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Prevalence of Primary Resistance at Baseline in Acutely and Recently Infected Subjects Enrolled in AIDS Clinical Trials Group Protocol 371

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To the Editor

The presence of antiretroviral drug-resistant HIV in therapy-naïve subjects has been documented in multiple cohorts around the world (reviewed in 1). The prevalence ranges from 2–30% depending on the cohort and the definition of resistance. While several studies have shown that drug resistant mutants persist for long periods after infection 2–4, some antiretroviral drug resistance mutations, such as M184V, which have been associated with diminished virus fitness 5, revert in the absence of drug in chronically HIV-infected individuals 3. The fitness and persistence of resistant virus isolates in newly infected individuals have been less well-studied 2,3. To date, there have been no comparisons of the frequency of antiretroviral resistance in acutely and recently infected subjects within the same cohort.

AIDS Clinical Trials Group Protocol 371 (ACTG 371) was designed to evaluate whether structured treatment interruption after 1 year of antiretroviral therapy leads to controlled viremia off treatment, and whether the frequency of virologic control differs in those with acute infection versus recent seroconversion. A description of the cohort and findings from the primary study have been published elsewhere 6. Subjects were enrolled from July 1999 to September 2003 and initiated a standardized ritonavir-boosted protease-inhibitor regimen. Informed consent was obtained from patients or their parents or guardians and human experimentation guidelines of the US Department of Health and Human Services and the University of Rochester Institutional Review Board were followed. Acute infection was defined as either seroconversion ≤ 30 days before entry or a viral load $\geq 2,000$ copies/mL and a negative or indeterminate HIV serology ≤ 14 days before entry. Recent infection was defined as either documented seroconversion 31–90 days before entry or a positive ELISA or western blot and a non-reactive detuned ELISA ≤ 21 days before entry. A single laboratory using the Trugene genotyping kit V3.3 performed genotypes on plasma collected from subjects at study entry in real-time (Bayer Healthcare, Tarrytown, NY). Resistance mutations used in this analysis were defined according to the International AIDS Society (IAS) 7. All IAS-defined resistance mutations were included in the primary analysis except for L63P, which has been shown to be a common polymorphism in protease 8 and was found in 60% (68/114) of subjects in this study. Samples were classified as resistant only if they had ≥ 1 nucleoside reverse transcriptase inhibitor (NRTI), ≥ 1 non-nucleoside reverse transcriptase inhibitor (NNRTI), and/or ≥ 1 primary protease inhibitor (PI) resistance mutations. We separately assessed ≥ 1 secondary PI resistance mutations. Atypical mutations (all non-wild type mutations) were also

included in a subset of analyses. Comparisons were performed using Fisher's exact test and the Wilcoxon rank-sum test.

ACTG 371 enrolled 121 subjects of which 50 were acutely infected and 71 recently infected. One subject had a viral load too low to determine a genotype. Three pairs of genotypes showed clustering in phylogenetic analyses as determined using PHYLIP (V3.57c) and KIMURA 2-parameter model and neighbor-joining method. For one pair there was an epidemiological link, but since contamination could not be definitively ruled out as a possible explanation for the similarity, all 3 pairs were excluded from the analysis. As a result, 114 of 120 subjects who had baseline genotypes performed were included, all of which were subtype B. The median viral loads at baseline were 5.3 and 4.6 log₁₀ copies/mL in the acutely and recently infected groups, respectively (p=0.01, Table-1). Median CD4+ T cell counts did not differ significantly between the groups (541 and 507 cells/mm³, p=0.50).

Table-1 shows the frequency of resistance to different classes of antiretroviral drugs. Fifteen percent (17/114) of study subjects harbored HIV with drug resistance mutations (95% CI 9–23%). Resistance was most frequently observed to nRTIs (11%), and less frequently to NNRTIs (5%) or PIs (4%). Twelve subjects' viruses were resistant to 1 class of drug only, 4 were resistant to 2 classes, and 1 was resistant to 3 classes of drugs. Virus from 14% (7/49) of the acutely infected subjects and 15% (10/65) of the recently infected subjects contained resistance mutations (Fisher's exact test p=0.99). There were no significant differences between acutely and recently infected individuals in the frequency of antiretroviral drug resistant HIV within individual classes of antiretroviral drugs.

Several drug resistance codons had atypical mutations that were not known to be associated with phenotypic drug resistance. For example, 3 subjects had 215D instead of 215Y or F. Other atypical mutations in RT included 69S, 69N, 103R, 106I, 210S, 210F, and 236S. Atypical mutations in protease included 10H and 82I. Because viruses with atypical mutations occur in some treatment-naïve subjects and because these mutations may confer drug resistance⁹, we determined the proportion of patients who would be drug resistant if these mutations were categorized as drug resistance mutations. Overall, 19% (22/114) of subjects had resistance if atypical mutations were included. Five subjects had atypical mutations with no other major resistance mutations except for secondary protease mutations. There was no difference in the proportion of recent or acutely infected subjects with all mutations including atypical mutations (Table-1).

Acute subjects were more likely to have primary HIV infection symptoms within one month prior to enrollment (acute with infection symptoms 92% versus recent with infection symptoms 31%; p<0.0001). However, there was no difference in primary HIV infection symptoms among acute and recent subjects who harbored resistant virus versus those who did not (p=0.99). There was also no difference in the prevalence of mutations in subjects from different trial sites or regions (data not shown). There was also no difference in the percentage of subjects with resistant versus non-resistant virus who achieved a viral load <50 copies between weeks 8 and 48 after treatment initiation (94% versus 91%; p=0.99), who underwent treatment interruptions (41% versus 62%; p=0.12), and who were virologically successful defined as plasma HIV RNA concentration < 5000 copies/mL after 24 weeks of treatment interruption (24% versus 23%; p=0.99).

This is the first study to compare the frequency of antiretroviral drug resistance in patients who were acutely infected versus those who were recently infected with HIV. No significant differences in the frequency of drug resistance, the frequency of resistance for each class of drug, or the frequency of atypical mutations were found between the two groups. This observation is compatible with previous studies demonstrating slow reversion of transmitted

drug resistant variants 10¹ 11. This slow reversion could reflect the lack of viral diversity early in infection, or may reflect selection for relatively fit viral variants during the process of transmission. Consistent with other studies in recently infected 12¹ 13, secondary protease resistance mutations were seen in more than half of the cohort, with 64% (73/114) of subjects having one or more mutations. These data suggest that secondary mutations may be more readily transmitted than primary mutations. Furthermore, there was no significant difference in clinical outcomes between subjects with and without drug resistant virus including the ability to control viremia upon treatment interruption.

The prevalence of antiretroviral drug resistance in this cohort overall (15%) was comparable to previous studies in recently infected subjects with prevalences ranging from 1–19% ¹. The prevalence of nRTI, NNRTI and PI resistance was similar to a previous study of similar size 14. However, Little *et al.* ² identified NNRTI resistance in 12 (86%) of 14 acutely and recently infected subjects with any primary drug resistance mutations compared to 6 (35%) of 17 in this study. One potential explanation for this difference is geographic variation in the prevalence of transmitted NNRTI resistance. While we observed no difference in prevalence of resistance by geographical region in our study, the sample size and number of geographical regions likely limited the statistical power of this analysis.

There was no difference in the CD4+ T cell counts, viral load or infection symptoms between subjects with resistant and those with non-resistant virus. There was also no difference in virologic suppression in subjects who had drug resistant virus versus those who did not, indicating that resistance did not have an impact on response to therapy in this study. In conclusion, there was no detectable difference in the frequency of antiretroviral drug-resistant virus between acutely and recently HIV-infected subjects. We conclude that reversion of transmitted drug-resistant variants occurred infrequently during acute and recent infection, perhaps because of a lack of viral diversity early in HIV infection, or because transmitted viral variants are inherently more fit than those selected for during therapy of chronically infected patients.

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Table 1

Viral Load, CD4+ T Cell Counts and Resistance Prevalence Data

	All Subjects ^a N=114	Subjects with Acute Infection N=49	Subjects with Recent Infection N=65
Median Viral Load (log ₁₀ copies/mL)	4.8	5.3	4.6
Median CD4+ T Cell Count (cells/mm ³)	517	541	507
Mutation Category^b			
Any nRTI, NNRTI or Primary PI mutation	17 (15%)	7 (14%)	10 (15%)
One or more primary PI mutations	5 (4%)	2 (4%)	3 (5%)
≥ 1 secondary mutation	73 (64%)	33 (67%)	40 (62%)
≥ 2 secondary mutations	19 (17%)	12 (24%)	7 (11%)
≥ 3 secondary mutations	4 (4%)	2 (6%)	2 (3%)
One or more NNRTI mutations	6 (5%)	1 (2%)	5 (8%)
One or more nRTI mutations	12 (11%)	6 (12%)	6 (9%)
Multi-drug class resistance			
Combination of primary PI and nRTI mutations	3 (3%)	2 (4%)	1 (2%)
Combination of primary PI and NNRTI mutations	1 (1%)	0 (0%)	1 (2%)
Combination of nRTI and NNRTI mutations	3 (3%)	0 (0%)	3 (5%)
Combination of primary PI, nRTI and NNRTI mutations	1 (1%)	0 (0%)	1 (2%)
All Mutations^c			
Any nRTI, NNRTI or Primary PI and ALL atypical mutations	22 (19%)	9 (18%)	13 (20%)

^aThe 3 pairs of subjects, whose genotypes clustered on phylogenetic analysis, and the 1 subject whose viral load was too low to sequence, were excluded.

^bOnly IAS-defined resistance mutations were included, with the exception of L63P in protease. L33I and V were classified as secondary mutations only.

^cAny coding change at any IAS resistance site that is not listed as a resistance mutation was included as an atypical mutation. Subjects who had atypical mutations at primary PI, nRTI, and NNRTI resistance sites were included.