175LB - Primer ID Sequencing to Study the Development of CXCR4-using HIV-1 Variants

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Background

HIV-1 uses CD4 as the main receptor and either CCR5 (R5 variants) or CXCR4 (X4 variants) as a coreceptor to enter the host cells. X4 variants are usually seen in the late stage of HIV-1 infection and are related to rapid disease progression. X4 and R5 variants have substantial sequence differences especially at the V3 region. However, the evolution pathways that drive development of X4 variants are unclear. We performed Primer ID deep sequencing using longitudinal plasma samples from the Women's Interagency HIV Study (WIHS) cohort to study the development of X4 variants.

Methods

We identified 23 subtype B HIV-seropositive WIHS participants meeting the following criteria: 1) CD4 T+ cell counts declined from >500 cells/μl to <100 cells/μl; 2) therapy-naïve or on failed therapy with unsuppressed viral load; and 3) plasma samples available biannually during the period of CD4 decline. Multiplexed Primer ID MiSeq sequencing targeted HIV-1 PR, RT, IN and V1/V3 regions on longitudinal specimens. X4 variants were determined by both criteria: 1) Geno2pheno coreceptor algorithm with a false positive rate below 2%, and 2) variants form a distinct lineage.

Results

- Seventeen participants (74%) had X4 variants at the last time point when CD4 was <100 cells/μl and 6 (26%) had R5 only viral populations.
- We sequenced all available longitudinal samples for eight X4 participants and two R5 participants with an average 6.6 biannual time points per participant. Seven of the eight X4 participants (87.5%) did not have X4 variants at baseline but developed X4 viruses as minority variants (mean abundance 21% in the viral population) at a median CD4 count of 538 cells/μL (IQR: 491-655). One participant had X4 variants at baseline with a CD4 count of 759 cells/μL.
- Three of the eight X4 participants (37.5%) had rapid CD4 decline (>300 cells/μL per year) after emerging of X4 variants and two of six R5 participants (33.3%) had rapid CD4 decline overall (p = 0.57, Fig 1).
- Phylogenetic analysis showed that X4 variants could emerge from multiple pre-existing lineages with distinct V3 sequence changes (Fig 2). Sequence changes at the V1/V2 region were associated with sequence changes in the V3 region during the development of X4 variants in most X4 participants.
- Pairwise diversity (pi) changed greatly across the disease course at V1/V3 regions but remained stable at PR, RT and IN regions (Fig 3). We found a positive linear correlation between the CD4 drop rate and the pi drop rate at V1/V3 region (r=0.82, p=0.003, Fig 4).

Fig 1. CD4 decline and coreceptor switch. One line per subject. Dotted lines for time points with X4 variants. Star marks are the 1st times X4 emerges.

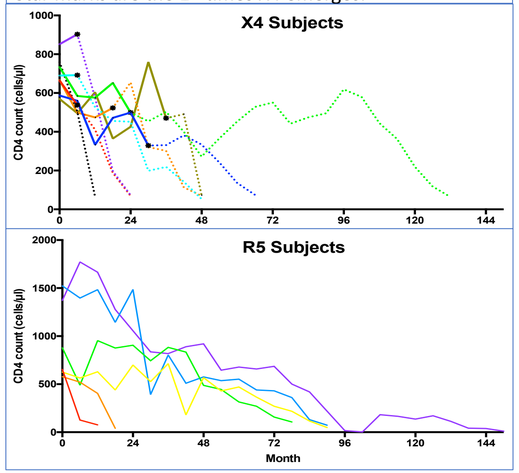


Fig 2. Phylogenetic analysis of subject W02.

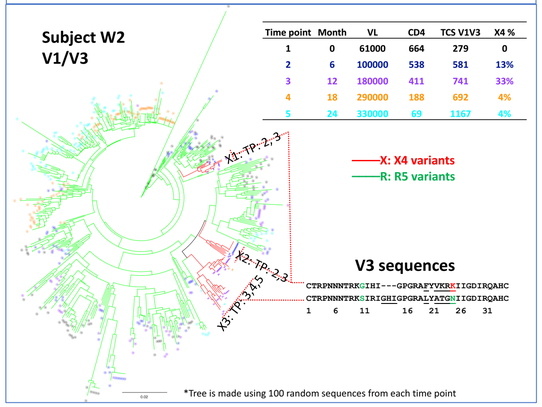


Fig 3. Change of pairwise diversity at 4 regions over the disease course. Purple arrows show when the X4 variants emerged.

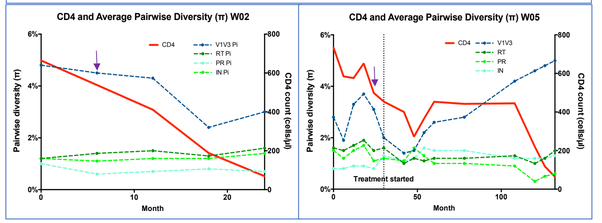
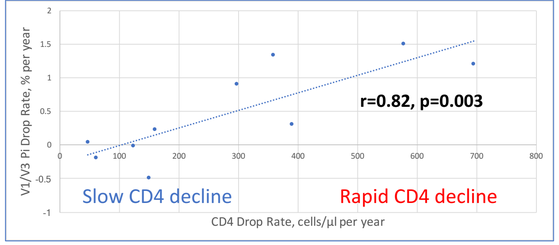


Fig 4. Positive linear correlation between the CD4 drop rate and pi drop rate at the V1/V3 region in 10 subjects sequenced.



Conclusions

X4 variants can emerge at early stage of infection as minor variants. The presence of X4 variants is not associated with rapid CD4 decline in our study subjects. The co-evolution of V1/V2 region and V3 region suggests that the compensatory mutations on the V1/V2 regions may be required to develop X4 tropism.

Acknowledgements

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