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HIV Treatment Failure: Testing for HIV Resistance in Clinical Practice

Luc Perrin and Amalio Telenti

In a recent commentary on AIDS therapy, the phrase "Failure isn't what it used to be . . . but neither is success" was coined (*1*). By now we should be used to issues concerning the human immunodeficiency virus (HIV) being always more complex than expected. Understanding of HIV pathogenesis indicated that early pharmacological intervention would give the best chance at preserving the integrity of the immune system and possibly eradicating the virus. These concepts provided the impetus to treat a large proportion of HIV-infected individuals with a combination of antiretroviral drugs [highly active antiretroviral therapy (HAART)], resulting in a dramatic reduction in AIDS-related morbidity and mortality (*2*). However, viral eradication is not achievable with current strategies (*3*), and the shift in treatment paradigm to one of long-term viral suppression has led to the challenge of ensuring continuous treatment benefit and avoiding failure (*4*).

Failure has generally been defined in virological terms—the inability to achieve complete suppression of viral replication. The factors leading to this type of failure are straightforward: poor adherence to HAART, prior exposure to antiretroviral drugs in mono- or bi-therapy, the sequential addition of drugs to a failing regimen, and counteractive interactions among the drugs used (*5*)—nothing new for those who witnessed the early days of antituberculosis chemotherapy in the 1950s and 1960s. However, treatment failure is not only viral resistance.

In fact, definition of failure or success of treatment is a far more complex phenomenon (Fig. 1). In real life, there are individuals who experience an optimal response to treatment, as shown by effective viral suppression and ensuing immune recovery (6) (Fig. 1A), but there are others with increasing CD4 cell counts in the presence of ongoing viral replication (*7*) (Fig. 1B), or blunted immune recovery despite viral control (Fig. 1C), and finally complete treatment failure (Fig. 1D). Analysis of the Swiss HIV cohort study database of HIV-1–infected individuals on HAART indicates that an estimated 40% of the participants present the constellation described in Fig. 1A, 40% in Fig. 1B, 5% in Fig. 1C, and 15% in Fig. 1D. We need to understand better what each situation represents clinically and what each implies for the current models of HIV immunopathogenesis (*8*). Finally, we have to learn more about the mechanisms by which current antiretroviral drugs exert their remarkable effect on HIV disease despite widespread drug resistance (*7, 9*). In particular, the frequent observation of increasing CD4 cell counts in individuals maintaining high viremia levels needs to be explained, because it may yield clues regarding issues such as viral fitness, resetting in the steady state of CD4 cell turnover, and the possible action of protease inhibitors on nonviral targets participating in the mechanisms of CD4 T cell depletion.

Resistance is a widespread problem. Although treatment failure is a complex phenomenon, viral resistance indeed remains a major issue. It affects up to 30 to 50% of all individuals under HAART (*7, 9*) and also might be transmitted (*10*). Once multidrug resistance is present, regaining control of viremia becomes difficult because no effective "salvage" strategy has been devised.

However, testing of HIV resistance is not straightforward because the best analysis strategies have not been defined and remain the topic of intense clinical investigation. Central to its complexity are the phenomenon of HIV quasi-species (the simultaneous presence in a patient of a swarm of viral variants), the extent of cross-resistance among antiviral drugs, the existence in each individual of archival HIV DNA

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copies representing all viruses that emerged under previous treatment, and the preexistence of resistant variants even without prior exposure to the drug (*11*). This implies that resistant clones may be present well below detection levels and emerge only when the specific antiretroviral drug is introduced. All antiviral drugs are not equal. Although a single mutation of the reverse transcriptase gene might confer a high degree of resistance to some drugs (for example, 3TC and nevirapine), multiple mutations of the protease gene are required for development of significant resistance to the antiproteases. Combining several drugs, as in HAART, remains the best option for limiting the emergence of preexisting or new resistant variants.

How can resistance be assessed? Two general approaches are being used: genotypic and phenotypic resistance testing. For genotypic analysis, sequences of viral reverse transcriptase, protease, and Gag-Pol cleavage sites are amplified by polymerase chain reaction and analyzed by sequencing or by other genetic approaches (*12*). Data will reflect the predominant virus species in the sample. Only by time-consuming subcloning experiments can a quantitative analysis of minority sequences be generated. Results can be obtained within 1 week at a minimum cost of U.S. $100 per sample. Genotypic testing may be extended to the analysis of proviral DNA in individuals with undetectable viremia, because it might provide some clues on archival (pro)viruses. In most instances, analysis of mutation patterns well exceeds our current ability to interpret them (*13*): mutation to one antiretroviral may restore susceptibility to a second drug; mutations selected in vitro to a given agent may never be identified in clinical isolates from patients failing treatment with that same antiretroviral drug; and prior exposure to antiretroviral drugs may result in uncharacteristic mutational pathways upon introduction of a new treatment (*14*).

Analysis of phenotypic resistance is now frequently performed by cloning the relevant genes into viral constructs to be analyzed via reporter cell lines (*15*). By doing this, issues such as cell tropism, growth variation, and quantification of virus can be standardized. Still, testing takes days to weeks at a cost of several hundred dollars, introduces an undefined modification of the quasi-species distribution, and does not necessarily identify minority resistant variants or detect a relevant decrease of susceptibility. Direct viral isolation and testing from plasma of infected individuals may soon be facilitated by the development of modified cell lines expressing CD4 and chemokine receptors (*16*).

Is there a role for resistance testing in clinical practice? The necessary information on how best to use these technologies and whether resistance testing will provide timely results leading to clinical benefit is missing. Intuitively, one may rank clinical situations according to the usefulness of resistance testing as follows: (i) during primary infection, when there is a limited viral heterogeneity and a definite possibility for transmission of resistant variants (*10*); (ii) in drug-naïve infected persons before treatment initiation; (iii) in antiviral experienced persons before modification or simplification of a successful treatment (*17*); and (iv) in situations of early or established viral rebound during treatment (*18*).

In the first three instances, a positive detection of mutant variants would be informative, whereas failure to identify mutants would not be conclusive. In any case, the data derived from resistance testing might help to select the most appropriate antiviral combination. The situation becomes more complex in the event of viremia rebound in treated individuals. Here, the physician faces all caveats discussed earlier: the inability to detect minority or archival copies of resistant virions, the fact that mutants can be rapidly overgrown by more fit wild-type viruses once that antiviral pressure is removed, and the complexity of cross-resistance among drugs. In this clinical situation, data are beginning to accumulate suggesting that the number of mutated positions as determined by genotypic testing might predict the likelihood of response to salvage treatment (*19*). However, the same information did not necessarily help in selection of the best salvage treatment, which might rather be based on past treatment history.

Treatment failure and emergence of drug-resistant variants constitute a great challenge. The success of HAART may be determined before treatment is started, and here is where investment is needed. Performing resistance testing might belong in this up-front investment. The first go is the best chance.

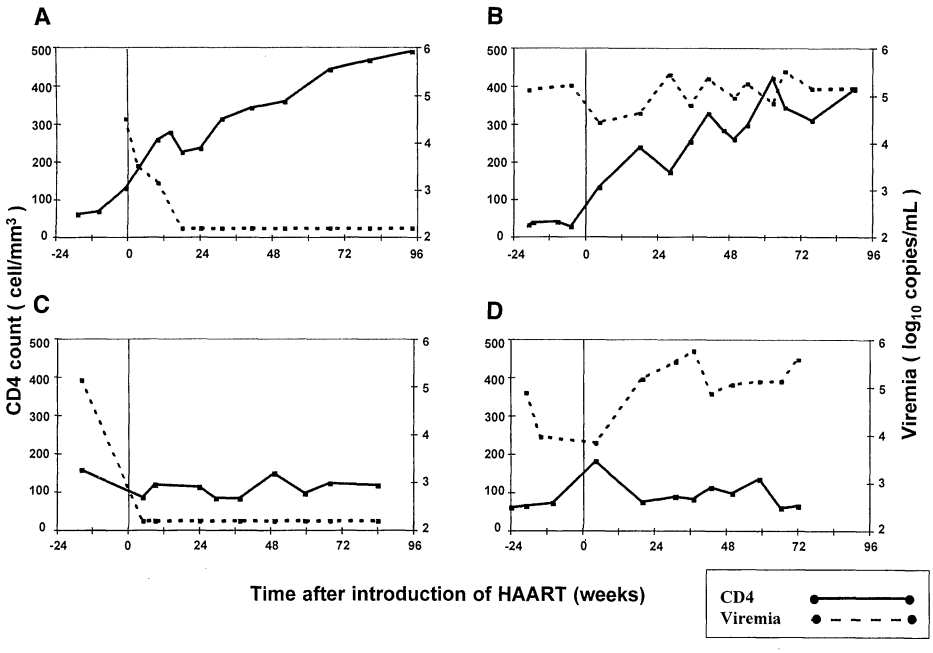

Fig. 1. Who is failing treatment? Data from four HIV-infected individuals on HAART.

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HIV/AIDS Prevention in Thailand: Success and Challenges

Wiput Phoolcharoen

Thailand's human immunodeficiency virus (HIV)/acquired immune deficiency syndrome (AIDS) epidemic is one of the most extensively documented of any developing country. Thailand has made substantial progress in the fight against HIV/AIDS because of strategies and policies for prevention that were initially based on research and evaluation and then received the necessary level of commitment to implementation and financing. Sexual behaviors have changed significantly, with condom use increasing and visits to sex workers decreasing (*1*). The spread of HIV has been slowed dramatically but not before close to a million people were infected (*2*).

A country's response to the epidemic is influenced to a great extent by the information available. Modifications of health and social services to cope with the evolving epidemiological trends of disease are vital to the success of HIV prevention. The first few AIDS cases in Thailand were in men who had sex with men, but by 1988, HIV was detected in intravenous drug users. Findings from the first round of HIV sentinel sero-surveillance in 1989 showed that heterosexual transmission (from commercial sex workers) would be the predominant mode of transmission. This first sentinel surveillance alerted the public so that HIV/AIDS control became national policy by 1990 (*3*).

In 1990, the first behavioral study at the national level, the Survey of Partner Relations and Risk of HIV Infection, was conducted, and it demonstrated the pervasive extent of risk behavior throughout Thai society (*4*). The result was that policy-makers allowed HIV/AIDS warning messages to be publicized through all kinds of media. They were aired regularly and repeatedly on television as part of the national strategy in 1991 to minimize transmission of HIV. The education and prevention messages were chosen to do more than just suggest measures to avoid infection. These messages also defined characteristics of people who were considered to be substantial risks for transmitting HIV (*5*).

In 1991, all government-sponsored sexually transmitted disease (STD) clinics began to promote condom use in the commercial sex setting. The "100% condom program" enlisted the cooperation of sex establishment owners and sex workers to encourage all clients to use condoms when obtaining sex. The government supplied almost 60 million free condoms a year to support this activity (*6*).

School education on AIDS was initiated in 1990. At this time, the Thai HIV/AIDS research community was also extremely active in conducting quantitative and qualitative studies of risk behavior and its determinants. These studies demonstrated that the idea of individual risk that had been dominant in the beginning of the epidemic was too narrow to address the underlying social, cultural, and economic forces driving the epidemic in Thailand (*7*–*9*). Thus, the concept of individual risk was broadened to include the influence of the social environment. Conventional AIDS education evolved to foster life-skills empowerment in Thai youth rather than behavior modification, so that their culture, peer pressure, and norms would promote safer sex behavior.

Also of note is the important role currently given to people with HIV/AIDS as an essential human resource for prevention and care, rather than viewing them as a potential reservoir or unfortunate consequence of the epidemic, as was often the case in earlier responses (*10*). The collaboration between groups of people with HIV/AIDS and the national program has been enhanced so that they can be active partners in the planning and implementation of a wide range of programs from national to community levels.

A major contributor to the Thai program's impact has been the willingness of the government to alter strategies and policy as knowledge of the extent of risk behavior grew and the social, economic, and cultural roots of the epidemic were understood. This willingness helped to illuminate the role that each sector of society had to play in the response. Thus, implementation has been expanded from the public health sector to the social and economic sectors. The strategic alliances have included nongovernmental organizations, private businesses, and community organizations that have worked as equal partners with the government (*10*).

There has also been an evolution in the funding of our efforts from international agencies to government and local funding. The Thai government's AIDS budget in 1996 expanded to cover 91% of all the expenses in AIDS programs in the country.

Evidence of Success

The changing trend of HIV infection in the general population is shown by two sets of data. First is the Royal Thai Army's information on the HIV infection rate among its roughly 60,000 annual military conscripts, selected by lottery from 21-year-old Thai males. The rate started to increase steadily from 0.5% in 1989 to a peak of 3.7% in mid-1993 before leveling off at 1.9% in 1997 (*11*). The second source of data is the sero-surveillance tests of the Division of Epidemiology in the Ministry of Public Health, which have been conducted on samplings of pregnant women in all 76 provinces yearly since 1989. The HIV infection rate in pregnant women was about

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