Differences in the Frequency of Resistance to Antiretroviral Drug Classes among Human Immunodeficiency Virus Type 1 Clinical Isolates

Rafael E. Campo,1* Paola N. Lichtenberger,1 Isabella Rosa,2 German Suarez,2 Fernando A. Rivera,2 Allan E. Rodriguez,1 Dushyantha T. Jayaweera,1 Natalie A. Wahlay,3 and Michael A. Kolber1

Division of Infectious Diseases,1 Department of Medicine,2 and Department of Epidemiology and Public Health,3 University of Miami School of Medicine, Miami, Florida

Received 24 October 2002/Returned for modification 26 February 2003/Accepted 7 April 2003

Genotypic resistance to all antiretroviral classes was widespread among human immunodeficiency virus type 1 isolates failing therapy. Resistance to nonnucleoside reverse transcriptase inhibitors was found most frequently and resistance to protease inhibitors was found least frequently, most likely due to differences in the number of enzymatic amino acid substitutions leading to resistance to each particular drug class.

Failure of highly active antiretroviral therapy (HAART) is associated with selection of human immunodeficiency virus type 1 (HIV-1) strains with decreased susceptibility to the failing agents and with cross-resistance to other agents in the same drug class (10). Genotypic antiretroviral resistance testing (GT) provides indirect, potentially difficult-to-interpret evidence of resistance by detecting resistance-associated mutations in the reverse transcriptase (RT) and protease (PR) genes; phenotypic testing (PT), on the other hand, directly measures susceptibility to antiretroviral drugs (9). Nonetheless, GT is technically less demanding, faster to perform, and less expensive than PT (9) and thus is more likely to be widely used. The utility of GT has been recently demonstrated in several prospective studies (2, 5, 10, 16).

The frequencies of genotypic resistance to specific antiretroviral classes among patients failing HAART and how they compare to one another continue to be defined in clinical practice. A better understanding of this phenomenon might provide valuable insights into the resistance patterns associated with treatment failure.

We conducted a cross-sectional analysis to compare the frequency of resistance by antiretroviral drug class among 200 randomly chosen HIV-1 strains that underwent GT from patients failing HAART at Jackson Memorial Hospital in Miami, Florida, between September 1999 and August 2000. This medical center mostly treats an inner-city, minority, indigent population. To be included in this study, patients needed to have been at 1,000 copies/ml after 6 months of HAART or vRNA that increased to \( \geq 1,000 \) copies/ml after being at \(<400 \) copies/ml at least once). This study was approved by the institution’s Office for the Protection of Human Subjects.

Patients meeting enrollment criteria were divided into groups of those failing a particular antiretroviral drug class at the time of GT and those who had previously failed a particular antiretroviral drug class but were no longer on therapy with it at the moment of GT, since it is known that antiretroviral drug resistance may cease being detectable when antiretroviral selective pressure is discontinued (3, 8, 17). Thus, it is possible that detectable resistance to an antiretroviral drug class could be less frequent among isolates with prior but not present treatment with that class than among isolates presently failing and under the selective pressure of that class. Consequently, only patients failing a specific drug class as part of their HAART at the moment of GT were included in this analysis.

Viral isolates underwent GT by the TRUGENE method (Bayer Diagnostics, Tarrytown, N.Y.); interpretation of drug resistance took into account expert opinion on interpretation of resistance-associated primary and secondary mutations (9, 10, 15). The proportions of isolates with resistance to a particular antiretroviral class were compared with the chi-squared test.

Among 146 patients who met inclusion criteria, most had extensive antiretroviral experience (Table 1). Most (62%) patients were male; almost all were members of an ethnic minority (40% Hispanic, 38% African-American, 20% Haitian, and 2% non-Hispanic white). No resistance to any antiretroviral drug class was found among 11% \((n = 16)\) of all isolates, resistance to one class was found among 27% \((\text{NRTI}, n = 19; \text{NNRTI}, n = 19; \text{PR}, n = 2)\) of all isolates, resistance to two classes was found among 36% \((\text{NRTI and NNRTI}, n = 23; \text{NRTI and PR}, n = 27; \text{NNRTI and PR}, n = 3)\) of all isolates, and resistance to three classes was found among 26% \((n = 37)\) of all isolates. After correcting for class exposure (number of isolates with resistance to a drug class/number of strains treated with that drug class), we found significant differences in the prevalence of resistance to the three antiretroviral drug
Resistance to PI was found least frequently (54%; 55 out of 101 isolates), resistance to NRTI was found with intermediate frequency (74%; 100 out of 135 isolates), and resistance to NNRTI was found most frequently (85%; 52 out of 61 isolates) ($P = 0.00006$).

The prevalence of antiretroviral drug resistance described here is sobering. Close to 90% of all individuals undergoing GT in a major metropolitan area in the United States had HIV-1 with resistance to at least one antiretroviral class. Furthermore, over a quarter of these patients had resistance to all three antiretroviral classes in use when this study was performed, even though less than half of the entire population had been exposed to those three classes. This finding of widespread antiretroviral resistance has important implications for the selection of subsequent therapies for these patients.

The differences in prevalence of resistance to antiretroviral drug classes are intriguing, although they are not entirely novel on the basis of previously reported studies. Among newly infected individuals without prior HAART but presumably infected with isolates from individuals failing therapy, resistance to PIs was found less frequently than resistance to NNRTI or NRTI (4, 18). Among 60 treatment-experienced patients undergoing resistance testing within 36 weeks of failing PI-NRTI or NNRTI-NRTI regimens, resistance to PIs was found least frequently and resistance to nevirapine was found most frequently, with resistance to zidovudine and lamivudine being found with intermediate frequency and at rates similar to those reported here (7).

Differences in the number of RT and PR enzymatic amino acid substitutions leading to loss of antiretroviral activity (the so-called genetic barrier of antiretroviral classes) may explain our findings. It is well known that multiple PR substitutions are needed before resistance to some PI emerges (6, 13). On the other hand, single RT substitutions will lead to marked loss of NNRTI activity (1). This is also the case for some NRTI, such as lamivudine (12), although for others, such as zidovudine, multiple RT substitutions are required for incremental and substantial loss of antiretroviral activity (11). Thus, it is not surprising that PI resistance was found less frequently than resistance to RT inhibitors. The extremely rapid fashion in which resistance is known to emerge to failing NNRTI therapy (14), perhaps in combination with differences in NRTI and NNRTI genetic barriers, may also explain the difference in rates of NRTI and NNRTI resistance.

Although these findings and the possible explanations for them require further study, we suggest that they should be taken into consideration when choosing initial HAART and thinking strategically with regards to subsequent therapies should initial HAART fail.

This study was supported by a Medical School Grant from Merck & Co., Inc., to R.E.C.

We gratefully acknowledge invaluable discussions and advice from John Szwumiloski and Jon Condra and the performance of the genotypic assays by Roberto Patarca.

### REFERENCES

1. Bacheler, L., S. Jeffrey, G. Hanna, R. D’Aquila, L. Wallace, K. Logue, B. Cordova, K. Hertogs, B. Larder, R. Buckery, D. Baker, K. Gallagher, H. Scarnati, R. Trich, and C. Rizzo. 2001. Genotypic correlates of phenotypic resistance to elavirenz in virus isolates from patients failing nonnucleoside reverse transcriptase inhibitor therapy. J. Virol. 75:4999–5008.
2. Baxter, J. D., T. C. Merigan, D. N. Wentworth, J. D. Neaton, M. L. Hoover, R. M. W. Hoetelmans, S. Piscitelli, W. H. A. Verbiest, and D. L. Mayers. 2002. Both baseline HIV-1 drug resistance and antiretroviral drug levels are associated with short-term virologic responses to salvage therapy. AIDS 16:1131–1138.
3. Biek, M., V. Svedhem, and A. Sonnerborg. 2001. Kinetics of HIV-1 RNA and resistance-associated mutations after cessation of antiretroviral combination therapy. AIDS 15:1359–1368.
4. Boden, D., A. Hurley, L. Zhang, Y. Cao, Y. Guo, E. Jones, J. Tsay, J. Ip, C. Feirthing, K. Limoli, N. Parkin, and M. Markowitz. 1999. HIV-1 drug resistance in newly infected individuals. JAMA 282:1135–1141.
5. Clevenbergh, P., J. Durant, P. Halfon, P. Delgiudice, V. Mondain, N. Montagne, J. M. Schapiro, C. A. Boucher, and P. Dellamonica. 2000. Persisting long-term benefit of genotypy-guided treatment for HIV-infected patients failing HAART. The Viradapat study: week 48 follow-up. Antivir. Ther. 5:65–70.
6. Condra, J. H., D. J. Holder, W. A. Schleif, O. M. Blahy, R. M. Danovich, L. J. Gabryelski, et al. 1996. Genetic correlates of in vivo viral resistance to indinovir, a human immunodeficiency virus type 1 protease inhibitor. J. Virol. 70:8270–8278.
7. Cozzi Lepri, A., C. A. Sabin, S. Staszewski, K. Hertogs, A. Muller, H. Rabena, A. N. Phillips, and V. Miller. 2000. Resistance profiles in patients with viral rebound on potent antiretroviral therapy. J. Infect. Dis. 181:1143–1147.
8. Deeks, S. G., T. Wrin, T. Liegler, R. H. Mohy, M. Hayden, J. D. Barbour, N. S. Hellmann, C. J. Petropoulos, J. M. McCune, M. K. Hellerstein, and R. M. Grant. 2001. Virologic and immunologic consequences of discontinuing combination antiretroviral-drug therapy in HIV-infected patients with detectable viremia. N. Engl. J. Med. 344:472–480.
9. Garcia-Lerma, J. G., and W. Heneine. 2001. Resistance of human immunodeficiency virus type 1 to reverse transcriptase and protease inhibitors: genotypic and phenotypic testing. J. Clin. Virol. 21:197–212.
10. Hirsch, M. S., F. Brun-Vezinet, R. T. D’Aquila, et al. 2000. Antiretroviral drug resistance testing in adult HIV-1 infection: recommendations of an International AIDS Society-USA panel. JAMA 282:2417–2426.
11. Hooker, J. D., J. C. Donchelian, A. E. Solomon, A. D. Gurusinghe, et al. 1996. An in vivo mutation from leucine to tryptophan at position 210 in human immunodeficiency virus type 1 reverse transcriptase contributes to high-level resistance to 3’-azido-3’-deoxy-2’,3’-dideoxythymidine. J. Virol. 70:8010–8018.
12. Kavlick, M. F., T. Shirasaka, E. Kojima, J. M. Pluda, F. J. Hui, R. Yarchoan, and H. Mitsuya. 1995. Genotypic and phenotypic characterization of HIV-1 isolated from patients receiving (+)-2’,3’-dideoxy-3’-thiacytidine. Antivir. Res. 28:133–146.
13. Molla, A., M. Korneyeva, Q. Gao, et al. 1996. Ordered accumulation of mutations in HIV protease confers resistance to ritonavir. Nat. Med. 2:760–763.
14. Richman, D. D., D. Havlir, J. Corbeil, D. Looney, C. Ignacio, S. A. Spector, J. Sullivan, S. Cheseman, K. Barringer, D. Pauletti, et al. 1994. Nevirapine resistance mutations of human immunodeficiency virus type 1 selected during therapy. J. Virol. 68:1660–1666.
15. Shaffer, R. W. 2002. Genotypic testing for human immunodeficiency virus type 1 drug resistance. Clin. Microbiol. Rev. 15:247–277.

### TABLE 1. Prior antiretroviral therapy, duration, and extent of treatment experience among 146 patients failing HAART and undergoing genotyping testing

| Antiretroviral drug classes received as components of prior ART or treatment experience | No. of patients (% of total) |
|---|---|
| PI and NRTI | 62 (42) |
| NNRTI and NRTI | 16 (11) |
| PI and NNRTI and NRTI | 68 (47) |
| Treatment experience | |
| Failing first HAART regimen prior to which no ART had been received | 38 (26) |
| Failing first HAART regimen prior to which non-HAART ART had been received | 16 (11) |
| Failing second HAART regimen | 34 (23) |
| Failing third HAART regimen | 17 (12) |
| Failing fourth HAART regimen | 17 (12) |
| Failing fifth to eighth HAART regimen | 24 (16) |

The mean (range) durations of prior antiretroviral therapy (ART) with the respective drug classes are the following: for PI, 97 (6 to 221) weeks; for NNRTI, 12 (23) weeks; for NRTI, 38 (24 to 124) weeks; and for NRTI, 143 (13 to 499) weeks.

1. For example, zidovudine and lamivudine.

15. Shafer, R. W. 1995. Genotypic and phenotypic characterization of HIV-1 isolated from patients receiving (+)-2’,3’-dideoxy-3’-thiacytidine. Antivir. Res. 28:133–146.

16. Molla, A., M. Korneyeva, Q. Gao, et al. 1996. Ordered accumulation of mutations in HIV protease confers resistance to ritonavir. Nat. Med. 2:760–763.

17. Richman, D. D., D. Havlir, J. Corbeil, D. Looney, C. Ignacio, S. A. Spector, J. Sullivan, S. Cheseman, K. Barringer, D. Pauletti, et al. 1994. Nevirapine resistance mutations of human immunodeficiency virus type 1 selected during therapy. J. Virol. 68:1660–1666.
16. Tural, C., L. Ruiz, C. Holtzer, J. Schapiro, P. Viciana, J. Gonzalez, P. Domingo, C. Boucher, C. Rey-Joly, and B. Clotet. 2002. Clinical utility of HIV-1 genotyping and expert advice: the Havana trial. AIDS 16:209–218.

17. Verhofstede, C., F. Van Wanzeele, B. Van der Gucht, N. De Cabooter, and J. Plum. 1999. Interruption of reverse transcriptase inhibitors or a switch from reverse transcriptase to protease inhibitors resulted in a fast reappearance of virus strains with a reverse transcriptase inhibitor-sensitive genotype. AIDS 13:2541–2546.

18. Weinstock, H., R. Respess, W. Heneine, C. J. Petropoulos, N. S. Hellmann, C.-C. Luo, C.-P. Pau, T. Woods, M. Gwinn, and J. Kaplan. 2000. Prevalence of mutations associated with reduced antiretroviral drug susceptibility among human immunodeficiency virus type 1 seroconverters in the United States, 1993–1998. J. Infect. Dis. 182:330–333.