

Transmission of Drug-Resistant HIV

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THE HIV MEDICAL COMMUNITY RECEIVED AN OFFICIAL WAKEUP CALL ON July 30, 1998, when Dr. Frederick M. Hecht—a frequent PRN lecturer—and his colleagues published the first documented case of high-level protease inhibitor resistance in a recently infected, treatment-naive, HIV-positive individual. Since this initial report, a number of researchers with close ties to primary HIV infection cohorts have not only confirmed that transmission of drug-resistant HIV is possible, but that it is occurring in up to 20% of all new HIV infections identified in North America in recent years (Little, 2002).

Monitoring the frequency and prevalence of transmitted drug-resistant virus in individuals with primary HIV infection (PHI) is only one component of this important field of study. There is still a need to better understand the mechanisms, pathogenesis, and outcomes of drug-resistant HIV transmission. These important questions have been a central concern to Dr. Viviana Simon, who has been working closely with other investigators at the Aaron Diamond AIDS Research Center to shed some light on this disconcerting issue.

Prevalence of Drug Resistance in Chronic and Acute HIV Infection

A CRITICAL ISSUE RAISED BY DR. SIMON IS THE DISPARITY IN PREVALENCE rates of drug resistance among chronically infected and acutely infected HIV-positive individuals. One of the most extensive evaluations of drug resistance among chronically infected HIV-positive individuals was reported by Dr. Douglas Richman at the 41st Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), held in Chicago in 2001 (Richman, 2001). The study reported by Dr. Richman involved plasma samples taken from 1906 men and women who were receiving antiretroviral therapy while participating in the HIV Cost and Service Utilization Study (HCSUS), a longitudinal study representative of all HIV-infected adults throughout the United States who received medical care in early 1996. Resistance assays were performed on banked frozen specimens of plasma collected from each subject between 1998 and 1999. Approximately 1/3 (36.5%) of the samples had HIV-RNA levels below 500 copies/mL and were therefore excluded from the resistance analysis employing ViroLogic's PhenoSense phenotypic assay. All of the remaining samples (63.5%) yielded viral loads above 500 copies/mL. Among these, 78% had evidence of decreased susceptibility to at least one antiretroviral drug.

The prevalence of HIV drug resistance in acutely infected individuals, while significant, is much lower. In one study published in the *New England Journal of Medicine* by Dr. Susan Little and her colleagues at the University of California, San Diego, plasma samples from 377 treatment-naive patients with primary HIV infection, identified between May 1995 and June 2000 in 10 North American cities, were analyzed (Little, 2002). High-level resistance to one or more drugs was documented in 12.4% of samples collected in 1999 and 2000. In an earlier study conducted by Dr. Sabine Yerly and her colleagues at Geneva University Hospital, plasma samples collected from 82 patients diagnosed with primary

HIV infection between January 1996 and July 1998 were analyzed using a genotypic resistance assay (Yerly, 1999). Zidovudine (Retrovir)-associated resistance mutations were detected in 7/82 (9%) patients. Mutations associated with resistance to other reverse transcriptase inhibitors were detected in two individuals. Primary resistance mutations associated with protease inhibitors were detected in 3/70 (4%) samples, with two of these samples also containing virus that harbored mutations to reverse transcriptase inhibitors. There are also data from McGill University AIDS Center, published in 2000 by Dr. Horacio Salomon and his colleagues, indicating that approximately 24% of newly infected intravenous drug users and 12% of acutely infected individuals exposed via sexual activity harbored strains of HIV resistant to at least one antiretroviral drug (Salomon, 2000).

"If we look at the cohorts available that summarize resistance data," Dr. Simon said, "we find a fivefold to tenfold lower prevalence of drug resistance in newly infected individuals compared to chronically infected individuals." With such a large number of chronically infected HIV-positive individuals currently receiving HAART—a sizeable percentage of them with detectable viral loads and drug-resistant mutants—one would expect to see a larger percentage of transmissions involving drug-resistant virus. "This leads us to wonder if resistance is a factor that selects against transmission."

To determine if transmission efficiency is influenced by the presence of certain drug resistance-associated mutations, Dr. Yerly and various colleagues throughout Switzerland and France performed genotypic analyses on samples collected from 225 recently infected Swiss patients and 373 chronically infected patients enrolled in the Geneva Swiss HIV Cohort Study with HIV-RNA levels above 1,000 copies/mL (Yerly, 2003). From there, the drug-resistance profiles of potential transmitters were estimated by weighting the resistance profiles of the chronically infected individuals according to the minimal and maximal estimates of HIV-infected individuals living in Switzerland (14,000 to 15,000 patients), to the proportion of the Swiss drug-exposed, chronically infected population (51% to 57%), and to the impact of recently infected individuals on new infections.

The prevalence of drug-resistance, defined as evidence of genotypic resistance to at least one drug, in recently infected patients was 10.5%. Among the chronically infected patients receiving treatment, the prevalence of drug-resistant virus was in the ballpark of 70%. "Dr. Yerly's group went ahead and calculated relative risks of transmission, depending on detected drug resistance to one, two or three drug classes and demonstrated that there is a tremendous selection against transmission of virus that exhibit resistance to two or more classes," explained Dr. Simon. "There was a 14-fold to 20-fold reduced probability of transmission of variants with reduced susceptibility to two or three drug classes, variants that we would say are multi-drug resistant. These are variants that we know are less replication competent, or less replication efficient, and therefore less likely to be transmitted."

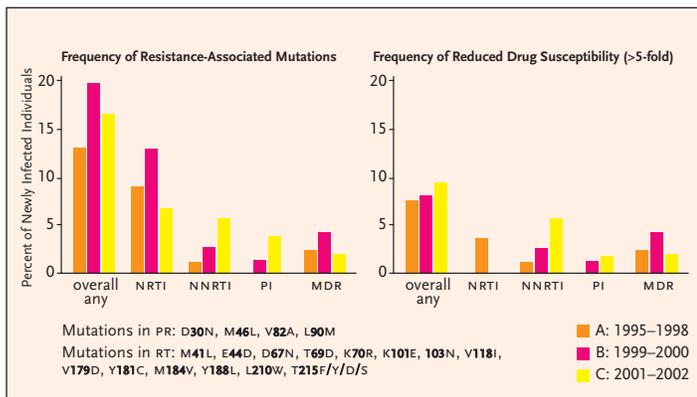


Figure 1. Changing Pattern of Transmitted Drug-Resistant HIV Strains

Evidence of genotypic and phenotypic drug resistance among 249 individuals joining a primary HIV infection cohort between 1995 and 2002. Patients were divided into three groups, according to the year of entry into the cohort. Group A consisted of 76 patients, enrolled between 1995 and 1998; group B consisted of 71 patients, enrolled between 1999 and 2000; and group C consisted of 102 patients, enrolled between 2001 and 2002. As illustrated in the bar graph on the left, the overall prevalence of any resistance-associated mutations in newly infected patients increased from 13% in group A to 20% in group B. Patients in group C actually reflected a downward trend in the prevalence, with approximately 17% presenting with at least one resistance-associated mutation. Phenotypic resistance profiles are illustrated in the bar graph on the right. Reduced susceptibility to nucleoside reverse transcriptase inhibitors was only seen in group A. The prevalence of resistance to non-nucleoside reverse transcriptase inhibitors increased steadily over the three observation periods and evidence of reduced susceptibility to protease inhibitors, which was not documented in group A, remained below 5% in groups B and C. Multiple-drug resistance, documented by phenotypic testing, also remained below 5%, which is consistent with data from other cohort studies.

Source: Viviana Simon, MD, PhD

Changing Pattern of Transmitted Drug-Resistant HIV Strains

WHILE THERE HAVE BEEN A NUMBER OF STUDIES DESCRIBING THE PREVALENCE of HIV-drug resistance in newly infected individuals, data have been limited with respect to temporal changes in transmitted drug resistance. To look a bit more closely at the epidemiology of transmitted drug resistance over time, Dr. Simon summarized her recent analysis of 249 individuals who joined a primary HIV infection cohort at ADARC between 1995 and 2002. The patients were divided into three groups, according to the year of entry into the cohort. Group A consisted of 76 patients, enrolled between 1995 and 1998; group B consisted of 71 patients, enrolled between 1999 and 2000; and group C consisted of 102 patients, enrolled between 2001 and 2002. All patients were naive to antiretroviral therapy at study entry, and genotypic and phenotypic resistance testing was performed on samples obtained before initiation of treatment.

As explained by Dr. Simon, the overall prevalence of any resistance-associated mutations in newly infected patients increased from 13% in group A to 20% in group B. Patients in group C actually reflected a downward trend in the prevalence, with approximately 17% presenting with at least one resistance-associated mutation (see Figure 1). “This was mainly because of the decrease of transmitted NRTI resistance,” Dr. Simon said. “However, if you look at NNRTI or PI resistance, we observed an increase in the last observation period.”

In terms of phenotypic resistance testing—resistance was defined as a greater than fivefold reduction in susceptibility to any antiretroviral drug—NRTI resistance was only seen in group A. “Over the last four years,” Dr. Simon commented, “no virus with more than fivefold reduced susceptibility to any NRTI was seen, with the exception of multi-drug resistant variants.” In other words, Dr. Simon and her colleagues observed the transmission of viruses with altered genotypes—compatible with previous thymidine analogue exposure (e.g., mutations conferring resistance to zidovudine)—but with drug-sensitive phenotypes. More specifically, 50% of the patients identified in 1999 and 2000 who had evidence of genotypic resistance to NRTIs, but no evidence of phenotypic resistance to this class of drugs, had virus harboring the T215D/S mutation in the reverse transcriptase gene. These changes, Dr. Simon pointed out, do not mediate phenotypic resistance to zidovudine, but reflect the viral evolution away from the primary mutation T215Y in the absence of treatment (discussed in greater detail in “Understanding Treatment-Resistant HIV,” published in the June 2003 issue of *The PRN Notebook*).

As for reduced phenotypic susceptibility to NNRTIs, the prevalence increased steadily over the three observation periods. Phenotypic resistance to protease inhibitors, which was not reported in group A, remained below 5% in groups B and C. Multiple-drug resistance, documented by phenotypic testing, also remained below 5%, which is consistent with data from other cohort studies.

“Something else we wanted to look at was the relationship between the viruses taken from the patients participating in our cohort,” Dr. Simon said. “Since all of these patients were identified in New York, and most lived in New York, we imagined that there could be chains or clusters of transmission events.” Phylogenetic analyses of 241 protease and reverse transcriptase sequences revealed 34 clusters containing 51 sequences. Nine transmission events were established by patient interview and confirmed by the relatedness of the viruses. “Interestingly,” Dr. Simon commented, “we also discovered 15 previously unknown linkages. These clusters consisted of twelve pairs, which were not identified in the same years. The biggest gap was up to four years, where virus taken from one patient in 1997 was matched with virus four years later.”

One interesting finding of this work was a cluster of eight sequences, “a mini epidemic,” explained Dr. Simon. “The parental virus was seen the first time in 1997. Three years later, we saw a related virus. Next there was a chain of transmission that took place in 2001 and 2003. In 2001, we noticed that the transmission events took place very close together. So, we’re currently trying to reinterview those patients in order to find out more about their possible linkages.”

Replication Characteristics of Viral Isolates Derived During Primary HIV Infection

ANOTHER AIM OF DR. SIMON’S WORK AT ADARC HAS BEEN TO DETERMINE if transmitted drug-resistant virus behaves similarly to drug-resistant variants typically seen in chronic HIV infection. “The paradigm of drug resistance says that the more drug resistant the virus is, the less replication competent it should be. However, we were speculating that, since some drug-resistant mutants are successfully transmitted, they might have different characteristics and are possibly more efficient in establishing infection.”

To test this hypothesis, Dr. Simon’s group cultured nine drug-resistant and nine drug-sensitive isolates from PBMCs, collected from 18 patients who presented within 10 to 79 days after onset of acute retroviral symptoms. Three *in vitro* assays were used: one single-cycle assay using reporter cell lines to measure infectivity of the isolates, a second in which par-

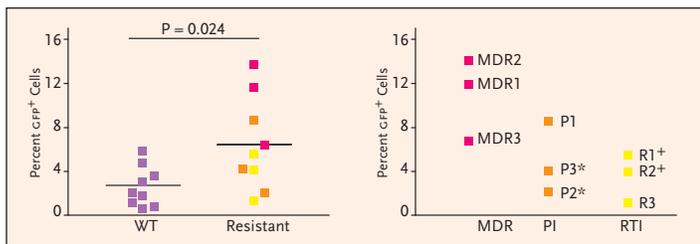


Figure 2. Infectivity of Drug-Resistant Isolates

Results from a study employing a single-cycle assay, using reporter cell lines to measure infectivity of wild-type and drug-resistant isolates, collected from 18 treatment-naïve patients in the primary stages of HIV infection. As illustrated here, drug-resistant variants were significantly more infectious than wild-type strains.

Source: Viviana Simon, MD, PhD

lel infection of CD4+ cells was performed to measure the growth kinetics of the isolates, and a third single-cycle assay, developed by ViroLogic, to measure the replication capacity of recombinant viruses encoding only the protease and reverse transcriptase regions from the patients' isolates.

Using the first assay, Dr. Simon's group found that the drug-resistant variants were significantly more infectious than wild-type strains (see Figure 2). "If we break these data up by drug classes," Dr. Simon explained, "it's easily seen that this difference is mainly driven by a subset of three viruses: two multiple-drug-resistant viruses and one protease inhibitor-resistant virus."

Using the growth kinetics assay, Dr. Simon's group found that drug-resistant isolates replicated as efficiently over multiple rounds of replication in CD4+ cells as wild-type virus. Again, the multiple-drug resistant variants and one of the protease inhibitor-resistant variants had better growth kinetics, while growth kinetics of the other drug-resistant isolates were similar to wild-type virus."

Finally, Dr. Simon's group looked at the replication capacity of recombinant viruses in which the only patient-derived sequences were the drug-resistance associated regions of protease and reverse transcriptase. "In this assay," Dr. Simon illustrated, "drug-resistant virus had a significantly lower assay value compared to wild-type virus." Dr. Simon speculated that functional regions outside of the mutated protease and reverse transcriptase genes essentially help the virus to overcome any limitations in infectivity and growth kinetics. "The conclusion here is that drug-resistant mutants derived shortly after transmission do not differ from drug-susceptible virus in terms of their ability to establish infection and proliferate." However, it is important to note that the assessment of viral fitness is complex and that *in vitro* observations may misrepresent the *in vivo* reality.

Impact of Transmitted Drug Resistance On Viral Set Point

A FINAL PIECE OF SCIENTIFIC DISCOURSE RAISED BY DR. SIMON WAS THE impact of transmitted drug resistance on viral set point. For this, Dr. Simon turned to a study conducted by Dr. Susan Little and her colleagues, the results of which were reported at the 10th Conference on Retroviruses and Opportunistic Infections earlier this year (Little, 2003). In this study, HIV isolates were collected from 2002 patients identified, on average, approximately 120 days after the estimated date of HIV infection and who remained naïve to antiretroviral treatment for at least a year. Analyses included plasma HIV-RNA levels, genotypic resistance, phenotypic resistance, and replicative capacity, at baseline and upon follow-up.

The median viral load at baseline was not significantly different among subjects infected with drug-resistant virus, defined as a tenfold reduction in phenotypic susceptibility to at least one drug. Among patients with drug-sensitive virus, the median baseline HIV-RNA level was 4.48 log copies/mL, compared to 4.58 log copies/mL among patients with drug-resistant virus. A noted difference in median baseline CD4 percentages (CD4%)—34% in the drug-resistant group and 28% in the drug-sensitive group—also failed to achieve statistical significance.

Across the board, the replication capacity of the drug-resistant isolates was only slightly lower (32%) than that of drug-sensitive virus (42%), but this difference was not statistically significant. Furthermore, higher replication capacity was not associated with higher baseline viremia.

At the later time points where HIV infection had reached its chronic phase, preliminary results from Dr. Little's group suggest that individuals infected with drug-resistant variants may be more likely to have a higher viral set point than those infected with drug-sensitive HIV. In particular, despite comparable baseline viremia, patients infected with variants resistant to NNRTIs were found to have a significantly higher viral set point than patients infected with drug-susceptible viruses. Protease inhibitor- and nucleoside analogue-resistant mutants tended to result in viral set points that did not differ significantly from the set points in individuals infected with drug-sensitive strains. Further studies are needed to confirm these important observations.

Conclusion

IN SUMMARIZING HER TALK, DR. SIMON OUTLINED THE PARADOXES associated with drug resistance in primary HIV infection. "We've learned that some viral variants, such as those associated with multiple-drug resistance, are less likely to be transmitted," she said. "However, we've also learned over the past year that if these MDR viruses are successfully transmitted, their ability to replicate in the absence of drugs might be different from MDR viruses derived from chronically infected patients. Indeed, the replication characteristics of drug-resistant viruses isolated during primary infection are comparable — in some cases even more efficient — than drug-susceptible viruses. And there is also the concern that drug-resistant variants, particularly those harboring mutations associated with NNRTI resistance, can result in a higher viral set point as primary HIV infection evolves into chronic HIV infection." Dr. Simon concedes that there's still much more to be learned about the mechanisms behind increased replication capacity and higher viral set points in primary HIV infection. "At the same time, I believe that newly infected patients who are found to have drug-resistant virus need to be treated appropriately," she said. "We need to determine the best and most appropriate treatment regimens for these patients." 

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