tropism of plasma virus, and thus R5 inhibitors cannot be used. The current study used cellular samples from A5102 to determine whether the viral tropism of emergent plasma virus could be accurately predicted by sequencing envelope from proviral DNA among subjects who are suppressed. These patients then interrupted treatment and had increasing viral loads at 2 to 8 weeks post interruption. We compared the predicted envelope tropism from amplified proviral DNA isolated from PBMCs before treatment interruption with a sensitive Trophile assay of plasma RNA samples post interruption.

Here we present the results from the first 18 patients for whom we have both sequence and Trophile data.

Table 1. Baseline characteristics of 18 patients from the ACTG A5102 study

	Overall (n=18)	X4 by Trophile (n=3)	R5 by Trophile (n=15)	P-value[a]
Baseline CD4 per cu mm	888	707	913	0.906
CD4 per cu mm at 2-8 wks	649	700	639	0.407
Nadir CD4 per cu mm	337.5	150	350	0.076
Change in CD4 count post interruption	-228	-47	-237	0.085
Plasma HIV RNA $\log_{10}$ copies/ ml[b]	4.27	4.79	4.22	0.236

[a] Mann Whitney test comparing X4 and R5 samples
[b] 1st detectable viral load after TI

Figure 1.
Comparison of CD4 counts at Baseline and after STI

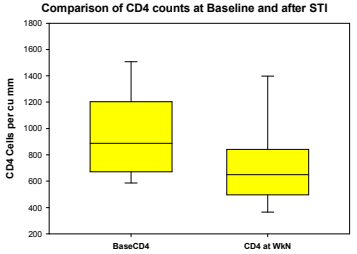

Table 2. Tropism of Samples using Trophile and PBMC env sequencing

ID	TROFILE	PBMC	X4 Clones?
2	D/M	X4/R5	14/21
3	R5	R5	
4	R5	R5	0/20
5	R5	R5	
9	R5	R5	
11	R5	R5	
21	R5	R5	
22	R5	X4/R5	5/20
23	R5	R5	
24	R5	R5	0/15
26	R5	R5	
29	R5	R5	
30	R5	R5	
31	R5	R5	
34	D/M	X4	Not Cloned
35	R5	R5	
36	R5	X4/R5	2/12
41	D/M	X4	Not Cloned

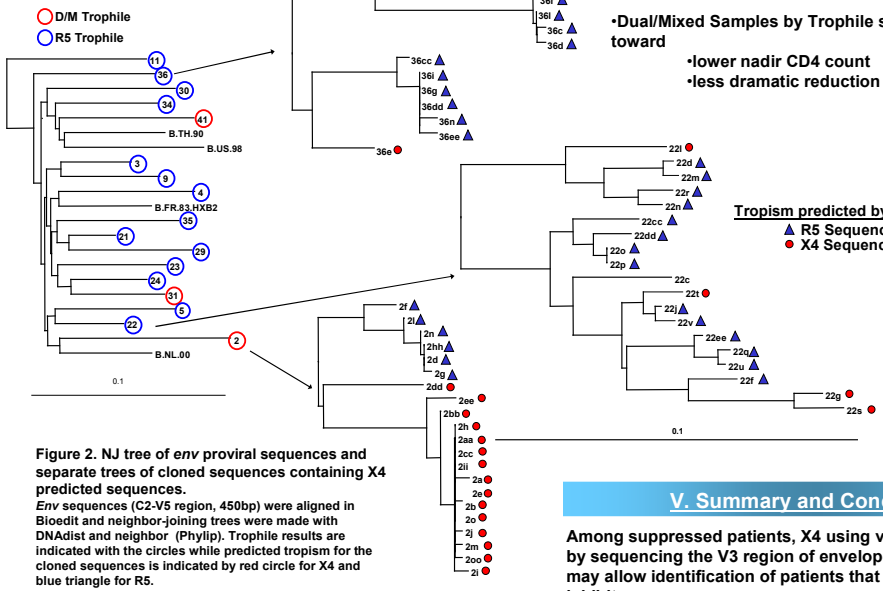

Figure 2. NJ tree of *env* proviral sequences and separate trees of cloned sequences containing X4 predicted sequences.
Env sequences (C2-V5 region, 450bp) were aligned in Bioedit and neighbor-joining trees were made with DNAdist and neighbor (Phylip). Trophile results are indicated with the circles while predicted tropism for the cloned sequences is indicated by red circle for X4 and blue triangle for R5.

III. Methods

- Envelope sequences (C2-V5) were amplified from proviral DNA isolated from PBMCs collected from suppressed patients before treatment interruption.

- Co-receptor usage was predicted for the population sequence using the WebPSSM program
  http://indra.mullins.microbiol.washington.edu/webpssm/

- 10-20 cloned *env* amplicons from the proviral DNA were also sequenced.

- Tropism was evaluated for plasma samples after treatment interruption with the Trofile assay (Monogram Biosciences) in the matched samples when the plasma HIV RNA level was > 1000 copies/ml.

IV. Results

- 2 to 8 wks after TI (mean =2.8 wks), viral loads were >1000 copies per ml and CD4 counts had significantly dropped from 888 to 649 CD4 cells per cu mm (p= 0.0008, Wilcoxon signed rank test)

- Comparison of tropism prediction from PBMCs vs Trophile assay
  R5 predicted/R5 by Trophile: 13
  X4 predicted/X4 by Trophile: 3
  X4 predicted/R5 by Trophile: 2

- Prediction of X4 from PBMCs but not Trophile: Clones predicted X4 but in less than 25% of clones

- Dual/Mixed Samples by Trophile showed a trend toward
  - lower nadir CD4 count
  - less dramatic reduction in CD4 count after TI

V. Summary and Conclusions

Among suppressed patients, X4 using viruses can be detected by sequencing the V3 region of envelope from proviral DNA and may allow identification of patients that could take an R5 inhibitor.

Predicting X4 usage based on proviral sequences can identify small populations of X4 tropic virus though these may or may not be from viable provirus

Surprisingly, patients with X4 virus identified by Trophile had less CD4 loss after stopping treatment

VI. Acknowledgements

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