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Potential limitation of CCR5 antagonists: drug resistance more often linked to CXCR4-utilizing than to CCR5-utilizing HIV-1

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Abstract

CCR5 antagonists are approved for treatment-experienced individuals, who are at risk of harboring both drug-resistant and CXCR4-utilizing (X4) HIV-1. If CXCR4 usage and drug resistance are linked, CCR5 antagonists may select for CXCR4-utilizing viruses resistant to antiretrovirals. Analysis of 117 individual viruses found that 69% of CXCR4-utilizing viruses versus 48% of R5 viruses had drug resistance mutations ($P = 0.025$). Linkage of X4 and drug resistance may limit the effectiveness of CCR5 antagonists.

CCR5 antagonists are a new class of antiretrovirals shown to have short-term virologic benefits in treatment-experienced HIV-1 infected individuals [1,2]. However, among individuals with only CCR5-utilizing (R5) viruses detected prior to therapy, CXCR4-utilizing (X4) viruses (that presumably existed below the limit of detection) emerged soon after the initiation of CCR5 antagonist therapy [3]. In trials of the CCR5 antagonist maraviroc in antiretroviral-experienced individuals, 52% of participants had virologic failure within 24 weeks, and 64% of these had X4 viruses [1,2]. Individuals who had drug-resistant viruses detected prior to maraviroc had a higher rate of treatment failure, but it is unclear whether the X4 viruses that emerged also harbored additional drug-resistance mutations.

In advanced HIV-1 disease, both X4 and drug-resistant viruses are prevalent, but whether these traits are linked is unknown. Routine sequencing to determine predicted X4/R5 phenotype and resistance to antiretrovirals requires amplification of different regions of the HIV-1 genome from a population of hundreds to thousands of viruses, making it impossible to determine whether the traits coexist within individual viruses. By analyzing the region of *env* encoding the X4/R5 phenotype and the regions of *pol* encoding resistance to nucleoside (NRTIs) and nonnucleoside reverse transcriptase inhibitors (NNRTIs), derived from single viruses, we sought to evaluate whether drug-resistant mutations were more prevalent in X4 viruses compared with R5 viruses. If these properties are linked, CCR5 antagonists may select for X4 variants that are also resistance to other classes of ARV.

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Thor Wagner generated a portion of the sequences, analyzed our database of single-genome sequences and abstracted the relevant data for this study. He performed the statistical analysis and wrote the manuscript. Lisa Frenkel provided ongoing guidance and feedback at all stages of the process.

Thor Wagner and Lisa Frenkel had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Neither of the authors have a commercial or other association that might pose a conflict of interest.

More than 1500 *pol* and *env* HIV-1 DNA single-genome sequences derived from 22 antiretroviral-treated children [4–6] and 18 HIV-infected adults ([7] and unpublished data) by multiplex PCR of peripheral blood mononuclear cells subjected to end-point dilution [4–6] were retrospectively evaluated. The majority of these participants were exposed to monotherapy or dual therapy prior to the advent of highly active combination antiretroviral therapy (ART). Mutations conferring resistance to protease were infrequent in these sequences and were not analyzed. Primary mutations associated with resistance to NRTI and NNRTI were identified in each sequence as defined by the International AIDS Society (www.iasusa.org) [8]. Each *env* sequence was classified as X4 or R5 based on a position specific scoring matrix [9]. A two-tailed Fischer's exact test evaluated the strength of the association between predicted coreceptor usage and drug-resistance mutations within viruses. A two-tailed (*t*-test) was used to compare the participants' mean proportion of drug resistance in X4 versus R5 viruses.

Of 40 participants, 30 had sequences with mutations conferring resistance to NRTI or NNRTI or both, 14 had sequences encoding for X4 usage, and seven had sequences encoding for both drug resistance and X4 usage. One of these seven participants had exclusively drug resistant sequences and therefore was excluded. The six remaining individuals each had a mixture of drug-resistant, wild-type, X4, and R5 viruses (Table 1). A total of 791 *pol* and *env* single-genome sequences were analyzed from these six individuals; however, only in 117 instances did both the *pol* and the *env* sequence come from the same limiting dilution PCR, and were thought to be derived from a single virion. The sequences analyzed from these six were collected over a median of 7.5 years (range 1.5–14) beginning when the median age of participants was 5.5 years (range 2–36). The antiretroviral history of W19 was not available. All other participants had mono or dual antiretroviral exposure prior to starting an effective ART regimen. Dual antiretroviral therapy included dual NRTI, one or more protease inhibitors, and in one participant (G2) nevirapine. The participants CD4 cell count nadir ranged from 9 to more than 500 cells/ml; however, participant W19's nadir was unavailable. A median of 43% of the sequences (range 20–100%) were from specimens collected prior to initiating effective ART.

Drug-resistance mutations occurred in 69% (41/59) of all the X4 viruses compared with 48% (28/58) of the all R5 viruses ($P = 0.025$). In the average participant, 81% (range 33–100%) of X4-viruses and 43% (range 17–58%) of R5-viruses had drug-resistance mutations ($P = 0.049$). The density of drug-resistance mutations was greater in X4 versus R5 sequences, with 2.1 versus 0.89 per sequence, respectively. Only one participant (B1) had a higher rate of drug-resistance mutations in R5 sequences, and the prevalence of mutations in her sequences was relatively low, with 0.5 drug resistance mutations per sequence.

Among individuals with both HIV-1 drug resistance mutations and sequences predicted to be X4, drug-resistance was linked more frequently to X4 than to R5 viruses. Because individuals who have failed antiretroviral regimens often have mutant viruses persisting as minority populations, which may not be detected by consensus sequencing [4], linkage of drug-resistant mutants to *env* encoding X4 in single virions could be selected as a consequence of treatment with CCR5 antagonists, and potentially diminish the benefits from CCR5 antagonists.

The present study has several limitations. First, the conclusions are based on only 117 viruses from six individuals. Nevertheless, the viral sequences analyzed come from a relatively unique database of more than 1500 multiplexed PCR of single viral genomes from over 5000 end-point dilutions of peripheral blood mononuclear cells. Second, the eligible sequences were mostly from pediatric participants; though there is no reason to suspect that the association between drug-resistant and X4 viruses would be different in adults. Third, only a limited number of screened individuals were eligible for this analysis based on the detection of a

mixture of drug-resistant and X4 viruses; and these individuals might not be a representative of those prescribed CCR5 antagonists.

To our knowledge, this is the first study to evaluate the linkage of drug-resistance mutations and CXCR4-tropism within single viruses. Because of the limitations of our study, additional studies examining the risks from linked drug-resistance and X4 in persons treated with CCR5 antagonists seem warranted to determine how best to utilize this new class of antiretrovirals. Until more information becomes available, our findings suggest caution in pairing CCR5-antagonists with recycled antiretrovirals, and one might assume that CCR5 antagonists may be more effectively utilized as part of an initial antiretroviral treatment regimen, prior to the selection of drug resistance and X4 variants.

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HIV-1 drug-resistance and coreceptor affinity from antiretroviral-experienced individuals, derived from single-genome sequences of *env* and *pol*.

Table 1

Participant	<i>pol</i> sequence classification	No. of X4 sequences	No. of R5 sequences	Total sequences	Drug-resistant reverse transcriptase codons
B1	Wild type	8	4	12	M41L, D67N, M184V/I, K219E
	Drug resistance (%)	4(33)	4(50)	8(40)	
C1	Total	12	8	20	M41L, D67N, K70R, K103N, T215Y, K219Q
	Drug resistance (%)	0	4	4	
G1	Wild type	4 (100)	3 (43)	7 (64)	M41 L, K69R, G190A, T215F
	Drug resistance (%)	4	7	11	
G2	Total	9 (100)	1 (17)	10 (67)	D67N, K70R, V75I, K103N, V108I, Y181C, M184V/I, T215Y/F
	Drug resistance (%)	0	10	10	
J1	Wild type	5 (100)	14(58)	19 (66)	M41L, A62V, L74V, K103N
	Drug resistance (%)	5	24	29	
W19	Total	9 (100)	4 (50)	13 (76)	K103N
	Drug resistance (%)	0	8	8	
Total	Wild type	10	3	13	
	Drug resistance (%)	10	2	12	
	Total	20	5	25	
	Drug resistance (%)	18	30	48	
	Wild type	41 (69) ^a	28 (48) ^a	69 (62)	
	Drug resistance (%)	59	58	117	

^aDrug resistance was more prevalent in X4 versus R5 viruses.

P = 0.024.