

Antiretroviral (ARV) Drug Resistance in the Developing World

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Preface

The Agency for Healthcare Research and Quality (AHRQ), through its Evidence-Based Practice Centers (EPCs), sponsors the development of evidence reports and technology assessments to assist public- and private-sector organizations in their efforts to improve the quality of health care in the United States. This report was requested by the Fogarty International Center at the National Institutes of Health (NIH). The reports and assessments provide organizations with comprehensive, science-based information on common, costly medical conditions and new health care technologies. The EPCs systematically review the relevant scientific literature on topics assigned to them by AHRQ and conduct additional analyses when appropriate prior to developing their reports and assessments.

To bring the broadest range of experts into the development of evidence reports and health technology assessments, AHRQ encourages the EPCs to form partnerships and enter into collaborations with other medical and research organizations. The EPCs work with these partner organizations to ensure that the evidence reports and technology assessments they produce will become building blocks for health care quality improvement projects throughout the nation. The reports undergo peer review prior to their release.

AHRQ expects that the EPC evidence reports and technology assessments will inform individual health plans, providers, and purchasers, as well as the health care system as a whole, by providing important information to help improve health care quality.

We welcome comments on this evidence report. They may be sent to: Director, Center for Outcomes and Evidence, Agency for Healthcare Research and Quality, 540 Gaither Road, Rockville, MD 20850.

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Structured Abstract

Objectives: To describe the overall prevalence of ARV resistance in the developing world, focusing on: (1) treatment naïve populations, (2) the resistance consequences of prevention of mother to child transmission (pMTCT) drug regimens, and (3) the relationship of medication adherence to resistance.

Data sources: We searched PubMed®, EMBASE, the Cochrane Controlled Clinical Trials Register Database, and the Cochrane Database of Reviews of Effectiveness (DARE). Additional sources of evidence included the Stanford University HIV Drug Resistance Database; reports of WATCH: Worldwide Analysis of Resistance Transmission over Time of Chronically and Acute Infected HIV-1 infected persons; a recent unpublished pMTCT overview; and various conference proceedings. Studies that did not report original research, that reported data already reported in another article, and case studies of fewer than 20 individuals were excluded. Of 1,122 titles identified, 117 journal articles and presentations were included.

Review methods: We abstracted data on geographic region, number of participants, subject demographics, HIV viral clade, medications taken (if any), years of data collection, how people were selected for resistance testing, and how and when resistance was assessed. Because of study heterogeneity, pooling was not possible; thus, the data are summarized qualitatively. Differences by region, population group, and HIV viral clade are described.

Results: The patterns of ARV resistance among treatment naïve populations worldwide appear to reflect geographic trends in use of ARV medications. A worldwide surveillance program (WATCH) found the rate of resistance (to any drug) among treatment naïve individuals was 5.5 percent in Africa, 7.4% in East Asia, 5.7 percent in Southeast Asia, and 6.4 percent in Latin America, lower than in North America (11.4 percent) and Europe (10.6 percent).

Resistance data on HIV clades other than A, B, C, and D were too scarce to permit reliable conclusions. We also identified very few studies designed to assess the effect of health services delivery factors or medication adherence on the development of resistance in patients in developing countries.

Evidence provided by longitudinal analyses suggests that, among women taking intrapartum single dose nevirapine (SD-NVP) to prevent mother-to-child transmission of HIV, both the overall prevalence of NNRTI resistance as well as the frequency of mutant virus in the overall viral population decreases with time since SD-NVP prophylaxis was received.

Conclusions: In future resistance studies, rare HIV clades should be over-sampled in order to provide statistically meaningful data. Resistance surveillance programs should be maintained *throughout* the developing world, and data should be reported and analyzed in a consistent and timely manner. Where resources permit, studies of adherence in developing regions should conduct resistance testing.

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Executive Summary

Introduction

The clinical management of HIV infection has greatly improved through the use of highly active antiretroviral therapy (HAART),¹ which comprises the following classes of agents: nucleoside or nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), and fusion inhibitors. The clinical effectiveness of these therapies is mediated by treatment-induced reduction of HIV viral replication as demonstrated by measurements of the amount of HIV RNA in the blood (the plasma viral load). However, successful suppression of HIV replication is influenced by the intrinsic potency of the prescribed regimen, patient adherence to treatment, and pre-existing or emerging resistance to antiretroviral (ARV) agents.²

Resistance of HIV to ARV agents was first reported within 2 years of the approval of the NRTI zidovudine (ZDV) for the treatment of persons with late-stage HIV infection.³ Subsequently, transmission of a ZDV-resistant isolate was first reported in 1992.⁴ The development of ARV resistance has since been reported with all other commercially available ARV agents within all classes.⁵ Because drug resistance mutations often decrease the activity of many ARV agents within an individual class, the emergence of a single major resistance mutation can have important effects on a patient's response to multiple ARV agents.⁵⁻⁷ Thus, assessment of the proportion of HIV-infected persons who have developed ARV resistance and characterization of the causes and factors associated with resistance development are critical steps in modifying treatment guidelines and regimens to improve their effectiveness.

The emergence of mutations that confer resistance to ARV therapy is an expected consequence of the high rates of viral replication and the inherent error rate of the reverse transcription of single-stranded RNA viruses such as HIV.⁷ Among individuals who are receiving ARV, the likelihood of developing resistance is increased if optimal drug doses (that is, doses that reduce viral load to less than approximately 50 to 200 HIV RNA copies/mL)^{8,9} are not maintained, either through poor adherence or the prescription of a suboptimal regimen (such as the administration of single-dose nevirapine [SD-NVP] to pregnant women infected with HIV for prevention of mother to child transmission [pMTCT]). A second method of acquiring a resistant strain of the virus is via transmission of a resistant strain to a treatment-naïve person.

The development of ARV resistance can be determined by the use of either genotypic or phenotypic assays.^{2,10} Genotypic testing assesses the viral genome for mutations in the genes that encode reverse transcriptase or protease; phenotypic testing assesses the ability of the virus to replicate in the presence of the drug in question. Interpretation of genotypic assays depends upon assessment of the probability that a given mutation pattern confers resistance to one or several specific ARV drugs.⁶ Despite agreement regarding the significance of most individual mutations, experts continue to show some disagreement in the interpretation of genotypes.¹¹ Although the interpretation of phenotypic HIV resistance tests is straightforward compared with that of genotypic tests, these assays are considerably more expensive, and their clinical utility is less well validated by clinical trials.⁶

Both assays can be applied to virus obtained from any tissue, but for routine clinical purposes, the assays are used to characterize HIV in plasma specimens. Both tests are relatively

insensitive for the detection of resistant viral variants (or quasispecies) that account for less than 20 percent of the circulating virus population in plasma, and neither assay detects intracellular, archival virus (virus that remains in a dormant state).⁶ In contrast, by the use of specific genetic probes and allele-specific real-time polymerase chain reaction (PCR, a method used to amplify genetic material for sequencing or assaying), it is possible to detect viral subpopulations that constitute as little as 0.1 to 0.2 percent of circulating virus in plasma.^{12,13}

HIV gene mutations may be classified as primary or secondary. Primary mutations alter the binding of a drug to its target, resulting in increased amounts of medications necessary to inhibit the target enzyme. Secondary mutations increase the level of resistance by improving the fitness of the virus carrying the primary mutations. Often, secondary mutations have little or no effect on the level of resistance in the absence of primary mutations. Other genetic variations, called polymorphisms, are found frequently in untreated HIV positive populations in the developing world. A polymorphism is a form of genetic variation (specifically a discontinuous variation), which occurs in an animal species in which distinct forms exist together in the same population. (The human blood groups are examples of a polymorphism.) The interpretation of these naturally-occurring polymorphisms, usually found in HIV protease, is difficult. Generally, these variations are not primary mutations which confer significant resistance, but may be secondary mutations which could contribute to resistance or improve the fitness of resistant virus.

Virologic treatment failure, and hence the emergence of ARV resistance, was particularly common in persons who received mono- or dual- drug therapy prior to the use of HAART regimens that included NNRTIs and PIs.¹⁴ As a consequence of prior inadequate therapy, the relatively poor potency of early HAART regimens, and poor adherence related to the complexity of dosing regimens and the frequency of adverse side effects, it was estimated that 50 percent of infected individuals who were receiving care for HIV infection in the United States (U.S.) in 1999 had some evidence of ARV resistance.¹⁵ More recently, despite the development of new ARV agents with unique profiles of resistance mutations and regimens with less complex dosing and fewer side effects,^{2,10} ARV resistance remains a serious problem for HIV-infected patients.^{16,17} Furthermore, up to 10 percent of newly infected, treatment-naïve individuals in the developed world are found to be infected by relatively fit, resistant virus that persists in the absence of treatment.¹⁸

The purpose of this report was to review and synthesize the literature that describes the overall prevalence as well as factors in the development of ARV resistance throughout the *developing* world, particularly studies that address resistance in non-clade B viral strains (a clade is a group of organisms believed to originate from a single common ancestor; non-clade B viruses are that subtype of virus that is more common outside of North America and Western Europe). According to International AIDS Society recommendations,⁶ evaluating susceptibility patterns among non-clade B persons should be a high priority because these viruses are by far the most prevalent world-wide. The first part of this report focuses on treatment-naïve populations in developing regions. The second focuses on the consequences of pMTCT regimens. Studies of pMTCT are particularly noteworthy, because they represent one of the most common uses of non-suppressive regimens of HAART. The third section focuses on adherence and its association with resistance.

Methods

This project was suggested by the Fogarty International Center (Fogarty) at the National Institutes of Health (NIH) on behalf of several institutes at NIH, with the aim of guiding the research agenda on the development of ARV resistance in developing countries by identifying critical gaps in the published research. A secondary aim was to identify issues for clinicians rolling out HIV medications in the developing world.

A technical expert panel (TEP) assisted in refining the methodology as well as the following key questions, which guided the review:

1. What is the prevalence of resistance? Does the prevalence of resistance differ between adults and children? Between men and women? Between groups with differing modes of transmission?
2. What are the factors associated with the development of resistance? How do the factors differ among varying population groups?
3. What are effective strategies to prevent or reduce the development of resistance in different population groups (e.g., post pMTCT intervention; people receiving ARV)?
4. What is the likely impact of low-level or partial resistance on subsequent therapy? What is the impact on response to subsequent pMTCT interventions?

The literature search was initiated in November, 2005 with an electronic search of PubMed® and Embase as well as the Cochrane Controlled Clinical Trials Register Database and the Cochrane Database of Reviews of Effectiveness (DARE) for reports on resistance to ARV. Search terms included the generic and trade names of every available NNTRI, NRTI, Protease Inhibitor (PI), and fusion inhibitor (FI), regardless of U.S. Food and Drug Administration (FDA) approval status. Additional sources of evidence included the Stanford University HIV Drug Resistance Database; reports of WATCH: Worldwide Analysis of Resistance Transmission over Time of Chronically and Acute Infected HIV-1 infected persons; a recent presentation on pMTCT by a technical expert; a recent overview;¹⁸ and various conference proceedings. The Stanford HIV RT and Protease Sequence Database is an on-line relational database that catalogs sequence variation in HIV reverse transcriptase (RT) and protease enzymes, the molecular targets of anti-HIV therapy. One part of the database provides copies of or links to published papers, meeting abstracts, and GenBank entries that have not yet been published as a paper or abstract.

Articles that did not report original research, that reported the results of animal or in vitro studies, that reported on case studies of fewer than 20 individuals, or that reported data already reported in another article were excluded (although we summarized the findings of several recent overviews). Although geographical setting was not initially considered a criterion for exclusion from the review, the TEP – after the first round of screening - recommended including only studies set in developing countries. The TEP also suggested that we focus on key questions one and two.

Results for treatment-naïve populations and pregnant women and their children were examined separately. Because of study heterogeneity, pooling was not possible; thus, the data were summarized qualitatively. Differences by region, population group, and HIV viral clade are described. Resistance studies that reported adherence data are also summarized separately.

Results

Of 1,122 titles identified by the literature search, only 117 met the inclusion criteria.

Review of Published Overviews on Incidence and Prevalence

Several overviews on the incidence and prevalence of ARV resistance were identified; however, most provided data only on the developed world. The WATCH study found that the rate of resistance (to any drug) was 5.5 percent in Africa, 7.4 percent in East Asia, 5.7 percent in Southeast Asia, and 6.4 percent in Latin America, lower than in North America (11.4 percent) and Europe (10.6 percent). The patterns of resistance world-wide appear to reflect trends in use of ARV medications: In developed areas, where medications have been more widely available, rates of resistance to NNRTI range from three to four percent, whereas, in developing regions with limited resources, the rates of resistance are much lower.

Resistance Among Treatment-naïve Persons in Developing Countries

We analyzed resistance prevalence separately for the following areas: India, other developing nations in Asia, Latin America, and the continent of Africa.

Studies of ARV resistance among treatment-naïve patients in India were rare. Published data were found from only three studies;¹⁹⁻²¹ these studies varied in their results. A study of 60 persons in Northern India found that at least 80 percent of patients had resistance to ZDV.¹⁹ A study of 50 persons in South India showed that fourteen percent were resistant to NNRTIs, 6 percent were resistant to NRTIs, and 20 percent were PI resistant although all had mutations that conferred partial resistance.²⁰ A study of 128 persons in Mumbai found that only two were resistant to NRTIs.²¹

Only three studies were found that reported on rates of resistance in other developing regions of Asia.²²⁻²⁴ None reported NNRTI resistance. NRTI resistance ranged from four to seven percent; primary resistance to PIs ranged from two to three percent.

In Latin America, eight studies identified rates of ARV resistance. Resistance to NRTIs ranged from 2 to 14 percent among treatment-naïve groups.²⁵⁻³² Reported rates of resistance to NNRTIs were low, ranging from zero to two percent. In a handful of studies that included Brazilian and Argentine populations, rates of secondary PI mutations were high, presumably among persons with HIV clade B. No studies assessed HIV resistance in Central America.

In sub-Saharan Africa, 14 studies identified rates of ARV resistance among the treatment naïve. NNRTI resistance rates ranged from 0 percent to 7.7 percent, and were associated with infection with most HIV clades.³³⁻⁴⁶ NRTI resistance rates ranged from zero percent to eight percent for all clades. Primary PI mutations were rare (less than three percent) among Africans; however, there were high levels of secondary PI mutations in most studies.

Resistance among Pregnant Women and Children in Developing Countries

Thirty-one studies were identified that addressed the emergence of ARV resistance in women in developing countries subsequent to the administration of therapy to prevent pMTCT of HIV-1 infection. These studies were highly heterogeneous with respect to study design and outcomes.

Studies of resistance following SD-NVP. Seven studies assessed the effects of SD-NVP on postpartum NNRTI resistance; resistance rates ranged from 9 to 69 percent.⁴⁷⁻⁵³ Seven studies that assessed the effect of SD-NVP among women infected with clade C on resistance reported rates of prevalence that varied from over 70 percent at 2 weeks postpartum to less than nine percent at six weeks postpartum; these findings suggest a decline in resistance over time.^{49,52,54-58} In studies of women infected with other clades,^{48,49,59-61} resistance ranged from 16 percent⁶⁰ to 36 percent.⁴⁹

Complex patterns of NNRTI resistance were observed following the receipt of SD-NVP. The most common mutations that resulted in amino acid changes were K103N and Y181C. Y181C was the most commonly detected resistance mutation in plasma collected 2 weeks postpartum from women infected by HIV-1 clade C.^{54,55} In contrast, the K103N resistance mutation was most common at later time points in persons infected by clade C and at all times in persons infected by other HIV-1 clades. Among clade-C women, from one study in South Africa, V106/A/M was the most common amino acid change.⁵⁴ One report found that the rates of detection of K103N, Y181C, and Y188C were significantly higher in women infected by HIV-1 clade C than by HIV-1 clades A or D.⁴⁹

Several longitudinal analyses support the observation of declines in NNRTI resistance with time and suggest that these declines may differ by clade.^{47,48,52,55,62}

Studies of resistance following other pMTCT regimens. Ten studies from the developing world were identified that studied the emergence of ARV resistance following other pMTCT regimens, including adding ARV therapy before or after SD-NVP. Two studies were done in Thailand,^{63,64} one was conducted in Argentina,⁶⁵ and the other eight studies were done in Sub-Saharan Africa.^{50,60,66-71} A randomized comparison of outcomes in women receiving pMTCT with SD-NVP alone with those of women who received SD-NVP followed by a “tail” of 3 or 7 days of ZDV and 3TC found that the prevalence of NNRTI resistance in these three groups was 57 percent, 13 percent, and 9 percent, respectively.⁴⁶

In one study, women who received either intrapartum SD-NVP or placebo following daily ZDV during their third trimester were put on three-drug ARV postpartum. Intrapartum SD-NVP was statistically associated with NRTI and NNRTI mutations at both 3 and 6 months. In another study, in which women were put on three-drug ARV after intrapartum SD-NVP or placebo, resistance was strongly associated with intrapartum SD-NVP among women who started ARV within 6 months postpartum, but not among women who started ARV 6 months or more postpartum,⁷² suggesting development of resistance may be associated with shorter interval between receipt of SD-NVP and initiation of full ARV.

Several trials suggest that when infants receive SD-NVP at birth in the background of ZDV, maternal SD-NVP does not provide any additional protection from HIV transmission.^{71,73}

Effect of test sensitivity. The rates of NNRTI resistance are consistently greater when more-sensitive assays are employed.^{51,58,65,74} For example, the prevalence detected with RT-PCR

(real time polymerase chain reaction) of plasma RNA can be up to double that detected with standard testing at six or seven weeks post-partum.

Resistance in HIV-positive Infants Born To Mothers Receiving pMTCT

Children are often born HIV infected despite pMTCT. Resistant viruses can be transmitted or can emerge independently in either mother or infant. Eight studies^{50 54 55 57 60 62 69 75} evaluated the emergence of ARV resistance in children born to mothers in sub-Saharan Africa who received pMTCT. Most studies examined the impact of SD-NVP. In most studies, infected infants were tested for resistance at 6 or 7 weeks of age. Rates of NNRTI resistance among untreated HIV-positive infants of mothers receiving only SD-NVP ranged from 36 percent to 50 percent. One study found that detectable resistance fades over time in infants exposed to SD-NVP.⁶²

Several studies compared the effects of multiple drugs on infant drug resistance. One study compared resistance rates among mother-infant pairs infected with various HIV clades who were treated with ZDV (N = 48) or SD-NVP (N = 61).⁶⁰ The HIV transmission rates were similar in the two groups: 16.4 percent and 16.7 percent respectively. Using the oligonucleotide DNA ligation assay for resistance at 6 weeks postpartum, the researchers found NNRTI mutations in half the infected pairs who received SD-NVP. No ZDV mutations were present in pairs treated with that drug. Resistance data for mothers and infants were not presented separately. In a second study, researchers gave mothers ZDV (300mg) twice daily starting at 36 weeks gestation in addition to oral SD-NVP at onset of labor.⁶⁹ Only 23 percent of their HIV-positive children were resistant to NNRTIs at 4 weeks postpartum. In a third study, researchers compared resistance among infants who received SD-NVP with those who received SD-NVP plus either 4 or 7 days of ZDV + 3TC after birth.⁵⁰ Infected infants who received the additional 7-day ZDV + 3TC showed no resistance at 6 weeks, compared to 78 percent of those who received SD-NVP only. Finally, a fourth study recently compared resistance rates in infants who received various combinations of intrapartum SD-NVP, SD-NVP (2 mg/kg) immediately postpartum, and ZDV (4 mg/kg) twice daily for one week.⁷⁵ At 6 to 8 weeks, the rate of resistance was much lower (27 percent) in the infants who received the postpartum SD-NVP and ZDV without the intrapartum SD-NVP. The study also showed that when infants received ZDV, the transmission rate did not differ between mothers who received SD-NVP and those who did not. When infants received SD-NVP plus one week of ZDV and the mother received SD-NVP, transmission was 17 percent and resistance was observed in 74 percent; when infants received SD-NVP plus 1 week of ZDV and mothers did not receive SD-NVP, transmission was 17 percent but resistance was only 27 percent. Data from the MASHI trial also suggest that when the infant receives SD-NVP at birth in the background of ZDV, maternal SD NVP does not provide any additional protection.^{51 65 72 74}

Predictors of Resistance. Several studies evaluated predictors of ARV resistance among persons receiving SD-NVP. A multivariate analysis demonstrated an increased risk of resistance detectable by standard genotyping assays in persons infected with clade C (compared with clade A or clade D) or with increased viral load at delivery, but not with increased age or number of births.⁴⁹ One additional study supports the predictive value of increased viral load.⁵⁴ Plasma HIV RNA level⁶⁴ has also been shown to be predictive of resistance, and increased duration of prepartum use of ZDV+3TC for pMTCT was associated with an increased maternal prevalence of the M184V resistance mutation.⁷⁶

Adherence and Resistance

Adherence to HIV treatment has long been associated with treatment outcomes. Most studies in the developed world have found evidence that at very low levels of adherence, there is insufficient selective pressure for mutations to evolve. At high levels of adherence, viral replication, and thus, viral evolution, should cease.

Few studies could be identified that were designed to assess the effect of health services delivery factors or medication adherence on the development of resistance in patients in developing countries. Three studies assessed the impact of treatment interruptions on ARV resistance in the developing world: two were conducted in Kampala, Uganda.^{77 78} In the first study, 137 patients received a three-drug regimen; most included NVP (77 percent) or efavirenz (14 percent). At a median of 38 weeks, 91 of the 137 patients had undetectable viral loads. Of the 137 patients, 32 experienced an unplanned treatment interruption of more than 4 days. In multivariate analysis, this treatment interruption was statistically associated with resistance, as was being treatment-naïve at study entry. Of 36 patients for whom genetic data were available, 26 were resistant to NNRTIs; the most common mutation was K103N. NRTI resistance was measured in 23 patients, attributable to the M184V/I mutation. A second study found that among 95 participants, the 33 without treatment interruption had no drug resistance, compared to eight of the 62 participants who did interrupt treatment. A third African study that assessed the relationship between adherence and the presence of resistance in an Ivory Coast AIDS clinic found no association between missed treatments and resistance.⁷⁹ Finally, one additional study from the UK studied black African children with non-clade B virus.⁸⁰ All children whose adherence was classified as good or intermediate had resistance, compared with five of eight children whose adherence was poor. Of critical importance, unlike many studies conducted in the developed world, the latter studies were not initially designed or powered to assess the relationship between adherence and resistance.

Discussion

This review had several limitations. The primary limitation was the quality and quantity of available studies. Other limitations related specifically to how the studies were conducted, including heterogeneity with respect to study design, the lack of inclusion of participants infected with more unusual viral clades, and failure to stratify treatment-naïve patients.

The paucity of quality research examining and characterizing ARV resistance among populations of developing nations leads us to make the following recommendations:

Given the relatively high prevalence of HIV infection in India, more research on resistance patterns should be a priority for that nation.

Few published studies stratified resistance data by host characteristics such as mode of HIV transmission, risk factors, or gender, although these data were collected in most of the studies. Studies with larger sample sizes would allow meaningful analyses of patterns among groups, for example MSM, heterosexuals, prostitutes, and IDUs.

Data on HIV clades other than A, B, C, D, or the recombinant types were too scarce to permit reliable conclusions. In future studies, rare HIV clades should be over-sampled in order to provide statistically meaningful data.

Evidence provided by longitudinal analyses suggests that the prevalence of NNRTI resistance may vary with the time elapsed since treatment among women taking SD-NVP for

pMTCT. In one study, fading of variants with Y181C was reported in women infected with clade A but not clade D; thus, Y181C may have less effect on viral fitness of clade D than on A. This observation warrants further investigation in future studies. The impact of the Y181C mutation should also be studied in clades other than D and A.

Research should be conducted regarding the need for maternal SD-NVP when an infant receives NVP at birth or when the mother receives ZDV regularly before birth.

Further research is needed on the optimum time to begin ongoing postpartum ARV treatment.

Where possible, future research projects on adherence should measure resistance in addition to routinely collected data such as CD4 count and viral load. In addition, observational studies of HIV treatment in developing regions should ask questions about factors that affect patient access to medication.

More studies should use ultrasensitive assays to determine not just the occurrence of resistance but also the quantitative frequency of resistance mutations and types of mutations over time, which may be important in determining response to therapy. Where possible, patients, including mothers and their HIV positive children, should be followed over several years to assess the extent and quantity of archiving of resistance mutations in cells.

Resistance surveillance programs should be established throughout the developing world. Data should be reported and analyzed in a consistent and timely fashion. It is expected that the Global HIV Drug Resistance Surveillance Network (HIV Resnet), recently launched by the World Health Organization, should fulfill this function.

Evidence Report

Chapter 1. Introduction

The clinical management of HIV infection has been greatly improved by the use of highly active antiretroviral (ARV) therapy (HAART)¹ HAART comprises the following classes of agents: nucleoside or nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), and fusion inhibitors. The clinical effectiveness of these therapies is mediated by treatment-induced reduction of HIV viral replication as demonstrated by measurements of the amount of HIV RNA in the blood (the plasma viral load). In turn, successful suppression of HIV replication is influenced by the intrinsic potency of the prescribed regimen, patient adherence to treatment, and pre-existing or emerging resistance to ARV agents.²

Resistance of HIV to ARV agents was first reported within 2 years of the approval of the NRTI zidovudine (ZDV) for the treatment of persons with late-stage HIV infection.³ Subsequently, transmission of a ZDV-resistant isolate was first reported 1992.⁴ The development of ARV resistance has since been reported with all other commercially available ARV agents within all classes.⁵ Because drug resistance mutations often decrease the activity of many ARV agents within an individual class, the emergence of a single major resistance mutation, e.g., the NRTI mutations M184V* or T215Y, the NNRTI mutation K103N, or any of a number of major PI mutations, can have important effects on a patient's response to multiple ARV agents.⁵ Thus, assessment of the proportion of HIV-infected persons who have developed ARV resistance and characterization of the causes and factors associated with resistance development are critical steps in modifying treatment guidelines and regimens to improve their effectiveness.

The emergence of mutations that confer resistance to ARV therapy is an expected consequence of the high rates of viral replication and the inherent error rate of the reverse transcription of single-stranded RNA viruses such as HIV.⁷ Because these mutations can decrease the growth rate or replication capacity of HIV,⁸¹ some drug-resistant viral strains do not compete successfully with wild-type virus in the absence of ARV therapy or the emergence of other compensatory mutations.⁷ However, whereas this phenomenon of decreased replication capacity has been demonstrated with some specific resistance mutations (e.g., K65R and M184V), other mutations such as the K103N mutations produce little if any impact on viral replication.⁸²

In contrast, if non-suppressive antiretroviral therapy is administered, emergent resistant viruses are selected as the most replication capable (fit) virus and quickly predominate.⁸³ In addition, prolonged non-suppressive ARV therapy may eventually select for the emergence of further compensatory mutations that increase fitness of resistant virus such that they compete successfully with wild-type virus even if ARV therapy is withdrawn. However, if ARV therapy is withdrawn prior to the emergence of compensatory mutations, overgrowth of more rapidly replicating wild-type viruses leads to subsidence of detectable resistant virus in the bloodstream within 4 to 6 weeks.⁸⁴ Even so, resistant virus persists at low levels and/or in latently infected cells^{85,86} and may later re-emerge, often leading to the failure of subsequent therapeutic

* M184V refers to a mutation that results in the replacement of the amino acid methionine (M) with valine (V) at position 184. The one-letter amino acid abbreviations are as follows: A=alanine, C=cysteine, E=glutamic acid; G=glycine, I=isoleucine, K=lysine, L=leucine, M=methionine, N=asparagine, P=proline, V=valine, Y=tyrosine.

regimens.⁸⁷ This phenomenon of re-emergence of drug resistance from cellular reservoirs has been best described in persons who have received prolonged courses of ARV therapy. Whether resistance that emerges after brief exposure (e.g., single-dose (SD) nevirapine (NVP) for preventing mother-to-child transmission (pMTCT) of HIV) has the same implications for the success of subsequent ARV therapy is less certain.⁸⁸

The plasma viral load directly correlates with the amount of viral replication and therefore serves as an indirect measure of the potential mutational rate of HIV and thus of the potential rate of acquisition of ARV resistance mutations. While any residual HIV replication during treatment may allow for continued viral evolution, the emergence of ARV resistance is unusual in persons who have isolated plasma viral loads no higher than 50 – 200 HIV RNA copies/mL.^{8,9} In contrast, failure to achieve intakes of HAART that are sufficient to maintain a plasma viral load of less than approximately 400 – 1,000 copies/mL is associated with an increased risk for developing ARV resistance.^{6,7} While patients may derive immunological and clinical benefit from a treatment regimen that does not fully suppress viral replication,⁸ such patients remain at substantial risk for the eventual emergence of drug resistance mutations.⁸⁹

The observation of resistance in treatment-naïve individuals raises the question of when resistance testing should be conducted. This question is informed, at least in part, by the rate at which resistant virus is overgrown by wild-type virus. Because of the frequent, rapid subsidence of resistance when therapy is withdrawn, expert groups recommend that resistance testing should be done while patients are on therapy or shortly after the discontinuation of therapy,^{2,10} as testing can detect more mutations during this interval. In treatment-naïve patients or persons who have long since discontinued ARV therapy, resistance testing retains specificity but has decreased sensitivity. Nevertheless, the high rate of transmitted resistance has led to recommendations for testing chronically infected treatment-naïve persons.

Frequency of ARV Resistance

Virological treatment failure, and hence the emergence of ARV resistance, was particularly common in persons who received mono- or dual- drug therapy prior to the use of HAART regimens that included NNRTIs and PIs.¹⁴ As a consequence of prior inadequate therapy, the relatively poor potency of early HAART regimens, and poor adherence related to the complexity of dosing regimens and the frequency of adverse side effects, it was estimated that 50 percent of infected individuals who were receiving care for HIV infection in the United States (U.S.) in 1999 had some evidence of ARV resistance.¹⁵ More recently, despite the development of new ARV agents with unique profiles of resistance mutations and regimens with less complex dosing and fewer side effects,^{2,10} ARV resistance remains a serious problem for HIV-infected patients.^{16,17} Furthermore, up to 10 percent of newly infected, treatment-naïve individuals in the developed world are found to be infected by relatively fit, resistant virus that persists in the absence of treatment.¹⁸

Determination of ARV Resistance

The development of ARV resistance can be determined by the use of either genotypic or phenotypic assays.^{2,10} Genotypic testing assesses the viral genome for mutations in the genes that encode reverse transcriptase or protease; phenotypic testing assesses the ability of the virus to replicate in the presence of the drug in question. Both assays can be applied to virus obtained

from any tissue, but for routine clinical purposes, the assays are used to characterize HIV in plasma specimens. Both tests are relatively insensitive for the detection of resistant viral variants (or quasispecies) that account for less than 20 percent of the circulating virus population in plasma, and neither assay detects intracellular, archival virus (virus that remains in a dormant state).⁶ In contrast, by the use of specific genetic probes and allele-specific real time polymerase chain reaction (PCR, a method used to amplify genetic material for sequencing or assaying), it is possible to detect viral subpopulations that constitute as little as 0.1 to 0.2 percent of circulating virus in plasma.^{12,13}

In clinically used genotypic assays, the sequences of the HIV-1 reverse transcriptase and protease genes are reverse-transcribed, and the resultant cloned DNA (cDNA) amplified and sequenced or otherwise assayed for known mutant sequences and compared to a list of mutations that have been associated with ARV resistance. An interpretive algorithm is then used to determine whether the identified mutations are likely to lead to decreased effectiveness of (i.e., resistance to) specific ARV agents. Interpretation of genotypic assays depends upon assessment of the probability that a given mutation pattern confers resistance to one or several specific ARV drugs.⁶ Despite agreement regarding the significance of most individual mutations, experts continue to show some disagreement in the interpretation of genotypes.¹¹ Numerous schemes have been developed for interpreting HIV resistance genotypes. Since these schemes differ in how evidence regarding resistance is interpreted, in how interactions between mutations are weighted, and in the frequency with which they are revised in the light of new scientific evidence, it is not surprising that some of these schemes predict the phenotypic response to treatment substantially better than do others.⁹⁰

Although the interpretation of phenotypic HIV resistance tests is straightforward compared with that of genotypic tests, these assays are considerably more expensive, and their clinical utility is less well validated by clinical trials.⁶ Phenotypic susceptibility assays report the drug concentration necessary to inhibit viral replication *in vitro* by 50 percent, the Inhibitory Concentration(IC)₅₀. Traditionally, phenotypic assays for HIV drug susceptibility have required the preparation of a high-titer viral stock in tissue culture, followed by infection of a second culture and determination of the inhibitory effect of the agents in question. Such multistage assays are not only time-consuming and expensive, but the resultant values suffer from relatively poor inter-laboratory reproducibility.⁹¹ More rapid and reproducible viral phenotypic assays have been developed using recombinant DNA technology. In one such assay, the reverse transcriptase and HIV protease genes of a target virus are cloned and recombined with a laboratory HIV DNA clone from which the the reverse transcriptase and protease genes have been deleted. Another assay uses luciferase indicator genes to track the expression of genes that have conferred resistance.^{92, 93}

HIV gene mutations may be classified as primary or secondary. Primary mutations alter the binding of a drug to its target, resulting in increased amounts of medications necessary to inhibit the target enzyme. Secondary mutations increase the level of resistance by improving the fitness of the virus carrying the primary mutations. Often, secondary mutations have little or no effect on the level of resistance in the absence of primary mutations. Other genetic variations, called polymorphisms, are found frequently in untreated HIV positive populations in the developing world. A polymorphism is a form of genetic variation (specifically a discontinuous variation), which occurs in an animal species in which distinct forms exist together in the same population. (The human blood groups are examples of a polymorphism.) The interpretation of these naturally-occurring polymorphisms, usually found in HIV protease, is difficult. Generally, these

variations are not primary mutations which confer significant resistance, but may be secondary mutations which could contribute to resistance or improve the fitness of resistant virus.

The purpose of this report is to review and synthesize the literature that describes the overall prevalence of ARV resistance throughout the world. We have emphasized studies conducted since 2000 and have particularly sought out studies in the developing world that address resistance in non-clade B virus (a clade is a group of organisms believed to originate from a single common ancestor; clade B viruses are that subtype of virus that is more common outside of the North America and Western Europe). According to the recommendations of the International AIDS Society,⁶ evaluating susceptibility patterns among non-clade B persons should be a high priority because these viruses are by far the most prevalent worldwide. The first part of our report focuses on treatment-naïve populations in developing regions. The second focuses on the consequences of pMTCT regimens. Studies of pMTCT are particularly noteworthy, because they represent one of the most common uses of non-suppressive regimens of HAART. A third section reviews the few studies that report both adherence and resistance data.

In these analyses, we examine whether the prevalence of resistance differs between adults and children, between men and women, and between groups with differing modes of transmission. We report factors associated with the development of resistance and how these factors differ among varying population groups. Factors of particular concern included viral clades (especially in the context of pMTCT studies in the developing world), region, treatment regimen, adherence, and host factors such as gender and mode of transmission.

Chapter 2. Methods

Original Proposed Key Questions

This project was suggested by the Fogarty International Center (Fogarty) at the National Institutes of Health (NIH) on behalf of several institutes at NIH with the aim of guiding the research agenda on the development of ARV resistance in developing countries by identifying critical gaps in the published research. A secondary aim was to identify issues for clinicians rolling out HIV medications in the developing world. The following key questions were proposed:

1. What is the prevalence of resistance? Does the prevalence of resistance differ between adults and children? Between men and women? Between groups with differing modes of transmission?
2. What are the factors associated with the development of resistance? How do the factors differ among varying population groups?
 - a. Viral factors
 - b. Host factors
 - c. Specific ARVs
 - d. Resistance assay (whether resistance identified depends on the type of test used)
 - e. Health service associated
3. What are effective strategies to prevent or reduce the development of resistance in different population groups (prevention of Mother To Child Transmission (pMTCT); people receiving ARV, etc.)?
4. What is the likely impact of low-level or partial resistance on subsequent therapy? What is the impact on response to subsequent PMTCT interventions?

Technical Expert Panel

In designing the study questions and methodology at the outset of this report, the EPC consulted several technical and content experts. Broad expertise and perspectives were sought. The following scientists and clinicians participated in the TEP for this report: David Bangsberg, John Baxter, Lisa Frenkel, David Katzenstein, Shahin Lockman, Douglas Richman, Robert Shafer, Steve Spector, and Jonathan Uy. Appendix A* provides a list of their names, degrees, and affiliations.

Divergent and conflicting opinions are common and perceived as healthy scientific discourse that results in a thoughtful, relevant systematic review. Therefore, the final study questions, design, and/or methodologic approaches do not necessarily represent the views of all technical and content experts.

The TEP's participation in the preparation of the report began with a meeting conducted via conference call on February 27, 2006. The purpose of this meeting was to obtain TEP input on the scope of the project. The TEP expressed the strongly held belief that we focus on the ARV

* Appendixes cited in this report are provided electronically at <http://www.ahrq.gov/clinic/tp/antiretftp.htm>

resistance picture in developing countries. During the course of the worldwide spread of HIV, the main virus (M) evolved into multiple clades. Most approved ARV medications were developed in studies of clade B, the most common clade in the Western world. Since clade B viruses account for only about 10 percent of HIV cases worldwide,⁹⁴ knowledge of how ARVs affect the other clades is of critical importance. Thus, the TEP suggested that we provide an overview of the prevalence of resistance world-wide and how it differs by region. They asked us to address both resistance acquired through taking ARV drugs as well as drug resistance transmitted to treatment-naïve persons through infection.

A second TEP meeting was held by telephone on May 26, 2006. To better match the scope of the project with resources, the panel agreed that we should focus on key question one and on parts a, b, c, and e of key question two. They expressed the belief that comparing types of resistance assays was beyond the scope of the project. They again emphasized the focus on developing countries. Specific concerns were MTCT and prevalence of ARV resistance in treatment-naïve populations.

Literature Search

Our search for studies began in November, 2005 with an electronic search of PubMed® and Embase for reports of resistance to ARV medications. We also searched the Cochrane Controlled Clinical Trials Register Database and the Cochrane Database of Reviews of Effectiveness (DARE). (The Cochrane Collaboration is an international organization that helps people make well-informed decisions about health care by preparing, maintaining, and promoting the accessibility of systematic reviews on the effects of health care interventions.)

We ordered all articles on resistance to ARVs, regardless of study design, language, or publication date. Search terms included the generic and trade names of every available NNTRI, NRTI, and Protease Inhibitor (PI), regardless of U.S. Food and Drug Administration (FDA) approval status. We also searched for studies of resistance to fusion inhibitors. We included only studies of humans; no animal studies or in vitro passage studies were included. Appendix B* shows our specific search terms.

Additional Sources of Evidence

Stanford University HIV Drug Resistance Database

The HIV RT and Protease Sequence Database is an on-line relational database that catalogs sequence variation in HIV reverse transcriptase (RT) and protease enzymes, the molecular targets of anti-HIV therapy. The database was developed in order to help physicians and researchers determine clinical cross-resistance among current and experimental anti-HIV drugs and to identify any drug regimens that retain their effectiveness against drug-resistant HIV-1 isolates. The database links HIV-1 RT and protease sequence data, drug treatment histories, drug susceptibility, and clinical parameters.

* Appendixes cited in this report are provided electronically at <http://www.ahrq.gov/clinic/tp/antiretptp.htm>

One part of the database provides copies of or links to published papers, meeting abstracts, and GenBank entries that have not yet been published as a paper or abstract. We searched the publications list in January, 2006, and downloaded relevant studies. We did not conduct our own analysis of the patient-level data stored in the RT and Protease Sequence database.

WATCH: Worldwide Analysis of Resistance Transmission over Time of Chronically and Acute Infected HIV-1 infected persons

Located in Europe, the WATCH project aims to collect HIV-1 RT and protease sequences from treatment-naïve participants all over the world and analyse them together in a standardised manner, in order to be able to make a good comparison of resistance figures. We found two WATCH conference abstracts and contacted the authors about additional research in progress.

Presentation – Preventing Mother-to-Child HIV Transmission and ARV Drug Resistance

Dr. Lynne Mofenson of the National Institute of Child Health and Human Development provided us with a copy of an overview she presented on March 16, 2006; this excellent overview contained nearly 100 slides, from which we obtained all relevant studies cited.

Article – The Global Status of Resistance to ARV Drugs

In 2005, Vella and Palmisano published an overview in the journal *Clinical Infectious Diseases*.¹⁸ They searched for published studies on the incidence and prevalence of drug resistance in persons recently infected with HIV in all regions of the world. We retrieved the one study from a developing country (Ivory Coast).

We also searched the proceedings of the following conferences for the past 2 years. (We chose a 2 year cut-off because we expected that in general, abstracts and presentations would be published in journals within 2 years of the conference.)

Conference on Retroviruses and Opportunistic Infections (CROI)

The most recent CROI conference was held in Denver, Colorado on February 5-8, 2006. The CROI is a scientifically based meeting of leading HIV/AIDS researchers from around the world, and the 2006 conference was sponsored by the Foundation for Retrovirology and Human Health, the National Institute of Allergy and Infectious Diseases, the Centers for Disease Control and Prevention, and the University of California, San Diego School of Medicine. Participants included scientists actively engaged in basic science or clinical studies examining retroviral diseases and their complications or full-time academic clinician-teachers responsible for HIV/AIDS training and research programs. The aim of the conference was to enable researchers to present, discuss, and critique their investigations with the ultimate goal of translating their research into progress in the fight against HIV/AIDS.

International AIDS Conference

The most recent International AIDS Conference was held in Toronto, Canada on August 13-18, 2006. The conference is the largest HIV/AIDS meeting in the world; participants include scientists; health care providers; political, community and business leaders; journalists; government, non-governmental and intergovernmental representatives; and people living with HIV/AIDS. The goal of the conference is to allow for the exchange of ideas, research, and knowledge to help strengthen HIV/AIDS programs worldwide, with an emphasis on evidence, outcomes, and best practices. The theme of the 2006 conference was *A Time to Deliver*, underscoring the still urgent need to bring effective prevention and treatment programs to communities worldwide.

IAS Conference on HIV Pathogenesis and Treatment

The International AIDS Society (IAS) is a professional society with over 6,000 members, including scientists, health care workers, and others involved in the prevention and treatment of HIV/AIDS. Every 2 years, IAS sponsors a conference to disseminate knowledge that can accelerate the response to HIV/AIDS worldwide. We examined the proceedings from the July 24-27, 2005 conference, which took place in Rio de Janeiro, Brazil and was co-sponsored with the Universidad de Federal do Rio de Janeiro and the Brazilian Society of Infectious Diseases. The specific aim of the conference was to focus on new insights into HIV disease development, prevention, and care and their practical applications in developing countries.

Meetings of the Infectious Diseases Society of America (IDSA)

The meetings of the Infectious Diseases Society of America (IDSA) are geared towards physicians, scientists, and other health care professionals involved in research, patient care, public health, disease prevention, and education in the field of infectious diseases (ID). The meetings are designed to disseminate current knowledge and advancements in the field, bridge the gaps among various medical disciplines, and promote multidisciplinary dialogues and communications that will facilitate the prevention and treatment of ID. The 2006 meeting of the IDSA took place on October 12-15 in Toronto, Canada, and included four program tracks: investigative infectious diseases (ID), adult ID, HIV, and pediatric ID. The IDSA website also provides a meetings archive that enabled us to examine the proceedings.

Article Review

Study Inclusion

Our initial search was unrestricted by study design. The studies included in the review are of one of the following types of designs.

Review articles identified by the search were classified as either *systematic* (including meta-analyses) or *nonsystematic*. Systematic reviews were identified by reading the methods section of the article to determine whether an acceptable method was employed to identify evidence (such as a description of the name of the computerized database searched and the full set of

search terms used, as well as details about the method for accepting and rejecting identified articles).

Randomized controlled trials (RCTs) are studies where the participants are definitely assigned prospectively to one of two (or more) alternative forms of intervention, using a process of random allocation (e.g., random number generation, coin flips).

Controlled clinical trials (CCT) s are studies where participants (or other units) are either

(a) definitely assigned prospectively to one of two (or more) alternative forms of health care using a quasi-random allocation method (e.g., alternation, date of birth, patient identifier)

OR

(b) possibly assigned prospectively to one of two (or more) alternative forms of health care using a process of random or quasi-random allocation.

Observational studies (such as cohort, case-control, and cases series) are those where the investigators do not control who gets the interventions. Much of the data included in this report comes from observational studies. To avoid reviewing potentially numerous case reports, we initially set a threshold of 50 or more participants per series for inclusion in our review. After discussion with our TEP, we reduced this number to 20 for studies of populations in developing countries, so as not to exclude potentially important data.

Screening

Using a single-page “screening form” (included in Appendix C*), we reviewed the studies retrieved from the various sources against our exclusion criteria. Two reviewers, each trained in the critical analysis of scientific literature, independently reviewed each study and resolved disagreements by consensus. The lead investigators resolved any disagreements that remained unresolved after discussions between the reviewers.

To be included in our report, a study had to focus on human resistance to ARV. Studies of cell lines or animals were excluded. There were no language limitations; we included many non-English studies. Studies of obsolete therapies were also excluded at this time, if it was very unlikely that such therapies would never be used again, even in resource-limited regions.

After the TEP further narrowed the scope of the project, we created a second one-page screening form. Using this form, we tracked articles on treatment-naïve patients, pregnant women and children. We also used this form to identify studies of entire geographic regions, large hospitals, and specific medications.

During this phase of screening, we were asked to exclude studies not set in the developing world. “Developing” generally refers to economic growth and social development in areas such as health care, literacy, life expectancy, and fertility. Some less-developed regions could actually be considered non-developing, as a number have experienced years of decline rather than development. However, the United Nations (UN) allows each country to decide for itself whether it will be designated as “undeveloped” or “developing.” For the purposes of our report, we chose to include the following “undeveloped” and “developing” regions: Latin America, sub-

* Appendixes cited in this report are provided electronically at <http://www.ahrq.gov/clinic/tp/antiretftp.htm>

Saharan Africa, India, and all other areas of Asia except Hong Kong, Macau, Singapore, and Japan.

Extraction of Study-Level Variables and Results

We abstracted data from the articles that passed our screening criteria onto a specialized Quality Review Form (included in Appendix C*). The form collects data on geographic region, number of participants, subject demographics, HIV viral clade (clade), medications taken (if any), years of data collection, how people were selected for resistance testing, and how and when resistance was assessed. Data for all abstracted studies are presented in Evidence Tables (Appendix D). Trained reviewers, working in groups of two, extracted data from the same articles and resolved disagreements by consensus. A senior researcher resolved any disagreements not resolved by consensus.

Synthesis of Results

Results for a) treatment-naïve populations and b) pregnant women and their children are presented in separate tables. Because of study heterogeneity, pooling was not possible; thus, we summarize the data qualitatively. Differences by region, population group, and HIV viral clade are described. We also include a separate summary of resistance studies that reported adherence data.

Peer Review

A draft of this report was prepared in December, 2006 and sent to the TEP members and eight additional national and international experts for review. Peer reviewer comments were considered by the EPC in preparation of this final report. A list of peer reviewers and TEP members is included as Appendix A. Synthesis of the scientific literature presented here does not necessarily represent the views of individual reviewers.

* Appendixes cited in this report are provided electronically at <http://www.ahrq.gov/clinic/tp/antirettp.htm>

Chapter 3. Results

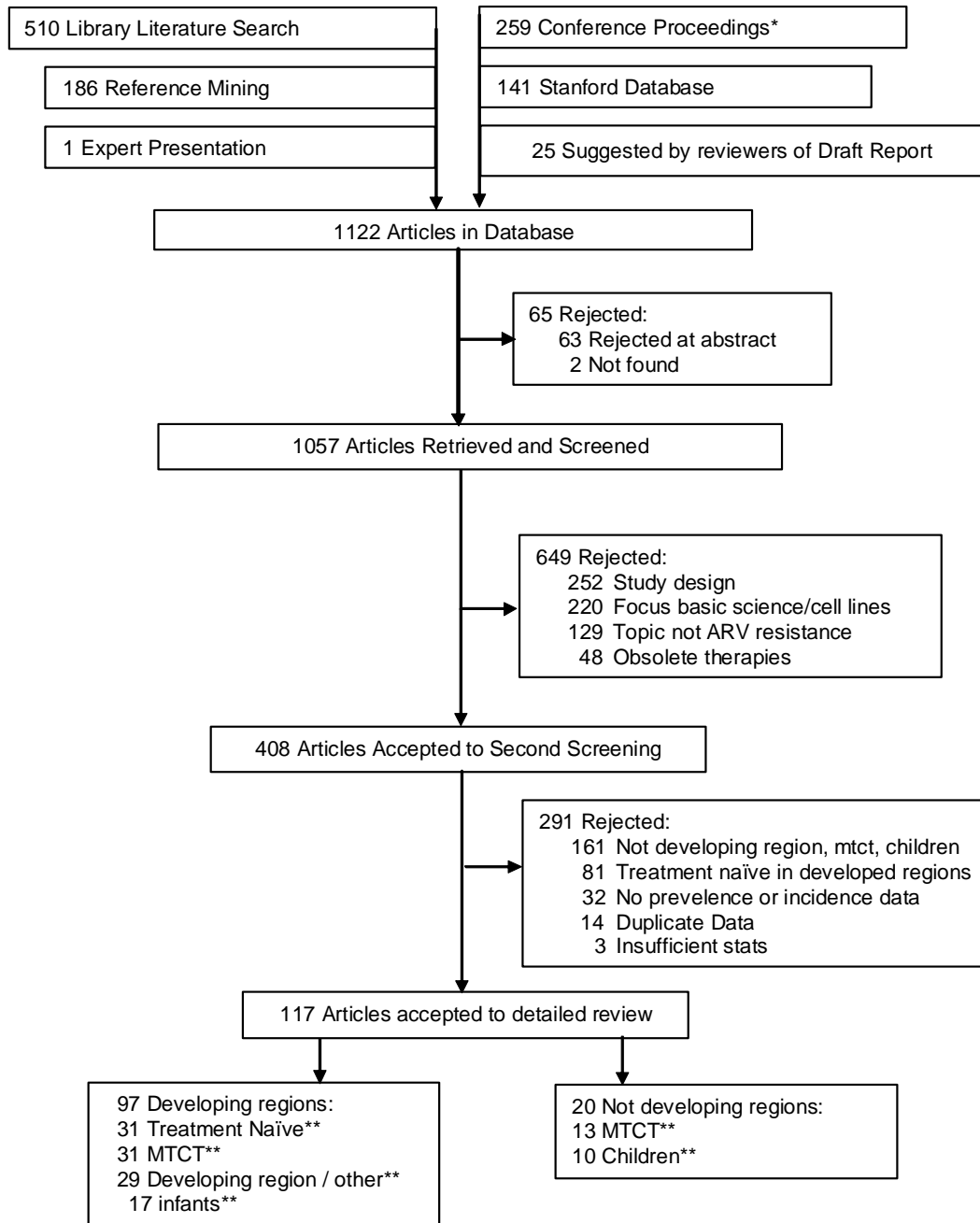
Description of the Studies

The literature search identified 1,122 titles. These references were culled from electronic library searches (510), conference proceedings (259), the Stanford University HIV Drug Resistance Database (141), and reference mining from retrieved articles (186). One content expert sent us an overview presentation on pMTCT. Twenty-five articles or abstracts were suggested by reviewers of a draft version of this report.

Of the 1,122 titles identified as possibly relevant to our topics, 63 were excluded at abstract review and two could not be found, leaving 1,057 to be retrieved. Of these 1,057 articles, screening resulted in exclusion of 649 articles: 252 due to study design (background articles, commentary, individual case reports, case series of fewer than 20 persons); 220 focused on basic science or cell lines; 129 were excluded because the topic was not ARV resistance; and 48 reported on obsolete therapies. The remaining 408 were then reviewed with a second screening form.

Of these 408 articles, 291 were excluded from further analysis: 242 either because they were not within developing regions or not on pregnant women or children; 32 had no prevalence or incidence data; 14 reported the same data already reported in other included studies (duplicate data); and three had insufficient statistics. Data were abstracted from the remaining 117 studies (Figure 1 Literature Flow).

Figure 1. Literature flow



* Including the Conference on Retroviruses and Opportunistic Infections (CROI), IAS Conference on HIV Pathogenesis and Treatment, International AIDS Conference and meetings of the Infectious Disease Society of America (IDSA).

** Categories not mutually exclusive. One article can contain data on treatment naïve patients, and/or MTCT

Published Overviews

We found several overviews on the incidence and prevalence of resistance to ARV medications. Most focused on the developed world.

Recent data on treatment naïve persons from ten large cities in the U.S.⁹⁵ showed that resistance was higher in men who have sex with men (MSMs, 12 percent) compared to other risk groups and higher among whites (13 percent) than among other races. Overall, 8.3 percent of treatment-naïve persons were resistant to at least one drug. Regarding treatment-experienced patients in the U.S., 1998 data from the HIV Cost and Services Utilization Study (HCSUS) showed that 76 percent of viremic patients (that is, patients with measurable blood levels of the virus) were resistant to at least one drug. Of the viremic patients, 71.4 percent were resistant to NRTIs, 25.2 percent to NNRTIs, and 40.5 percent to PIs.¹⁵ In univariate analyses, men had a higher rate of resistance than women; there were no differences by race. Those with heterosexually acquired HIV or unknown mode of transmission had lower levels of resistance than MSMs and injection drug users (IDUs). The patterns likely reflect historical access to medications among population groups, that is, populations with earlier access to medications were more likely to be resistant. However, none of these variables were significant predictors of resistance in a multiple logistic regression that included CD4 count, viral load, and treatment variables.

The CATCH study (Combined Analysis of Resistance Transmission over Time of Chronically and Acutely Infected HIV Patients in Europe) presented an overview of resistance in Europe. Authors analyzed data from 2,208 treatment-naïve individuals. Prevalence of resistance to any medication class was 10.4 percent: 7.6 percent were resistant to NRTIs, 2.9 percent to NNRTIs, 2.5 percent to PIs.^{96,97} Clade B viruses were more likely to carry resistance mutations than non-B clades (12.9 percent vs. 4.8 percent). Baseline resistance in new cases of non-B infection increased over time, from 2.0 percent in 1996-1998 to 8.2 percent in 2000-2001.

The WATCH study (described in the methods section) collected data on resistance from treatment-naïve participants from around the world. In 2006, researchers presented two abstracts {#2102}⁹⁸ on 4,714 patients for whom the viral protease and/or reverse transcriptase sequences had been determined. The patients were primarily male (69 percent) with mean age 35.3 years. Thirty-one percent of the patients identified as MSMs; another 9 percent were IDUs. Data on route of transmission were unavailable for 20 percent. Again, the rates of resistance reflect the patterns of drug availability over the course of the epidemic. The rate of resistance (to any drug) was 5.5 percent in Africa, 7.4 percent in East Asia, 5.7 percent in Southeast Asia, and 6.4 percent in Latin America, lower than in North America (11.4 percent) and Europe (10.6 percent). WATCH data suggest that the use of mono- and dual therapy in the developed world resulted in extensive transmission of NRTI resistance; Western regions had higher levels of resistance to NRTIs than to NNRTIs or PIs, whereas developing regions tended to have a relatively equal distribution of resistance over the three drug classes. Multi-class resistance was very low in both east and Southeast Asia (close to zero) compared to 3.1 percent in North America. The WATCH authors also calculated odds ratios - they were higher for NNRTI resistance in Africa (1.67) and Latin America (1.93) than in Europe, but these results did not meet conventional levels of statistical significance ($p=0.16$ and 0.09 , respectively).

In 2005, a paper by Vella and Palmisano presented previously published data on the prevalence of resistance in recently infected persons in various countries. The analysis included

only one study from the developing world; it showed no drug resistance in 99 patients from Africa's Ivory Coast.

In sum, according to published overviews, the patterns of resistance world-wide appear to reflect trends in use of ARV medications. In developed areas, where medications were available to many HIV patients, rates of resistance to NNRTI ranged from three to four percent. In developing regions with limited resources, the rates of resistance were much lower among treatment naïve persons. Resistance to NRTIs ranged from seven to eight percent in the developed world.

Treatment-naïve Persons in Developing Regions

Tables 1 through 4 show the prevalence of drug resistance in treatment-naïve persons in developing countries. Because the studies are heterogeneous with respect to sample size, sampling criteria, selection for testing, type of assessment conducted, time from seroconversion, and population characteristics, they report conflicting results. Nevertheless, we believe this overview is an important starting point for discussion.

Of the 30 studies retrieved, 25 reported clade data, although some of the studies grouped results from different clades together. Three studies included patients in India, three included patients across Asia, nine included patients in Latin America, and fifteen included patients in Africa. Data were collected between 1995 and 2004. The majority conducted genotypic testing only.

India

In recent years, ARV treatment in India has expanded. However, studies on resistance are quite rare. We found only three studies conducted in India that reported resistance in treatment-naïve HIV positive persons, all published in peer-reviewed journals (Table 1). All studies conducted genotypic testing; none conducted phenotyping. None of the studies stratified data by mode of transmission, and none estimated time from seroconversion to resistance testing. Each study tested consecutive patients entering treatment.

One study¹⁹ from North India assessed 60 men, women, and children from 2000-2002 using a sensitive nested amplification refractory mutation (ARMS) PCR test as well as a sequence-based mechanism. Of the NRTI mutations, about 32 percent occurred at codon 184, 80 percent at codon 70, and fewer than 2 percent at codon 215. NNRTI and PI mutations were not reported. Most participants showed a mixture of wild-type and mutant virus. CD4 count and gender were not statistically associated with mutations. Frequency of mutations at codon 70 was higher in adults than children.

A second study²⁰, collected data on 50 male and female adults in South India in 2002 and 2003. All were infected with HIV clade C. Fourteen percent were resistant to NNRTIs and 6 percent were resistant to NRTIs. The most common PI mutations were naturally occurring polymorphisms, including M36, L63, and I93. Twenty percent had PI mutations at major positions; the most common was V82.

The final study collected data in 2003 from 128 participants in Mumbai (mean age 30.4 years).²¹ Over 96 percent were infected with HIV clade C. Two participants were resistant to

NRTIs, as the M184V mutation was present. Polymorphisms such as M36 and L63 were common, but no major PI mutations were reported.

In sum, the populations assessed were very small compared to the number of persons living with HIV in India. We are not sure how well they represent the regional populations. Results varied, and data were not usually stratified by variables of interest. More research on ARV resistance in India is recommended.

Table 1. Treatment Naïve Data - India

Reference	Country	Population	Data collected	N	Clade	When assessed	Type of assessment	NNRTI	NRTI	PI	Any
Sachdeva, 2005 ¹⁹	India	Children, women, men	2000-2002	60	B,C	NR	Genotypic other, Nested PCR	NR	31.67% at 184, 1.67% at 215, 80% at 70	NR	
Balakrishnan P, 2005 ²⁰	India	Women, men	2002-2003	50	C	NR	Genotypic sequence-based mechanism, Nested PCR	14%	6%	100% at minor positions, 20% at major positions	
Deshpande A, 2004 ²¹	India	Population NR	2003	128	(96.1%) C, (2.3%) A/C	NR	Genotypic sequence-based mechanism	NR	1.6%	0% at major positions	

Other Developing Nations in Asia

We found only three studies that reported resistance patterns in treatment-naïve persons in other developing regions of Asia (we excluded Japan, Singapore, and Hong Kong) (Table 2). The first two studies did not stratify results by gender; none of the studies stratified by mode of transmission. All studies conducted genotypic testing; no phenotyping was conducted. All were published in peer-reviewed journals.

The first study²² reported data for 50 adults, including MSMs and IDUs, in South Korea. Data were collected between 1998 and 2002. It is unclear how participants were selected for testing. Ninety-four percent of the sample was infected with HIV clade B. Participants were tested for resistance an average of 2 months after testing positive for HIV. None were resistant to NNRTIs, 6.5 percent were resistant to NRTIs, and 2.6 percent were resistant to PIs. The most common NRTI mutations were D67N, K70R, V118I, T215F, and T219Q.

Another study²³ reported data on 200 adults and adolescents in Vietnam; 42.5 percent were IDUs. Data were collected in 2001 and 2002. HIV clade was primarily CRF01_AE. None of the participants were resistant to NNRTIs; 4.5 percent were resistant to NRTIs, and 2.0 percent were resistant to PIs. The most common NRTI mutations were M41L, K219Q, and M184I. PI mutations found were D30N and L90M.

The last study²⁴ collected data on all pregnant women (N = 124) and other adults (N=22) who consecutively entered a treatment program in Cambodia in 2003 and 2004. They were tested for resistance within 1 year of a known date of seroconversion. HIV clade was primarily CRF01_AE. NRTI mutations were found in 3.4 percent; the most common mutations were K70R, V75M, and K101E. Major PI mutations were found in two participants (1.4 percent); one had M46I, the other N88D.

The samples in these studies were very small compared to the numbers of persons living with HIV in the region. Resistance data were not stratified by HIV risk factors, although that information was collected in at least two of the studies. Rates of resistance were fairly low among both clade B and recombinant clade CRF01_AE; rates were similar to those reported in Southeast Asia by the WATCH study. {Bowles, Wensing, et al. August 11-12 #2102}⁹⁸ The specific mutations in the clade B South Korean participants were quite different from those found in the Southeast Asian CRF01_AE participants.

Table 2. Treatment-Naïve Data – Other Nations of Asia

Reference	Country	Population	Data collected	N	Clade	When assessed	Type of assessment	NNRTI	NRTI	PI	Any
Park, S W, 2003 ²²	South Korea	IDU , MSM, women, other men	1998-2002	50	94% B, 4% A, 2% G	(median) 2 months after HIV infection documented	Genotypic sequence-based mechanism	0%	6.40%	2.60%	8.0%
Lan N T, 2003 ²³	Viet Nam	IDU, adolescents (12-18 yrs), women, men	2001-2002	200	99% CRF01_AE	NR	Genotypic sequence-based mechanism	NR	4.5%	2.0%	6.5%
Ly N, 2005 ²⁴	Cambodia	Pregnant women, other adults	2003-2004	146	99% CRF01_AE	Known date of seroconversion of less than 1 year	Genotypic sequence-based mechanism	NR	3.4%	1.4%	4.9%

Latin America

Nine studies reported data on resistance to ARV medications among treatment-naïve populations in Latin America. We found one study from Argentina, three from Brazil, one from Peru, one from Venezuela, and two from Mexico (one study⁹⁹ combined data from several countries—Brazil, Portugal, Ivory Coast, Nigeria, Guinea Bissau, Lebanon, and the U.S. —but did not stratify data by region or HIV clade, so it was excluded). Data were collected between 1997 and 2004. None of the studies reported time from seroconversion to assessment. The Peru and Argentina studies did not report HIV clade, nor did one of the studies from Mexico. None of the studies stratified resistance data by gender or mode of transmission, although one study included only MSM. All studies conducted genotypic assessment; two conducted additional phenotyping. Many studies did not report how participants were selected for resistance testing; statements were often vague as to whether all, or only a sample of patients entering treatment, were tested.

The study from Argentina²⁵ assessed resistance among adults, some of whom were IDUs. HIV clade was not reported. The authors compared persons with *primary* infection (N=13) to those with *established* infection (N=86). Of those with primary infection, one person was resistant to NRTIs and one was resistant to PIs. Of those with established infection, 1.2 percent had mutations to NRTIs and 1.2 percent had primary PI mutations (83.7 percent had secondary PI mutations). Line probe assays (LiPA), which identify specific mutations by a reverse hybridization strategy, were conducted on 52 of the established infection samples;¹⁰⁰ 11.5 percent were resistant to NRTIs.

In Brazil, a study of 409 treatment-naïve women (mostly infected with HIV clade C), MSMs, IDUs, and others showed very low resistance levels.²⁶ Of the group, 2.06 percent were resistant to NNRTIs, 2.36 percent were resistant to NRTIs, and 2.24 percent were resistant to PIs. Another study of 49 adults, mostly infected with clade B HIV, reported that none were resistant to NNRTIs, 2.0 percent were resistant to NRTIs, and 85 percent had secondary PI mutations.²⁷ Another study of 50 adults (mostly infected with clade B), including MSMs, reported that 14 percent were resistant to NRTIs and 85.7 percent had secondary PI mutations.²⁸ The secondary mutations in these studies were most likely naturally occurring polymorphisms with no known relationship to resistance.

A study of MSM in Peru²⁹ compared resistance in 359 treatment-naïve and 16 treatment-experienced patients. Resistance to drugs was low among the treatment naïve – 3.3 percent overall, compared to 31.3 percent overall among the treatment experienced. HIV clade was not reported.

A United Nations (UN) AIDS clinic in Venezuela³⁰ reported data on adults including IDUs, MSM, and women. All but two persons had HIV clade B. The authors compared resistance rates of PI-naïve (N=56) and PI-experienced (N=44) patients. Some of the PI-naïve patients had experience with mono- and dual therapy. The rate of resistance was low among the PI naïve – only one person was resistant to any drug. Among the PI-experienced patients, 40.9 percent were resistant to NRTIs and 9.1 percent were resistant to PIs.

Finally, studies in Mexico reported that 5.0 percent of 20 adults infected with clade B who enrolled in treatment at a Jalisco clinic were resistant to PIs,³¹ while of 45 patients who enrolled for treatment in Central Mexico, 11.0 percent were resistant to NRTIs and 2.0 percent were resistant to PIs.³² Again, stratified data were not reported.

In sum, rates of resistance to NRTIs ranged from 2.0 percent to 14 percent among treatment-naïve groups in Latin America. Rates of resistance to NNRTIs were low, ranging from zero to two percent. Rates of PI polymorphisms were high in Brazil and Argentina, presumably among persons infected with HIV clade B. Resistance data were rarely stratified by mode of transmission or gender, although those data were available in many cases.

The number of persons tested for resistance in these studies is small relative to the number of people taking HIV ARVs in Latin America. Some studies tested all consecutive patients entering clinic^{26,29}, while others did not explicitly state how participants were selected. Therefore, it is difficult to judge how representative the data are. We found no published reports of resistance rates in Central American countries, despite the high HIV prevalence rates in some of those nations.

Table 3. ARV resistance in treatment-naïve patients in Latin America

Reference	Country	Population	Data collected	N	Clade	When assessed	Type of assessment	NNRTI	NRTI	PI	Any
Kijak, 2001 ²⁵	Argentina	IDU, women, men - with <i>established</i> HIV infection	1997-2000	86	NR	NR	Genotypic sequence-based mechanism and probe based system		1.20%	(1.2%) primary, (83.7%) secondary	
		IDU, women, men - subset of above, with LiPA tests	1997-2000	52	NR	NR	Genotypic sequence-based mechanism and probe based system		11.54%		
		IDU, women, men - with <i>primary</i> HIV infection	1997-2000	13	NR	NR	Genotypic sequence-based mechanism and probe based system		7.69%	7.69%*	
Dumans A T, 2002 ²⁷	Brazil	Population NR	1998	49	Mostly B	NR	Genotypic sequence-based mechanism	0%	2%	85% (secondary)	
Pires I L, 2004 ²⁸	Brazil	Women, MSM	2000-2002	50	Mostly B	NR	Genotypic sequence-based mechanism		14%	0% (primary), 85.7% (secondary)	
Brindeiro R M, 2003 ²⁶	Brazil	Women, IDU, MSM	2001	409	(Mostly) C and F	NR	Genotypic sequence-based mechanism, Nested PCR	2.06%	2.36%	2.24%	

NR = Not Reported; IDU = Injection Drug Users; MSM = men who have sex with men; * represents primary PI mutations.

Reference	Country	Population	Data collected	N	Clade	When assessed	Type of assessment	NNRTI	NRTI	PI	Any
Lama, 2005 ²⁹	Peru	Tx-naïve - MSM, other adults	2002-2003	359	NR	NR	Genotypic sequence-based mechanism				3.3%
		Tx-experienced - MSM, other adults	2002-2003	16	NR	NR	Genotypic sequence-based mechanism				31.3%
Andrade-Villanueva, 2004 ³¹	Mexico	Women, men	2003-2004	20	B		Genotypic sequence-based mechanism			5%	
Delgado E, 2001 ³⁰	Venezuela	PI naïve; Women, IDU, MSM	NR	56	Primarily B	NR	Genotypic sequence-based mechanism, Nested PCR	1.80%	1.8%	1.8%	
	Venezuela	PI treated; Women IDU, MSM	NR	44	Primarily B	NR	Genotypic sequence-based mechanism, Nested PCR	0%	40.9%	9.1%	
Fuentes-Romero, 2004 ³²	Mexico	Population NR	NR	45	NR	at enrollment	Genotypic sequence-based mechanism	0%	11%	2%	

Africa

We found 14 studies that reported resistance data on treatment-naïve populations in sub-Saharan Africa. They are displayed in Table 4. Data were collected between 1995 and 2002. Studies were conducted in Cameroon, Senegal, Republic of Congo, Ivory Coast, Zambia, Gabon, Ghana, Malawi, South Africa, Zimbabwe, and Botswana; all reported HIV clade. The most common clades were CRF02 AG, which predominates in west Africa, and C, which is predominant in southern Africa. All studies conducted genotypic testing; three conducted additional phenotypic assessments³³⁻³⁵. Two studies included only pregnant women^{36,37}; another included only men³⁵. The other studies did not stratify results by gender. None of the studies stratified results by mode of transmission; the majority of studies described their participants as heterosexual adults.

Of the studies that described participant selection, two studies^{38,39} tested random samples of patients, and one study tested all pregnant women entering a clinical trial.³⁷ The other studies did not clarify how they selected participants for resistance testing. Many implied that all incoming HIV positive persons were tested; however, none explicitly stated this.

Regarding treatment-naïve pregnant women, one study³⁶ performed genotypic testing on 28 women who entered treatment in Zambia in 2000; 92.8 percent of the women were infected with HIV clade C. None had any primary mutations; 89.3 percent had secondary NRTI mutations, and all had secondary PI mutations. Most secondary mutations were polymorphisms. Testing for RT mutations was also performed on samples from 37 pregnant women who entered a clinical trial in South Africa the same year.³⁷ No women had resistance to NRTIs or NNRTIs; all were infected with clade C.

The study that included only males³⁵ found that although none of the 37 Ghanaian men (mostly infected with mostly clade CRF02 AG) had primary PI mutations, secondary mutations were “commonly observed.” Exact figures were not reported. Testing for RT mutations was not performed.

Two studies compared resistance among small samples of people infected with rarer HIV clades. Vergne⁴⁰ reported data collected from 1995 to 1999 in Cameroon, Senegal, the Congo, and France. Regarding NNRTIs, one of five samples from participants infected with type J showed resistance, as did all four type O samples, compared to none of the type A, B, C, D, F, G, K, or recombinant types. Other than one participant infected with clade B, none of the others were resistant to NRTIs. No participants had primary PI mutations; all samples except those from participants infected with clade B and D had secondary PI mutations. Another study³⁹ reported on resistance among participants infected with clades A, C, D, F2, G, H, and J in addition to the dominant CRF02-AG clade. Data were collected from adults and adolescents in Cameroon from 2000 to 2002. All samples had secondary PI mutations. Tests for RT mutations were not reported.

Many of the studies conducted among individuals with highly prevalent HIV clades showed a high rate of secondary PI mutations in CRF02-AG. The same was true for HIV clade C. Rates of resistance to NNRTIs ranged from 0 percent to 7.7 percent in clade C-infected individuals and 0 percent to 2 percent in those infected with CRF02-AG. Resistance to NRTIs also ranged from 0 percent to 7.7 percent in clade C and from 0 percent to 5.1 percent in CRF02-AG.

In summary, rates of NNRTI resistance ranged from 0 percent to 7.7 percent in most HIV clades. Prevalence was higher in clades B and O in one study, but samples were too small to allow generalization to the population at large. NRTI resistance rates ranged from 0 percent to 8

percent for all clades. Primary PI mutations were rare (<3 percent) among Africans. There were high levels of secondary PI mutations reported in most studies; there is no evidence that these affect resistance in the absence of primary mutations. It was unclear how some studies selected persons for genetic testing and most studies had small samples. Therefore, the generalizability of the data is limited.

Table 4. ARV Resistance Among Treatment-Naïve Patients in Africa

Reference	Country	Population	Data collected	N	Clade	When assessed	Type of assessment	NNRTI	NRTI	PI	Any
Vergne L, 2000 ⁴⁰	Cameroon, Senegal, the Democratic Republic of Congo, France	NR	1995-1999	9	A	NR	Genotypic sequence-based mechanism	0%	0% (primary)	0% (primary)	
		NR	1995-1999	13	B	NR	Genotypic sequence-based mechanism	0%	8% (primary)	0% (primary)	
		NR	1995-1999	2	C	NR	Genotypic sequence-based mechanism	0%	0% (primary)	0% (primary)	
		NR	1995-1999	5	D	NR	Genotypic sequence-based mechanism	0%	0% (primary)	0% (primary)	
		NR	1995-1999	11	F	NR	Genotypic sequence-based mechanism	0%	0% (primary)	0% (primary)	
		NR	1995-1999	4	G	NR	Genotypic sequence-based mechanism	0%	0% (primary)	0% (primary)	
		NR	1995-1999	2	K	NR	Genotypic sequence-based mechanism	0%	0% (primary)	0% (primary)	
		NR	1995-1999	5	J	NR	Genotypic sequence-based mechanism	20%	0% (primary)	0% (primary)	

NR = Not Reported

Reference	Country	Population	Data collected	N	Clade	When assessed	Type of assessment	NNRTI	NRTI	PI	Any
Vergne L, 2000 ⁴⁰ – cont'd		NR	1995-1999	67	CRF02_AG	NR	Genotypic sequence-based mechanism	0%	0% (primary)	0% (primary)	
		NR	1995-1999	3	CRF01_AE	NR	Genotypic sequence-based mechanism	0%	0% (primary)	0% (primary)	
		NR	1995-1999	6	A/G	NR	Genotypic sequence-based mechanism	0%	0% (primary)	0% (primary)	
		NR	1995-1999	2	G/A/G	NR	Genotypic sequence-based mechanism	0%	0% (primary)	0% (primary)	
		NR	1995-1999	2	G/K	NR	Genotypic sequence-based mechanism	0%	0% (primary)	0% (primary)	
		NR	1995-1999	1	D/G/D	NR	Genotypic sequence-based mechanism	0%	0% (primary)	0% (primary)	
		NR	1995-1999	4	?/K	NR	Genotypic sequence-based mechanism	0%	0% (primary)	0% (primary)	
		NR	1995-1999	2	unknown	NR	Genotypic sequence-based mechanism	0%	0% (primary)	0% (primary)	
		NR	1995-1999	4	O	NR	Genotypic sequence-based mechanism	100%	0% (primary)	0% (primary)	

Reference	Country	Population	Data collected	N	Clade	When assessed	Type of assessment	NNRTI	NRTI	PI	Any
Petch L A, 2005 ⁴¹	Malawi	NR	1996-2001	21	C	NR	Genotypic sequence-based mechanism	0%	0%(major), 100%(minor)	0% (primary)	
Toni T, 2002 ³³	Ivory Coast	Primarily men, heterosexual	1997-2000	99	83% CRF02_AG, 9% A, 4% CRF06	median - 9 mos from seroconversion	Genotypic sequence-based mechanism	0%	0%	0% (primary)	
Harrigan, 2001 ³⁴	South Africa	NR	1997	358	B and C	NR	Phenotypic VIRCO	< 2%	< 2%	< 2%	
Laurent, 2002 ⁴²	Senegal	Women, men	1998-2000	39	Mostly CRF02_AG, O, A, B, C, G, G/K		Genotypic sequence-based mechanism		5.1%	2.6%	
Handema R, 2003 ³⁶	Zambia	Pregnant women	2000	28	92.8% C	NR	Genotypic sequence-based mechanism	0%	89.3% (secondary), 0% (primary)	0% (primary)	
Konings F A, 2004 ³⁹	Cameroon	Adolescents (12-18 yrs), women, men	2000-2002	14	A	NR	Genotypic sequence-based mechanism			100% (secondary)	
		Adolescents (12-18 yrs), women, men	2000-2002	77	CRF02-AG	NR	Genotypic sequence-based mechanism			100% (secondary)	
		Adolescents (12-18 yrs), women, men	2000-2002	3	C	NR	Genotypic sequence-based mechanism			100% (secondary)	

Reference	Country	Population	Data collected	N	Clade	When assessed	Type of assessment	NNRTI	NRTI	PI	Any
Konings F A, 2004 ³⁹ - cont'd		Adolescents (12-18 yrs), women, men	2000-2002	8	D	NR	Genotypic sequence-based mechanism			100% (secondary)	
		Adolescents (12-18 yrs), women, men	2000-2002	4	F2	NR	Genotypic sequence-based mechanism			100% (secondary)	
		Adolescents (12-18 yrs), women, men	2000-2002	8	G	NR	Genotypic sequence-based mechanism,			100% (secondary)	
		Adolescents (12-18 yrs), women, men	2000-2002	1	H	NR	Genotypic sequence-based mechanism			100% (secondary)	
		Adolescents (12-18 yrs), women, men	2000-2002	7	J	NR	Genotypic sequence-based mechanism			100% (secondary)	
Vergne L, 2002 ⁴³	Gabon	Tx naïve adults	2000	13	CRF02_A G, A, G	NR	Genotypic sequence-based mechanism	7.7%	7.7%	100% (secondary)	
		ARV treated adults	2000	19	CRF02_A G, A, D	NR	Genotypic sequence-based mechanism	5.3%	78.9%	5.3% (primary), 94.7% (secondary)	

Reference	Country	Population	Data collected	N	Clade	When assessed	Type of assessment	NNRTI	NRTI	PI	Any
Kinomoto M, 2005 ³⁵	Ghana	Adult males	2001-2002	39	Most CRF02_AG		Genotypic sequence-based mechanism, Phenotypic assay			No primary mutation observed, secondary commonly observed	
Bussman, 2005 ³⁸	Botswana	Women, men	2001	70	C	NR	Genotypic sequence-based mechanism	2.00%	0%	100%	
Toni T D, 2003 ⁴⁴	Ivory Coast	Pregnant women, women, men	2001-2002	107	(mostly)CRF02_AG, A, K, CRF06_CPX	NR	Genotypic sequence-based mechanism	4%	1%	1% (primary), 94% of CRF02_AG (secondary)	5.6%
Bessong P, 2005 ⁴⁵	South Africa	NR	NR	40	C	NR	Genotypic sequence-based mechanism			0% (primary), >90% (secondary)	
Doualla-Bell, 2005 ⁴⁶	Botswana	Tx-naïve adults	NR	33	C	NR	Genotypic sequence-based mechanism			>39% (secondary)	
		PI-treated adults	NR	15	C	NR	Genotypic sequence-based mechanism			>60% (secondary), primary commonly exists	
Pillay C, 2002 ³⁷	South Africa	Pregnant Women	2000	37	C	Before entering clinical trial	Genotypic sequence-based mechanism; Phenotypic assay	0%	0%	NR	

Pregnant Women and Their Children in Developing Countries

Overview

We identified 31 studies in developing countries that addressed the emergence of ARV resistance in women subsequent to the administration of ARV therapy to prevent mother to child transmission (pMTCT) of HIV-1 infection. These studies were heterogeneous in terms of the duration of ARV treatment, the selection of the ARV regimen, the timing and frequency with which ARV resistance tests were done, the sensitivity of the assay for ARV resistance, and whether clade-specific data were reported. Many studies were reported only as conference abstracts; these studies tend to include less information than articles in peer-reviewed journals.

In this section, we review the data on the prevalence of resistance in this group according to the prophylactic regimen, the infecting viral clade, the timing of the resistance assay (i.e., how long after the cessation of ARV therapy), and the assay that was used to detect ARV resistance.

Resistance and pMTCT regimen

Single dose Nevirapine (SD-NVP) alone. The greatest number of evaluable reports studied the impact of SD-NVP alone on the emergence of resistance (N= 14). Several of these reports provided data derived from the HIV-1NET 012 trial, which left three evaluable reports that provided non-overlapping data from the study,⁴⁷⁻⁴⁹ two reports that provided non-overlapping data from one South African study,⁵⁰⁻⁵¹ and two reports that provided non-overlapping data from another South African study.⁵²⁻⁵³ All studies were done in sub-Saharan Africa.

The earliest time that resistance was assessed was 7 days postpartum.⁴⁷ Most studies assessed resistance 6 – 8 weeks postpartum (N = 10). Two studies evaluated the prevalence of resistance approximately 6 months postpartum. Eleven of the fourteen reports provided information by viral clade and three did not. The rate of NNRTI resistance 6 to 8 weeks post SD-NVP ranged from 8.5 percent to 69.2 percent.

Table 5 shows the results of standard genotypic resistance assays in the main evaluable studies. Studies that address specific questions in subpopulations of the main study (e.g., data comparing the results of highly sensitive assays for the detection of ARV resistance with standard genotypic resistance assays or studies of resistance over time¹³) are not included in this table.

Table 5. NNRTI Resistance among pregnant women given single-dose nevirapine alone

Reference	Country	When data collected	N	Clade	When assessed (weeks post-partum)	Type of assessment	NNRTI Resistance
Eshleman, 2005 ⁴⁸	Uganda	1997-1999	83	A	1	Genotypic sequence-based mechanism	24.0%
Eshleman, 2005 ⁴⁸	Uganda	1997-1999	57	D	1	Genotypic sequence-based mechanism	19.0%
Loubser, 2004 ⁵⁴	South Africa	NR	35	C ^b	2	Genotypic sequence-based mechanism	71.0%
Kantor, 2003 ⁵⁵	Zimbabwe	NR	28	C	2	Genotypic sequence-based mechanism	75%
Toni, 2005 ⁵⁹	Cote d'Ivoire	NR	29	CFR02-AG*	4	Genotypic sequence-based mechanism	20.7%
Lehman, 2005 ¹⁰¹	Kenya	NR	30	NR	4	Genotypic other, Allele-specific RT-PCR assay	40.0%
Mcintyre, 2005 ⁵⁰	South Africa	NR	68	NR	6	Genotypic sequence-based mechanism	57.0%
Loubser, 2006 ⁵⁸	South Africa	NR	66	C ^{***}	6	Genotypic sequence-based	54.0%

* 7% of isolates clade A

** 4 participants were not infected by HIV-1 clade C

*** 1 subject infected by HIV-1 clade A

**** 23% of participants were infected by HIV-1 clades D, 3% by clade C and 15% by recombinant clades

^a 61 women plus 10 infected infants; duration of NVP usage not clearly stated

^b 1% of participants were not infected by HIV-1 clade C

^c 2% of isolates were not infected by HIV-1 clade C

Reference	Country	When data collected	N	Clade	When assessed (weeks post-partum)	Type of assessment	NNRTI Resistance
						mechanism	
Morris, 2003 ⁵⁶	South Africa	2002	141	C	6	Genotypic sequence-based mechanism	8.5%
Gordon, 2004 ⁵⁷	South Africa	NR	30	C ^c	6	Genotypic sequence-based mechanism	40.0%
Loubser, 2004 ⁵⁴	South Africa	NR	164	C ^b	6	Genotypic sequence-based mechanism	48.0%
Troyer, 2005 ⁶⁰	Uganda	NR	71 ^a	A****	6	Genotypic other, Radio labeled oligonuc. DNA ligation (OLA)	16.0%
Martinson, 2004 ⁵²	South Africa	NR	456	C**	7	Genotypic other, NR	38.8%
Eshleman, 2005 ⁴⁹	Uganda	1997-1999	97	D	6 to 8	Genotypic sequence-based mechanism	36.1%
Eshleman, 2005 ⁴⁹	Uganda	1997-1999	144	A	6 to 8	Genotypic sequence-based mechanism	19.4%
Eshleman, 2005 ⁴⁹	Malawi	1997-1999	65	C	6 to 8	Genotypic sequence-based mechanism	69.2%

Reference	Country	When data collected	N	Clade	When assessed (weeks post-partum)	Type of assessment	NNRTI Resistance
Kantor, 2003 ⁵⁵	Zimbabwe	NR	32	C	8	Genotypic sequence-based mechanism	34%
Eshleman, 2004 ⁶¹	Uganda	1997-1999	28	Recombinant	6 to 8	Genotypic sequence-based mechanism	17.9%
Author not available, 2003 ¹⁰²	Zimbabwe	NR	33	NR	8		24.2%
Kantor, 2003 ⁵⁵	Zimbabwe	NR	8	C	24	Genotypic sequence-based mechanism	13%
Morris, 2004 ⁵³	South Africa	NR	155	C**	26	Genotypic sequence-based mechanism	35.0%

Analysis of SD-NVP resistance data by clade. Thirteen studies of SD-NVP contrasted the emergence of ARV resistance in mothers infected by differing clades of HIV-1. Aside from clade C, for which 8 reports provided information regarding the emergence of resistance in seven clinical trials (data from one South African study of persons infected by clade C were provided in two reports^{52 53}), no other clade was analyzed in more than two clinical trials.

As shown in Table 6, the reported prevalence of NNRTI-resistance as assessed by standard genotypic resistance tests, is highly variable, ranging from over 70 percent at 2 weeks postpartum^{54 103} to 8.5 percent at 6 weeks postpartum in one study performed in South African women.⁵⁶ Many studies showed a decline in resistance over time; this phenomenon will be discussed in more detail in a separate section.

Table 6. Resistance among pregnant women infected with HIV clade C after single-dose nevirapine

Reference	N	Clade	When assessed (weeks post-partum)	Type of assessment	NNRTI Resistance
Loubser, 2004 ⁵⁴	35	C*	2	Genotypic sequence-based mechanism	71.0%
Kantor, 2003 ⁵⁵	28	C	2	Genotypic sequence-based mechanism	75%
Morris, 2003 ⁵⁶	141	C	6	Genotypic sequence-based mechanism	8.5%
Gordon, 2004 ⁵⁷	30	C****	6	Genotypic sequence-based mechanism	40.0%
Loubser, 2004 ⁵⁴	164	C*	6	Genotypic sequence-based mechanism	48.0%
Loubser, 2006 ⁵⁸	66	C***	6	Allele-specific real-time PCR assay	54.0%
Martinson, 2004 ⁵²	456	C**	7	Genotypic other, NR	38.8%
Eshleman, 2005 ⁴⁹	65	C	6 to 8	Genotypic sequence-based mechanism	69.2%
Kantor, 2003 ⁵⁵	32	C	8	Genotypic sequence-based mechanism	34%
Kantor, 2003 ⁵⁵	8	C	24	Genotypic sequence-based mechanism	13%
Morris, 2004 ⁵⁵	155	C**	26	Genotypic sequence-based mechanism	35%

* 1% of participants were not infected by HIV-1 clade C

** 4 participants were not infected by HIV-1 clade C

*** 1 subject was infected by HIV-1 clade A

****2% of participants were not infected by HIV-1 clade C

Following SD-NVP, the prevalence of ARV resistance in women infected by HIV clades other than clade C ranged from 16 percent (6 weeks postpartum, clade A⁶⁰) to 36 percent (6 – 8 weeks postpartum, clade D⁴⁷). A summary of results from studies of ARV resistance following administration of SD-NVP for pMTCT to women infected by other clades of HIV-1 is provided in Table 7.

Table 7. ARV resistance after single-dose nevirapine, among women infected with other HIV clades

Reference	N	Clade	When assessed (weeks postpartum)	Type of assessment	NNRTI Resistance
Eshleman, Sept 2005 ⁴⁸	83	A	1	Genotypic sequence-based mechanism	24.0%
Eshleman, Sept 2005 ⁴⁸	57	D	1	Genotypic sequence-based mechanism	19.0%
Toni, 2005 ⁵⁹	29	CFR02-AG*	4	Genotypic sequence-based mechanism	20.7%
Troyer, 2005 ⁶⁰	71**	A	6	Genotypic other, Radio labeled oligonuc. DNA ligation (OLA)	16.0%
Eshleman, July 2005 ⁴⁹	144	A	6 to 8	Genotypic sequence-based mechanism	19.4%
Eshleman, July 2005 ⁴⁹	97	D	6 to 8	Genotypic sequence-based mechanism	36.1%
Eshleman, Feb 2004 ⁶¹	28	Recombinant	6 to 8	Genotypic sequence-based mechanism	17.9%

* 93% infected by CFR02-AG, 7% by clade A

** Resistance results are from 61 women plus 10 infected infants; 23% of patients were infected by HIV-1 clades D, 3% by clade C and 15% by recombinants

One formal cross-clade analysis of the prevalence of ARV resistance in female recipients of SD-NVP was identified. Using the results of the HIV-1NET 012 and NVAZ trials, Eshleman demonstrated that the proportion of women with clade C with at least one NVP resistance mutation (69 percent) 6 – 8 weeks postpartum was significantly higher than found in women infected by clade A (19 percent, $p < 0.0001$) or clade D (36 percent, $p < 0.001$).⁴⁹ In the HIV-1NET 012 trial, nevirapine resistance was greater in clade A than clade D.

Specific NNRTI amino acid changes associated with resistance in persons receiving SD-NVP. Complex patterns of NNRTI resistance were observed following the receipt of SD-NVP. The most common mutations that resulted in amino acid changes were K103N (lysine to asparagine at position 103) and Y181C (tyrosine to cysteine). Y181C was the most commonly detected resistance mutation in plasma collected 2 weeks postpartum from women infected by

HIV-1 clade C.^{54,55} In contrast, the K103N resistance mutation was most common at later time points in persons infected by clade C and at all times in persons infected by other HIV-1 clades. Among clade-C women from South Africa, V106/A/M (valine to alanine or methionine) was the most common amino acid change.⁵⁴ One report found that the rates of detection of K103N, Y181C, and Y188C were significantly higher in women infected by HIV-1 clade C than by HIV-1 clades A or D.⁴⁹ Still, there has not been enough research to assess whether there are clade-specific differences in the prevalence of resistance.

The findings from studies that provided data on the prevalence of various NNRTI resistance mutations are displayed in Table 8.

Table 8. Specific mutations observed in mothers receiving single-dose nevirapine

Reference	N	Clade	Weeks Post-partum	Any	Proportion of women with specific, indicated resistance mutation								
					K103N	Y181C	Y188C	G190A	V106A/M	K101E	V108A/I	L100I	P236L
Loubser, 2004 ⁵⁴	35	C	2	71%	29%	49%	34%	23%	43%				
Kantor, 2003 ⁵⁵	28	C	2	75%	25%	57%	14%		19%				
Loubser, 2004 ⁵⁴	164	C	6	48%	31%	23%	20%	14%	15%				
Gordon, 2004 ⁵⁷	30	C	6	40%	33%	10%	10%	3%	7%				
Eshleman, 2005, ⁴⁹	65	C	6 - 8	69%	58%	32%	17%	9%	3%	2%	2%		
Kantor, 2003 ⁵⁵	32	C	8	34%	28%	6%			6%				
Eshleman, 2005, ⁴⁹	144	A	6 - 8	19%	17%	6%	1%	4%	1%	1%	0%		
Eshleman, 2005, ⁴⁹	98	D	6 - 8	36%	29%	16%	3%	6%	0%	4%	1%		
Toni, 2005 ⁵⁹	29	CRF02_AG	4	21%	10%				3%	3%		3%	3%
Eshleman, 2004 ⁶¹	28	Recomb	6 - 8	18%	14%	7%				4%			
Shapiro, 2006 ^{71*}	155	NR	4	45%	33%	8%	1%	5%	8%				

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* mothers also received ZDV for one month. Abbreviations: A=alanine, C=cysteine, E=glutamic acid; G=glycine, I=isoleucine, K=lysine, L=leucine, M=methionine, N=asparagine, P=proline, V=valine, Y=tyrosine.

Effect of single-dose nevirapine on resistance over time. A number of considerations suggest that the prevalence of readily detectable ARV resistance, particularly resistance that results from the use of NNRTIs, may vary after the discontinuation of ARV administration. First, significant serum concentrations of NVP may persist for prolonged periods of time after a single dose, leading to delayed (i.e., beyond 7 days) emergence of resistance. The prolonged presence of subtherapeutic serum concentrations may lead to ongoing selection of mutants for 2 to 3 weeks. Conversely, if resistant virus is less fit than wild type virus, the prevalence of readily detectable resistant virus may gradually decline. Therefore, resistance testing done immediately after the administration of single dose NVP may underestimate the subsequent emergence of NNRTI-resistance due to this treatment strategy, whereas delayed ARV resistance testing may underestimate the prevalence of previously detectable resistance.

Several studies, using a variety of assays including very sensitive ones, assessed the presence of NNRTI resistance over time among women who took SD-NVP to prevent mother-to-child transmission of HIV. Study data are presented in Table 9.

Eshleman⁴⁸ conducted genotypic testing on participants in randomized trial HIV-1 NET 012 at 7 days postpartum. She found that 24 percent of women infected with HIV clade A had NNRTI resistance, compared to 19 percent of women infected with clade D. The same women were tested at 6 to 8 weeks postpartum. At that point, 28 percent of clade A women had evidence of resistance, compared to 42 percent of clade D women. The authors reported rapid fading of variants with Y181C in clade A but not in clade D. In the developed world, studies in individuals infected with clade B have shown that Y181C confers high-level resistance to NVP and impairs viral fitness. These results suggest that Y181C may have less effect on viral fitness of clade D than on fitness of clade A. Eshleman⁴⁷ also did follow up on 11 women who had resistance at the 6- to 8-week time point. When genotyping was conducted between one and two years postpartum; none of the women had evidence of NNRTI resistance.

Flys¹⁰⁴ also reported recently on women from HIV-1 NET 012. (It is unclear how her sample overlaps with those in the previous publications.) She reported that 34.1 percent of 88 clade A participants had the K103N mutation at 6 to 8 weeks, compared to 53.6 percent of 56 clade D participants. Using follow-up tests and survival analysis, she estimated that the prevalence of the mutation declined to 3.6 percent in clade A and 29.8 percent in clade D at 2 years. By 4 years, she estimated that no clade A participants and only 2.4 percent of clade D participants had the mutation.

The prevalence and decline of the most common NVP mutant, K103N, were also examined using allele-specific PCR in 65 women infected with HIV clade C in South Africa⁵⁸ after SD-NVP. The rate of resistance, assessed through a highly sensitive assay, declined steadily over 1 year, from 87 percent at 6 weeks post-partum to only 11 percent at 1 year, according to RT-PCR of plasma RNA. PCR was used to detect cellular proviral DNA; these results were used to generate estimated rates of resistance of 52 percent at 6 weeks and only 4.2 percent at 1 year. Of note, the number of women available for testing at each follow-up ranged from 26 to 53 of the original 65. Samples were available at all time points for only 16 women.

We also found two conference abstracts that assessed the association of resistance with time following SD-NVP in a pMTCT cohort. Martinson⁵² followed up on 456 women; all but four were infected with HIV clade C. NNRTI resistance was defined as any of the following mutations: K103N, V106 A/M, Y181C, Y188C, and G190A. It appears that each woman was tested once for resistance within 36 weeks of birth. Compared to women tested within 10 weeks postpartum, far fewer women who were tested between 10 and 36 weeks showed evidence of

resistance (44 percent versus 24 percent, respectively). Baseline CD4 cell count, viral load, time from birth to resistance testing, and number of times a mother took NVP during pregnancy were significantly associated with development of resistance. Martinson also measured resistance in infants;⁶² the results of this assessment are discussed below in the section on infants.

Kantor⁵⁵ assessed resistance over time in 34 women in Zimbabwe who received SD-NVP. All were infected with HIV clade C. NNRTI resistance rates dropped from 75 percent at two weeks postpartum to 34 percent at 8 weeks and 13 percent at 24 weeks. (The number of women available for testing dropped from 32 at eight weeks to only eight at 24 weeks, limiting the validity of the findings.) Prevalence of the Y181C mutation dropped from 57 percent at two weeks to six percent at week eight, while the K103N mutation was present in 25 percent of women at 2 weeks and 28 percent at 8 weeks. Similarly, Loubser⁵⁴ studied a group of women in south Africa who had received SD-NVP; all but two were infected by HIV clade C. These investigators found that the prevalence of Y181C decreased from 49 percent at week 2 (N = 35) to 23 percent at week 6 postpartum (N = 164). The prevalence of the K103N mutation was 29 percent at 2 weeks and 31 percent at 6 weeks.

In sum, evidence provided by longitudinal analyses suggests that the prevalence of detectable NNRTI resistance may vary with time since treatment. Fading of variants with Y181C were reported in clade A but not in clade D. Prior studies show that Y181C confers high-level resistance to NVP and impairs viral fitness in persons infected by HIV clade B. Thus it is possible that Y181C may have less effect on viral fitness of clade D than of A. This observation warrants further investigation in future studies.

Table 9. Abatement of resistance over time in women taking single-dose nevirapine to prevent mother-to-child transmission

Reference	Country	N	HIV Clade	Type of assessment	NNRTI Resistance Results			
Martinson, 2004 ⁵²	South Africa	456	C	Genotypic other, NR	4-6 weeks 43% (N-unclear)	6-7 weeks 44% (N-unclear)	7-10 weeks 44% (N-unclear)	10-36 weeks 24% (N-unclear)
Eshleman, 2005 ⁴⁸	Uganda	65	A	Genotypic sequence-based mechanism	7 days 24%	6-8 weeks. 28%		
			D	Genotypic sequence-based mechanism	7 days 19%	6-8 weeks. 42%		
Eshleman, 2001 ⁴⁷	Uganda	311 in NVP arm	A, D	Genotypic sequence-based mechanism		6-8 weeks 19% (21 of 111 tested)	12-24 months 0 (of 11 of those w/resistance at 6-8 weeks, who were re-tested)	
Flys, 2007 ¹⁰⁴	Uganda	88	A	Lig Amp assay	6-8 weeks 34.1%	2 years 3.6%	3 years 1.3%	4 years 0%
		56	D	Lig Amp assay	6-8 weeks 53.6%	2 years 29.8%	3 years 18.9%	4 years 2.4%
Loubser, 2006 ⁵⁸	South Africa	67	C	Allele-specific real-time PCR	6 weeks 87% (27 of 31 RNA tests)	3 months 65% (17 of 26 RNA tests)	7 months 39% (14 of 36 RNA tests)	12 months 11% (6 of 53 RNA tests)
Kantor, 2003 ⁵⁸	Zimbabwe	34	C	Genotypic sequence-based mechanism	2 weeks 75% (21 of 28 tests)	8 weeks 34% (11 of 32 tests)	24 weeks 13% (1 of 8 tests)	
Loubser, 2004 ⁵⁴	South Africa	164 ^a	C ^b	Genotypic sequence-based mechanism	2 weeks 71% (25 of 35 tests)	6 weeks 48% (78 of 164 tests)		

^a 35 patients were studied 2 weeks after delivery and 164 patients were studied 6 weeks after delivery

^b 2 patients were infected by HIV clade A

Other pMTCT regimens, including therapy pre and post SD-NVP. Ten studies from the developing world were identified that studied the emergence of ARV resistance following other pMTCT regimens, including adding ARV therapy before or after SD-NVP. Two studies were done in Thailand,^{63,64} one was conducted in Argentina,⁶⁵ and the other eight studies were done in Sub-Saharan Africa. The findings are presented in Table 9.

In women using NRTIs alone,^{60,63,66,67} the rates of NRTI resistance ranged from zero to four percent. Rates of HIV transmission to infants varied by regimen. In a study of pregnant women taking ZDV and DDI from 36 weeks gestation, 5.4% of the infants were HIV positive. In two studies of ZDV alone, transmission rates were 16.5% and 16.7%. (In the former study, women were breastfeeding.) Another study of ZDV alone did not report transmission rates.

An RCT conducted in the U.S. and France (not displayed) assessed whether NVP resistance would occur after intrapartum NVP (or placebo) in women receiving standard ARV treatment during pregnancy.¹⁰⁵ The study included mostly (62 percent) non clade B women, who were of African origin. 15 percent of the women in the NVP group developed a new NVP mutation at 6 weeks postpartum. The development of a new NVP mutation did not correlate with CD4 cell count or viral load at delivery, or with the type of ARV after delivery. Similar trials were conducted in the developing world; rates of 32 percent and 33 percent for NNRTI resistance were observed in two trials using ZDV monotherapy in the pre-partum period followed by SD-NVP during labor.⁶⁹ In another study⁷⁰ 6.3 percent of women who took ARV followed by SD-NVP showed NVP resistance at a mean of 48 days. A very recent study by Shapiro⁷¹ randomized pregnant women on ZDV-based ARV to either SD-NVP or placebo at birth. 45 percent of the SD-NVP group had NVP resistance at one month post-partum. Importantly, the difference in HIV transmission from mother to child was insignificant between the SD-NVP and placebo groups, suggesting that SD-NVP may be unnecessary in the context of ARV treatment of pregnant women

The World Health Organization (WHO)'s revised guidelines recommend a combination of ZDV and lamivudine (3TC) at the "tail" end (post-partum) be used to reduce the risk of resistance from NVP.¹⁰⁶ We found two trials that support these new guidelines. One prospective randomized trial compared the prevalence of NVP resistance in persons given SD-NVP followed by 0, 4 or 7 days of ZDV plus 3TC.⁴⁶ The rates of NNRTI resistance in these three groups, as detected by standard genotypic resistance assays, were 57 percent, 13 percent, and 9 percent respectively. Similarly, in a non-controlled trial, Chaix et al. found that the rates of NNRTI resistance were less when SD-NVP was followed by the administration of ZDV plus 3TC for 3 days post-partum than had been observed in previous trials of SD-NVP alone.⁶⁸

Recent reports from sub-Saharan Africa indicate that risk of virologic failure (the failure to achieve measurable decreases in serum viral load after treatment) in women initiating ARV after SD-NVP is associated with the interval between receipt of SD-NVP and initiation of full ARV. Lockman⁷² showed that viral response was similar between women who had SD-NVP and those who received no SD-NVP (placebo) if they started treatment more than 6 months after SD-NVP, while viral failure was more common with SD-NVP if ARV was started less than 6 months after exposure. The CD4 response was the same with or without SD-NVP, regardless of time of initiation. Data from Coovadia,¹⁰⁷ where women started ARV a median of 2 years after SD-NVP exposure, found no difference in viral response between women with SD-NVP exposure and those without. Chi⁷³ also found no difference between SD-NVP and no SD-NVP in viral response or CD4 count response. In this study, median time from SD-NVP to ARV initiation was 503 days; 81% started ARV at least six months post-partum. Additionally, several

abstracts have found that SD-NVP is as effective in second pregnancy as in first pregnancy.¹⁰⁸
¹⁰⁹ None of these abstracts reported resistance rates. We strongly suggest that future studies of this nature include resistance testing.

There are currently two RCTs funded by the NIH that will assess response to NNRTI- and PI-based therapy in persons with and without prior SD-NVP exposure. These trials, one in women and one in infants, will add significantly to our body of knowledge on this issue.

Table 10. Other pMTCT regimens, including pre- and post-SD-NPV therapy

Reference	Regimen	N	Clade	When assessed (weeks post-partum)	Type of assessment	New NNRTI	New NRTI
Giuliano 2005 ^{b66}	ZDV+DDI	22	NR	0	Genotypic other, NR	0%*	0%*
Ekpini, 2002 ⁶⁷	ZDV	20	NR	0	Genotypic sequence-based mechanism	NA	0.0%
Sutthent, 2005 ⁶³	ZDV	24 ^d	NR	0	Genotypic sequence-based mechanism	0.0%	0.0%
Sutthent, 2005 ⁶³	ZDV+3TC	21 ^e	NR	0	Genotypic sequence-based mechanism	0.0%	0.0%
Jourdain, 2004 ⁶⁴	ZDV	47	CRF01_AE**	1.5	Genotypic sequence-based mechanism	NA	4.0%
Jourdain, 2004 ⁶⁴	ZDV + SD-NVP	209	CRF01_AE**	1.5	Genotypic sequence-based mechanism	32.0%	5.0%
Chaix, 2005 ⁶⁸	ZDV+3TC+SD-NVP ^a	96	CRF02***	4	Genotypic sequence-based mechanism	1.1%	8.3%
Chaix, 2004 ⁶⁹	ZDV+SD-NVP	74	NR	4	Genotypic sequence-based mechanism	33.0%	NR
McIntyre, 2005 ⁵⁰	SD-NVP	68	NR	6	Genotypic sequence-based mechanism	57.0%	0%
McIntyre, 2005 ⁵⁰	SD-NVP+ 4 days ZDV & 3TC ^c	67	NR	6	Genotypic sequence-based mechanism	13.0%	0%

* 14.5% of participants had NNRTI resistance mutations and 4.5% had NRTI resistance mutations prior to administration of pMTCT regimen; no new mutations emerged in any participant

** 3.5% of participants were infected by other HIV-1 clades

*** An unspecified minority of patients were infected by other HIV-1 clades

**** 4 persons were infected by clade B virus

***** 23% of patients were infected by HIV-1 clades D, 3% by clade C and 15% by recombinants

^a ZDV and 3TC continued for 3 days post-partum

^b All mothers had transmitted HIV-1 to their infants

^c ZDV & 3TC initiated during labor

^d 24 women plus an unspecified number of infected infants

^e 21 (or 28?) women plus an unspecified number of infected infants

^f 48 women plus 8 infected infants

Reference	Regimen	N	Clade	When assessed (weeks post-partum)	Type of assessment	New NNRTI	New NRTI
McIntyre, 2005 ⁵⁰	SD-NVP+7 days ZDV & 3TC ^c	68	NR	6	Genotypic sequence-based mechanism	9.0%	0%
Troyer, 2005 ⁶⁰	ZDV	56 ^f	A****	6	Genotypic other, Radio labeled oligonuc. DNA ligation	0.0%	0%
Lyons, 2005 ⁷⁰	ZDV-based HAART	32	Non-B (69%)	7	Genotypic sequence-based mechanism	6.3%	12.5%
Shapiro, 2006 ⁷¹	ZDV + SD-NVP	155	NR	4	Genotypic sequence-based mechanism	45%	NR
Perez, 2006 ⁶⁵	3TC+ZDV+NVP	20	B/F****	12	Genotypic sequence-based mechanism, Real time PCR	0.0%	5.0%

Measuring the presence of resistance using standard vs. more-sensitive techniques. HIV resistance is usually assessed by performing population-based genetic sequencing of HIV RNA collected from virus found in plasma and determining the presence of specific mutations. These assays are insensitive to minor viral sub-populations that account for less than 20 – 30 percent of circulating virus. In contrast, by the use of specific genetic probes, RT-PCR, allele-specific PCR, or multiple single genomes, it is possible to detect viral subpopulations that constitute as little as 0.1 – 0.2 percent of circulating virus in plasma. Other, more sensitive, assays include tests for the presence of HIV proviral DNA in mononuclear cells.

As shown in Table 11, the rates of NNRTI resistance are consistently greater when more-sensitive assays are employed.^{51,58,65,74} For example, Eshleman first reported rates of NNRTI resistance of 69.2 percent, 36.1 percent, and 19.4 percent, respectively for women infected with clade C, D, and A, 6 to 8 weeks after SD-NVP.⁴⁹ According to Flys,¹¹⁰ Lig Amp assays on the same participants showed that in reality, 69.8 percent, 54.3 percent, and 41.7 percent of women with clade C, D, and A, respectively, had the K103N resistance mutation.

Some of the studies also show the quantitative frequency of viruses with resistance as part of the quasispecies (closely related but non-identical mutant and recombinant viral genomes subjected to continuous genetic variation, competition, and selection; thought to be the mechanism by which RNA viruses persist).¹¹¹ Loubser, 2006⁵⁸ showed that the actual *frequency* of resistant viruses is low and decreases further over time. Twenty seven of 31 women had the K103 N mutation at 6 weeks postpartum, but the median proportion of the virus that contained the K103N variant was 11 percent. At 3 months, in women who still had detectable resistance, the frequency was 6 percent; at 7 months, frequency was 1.2 percent; and at 12 months, the frequency was 0.7 percent. Thus, it is likely that time following SD-NVP exposure is very important in determining response to additional ARV therapy.

Table 11. Standard versus more sensitive tests

Reference	N	Regimen	Clade	When assessed (time post-partum)	NNRTI Resistance	Technique
Loubser, 2006 ^{a58}	66	SD-NVP	C	6 weeks	54.0%*	standard genotyping
Loubser, 2006 ^{a58}	31	SD-NVP	C	6 weeks	87.0% ^{g*}	RT-PCR of plasma RNA
Loubser, 2006 ^{a58}	26	SD-NVP	C	3 months	67.0% ^{g*}	RT-PCR of plasma RNA
Loubser, 2006 ^{a58}	36	SD-NVP	C	7 months	39.0% ^{g*}	RT-PCR of plasma RNA
Loubser, 2006 ^{a58}	53	SD-NVP	C	12 months	11.0% ^{g*}	RT-PCR of plasma RNA
Loubser, 2006 ^{a58}	44	SD-NVP	C	6 weeks	52.0% ^{g*}	RT-PCR of mononuclear DNA
Loubser, 2006 ^{a58}	48	SD-NVP	C	48 weeks	4.2% ^{g*}	RT-PCR of mononuclear DNA
Palmer, 2005 ⁵¹	10	SD-NVP	NR	6 weeks	50%*	standard genotyping
Palmer, 2005 ⁵¹	10	SD-NVP	NR	6 weeks	75% ^{l*}	RT-PCR of plasma RNA
Palmer, 2005 ⁵¹	22	SD-NVP & ZDV+3TC ^b	NR	6 weeks	0%*	standard genotyping
Palmer, 2005 ⁵¹	22	SD-NVP & ZDV+3TC ^b	NR	6 weeks	27% ^{l*}	RT-PCR of plasma RNA
Troyer, 2005 ⁶⁰	71 ^c	NVP	A ^e	6 weeks	16%*	standard genotyping
Troyer, 2005 ⁶⁰	71 ^c	NVP	A ^e	6 weeks	50% ^{l*}	Oligonucleotide DNA ligation assay
Troyer, 2005 ⁵¹	56 ^d	ZDV	A ^e	6 weeks	0%**	standard genotyping
Troyer, 2005 ⁶⁰	56 ^d	ZDV	A ^e	6 weeks	0% ^{l**}	Oligonucleotide DNA ligation assay
Johnson JA, 2005 ^{f13}	50	SD-NVP	C	7 weeks	20%* ^g	standard genotyping
Johnson JA, 2005 ^{f13}	50	SD-NVP	C	7 weeks	52%* ^g	RT-PCR of plasma RNA
Perez, 2006 ⁶⁵	20	ZDV/3TC/NVP	B/F ^h	3 months	0%* ^g	standard genotyping
Perez, 2006 ⁶⁵	20	ZDV/3TC/NVP	B/F ^h	3 months	15%* ^g	RT-PCR of plasma RNA
Eshleman, 2005 ⁴⁹	65	SD-NVP	C	6 - 8 weeks	69.2%*	standard genotyping
Eshleman, 2005 ⁴⁹	97	SD-NVP	D	6 - 8 weeks	36.1%*	standard genotyping
Eshleman, 2005 ⁴⁹	144	SD-NVP	A	6 - 8 weeks	19.4%*	standard genotyping
Flys, 2006 ¹¹⁰	63	SD-NVP	C	6 - 8 weeks	69.8%*	Lig Amp assay

* NNRTI resistance

** NRTI resistance

^a Loubser, 2006 (AIDS 2006; 20:995-1002), full publication of data in Loubser, 2005^b Patients received either 4 or 7 days of ZDV plus 3TC postpartum; substudy of McIntyre, 2005^c 61 mothers + 10 infected children^d 48 mothers + 8 infected children^e 23% of patients were infected by HIV-1 clades D, 3% by clade C and 15% by recombinants^f Substudy of Martinson, 2004^g Detection of K103N resistance mutation only^h 4 persons infected by clade B virusⁱ Detection of K103N or Y181C resistance mutations^j Detection of K70R resistance mutation only

Reference	N	Regimen	Clade	When assessed (time post-partum)	NNRTI Resistance	Technique
Flys, 2006 ¹¹⁰	94	SD-NVP	D	6 -8 weeks	54.3%*	Lig Amp assay
Flys, 2006 ¹¹⁰	144	SD-NVP	A	6 – 8 weeks	41.7%*	Lig Amp assay

Resistance in HIV-positive infants born to mothers receiving pMTCT. Children are often born HIV positive despite pMTCT. Resistant viruses can be transmitted or can emerge independently in either mother or infant. Eight studies^{50,54,55,57,60,62,69,75} evaluated the emergence of ARV resistance in children born to mothers in sub-Saharan Africa who received pMTCT. Most studies examined the impact of SD-NVP. Three studies reported on participants infected by HIV clade C; one other combined data from those with clades A, D, C, and recombinant types. The other studies did not report clade.

We also found a study conducted in Thailand that reported 12-month postpartum resistance rates in children who, themselves, received ART for unspecified lengths of time.⁶³ As the resistance could not be attributed to pMTCT alone, and specific pMTCT regimens were not mentioned, this study will not be discussed.

In most studies, HIV-infected infants were tested for resistance at 6 or 7 weeks of age. Rates of NNRTI resistance among untreated infants of mothers receiving only SD-NVP ranged from 36 percent to 50 percent. The most common mutations were Y181C and K103N.

Martinson⁶² showed that detectable resistance fades over time in infants exposed to SD-NVP. Of the 53 HIV-positive infants studied, 45.3 percent had NNRTI resistance at their first visit (range 4 to 12 weeks). Those with resistance were tested again every 6 months. At 18 months, only one still had the Y181C mutation.

Troyer⁶⁰ compared resistance rates among mother-infant pairs infected with various HIV clades, who were treated with ZDV (N = 48) or SD-NVP (N = 61). The HIV transmission rates were similar in the groups: 16.4 percent and 16.7 percent respectively. Using a sensitive assay for ARV resistance (oligonucleotide DNA ligation assay) at 6 weeks, NNRTI mutations were found in half of the infected pairs who received SD-NVP. ZDV mutations were not present in pairs treated with that drug. Resistance data for mothers and infants were not presented separately.

Chaix⁶⁹ gave mothers ZDV (300mg) twice daily starting at 36 weeks gestation in addition to oral SD-NVP at onset of labor. Only 23 percent of their HIV-positive children were resistant to NRTIs at 4 weeks postpartum. McIntyre⁵⁰ compared resistance among infants who received SD-NVP with those who received SD-NVP plus either 4 or 7 days of ZDV + 3TC after birth. Infected infants who received the additional 7-day ZDV + 3TC showed no resistance at 6 weeks, compared to 78 percent of those who received SD-NVP only.

Finally, Eshleman⁷⁵ recently compared resistance rates in infants who received various combinations of intrapartum SD-NVP, SD-NVP (2 mg/kg) immediately postpartum, and ZDV (4 mg/kg) twice daily for one week. At 6 to 8 weeks, the rate of resistance was much lower (27 percent) in the infants who received the postpartum SD-NVP and ZDV without the intrapartum SD-NVP. The Eshleman study also showed that when the infant received ZDV, the transmission rate did not differ between mothers who received SD-NVP and those who did not. When the baby received SD-NVP plus one week of ZDV and the mother got SD-NVP, transmission was 17 percent and resistance was seen in 74 percent; when the baby got SD-NVP plus 1 week of ZDV and the mother did not get SD-NVP, transmission was 17 percent but resistance was only 27 percent. Data from the MASHI trial also suggest that when the infant receives SD-NVP at birth in the background of ZDV, maternal SD NVP does not provide any additional protection.⁷² As mentioned earlier in this report, Shapiro⁷¹ randomized pregnant women on ZDV-based treatment to either SD-NVP or placebo at birth; the difference in HIV MTCT between the SD-NVP and placebo groups was insignificant at one month.

These findings imply that research should be conducted into the need for maternal SD-NVP when an infant gets NVP at birth, or when the mother receives ZDV regularly before birth.

Table 12. Resistance in HIV positive infants born to mothers receiving pMTCT

Reference	Country	Clade	Regimen	Type of assessment	Resistance in HIV+ infants
Loubser, 2004 ⁵⁴	South Africa	Mostly C	SD-NVP	Genotypic sequence-based mechanism	36% NNRTI resistant at 6 weeks
Gordon, 2004 ⁵⁷	South Africa	C	SD-NVP	Genotypic sequence-based mechanism	40% NNRTI resistant at 6 weeks
Martinson, 2004 & Morris, 2004 ⁵²	South Africa	Mostly C	SD-NVP	Genotypic other; Genotypic sequence-based mechanism	42% NNRTI resistant at 7 weeks; 65% of a subgroup resistant at 6 months
Troyer, 2005 ⁶⁰	Uganda	A, C, D, recombinants	ZDV	Genotypic other, Radio labeled oligonuc. DNA ligation	No resistance at 6 weeks*
		A, C, D, recombinants	SD-NVP	Genotypic other, Radio labeled oligonuc. DNA ligation	50% NNRTI resistant at 6 weeks*
Chaix, 2004 ⁶⁹	Ivory Coast	NR	ZDV + SD-NVP	Genotypic sequence-based mechanism	23% NRTI resistant at 4 weeks
McIntyre, 2005 ⁵⁰	South Africa	NR	SD-NVP	Genotypic sequence-based mechanism	78% NRTI resistant at 6 weeks
		NR	SD-NVP + 4 days ZDV+3TC	Genotypic sequence-based mechanism	13% NRTI resistant at 6 weeks
		NR	SD-NVP + 7 days ZDV+3TC	Genotypic sequence-based mechanism	No resistance at 6 weeks
Eshleman, 2006 ⁷⁵	Malawi	NR	Infant SD-NVP IP SD-NVP	Genotypic sequence-based mechanism	87% NNRTI resistance at 6-8 weeks
			Infant SD-NVP+ ZDV IP SD-NVP	Genotypic sequence-based mechanism	57% NNRTI resistance at 6-8 weeks
			Infant SD-NVP No IP SD-NVP	Genotypic sequence-based mechanism	74% NNRTI resistance at 6-8 weeks
			Infant SD-NVP+ ZDV No IP SD-NVP	Genotypic sequence-based mechanism	27% NNRTI resistance at 6-8 weeks
Martinson, 2006 ⁶²	South Africa	NR	SD-NVP	Genotypic sequence-based mechanism	45.2% NNRTI resistant at 4-12 weeks, only 4.2% resistant at 18 weeks

SD-NVP = Single dose Nevirapine

ZDV = Zidovudine

IP = Intrapartum

* Resistance rates for mothers and infants combined

Predictors of resistance. Several studies evaluated the predictors of ARV resistance among persons receiving SD-NVP. In the most comprehensive analysis, a multivariate analysis performed by Eshleman and colleagues demonstrated an increased risk of resistance detectable by standard genotyping assays in persons infected with clade C vs. clade A or clade D, or increased viral load at delivery, but not with increased age or number of births.⁴⁹ Similarly, Loubser found that an increased viral load was associated with an increased rate of resistance.⁵⁴ Jourdain also showed that plasma HIV RNA level was predictive of likelihood of ARV failure and NPV resistance.⁶⁴

Chaix and colleagues found that increased duration of prepartum use of ZDV+3TC for pMTCT was associated with an increased maternal prevalence of the M184V resistance mutation.⁷⁶ Another study⁵⁰ found a decrease in the rate of NNRTI resistance with longer duration of ZDV+3TC post-partum. In both studies, SD-NVP was given during birth.

The role of adherence in resistance is discussed in the next section.

Adherence and Health Service Issues

Adherence to HIV treatment has long been associated with treatment outcomes. Most studies in the developed world have found evidence that at very low levels of adherence, there is insufficient selective pressure for mutations to evolve. At high levels of adherence, viral replication, and thus, viral evolution, should be stopped. Recently, Bangsberg¹¹² has suggested that the relationship between adherence and resistance differs for the three drug classes (NNRTI, NRTI, and PI). He also suggests that the different half-lives of medications play a significant role (in regard to mutations) when combination therapy is discontinued or interrupted. Thus, health service issues such as unreliable delivery, low stock, and financial constraints are important concerns in the development of ARV resistance in developing regions.

We found three studies that assessed the impact of treatment interruptions on ARV resistance in the developing world; two of these took place in Kampala, Uganda.^{77 78} We also found another African study that assessed the relationship between adherence and the presence of resistance in selected populations.⁷⁹ We found one additional study from the UK that studied black African children with non-clade B virus. Of critical importance, unlike many studies conducted in the developed world, the latter studies were not initially designed or powered to assess the relationship between adherence and resistance.

The first study of treatment interruption included patients attending the HIV clinic at Mulago Hospital, Kampala, from August to December 2003. All patients received a three-drug regimen; most included NVP (77 percent) or efavirenz (14 percent). At a median of 38 weeks, 91 of the 137 patients had undetectable viral loads. Of the 137 patients, 32 experienced an unplanned treatment interruption of more than 4 days. In multivariate analysis, this treatment interruption was statistically associated with virologic failure, as was being treatment-naïve at study entry. The most common reasons for treatment interruption were lack of money to buy medications (63 percent) and toxicity/illness (31 percent). Of the 137 patients, 11 percent reported that, despite having money to buy medications, the medications were sometimes unavailable.

Genotypic and phenotypic data were available from 36 of these patients with detectable viral load. Of these patients, 26 were resistant to NNRTIs; the most common mutation was K103N. NRTI resistance was measured in 23 patients, due to the M184V/I mutation.

The second study in Kampala was a 24-week prospective observational study conducted between September, 2002 and April, 2004. Participants were recruited from pharmacies; they were on a generic fix-dosed combination, including NVP, lamivudine, and stavudine. Adherence was assessed through interviews, electronic medical monitors (EMMs), and unannounced visits to count pills. Treatment interruption was failing to open the EMM for at least 48 hours. Genotypic tests were conducted at 24 weeks. Adherence was good (82 percent - 95 percent) but declined significantly over time. At 24 weeks, 62 (65 percent) of the 95 participants with continuous EMM data had experienced at least one treatment interruption. Of the 33 participants without treatment interruption, none had drug resistance, compared to eight of the 62 participants who did have interruption. The most common mutation was Y181C.

A UNAIDS clinic in the Ivory Coast tested all ARV treatment patients who had a rebound in plasma viral load between August, 1998, and April 1, 2000.⁷⁹ Of these 97 patients, 86 had usable sequences. Thirty eight percent of the patients were female; the majority (89 percent) had A/G recombinant viruses. The rest were clade A, except for one clade G patient. Of the 86 patients, 84 percent had received 2 drug therapies, and 15 percent had received 3 drug therapies that included a PI or NNRTI. Forty percent of the patients had interrupted therapy for at least

one day prior to resistance testing; the mean number of days interrupted was 36. Fifteen percent had switched drug regimens. Fifty percent reported missing a least one dose; the mean number of pills missed was six.

Seventy-nine percent of the 86 patients had genotypic resistance to at least one NRTI, NNRTI, or PI. Phenotypic resistance testing was conducted in 80 patients. Of these, 56 percent were resistant to at least one NRTI, 10 percent were resistant to at least one NNRTI, and 1.3 percent were resistant to PIs. The authors conducted a logistic regression analysis of factors associated with drug resistance in patients with rebound in viral load, by comparing the 80 patients with phenotypic and genotypic resistance to the 6 patients without evidence of resistance. The logistic model included variables for age group, gender, and various treatment characteristics. The variable “skipped pills” was not associated with resistance (adjusted OR = 0.7, 95 percent CI 0.1 to 6.2). Likewise, “interrupt therapy \geq 1 day” was not associated with resistance (adjusted OR = 0.3, 95 percent CI 0.1 to 2.9). The only variables associated with resistance were “use of dual therapy” (adjusted OR = 9.7, 95 percent CI 1.2-81.6) and the patient’s maximum initial plasma viral load response, measured at 30 days (adjusted OR = 2.8, 95 percent CI 1.3-5.9). Given the small sample, confidence intervals are very wide, precluding conclusions about the relationship between adherence and resistance.

In another study, researchers tested 80 patients enrolled in an initiative for access to ARV in Senegal¹¹³ between August, 1988 and February, 2001. The method of selection of these 80 patients (almost equally split by gender) was not reported, except that patients had to have a biological follow-up of at least 6 months. The report also did not describe the degree to which these 80 patients represented the larger population of Senegalese with HIV. Among the patients studied, 68 were treatment-naïve at baseline. HIV clades were primarily recombinant forms. Of the patients, 66 received HAART and 14 received dual therapy. Median length of treatment at resistance testing was 18.4 months for those who were treatment-naïve at baseline and 30.0 months for those who were treatment-experienced. Thirteen of the 80 patients were resistant to at least one NRTI, NNRTI, or PI. In bivariate analyses, the average monthly adherence for previously treatment-naïve patients did not differ significantly between resistant (n=8, 96.5 percent) and non-resistant patients (n=60, 96.0 percent). In patients with prior ARV, adherence was 99.7 percent in both resistant (n=5) and non-resistant patients (n=7). In addition, adverse events that may cause adherence difficulties did not differ. Resistance also did not differ by gender, age, viral load, body mass index, or length of follow-up. Because of the small sample, no strong conclusions can be drawn.

Researchers assessed the association between adherence and drug resistance in 26 children from a clinic in south London who had experienced virological failure between January, 1996, and June, 2000.⁸⁰ These 26 patients were selected from 34 children who experienced one or more virologic failures during this same time and were part of a group of 105 HIV-1 infected children identified in South London. Twenty-four of the children were black African; 23 were infected with a non-B clade virus. Physicians reviewed medical records and estimated adherence for the six-month period prior to the onset of treatment failure. Adherence was categorized as good (>90 percent), intermediate (80-90 percent), or poor (<80 percent).

HIV RNA sequence data were available for 21 of 26 children at treatment failure. Thirty three percent had resistance to PIs; 90 percent had RT mutations. Genotypic resistance was detected in ten of the eleven children who were treated with lamivudine, six of eight treated with NVP, and seven of eleven treated with ZDV. All children whose adherence was classified as

good or intermediate had resistance, compared with five of eight children whose adherence was poor.

In summary, we identified few studies originally designed to assess the effect of health services delivery factors or medication adherence on the development of resistance in patients in developing countries. Results were mixed. Where possible, future research projects on adherence should measure resistance in addition to routinely collected data such as CD4 count and viral load.

Chapter 4. Discussion

Limitations

The primary limitations of this review are the quality and quantity of the available studies. Other limitations related specifically to how the studies were conducted, including heterogeneity with respect to study design, the lack of inclusion of participants infected with more unusual viral clades, and failure to stratify data on treatment-naïve patients by demographic or risk variables.

The quantity of studies we were able to include was limited by the fact that we considered only studies whose results were published or were presented at major scientific conferences. Our search strategies were comprehensive, but it is possible we did not identify all published work. One function of the peer review process is to identify published studies we missed. In addition to the information obtained from published studies, it is possible that substantially more information regarding prevalence and predictors of resistance might have been available via new analyses of existing large datasets such as WATCH or the Stanford University HIV RT and Protease Sequence Database.

The quality of the studies we identified was affected by two types of problems. The first is basic to all epidemiologic studies. Of high importance in studies that are primarily concerned with estimation – as are these studies of the frequency of resistance- is the representativeness of the studied population relative to some larger population of interest. In this case, the larger population of interest is usually something like “all treatment-naïve patients in the country [or some defined geographic area]” or “all patients infected with HIV clade C who have failed to respond to an initial treatment by time T with drugs X, Y, and Z.” Most identified studies either did not state how participants were selected for resistance-testing or spelled out methods that did not allow the reader to understand how representative the studied population was relative to a larger population of interest. Without such information, generalizing the results of identified studies to the inhabitants of a specific region is hazardous. The second problem is the small sample sizes of most of the identified studies, relative to the numbers of patients estimated to have HIV in the countries of interest. Given plausible assumptions regarding variability within populations, data derived from samples of 100 or 200 HIV-infected patients in a country with perhaps millions of infected persons cannot be extrapolated to that whole population with any confidence.

The studies were heterogeneous regarding the timing and frequency of ARV resistance tests, the sensitivity of the assay for ARV resistance, and whether clade-specific analyses of ARV resistance were performed. This makes direction comparisons between groups difficult. The pMTCT studies were also quite heterogeneous in terms of the duration and type of ARV treatment.

Data on HIV clades other than A, B, C, D, or the recombinant types were scarce. Only two studies which stratified results by clade reported data on clades F, G, K, J, and others. The samples were very small for those clades; the largest was 11 persons for clade F. Thus, results should be interpreted with caution.

Finally, many studies of treatment naïve persons did not stratify the resistance data by variables of interest such as gender and mode of transmission, although that data was often

available. That being said, many sample sizes were small enough that stratification would have lacked statistical power to detect real differences.

Conclusions and Recommendations for Future Research

In Latin America, nine studies identified rates of resistance to NRTIs ranging from 2 to 14 percent among treatment-naïve groups. Reported rates of resistance to NNRTIs were low, ranging from zero to two percent. In a handful of studies that included Brazilian and Argentinian populations, PI polymorphisms were common, presumably among persons with HIV clade B. We found no studies of HIV resistance in Central America.

In Africa, studies identified NNRTI resistance rates from 0 percent to 7.7 percent associated with infection with most HIV clades. NRTI resistance rates ranged from 0 percent to 8 percent for all clades. Primary PI mutations were rare (<3 percent) among Africans; polymorphisms were widespread.

We found published data from only three studies in India; these studies varied in their results. As India has a relatively high prevalence of HIV infection, more research on resistance patterns should be a priority. We found only three studies from other developing regions of Asia. None reported NNRTI resistance. NRTI resistance ranged from 4 to 7 percent; primary resistance to PIs ranged from 2 to 3 percent.

Few published studies stratified resistance data by host characteristics such as mode of HIV transmission, risk factors, or gender, although these data were collected in most of the studies. Studies with larger sample sizes would allow meaningful analyses of patterns among groups, for example MSM, heterosexuals, prostitutes, and IDUs. In addition, data on HIV clades other than A, B, C, D, or the recombinant types were too scarce to permit reliable conclusions. In future studies, rare HIV clades should be over-sampled in order to provide statistically meaningful data.

Evidence provided by longitudinal analyses suggests that the prevalence of NNRTI resistance may vary with time since treatment among women taking SD-NVP to prevent mother-to-child transmission of HIV. In one study, fading of variants with Y181C was reported in women infected with clade A but not clade D. Y181C may have less effect on viral fitness of clade D than on A; this observation warrants further investigation in future studies. The impact of the Y181C mutation should also be studied in clades other than D and A.

A randomized comparison of outcomes in women receiving pMTCT with SD-NVP alone, versus SD-NVP followed by a “tail” of 3 or 7 days of ZDV and 3TC found that the prevalence of NNRTI resistance in these three groups was 57 percent, 13 percent, and 9 percent, respectively. In a substudy of this trial, highly sensitive allele-specific RT-PCR assays detected the K103N mutation or Y181C resistance mutations in 75 percent of women receiving SD-NVP versus 27 percent of women receiving SD-NVP plus 3 or 7 days of ZDV and 3TC. The ideal “tail” regimen and duration needs to be researched; drugs other than ZDV and 3TC could be studied in both mothers and infants. The use of a “tail” to prevent resistance needs to be evaluated with SD-NVP alone but also with SD-NVP plus a short course of ZDV, because resistance can occur even when ZDV is given. At least two trials evaluating the use of “tails” are currently in progress.

In one study, women who received either intrapartum SD-NVP or placebo following daily ZDV during their third trimester were put on three-drug ARV postpartum. Intrapartum SD-NVP was statistically associated with virologic failure at both 3 and 6 months, as were NRTI and NNRTI mutations. In another study, in which women were put on three-drug ARV after

intrapartum SD-NVP or placebo, virologic failure was strongly associated with intrapartum SD-NVP among women who started ARV within 6 months postpartum, but not among women who started ARV 6 months or more postpartum. Importantly, several trials suggest that when the infant receives SD-NVP at birth in the background of ZDV, maternal SD-NVP does not provide any additional protection. This finding should be researched further, as should the ideal time to start ongoing postpartum ARV treatment.

We identified few studies designed to assess the effect of health services delivery factors or medication adherence on the development of resistance in patients in developing countries. Where resources permit, studies of adherence in developing regions should conduct resistance testing and report results. In addition, observational studies of HIV treatment in developing regions should ask questions about factors affecting patient access to medication.

Studies using phenotype assessments were rare in developing regions. Funding of such studies is recommended due to their increased specificity. More studies should use ultrasensitive assays to determine not just frequency of resistance as “all or none” but also the quantitative frequency of resistance mutations and types of mutations over time, which may be important in terms of response to therapy. Patients, including mothers and their HIV positive children, should be followed over several years where possible, to assess the extent and quantity of archiving of resistance mutations in cells.

Published data on resistance in developing regions, particularly India, Southeast Asia, and Central America, are scarce. Resistance surveillance programs should be established throughout the developing world. Data should be reported and analyzed in a consistent and timely fashion. To this end, the World Health Organization (WHO) recently launched the Global HIV Drug Resistance Surveillance Network (HIV Resent).

The Stanford University database and the WATCH program based in the Netherlands collect resistance data from all over the world. It is critical that their data be continually updated and analyzed so that trends in resistant mutations can be recognized quickly and addressed. The WATCH program recently reported that only 58 percent of the researchers asked to share individual-level protease and RT sequences agreed. It is crucial that this level of collaboration be improved.

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