Deep Sequencing of Plasma and Proviral HIV-1 to Establish Coreceptor Usage: What Is the Clinical Impact of the Quasispecies Distribution?

To The Editor—We have read with great interest the recent article by Swenson et al [1], as well as the commentary by Kuritzkes [2], concerning the use of deep sequencing to establish human immunodeficiency virus type 1 (HIV-1) coreceptor usage. We agree that recently introduced genotypic tests based on ultradeep pyrosequencing (UDPS), used in combination with genetic algorithms to infer HIV-1 coreceptor usage, may fill the gap of low sensitivity of genotypic tests in detecting X4 variants, as compared with phenotypic tests. In fact, UDPS may highlight the presence of X4 minority variants in the viral quasispecies that are missed by bulk sequencing. Based on the combined use of UDPS and position specific score matrix (PSSM) analysis [3], we obtained a sensitivity in detecting X4 variants that is virtually identical to that reported by Swenson et al [1] (ie, 83%), whereas the sensitivity reported for bulk sequence analysis does not exceed 70% [4–6]. In addition, the concordance of UDPS and the Trofile assay observed in our study was identical to that reported by these authors (82%), whereas the specificity was slightly higher (91% vs 81%, respectively).

A major finding of the study reported by Swenson et al [1] is that genotyping predicted virological response to maraviroc as well as did phenotyping, even if an inverse gradient of response based on percentage of CXCR4-using virus could be observed. Swenson et al [1] compared UDPS and the phenotypic test using the original Trofile version, of which the sensitivity for X4 detection is 5%–10%. For UDPS analysis only >2% of X4 variants were considered non-R5-using virus, because the authors stated that detection of non-R5 levels of <2% may have low reproducibility.

In our studies [3, 7], a very stringent correction algorithm was used to minimize the noise due to the high throughput pyrosequencing platform, enhancing the accuracy of detection of rare variants by UDPS. By this method, we could measure a very wide range of intrapatient X4 frequencies in plasma HIV-1 (0.3%–56.3% in early HIV-1–infected patients and 0.3%–89.4% in chronic HIV-1–infected patients). When peripheral blood mononuclear cell (PBMC)–associated proviral DNA quasispecies were investigated in parallel with plasma HIV, we could observe, especially in chronically infected patients, that X4 variants were present at higher frequencies with respect to those present in plasma. These variants sometimes showed a bimodal distribution (rather than a continuous spectrum) of PSSM scores, with different intrinsic diversity of each subgroup of variants (which is well illustrated by 3-dimensional surface plots) (Figure 1), suggesting the existence of a very heterogeneous quasispecies made by distinct groups of R5 and X4 variants. This higher variability of proviral DNA quasispecies is probably due to proviral DNA representing an archive of all the variants present in an infected patient during the natural history of the infection.

We agree with Kuritzkes [2] that establishing an appropriate cutoff for detecting CXCR4-using virus is the greatest challenge to understanding the real clinical significance of low amounts of X4 variants. This may be particularly relevant for proviral X4 variants that may not reflect actually replicating virus variants. In a previous study from our group [3], we observed that, among 5 patients who received maraviroc, 3 harbored X4 variants in PBMC proviral DNA at variable frequencies (up to 52.7%), but only 1 patient (with only 1% X4 variants in proviral DNA) developed virological failure. In this patient, the absolute number of X4 genomes harbored in proviral DNA at baseline was lower than that detected in DNA of the other 2 responding patients.

On the basis of the number of studies that have described a good correlation between plasma and proviral HIV-1 tropism [3, 8–11], it has been suggested that coreceptor usage established on proviral genome sequence should be required before CCR5 antagonists are used in patients with a fully suppressed plasma viral load [11]. However, the discrepant quasispecies composition between circulating and archived viral genomes observed in our studies [3, 7] may suggest caution in using only proviral DNA tropism determination. With regard to the clinical significance of minority X4 variants present both in plasma HIV-1 and in proviral PBMCs, effective optimized background therapy could minimize their negative impact on response to treatment to CCR5 antagonists, and we agree that it is possible that a threshold of non-R5 HIV-1 frequency must be exceeded before treatment is compromised. Thus, specific studies conducted with very sensitive technologies aimed to establish X4 frequency thresholds that can be tolerated in respect of effective CCR5 antagonist treatment are urgently needed.
Figure 1. Three-dimensional surface plots of the human immunodeficiency virus type 1 (HIV-1) V3 quasispecies distribution of HIV-1 variants present in circulating plasma RNA (A) and peripheral blood mononuclear cell–associated proviral DNA (B) in a representative patient with chronic infection. The x-axis describes the position specific score matrix (PSSM) score, the y-axis represents the distance of each sequence from a consensus V3 sequence, and the z-axis describes the percentage of reads on total quasispecies.
Funding

This work was supported by the Italian Ministry of Health (Ricerca Corrente to INMI L. Spallanzani and National AIDS project; grant 40H54 to M. R. C. and grant 40H59 to I. A.).

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Received 28 February 2011; accepted 18 April 2011.
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The Journal of Infectious Diseases 2011;204:971–73
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